The ASCEND-Eye Study

Effect(s) of Aspirin and Omega-3 Fatty Acids on Eye Disease



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Declaration

This thesis is my own work. All primary and secondary sources have been appropriately acknowledged, and permission to use the third-party copyright works was obtained. It has not been submitted to any other institution as part of another academic qualification.

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Acknowledgements and Affiliations

This work was carried out by myself[1] with the help of the people listed below. My role included:

i) the identification and critical appraisal of existing literature;

ii) the preparation and management of all study materials, publications, grant applications and regulatory submissions;

iii) establishing collaborations and organising the data collection from controllers of the NHS Diabetic Eye Screening Programme in England and Wales;

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Thesis Style

This thesis was formatted to conform with the style conventions of Cochrane.org.[1] The date range of named studies is presented at their first mention to orient the reader to the chronology of landmark research or evidence relevant to the thesis. Wherever possible, this is reported as the first year of enrolment to either the end of the follow-up period or the end of a programme of work by some of the larger research groups. I have consistently used the term “arm” when referring to either the aspirin or omega-3 fatty acid (FA) factorial randomisations and “group” in reference to the active or placebo allocations in each arm. Within the literature, the term age-related maculopathy (ARM) is used to describe the presence of early macular lesions, whereas age-related macular degeneration (AMD) is reserved for just the late stages of the disease. In places, I have used the term “senior maculopathy” to mean both ARM and AMD to enhance the writing.[*] For the same reason, in Chapter I, I have used “diabetic retinopathy” and “retinopathy” interchangeably. The Visual Function Questionnaire that was sent to some ASCEND participants consisted of a page of questions that specifically asked about incident diagnoses of serious eye conditions, followed by the National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ-25).[2] When referring to the document that was mailed as a whole, I have used the “VFQ” abbreviation. When referring to the standard instrument, without the additional page, I have used the “NEI-VFQ-25” abbreviation. Finally, in the results chapter, plots for the aspirin arm have a red palette, whereas a blue palette is used for the omega-3 FAs arm.

[*] In older literature, “senile maculopathy” is often used to refer to both ARM and AMD. This is now considered a pejorative word, so I have chosen not to use it here.

Acronyms and Abbreviations

(1)DD	(One) disc diameter
(e)GFR	(Estimated) glomerular filtration rate
(F)FU	(Final) follow-up
($_{LC}$)PUFA(s)	(Long chain) polyunsaturated fatty acid(s)
(M)REC	(Multi-centre)Research Ethics Committee
2P	Two-tailed P value
4HDHA	4-hydroxydocosahexaenoic acid
AA	Arachidonic acid
ACCEPT-D	Aspirin and Simvastatin Combination for Cardiovascular Events Prevention Trial in Diabetes
ACCORD	Action to Control Cardiovascular Risk in Diabetes Study
ACE-inhibitor (or ACEi)	Angiotensin converting enzyme inhibitor
ALA	α-linolenic acid
ALIENOR	Antioxydants, Lipides Essentiels, Nutrition et maladies OculaiRes Study
AMD	Age-related macular degeneration
ARB	Angiotensin II receptor blocker
AREDS	Age-Related Eye Disease Study
ARM	Age-related maculopathy
ARRIVE	Aspirin to Reduce Risk of Initial Vascular Events
ASCEND	A Study of Cardiovascular Events iN Diabetes
ASPREE	ASPirin in Reducing Events in the Elderly
BCVA	Best corrected visual acuity
BDES	Beaver Dam Eye Study
BL	Baseline
BMES	Blue Mountains Eye Study
BMI	Body mass index
BP	Blood pressure
BRAMD	The Bevacizumab to Ranibizumab in Age-Related Macular Degeneration Study
CARDS	Collaborative Atorvastatin Diabetes Study
CAREDS	Carotenoids in Age-Related Eye Disease Study
CARMS	The Clinical Age-Related Maculopathy grading System
CCC	Confirmation of Capacity and Capability
CF	Used to denote a person able to count fingers only during their visual acuity assessment
CFH	Complement Factor H
CHARM	The Cardiovascular Health and its Association with Prevalence and Progression of Age-Related Macular Degeneration Study
CI	Confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CL	Clinical Lead
COMET	Core Outcome Measures in Effectiveness Trials initiative
COSMIN	COnsensus-based Standards for the selection of health Measurement Instruments initiative
COX	Cyclooxygenase

CSMO	Clinically significant macular oedema
CTSU	Clinical Trial Service Unit & Epidemiological Studies Unit, University of Oxford
CVD	Cardiovascular disease
CWS	Cotton wool spots
DAMAD	Dipyridamole Aspirin Microangiopathy of Diabetes
DAP	Data Analysis Plan
DCCT	Diabetes Control and Complications Trial
DESP	Diabetic Eye Screening Programme
DESW	Diabetic Eye Screening Wales
DHA	Docosahexaenoic acid
DKD	Diabetic kidney disease
DM	Diabetes Mellitus
DMC	Data Monitoring Committee
DMO	Diabetic macular oedema
DPIA	Data Protection Impact Assessment
DR	Diabetic Retinopathy
DRS	Diabetic Retinopathy Study
DSA	Data Sharing Agreement
EPA	Eicosapentaenoic acid
ETDRS	Early Treatment Diabetic Retinopathy Study
EUREYE	European Eye Study
FA(s)	Fatty acid(s)
FFQ	Food Frequency Questionnaire
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes
GDPR	General Data Protection Regulation
GP(s)	General Practice(s)/General Practitioner(s)
GPRD	General Practice Research Database
HbA1c	Glycosylated haemoglobin
HDL	High-density lipoprotein
HM*	Haemorrhage(s)
HPFS	Health Professionals Follow-up Study
HR	Hazard ratio
HRA	Health Research Authority
HTRA1	High-Temperature Requirement Factor A1
IARMG	International Age-Related Maculopathy Grading System
ICD-10	International Classification of Diseases 10th revision
ICH	Internal Council for Harmonisation Requirements for Pharmaceuticals for Human Use
IFCC	International Federation of Clinical Chemistry
IQR	Interquartile range
IRAS	Integrated Research Application System
IRMA	Intraretinal microvascular abnormalities
IT	Information Technology

* In Appendix 14, this abbreviation has also been used to denote the perception of hand movements-only during a visual acuity assessment.

JPAD	Japanese Primary Prevention of Atherosclerosis with Aspirin for Diabetes
LA	Linoleic acid
LCI	Lower confidence interval
LDL	Low-density lipoprotein
LOX	Lipoxygenase
M	Maculopathy grade (based on the National Screening Committee criteria for England and Wales)
MA	Microaneurysm(s)
MADIABETES	The Madrid Diabetes Study
Melbourne VIP	The Melbourne Visual Impairment Project
META-EYE	The Meta-Analysis for Eye Disease Study Group
NA	Not applicable
NADPH	Nicotinamide adenine dinucleotide phosphate
NAT-2	Nutritional AMD Treatment-2
NDA	National Diabetes Audit
NEI-VFQ-25	National Eye Institute Visual Function Questionnaire-25
NHANES III	The Third National Health and Nutrition Examination Survey
NHS*	National Health Service
NICE	The National Institute for Health and Care Excellence
NOD	National Ophthalmology Database
NPDR	Non-Proliferative Diabetic Retinopathy
NPL	Used to denote no perception of light during a visual acuity assessment
NSAID	Non-steroidal anti-inflammatory drug
NSC	National Screening Committee
NVD	Neovascularisation of the disc
NVE	Neovascularisation elsewhere
OCR	Optical character recognition
OCT	Optical coherence tomography
OD	Once daily
OPCS	Operating Procedure Codes Supplement
ORIGIN	Outcome Reduction with an Initial Glargine Intervention
P	Photocoagulation grade (based on the National Screening Committee criteria for England and Wales)
PDR	Proliferative Diabetic Retinopathy
PHE	Public Health England
PHW	Public Health Wales
PIC	Participant Identification Centre(s)
PIS	Participant Information Sheet
PL	Used to denote the perception of light only during a visual acuity assessment
PLA2	Phospholipase A2
PM	Programme Manager
POPADAD	The Prevention Of Progression of Arterial Disease and Diabetes Trial
PPAR-γ	Peroxisome Proliferator Activated Receptor-γ

* In Table 6 only, 'NHS' is also used to abbreviate the Nurses' Health Study.

PREDIMED	Primary Prevention of Cardiovascular Disease with a Mediterranean Diet study
PROC	Procedure code within SAS software
PRP	Pan-retinal laser photocoagulation
Pt	Participant
py	Person-years
R	Retinopathy grade (based on the National Screening Committee criteria for England and Wales)
R&D	Research and Development
RBCM	Red blood cell membrane
RCT	Randomised Controlled Trial
RES	Rotterdam Eye Study
REVEAL	Randomised EValuation of the Effects of Anacetrapib through Lipid modification
RMP	Used to denote the retinopathy, maculopathy and photocoagulation grade (based on the National Screening Committee criteria for England and Wales)
RPE	Retinal Pigment Epithelium or Retinal Pigment Epithelial cells
RR	Relative risk (or risk ratio)[*3]
SBP	Systolic blood pressure
SD	Standard deviation
SES	Socioeconomic status
SLB	Slit-lamp biomicroscopy
SOP	Standard Operating Procedure
STDR	Sight-Threatening Diabetic Retinopathy
SubAm	Substantial amendment
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TDS	Three times daily
TIMAD	Ticlopidine Microangiopathy of Diabetes study
TPSA	Third-Party Security Assessment
UCI	Upper confidence interval
UK	United Kingdom
UKPDS	UK Prospective Diabetes Study
UnioOx	University of Oxford
VA	Visual Acuity
VAS	Visual Acuity Score
VECAT	Vitamin E, Cataract and Age-related Macular Degeneration Study
VEGF	Vascular Endothelial Growth Factor
VFQ	Visual Function Questionnaire
VITAL-AMD	The Age-Related Macular Degeneration ancillary study of the VITamin D and omega-3 triAL
WARMGS	Wisconsin Age-Related Maculopathy Grading System
WHI-OS	Women's Health Initiative-Observational Study
yr	Year

[*] RR is also a standard abbreviation for rate ratios; however, within this thesis, RR is consistently used when referring to relative risk (or risk ratio), and rate ratio has always been written in full.[3]

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Abstract

Background

Aspirin and omega-3 fatty acids (FAs) have potential disease-modifying roles in diabetic retinopathy (DR) and age-related macular degeneration (AMD), but randomised evidence of these effects is limited. This thesis describes the rationale, methods and results of ASCEND-Eye; a sub-study of the ASCEND double-blind, 2x2 factorial design, randomised, placebo-controlled trial, which compared the effects of 100mg aspirin daily and, separately, 1g omega-3 FAs daily for the primary prevention of serious cardiovascular events, in 15,480 UK adults with diabetes.

Methods

Events were derived from three sources: 1) six-monthly follow-up questionnaires for ASCEND or a one-off eye questionnaire sent to a subset of participants after the parent trial ended, 2) electronic NHS Diabetic Eye Screening Programme (DESP) data and 3) responses to the National Eye Institute's Visual Function Questionnaire-25 (NEI-VFQ-25). The primary efficacy outcome was the first occurrence of referable disease in either eye during the scheduled treatment period, a composite of referable diabetic retinopathy ($\geq R_2$) or referable diabetic maculopathy (M_1), based on grading criteria defined by the National Screening Committee. The primary safety outcome was the first sight-threatening eye bleed. Secondary outcomes included incident diagnoses of AMD and the NEI-VFQ-25 composite score. Logrank methods were used for intention-to-treat analyses of time until the primary efficacy, safety, and AMD outcomes, between groups in each treatment arm. Ordinal data from the NEI-VFQ-25 were analysed using proportional odds regression methods.

Results

DESP data were obtained for 48% of those randomised in ASCEND (n=7360). During their 6.5 years of follow-up, 539 [14.6%] participants assigned to active aspirin developed referable disease compared to 522 [14.2%] in the placebo group (rate ratio 1.03; 95% confidence interval: 0.91-1.16; P = 0.64). There was no statistically significant difference in the proportion of primary efficacy events by omega-

3 FA allocation. Similarly, neither treatment had a statistically significant effect on incident AMD events (n=15,480) or composite scores from the NEI-VFQ-25 (n=8839); aspirin did not increase the risk of sight-threatening eye bleeds (n=15,480).

Conclusions

ASCEND-Eye overcomes some of the methodological limitations of previous studies, providing reliable, randomised evidence of the eye-related effects of two inexpensive treatments commonly taken by those with diabetes, older people, or both. Overall, the data exclude any clinically meaningful benefits of aspirin or omega-3 FAs for DR or AMD but provide some reassurance regarding the ophthalmological safety of aspirin.

Introduction

The Royal National Institute of Blind People Sight Loss Data Tool represents the largest, freely-available repository of eye health data in the UK.[4] Comprised of administrative, demographic and clinical data from the Office for National Statistics, UK Health Security Agency, and National Health Service (NHS) Digital, as well as modelled estimates based on existing study publications, the compilation is a valuable resource for research, fundraising and service improvement activity, at local, regional and national levels of authority.[4] The most recent available report, which included data up to September 2022, estimated that 4.5 million people live with visual impairment or sight loss, which affects their daily lives.[4] The financial impact this has is substantial. In 2021, NHS expenditure on visual services accounted for 2.9% of the Clinical Commissioning Group's total budget.[5] Combined with some indirect costs, the burden of sight loss to the UK economy was estimated to exceed £36 billion annually.[5]

Diabetic retinopathy (DR) and age-related macular degeneration (AMD) are leading causes of registerable blindness among adults in the UK and elsewhere.[6,7] Both are expected to become more common, in parallel with a growing diabetes prevalence and ageing population, respectively.[4,8,9] Although there are effective interventions to stabilise the retinae of some affected people, many continue to lose their sight.[8,9] Therefore, establishing the ocular risks and benefits of drugs commonly taken by those with diabetes, older people or both and finding new treatments is important. The large, double-blind, 2x2 factorial design, randomised, placebo-controlled ASCEND (A Study of Cardiovascular Events iN Diabetes; 2005-2017) trial provided an opportunity to undertake comparisons of aspirin and, separately, omega-3 FA supplementation on DR and AMD.[10-13] Both are inexpensive therapies with theoretical disease-modifying roles on these conditions; however, randomised evidence of their effects is scarce and inconclusive. The aims of this sub-study, called ASCEND-Eye, are:

i) to determine whether aspirin or omega-3 FAs alter the course of DR or diabetic maculopathy;

ii) to characterise the occurrence and severity of participant-reported eye bleeding events by treatment allocation in the aspirin arm;

iii) to determine the role of aspirin and, separately, omega-3 FAs on incident diagnoses of AMD;

iv) to compare differences between group assignments in each study treatment arm on composite and subdomain scores derived from the National Eye Institute's Visual Function Questionnaire-25 (NEI-VFQ-25)[2];

v) to explore longitudinal between-group differences in visual acuity in each study treatment arm; and

vi) to describe incident reports of other eye diseases in each treatment arm, such as but not limited to cataracts, glaucoma, retinal vein thrombosis, eye infections and ocular nerve palsies.

A separate ambition of ASCEND-Eye was to identify the clinical and demographic characteristics associated with lower composite and subdomain scores on the NEI-VFQ-25 and to consider how concerns about eye health impact activities of daily living, social interactions and emotional well-being. The context for these observational analyses is presented in Appendix 1; however, given the scale of work involved and a desire to focus the narrative on the randomised comparisons only, a pragmatic decision was made to conduct this work as a post-doctoral research project.

Subsequent chapters in this thesis are intended to describe each stage of the research process followed to meet aims i) to vi). The first two chapters comprehensively overview the epidemiology, clinical features and management of DR and AMD, respectively, and situate ASCEND-Eye in the context of four systematic literature reviews (one for each intervention and each eye condition). Chapter III describes the methodologies chosen to answer the research questions, including activities to obtain regulatory approvals, some blinded preliminary analyses of the linkage data, and a detailed Data Analysis Plan. Chapter IV begins with a presentation of the study participants' baseline demographic and clinical characteristics, follow-up duration and completeness, and adherence to study treatments in three discrete analysis cohorts. Tabulations and figures of the main efficacy and safety results follow this. The last chapter summarises the findings and how they relate to existing studies. A discussion regarding the implications of the results for theory development, practice, public policy and future

research, and an appraisal of the strengths and limitations of ASCEND-Eye follow this. The final sections of the thesis include a list of references used in all chapters of this work and appendices, which include the systematic literature search proformas and quality assessments, materials used for data capture, and items relevant to data processing and the analyses.

Chapter I - Diabetes and the Eye

Diabetes mellitus is a global epidemic which causes severe disability and premature mortality in working-age and older people.[14] Three-quarters of those diagnosed with diabetes for more than 20 years develop some form of DR[15]; a condition responsible for 4.8% of the 37 million people who are estimated to be blind worldwide.[16,17] Existing treatments for advanced DR are inconvenient to administer, and their efficacy is short-lasting.[8] Therefore, therapies to delay the onset of disease or to be used alongside current standards of care are needed.[7,8]

This chapter begins with a summary of diabetes and its prevalence, followed by detailed descriptions of the clinical features, epidemiology, health economics, and management of DR. Finally, two systematic reviews on the role of aspirin and, separately, omega-3 FAs for DR are presented.

1.1 Diabetes Mellitus

Diabetes mellitus (henceforth just referred to as diabetes) is a disease in which there is impaired production of- or responsiveness to pancreatic insulin, leading to altered carbohydrate, fat and protein metabolism and chronically elevated blood glucose levels.[18] Traditionally, diabetes has been classified into two main aetiological forms. Type 1 diabetes, responsible for 5-10% of all diabetes diagnoses, is mediated by the autoimmune destruction of beta-islet cells in the pancreas, resulting in an absolute deficiency of insulin and a predisposition towards ketoacidosis.[18] Type 2 diabetes, which accounts for the remaining majority of confirmed cases, is associated with resistance to the effects of insulin, a relative reduction in the secretion of pancreatic insulin, or both.[18] The pathogenesis of type 2 diabetes is not fully understood; however, it occurs more frequently in older people, those who are overweight, women with prior gestational diabetes, those with hypertension or dyslipidaemia, and those of South-East Asian, Hispanic or African American ethnicity.[18] Smoking and over-consuming foods or beverages with a high sugar or saturated fat content also appear to increase the risk.[18]

Diabetes is a common disease. The global prevalence among adults aged 20 to 79 years old in 2021 was estimated to be 537 million (10.5%), but this is predicted to increase to 783 million (12.2%) by 2045

due to the urbanisation of lower-income countries, ageing populations, and the adoption of more sedentary lifestyles.[14,19] Left unmanaged, diabetes is associated with complications that require frequent hospitalisation and may be life-threatening. The latter are typically divided into those which present acutely and those which are chronic. Acute complications include hypoglycaemia, diabetic ketoacidosis, hyperglycaemic hyperosmolar state, recurrent infections and pregnancy-related problems.[14] Chronic complications are usually divided into those affecting the macrovascular circulatory system, including coronary heart disease[20-27], cerebrovascular disease[24,28] and peripheral artery disease[29-33], and those affecting the microvascular system, such as diabetic eye disease (see Sections 1.2 and 1.3), diabetic kidney disease[34-38] and diabetic neuropathy.[39-41] Having diabetes is also associated with a disproportionate risk of premature mortality.[42-47] The economic burden these complications put on national healthcare systems is substantial. In 2021 the total amount spent on healthcare for adults with diabetes worldwide was $966 billion, and a 9.1% growth in expenditure by 2045 has been predicted.[19]

1.2 Diabetic Retinopathy

Every person with diabetes is at risk of developing DR; therefore, the prevalence of this sight-threatening complication is likely to increase in parallel with the growing diabetes epidemic.[8] This section is split into several subsections. Section 1.2.1 provides an overview of the macroscopic and microscopic anatomy of the eye because having a sound knowledge of normal retinal histology and function is essential for understanding the clinical presentations of DR, which is presented in this chapter, and AMD, which is presented in Chapter II. Sections 1.2.2 to 1.2.5 introduce the clinical presentation, pathogenesis, epidemiology, and socioeconomic impact of DR. Finally, Sections 1.2.6 and 1.2.7 outline the NHS Diabetic Eye Screening Programme in the UK and the current approach taken to manage the disease, respectively.

1.2.1 Macroscopic and Microscopic Anatomy of the Eye

The orbital cavity is a pyramidal-shaped bony socket in the midline of the anterior skull in which the eye and its appendages are situated.[48] An intricate arrangement of seven facial bones creates fissures and foramina that permit entry of neurovascular bundles serving the eye and provide attachments and support for extraocular muscles and the lacrimal apparatus.[48] However, it is beyond the scope of this work to describe the gross anatomy of the eye and its adnexa in detail.

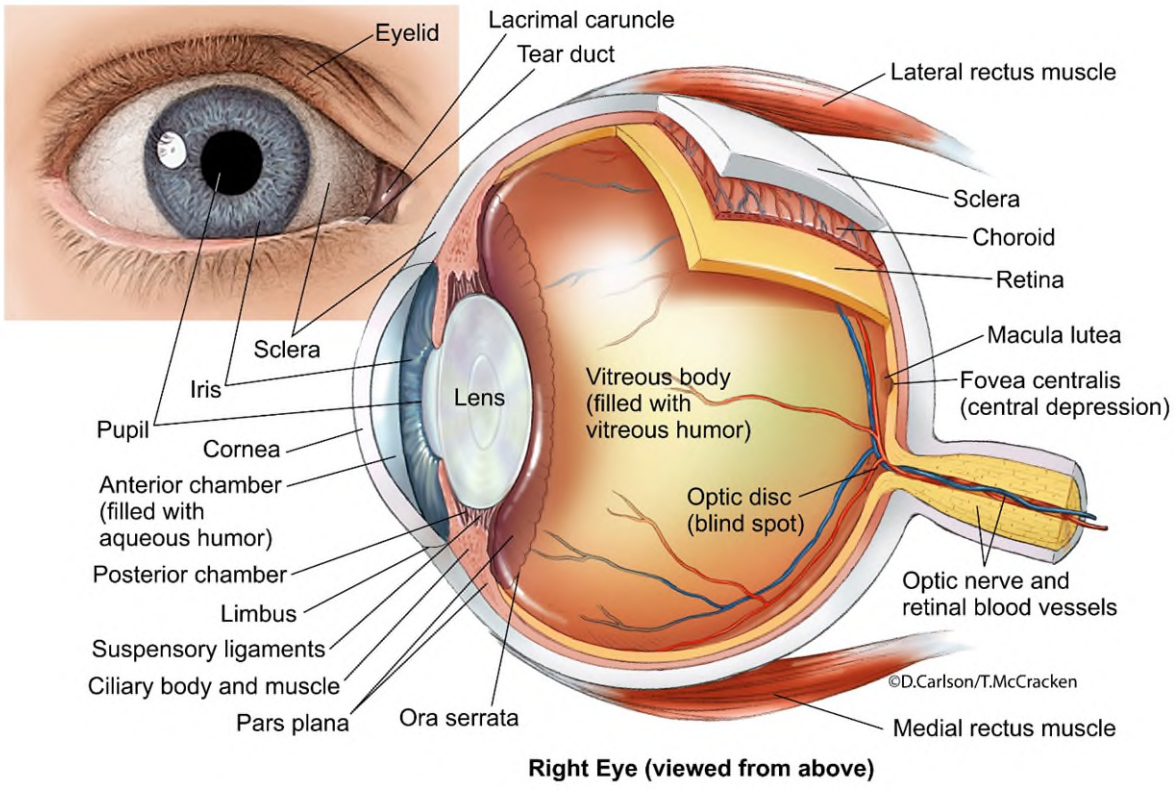
Figure 1 shows the external appearance and corresponding internal cross-sectional structure of the human eye, whose microarchitecture is highly organised and specific to the unique functions of each tissue layer, which are now described.[49] Collectively, the eyeball is described as having three discrete layers of different embryological origin: the sclera, uveal tract and retina.[48] The first, outermost layer of the eyeball, called the sclera, consists of densely packed collagen bundles that provide an attachment for extraocular muscles.[48] Over the anterior curvature of the globe, the sclera merges with the cornea, a transparent membrane in front of the anterior chamber of the eye, the pupil and the lens.[48] The cornea transmits rays of light through the pupil and towards the lens, which focuses them on the retina at the back of the eye.[49] Here, specialist rod and cone photoreceptor cells convert the energy from light into an electrical signal, which gets transmitted via the optic nerve to the brain in a process known as phototransduction (see Section 1.2.1.1).[48]

The next layer beneath the sclera, the uveal tract, is divided into three more areas: the iris, ciliary body and choroid.[48] The iris is a highly innervated, pigmented diaphragm made up of myoepithelial cells, whose contractile properties control the size of the pupillary aperture.[48] The ciliary body is comprised of smooth muscle, which changes the shape of the lens and is continuous with the iris anteriorly, and choroid posteriorly.[48] Finally, the choroid is a vascular layer between the sclera and retina, which carries oxygen and nutrients to, and takes carbon dioxide and spent metabolites away from, retinal pigment epithelial (RPE) cells and photoreceptors.[48] With its high melanin content, the choroid layer also reduces reflections of light that would otherwise distort the images we perceive.[48] The choroid consists of an external layer containing arterial branches of the short posterior ciliary arteries and veins, which coalesce into a network of large vortex veins; a choriocapillaris layer of wide-bore capillaries that drain

into the external layer; and Bruch's membrane.[48] The latter provides physical support for RPE cells and is involved in transferring metabolic waste between photoreceptors and the choriocapillaris.[48] The significance of Bruch's membrane and RPE cells in the pathogenesis of AMD is described in more detail in Chapter II.

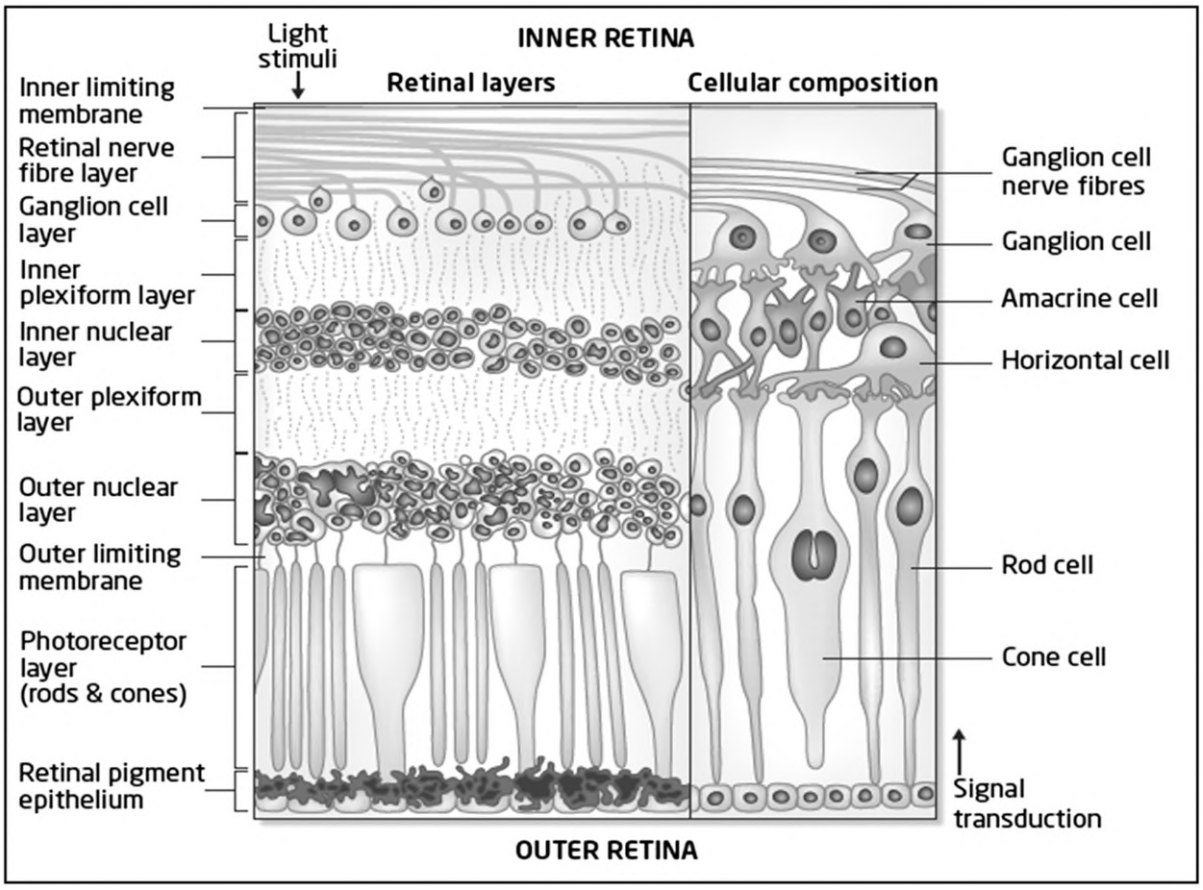
The final layer of the eye is the innermost, light-sensitive retina, which has two main layers from outer to inner: an RPE layer and a neurosensory layer.[48] Some also divide the retina into ten distinct histological layers, which are shown diagrammatically in Figure 2.[50] The constituents of each layer are presented in Table 1.[48] The distinct macula region of the retina and its central foveal depression contains the greatest concentration of photoreceptors, which support the highest level of visual acuity for tasks requiring detailed vision, such as reading, writing, driving and recognising people or objects.[48] Finally, the posterior hyaloid membrane separates the retina from the vitreous body; a chamber between the lens anteriorly and the retina posteriorly that is filled with a clear gel called vitreous humor, which maintains the shape of the eye.

Figure 1 Cross-Sectional Structure of the Human Eye



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Figure 2 Histological Layers of the Retina



Reprinted by permission of Oxford University Press: Saidha S, Syc SB, Ibrahim MA, *et al.* Primary retinal pathology in multiple sclerosis as detected by optical coherence tomography. *Brain* 2011; 134(2): 518-33[50]

Table 1 Summary of the Retinal Microarchitecture

Layer (innermost to outermost)	Description
Internal limiting membrane	The innermost basement membrane separates the retina from the vitreous. It is formed by the terminal expansions of Muller cells (glial cells), which provide metabolic support to other neurones and are involved in the formation of myelin and removal of dead cells.
Nerve fibre layer (stratum opticum)	This layer consists of the axons of the ganglion cells, which conduct electrical impulses through the internal limiting membrane to the optic nerve.
Ganglion cell layer	This layer consists of neurones, called ganglion cells, which receive visual information from the photoreceptors.
Inner plexiform layer	This layer consists of connections between the axons of bipolar cells, dendrites of ganglion cells, horizontal cells and the processes of amacrine cells, which help modify electrical stimulation or convey it laterally across the retina.
Inner nuclear layer	This layer consists of the cell bodies (nuclei) of Muller, horizontal, bipolar and amacrine cells.
Outer plexiform layer	This layer contains synapses between photoreceptor cells and dendrites of horizontal and bipolar cells.
Outer nuclear layer	This layer contains the cell bodies (nuclei) of rod and cone photoreceptor cells.
Outer limiting membrane	This layer has the appearance of a fenestrated membrane that processes of rod and cone photoreceptor cells pass through.
Photoreceptor layer	This layer contains outer and inner segments of rod and cone photoreceptor cells, responsible for sensing light that reaches the retina.
Retinal pigment epithelium (RPE)	RPE cells help to maintain a strict blood-retinal barrier and offer structural support to the outer segments of photoreceptor cells. The outer basement membrane of RPE cells forms the inner layer of Bruch's membrane.

The information in this table was compiled from descriptions of the retinal histology presented in chapter 2 of van Bijsterveld OP. Diabetic Retinopathy. London, UK: Martin Dunitz Ltd; 2000.[48]

1.2.1.1 Phototransduction

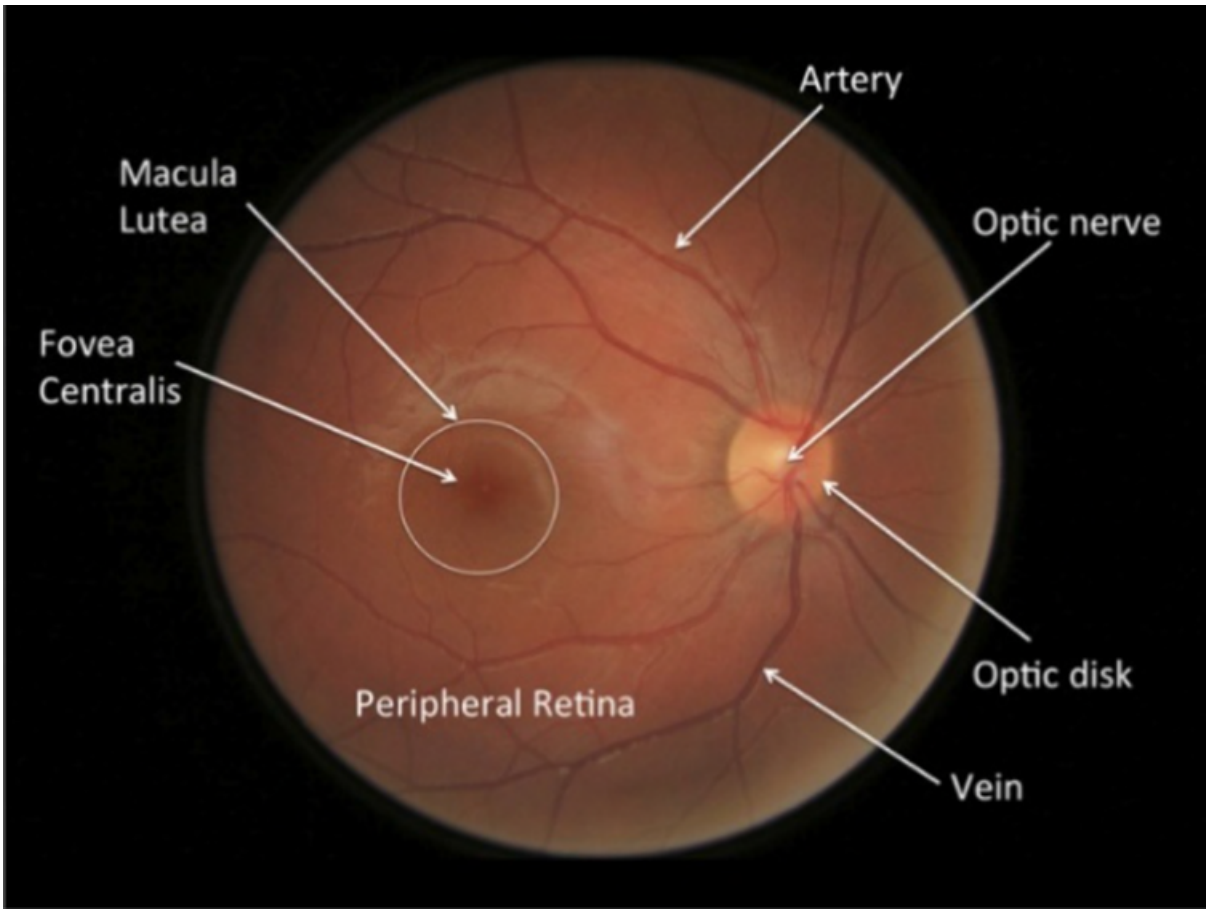
Each photoreceptor contains a pigment comprised of a vitamin-A-derived chromophore, called 11-cis-retinal, and transmembrane apoproteins, called opsins, which transduce different wavelengths and intensities of light.[48] For example, rod cells express rhodopsin, which conveys information about light and dark conditions, whereas cone cells express photopsins, which perceive the red, blue and green light integral to our colour vision.[48] Upon sensing a photon of light, opsins isomerise into active all-transretinoid forms and stimulate a G-protein-coupled receptor called transducin.[48] The latter increases the activity of a phosphodiesterase, resulting in the closure of ion channels and membrane hyperpolarisation.[48] RPE cells recycle all-transretinoids to their 11-cis-retinal forms, then transport them back into photoreceptor cells before the next visual cycle.[48] During the recycling process, photoreceptor cells *in vitro* exhibit highly specialised receptor-mediated uptake and conservation of docosahexaenoic acid, a key derivative of omega-3 FAs (see Section 1.4.2).[51]

1.2.1.2 The Retinal Vasculature

The metabolic demand for oxygen by the retina exceeds that of most other tissues.[48] Up to a third of all retinal blood supply comes from the ophthalmic artery, the first branch of the internal carotid artery, as it emerges through the cavernous sinus.[48] The ophthalmic artery enters the orbit alongside the optic nerve. At the optic nerve head, it branches into a central retinal endartery, which divides into the superior and inferior, nasal and temporal arcades, which are familiar clinically during fundoscopy examinations (Figure 3).[48,52] The remaining two-thirds of all retinal blood flow is derived from the choriocapillaris.[48]

Retinal neurones cannot survive prolonged periods of ischaemia.[48] Therefore, retinal perfusion must be carefully controlled, but paradoxically, blood vessels must also be relatively spaced apart to permit light transmission.[48] In the absence of autonomic input, pericytes with specialist contractile properties surround vascular endothelial cells to autoregulate the retinal blood supply.[48] The relevance of retinal pericytes in the pathogenesis of DR is discussed in Section 1.2.2.

Figure 3 Fundoscopy Image with Labelled Landmarks



Reprinted by permission of Springer Nature. Kumar ES, Bindu CS. Two-stage framework for optic disc segmentation and estimation of cup-to-disc ratio using deep learning technique. *Journal of Ambient Intelligence and Humanized Computing* 2021. Copyright 2021.[52]

1.2.2 Clinical Features and Pathogenesis

DR is a chronic, progressive complication of all aetiological forms of diabetes, characterised by retinal microvasculature damage and neural tissue degeneration.[8] Retinal capillaries comprise an endothelial cell layer interspersed with pericytes and anchored by a basement membrane.[48] In persistently hyperglycaemic environments, either cell type may be damaged via one or more of the following mechanisms[53]:

- Glycation of extracellular matrix materials.
- Direct oxidative injury caused by excessive activation of a polyol pathway in which aldose reductase reduces intracellular glucose to sorbitol, with the consumption of NADPH, an essential cofactor in the metabolic pathway maintaining the antioxidant glutathione.

- Activation of protein kinase C, which subverts gene expression towards the production of pro-inflammatory mediators and vasoconstrictors, and away from vasodilators such as endothelial nitric oxide synthase.
- Dysregulation of glial cells, which allows leukocytes to adhere to the retinal endothelium.

The net effect of these maladaptive changes is apoptosis of retinal pericytes and the vascular endothelium, with resultant outpouching of retinal capillary walls, called microaneurysms, and ischaemic injury to the underlying ganglion cell layer.[53,54] This neurodegeneration may be identified using an imaging modality called optical coherence tomography (OCT), to measure the thickness of the nerve fibre layer in cross-sectional pictures of the fundus.[54] As the disease progresses, further damage to the capillary wall and basement membrane compromises the integrity of the blood-retinal-barrier, leading to extravasation of plasma lipoproteins, fluids and erythrocytes, which are recognised clinically as hard exudates, retinal oedema, cotton wool spots[*], and dot and blot haemorrhages[†].[48] To counter the effects of ischaemia and restore perfusion, there is up-regulation of hypoxia-inducible factor-1 and Vascular Endothelial Growth Factor (VEGF), which promote angiogenesis from venules near the optic disc, and elsewhere in the retina.[48,54] However, the growth of these new vessels is morphologically abnormal, forming along the posterior hyaloid membrane in front of the retinal tissue they are meant to supply.[48] They also loop back to their venous origin without connecting to the arterial side of the circulation.[48] Such vessels tend to be fragile and more prone to bleeding, resulting in haemorrhage into the vitreous body.[53] During the physiological process of resorbing leaked blood, fibrous scar tissue may form between the retina and the vitreous.[53] Tractional forces along this scar tissue may subsequently cause vitreoretinal detachment and visual loss.[8] Sight can also be threatened if oedema or hard exudates localise to the central fovea area, in a specific form of retinopathy called diabetic maculopathy.[8] The latter can present in an eye without other retinopathy features.[8] Therefore, different approaches to grading retinal changes have emerged.[8] The first of these was developed in 1968 when a group of ophthalmologists met in Airlie House, Virginia.[55] Modified versions of their approach were later used

[*] Cotton wool spots represent swelling of ischaemic retinal nerve fibres.[48]
[†] Dot and blot haemorrhages represent microaneurysms that have ruptured into the deeper inner nuclear and outer plexiform layers of the retina.[48]

in two landmark randomised trials[56,57] and are widely accepted as gold standard grading tools in DR research.[8] The modified Airlie House classification grades lesions seen in 7 stereoscopic fields of a fundus photograph and classifies the severity of retinopathy into 13 levels.[8] The Early Treatment Diabetes Retinopathy Study (ETDRS; 1979-1990) group took this a step further by introducing a description of "clinically significant macular oedema (CSMO*)", defined as "*retinal thickening or hard exudates at or within 500µm of the central fovea*."[58] However, the complexity of both classification systems limits their use in routine clinical practice and population screening.[8] Hence, the National Screening Committee (NSC) for England and Wales has adopted a more straightforward approach based on features that a non-Ophthalmologist, but accredited photographic grader, can more reliably identify.[59-61] In the NSC grading system, DR is broadly classified into non-proliferative and proliferative disease stages. Non-proliferative diabetic retinopathy (NPDR) is characterised by clinical signs up to the point of neovascularisation, whereas proliferative diabetic retinopathy (PDR) is defined by abnormal angiogenesis, intraocular bleeding, or both.[59] The NSC classification grades individuals according to separate components of their diabetic eye disease, including the severity of their retinopathy (R grade), maculopathy (M grade), and the presence or absence of photocoagulation burns (P grade). The clinical features of each grade and their equivalence to the ETDRS classification system are shown in Table 2.[60,61] Reference is made to these RMP grades later on in the thesis.

* Or CSME if the American spelling of oedema (edema) is used.

Table 2 Feature-Based Grading System used by the National Screening Committee in England and Wales and its Corresponding ETDRS Term

NSC grade	International Term (ETDRS Term)	Clinical Features	Treatment Pathway*
Retinopathy Grade (excluding the macula)			
R_0	No DR (No DR)	Normal retina	Annual rescreen
R_1	Mild NPDR (background DR)	Microaneurysms and intraretinal microvascular abnormalities (IRMAs)	Annual rescreen
R_2	Moderate-severe NPDR (pre-proliferative DR)	Extensive microaneurysms, hard exudate, IRMAs, blot haemorrhages, cotton wool spots, venous beading	Refer to Ophthalmology
R_{3s}	PDR (stable treated PDR)	No haemorrhages, exudates or new vessels and evidence of treated disease	Annual rescreen or Digital Surveillance pathway
R_{3a}	PDR (active PDR)	Neovascularisation of the optic disc (NVD) or elsewhere (NVE), vitreous haemorrhage, glaucoma and/or retinal detachment	Refer to Ophthalmology
Maculopathy Grade			
M_0	No maculopathy	No diabetic maculopathy	Annual rescreen
M_1	Diabetic maculopathy	Macular oedema within 500μm of the foveal centre, hard exudates within one disc diameter (1DD) of the fovea, retinal thickening within 1DD of the fovea, or a group of exudates that is greater than or equal to half the disc area inside the macular field. The macular field includes the area of retina within a circle that originates from the fovea at its centre to the temporal edge of the optic disc.	Refer to Ophthalmology
Photocoagulation Status			
P_0	No photocoagulation	Photocoagulation scars absent	-
P_1	Photocoagulation	Photocoagulation scars present	-

*Please also refer to Figure 4 in Section 1.2.6, where the care pathways followed by NHS Diabetic Eye Screening Services in England and Wales are described in more detail.

1.2.3 Risk Factors

Landmark studies spanning several decades have increased our understanding of the risk factors for DR and its clinical management. These findings are also relevant to the stratification of baseline characteristics in ASCEND-Eye, specified in Chapter III.

1.2.3.1 Duration of Diabetes

The Wisconsin Epidemiologic Study of Diabetic Retinopathy (1979-2004) compared the effect of diabetes duration on the severity of DR in people with type 1 and type 2 diabetes.[15] In both cohorts, more advanced eye disease was strongly correlated with longer durations of diabetes.[15] Early changes were seen 5-10 years after diagnosis in those with type 1 diabetes, nearly all of whom had some degree

of retinopathy and maculopathy after 20 years.[15] Whilst lesions were uncommon at the time of diagnosis of type 1 diabetes, they were more frequently present in those with newly-diagnosed type 2 diabetes, reflecting a more insidious onset of this aetiological form.[15]

1.2.3.2 Glycaemic Control

The Diabetes Control and Complications Trial (DCCT; 1983-1993) assessed the effect of randomisation to intensive glycaemic control, compared with standard control, on microvascular outcomes in 1441 adults with type 1 diabetes.[34] Allocation to intensive control, in which the mean glycosylated haemoglobin (HbA1c) was 7.3% (56 mmol/mol), was associated with a significant 47% reduction in incident NPDR and, separately, improved renal and neuropathy outcomes, compared to those in the conventional glycaemic control group, in which the mean HbA1c was 9.1% (76 mmol/mol).[34] DCCT was stopped early, after 6.5 years of follow-up, upon the advice of an independent Data Monitoring Committee, because of these overwhelming benefits.[34] Similar results were reported for people with newly-diagnosed type 2 diabetes in the United Kingdom Prospective Diabetes Study (UKPDS; 1977-1999; n=5102; median follow-up =10 years), which had a similar design.[62] In both trials, retinal lesions initially worsened before any benefits emerged in the intensive glycaemic control groups.[34,62]

1.2.3.3 Type of Diabetes

The Meta-Analysis for Eye Disease (META-EYE) collaboration compared the prevalence and major risk factors for DR in a pooled analysis of individual participant data (n=22,896) from 35 population-based studies conducted in the US, Europe, Australia and Asia that were published between 1980 and 2008.[63] After adjusting for race, hypertension, hypertension duration, HbA1c and diabetes duration, prevalence estimates for any DR that were directly age-standardised (per 100 population) to the 2010 World Diabetes Population aged 20-79 years, were higher in those with type 1 diabetes (77.3%; 95% CI 25.0-25.4%) compared to those with type 2 diabetes (25.2%; 95% CI 25.0-25.4).[63] Type 1 diabetes was also found to be an independent risk factor for DMO.[63] Similarly, a more recent Cochrane systematic review of prospective cohort studies associated type 1 diabetes, particularly those with larger

central retinal vein diameters, with an increased risk of progression from NPDR to PDR.[64] This finding is consistent with studies evaluating haemodynamic changes of the retina before and after the onset of overt disease.[65] These showed arteriolar vasoconstriction in early DR followed by compensatory arteriolar and venular dilation to hyperperfuse the ischaemic tissue.[65] It has been suggested that a loss of autoregulatory capacity happens earlier in people with type 1 diabetes for reasons that are still uncertain, which hastens the progression of DR to PDR or DMO.[65]

1.2.3.4 Blood Pressure

Hypertension is another important risk factor for the development of DR. In an embedded sub-study of UKPDS, called the Hypertension in Diabetes Study (n=1148), there was a statistically significant slower deterioration of the disease and preserved visual acuity, in those assigned to strict control of their blood pressure (BP), whose mean BP was 144/82 mmHg, compared to those with more relaxed control, whose mean BP was 152/87 mmHg.[62] Conversely, the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Eye study (2003-2006; n=1263) did not find the same benefits of intensive BP control on their composite endpoint of incident laser photocoagulation, vitrectomy, or an increase of 3 or more steps on a DR severity scale defined by the ETDRS research group (odds ratio (OR) 1.23, 95% confidence interval (CI) 0.84-1.79, P = 0.29).[66] However, over four years of follow-up, the median systolic BP in their intensive group was 117 mmHg compared to 133 mmHg in the standard treatment group.[66] Thus, better BP control in the standard treatment group, compared to UKPDS, could explain these inconsistent findings.

Following UKPDS and ACCORD-Eye, angiotensin was identified as a pro-angiogenic growth factor in the retina.[8] Hence, there has been an interest in renin-angiotensin inhibitors to manage individuals with diabetes and high BP.[8] To date, randomised controlled trials have reported slower DR progression in normo- or microalbuminuric adults treated with angiotensin converting enzyme (ACE)-inhibitors[67,68], but not angiotensin receptor blockers (ARBs).[69] Based on this evidence, people with diabetes and retinopathy are advised to maintain their systolic BP below 130 mmHg using an ACE-inhibitor.[8]

1.2.3.5 Diabetic Kidney Disease

Diabetic kidney disease is independently associated with DR and macula oedema, irrespective of the duration of diabetes or glycaemic control.[8] Hypertension secondary to renal impairment can also cause retinopathy to deteriorate further.[8] Large placebo-controlled trials have shown that sodium-glucose cotransporter-2 inhibitors[70-72], and the non-steroidal mineralocorticoid receptor antagonist finerenone[73,74], reduce the risk of progression to end-stage kidney failure in people with diabetic kidney disease. However, the effect this has on DR is yet to be studied.

1.2.3.6 Sex

The risk of DR is generally not considered to be influenced by sex, although pregnancy and the first year post-partum are associated with a higher risk of disease progression in diabetic women with pre-existing retinal changes, which might be why, in the UK, more women are registered as severely sight impaired (legally blind) due to diabetes than men.[8]

1.2.3.7 Age

Children below 12 years of age rarely develop DR, but rising growth hormone levels during puberty have an adverse effect on insulin sensitivity, and hence retinal lesions may start to appear.[8] However, older people with diabetes have a much higher risk of the disease overall, reflecting a longer history of exposure to hyperglycaemia and other cardiovascular risk factors.[8]

1.2.3.8 Ethnicity

Differences in insulin resistance, anthropometric measures, genetics, access to healthcare, or both, may impart a greater risk of diabetes and associated complications like DR to certain ethnic groups.[75] For example, type 1 diabetes predominantly affects people of European ancestry, whereas type 2 diabetes is more common in South Asian, African and Caribbean island populations.[75] In the UK, the prevalence of sight-threatening retinopathy and maculopathy is higher in non-White persons, especially those with

a lower socioeconomic status.[76] A reluctance to integrate hospital eye services with primary health care or specialist diabetes clinics has been cited as a barrier to tackling these inequalities.[7,76]

1.2.3.9 Lipid Modification

Lipid modification has been studied as a possible way to reduce the extravasation of lipoproteins, which become hard exudate deposits in diabetic retinae.[77] The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD; 1998-2005) randomised placebo-controlled trial was designed to compare the effect of fenofibrate on cardiovascular outcomes in 9795 men and women aged 50-75 years old with type 2 diabetes.[78] In a pre-specified analysis of a tertiary endpoint, allocation to the active treatment was associated with a significantly lower requirement for incident laser therapy compared with the placebo (164 [3.4%] *vs.* 238 [4.9%]; hazard ratio (HR) 0.69, 95% CI: 0.56-0.84; P = 0.0002).[78] Similarly, two other fenofibrate trials have also reported trends towards a beneficial effect.[66,79] However, no statin trials have found any benefits of low-density lipoprotein (LDL)-cholesterol lowering on retinopathy outcomes.[8] This could mean fibrates mediate their protective effects directly within the eye instead of modifying systemic lipid concentrations.[8] Randomised trials designed to compare the effects of fenofibrate on progressive DR as their primary outcome are ongoing.[80,81]

1.2.3.10 Smoking and Alcohol

Cigarette smoking has not been identified as a strong risk factor for developing DR; however, persons with diabetes who smoke are advised to stop, given its harmful effects on cardiovascular health in general.[8,82,83] There is no consensus regarding the effects of alcohol consumption on the disease.[8]

1.2.3.11 Ocular History

In proliferative DR, the posterior hyaloid membrane acts as a scaffold for new vessels to grow onto (as described in Section 1.2.2).[48] Therefore, individuals with a posterior vitreous detachment are less likely to develop pathological angiogenesis.[8] By contrast, having a cataract operation has been associated with

worsening maculopathy and retinopathy, but other risk factors, such as age, may confound this observation.[8]

1.2.4 Epidemiology

A systematic review of population-based studies of eye disease, published in 2021, estimated the worldwide age-standardised prevalence and causes of severe visual impairment and blindness between 1990 and 2020.[84] The leading causes in adults over 50 years of age were cataracts (45.5% of all cases; 15.2 million age-standardised prevalence; 95% CI 12.7-18.0), followed by glaucoma (11.0%; 3.6 million; 95% CI 2.8-4.4), under-corrected refractive errors (6.6%; 2.3 million; 95% CI 1.8-2.8), AMD (5.6%; 1.8 million; 95% CI 1.3-2.4) and finally, DR (2.5%; 0.9 million; 95% CI 0.6-1.2).[84] Although DR may not be the biggest contributor of sight loss on a global level, it is arguably the most important one to address. Of the leading five conditions, DR was the only one becoming more common because of the growing prevalence of diabetes and improved life expectancies of those with diabetes.[84] Unlike cataracts, glaucoma and refractive errors, which are remedied with singular operations, medication, glasses, or both, DR uses a disproportionate amount of highly-trained resources to monitor the disease at regular intervals and administer laser therapy, intravitreal injections or surgery when necessary.[84] It also afflicts economically active, working-age adults, whereas the other leading causes of blindness tend to affect retired, older persons more.[84] The analyses did not consider younger individuals or less severe visual impairment due to the sparsity of available data. However, a separate pooled analysis of the participant-level data from 35 population-based studies conducted in the US, Europe, Australia and Asia (n=22,896), estimated that approximately one-third of all individuals with diabetes develop DR in their lifetime, of whom 10% have sight-threatening disease.[63] Therefore, finding new treatments to prevent advanced disease is imperative.

1.2.5 Socioeconomic Impact in the UK

4.9 million UK adults were estimated to have diabetes in 2022, equivalent to one in 14 people.[85] From a separate study conducted in the same year, 1.3 million lived with DR, of whom 9.5% had proliferative disease.[4] A 9% increase in these figures is expected by 2032.[4] DR is also responsible for 8% of approximately 24,000 people registered legally blind or partially sighted in England and Wales each year.[86] The socioeconomic burden of which is considerable. The average lifetime cost of managing DR, including income losses, in the UK has been estimated to be £237,000 per person.[87] However, timely detection of early lesions can reduce the need for expensive therapies used to treat proliferative disease.[87] Such compelling statistics lead to investment in a screening programme as part of the National Service Framework for Diabetes (see Section 1.2.6).[88] In 2017, the annual cost of delivering the NHS Diabetic Eye Screening Programme (DESP) in England was estimated to be £85.6 million, equivalent to approximately £40 per person per screening examination.[88] Although some economic evaluations suggest that the interval between screening assessments could be safely extended in low-risk individuals[87,89], it remains a demonstrably cost-effective service compared to no screening.[90]

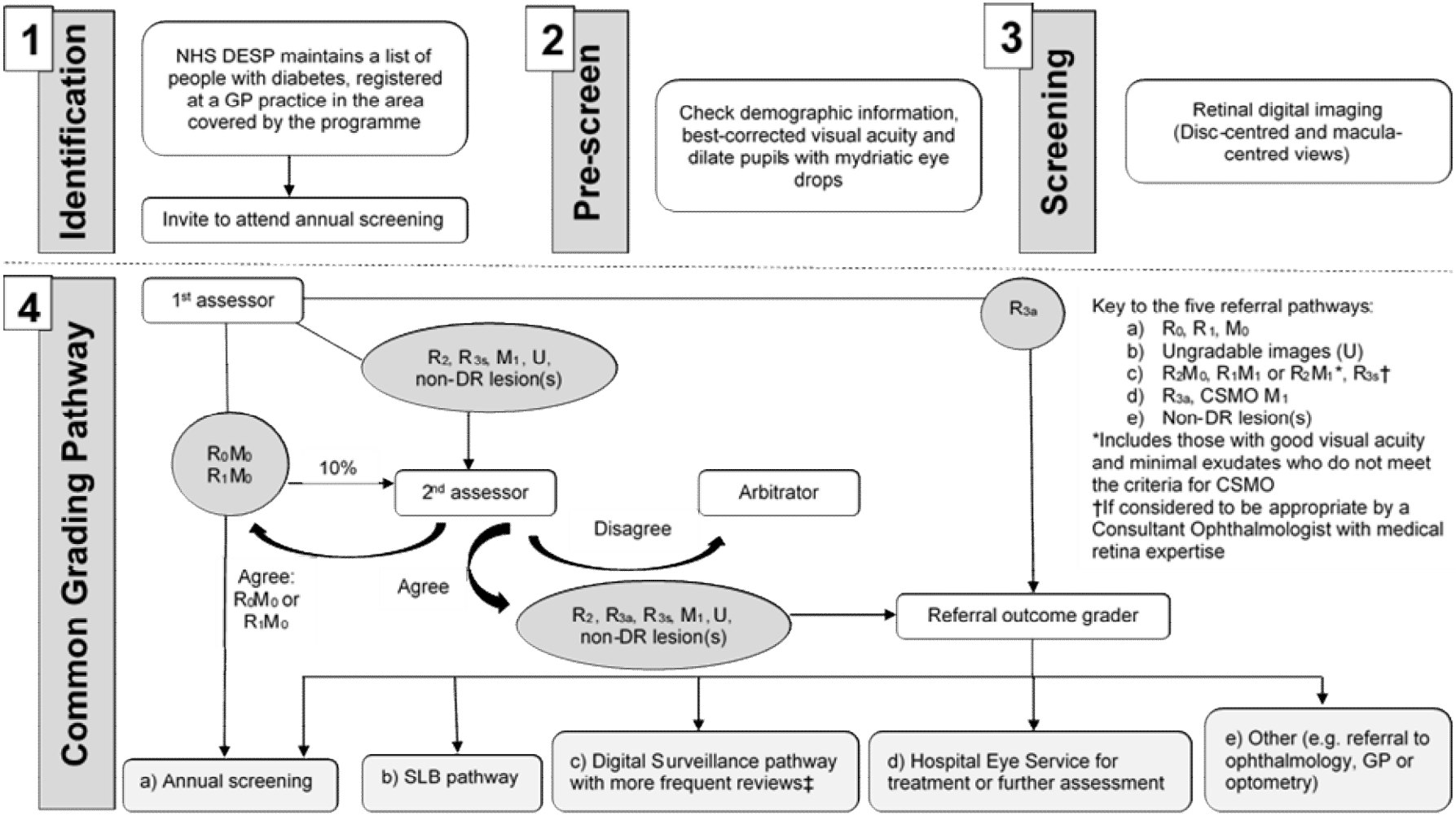
Diabetic retinopathy has wide-ranging consequences beyond the direct financial costs to health and social care services.[7] Visual impairment may force an early retirement or career change, reducing earning potential and increasing reliance on state-provided programmes or family members for financial assistance.[91] It might also limit the capacity to safely execute everyday tasks such as cooking, cleaning, driving, socialising and other recreational activities.[91] Finally, by hampering the ability to monitor blood glucose levels or read information about how to take medications safely, sight loss is detrimental to the management of diabetes and other comorbidities more generally.[91] Such losses of economic and personal autonomy have profoundly detrimental consequences for both the physical and emotional health of people with diabetes, and therefore, preserving vision is essential.[8]

1.2.6 Screening

A pivotal moment in the history of DR followed the publication of two key randomised trials. The Diabetic Retinopathy Study (DRS; 1971-1975)[56] and ETDRS[57] showed that intervening at an earlier

stage with pan-retinal laser photocoagulation (PRP) could reduce the risk of visual loss in those with proliferative disease and diabetic macular oedema (DMO), respectively. In response, individuals from the International Diabetes Federation, the World Health Organisation and a multidisciplinary team of Ophthalmologists, government officials and patients met to discuss the feasibility of initiating screening services in their representative countries.[8] Following a successful pilot of mobile fundoscopy units, a national programme of diabetic eye screening was introduced in England, Scotland and Wales in 2003.[8] Currently, all GP-registered persons aged 12 years and above with diabetes are invited to attend an annual screening appointment.[8] The latter entails digital retinal photography or slit-lamp biomicroscopy through a dilated pupil.[8] Images are graded using standardised coding practices by highly-trained and quality-controlled graders, as defined by the NSC.[88] Those with no retinopathy or background DR (graded R_0 or R_1, respectively) or no maculopathy (M_0) maintain a yearly screening schedule, whereas those with pre-proliferative or proliferative lesions (graded R_2 or $R_{3a/s}$, respectively) or maculopathy (M_1) are referred to care pathways overseen by a Consultant Ophthalmologist with medical retina expertise for further assessment and treatment.[59,61] Since 2015, individuals who do not meet treatment thresholds for retinopathy or CSMO have been referred to Digital Surveillance clinics for more frequent review; only those who may require an intervention are referred to Hospital Eye Services.[92,93] Figure 4 gives an overview of the care pathways currently followed by NHS Diabetic Eye Screening Services in England and Wales from end-to-end. The diagram, designed by the author, was based on guidelines published by Public Health England.[59,92]

Figure 4 Care Pathways in the NHS Diabetic Eye Screening Service in England and Wales



CSMO = clinically significant macular oedema; NHS DESP = NHS Diabetic Eye Screening Programme; SLB = slit-lamp biomicroscopy
‡Pregnant women with diabetes are also referred directly into the Digital Surveillance pathway.
Routine digital screening is suspended in those referred to the Digital Surveillance pathway or Hospital Eye Services. However, their data is recorded on the same screening software [personal communication with Professor Peter Scanlon].

1.2.7 Management

Stringent control of cardiovascular risk factors and screening for early retinal changes are proven to reduce the risk of blindness in people with diabetes.[88] Those referred to Hospital Eye Services for progressive disease are likely to be treated according to guidelines produced by the International Council of Ophthalmology.[94] The three mainstays of treatment include laser photocoagulation, intraocular pharmacotherapy and vitrectomy, which are now discussed.

1.2.7.1 Treatment of Diabetic Retinopathy

As described in Section 1.2.6, DRS showed that administering PRP earlier could reduce visual loss in those with proliferative disease.[56] Making thermal burns to an ischaemic retina reduces the production of pro-angiogenic mediators and causes newly-formed vessels to regress, thereby lowering the risk of intravitreal bleeding and tractional membranes.[56] Since it was first introduced, PRP has been associated with a higher risk of DMO, and damage to posterior ciliary nerves, which causes pain, mydriasis and impaired visual accommodation.[95] Therefore, adjuncts and alternatives to PRP have been researched, including biological agents targeting VEGF. A few small, open-design, non-inferiority, randomised trials have reported favourable outcomes from monthly injections of ranibizumab (Lucentis), aflibercept (Eylea) or bevacizumab (Avastin), compared with PRP, but all three drugs are expensive, their efficacy is short-lasting, they are inconvenient to administer, and each injection confers a risk of endophthalmitis and raised intraocular pressure.[95-97] Trials of anti-VEGF therapies to sham injections in NPDR are ongoing.[98,99] Therefore, the current standard of care in those with high-risk non-proliferative or proliferative disease is laser therapy within three months from the screening date.[94] However, injectable agents may also be given to those with persistent neovascularisation, or if that is the patient's preference[*].[94]

[*] Earlier treatment may be necessitated in those with poor visual potential in the fellow eye, those who are non-adherent with insulin or oral hypoglycaemic, lipid-modifying or blood pressure medications, pregnant women and those with impending cataract operations.[92]

1.2.7.2 Treatment of Diabetic Maculopathy

The British Multicentre Study Group (1970-1974)[100] and ETDRS[57] studies reported that laser photocoagulation could reduce visual loss secondary to DMO in those with co-existing PDR and pre-treatment visual acuities of 6/12 or worse. Those with better visual acuity or fovea-sparing oedema did not benefit as much. Therefore, adjunct and alternative treatments have been considered, including intravitreal injections of the corticosteroid triamcinolone or anti-VEGF drugs.[101-109] Triamcinolone was found to be superior to laser therapy in the short-term, but its long-term administration imparts a greater risk of cataract formation and raised intraocular pressures.[110,111] Therefore, the current guidelines recommend that those with DMO without central foveal involvement should receive monitoring-only.[94] Those with central-involved oedema should be offered intravitreal anti-VEGF monotherapy, such as ranibizumab, bevacizumab, aflibercept or faricimab.[94,112] Individuals whose eyes show persistent retinal thickening on OCT after 24 weeks of anti-VEGF therapy should then be offered focal laser therapy, and triamcinolone is to be reserved as a salvage therapy in pseudophakic eyes refractory to laser treatment.[94] Most recently, there has been interest in slow-release biodegradable intravitreal implants of anti-VEGF drugs for managing DMO.[94] However, reviewing those in detail is beyond this work's scope.

1.2.7.3 Indications for Vitrectomy

Vitrectomy should be considered in those with a sight-threatening vitreous haemorrhage that does not clear spontaneously after three months, those with tractional or rhegmatogenous retinal detachments, and those with vitreomacular traction causing diffuse macular oedema*.[94,113]

1.3 Other Ocular Complications of Diabetes

Having diabetes may exacerbate or be causally associated with several other eye diseases, including cataracts, refractive errors, rubeosis iridis and rubeotic glaucoma, retinal detachment, retinal vein occlusion and ischaemic optic neuropathy, corneal epitheliopathy, ocular motor nerve palsies and delayed healing after ocular surgery.[8] However, it is beyond the scope of this work to describe these in detail.

1.4 Systematic Literature Reviews

Although effective treatments and screening for DR are widely available, achieving adequate control of cardiovascular risk factors is not always possible.[114] Therefore, a significant proportion of people with diabetes continue to lose their sight.[114] Hence, there is a need to research other treatments that could be used alongside existing standards of care to prevent DR and preserve vision. The ASCEND randomised controlled trial provided an opportunity to undertake comparisons of aspirin and, separately, omega-3 FA supplementation on DR.[10-13] To contextualise the importance of this work, the

* A retinal detachment is said to have occurred when the neurosensory retina is pulled away from the supporting RPE layer. There are three types of retinal detachment: tractional, rhegmatogenous and exudative (or serous). In tractional retinal detachment, contractures along scar tissue, which DR often creates, separate the retinal tissue layers. In rhegmatogenous retinal detachment, vitreous humour translocates behind the retina via a tear in the retinal surface. In exudative or serous retinal detachment, fluid accumulates in the subretinal space between the neurosensory retina and its supporting structures.
In younger eyes, microscopic fibres attach the vitreous body to the retina. As we age, the vitreous shrinks, imparting tractional forces along these fibres through to the retina. The vitreous body and retina might also separate entirely, termed a "posterior vitreous detachment". In some people, the vitreous continues to adhere over the macula region; further traction may cause macular holes, puckering or swelling (termed "cystoid macular oedema"). [Information in this footnote was compiled from the Royal National Institute of Blind People's Eye Condition Summaries. Available at: https://www.rnib.org.uk/your-eyes/eye-conditions-az (accessed 20/04/2023)][111]

author conducted two systematic literature reviews, first in February 2018 and updated in July 2022. Four databases were systematically searched for articles written in English, with no limits on publication year, up to July 2022, using standardised criteria (a detailed search proforma and list of studies excluded after they were assessed for eligibility are presented in Appendices 2 and 3, respectively). Briefly, randomised controlled trials, meta-analyses and observational studies that evaluated the effects of aspirin and, separately, omega-3 FAs on incident DR, diabetic maculopathy, visual acuity, change in the size, count or morphology of lesions associated with DR, safety outcomes and vision-specific quality of life, were included in the review. Experimental and narrative review articles were also sought to broaden an understanding of the topic, but these have not been tabulated. The initial search identified 484 abstracts for the aspirin review and 439 for the omega-3 FAs review. Studies were then systematically excluded according to pre-specified criteria. Consort diagrams[115] and tabulations from each search are presented at the end of this chapter[*]. The methodological quality of each study was assessed using the Downs and Black instrument (Appendix 4)[†].[116] A core outcome set, representing the endpoints deemed most important to stakeholders for a specific health condition, was sought for DR but none was found.[117] The resultant heterogeneity between the definition of outcomes and event ascertainment from only a few studies precluded meta-analyses of the existing data. Therefore, Sections 1.4.1 and 1.4.2 are narrative reviews only. Establishing a core set of outcomes for DR and standardising the way they are measured, as recommended by the Core Outcome Measures in Effectiveness Trials (COMET) [117] and COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN)[118] initiatives, respectively, would improve the quality of evidence produced by future studies and help clinicians make better recommendations for their patients. The same argument applies to the systematic literature review for AMD, presented in the next chapter.

[*] Figures 6-9 were based on the PRISMA 2020 Flow Diagram template in: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al.* The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71[113]

[†] The systematic review did not include the recommended dual review process. Single screening by the author alone was deemed sufficient, given the lack of randomised evidence identified from a preliminary literature search.

1.4.1 Aspirin and Diabetic Retinopathy

Aspirin has been considered of potential benefit for DR because of its anti-thrombotic and anti-inflammatory properties, which also confer its therapeutic applications for cardiovascular protection and rheumatoid pain management.[119] However, aspirin is also associated with a higher risk of bleeding, which raises concerns about its safety when taken by those with proliferative disease.[120,121] The proposed mechanisms and evidence of aspirin's effects on DR are now described.

One of the earliest recognisable features of DR is retinal capillary occlusion, which results in areas of non-perfusion and microaneurysm formation in neighbouring vessels.[53] There follows the cascade of maladaptive changes described in Section 1.2.2. Therapies to stop this triggering event are needed and have long been sought. Previous studies have reported increased platelet adhesiveness in people with diabetes, which is thought to be due to higher circulating concentrations of pro-aggregating arachidonic acid metabolites, such as prostaglandin E2 and thromboxane A2.[122,123] As well as conferring a higher risk of cardiovascular events, this may also increase the risk of blood clots within the eye. Indeed, two small but detailed post-mortem studies found more platelet thrombi[124], and a higher immunostaining affinity for platelet glycoprotein IIIa receptors[125], in the retinal capillaries of diabetic individuals compared to controls. Therefore, if abnormal platelet activity were involved in the early development of DR, it was hoped that the anti-platelet effects of aspirin would be a beneficial treatment for the disease.[121] In support of this hypothesis, in dogs rendered diabetic, the use of aspirin for five years was found to reduce microaneurysm formation compared with untreated controls.[126] A small case series in humans (n=34) also observed a lower incidence of retinal lesions in individuals with arthritis and diabetes who took salicylates compared to those with diabetes-only who did not.[127]

Others have been interested in the anti-inflammatory effects of aspirin on the retina. In healthy eyes, the blood-retinal-barrier separates neural tissue of the retina from the rest of the circulation, conferring protection from systemic immune cells and cytokines.[128] In diabetes, a milieu of pro-inflammatory prostanoids and eicosanoids upregulates the expression of endothelial adhesion molecules, facilitating leucocyte binding to retinal capillary walls, which results in their injury or apoptosis and subsequent ischaemia of the underlying neural tissue.[128,129] Such mediators derive from the action of

cyclooxygenase-2 on arachidonic acid, an enzyme pathway that is irreversibly blocked by aspirin.[119] In four rodent models, administering aspirin was associated with lower inflammatory markers, preservation of the blood-retinal-barrier, and a delayed onset of DR.[128,130-132] However, there is no guarantee that what works in mice or dogs will work well in humans, and currently, that evidence is much more limited.

A large cohort study, which followed 73,964 individuals with type 2 diabetes for five years using the Taiwanese National Health Insurance Research Database, associated the use of dual anti-platelet agents or anti-platelet plus anti-coagulant therapy for more than 28 days with a lower risk of incident NPDR.[133] In sensitivity analyses, in which the contribution of aspirin in combination with either clopidogrel, ticlopidine, dipyridamole or warfarin was compared, aspirin had a significant protective effect at $P<0.01$, compared to those who had not been exposed to aspirin or the comparator drug. However, there were no analyses in which aspirin alone was compared to those without exposure to any of the five anti-thrombotic drugs. Therefore, this study has not been included in Table 3. Similarly, the effects of aspirin on the risk of intraocular bleeding could not be isolated from other anti-platelet or anti-coagulant therapies in two other observational studies.[134,135] Hence, these have not been tabulated either.

The Madrid Diabetes Study (MADIABETES; 2007-2011) sought to examine risk factors for the development of DR in a cohort of 3443 adults over 30 years of age with type 2 diabetes recruited from general practices across Spain.[136] Demographic, clinical and biochemical information about each participant was recorded at a baseline interview.[136] DR events were ascertained using annual slit-lamp biomicroscopy over four years of follow-up.[136] A higher proportion of those who developed DR were concurrently taking aspirin compared to those who did not develop the disease: 117 out of 194 [60.1%] individuals with DR *vs.* 1123 out of 2211 [50.8%] with no DR; HR 1.65; 95% CI 1.22-2.24; $P<0.001$.[136] However, the regression model was not adjusted for major cardiovascular events. In two subsequent cross-sectional studies, with approximately 2000 participants in each, which did make this adjustment, there was no independent association between aspirin and DR.[137,138] Thus, aspirin use is more likely to be an indicator of cardiovascular disease, which often co-presents with DR.[8] All three observational studies lacked detail regarding their definition of aspirin exposure, and all may have had residual

confounding from unmeasured or unknown variables that were not included in the analysis model. Hence, their ability to estimate causality was limited.

Despite extensive randomised data on the effects of aspirin on cardiovascular disease[139], only two trials with very different study designs have specifically reported on DR outcomes. The ETDRS study randomised 3711 men and women to 650mg of aspirin daily or a matching placebo and followed them up clinically at a study visit every four months[*], and photographically with annual fundus imaging.[140,141] In their 2x2 factorial design, one eye of each participant was randomly assigned to immediate photocoagulation and the other to deferred laser therapy if high-risk proliferative features of a composite primary endpoint developed. The latter was defined as the first occurrence of neovascularisation of the optic disc or neovascularisation elsewhere, with or without a vitreous or pre-retinal haemorrhage.

ETDRS selected individuals who were likely to progress to advanced disease. At baseline, 84% of the participants had been diagnosed with diabetes (30% type 1) for more than ten years, 44% were current smokers, and 59% had DR equivalent to R_2 disease or higher.[141] In addition, the majority had poor blood pressure and glycaemic control compared with modern standards; 86% exceeded the treatment target for systolic blood pressure of 130mmHg, and 30% had an HbA1c greater than 10% (85.8 mmol/mol).[141]

The effects of aspirin were analysed in eyes assigned to early versus deferred photocoagulation separately. Overall, the incidence of high-risk proliferative disease and bleeding was lower in eyes treated earlier. Although data for early-lasered eyes were not shown, the proportional effects of aspirin were reported to be consistent with findings in deferred eyes, which are now described. Over seven years of follow-up, allocation to aspirin did not reduce progression to proliferative disease, which occurred in 756 [40.7%] participants in the active treatment group and 768 [41.4%] in the placebo group (relative risk (RR) 0.97; 99% CI 0.85-1.11).[140] An incident pre-retinal or vitreous haemorrhage occurred in 585 participants [32%] assigned to aspirin and 564 [30%] assigned to the placebo; a χ^2 test of these proportions was not statistically significant (P = 0.48).[142] Further analyses were conducted to see whether aspirin exacerbated the extent and duration of bleeding in the eye. Eye bleeds were categorised

[*] Each follow-up visit in ETDRS entailed an assessment of best-corrected visual acuity and an ophthalmoscopy examination by a Consultant Ophthalmologist.[136,137]

into those covering an area less than the size of the optic disc, more than one disc area but less than half the macular field, or over half the macular field.[142] The severity of bleeding across this grading scale was also assessed using a χ^2 test.[142] Overall, there were no significant differences between the treatment groups (P for linear trend = 0.12).[142] Similarly, there were no significant differences in the cumulative rate of blood reabsorption after a bleed between those assigned to aspirin compared to placebo: RR of persistent blood (Cox proportional hazards model) 1.00; 99% CI 0.8-1.3; P = 0.86.[142] Finally, there was no increased risk of intraocular haemorrhage in those assigned to aspirin compared with the placebo, in a pre-specified analysis of the subgroup who developed neovascularisation (n=666): 190 [58.6%] *vs.* 203 [59.4%], respectively; RR 1.05; 99% CI 0.81-1.36.[140]

In contrast to the efficacy findings in ETDRS, the Dipyridamole Aspirin Microangiopathy of Diabetes (DAMAD; 1976-1984) reported favourable effects of aspirin.[143] In this double-blind trial, 475 people were randomised to aspirin alone (330mg taken three times daily), aspirin in combination with dipyridamole, or a matching placebo, for three years.[143] The primary endpoint was based on the mean number of microaneurysms per year seen in the macular field of fluorescein angiograms.[143] Those who took aspirin alone, or dual anti-platelet therapy, had marginally fewer microaneurysms per year than those in the placebo group (0.69 ± 5.09 *vs.* 1.44 ± 4.49), but significance testing was not performed for this comparison, and it was not an intention-to-treat analysis; data were excluded for 11.6% of the cohort who were lost to follow-up.[143]

The findings in DAMAD were consistent with the similarly designed Ticlodipine Microangiopathy of Diabetes Study (TIMAD; 1979-1985) of another anti-platelet drug, ticlopidine, which may provide some proof of concept for an anti-thrombotic mechanism of protection.[144] Both DAMAD and TIMAD primarily sought to establish the feasibility of using microaneurysm counts as a new, objective endpoint in early DR, and hence, neither study followed their participants beyond the on-treatment periods. However, others have since correlated larger numbers of microaneurysms to a higher risk of developing sight-threatening maculopathy later on, which might be relevant.[145-147]

Despite their contradictory results, ETDRS and DAMAD may be complementary. Whereas participants in DAMAD were required to have minimal retinal changes at baseline, those in ETDRS had to have at

least non-proliferative disease. DR tends to take an unremitting course after it has begun.[15] Therefore, aspirin may have been administered too late for it to benefit those with more advanced disease in ETDRS. In contrast, the findings in DAMAD were consistent with the experimental studies described earlier, which concluded that aspirin was more likely to be effective when initiated at an earlier stage.[124,125,127-131] However, DAMAD was underpowered to determine whether aspirin slows the progression of retinopathy in the long term because the number of people who took part was too small, and they were followed up over a relatively short period. Hence, a larger study, conducted in the context of contemporary standards of care for glycaemic and blood pressure control, with a longer follow-up and clinically-meaningful endpoints, is needed to confirm or refute the effects of aspirin in those with less severe DR.

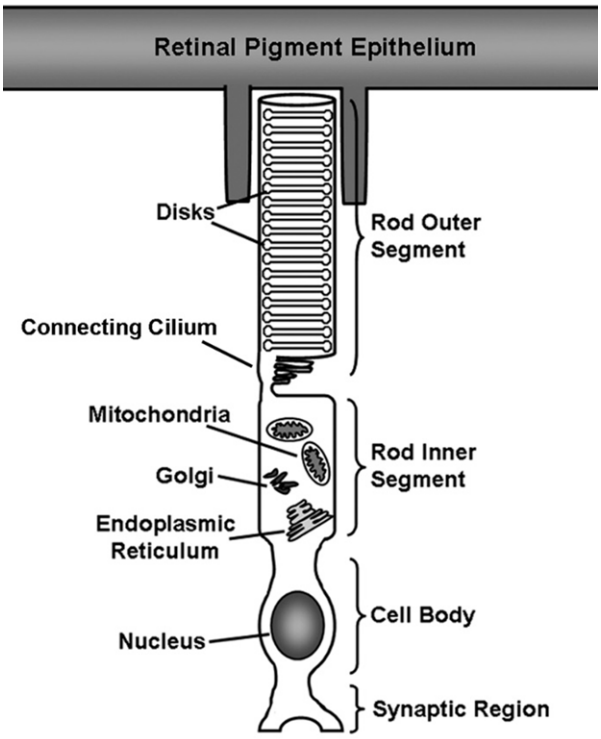
1.4.2 Omega-3 Fatty Acids and Diabetic Retinopathy

Although humans can synthesise saturated FAs and some monounsaturated FAs from carbohydrates and proteins, they lack the Δ-15 and -12 desaturase enzymes required to insert more than one double bond near the methyl (or omega) terminal of a FA hydrocarbon chain.[51] Thus, omega-3 and omega-6 FAs are essential nutrients that can only be obtained from dietary sources such as vegetable oils, nuts, oily fish and egg yolks.[51] The parent compounds of the omega-3 and omega-6 FA families are α-linolenic acid (ALA) and linoleic acid (LA), respectively.[51] Once consumed, humans can elongate ALA or LA chains into compounds with a structural role in cell membranes and functional roles in immune regulation.[51] The main derivative of LA is arachidonic acid (AA), whereas eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are synthesised from ALA.[51]

Figure 5 is a schematic showing the structure of a photoreceptor cell relative to the RPE layer of cells.[148] After its absorption in the small intestine and further metabolism by the liver, DHA is delivered to the RPE via very low-density lipoproteins and chylomicrons in blood flowing through the choriocapillaris.[51] Specialised cytoplasmic transport systems, binding proteins and receptors, then transfer DHA to the outer segments of photoreceptor cells.[51,149] The latter contain multiple membranous shelves called disks, which support visual pigments such as rhodopsin and are replaced every ten

days.[149] During the phagocytosis of spent visual pigment and disks by RPE cells, a dedicated pathway ensures that DHA is preferentially recycled and highly conserved.[149,150] As such, the retina is said to have a uniquely high composition of long-chain polyunsaturated fatty acids ($_{LC}$PUFA(s) or PUFA(s)).[51,149,150]

Figure 5 Photoreceptor Structure



Reprinted by permission of Informa UK Limited, trading as Taylor & Taylor & Francis Group, http://www.tandfonline.com: Anne R. Murray, Steven J. Fliesler & Muayyad R. Al-Ubaidi. Rhodopsin: The Functional Significance of Asn-Linked Glycosylation and Other Post-Translational Modifications, Ophthalmic Genetics © copyright 2009[148]

In contrast to other tissues, which mainly use lipids for energy or structural purposes, there is amassing evidence supporting a role for omega-3 and omega-6 FAs in visual transduction pathways, photoreceptor differentiation and survival, and protection against oxidative stress.[151-155] Our eyes are intermittently exposed to adverse conditions such as high oxygen tensions, excessive light exposure, and systemic inflammation.[153] A ubiquitous response to these stressors is the activation of phospholipase A_2 (PLA_2), which releases free omega-3 and omega-6 FAs from retinal cell

membranes.[153] Free FAs activate cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 oxidase enzymes, which catalyse the synthesis of mediators with localised vasoactive and immunogenic actions.[51] In general, AA-derived eicosanoids are said to have pro-inflammatory, pro-coagulant, or vasoconstricting effects on the local microarchitecture, whereas ALA-derived docosanoids tend to be anti-inflammatory, vasodilating or anti-angiogenic.[51] The composition of retinal membranes determines which free FAs are available as substrates in these processes.[51] Hence, it has been hypothesised that supplementing omega-3 FAs could subvert lipid pathways towards the production of more protective docosanoid intermediaries.[150,156]

The specific mechanisms through which the bioactive metabolites of omega-3 FAs could modulate retinal neovascularisation in DR were first described by Sapieha and his colleagues in 2011.[157] Diets rich in omega-3 FAs were fed to genetically altered mice that were deficient in one of four enzymes involved in their conversion into vasoactive compounds: COX-1, COX-2, 5-LOX and 12/15-LOX.[157] Hyperoxia was used to induce retinopathy in developing mouse pups, a technique that causes lesions said to be analogous to DR.[157] Loss of COX-1, COX-2, or 12/15-LOX activity did not affect the extent of retinal neovascularisation in the knockout mice compared to their wild-type controls.[157] By contrast, the 5-LOX knockout mice developed more severe proliferative disease, suggesting this enzyme mediates the anti-angiogenic effects of omega-3 FAs.[157] Importantly, by ruling out the contribution of other enzymes, concurrent use of aspirin, as in ASCEND's 2x2 factorial design (see Chapter III), with its inhibitory effects on the COX enzymes, is not expected to interact with the potential benefits of omega-3 FAs.

The model designed by Sapieha *et al.* specifically showed that 5-LOX adds a hydroxyl group to DHA, forming 4-hydroxydocosahexaenoic acid (4HDHA), which, in turn, activates the peroxisome proliferator-activated receptor-γ (PPAR-γ) in RPE cells.[157] PPAR-γ is thought to directly inhibit endothelial cell proliferation, increase matrix metalloproteinase inhibitor activity, and lower VEGF expression.[157] The oral anti-diabetic, insulin-sensitising thiazolidinediones are agonists for PPAR-γ, and hence research into their effects on DR may be relevant to omega-3 FAs. To date, intraocular injections of thiazolidinediones have been shown to reduce neovascularisation in animal models[158] and human tissue *in vitro*[159], but this has not translated into clear results *in vivo*.[66,160,161] However, thiazolidinediones

are not first-line therapies for diabetes, thus using one may be a surrogate indicator of worse glycaemic control, which itself confers a higher risk of PDR.[34,62]

PPAR-γ activation is but one of the proposed mechanisms through which omega-3 FAs might mediate a beneficial effect in DR. In animal models of streptozocin-induced DR, omega-3 FAs also reduced the production of inflammatory markers associated with the disease progression, such as tumour necrosis factor-α, angiopoietin-2 (Ang-2) and interleukin-6, as well as decreasing the expression of intercellular adhesion molecules (such as ICAM-1, VCAM-1 and endothelial selectin), and leucocyte integrins (such as very late antigen-4, integrin α4β1 and β2).[51] Hence, it has been hypothesised that supplementing omega-3 FAs would be a useful adjunct to current interventions for DR; most of which are only given after the presentation of retinopathic features, and all incur substantial costs and unwelcome complications (see Section 1.2.7). The evidence supporting this approach is limited but is now discussed.

One cross-sectional study used multivariate regression techniques to compare energy-adjusted PUFA intake per day estimated using a food frequency questionnaire (FFQ), with the presence and severity of DR on fundus photography in adults with well-controlled (n=123) and poorly-controlled diabetes (n=256).[162] After adjusting for age, sex, glycosylated haemoglobin, mean arterial blood pressure, and duration of diabetes, those with well-controlled diabetes (HbA1c < 7.0%) had an inverse association between PUFA intake and the presence of DR (OR 0.18; 95% CI: 0.06-0.59), and severity of DR (the OR for PDR relative to no DR per PUFA consumption adjusted for total energy intake, was 0.13; 95% CI 0.02-0.74; significance testing for trend across subgroups with no DR, NPDR or PDR was not performed).[162] In those with poorly-controlled diabetes, there were no significant effects (see Table 4).[162] Overall, the possibility of residual confounding from variables not included in the analysis model and the study's small size limits the utility of these results.

Studies in which the effects of omega-3 FAs on DR were specifically isolated are scarce, and their findings are inconsistent. A post-hoc observational analysis of the Primary Prevention of Cardiovascular Disease with a Mediterranean Diet (PREDIMED; 2003-2013) trial[163] (n=3482) found that participants from either of its three dietary arms, who consumed at least 500mg of omega-3 FAs per day, or two or more servings of oily fish per week, based on an FFQ, had a 48% proportional

reduction in their risk of sight-threatening DR compared with those who ate less (27 *vs.* 42; HR 0.52; 95% CI: 0.31-0.88; P = 0.001)*.[164] However, the estimate was imprecise because there were too few events. In addition, results from the main PREDIMED trial were retracted and later republished after serious irregularities were identified during an audit regarding the legitimacy of its randomisation and subsequent analyses.[165] Therefore, the DR results might also be less reliable.

As for aspirin, despite several large trials to assess the effects of omega-3 FA supplementation on cardiovascular outcomes, none have reported specifically on DR outcomes.[166-176] The 2x2 factorial design, Outcome Reduction with an Initial Glargine Intervention (ORIGIN; 2005-2012) trial, randomised 12,536 people with type 2 diabetes, impaired fasting glucose or impaired glucose tolerance to 1g omega-3 FA ethyl ester capsules† daily or a matching placebo and, separately, to titrated basal insulin glargine *versus* standard care, and followed them up for a median of 6.2 years for the secondary development of major vascular events.[172,177,178] Although incident blindness, laser photocoagulation or vitrectomy for DR or DMO were components of a pre-specified composite microvascular (kidney and eye disease) efficacy outcome, randomised comparisons have only been published for the basal insulin arm.[177]

The only randomised evidence of omega-3 FA supplementation on DR comes from the RETIPON trial (2008-2011), in which 467 adults with insulin-treated type 2 diabetes, NDPR and microalbuminuria >30 mg/l were assigned to 600mg ALA daily or a matching placebo.[179] The primary outcome was the first detection of CSMO‡ on fundus photographs taken every six months.[179] When CSMO was found, both treatment groups were stabilised on a loading schedule of intravitreal ranibizumab, with *pro re nata* maintenance injections thereafter.[179] During two years of follow-up, 26 out of 235 [11.1%] participants in the active treatment group and 30 out of 232 [12.9%] participants in the placebo group developed CSMO: HR 0.91; 95% CI: 0.54-1.53; P = 0.71.[179] However, RETIPON was underpowered to detect small, but potentially important treatment effects. In addition, the trial selected individuals

* Participants of the PREDIMED trial were assigned to one of three diets: a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts, or a control diet in which they were advised to reduce their saturated fat intake.[159] Sight-threatening diabetic retinopathy was defined as any DR requiring laser photocoagulation, vitrectomy, anti-angiogenic therapy, or both.[160]

† Containing 465mg EPA and 375mg DHA.

‡ As defined by the ETDRS trialists (see Section 1.2.2).

with a high risk for developing CSMO and therefore, the results may not generalise to diabetic populations with a lower risk. Finally, although stereoscopic fundus photography is the current gold standard imaging modality for CSMO, OCT may perform better in some individuals, but this was not included in the trial protocol.[180]

Overall, the conceptual role of omega-3 FAs has led to the commercialisation of supplements intended to maintain or increase retinal DHA in those with diabetes or to help other diseases of the medical retina such as AMD, retinitis pigmentosa and retinopathy of prematurity. However, more reliable, large-scale, randomised evidence is needed to justify their use.

1.5 Chapter Summary

In this chapter, the clinical features of DR and the landmark trials which informed its current management were presented in the context of a growing diabetes epidemic. Two systematic literature reviews identified potential disease-modifying effects of aspirin and, separately, omega-3 FAs on the disease and a need for more reliable randomised evidence. Capitalising on the ASCEND trial provides a sufficient sample size and follow-up duration to make these comparisons in a population with contemporary standards of glycaemic and blood pressure control. ASCEND-Eye also represents the first large-scale, prospective test of omega-3 FAs on DR.

Figure 6 Aspirin and Diabetic Retinopathy Systematic Literature Search

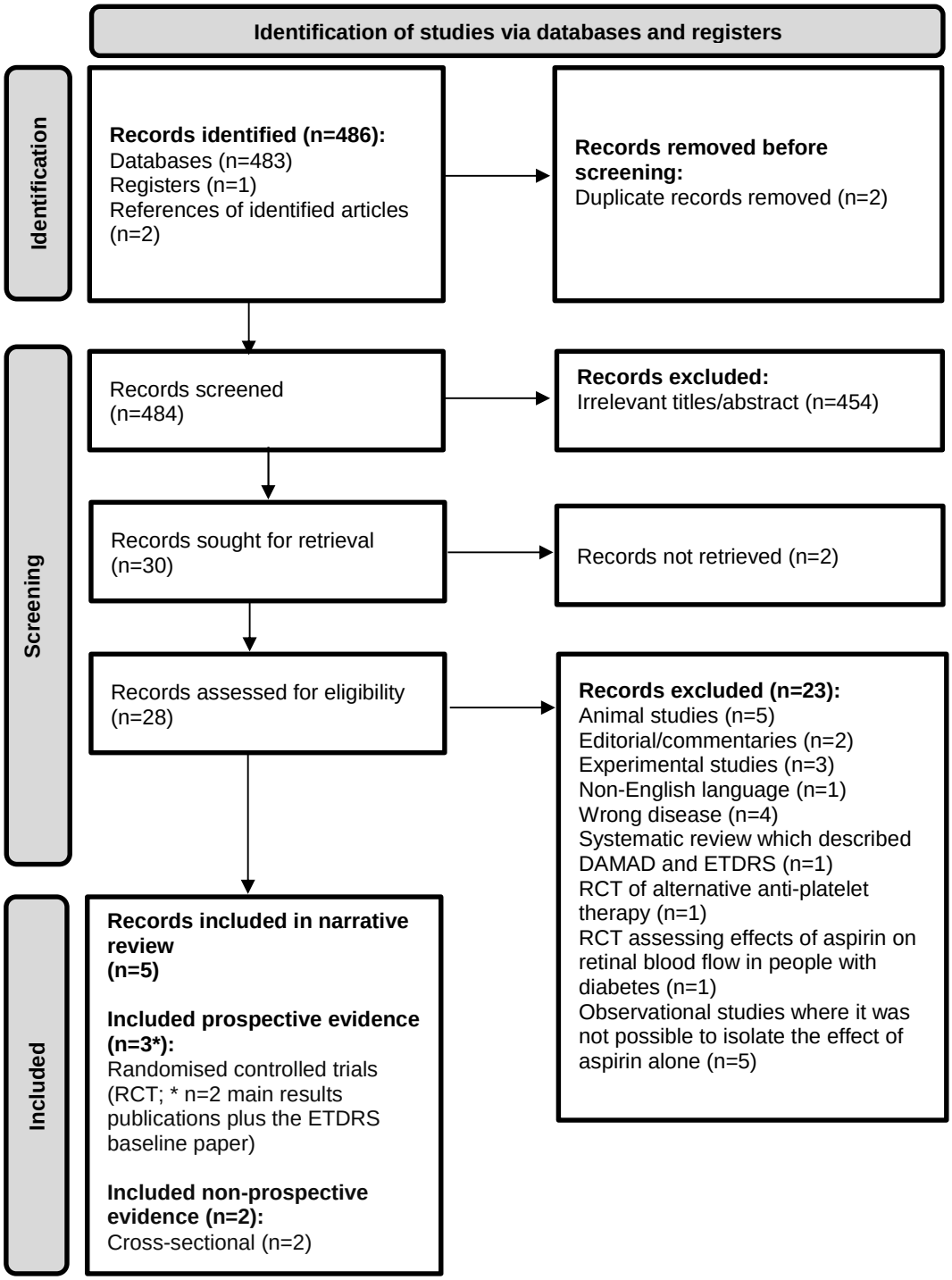


Table 3 Aspirin and Diabetic Retinopathy Systematic Literature Search

Where reported, and if it were appropriate to do so, the summary statistic in this table is presented as the number [%] of cases in the aspirin group *vs*. the number [%] of cases in the comparison group, followed by the risk estimate and the lower and upper bounds of the confidence interval around that risk estimate. The summary statistics are in bold, and P values are shown if the result was statistically significant.

Variables that were adjusted in the analysis models of studies with an observational design are listed in rows underneath the relevant study. Shading is used to demarcate rows of data belonging to separate studies.

Characteristics of the study			**Characteristics of the participants**		**Characteristics of the aspirin exposure**		**Characteristics of the outcome**		**Summary Statistics**
Source, year of main publication, country, quality assessment, study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1º Outcome(s)**	**Event ascertainment**	
Efficacy									
ETDRS Research Group Report No. 8 1991, US[140] Excellent The ETDRS Study	Double-blind, 2x2 factorial design	3711 (7)	18-70 (56.5)	Adults with NDPR or early PDR	-	650mg OD *vs.* placebo OD*	Progression to high-risk retinopathy†	4-monthly ophthalmoscopy review Annual fundus photography ETDRS grading system	756 [40.7%] *vs.* 768 [41.4%]; RR 0.97; 99% CI: 0.85-1.11
DAMAD Study Group 1989, France and UK[143] Good The DAMAD Study	Double-blind, parallel groups	475 (3)	17-87 (70.1)	Adults with no DR or background DR	-	330mg aspirin TDS *vs.* 330mg aspirin TDS plus 75mg dipyridamole OD *vs.* placebo	Mean (±SD) no. of micro-aneurysms per year	Annual fluorescein angiograms	0.69 ± 5.09 *vs.* 1.44 ± 4.49; (significance testing was not performed)
Salinero-Fort *et al* 2013, Spain[136] Poor The MADIABETES Study	Cohort	3443 (4)	67.8 (50.4)	Adults aged ≥30 with T2DM	BL interview	"Yes" *vs*. "No" (dose, frequency and duration not defined)	Incident DR	Annual slit lamp biomicroscopy	***-/- vs. -/-; HR 1.65; 95% CI: 1.22-2.24; P<0.001***
Adjustment for confounding: Age, sex, duration of diabetes, HbA1c, microalbuminuria, LDL-cholesterol and BP									
Shi *et al* 2017, Singapore[138] Poor The Singapore Epidemiology of Eye Disease Study	Cross-sectional	2061 (-)	≥40 (50.4)	Population-based study	Interview by study personnel	"Current use" of aspirin or "aspirin-type" medication *vs.* no use (dose, frequency and duration not defined)	Any DR	Fundus photography ETDRS grading system	-/- *vs.* -/-; OR 1.23; 95% CI: 0.90-1.67
Adjustment for confounding: Age, sex, ethnicity, SES, smoking, BMI, HbA1c, SBP, TC, statin therapy, anti-hypertensive therapy, prior cardiovascular disease and DKD									

Characteristics of the study			Characteristics of the participants		Characteristics of the aspirin exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1º Outcome(s)**	**Event ascertainment**	
Efficacy									
Pafundi *et al* 2020 Italy[137] Poor The NO BLIND Study	Cross-sectional	2068 (-)	≥14 (-)	Clinic-based Individuals withT2DM	Interview by study personnel	100mg aspirin per day *vs*. no use (duration not defined)	Any DR	Mydriatic ophthalmoscopy examination	-/- vs. -/-; OR 0.93; 95% CI: 0.71-1.22
Adjustment for confounding: Age, sex, smoking, BMI, HbA1c, SBP, TC, statin therapy, prior cardiovascular disease and eGFR									
Safety									
ETDRS Research Group Report No. 8 1991, US[140] Excellent The ETDRS Study	Double-blind, 2x2 factorial design	3711 (7)	18-70 (56.5)	Adults with NDPR or early PDR	-	650mg OD *vs.* placebo OD*	Incident vitreous or pre-retinal haemorrhage in those with high risk retinopathy†	4-monthly ophthalmoscopy review Annual fundus photography	190 [10.2%] *vs*. 203 [10.9%]; RR 1.05; 99% CI: 0.81-1.36
ETDRS Research Group Report No. 20 1995, US[142] Excellent The EDTRS study	Double-blind, 2x2 factorial design	3711 (7)	18-70 (56.5)	Adults with NDPR or early PDR	-	650mg OD *vs.* placebo OD*	Incident vitreous or pre-retinal haemorrhage‡	4-monthly ophthalmoscopy review Annual fundus photography	585 [32%] vs. 564 [30%]; P = 0.48
							Extent of vitreous or pre-retinal haemorrhage§		"No significant differences" (P for trend = 0.12)
							Duration of persistent blood after an incident vitreous or pre-retinal bleed¶		RR 1.0; 99% CI 0.8-1.3

ACEi = Angiotensin Converting Enzyme inhibitor; BL = baseline; BMI = body mass index; CSMO = clinically-significant macular oedema; DKD = diabetic kidney disease; DR = diabetic retinopathy; ETDRS = Early Treatment Diabetic Retinopathy Study; HbA1c = glycosylated haemoglobin; OD = once daily; NDPR = non-proliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; SBP = systolic blood pressure; SES = socioeconomic status; TC = total cholesterol; TDS = three times daily; T2DM = type 2 diabetes mellitus

*Randomised factorial comparison: early *vs.* deferred photocoagulation.

†A composite of:

- Neovascularisation of the optic disc, defined as new vessels covering 1/4th to1/3rd of the disc area, within 1 disc diameter of the optic disc on a standard 10A stereoscopic fundus photographic field; or
- A pre-retinal or vitreous haemorrhage accompanied by neovascularisation of the disc, neovascularisation of the disc less than 1/4th of the disc area, or new vessels elsewhere covering an area ≥1/4 of the disc area.

‡Among the full randomised population, in eyes assigned to deferred photocoagulation. The same analyses were performed for eyes assigned to early photocoagulation, and no statistically significant differences were found. These results were narratively described but the numbers were not presented.

§The extent of vitreous/pre-retinal haemorrhage was categorised as those which involved ≤1 disc area, >1 disc area< half macular field,≥ half macular field. The severity of bleeding across this grading scale was compared using the χ^2 test.
¶The Cutler-Ederer life-table method of survival analysis was used to estimate the cumulative rate of blood resorption in those assigned to aspirin or placebo. This was based on a binary presence or absence of blood identified with ophthalmoscopy during 4-monthly clinic visits, or photographically at each annual visit. Survival curves over the full treatment period were compared using the Mantel-Cox statistic. A Cox proportional hazards model was then used to estimate the relative risk of persistent blood between the aspirin and placebo treatment groups. This analysis included those who developed the primary endpoint for ETDRS (i.e. high-risk retinopathy features) and those who did not.

Figure 7 Omega-3 Fatty Acids and Diabetic Retinopathy Systematic Literature Search

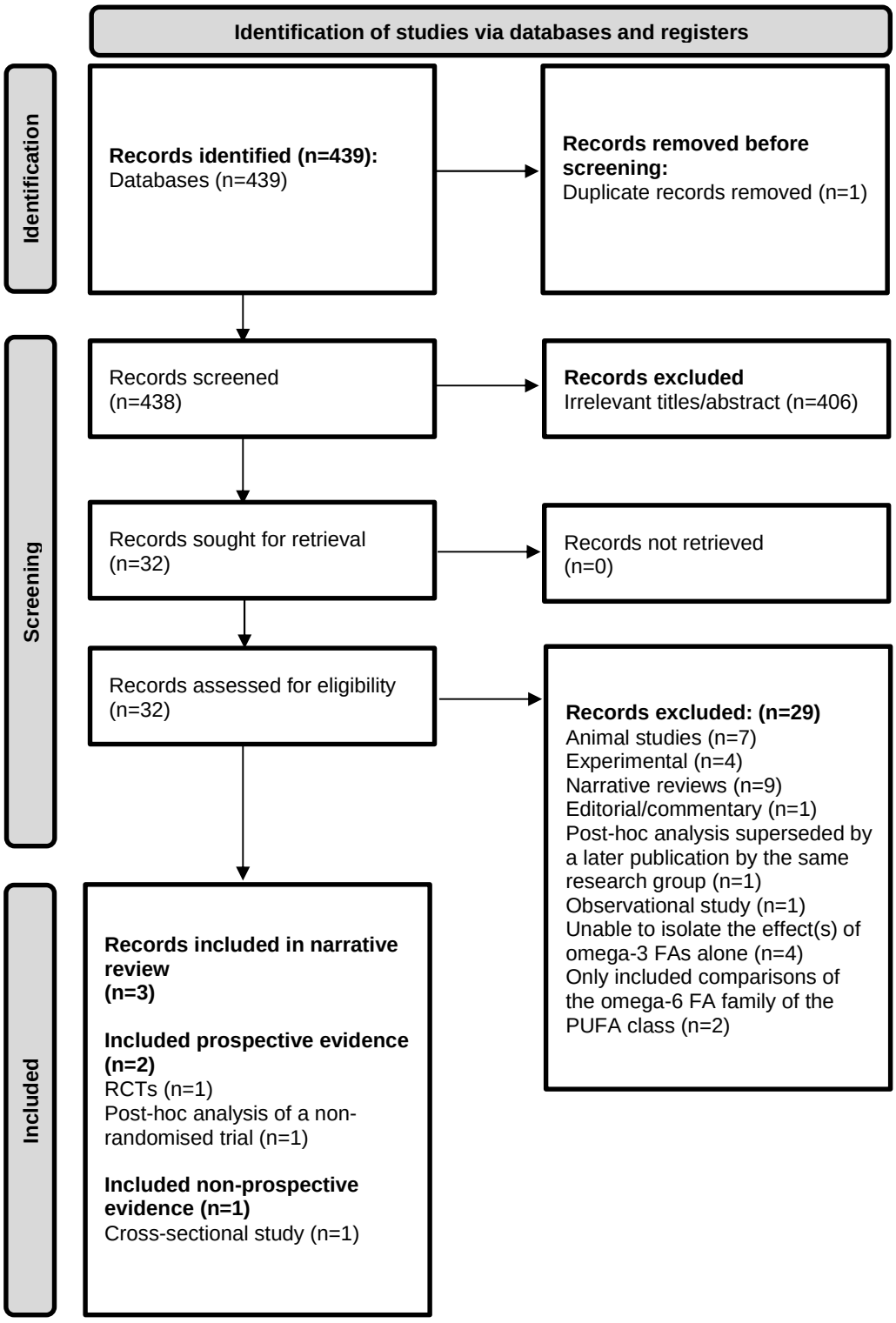


Table 4 Omega-3 Fatty Acids and Diabetic Retinopathy Systematic Literature Search

Where reported, and if it were appropriate to do so, the summary statistic in this table is presented as the number [%] of cases in the omega-3 FA group *vs*. the number [%] of cases in the comparison group, followed by the risk estimate and the lower and upper bounds of the confidence interval around that risk estimate. The summary statistics are in bold, and P values if the result was statistically significant and if it was reported.

Variables that were adjusted in the analysis models of studies with an observational design are listed in rows underneath the relevant study. Shading is used to demarcate rows of data belonging to separate studies.

Characteristics of the study			**Characteristics of the participants**		**Characteristics of the omega-3 FAs exposure**		**Characteristics of the outcome**		**Summary Statistics**
Source, year of main publication, country, quality assessment, study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1º Outcome(s)**	**Event ascertainment**	
Haritoglou *et al* 2011, Germany[179] Fair The RETIPON Study	Double-blind, parallel groups	467 (2)	58 (41.8)	Adults with insulin-dependent T2DM, NDPR and microalbuminuria >30mg/l	-	600mg ALA OD *vs.* placebo (plus standard care ranibizumab therapy)	Incident CSMO in at least one eye	6-monthly fundus photography ETDRS grading system	26 [11.1%] *vs*. 30 [12.9%]; HR 0.91; 95% CI: 0.54-1.53
Sala-Vila *et al* 2016, Spain[164] Fair (Participants of the PREDIMED trial)	Post-hoc analysis of a non-randomised trial*	3482 (6)	67 (48)	Men aged ≥55 and women aged ≥60, with T2DM or 3 or more metabolic risk factors†	BL and annual FFQ	Dietary omega-3 FAs of at least 500mg per day or ≥2 weekly servings of oily fish	Incident STDR‡	Medical record review	***27/- vs. 42/-; HR 0.52; 95% CI: 0.31-0.88; P = 0.001***
Adjustment for confounding: Age, sex, BMI, intervention group in PREDIMED, duration of diabetes, insulin use, oral hypoglycaemic agent use, smoking, SBP, hypertension, ACEi or ARB use, and adherence in PREDIMED									
Sasaki *et al* 2015, Australia[162] Poor	Cross-sectional	123 (-)	67 (69.1)	Adults ≥18 years with well-controlled DM§	FFQ	Energy-adjusted PUFA intake per day (Dose and duration not defined)	Presence of any DR	Fundus photography ETDRS grading system	***-/- vs. -/-; OR 0.18; 95% CI: 0.06-0.59***
		256 (-)	65 (64.5)	Adults ≥18 years with poorly-controlled DM§					-/- *vs.* -/-; OR 1.55; 95% CI: 0.67-3.56
Adjustment for confounding: Age, sex, HbA1c, mean arterial blood pressure and duration of diabetes									

ACEi = angiotensin-converting enzyme inhibitor; ALA = α-linolenic acid; ARB = angiotensin-II receptor blocker; BL = baseline; CSMO = clinically significant macular oedema; DM = diabetes mellitus; ETDRS = Early Treatment Diabetic Retinopathy Study; FFQ = food frequency questionnaire; NPDR = non-proliferative diabetic retinopathy; OD = once daily; PUFA = polyunsaturated fatty acids; STDR = sight-threatening diabetic retinopathy

*Due to the serious protocol violations that were identified during an audit of the PREDIMED trial (as discussed in Section 1.4.2), this study has been listed as a non-randomised trial.
†The metabolic risk factors included: being a current smoker, history of hypertension, LDL-cholesterol >160mg/dl or treatment with LDL-lowering therapy, HDL-cholesterol ≤40mg/dl, BMI ≥25kg/m^2 or a family history of premature coronary heart disease.
‡Defined as incident DR requiring laser photocoagulation, vitrectomy and/or anti-angiogenic therapy.
§Well-controlled was defined as having an HbA1c < 7.0% (53 mmol/mol); poorly controlled: HbA1c ≥7.0% (53 mmol/mol).

Chapter II - Age-Related Macular Degeneration

Between 2018 and 2020, the average life expectancy in the UK was 79 years for men and 83 years for women.[181] The quality of those advancing years is greatly diminished by degenerative conditions such as AMD, a condition characterised by the progressive loss of central vision, which profoundly compromises the ability to read, drive or recognise the facial features of loved ones. Understanding the effects of aspirin and, separately, omega-3 FAs on AMD is important, given their potential to modify the disease process and widespread use by older persons for cardiovascular disease or as a dietary supplement, respectively. The ASCEND trial of a diabetic population provided the opportunity to make these comparisons; however, it is important to state at the beginning of this chapter that diabetes and senior maculopathy are not considered to be pathologically related to each other.[182-185]

This chapter introduces the terminology, disease definition and grading systems used by former observational studies of senior maculopathy. It will appraise the limitations of existing epidemiologic data, describe the pathological mechanisms behind AMD, and discuss current approaches to managing the disease. Finally, two systematic reviews of the literature on the roles of aspirin and omega-3 FAs on AMD are presented.

2.1 Disease Introduction

2.1.1 Disease Definitions and Terminology

The earliest descriptions of senior changes in the macula area of the retina appeared around 1850[186], but a more precise concept of the disease came from a published case series of patients attending specialist Ophthalmology clinics in the 1970s.[187] Later population-based studies helped define and quantify the disease's pathognomonic features, natural history and portending risk factors. However, inter-study comparisons were limited by the absence of a universally-accepted method to detect, grade, and define the presence, severity or progression of lesions in a standardised way.[188-190] With this in mind, the International Age-Related Maculopathy Epidemiological Studies Group (IARMG) was established.[189] After a series of meetings between delegates from six leading research groups, a consensus was reached

regarding the gold standard way of photographing, processing and grading senior maculopathy to harmonise future research protocols.[189] The group defined early disease as:

"A disorder of the macula area of the retina, most often clinically apparent after 50 years of age, characterised by any of the following pathological features: soft distinct or soft indistinct drusen with areas of either increased pigmentation or depigmentation of the retinal pigment epithelium. There should be no indication that changes are secondary to another cause, e.g. ocular trauma, retinal detachment, high myopia, chorioretinal infective or inflammatory processes, or choroidal dystrophy."[189]

Where these conditions are met, the term "Age-Related Maculopathy (ARM)" or "early ARM" should be used.[189] Only late changes of the disease should be referred to as "Age-Related Macular Degeneration" or "AMD".[189] The latter includes two discrete presentations of the disease known as "Neovascular (or exudative) AMD" and "Geographic Atrophy", which are colloquially referred to as "Wet" and "Dry" AMD, respectively.[189] Dry AMD is defined by the presence of:

"Any sharply delineated roughly round or oval area of hypopigmentation or depigmentation, or apparent absence of the retinal pigment epithelium in which choroidal vessels are more visible than in the surrounding areas – that must be at least 175µm in diameter on a colour slide."[189]

Meanwhile, wet AMD is defined by the presence of one or more of the following pathological features:

- *"RPE detachment with or without neurosensory retinal detachment;*
- *subretinal or sub-RPE neovascular membranes;*
- *epi-, intra- or sub-retinal or sub-RPE scarring, glial tissue or fibrin-like deposits;*
- *subretinal haemorrhages that are not caused by other retinal vasculopathy; or*
- *hard exudates within the macular area related to other neovascular changes that are not caused by other retinal vascular diseases."*[189]

The pathogenesis behind these disease definitions will now be described in more detail.

2.1.2 Pathogenesis

The organisation of microscopic retinal anatomy ensures a strict segregation between its vascular and avascular layers to protect the neural retina's functional integrity. The RPE and Bruch's membrane are essential to this. In healthy eyes, they work together to regulate the transfer of fluid and ions between photoreceptor cells and the choriocapillaris. With advancing age, the lumina of these vessels narrow, and Bruch's membrane thickens due to an accumulation of lipid and collagen debris, known as basal laminar deposits.[190,191] Both changes reduce fluid permeability and nutrient transport, threatening the survival of nearby RPE and choriocapillaris cells.[190] The RPE of ageing eyes also accumulate more lipofuscin, a retinoid produced during the recycling of photoreceptor outer segments, which contains fluorophores associated with causing oxidative damage.[192] The combination of epithelial injury and basal laminar deposits is thought to promote drusen formation in the extracellular space between the RPE and choroid tissue layers.[191,193] Drusen comprises glycoproteins, crystallins, apolipoproteins B and E, and proteins associated with inflammation, such as amyloid P and β, C5 and C5b-9 complement complexes.[193] Whilst small drusen (<63μm in diameter) are ubiquitous in older persons, larger confluent drusen are considered pathognomonic of AMD because they cause more RPE disruption.[191,194] For example, there may appear to be areas of depigmentation due to atrophied RPE cells, or hyperpigmentation, where damaged cells clump together.[190] Compromise of the RPE-Bruch's complex in this way, impedes normal photoreceptor support systems and eventually causes a loss of the outer plexiform and inner nuclear retinal layers.[195] Such changes create favourable conditions for neovascularisation from the choroid vessels, which may breach Bruch's membrane and spread into the sub-RPE or sub-neurosensory space.[195] Neovascularisations can also originate from the macula area of the retina and join up with choroid vessels, referred to as 'retinal angiomatous proliferation with chorioretinal anastomoses'.[195] Unlike normal capillaries, the new vessels tend to be fenestrated and thinner, allowing blood constituents to leak out.[195] An accumulation of intraretinal fluid and exudates further separates the RPE and innermost aspects of Bruch's membrane from each other (called 'pigment epithelial detachments'), causing more misalignment and eventual apoptosis of the photoreceptors.[195] The end result is an area of fibrous scarring behind which the larger choroidal vessels can be seen, and eventually, involution of the pathological angiogenesis.[190]

2.1.3 Epidemiological Studies

After cataracts, under-corrected refractive errors and glaucoma, AMD is the fourth leading cause of central visual loss among older people worldwide.[84,196-198] However, most epidemiological studies of the disease are limited to White populations in America, Europe and Australia because data on different ethnic groups in other parts of the world is scarce.[84] In population-based studies with a cross-sectional design, the prevalence of late AMD has been reported to range between 0.1% and 3.5%[184,185,188,199-223], whereas early lesions are more common, with estimates ranging between 2.5% and 43.1%.[183,185,199-203,205-216,218,219,221,222,224,225] Since the condition becomes more common with advancing age, differing age distributions probably explain the breadth of these ranges; however, dissimilarities in ethnicity, the definition of ARM, non-participation rates, unilateral imaging protocols, and the proportion of ungradable images could also be contributory.[189] In the only meta-analysis of studies that used comparable, standardised protocols and comprised data on nearly 25,000 individuals from high-income countries, who were predominantly White, the estimated age-specific prevalence of AMD was less than 0.5% in those aged between 50 to 60, but this increased to 12% and 16% in men and women over 80 years of age, respectively.[226]

A disadvantage of cross-sectional studies is their limited ability to show what is antecedent and what is consequent. Therefore, cohorts from some of the larger studies have been followed up over several years to understand the progression of lesions over time and to estimate the incidence of senior maculopathy in their population.[227-238] The Beaver Dam Eye Study (BDES; 1987-2012) is one of the largest and longest-running epidemiological studies of a mostly White cohort in Wisconsin, USA, aged 43 to 86 years old (n=3917).[201,230-232] Over 15 years of follow-up, the cumulative incidence of early ARM was 14.3%, and for late AMD, it was 3.1%.[232] A quarter of those with early ARM at baseline progressed to late disease, a rate of 1.7% per year.[232] Other population-based studies have reported similar annual progression rates of between 2% and 5% in at-risk people.[227-238] The Copenhagen City Eye Study (1986-2002; n=946) is the only incidence study that predates the use of antioxidant, photodynamic or anti-VEGF therapy* in the participant's country of residence, and it therefore offers

* See Section 2.1.7.

an insight into the natural history of untreated AMD.[235] Over 14 years of follow-up, 5.1% of the cohort aged 60-80 years at baseline became legally blind, corresponding to a calculated incidence of blindness of 0.37% per year.[235] For comparison, in the BDES population, which did have access to anti-VEGF therapies for wet AMD, the 10-year incidence of legal blindness was 2.5% or 0.25% per year, thus demonstrating the success of this intervention.[231]

The BDES first reported that senior macular changes follow a continuum from several small hard drusen to soft indistinct drusen and pigmentary abnormalities in early ARM.[239] In the later stages, dry AMD may transform into the wet form of the disease.[232] Thus, dry AMD is the more common phenotype of late senior maculopathy, accounting for 85% to 90% of all cases; however, 90% of AMD-related blindness registrations are attributed to the wet form because it progresses much more rapidly.[195]

Most incidence studies have described a binocular nature of the disease, in which the phenotype in the first eye is likely to predict AMD type in the second.[229-231,233,234,237] Macular areas with over 10% drusen coverage and ten or more small drusen seem to have a predilection for the development of dry AMD, whereas the relationship between earlier lesions and wet AMD is less clear.[232]

Overall, former incidence studies have similar methodological limitations as the cross-sectional prevalence studies, such as differences in disease definitions and variable proportions of ungradable images. However, longer durations of follow-up incur other problems, such as higher losses to follow-up, survival bias and comparing images as camera technologies improve over time.

2.1.4 Socioeconomic Impact in the UK

The Royal National Institute of Blind People Sight Loss Data Tool estimated that 3 million people were living with ARM, and 653,000 had late disease in the UK in 2022.[4] Based on the United Nation's five-yearly population estimates for the UK, by 2032, the prevalence of early disease is predicted to increase by 16% and by 24% for AMD, totalling 3.5 million and 815,000 people, respectively.[4] Despite being a comparatively rare eye condition, AMD is disproportionately responsible for nearly half (48%) of all Certifications of Visual Impairment registrations (legal blindness) in the UK each year.[4] The disease is also inordinately expensive. In 2020, the annual NHS expenditure on AMD exceeded £1.2 billion, most

of which was attributed to the cost of anti-VEGF medications for the wet form, which are among the top five highest costing items in the national drug budget.[240,241] A further £1.4 billion was spent on visual devices and social care for those with AMD-related sight loss.[240] Overall, the average lifetime cost of AMD per person is estimated to be £73,350.[240] However, good vision plays a bigger role in healthy ageing, including preserving cognitive function and preventing falls.[242-244] The attributable costs of these are unknown but likely to be substantial. Therefore, finding new treatments to prevent AMD or delay visual loss is imperative.

2.1.5 Risk Factors

In an early case series published in the 1970s, advancing age and family history were described as key risk factors for developing senior maculopathy.[187] Later observational studies have also found a consistent adverse association with smoking (see Section 2.1.5.4). However, the influence of other sociodemographic, behavioural, genetic and medical risk factors is less clear, suggesting that if other disease modifiers exist, their effect may not be very strong. Nevertheless, the association between AMD and common risk factors are now discussed.

2.1.5.1 Sociodemographic Factors

Most observational studies have found a consistent association between macular lesions and advancing age.[184,201,203] The relationship with sex is unclear. Whilst some observational studies have reported a higher risk of early disease[201,203,207,233] and wet AMD[201,230] in women, others have found no association.[183,200,208,209,213,227,236] Conflicting associations have also been reported for surrogate indicators of oestrogen exposure in women, such as age at menarche, menopause and use of hormone replacement therapy.[232,245,246] Such findings could reflect differences in the way research groups have adjusted for stronger risk factors such as age and smoking status, or they may reflect the increased longevity of women and, therefore, a survival bias in the studies that included older age men.[207,229]

The prevalence of early lesions is considered to be similar between ethnic groups; however, late disease is reported to be less frequent in those with darker skin, which would imply that the condition progresses

more quickly in White people than Black people.[202,205,213,214] Some have proposed that melanin acts as a free radical scavenger that protects the RPE and Bruch's membrane[247], consistent with an animal model in which some albino mice given corneal angiogenic growth factors showed a higher rate of iris neovascularisation and hyphaema than their pigmented relatives.[248] However, other human observational studies have found no differences by ethnicity[212,216,217], leading some to question if there was a misclassification of AMD-mimics in some studies.[202,205,210] Finally, no clear associations have been established between educational attainment or socioeconomic deprivation.[195]

2.1.5.2 Ocular History

The effect of other eye problems on the risk of age-related macular disease is also unclear. Some have associated higher risks of AMD with hypermetropic individuals, whereas others have not.[9] Previous cataract surgery has also been reported to have a disease-conferring role, but AMD and senior cataracts have the shared risk factor of age, so this may be a confounded observation.[199,249-253] Indeed, a Cochrane Review of randomised trials concluded that there was insufficient evidence to associate cataract surgery with a higher likelihood of developing AMD.[254] Finally, darker iris colour was protective in one study[233], but not another.[255]

2.1.5.3 Cardiovascular Disease

Atherosclerotic cardiovascular disease has been proposed as a potential risk factor for developing senior maculopathy because of its deleterious effects on the choroidal circulation, such as retinal arteriolar narrowing and deposition of lipids in Bruch's membrane.[185] Although carotid stenosis was correlated to a higher risk of early ARM in two studies[184,185], no association was found in one other.[183] Nevertheless, all three studies reported an inverse relationship between plasma LDL-cholesterol levels and AMD, which is counter-intuitive to what one would expect if drusen deposits contain extravasated serum lipids.[183-185] It has been hypothesised that higher circulating LDL-cholesterol levels protect against AMD by down-regulating RPE LDL receptors.[256] However, lowering LDL-cholesterol

pharmacologically with statins did not increase AMD risk in a Cochrane review of randomised trials.[257] Conversely, a proportional relationship may exist between high-density lipoprotein (HDL)-cholesterol levels and AMD risk.[256,258] HDL-cholesterol is thought to convey lipoproteins involved in the turnover of photoreceptor discs between the interphotoreceptor matrix, RPE cells and Bruch's membrane.[256,258] In ageing eyes, these mechanisms may become less efficient, leading to collections of different lipids in the sub-RPE space.[256,258] HDL moieties could also have a role in maintaining homeostasis of the complement system within the retina.[256,258] However, no hazard was identified from pharmacologically raising HDL-cholesterol using the cholesterol ester transfer protein inhibitor, anacetrapib, during 4.1 years of follow-up, in the large (n=30,449), phase III randomised, placebo-controlled Randomised EValuation of the Effects of Anacetrapib through Lipid modification (REVEAL; 2011-2017*) trial[259] or during a two-year, off-treatment, post-trial follow-up of surviving participants [unpublished analysis conducted by Dr William Stevens of the REVEAL data analysis team at Nuffield Department of Population Health, University of Oxford in 2022]. Finally, there have been inconsistent associations with other cardiovascular risk factors, such as hypertension[183-185,209,210] and obesity.[9]

2.1.5.4 Lifestyle Factors

After age and family history, smoking has been most consistently associated with a two to three-fold increased risk of wet and dry AMD in both sexes and all ethnic groups.[195,260-265] The mechanisms through which smoking causes harm may include 1) a reduction in choroidal blood flow, 2) consumption of systemic antioxidants and 3) a reduction of macular carotenoid pigments.[266,267] The relationship between alcohol and AMD is inconclusive.[195,268,269] Finally, in comparisons of ethnic groups residing at different latitudes or altitudes, some studies have correlated a higher prevalence of AMD to prolonged light exposure[250,270,271], whereas others have not.[182,219] Therefore, occupational or recreational sunlight exposure may confound some of these studies.[182,219]

* Longer-term follow-up of the UK REVEAL participants is ongoing.

2.1.5.5 Genetic Factors

Specific genes could affect the likelihood of developing AMD. In linkage studies, greater disease concordance has been reported among homozygous twins than heterozygous twins and between siblings and parents more than extended family members.[182,272] However, the strength of genetic association is low, suggesting that the effect genes have mainly manifests when susceptible individuals are exposed to more potent risk factors such as smoking.[182] Nevertheless, the genetic associations with AMD are now described.

Complement Factor H (CFH) is an alternative complement pathway inhibitor encoded by a gene on chromosome 1 (q23-32).[182] The following single nucleotide polymorphisms of the CFH gene have been associated with a higher vulnerability to AMD in White populations: rs1061170, rs3753394, rs800292, rs1061147, rs380390 and rs1329428.[182] Of these, variant rs1061170, also called the CFH Y402H allele, has been correlated with the highest risk.[182] CFH also appears to have two common protective alleles: CFHR1 and CFHR3.[195] Interestingly, the frequency of both CFHR1 and CFHR3 alleles is higher in Black and Hispanic populations, which may also have a lower prevalence of late disease (see Section 2.1.5.1).[195] Next, genome-wide association studies have identified three tightly-linked genes on chromosome 10 (q26) which harbour the following high-risk haplotypes: PLEKHA1, LOC387715/ARMS2 (Age-Related Maculopathy Susceptibility gene 2) and HTRA1 (High-Temperature Requirement Factor A1).[273] Finally, the apolipoprotein E genes on chromosome 19 (q13.2) have three common isoforms: ApoE2, ApoE3 and ApoE4.[195] Previous studies have reported a protective role for the ApoE4 allele and a risk-conferring role for ApoE2.[195]

2.1.6 Diagnostic Techniques

The presentation of AMD is insidious. Whilst some individuals notice difficulties reading small print sizes, or a distorted appearance of objects expected to have straight edges, such as door frames, others may not appreciate subtle changes, such as impaired contrast sensitivity and scotopic vision, unless they are directly prompted.[9] At the point of referral, some signs of late disease can be detected on routine fundus examination.[9] However, other retinal imaging techniques are used for more formal diagnoses of

the AMD phenotype *versus* disease mimics and to monitor the response to treatment.[9] These include high-resolution digital fundus photography, fundus fluorescein angiography, OCT, OCT-angiography, or indocyanine green angiography, but it is beyond the scope of this work to describe these investigations in detail.[9]

2.1.7 Management

The management of both phenotypic forms of senior maculopathy focuses on practical measures to help individuals cope with the effects of visual loss and lifestyle modification, such as avoiding cigarette smoking and wearing sunglasses to reduce light exposure.[9] Addressing the nutritional status of individuals might also be beneficial.[274,275] The Age-Related Eye Disease Study 1 (AREDS1; 1992-2001) randomised 3640 men and women aged 55-80 with existing macular disease to antioxidant multi-vitamins containing vitamin C, vitamin E, beta-carotene, zinc and copper, or a matching placebo.[276] During their mean follow-up of 6.3 years, the AREDS1 supplement delayed progression from early ARM to late AMD by 28% (OR 0.72; 99% CI 0.52-0.98; P=0.007).[276] Substituting beta-carotene for the alternative carotenoids, lutein and zeaxanthin, did not confer additional benefits in the subsequent AREDS2 trial (2006-2013; described in detail in Section 2.2.2), but post-hoc comparisons of the two studies found a higher incidence of lung cancer in current and former smokers who were supplemented with beta-carotene.[277] Therefore, most commercially available compounds contain the AREDS2 multi-vitamins, which are recommended for all individuals with ARM or AMD.[9]

2.1.7.1 Management of the Wet Disease

Three main treatments are available for individuals with wet AMD: laser photocoagulation, photodynamic therapy, and intravitreal anti-VEGF injections.[9] In the 1980s, randomised trials by the Macular Photocoagulation Study Group helped define the angiographic characteristics of neovascular choroidal lesions most amenable to laser therapy.[278,279] Favourable outcomes were reported in those eyes with small extra- or juxta-foveal vessels, whilst (more frequent) sub-foveal lesions were less

responsive.[278,279] Laser therapy may be complicated by permanent scotomata, and therefore in the 1990s, photodynamic therapy was introduced for the treatment of sub-foveal vessels.[280] During this treatment, an intravenously-administered photosensitising dye called verteporfin binds to circulating LDL-cholesterol particles.[280] Uptake of verteporfin-LDL complexes occurs more readily in proliferating endothelial cells because they have upregulated LDL-receptor expression.[280] A laser is then used to activate the verteporfin, which destroys pathologically abnormal vessels.[280] Verteporfin photodynamic therapy has been associated with idiosyncratic back pain in up to 2% of patients who receive it and severe visual loss in 1-4%.[9] Therefore, the procedure is mainly reserved for a mimic of AMD called idiopathic polypoidal choroidopathy.[9]

Next, three important phase III clinical trials called MARINA[281,282], ANCHOR[283] and FOCUS[284] (all conducted between 2003 and 2006) established intravitreal ranibizumab as a first-line therapy for wet AMD in eyes within the visual acuity range 6/12 to 6/96, and no evidence of permanent structural damage to the central fovea.[241] Monthly injections given over a 12-month period significantly improved visual acuity compared to photodynamic therapy or a sham injection.[281,283,284] Later trials helped determine the dosing intervals capable of maintaining visual gains with fewer injections.[285-290] Most of the adverse events reported by these studies were related to the injection procedure, including endophthalmitis, traumatic cataract formation, and retinal detachment.[281-290] Other anti-VEGF agents, including pegaptanib, aflibercept, bevacizumab and brolucizumab, have also been used to treat wet AMD; however, the National Institute for Health and Care Excellence (NICE) only recommends using these when ranibizumab is contraindicated, and under specific criteria, which are beyond the scope of this work to describe in detail.[241]

2.1.7.2 Management of the Dry Disease

Unlike its wet counterpart, there is currently no proven drug treatment or intervention for the dry form of AMD.[9] Although some studies observed drusen regression after focal laser therapy for choroidal neovascularisation, a subsequent Cochrane Review of nine randomised trials found no evidence that prophylactic laser therapy conferred any benefits.[291] Therefore, managing dry AMD mainly involves

signposting patients to measures that could help to mitigate the effects of sight loss, such as adaptive lighting and low vision aids, as well as psychological support.[292]

2.2 Systematic Literature Reviews

Despite the successes of anti-angiogenesis therapy for wet AMD, the treatment is not curative; it is only intended to delay visual loss before the disease evolves into an inactive state, and there are no effective treatments for dry AMD.[191] Therefore, finding new treatments for both phenotypes that are effective at an earlier stage remains necessary before irreversible damage occurs. Establishing the balance of risks and benefits of drugs commonly taken by older people is also essential. This section describes two systematic literature searches conducted by the author in February 2018 and updated in July 2022 to understand the existing evidence for aspirin and omega-3 FAs' effects on senior maculopathy separately. Four databases were systematically searched for articles written in English, with no limits on publication year, up to July 2022, using standardised criteria (a detailed search proforma and list of studies excluded after they were assessed for eligibility are presented in Appendices 2 and 3 respectively). Briefly, randomised controlled trials, meta-analyses and observational studies evaluating the effects of aspirin and, separately, omega-3 FAs on incident or progressive ARM or AMD, change in the size, count or morphology of pathological lesions associated with senior maculopathy, safety outcomes, and vision-specific quality of life were included in the review. Experimental and narrative review articles were also sought to broaden an understanding of the topic, but these have not been tabulated. The initial search identified 203 abstracts for the aspirin review and 331 for the omega-3 FAs review. Studies were then systematically excluded according to pre-specified criteria. Consort diagrams and tabulations from these are presented at this chapter's end. The methodological quality of each study was assessed using the Downs and Black instrument (Appendix 4)[*].[116] In the case of both reviews, existing meta-analyses negated the need for syntheses to be repeated. Moreover, significant

[*] The systematic review did not include the recommended dual review process. Single screening by the author alone was deemed sufficient, given the lack of randomised evidence identified from a preliminary literature search.

heterogeneity in outcome definitions, event ascertainment, and a lack of randomised evidence made meta-analyses of the data inappropriate. Therefore, Sections 2.2.1 and 2.2.2 are narrative reviews only.

2.2.1 Aspirin and Age-Related Macular Degeneration

The use of aspirin by older people who are at risk of AMD has been controversial since the late 1980s when the authors of a case series, which described 109 Ophthalmology outpatient attendees, warned that its over-the-counter availability would "*usher in a new wave of blindness*".[293] Since then, aspirin has also been considered of potential benefit in AMD owing to its complementary, non-selective and irreversible inhibitive effects on two isoforms of the COX enzyme: COX-1 and COX-2. The long-term suppression of platelet aggregation via acetylation of COX-1 is thought to slow atherosclerotic cardiovascular disease progression, which might also protect against retinal arteriolar narrowing and the deposition of lipids in Bruch's membrane.[294,295] Meanwhile, COX-2 inhibition may reduce the platelet-mediated release of VEGF[295-297] and the expression of pro-inflammatory prostaglandins involved in the biogenesis of drusen.[193,298-302] Collectively, these actions could confer protection against choroidal neovascularisation and RPE disruption. On the other hand, the vasoconstrictive effect of COX-2-suppressed prostacyclin synthesis might cause hypoxia in older people with narrowed choroidal blood vessels, becoming the stimulus for neovascularisation and the development of wet AMD.[303]

Previous research into the association between senior maculopathy and aspirin has produced mixed findings. Most observational studies used multivariate regression methods to compare the risk of developing the disease based on different classifications of aspirin exposure. Of eleven studies with a non-prospective design, three reported protective effects of aspirin use[304-306], two observed a risk-conferring role for wet AMD[307,308], one reported a higher incidence of sub-retinal haemorrhage in aspirin-prescribed AMD patients[293], whilst the remaining five found no association at all.[309-313] Among ten prospective cohort studies, one examined associations with early maculopathy only[314], four analysed their data by wet and dry phenotypes separately[303,315-317], one compared wet AMD alone[318], and the remaining four compared both phenotypes as a combined 'late AMD' endpoint.[319-322] Of these, one found no association with ARM[314], two reported detrimental associations with wet AMD but not early

maculopathy or dry AMD[303,316], one found harm for late AMD[322], two reported protective effects for late AMD[317,319], and four found no association with AMD at all.[315,318,320,321] Next, two randomised trials, which represent the most robust study design to assess the relationship, found no hazard and a non-significant trend towards a protective benefit of aspirin but had too few events among their relatively young study population to draw firm conclusions*.[301,323] Finally, out of six meta-analyses, three found no statistically significant association between aspirin and AMD[324-326], and three reported a weak harmful relationship.[327-329] The studies included in the meta-analyses predominantly represented White Europeans or Americans, so the results may not be generalisable to other populations. There was also substantial heterogeneity between the exposure definitions, event ascertainment methods, and adjustment for confounders in the pooled studies; the inclusion of data from observational studies further limited their ability to estimate causality.

Several reasons could explain the inconsistent results of previous studies. The inability to account for residual confounding from unmeasured or unknown variables, even after applying advanced statistical methods, is an inherent limitation of all observational studies.[330] This problem is heightened in complex, multifactorial diseases such as AMD, where the level of risk conferred by different genetic, inflammatory, vascular or environmental variables is difficult to quantify. These issues, and the small number of participants with AMD, further affected the stability and accuracy of the risk estimates they provided. Most of the non-randomised studies also lacked detail regarding their dose, duration and frequency definitions of aspirin exposure, and few could evaluate cumulative dose-response relationships in participants whose medication use changed over time. Lastly, the possibility of reverse causality was rarely addressed, for example, if an Ophthalmologist advised their patient to avoid aspirin to reduce AMD progression. Nevertheless, evidence from the bigger cohort studies suggests that the duration of aspirin therapy and AMD subtype could be relevant. The cumulative exposure to aspirin associated with a higher risk of wet AMD in two of the largest longitudinal studies was 15 and 20 years[303,316], respectively, whereas the scheduled treatment periods in the two randomised trials were shorter than this (5 and 10 years), and neither compared their data by late AMD phenotype

* Both randomised trials are described in more detail in Chapter V: Discussion, Section 5.4.

separately.[301,323] Both trials used an alternate-day aspirin regimen, but this may not have been enough to suppress thromboxane pathways in everyone.[331] Finally, the trials used participant-reported diagnoses confirmed by reviewing their medical records; however, event ascertainment via retinal photography is the gold standard.[189] An observational extension of the ASPirin in Reducing Events in the Elderly (ASPREE 2010-2018) randomised trial, called ASPREE-AMD, used retinal photography to examine the incidence and progression of AMD in approximately 5000 healthy people aged 70 years or older who were assigned to 100mg daily aspirin or a placebo and followed up over five years.[332] In their baseline publication, 52% of participants were women with a mean age of 73.4 years, the prevalence of early and late maculopathy at randomisation was 20.6% and 1.1%, respectively, and treatment arms were well-balanced for AMD risk factors including age and smoking status.[332] The main results of this trial are yet to be published.

Thus, more research into the relationship between aspirin and senior maculopathy is needed, given its widespread use by older persons to treat cardiovascular disease or relieve pain. ASCEND-Eye is likely to be underpowered to confirm any beneficial or harmful effects on its own because the participants in ASCEND were relatively young (mean baseline age = 63.3; SD 9.2)[11], and AMD is a comparatively rare endpoint. However, with its randomised design, well-defined dose and long treatment duration, evidence from ASCEND-Eye can contribute more reliable data to help future meta-analyses resolve the remaining uncertainty.

2.2.2 Omega-3 Fatty Acids and Age-Related Macular Degeneration

AMD shares some of the processes implicated in the pathogenesis of DR, except the main vascular network affected is the choriocapillaris instead of the retinal capillaries.[51] Thus, omega-3 FAs have been considered of potential benefit in AMD for the same reasons they have been proposed for DR. Namely, by maintaining levels of DHA in the outer segments of photoreceptor cells and by subverting the metabolism of fatty acids towards anti-inflammatory, vasodilating or anti-angiogenic docosanoid intermediaries, as described in Section 1.4.2. [50,136,137,139,141,168,303-308] DHA might also have a specific role in maintaining the density of macular pigments that protect against oxidative damage caused by shorter

wavelengths of light, which some have implicated in the development of AMD.[191,333] Finally, the chemical structure of omega-3 FAs may preserve the elasticity of choriocapillaris vessels, thereby maintaining perfusion, fluid permeability and nutrient transport to RPE cells, which could be important in elderly eyes.[51]

Most observational studies of omega-3 FAs and senior maculopathy have used multivariate regression methods to compare the risk of developing the disease according to the highest and lowest quantiles of dietary lipid intake, estimated using an FFQ and national food composition data. Using this approach, most, but not all, have found that individuals affected by AMD recall a lower consumption of fish or omega-3 FAs. The studies with a non-prospective design included seven cross-sectional[334-340] and seven case-control studies.[341-347] Of those, eight reported statistically significant beneficial effects of consuming more fish or omega-3 FAs.[334,340,341,343,345-347] However, cohort studies with a prospective design mitigate the risk of reverse causality and may be a more reliable way to confirm associations than case-control or cross-sectional studies.[330] Among nineteen cohort studies identified in the systematic search, there was considerable variation between the outcomes and exposures of interest.[348-366] Some compared the effects of omega-3 FAs on late AMD-only, whilst others subdivided their study population into those with early ARM, wet AMD and dry AMD. However, the grading systems used to define these phenotypes differed. There was also dissimilarity in the extent to which different lipid components were stratified. Some analysed the effects of PUFAs as an overarching class of lipids, whilst others distinguished the omega-3 and omega-6 families, their component fatty acids, or both. Therefore, the results are difficult to interpret as a collective body of evidence. Nevertheless, in five cohort studies, there was a detrimental effect of higher fish consumption[363], PUFA intake[361], omega-3 FA intake[357], or ALA intake[354,362] on the development of senior maculopathy, whilst four others found no statistically significant associations.[352,355,359,366] Meanwhile, eleven cohort studies found significant beneficial associations with consuming more fish[351], omega-3 FAs[360,364,365], DHA[348,350,353,362], EPA[349,356], or DHA and EPA[358], on ARM-alone[353,360], AMD-alone[350,351,358,362,365], dry AMD-alone[348,349], wet or dry AMD[356], or wet AMD-alone.[364] It has been suggested that those analysis models which did not adjust for dietary omega-6 intake, could explain the non-significant associations of the other cohort studies;

as introduced in Section 1.4.2, omega-3 and omega-6 FAs compete for the same COX and LOX enzymes in response to adverse systemic or environmental conditions.[191]

Several criticisms can be made of the existing observational research. Most of the cohort studies assessed lipid exposures just once at baseline. AMD events were then ascertained at the final visit, often several years later (range 3-12 years). Therefore, it is plausible that the participants' dietary habits changed over that time. Among the studies that repeated an FFQ during the follow-up period, none described the movement of participants between quantiles or how they were categorised into the average quantile used in the primary analyses.[351,353,354,362,363] Additionally, too few provided the number of events and the number at risk contributing to risk ratio estimates in each quantile. When they did, the numbers were often small, so the confidence interval around the risk estimate was imprecise. Moreover, FFQs may be prone to recall inaccuracies, and most studies lacked detail regarding the dose, duration and frequency of omega-3 FA exposure. Finally, the ability to control for confounding factors without a control group limits the strength of evidence provided by these observational studies.[330] Most of these issues are overcome by well-designed randomised controlled trials.

To date, three trials of omega-3 FA supplementation for the secondary prevention of AMD have been conducted. AREDS2 randomised 4203 men and women aged 50-85 with bilateral ARM or late AMD in only one eye, in a 2×2 factorial blinded design between 10mg lutein plus 2mg zeaxanthin daily or a matching placebo and, separately, between omega-3 FA capsules (containing 650mg EPA and 350mg DHA) daily or a matching placebo.[367] Every participant also received the AREDS1 formulation of antioxidant vitamins and minerals.[367] The primary outcome was incident wet or dry AMD identified using annual mydriatic fundus photographs.[367] During 4.9 years of follow-up, being allocated to active omega-3 FAs did not prevent the progression to AMD: 979 out of 3491 eyes [28.0%] in the active treatment group developed late disease, compared with 961 out of 3400 eyes [28.3%] in the placebo group (HR 0.98; 95% CI: 0.89-1.08; P = 0.74).[367] However, the study was limited by its complicated design, which included a secondary randomisation to the AREDS1 formulation with no beta-carotene, with low-dose zinc, or both. The potential for interactions between single specific ingredients or the combination of ingredients in the AREDS1 formulation when administered simultaneously with omega-

3 FAs was not taken into account. There was also no correction for inter-eye correlation in their per-eye unit of analyses; therefore, the variance, confidence intervals and P values could have been underestimated.

The Nutritional AMD Treatment-2 (NAT-2; 2003-2008) study compared the efficacy of DHA-enriched supplementation for preventing wet AMD.[368] In total, 263 men and women aged 55-85 years with bilateral drusen or wet AMD in just one eye were randomised to a daily omega-3 FA supplement (containing 270mg EPA and 840mg DHA) or a matching placebo.[368] Participants underwent baseline and annual fluorescein angiography examinations over a three-year follow-up period.[368] There were no beneficial effects of omega-3 FAs on the primary efficacy outcome of time to the first occurrence of wet AMD, which was 19.5±10.9 months in the active group compared to 18.7±10.6 months in the placebo group (HR 0.89; 95% CI: 0.55-1.42).[368] However, the trialists performed a per-protocol analysis instead of intention-to-treat, which invalidates the assumptions underlying randomisation and may be prone to bias.[369] In a separate observational analysis that only included participants allocated active omega-3 FAs, there was a marginally significant association between red blood cell membrane (RBCM) EPA and DHA levels, measured using gas chromatography, and the three-year incidence of wet AMD.[368] Of the 118 individuals included in this analysis, 14.3% in the highest tertile of RBCM fatty acids developed the disease, compared to 32.5% in the lowest tertile (HR 0.32; 95% CI: 0.10-0.99; P = 0.047).[368] The composition of RBCMs has been reported to reflect lipid uptake in retinal tissues[154], which might be relevant; however, causality cannot be inferred from these results, which have not been replicated in a larger study with a placebo control group.

The VITamin D and omega-3 triAL (VITAL-AMD; 2011-2017) randomised 25,871 men over 50 and women over 55 years of age, in a 2x2 factorial blinded design, to 2000 units of cholecalciferol daily or a matching placebo and, separately, to an omega-3 FA supplement (containing 460mg EPA and 380mg DHA) daily or a matching placebo.[370] The primary outcome of total AMD was a composite of incident AMD or AMD progression based on participant-reported events confirmed by medical record review.[370] Over 5.3 years of follow-up, allocation to active omega-3 FAs did not reduce the total number of

participants who developed AMD compared to the placebo: 157 [1.2%] *vs.* 167 [1.3%], respectively; HR 0.93; 95% CI: 0.73-1.17.[370]

The headline results of these trials were complementary, but they had important methodological differences. The sample size in AREDS2 had 90% power to detect a 25% reduction in the primary endpoint, based on the disease progression rates observed in the AREDS1 study.[277] Hence it was underpowered to detect any benefits of omega-3 FAs smaller than this, which might nevertheless be important given the current lack of effective treatments for dry AMD. Similarly, the event rate in the placebo arm of NAT-2 was less than they expected when the study was planned.[368] It could also be relevant that 11.1% of participants in the placebo arm of AREDS2 took omega-3 FAs in contravention of the study protocol.[371] These issues increase the likelihood of shifting the overall result towards the null in both the AREDS2 and NAT-2 studies. Finally, the formulations in AREDS2 and VITAL-AMD differed from NAT-2, which could affect their bioavailability. Both AREDS2 and VITAL-AMD used the ethyl ester form of omega-3 FAs, with a DHA:EPA ratio of 1:2, compared to the triglyceride form at a 3:1 ratio in NAT-2. Therefore, even though a limited number of AMD events are expected in ASCEND-Eye, for the reasons discussed in Section 2.2.1, the contribution of more randomised evidence with a well-defined and long exposure to omega-3 FAs is of value.

2.3 Chapter Summary

This chapter described the population studies that characterised AMD's clinical features and risk factors. Two systematic literature reviews identified the theoretical beneficial and adverse effects that aspirin and omega-3 FAs might have on the development of senior maculopathy and the need for more reliable, randomised evidence of these. The ASCEND trial provides an opportunity to make the necessary comparisons.

Figure 8 Aspirin and Age-Related Macular Degeneration Systematic Literature Search

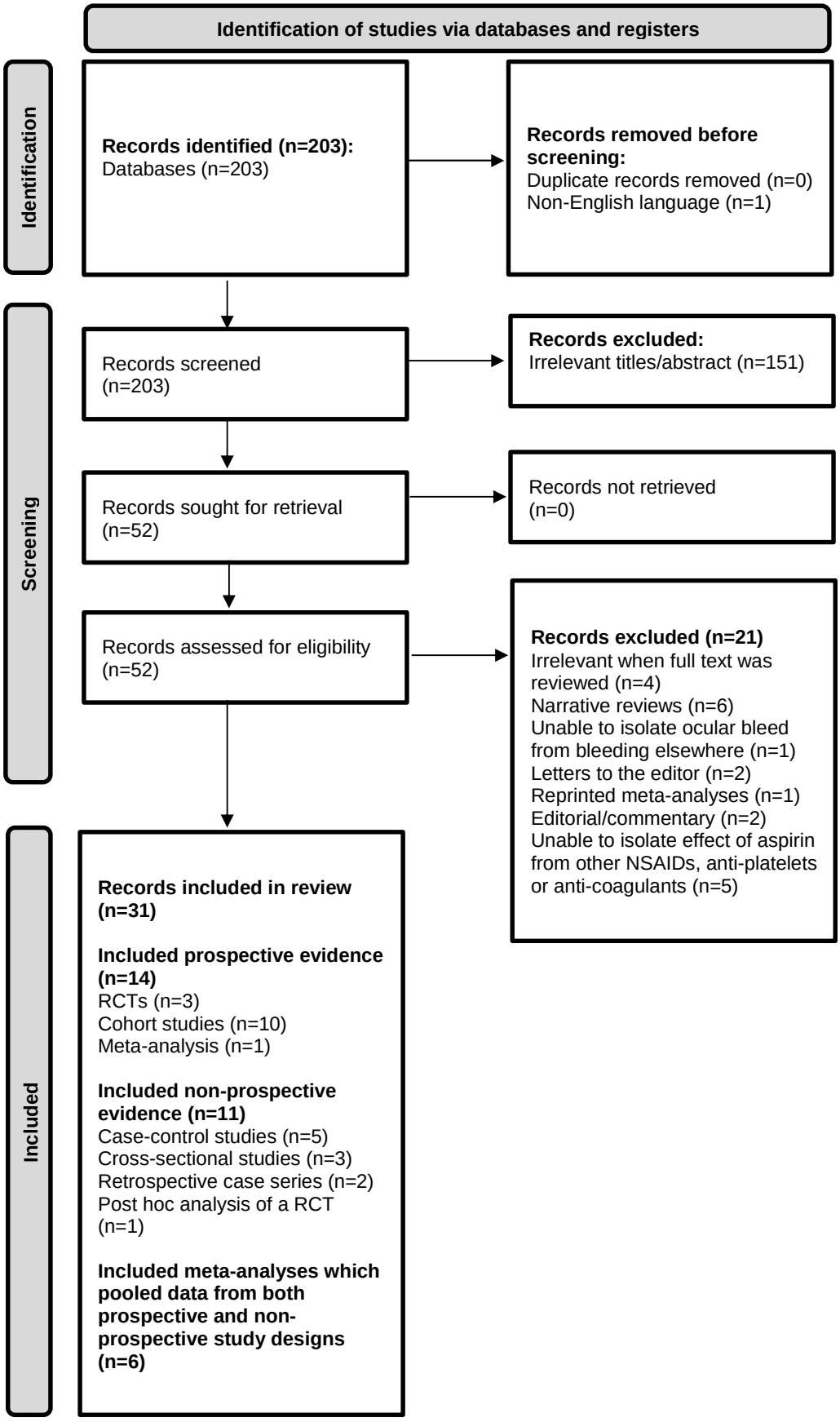


Table 5 Aspirin and Age-Related Macular Degeneration Systematic Literature Search

For the randomised trials, the summary statistic in this table is presented as the number [%] of cases in the aspirin group *vs*. the number [%] of cases in the comparison group. Where reported in the observational studies, the summary statistic is presented as the number of cases/the number at risk [%] in those with the highest exposure to aspirin *vs.* the lowest exposure. For both study designs, this is followed by the risk estimate and the lower and upper bounds of the confidence interval around that risk estimate. The summary statistics are in bold, and P values if the result was statistically significant and if it was reported.

Variables that were adjusted in the analysis models of studies with an observational design are listed in rows underneath the relevant study. Shading is used to demarcate rows of data belonging to separate studies.

Characteristics of the study			**Characteristics of the participants**		**Characteristics of the aspirin exposure**		**Characteristics of the outcome**		**Summary Statistics**
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1º Outcome(s)**	**Event ascertainment**	
Efficacy									
Randomised controlled trials									
Christen *et al* 2001, US[323] Excellent Physicians' Health Study	Double-blind, 2x2 factorial design	22,071 (5)	52.8 (100)	Volunteer male physicians	-	325mg aspirin on alternate days *vs.* placebo*	Incident AMD causing BCVA loss to 20/30 or worse	Self-report confirmed by medical record review	51 [0.5%] *vs.* 66 [0.6%]; RR 0.77; 95% CI: 0.54-1.11
Christen *et al* 2009, US[301] Excellent Women's Health Study	Double-blind, 2x2 factorial design	39,421 (10)	54.5 (0)	Volunteer female healthcare professionals	-	100mg aspirin on alternate days *vs.* placebo†	Incident AMD causing BCVA loss to 20/30 or worse	Self-report confirmed by medical record review	111 [0.68%] *vs.* 134 [0.68]; HR 1.03; 95% CI: 0.88-1.21
Robman *et al* 2017, Australia[332,372] Excellent ASPREE-AMD	Double-blind, parallel groups	4993 (5)	73.4 (48)	Healthy elderly volunteers aged ≥70	-	100mg daily *vs.* placebo	Incident AMD	Non-mydriatic fundus photography (grading system to be confirmed)	The results of this study are yet to be published
Cohort studies									
Klein *et al* 2001, US[314] Fair Beaver Dam Eye Study	Cohort	3684 (5)	43-86 (-)	Population-based. Adults aged ≥43	BL and FFU questionnaire	Current users *vs.* non-users (dose, duration and frequency not defined)	Incident ARM	BL and FFU fundus photography WARMGS	62/628 [9.9%] *vs.* 167/2198 [7.7%]; OR 1.08; 95% CI: 0.79-1.48
Adjustment for confounding: Age, sex, smoking and beer consumption									

Characteristics of the study			Characteristics of the participants		Characteristics of the aspirin exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
Klein *et al* 2012, US[303] Good Beaver Dam Eye Study	Cohort	3206 (20)	43-86 (44)	Population-based. Adults aged ≥43	BL and 5-yearly questionnaire	Regular use 5 years prior to FU in year 20‡ *vs*. never used	Incident ARM Incident wet AMD Incident dry AMD	BL and 5-yearly fundus photography WARMGS	ARM: 117/1330 [8.8%] *vs*. 194/2531 [7.6%]; HR 0.93; 95% CI: 0.70-1.23
									Wet AMD: 27/2008 [1.3%] *vs*. 34/3519 [1.0%]; HR 1.46; 95% CI: 0.81-2.60
									Dry AMD: 21/1968]1.1%] *vs*. 16/3460 [0.5%]; HR 2.25; 95% CI: 0.75-6.72
						Regular use 5 and 10 years prior to FU in year 20‡ *vs*. never used			ARM: 81/963 [8.4%] *vs*. 230/2898 [7.9%]; HR 0.86; 95% CI: 0.65-1.13
									Wet AMD: 30/1462 [2.1%] vs. 31/4065 [0.8%]; HR 2.20; 95% CI: 1.20-4.15; P = 0.01
									Dry AMD: 14/1417 [1.0%] *vs*. 23/4001 [0.6%]; HR 0.66; 95% CI: 0.25-1.95
Adjustment for confounding: Age, arthritis history and education level									
Lee *et al* 2021, Taiwan[322] Fair	Retrospective cohort	682,133 (11)	≥45 (50.5)	Population-based. Adults aged ≥45 who started aspirin between 2002-2012 were age and sex-matched to non-users§	Linkage data between 2002-2012¶	Consecutive users of 100mg aspirin per day for 180 days before incident AMD diagnosis *vs*. non-users	Incident AMD	Linkage to the National Health Insurance Research Database in 2013	***AMD: 8108/204,085 [3.9%] vs. 10,526/478,048 [2.2%]; HR 2.54; 95% CI: 2.44-2.65***
							Incident wet AMD		***Wet AMD: 570/204,085 [0.3%] vs. 684/204,085 [0.1%]; HR 2.82; 95% CI: 2.43-3.27***
Adjustment for confounding: Age, dyslipidemia, other eye conditions, arthritides, cardiovascular disease, liver disease, renal disease, neuroses, eczema, dementia drugs, antimicrobials, anti-inflammatory drugs, nasal decongestants, laxative use and anti-diarrhoeal drug use									

Characteristics of the study			Characteristics of the participants		Characteristics of the aspirin exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
Wang *et al* 2003, Australia[321] Fair Blue Mountains Eye Study	Cohort	2334 (5)	≥49 (-)	Population-based. Adults aged ≥49	BL interview	≤ Once a month *vs.* ≥ once a week in the preceding year (Dose not defined)	Incident ARM	BL and FFU fundus photography WARMGS	ARM: -/- *vs.* -/-; OR 1.0; 95% CI: 0.6-1.6
							Incident AMD		AMD: -/- *vs.* -/-; OR 2.0; 95% CI: 0.8-4.9
Adjustment for confounding: Age, sex, family history of AMD and smoking									
Liew *et al* 2013, Australia[316] Fair Blue Mountains Eye Study	Cohort	2389 (15)	64.1 (42.4)	Population-based. Adults aged ≥49	BL interview	150mg ≥ once a week in the preceding year *vs*. occasional users *vs.* non-users	Incident wet AMD	BL and 5-yearly fundus photography WARMGS	***Wet AMD: 15/257 [5.8%] vs. 34/1524 [2.2%]; OR 2.46; 95% CI: 1.25-4.83; P (trend) = 0.01***
							Incident dry AMD		Dry AMD: -/- *vs.* -/-; OR 0.99; 95% CI: 0.59-1.65
Adjustment for confounding: Age, sex, smoking, BMI, cardiovascular disease and SBP									
Aronow *et al* 2014, US[315] Fair	Cohort	2442 (6)	50-85 (-)	Adults aged ≥50, with bilateral ARM or AMD in one eye (AREDS2 RCT population)	BL and annual follow-up interviews	Users taking aspirin ≥ 5 x per week *vs.* non-users (Dose and duration not defined)	Incident AMD	Annual fundus photography AREDS grading system	AMD: -/- *vs.* -/-; OR 0.80; 95% CI: 0.41-1.56
							Incident wet AMD		Wet AMD: -/- *vs.* -/-; OR 1.31; 95% CI: 0.52-3.32
							Incident dry AMD		Dry AMD: -/- *vs.* -/-; OR 0.60; 95% CI: 0.23-1.58
Adjustment for confounding: Propensity-score matching of users and non-users by age, sex, ethnicity, education, smoking, hypertension, diabetes and angina									
Keenan *et al* 2019, US[320] Fair	Cohort	AREDS1: 1950 (10.1) AREDS2: 1694 (5)	50-85 (-)	Adults aged ≥50, with bilateral ARM or AMD in one eye (AREDS1 and AREDS2 RCT populations)	BL and annual follow-up interviews	Users taking aspirin ≥ 5 x per week *vs.* non-users (Dose and duration not defined)	Progression to AMD in quintile 5 of propensity for aspirin use *vs.* quintile 1	Fundus photography AREDS grading system	AREDS1: -/- *vs.* -/-; HR 1.17; 95% CI: 0.84-1.63
									AREDS2: -/- *vs.* -/-; HR 1.26; 95% CI: 0.96-1.66
Adjustment for confounding: Propensity-score matching of users and non-users by age, sex, ethnicity, education, smoking, hypertension, diabetes, hypercholesterolaemia, coronary artery disease and congestive heart failure									

Characteristics of the study			Characteristics of the participants		Characteristics of the aspirin exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
Modjtahedi *et al* 2018, US[317] Fair California Men's Health Study	Cohort	51,371 (7.4)	≥45 (100)	Population-based. Men aged ≥45	BL and FFU participant questionnaires	Longer-term, short-term, and former users *vs.* non-users** (dose, duration and frequency not defined)	Incident wet AMD	Self-report confirmed with electronic medical record review	***32/- vs. 30/-; HR 0.82; 95% CI: 0.70-0.97; P = 0.018***
Adjustment for confounding: Age, ethnicity, smoking, education, income and alcohol use									
Xu *et al* 2021, US[319] Fair California Teacher's Study	Cohort	88,481 (17)	50-80 (0)	Female teachers aged ≥50	BL questionnaire	Aspirin use ≥3x per week for ≥5 years *vs*. never used (Dose not defined)	Incident outpatient attendance for AMD	Linkage to hospital discharge records	312/12,650 [2.5%] *vs*. 1194/67,269 [1.8%]; HR 1.22; 95% CI: 0.96-1.55
					Additional questionnaire sent to n=50,202, between 2005-2012	81mg aspirin per day on ≥3 days a week *vs*. non-users (Duration not defined)			***-/- vs. -/-; HR 0.81; 95% CI: 0.70–0.95***
						325mg aspirin per day on ≥3 days a week) *vs*. non-users (Duration not defined)			-/- vs. -/-; HR 1.16; 95% CI: 0.95-1.48
Adjustment for confounding: Age, smoking, diabetes, ethnicity, BMI, physical activity, alcohol use, hospitalisation for musculoskeletal or connective tissue disease, hospitalisation for circulatory disease, asthma, clotting disorders and antihypertensive medication use									
Rim *et al* 2019, South Korea[318] Poor	Retrospective cohort	482,613 (5)	≥45 (47.9)	Population-based. Adults aged ≥45	Linkage data between 2010-2015	≤100mg aspirin per day for ≥1044 days *vs*. no use	Incident wet AMD	Linkage to the South Korean Health Insurance Database for prescription, diagnostic and treatment data	49/12,366 [0.4%] *vs*. 257/61,830 [0.4%]; HR 0.94; 95% CI: 0.70-1.28
Adjustment for confounding: Propensity score matching of users and non-users by age, sex, income and residential area									

Characteristics of the study			Characteristics of the participants		Characteristics of the aspirin exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
Cross-sectional studies									
De Jong *et al* 2012, Holland[307] Fair EUREYE Study	Cross-sectional	4691 (-)	≥65 (44.3)	Population-based. Adults aged ≥65 in 7 European countries	Interview by study personnel	Daily, at least once per week but not daily, monthly or less *vs*. never used (Dose and duration not defined)	Prevalent dry AMD	Fundus photography RES grading system	Dry AMD: 21/- *vs*. 28/- ; OR 0.79; 95% CI: 0.52-1.21
							Prevalent wet AMD		***Wet AMD: 57/- vs. 51/-; OR 2.26; 95% CI: 1.66-3.08; P (trend) <0.001***
Adjustment for confounding: Age, sex, BMI, SBP, cholesterol, smoking, education, cardiovascular disease and diabetes									
Hirvela *et al* 1996 Finland[309] *Poor*	Cross-sectional	478 (-)	≥70 (37.4)	Population-based. Adults aged ≥70	Interview by study personnel	Taking *vs*. not taking aspirin (Dose, frequency and duration not defined)	Age-standardised prevalence of AMD	Fundus photography IARMGS	44% *vs*. 43%; "Not significant"
Adjustment for confounding: Age, diabetes, hypertension, BMI, smoking, occupation, time spent outdoors, glaucoma, cataract and retinal arteriosclerosis									
Cheung *et al* 2013, Singapore[310] Poor Singapore Indian Eye Study	Cross-sectional	3207 (-)	- (-)	Population-based. Adults aged ≥40	Interview by study personnel	Current use *vs*. no use (Dose, frequency and duration not defined)	Prevalent ARM	Fundus photography WARMGS	40/366 [10.9%] *vs*. 139/2841 [4.9%]; OR 1.38; 95% CI: 0.89-2.14
Adjustment for confounding: Age , sex, smoking, education, BMI, SBP and cardiovascular disease									
Case-control studies									
Blumenkranz *et al* 1986, US[311] Poor	Case-control	49 (-)	75.7 (-)	Clinic-based. Age and sex-matched controls	Interview by study personnel	Use *vs*. no use (Dose, frequency and duration not defined)	Incident wet AMD	Fundus photography (grading system not specified)	"Not significant"
Adjustment for confounding: Age, white blood cell count and lens opacities									
Rudnicka *et al* 2010, UK[312] Poor	Case-control	158 (-)	≥45 (-)	Clinic-based. Age and sex-matched controls	Interview by study personnel	Use *vs*. no use (Dose, frequency and duration not defined)	Incident AMD	Fundus photography IARMGS	19/81 [23%] *vs*. 27/77 [35%]; OR 0.61; 95% CI: 0.23-1.57
Adjustment for confounding: Sex, cataract, glaucoma, cardiovascular disease, hypertension, smoking and alcohol intake									

Characteristics of the study			Characteristics of the participants		Characteristics of the aspirin exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
Swanson and McGwin 2008, US[304] Fair	Case-control	5140 (-)	≥50 (100)	Male veterans aged ≥50 who attended a Veteran's Medical Centre in the US	Linkage to the Veteran's Administration Health Care System registry data	Ever filed a prescription for aspirin *vs*. never used (Dose, frequency and duration not defined)	Incident AMD	Linkage to the Veteran's Administration Health Care System registry data	***104/614 [17%] vs. 2115/4526 [47%]; OR 0.15; 95% CI: 0.12-0.18; P<0.001***
Adjustment for confounding: Arthritis, diabetes, hypertension, coronary heart disease, cerebrovascular disease and peripheral arterial disease									
DeAngelis *et al* 2004, US[313] Poor	Case-control	136 (-)	≥50 (-)	Clinic-based. Sibling cases and controls aged ≥50	Telephone interview	Use for 6 months prior to interview *vs*. never taken aspirin regularly (Dose, frequency and duration not defined)	Incident wet AMD	Fundus photography (± fluorescein angiography where phenotype was unclear)	-/- vs. -/-; RR 1.37; 95% CI: 0.57-3.32
Adjustment for confounding: Alcohol consumption, BMI and smoking									
Douglas *et al* 2007, UK[308] Fair	Case-control	104,176 (-)	77.4 (34.1)	Age and sex-matched cases and controls identified from the UK GPRD between 1987-2002	Linkage to GPRD prescription records	Ever *vs.* never prescribed aspirin	Association with incident AMD	Linkage to GPRD diagnostic records	***4277/18,007 [23.8%] vs. 17,729/86,169 [20.6%]; OR 1.22; 95% CI: 1.17-1.27***
Adjustment for confounding: GP consultation rate									
Case-series									
Wilson *et al* 2004, US[305] Poor San Francisco Veterans Affairs Hospital Study	Case-series	326 (-)	≥60 (-)	Veterans aged ≥60	Medical record review of clinical and demographic risk factors (proximity to AMD diagnosis was unclear)	Use *vs*. no use (Dose, frequency and duration not defined)	Risk factors associated with AMD	Fundus photography or fluorescein angiograms	***-/- vs. -/-; HR 0.63; 95% CI: 0.40-0.98***
Adjustment for confounding: Age, sex, smoking, ethnicity, hypertension, diabetes, cardiovascular disease, family history of coronary artery disease, statin use and serum lipid profile									

Characteristics of the study			Characteristics of the participants		Characteristics of the aspirin exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
Post-hoc analysis of a randomised controlled trial									
Buitendijk *et al* 2018, Denmark[306] Poor	Post-hoc analysis of an RCT	330 (1)	≥60 (47.4)	BRAMD RCT†† participants. Adults ≥60 with wet AMD	BL interview	Users *vs*. non-users (dose and duration of exposure not defined)	Visual impairment (not defined)	FFU fundus photography	-/- *vs*. -/-; OR 0.58; 95% CI: 0.34-1.09
Adjustment for confounding: Age, sex, study centre, smoking, lens opacities, cardiovascular disease, diabetes and hypertension									
Meta-analyses									
Li *et al* 2015, China[327]	Meta-analysis	180,834 (-)	**Studies included:** Cheung *et al* 2013, Christen *et al* 2009, Christen *et al* 2001, DeAngelis *et al* 2004, De Jong *et al* 2012, Douglas *et al* 2007, Klein *et al* 2012, Liew *et al* 2013, Rudnika *et al* 2010, Blumenkranz *et al* 1986 **1° Outcomes:** Incident ARM Incident AMD Incident Wet AMD						ARM: RR 1.19; 95% CI: 0.92-1.53 AMD: RR 1.22; 95% CI: 0.87-1.72 ***Wet AMD: RR 1.95; 95% CI: 1.40-2.72*** Dry AMD: RR 0.84; 95% CI: 0.62-1.15
Ye *et al* 2014, China[328]	Meta-analysis	177,683 (-)	**Studies included**: Klein *et al* 2012,Blumenkranz *et al* 1986, Aronow *et al* 2014, Liew *et al* 2013,Rudnicka *et al* 2010, Douglas *et al* 2007, DeAngelis *et al* 2004, Christen *et al* 2001 **1° Outcomes:** Incident AMD Incident Wet AMD Incident Dry AMD						AMD: RR 1.00; 95% CI: 0.96-1.04 ***Wet AMD: RR 1.59; 95% CI: 1.09-2.31*** Dry AMD: RR 0.84; 95% CI: 0.58-1.22
Small *et al* 2017, UK[324]	Meta-analysis	12,006 (-)	**Studies included:** Wang *et al* 2003, Klein *et al* 2012, De Jong *et al* 2012 **1° Outcome:** Incident AMD						RR 1.09; 95% CI: 0.96-1.24
Yan *et al* 2022, China[325]	Meta-analysis	1,002,092 (-)	**Studies included:** Blumenkranz *et al* 1986, Christen *et al* 2001, Klein *et al* 2001, DeAngelis *et al* 2004, Clemons *et al* 2005, Douglas *et al* 2007, Christen *et al* 2009, Rudnicka *et al* 2010, de Jong *et al* 2012, Klein *et al* 2012, Cheung *et al* 2013, Liew *et al* 2013, Modjtahedi *et al* 2018, Keenan *et al* 2019, Rim *et al* 2019, Lee *et al* 2021 **1° Outcome:** Incident AMD						RR 1.11;95% CI: 0.87–1.39

Characteristics of the study			Characteristics of the participants		Characteristics of the aspirin exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1º Outcome(s)**	**Event ascertainment**	
Zhu *et al* 2013, China[326]	Meta-analysis	171,729 (-)	**Studies included:** Rudnicka *et al* 2010, Christen *et al* 2001, De Jong *et al* 2012, Hirvela *et al* 1996, Wang *et al* 2003, DeAngelis *et al* 2004, Moon *et al* 2012, Klein *et al* 2012 **1º Outcomes:** Incident ARM Incident AMD						ARM: RR 1.02; 95% CI: 0.87-1.20 AMD: RR 1.11; 95% CI: 0.7-1.60
Kahawita *et al* 2015, Canada[329]	Meta-analysis	10,292 (-)	**Studies included:** De Jong *et al* 2012, Klein *et al* 2012, Klein *et al* 2001, Cheung *et al* 2013 **1º Outcome:** Incident ARM						***OR 1.43; 95% CI: 1.09-1.88; P = 0.009***
Safety									
Case-series									
Kingham *et al* 1988, UK[293] *Poor*	Case-series	109 (-)	≥60 (-)	Clinic-based observations of late AMD patients	Between 1983-1988	Current use (Dose, frequency and duration not defined)	Incidence of sub-retinal bleeding	Fluoroscein angiogram	42/109 [39%] participants who bled were "currently" taking aspirin
Post-hoc analysis of a randomised controlled trial									
Buitendijk *et al* 2018, Denmark[306] *Poor*	Post-hoc analysis of an RCT	330 (1)	≥60 (47.4)	BRAMD RCT†† participants. Adults ≥60 with wet AMD	BL interview	Users *vs*. non-users (dose and duration of exposure not defined)	Any retinal bleed	FFU fundus photography	***22/99 [22.%] vs. 77/231 [33.3%]; OR 0.36; 95% CI: 0.18-0.71***
Adjustment for confounding: Age, sex, study centre, smoking, lens opacities, cardiovascular disease, diabetes and hypertension									

AREDS = Age-Related Eye Disease Study; ASPREE-AMD = ASPirin in Reducing Events in the Elderly-Age Related Macular Degeneration Study; BCVA = best corrected visual acuity; BL = baseline; BRAMD = The Bevacizumab to Ranibizumab in Age-Related Macular Degeneration Study; EUREYE = European Eye Study; FFU = final follow-up; GPRD = General Practice Research Database; IARMGS = International Age-Related Maculopathy Grading System; RCT = randomised controlled trial; RES = Rotterdam Eye Study (grading system); WARMGS = Wisconsin Age-Related Maculopathy Grading System

*Randomised factorial comparison: beta-carotene *vs.* placebo.
†Randomised factorial comparison: vitamin E *vs.* placebo.
‡Defined as taking aspirin twice a week for ≥3 months, although the dose was not specified.
§Patients were excluded from taking part if they had less than one year of data in the National Health Insurance database, if they had a history of AMD prior to 2002 or if they were prescribed aspirin ≥180 days before the incident AMD diagnosis.
¶Timings of the collected demographic and clinical data relative to each aspirin exposure were unclear.
**Long-term users were those who reported they were taking aspirin on their baseline and final follow-up questionnaires; short-term users included those who reported they were taking aspirin on the final follow-up questionnaire-only; former users were those who reported taking aspirin on their baseline but not the final follow-up questionnaire; non-users were those who did not report taking aspirin on either their baseline or final follow-up questionnaires.
††BRAMB (Bevacizumab to Ranibizumab in Age-Related Macular Degeneration Study) was a multi-centre, double-blind, non-inferiority trial of bevacizumab and ranibizumab to treat wet AMD.

Figure 9 Omega-3 Fatty Acids and Age-Related Macular Degeneration Systematic Literature Search

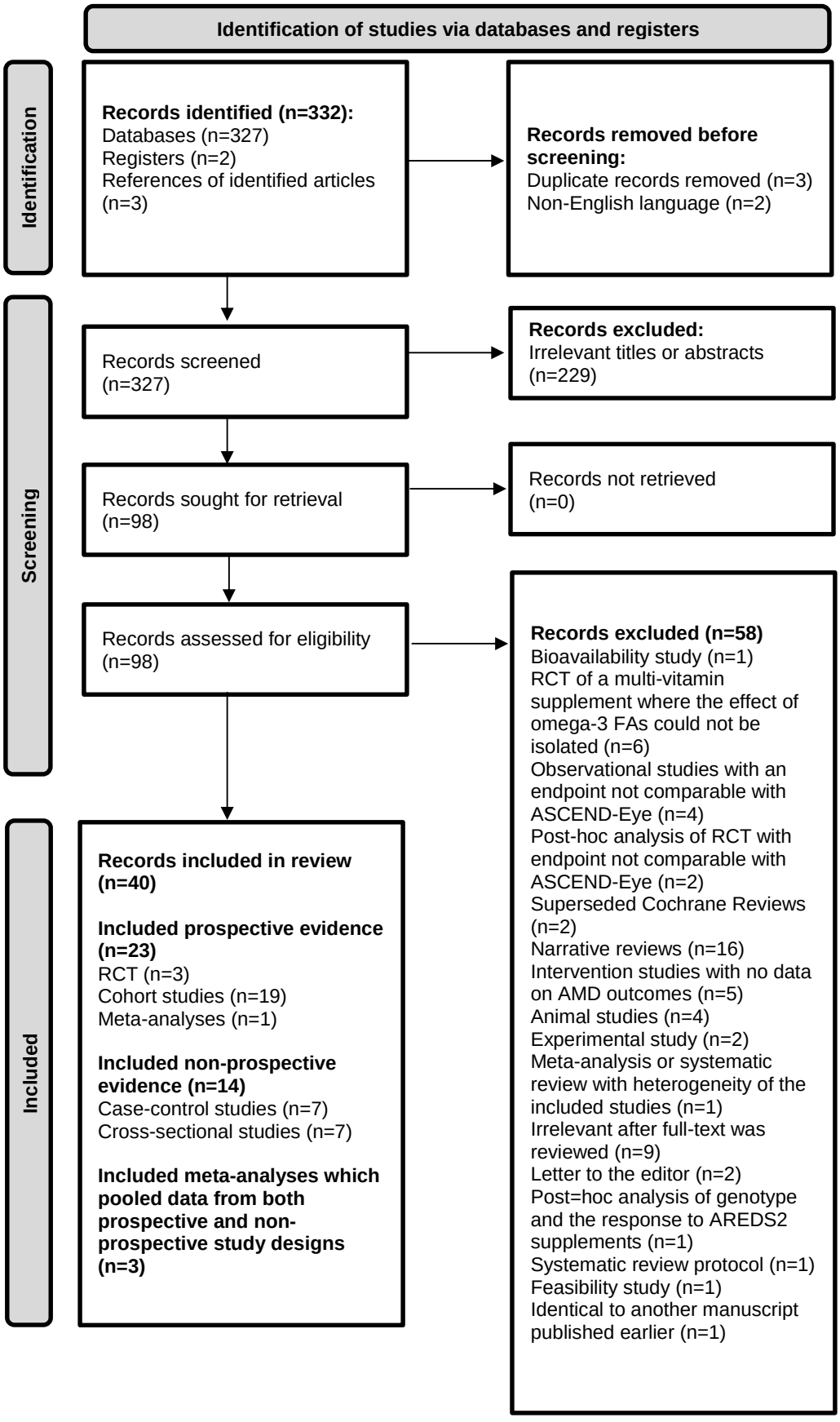


Table 6 Omega-3 Fatty Acids and Age-Related Macular Degeneration Systematic Literature Search

For the randomised trials, the summary statistic in this table is presented as the average time to, or the number [%] of cases in the omega-3 FA group *vs*. the number [%] of cases in the comparison group. Where reported in the observational studies, the summary statistic is presented as the number of cases/the number at risk [%] in those with the highest exposure to omega-3 FAs *vs.* the lowest exposure. For both study designs, this is followed by the risk estimate and the lower and upper bounds of the confidence interval around that risk estimate. The summary statistics are in bold, and P values if the result was statistically significant and if it was reported.

Variables that were adjusted in the analysis models of studies with an observational design are listed in rows underneath the relevant study. Shading is used to demarcate rows of data belonging to separate studies.

Characteristics of the study			**Characteristics of the participants**		**Characteristics of the omega-3 FA exposure**		**Characteristics of the outcome**		**Summary Statistics**
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1º Outcome(s)**	**Event ascertainment**	
Efficacy									
Randomised Controlled Trials									
AREDS2 Research group, 2013, US[367] Excellent AREDS2	Double-blind, 2x2 factorial design	2080 (4.9)	74 (45)	Adults aged ≥50, with bilateral ARM or AMD in one eye	-	650mg EPA plus 350mg DHA OD *vs.* placebo*	Progression to late AMD in either eye (per eye unit of analysis)	Annual fundus photography; AREDS grading system	979 [28.0%] of 3491 eyes *vs*. 961 [28.3%] of 3400 eyes; HR 0.97; 98.7% CI: 0.82-1.16
Souied *et al* 2013, France[368] Good NAT-2	Double-blind, parallel groups	263 (3)	74 (35)	Adults aged ≥55, with bilateral ARM or wet AMD in one eye	-	840mg DHA plus 270mg EPA daily *vs.* placebo†	Time to first occurrence of Wet AMD in one eye	Annual fluorescein angiography	19.5±10.9 months *vs*. 18.7±10.6 months; HR 0.89; 95% CI: 0.55-1.42
Christen *et al* 2020, US[370] Excellent VITAL-AMD	Double-blind, 2x2 factorial design	25,871 (5.5)	67 (49)	Men aged ≥50 and women aged ≥55	-	460mg EPA plus 380mg DHA OD *vs*. placebo	Total late AMD events in one eye	Annual fundus photography; AREDS grading system	157 [1.2%] *vs.* 167 [1.3%]; HR 0.94; 95% CI: 0.76-1.17
Cohort Studies									
Parekh *et al* 2009, US[357] Poor CAREDS	Prospective cohort	1787 (4)	50-79 (All female)	Women aged 50-79, from WHI-OS with carotenoid intake above the 78th percentile or below the 28th percentile on a BL FFQ	BL 122-item FFQ	Quintiles of omega-3 FA intake before baseline (duration not defined)	4-yr incidence of ARM	FFU fundus photography; AREDS grading system	***63/209 [30.1%] vs. 26/232 [11.2%]; OR 1.8; 95% CI: 1.2-2.6; P = 0.003***
Adjustment for confounding: Age, smoking, alcohol intake, BMI, post-menopausal hormone therapy, physical activity, antioxidant vitamin use, self-reported hypertension, diabetes or cardiovascular disease, family history of AMD and iris colour									

Characteristics of the study			Characteristics of the participants		Characteristics of the omega-3 FA exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
Arnarsson *et al* 2006, Iceland[363] Fair Reykjavik Eye Study	Prospective cohort	846 (5)	≥50 (-)	Adults aged 50+ from the Reykjavik Population Census	16-iteam FFQ at BL and at 5 years	Average tertiles of herring intake/month from BL and 5-yr follow-up FFQs	5-yr incidence of ARM	BL and FFU fundus photography; IARMGS	***-/- vs. -/-; OR 1.64; 95% CI: 1.00-2.68 P <0.05***
Adjustment for confounding: Age, sex and smoking									
Cho *et al* 2001, US[362] Good Nurses' Health Study and Health Professionals Follow-up Study	Prospective cohort	72,489 (12)	30-75 (41)	Adults aged 30-75	BL and biennial 130-item FFQ	Average quintiles of lipids as a percentage of the total energy intake during the follow-up period: PUFA, LA, ALA, AA, EPA, DHA§	Incident AMD with visual loss of 20/30 or worse	Self-report confirmed with medical record review	PUFA: 126/- *vs*. 128/-; RR 1.13; 95% CI: 0.88-1.44 LA: 123/- *vs*. 126/-; RR 0.84; 95% CI: 0.58-1.22 ***ALA: 145/- vs. 100/-; RR 1.41; 95% CI: 1.00-1.98; P(trend) = 0.03*** AA: 105/- *vs*. 120/-; RR 0.89; 95% CI: 0.68-1.18 EPA: 119/- *vs*. 120/-; RR 0.77; 95% CI: 0.59-1.01 ***DHA: 109/- vs.121/-; RR 0.70; 95% CI: 0.52-0.93; P(trend) = 0.05***
Adjustment for confounding: Age, sex, smoking, BMI, occupation, physical activity and alcohol intake									
Christen *et al* 2011, US[358] Fair Follow-up of the Nurses' Health Study trial	Prospective cohort	38,022 (10)	54.6 (All female)	Women aged 45+	BL 131-item FFQ	Tertiles of DHA intake in the year before BL	Incident AMD with BCVA ≤20/30	Self-report confirmed with medical record review	***60/12,499 [0.48%] vs. 103/13,600 [0.76%]; RR 0.62; 95% CI: 0.44-0.87; P = 0.005***
						Tertiles of EPA intake in the year before BL			***62/12,894 [0.47%] vs. 98/13,716 [0.71%]; RR 0.66; 95% CI: 0.48-0.92; P = 0.012***
Adjustment for confounding: Age, smoking, alcohol intake, BMI, post-menopausal hormone use, history of hypertension, history of hyperlipidaemia, history of diabetes mellitus, saturated fat intake, monounsaturated fat intake and trans-unsaturated fat intake									

Characteristics of the study			Characteristics of the participants		Characteristics of the omega-3 FA exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
Wu *et al* 2017a, US[354] Fair Follow-up of the Nurses' Health Study and Health Professionals Follow-up Study	Prospective cohort	114,850 (28 years (NHS) and 24 years HPFS)	50-90 (33.9)	Healthcare professionals aged 50-90	BL and every 4-yr thereafter 130-item FFQ	Quintiles of ALA intake during the year before each FFQ assessment	Incident ARM; incident AMD	Self-report confirmed with medical record review	***ARM: 373/19,425 [1.9%] vs. 285/19,405 [1.5%]; HR 1.28; 95% CI: 1.05-1.56; P = 0.01*** AMD: 292/19,425 [1.5%] *vs*. 255/19,405 [1.3%]; HR 1.05; 95% CI: 0.85-1.30
Adjustment for confounding: Age, sex, ethnicity, BMI, smoking, physical activity, aspirin use, hypertension, hypercholesterolaemia, LA quintiles, monounsaturated FA quintiles, saturated fat quintiles and trans-fat quintiles									
Wu *et al* 2017b, US[353] Fair Follow-up of the Nurses' Health Study and Health Professionals Follow-up Study	Prospective cohort	114,850 (28 years (NHS) and 24 years HPFS)	50-90 (33.9)	Healthcare professionals aged 50-90	BL and every 4-yr thereafter 130-item FFQ	Quintiles of EPA intake during the year before each FFQ assessment	Incident ARM; incident AMD	Self-report confirmed with medical record review	ARM: 309/19,379 [1.6%] *vs*. 316/19,461 [1.6%]; HR 0.89; 95% CI: 0.75-1.05 AMD: 278//19,379 [1.4%] *vs*. 262/19,461 [1.4%]; HR 1.07; 95% CI: 0.89-1.28
						Quintiles of DHA intake during the year before each FFQ assessment			***ARM: 289/19,379 [1.5%] vs. 343/19,461 [1.8%]; HR 0.78; 95% CI: 0.66-0.92; P(trend) = 0.008*** AMD: 266/19,379 [1.4%] *vs*. 269/19,461 [1.4%]; HR: 1.01; 95% CI: 0.84-1.21
Adjustment for confounding: Age, sex, ethnicity, BMI, smoking, physical activity, aspirin use, hypertension, hypercholesterolaemia, LA quintiles, monounsaturated FA quintiles, saturated fat quintiles, trans-fat quintiles, post-menopausal status and menopausal hormone use									
Seddon *et al* 2003, US[361] Fair Progression of Age-Related Macular Degeneration Study	Prospective cohort	261 (4.6)	72.8 (-)	Adults with ARM in at least one eye	BL FFQ	Quartiles of PUFA intake/week in the year before BL	Progression to AMD	BL and FFU fundus photography AREDS grading system	***65/- vs. 64/-; RR 2.28; 95% CI: 1.04-4.99; P(trend) = 0.04***
Adjustment for confounding: Age, sex, smoking, BMI, alcohol intake, protein intake, cardiovascular disease, educational attainment, physical activity and components of the AREDS1 multi-nutrient supplement									

Characteristics of the study			Characteristics of the participants		Characteristics of the omega-3 FA exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1º Outcome(s)**	**Event ascertainment**	
Chong *et al* 2009, Australia[366] Fair Melbourne Collaborative Cohort Study	Prospective cohort	6734 (3)	63.7 (39)	Population-based study of adults aged ≥40	BL 121-item FFQ‡	Quartiles of omega-3 FA intake in the year before BL	3-yr incidence of ARM; 3-yr incidence of AMD	FFU fundus photography IARMGS	ARM: 404/1391 [29.0%] *vs.* 402/1396 [28.8%]; OR 0.85; 95% CI: 0.71-1.02 AMD: 24/1391 [1.7%] *vs.* 21/1396 [1.5%]; OR 0.98; 95% CI: 0.52-1.86
Adjustment for confounding: Age, sex, components of the AREDS1 multi-nutrient supplement and use of cod liver oil or omega-3 fatty acids at baseline									
Chua *et al* 2006, Australia[359] Good Blue Mountains Eye Study II	Prospective cohort	2895 (5.1)	≥49 (-)	Population-based study of adults aged ≥49	BL 145-item FFQ	Quintiles of omega-3 FA intake in the year before baseline	5-yr-incidence of ARM; 5-yr incidence of AMD	BL and 5-yr fundus photography WARMGS	ARM: -/- *vs.* -/-; OR 0.92; 95% CI: 0.57-1.51 Total no. of participants with an ARM event = 130 AMD: -/- *vs.* -/-; OR 0.18; 95% CI: 0.02-1.38 Total no. of participants with an AMD event = 22
Adjustment for confounding: Age, sex, smoking and components of the AREDS1 multi-nutrient supplement									
Tan *et al* 2009, Australia[360] Good Blue Mountains Eye Study III	Prospective cohort	2454 (10.5)	≥49 (-)	Population-based study of adults aged ≥49	BL 145-item FFQ	Quartiles of omega-3 FA intake in the year before BL	10yr-incidence of ARM; 10-yr incidence of AMD	BL, 5-yr and 10-yr fundus photography WARMGS	***ARM: 44/484 [9.1%] vs. 68/484 [14.0%]; OR 0.63; 95% CI: 0.42-0.95; P(trend) = 0.04*** AMD: 9/507 [1.8%] *vs.* 15/511 [2.9%]; OR: 0.54; 95% CI: 0.22-1.31
Adjustment for confounding: Age, sex, smoking and components of the AREDS1 multi-nutrient supplement									
Joachim *et al* 2015, Australia[351] Blue Mountains Eye Study IV	Prospective cohort	1149 (15.6)	64.3 (42.4)	Population-based study of adults aged ≥49	BL and FFU 145-item FFQ	≥1 serving of fish per week (duration not defined)	15-yr incidence of ARM; 15-yr incidence of AMD	BL, 5-yr, 10-yr and 15-yr fundus photography WARMGS	ARM: -/- *vs.* -/-; OR 0.92; 95% CI: 0.68-1.24 ***AMD: -/- vs. -/-; OR 0.48; 95% CI: 0.29-0.79***
Adjustment for confounding: Age, sex, smoking, CFH and ARMS2 polymorphisms									

Characteristics of the study			Characteristics of the participants		Characteristics of the omega-3 FA exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
Robman *et al* 2007, Australia[355] *Fair* CHARM Study	Prospective cohort	252 (7)	74 (47)	Adults aged 50+ with ARM in at least one eye, from the VECAT Melbourne VIP studies	BL 38-item FFQ	Quintiles of omega-3 FA intake per day (duration not defined)	Progression to AMD	FFU fundus photography IARMGS	-/- *vs*. -/-; OR 2.44; 95% CI: 0.98-6.04 Total no. of participants who progressed to AMD = 61
Adjustment for confounding: Age, smoking, family history of AMD, source study and duration of follow-up									
Karger *et al* 2022, US[350] Fair -	Prospective cohort	3772 (10)	45-84 (-)	Adults ≥45 from the Multi-Ethnic Study of Atherosclerosis study	BL plasma phospholipid FA measured by gas chromatography of fasting blood samples	Quartiles of DHA as a % of the total plasma phospholipid FAs	Incident AMD	FFU fundus photography AREDS grading system	***48/940 [5.1%] vs. 66/967 [6.8%]; HR 0.54; 95% CI: 0.32-0.89; P(trend) = 0.014***
						Quartiles of EPA as a % of the total plasma phospholipid FAs			58/955 [6.1%] *vs*. 62/906 [6.8%]; HR 0.78; 95% CI: 0.49–1.21
Adjustment for confounding: Age, sex, ethnicity, smoking, total cholesterol, triglycerides, body mass index and statin use									
SanGiovanni *et al* 2008, US[349] Fair -	Observational follow-up of the AREDS1 trial participants	4519 (6.3)	55-80 (46)	Adults aged ≥50, with bilateral ARM or AMD in one eye who took part in AREDS1	BL 90-item FFQ	Quintiles of DHA as % total daily energy before baseline (duration not defined)	Progression to wet AMD; progression to dry AMD	Annual fundus photography during the in-trial period of AREDS1. Repeat examination at the FFU	Dry AMD: 28/423 *vs*. 31/406; OR 0.55; 95% CI: 0.28-1.10
									Wet AMD: 36/423 *vs*. 35/406; OR 0.97; 95% CI: 0.54-1.72
						Quintiles of EPA as % total daily energy before baseline (duration not defined)			***Dry AMD: 32/429 vs. 27/390; 0.44; 95% CI: 0.23-0.87; P ≤0.05***
									Wet AMD: 39/429 *vs*. 34/390; OR 0.99; 95% CI: 0.57-1.73
Adjustment for confounding: Age, sex, ethnicity, antacid use, iris colour, educational attainment, BMI, total energy intake, smoking and the AREDS1 clinical trial treatments									

Characteristics of the study			Characteristics of the participants		Characteristics of the omega-3 FA exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
SanGiovanni *et al* 2009, US[356] Fair -	Observational follow-up of the AREDS1 trial participants	1837 (12)	55-80 (-)	Adults aged ≥50, with bilateral ARM or AMD in one eye who took part in AREDS1	BL 90-item FFQ	Quintiles of DHA as % total daily energy before baseline (duration not defined)	Progression to wet AMD; progression to dry AMD	Annual fundus photography during the in-trial period of AREDS1. Repeat examination at the FFU	Dry AMD: 54/288 [18.8%] *vs.* 78/303 [25.7%]; OR 0.68; 95% CI: 0.47-0.99
									Wet AMD: 86/256 [33.6%] vs. 144/237 [60.8%]; OR 0.66; 95% CI: 0.47-0.92; P(trend) = 0.026
						Quintiles of EPA as % total daily energy before baseline (duration not defined)			***Dry AMD: 63/285 [22.1%] vs. 78/286 [27.3%]; OR 0.70; 95% CI: 0.49-1.00; P(trend) = 0.024***
									Wet AMD: 100/248 [40.3%] vs. 129/235 [54.9%]; OR 0.71 95% CI: 0.51-0.98; P(trend) = 0.068
Adjustment for confounding: Age, sex, total energy intake, smoking, AMD status at enrolment and the AREDS1 study treatments									
Agron *et al* 2021, US[348] *Fair* -	Observational follow-up of the AREDS1 and AREDS2 trial participants	8130 (10.4)		Adults with bilateral ARM or unilateral AMD who took part in AREDS1 or AREDS2	90-item FFQ at BL in each trial	Quintiles of DHA in the year before randomisation in each trial	Progression to AMD; progression to wet AMD; progression to dry AMD	Fundus photography at baseline and annually thereafter in both trials. Additional examination in AREDS2 participants at 10-yr AREDS grading system	AMD: -/- *vs.* -/-; HR 0.83; 95% CI: 0.73-0.93
									Wet AMD: -/- *vs.* -/-; HR 0.93; 95% CI: 0.79- 1.09
									Dry AMD: -/- vs. -/-; HR 0.74; 95% CI: 0.63-0.88; P(trend) = 0.05
						Quintiles of EPA in the year before randomisation in each trial			AMD: -/- *vs.* -/-; HR 0.83; 95% CI: 0.74-0.93
									Wet AMD: -/- *vs.* -/-; HR 0.92; 95% CI: 0.79-1.07
									Dry AMD: -/- *vs.* -/-; HR 0.76; 95% CI: 0.65-0.89
Adjustment for confounding: Treatment assignments in each trial, age, sex, smoking, BMI and correlation between eyes									

Characteristics of the study			Characteristics of the participants		Characteristics of the omega-3 FA exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
Chew *et al* 2022, US[352] *Good* -	Observational follow-up of the AREDS2 trial participants	8130 (10.4)	71 (44)	Adults with bilateral ARM or unilateral AMD who took part in AREDS1 or AREDS2	BL 90-item FFQ	Off-treatment follow-up of the AREDS2 randomisation: 650mg EPA plus 350mg DHA OD *vs.* placebo	Progression to late AMD in either eye (per eye unit of analysis)	Self-report confirmed with medical record review	1523/3203 eyes [47.5%] *vs*. 1517/3148 eyes [48.2%]; HR 1.01; 95% CI: 0.93-1.09
Adjustment for confounding: Age, sex, smoking and the AREDS2 study treatments									
Merle *et al* 2011, France[364] Poor Follow-up of the ALIENOR study participants	Prospective cohort	666 (1.5)	80 (37.7)	Population-based study. Adults ≥65	BL FFQ	Quintiles of omega-3 FA intake in the 24-hr before BL in the ALIENOR study 5 years earlier.	Incident ARM; incident dry AMD; incident wet AMD	FFU fundus photography Multi-Ethnic Study of Atherosclerosis grading system	***ARM: -/- vs. -/-; OR 0.83 95% CI: 0.71-0.98; P = 0.03*** Total no. of participants with ARM = 163 Dry AMD: -/- *vs*. -/-; OR 0.74; 95% CI: 0.52-1.06 Total no. of participants with dry AMD = 33 ***Wet AMD: -/- vs. -/-; OR 0.26; 95% CI: 0.08-0.83; P = 0.02*** Total no. of participants with wet AMD = 29
Adjustment for confounding: Age, sex, smoking, omega-6 FAs, monthly income, educational level, physical activity, BMI, dietary vitamins C and E, zinc, plasma HDL-cholesterol and CFH Y402H, ApoE4, ApoE2, and ARMS2 A69S polymorphisms									
Merle *et al* 2013, France[365] Poor Follow-up of the ALIENOR study participants	Prospective cohort	605 (2.5)	80 (37.7)	Population-based study. Adults ≥65	BL plasma phospholipid FA measured by gas chromatography of fasting blood samples	Quintiles of plasma omega-3 FA obtained 5 years earlier, at BL in the ALIENOR study, as % of total plasma FAs	Incident AMD	FFU fundus photography Multi-Ethnic Study of Atherosclerosis grading system	***-/- vs. -/-; OR 0.62; 95% CI: 0.44-0.88; P = 0.008*** Neither the no. of participants with AMD nor the total no. of participants in each quintile was provided
Adjustment for confounding: Age, sex, smoking, omega-6 FAs, monthly income, educational level, physical activity, BMI, dietary vitamins C and E, zinc, plasma HDL-cholesterol and CFH Y402H, ApoE4, ApoE2, and ARMS2 A69S polymorphisms									

Characteristics of the study			Characteristics of the participants		Characteristics of the omega-3 FA exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1º Outcome(s)**	**Event ascertainment**	
Cross-sectional Studies									
Roh *et al* 2020, US and Portugal[335] Fair -	Cross-sectional	549 (-)	≥50 (-)	Adults aged 50+ with and without AMD recruited from general medical clinics	86-item FFQ in Portugal; 61-item FFQ in US	Quintiles of omega-3 FA intake over the preceding year	Risk of AMD	Fundus photography AREDS grading system	81/95 [85.2%] *vs*. 78/95 [82.1%]; OR 1.21; 95% CI: 0.53–2.78
Adjustment for confounding: Age, sex, ethnicity, smoking, physical activity, hypertension and country									
Smith *et al* 2000, Australia[336] Fair Blue Mountains Eye Study I	Cross-sectional	2915 (-)	≥49 (-)	Population-based. Adults ≥49	145-item FFQ	Quintiles of PUFA intake during the preceding week	Risk of ARM	Fundus photography WARMGS	-/- *vs*. -/-; OR 1.18; 95% CI: 0.75-1.87
Adjustment for confounding: Age, sex, smoking and family history of AMD									
Mares-Perlman *et al* 1995, US[338] Poor Beaver Dam Eye Study	Cross-sectional	1968 (-)	45-84 (-)	Population-based. Adults aged 45-84	FFQ	Usual daily intake of seafood (duration not defined)	Risk of ARM; risk of AMD	Fundus photography WARMGS	ARM: -/- *vs*. -/-; OR 0.9; 95% CI: 0.6-1.3 AMD: -/- *vs*. -/-; OR 0.8; 95% CI: 0.2-2.5
Adjustment for confounding: Age and alcohol intake from beer									
Swenor *et al* 2010, US[340] Poor Salisbury Eye Evaluation study	Cross-sectional	2391 (-)	65-84 (42.8)	Population-based. Adults aged ≥65	91-item FFQ	Quintiles of weekly intake of fish high in omega-3 FAs	Risk of AMD	Fundus photography (grading system was not specified)	***OR: -/- vs. -/-; 0.4; 95% CI: 0.2-0.8***
Adjustment for confounding: Age, ethnicity, smoking and correlation between eyes									
Delcourt *et al* 2007, France[339] Poor POLANUT study	Cross-sectional	701 (-)	≥70 (-)	Population-based. Adults aged ≥70	165-item FFQ	Tertiles of PUFA intake per day (duration not specified)	Prevalent AMD	Fundus photography IARMGS	-/- *vs*. -/-; OR 1.02; 95% CI: 0.29–3.53
Adjustment for confounding: Age, sex, smoking, BMI and history of cardiovascular disease									

Characteristics of the study			Characteristics of the participants		Characteristics of the omega-3 FA exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1° Outcome(s)**	**Event ascertainment**	
Augood *et al* 2008, UK[334] Fair EUREYE study	Cross-sectional	2776 (-)	≥65 (44.9)	Population-based. Adults aged ≥65	130-item FFQ	Quartiles of DHA as a proportion of the total energy intake over the preceding year	Prevalent wet AMD	Fundus photography IARMGS	***13/542 [2.4%] vs. 37/544 [6.8%]; OR 0.32; 95% CI: 0.12-0.87; P(trend) = 0.01***
						Quartiles of EPA as a proportion of the total energy intake over the preceding year			***12/542 [2.2%] vs. 38/543 [7.0%]; OR 0.29; 95% CI: 0.11-0.73; P(trend) = 0.01***
Adjustment for confounding: Age, sex, diabetes status, smoking and aspirin use									
Heuberger *et al* 2001, US[337] Poor NHANES III Study	Cross-sectional	7405 (-)	40-79 (-)	Population-based. Adults aged ≥ 40	66-item FFQ	Tertiles of fish servings over the previous month	Prevalent ARM in those aged ≥60; prevalent AMD in those aged ≥60	Fundus photography WARMGS	ARM: 157/1849 [8.5%] *vs*. 208/2064 [10.1%];OR 1.0; 95% CI: 0.7-1.4
									AMD: 9/1003 [0.9%] *vs*. 21/1281 [1.6%]; OR 0.4; 95% CI: 0.2-1.2
Adjustment for confounding: Age and ethnicity									
Case-Control Studies									
Seddon *et al* 2001, US[373] Fair Dietary Ancillary Study of the Eye Disease Case Control	Case-control	853 (-)	55-81 (42)	Cases and controls were recruited from ophthalmology clinics. Cases were required to have wet AMD	FFQ	Quintiles of omega-3 FA intake during the preceding year	Relative risk of wet AMD	-	63/108 [58.3%] *vs*. 76/95 [80.0%]; OR 0.78; 95% CI: 0.39-1.56
Adjustment for confounding: Age, sex, smoking, educational attainment, physical activity, alcohol intake, BMI, SBP and carotenoid intake									
Seddon *et al* 2006, US[341]	Case-control	681 (-)	75.9 (100)	Cases and controls were	FFQ	Quartiles of omega-3 FA	Relative risk of AMD	Fundus photography	***48/123 [39.0%] vs. 64/102 [62.7%]; OR 0.55;***

Characteristics of the study			Characteristics of the participants		Characteristics of the omega-3 FA exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1º Outcome(s)**	**Event ascertainment**	
Fair US Twin Study of AMD				identified from the US National Academy of Sciences–National Research Council World War II Veteran Twin Registry		intake per week (duration not defined)		CARMS grading system	***95% CI: 0.32-0.95; P(trend) = 0.02***
Adjustment for confounding: Age, smoking, educational attainment, SBP, history of cardiovascular disease, protein intake, beta-carotene intake, alcohol intake, physical activity and intake of vitamin C, vitamin E and zinc									
SanGiovanni *et al* 2007, US ¶[343] Fair -	Case-control	4519 (-)	55-80 (43.9%)	Cases and controls were identified at recruitment for the AREDS1 trial	90-item FFQ	Quintiles of EPA intake during the preceding year	Relative risk of wet AMD; relative risk of dry AMD	Fundus photography AREDS grading system	Dry AMD: -/- *vs*. -/-; OR 0.88; 95% CI: 0.37-2.09 Total no. of participants with dry AMD = 118
									Wet AMD: -/- vs. -/-; OR 0.62; 95% CI: 0.45-0.85; P(trend) <0.01 Total no. of participants with wet AMD = 658
						Quintiles of DHA intake during the preceding year			Dry AMD: -/- *vs*. -/-; OR 0.99; 95% CI: 0.54-1.80 Total no. of participants with dry AMD = 118
									Wet AMD: -/- vs. -/-; OR 0.60; 95% CI: 0.42-0.85; P(trend) <0.01 Total no. of participants with dry AMD = 658
Adjustment for confounding: Age, sex, angina, arthritis, ethnicity, smoking, educational attainment, hypertension, cataracts, hydrochlorothiazide use, refractive error, antacid use and thyroid hormone use									
Sanders *et al* 1993, UK[344] Poor -	Case-control	130 (-)	74 (32.2)	Adults aged 65+ from a general practice	Plasma phospholipid FAs measured by gas	Tertiles of plasma omega-3 FA levels	Relative risk of senior maculopathy	Fundus photography (to confirm cases and controls in	-/- *vs*. -/-; OR 0.82; 95% CI: 0.35-1.93

Characteristics of the study			Characteristics of the participants		Characteristics of the omega-3 FA exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1º Outcome(s)**	**Event ascertainment**	
				population in London, UK	chromatography of fasting blood samples	Tertiles of RBCM omega-3 FA levels	(ARM and AMD)	the invited sample)	-/- *vs*. -/-; OR 0.92; 95% CI: 0.37-2.32
Adjustment for confounding: None									
Merle *et al* 2014, France[345] Poor -	Case-control	290 (-)	55-80 (37)	NAT-2 trial cases and population-based controls	Plasma phospholipid FAs measured by gas chromatography of fasting blood samples	Tertiles of plasma omega-3 FA levels	Relative risk of wet AMD	-	-/- *vs*. -/-; OR 0.74; 95% CI: 0.40-1.38 Total no. of cases = 290; total no. of controls = 144
						Tertiles of RBCM omega-3 FA levels			***OR 0.52; 95% CI: 0.29-0.94; P(trend) = 0.03*** Total no. of cases = 290; total no. of controls = 144
Adjustment for confounding: Age, sex, CFH Y402H, ARMS2 A69S, and ApoE4 polymorphisms, plasma triglycerides, hypertension, hypercholesterolemia and family history of AMD									
Aoki *et al* 2016, Japan[346] Fair -	Case-control	526 (-)	≥50 (-)	Hatoyama Cohort Study (controls) and macular clinic (cases)	58-item FFQ	Quintiles of omega-3 FA intake over the preceding month	Relative risk of AMD	-	***13/92 [14.1%] vs. 44/61 [72.1%];OR 0.2; 95% CI: 0.1-0.4; P< 0.0001***
Adjustment for confounding: Age, sex, smoking, alcohol use, hypertension, diabetes, hyperlipidemia, stroke and cardiovascular disease									
Leung *et al* 2019, Hong Kong[347] Fair -	Case-control	297 (-)	73.7 (-)	Cases and controls were identified from outpatient departments in two hospitals in Hong Kong. Eligibility criteria were unclear	Liquid chromatography and tandem mass spectrometry of fasting blood and urine samples	Plasma omega-6:omega-3 FAs ratio	Relative risk of wet AMD	-	***11:1 in cases (n=99) vs. 8.3:1 in controls (n=198) (P<0.001)***
						DHA-derived urinary lipid oxidation products**			***-/- vs. -/-; OR 0.55; 95% CI: 0.36-0.86; P = 0.008***
						EPA- derived urinary lipid oxidation products**			***-/- vs. -/-; OR 0.16; 95% CI: 0.04-0.69; P = 0.014***
Adjustment for confounding: Age and sex									
Meta-analyses									
Lawrenson and Evans 2015, UK[374]	Cochrane meta-analysis	2343 (-)	**Studies included:** AREDS Research Group 2013, Souied *et al* 2013 **1º Outcome**: Progression to AMD						Pooled HR 0.96; 95% CI: 0.84-1.10

Characteristics of the study			Characteristics of the participants		Characteristics of the omega-3 FA exposure		Characteristics of the outcome		Summary Statistics
Source, year of main publication, country, quality assessment, Study name	**Design**	**Sample size (follow-up (years))**	**Mean age or age range (years) (% Male)**	**Eligibility**	**Exposure assessment and timing**	**Exposure**	**1º Outcome(s)**	**Event ascertainment**	
Jiang *et al* 2021, China[375]	Meta-analysis	-	**Studies included:** Wu *et al* 2017a, Wu *et al* 2017b, Joachim *et al* 2015, Christen *et al* 2011, Tan *et al* 2009, Arnasson *et al* 2006, Seddon *et al* 2006, Seddon *et al* 2001, Roh *et al* 2020, Sasaki *et al* 2020, Swenor *et al* 2010, Chong *et al* 2009, Augood *et al* 2008, Delcourt *et al* 2007, Heuberger *et al* 2001, Mares-Perlman *et al* 1995, Sangiovanni *et al* 2007 **1º Outcomes:** Incident ARM and, separately, incident AMD (The number of participants included in each analysis was unclear)						***ARM: Pooled RR 0.86; 95% CI: 0.77-0.96*** ***AMD: Pooled RR 0.71; 95% CI: 0.55- 0.9***
Zhong *et al* 2021, China[376]	Meta-analysis	167,581 (-)	**Studies included:** Seddon *et al* 2003, Delcourt *et al* 2007, Chong *et al* 2009, Parekh *et al* 2009, Tan *et al* 2009, Christen *et al* 2011, Ho *et al* 2011, Wu *et al* 2017a, Wu *et al* 2017b, Merle *et al* 2013, Merle *et al* 2014 **1º Outcomes:** Incident ARM per 1g/day dose increment of DHA and, separately, incident ARM per 1g/day dose increment of EPA						***DHA: Pooled RR 0.50; 95% CI: 0.32-0.78*** ***EPA: Pooled RR 0.40; 95% CI: 0.18-0.87***
Chong *et al* 2008, Australia[377]	Meta-analysis	88,974 (-)	**Studies included:** Arnasson *et al* 2006, Cho *et al* 2001, Chua *et al* 2006, Sangiovanni *et al* 2007, Seddon *et al* 2001, Seddon *et al* 2006, Delcourt *et al* 2007, Heuberger *et al* 2001, Mares-Perlman *et al* 1995 **1º Outcome:** Incident AMD						***OR 0.62; 95% CI: 0.50-0.80***

AA = arachidonic acid; ALA = alpha linolenic acid; ALIENOR = Antioxydants, Lipides Essentiels, Nutrition et maladies OculaiRes study; AMD = Age-Related Macular Degeneration; AREDS = Age Related Eye Disease Study; ARM = Age-Related Maculopathy; BL = baseline; CAREDS = Carotenoids in Age-Related Eye Disease Study; CARMS = the Clinical Age-Related Maculopathy grading System; CHARM = Cardiovascular Health and its association with prevalence and progression of Age-Related Macular degeneration; DPA = docosahexaenoic acid; EPA = eicosapentaenoic acid; FFQ = Food Frequency Questionnaire; FFU = final follow-up; IARMGS = International Age-Related Maculopathy Grading System; LA = linolenic acid; Melbourne VIP = the Melbourne Visual Impairment Project; NAT-2 = Nutritional AMD Treatment-2; NHANES III = The Third National Health and Nutrition Examination Survey; PUFA = Polyunsaturated fatty acid; RBCM = red blood cell membrane; VECAT = Vitamin E, Cataract and Age-related Macular Degeneration Study; VITAL = VITamin D and omega-3 triAL; WARMGS = Wisconsin Age-Related Maculopathy Grading System; WH-OSI = Women's Health Initiative Observational Study

*Randomised factorial comparison: 10mg lutein plus 2mg zeaxanthin daily or placebo.
†Randomised factorial comparison: 2000 units of cholecalciferol daily or placebo.
‡In a separate validation study of n=4439 randomly-selected participants, the plasma phospholipid fatty acid levels were reported to correlate with those estimated from the FFQ.
§The movement of participants between quintiles at each biennium was not presented.
¶The severity of senior maculopathy on fundus photographs was graded 1-5 during recruitment into the AREDS1 trial. Those with bilateral AREDS grade 3 (ARM) disease or unilateral grade 4 (dry AMD) or grade 5 (wet AMD) were eligible to participate. In the analyses presented, ineligible adults with grades 1 or 2 disease acted as age- and sex-matched controls.
**The stratification of urinary variables in this risk assessment was unclear.

Chapter III - Methods

This chapter describes the strategy, rationale and statistical methods that will guide assessments of the clinical efficacy and safety of aspirin and omega-3 FAs for DR and AMD, and some related areas of eye health, in the sub-study of the ASCEND randomised, placebo-controlled trial, called ASCEND-Eye.

3.1 ASCEND

3.1.1 Design, Recruitment and Follow-Up

The ASCEND trial, coordinated by the Clinical Trial Service Unit & Epidemiological Studies Unit (CTSU) at the University of Oxford, compared the safety and efficacy of aspirin and omega-3 FAs for the primary prevention of serious cardiovascular events in people with diabetes. Details of the study design have been published elsewhere.[10,11] Briefly, the trial used mail-based methods to recruit and follow up 15,480 participants from the UK, who were identified from DR screening registers, hospital diabetes clinics and general practices between 2005 and 2011. They comprised men and women with type 1 or type 2 diabetes, who were aged 40 years or older, with no previous history of cardiovascular disease, no clear contraindication to aspirin (e.g. peptic ulcer disease within the previous six months, chronic liver disease, haematological conditions predisposing a high risk of bleeding, or concurrent use of anti-coagulant therapy), no allergy to either study treatment, and no pre-existing life-limiting medical conditions. The likelihood of adherence to the study regimen was evaluated during a 2-month pre-randomisation, single-blind, placebo run-in period, during which general practitioners (GPs) were notified of the participant's interest in the trial and asked if they knew of any reason why their patient should not be randomised. Participants were then sent a blood and urine sampling kit and a physical measurement form for BP, weight and height to be recorded. They were asked to take these to their GP surgery, where a practice nurse or phlebotomist would perform the tests. Each sample and completed form was mailed back to the Wolfson Laboratory at CTSU for central analysis. A total of 11,685 participants (75%) who were later randomised returned samples; however, they were only deemed

usable for 9813 of these (63% of the randomised population). Samples were discarded if there was an inadequate volume, incomplete consent or a significant delay in transit to the laboratory.

Eligible participants who completed the run-in were then randomised in a 2×2 factorial blinded design, between enteric-coated aspirin at a dose of 100mg daily or a matching placebo and, separately, between 1g omega-3 FA ethyl ester capsules (containing 460mg EPA and 380mg DHA) daily or a matching placebo. Randomisation was in a 1:1 ratio in both the aspirin and omega-3 FA arms; a minimization algorithm was used to ensure an equal balance of prognostic variables in each arm, which included age, sex, duration of diabetes, history of treated hypertension, smoking status, ethnic origin, total cholesterol, HbA1c, and urinary albumin/creatinine ratio.[378,379] A copy of the randomisation questionnaire is provided in Appendix 5. All randomised individuals were then sent a double-sided A3 follow-up questionnaire once every six months, with a further supply of their allocated treatment via the post. Follow-up questionnaires explicitly asked about the trial's cardiovascular endpoints, adherence to the study medications, initiation of contraindicated anti-thrombotic therapies, other serious adverse events such as new cancer diagnoses, and any major bleeding (Appendix 6). Participants could also directly report adverse events or concerns to the central coordinating office via a dedicated free 24-hour telephone service. Those who had a serious bleed[*] or a new indication for anti-thrombotic therapy were asked to discontinue the aspirin arm of the trial, but the omega-3 FAs arm was not interrupted. Those with other reasons for stopping were free to do so, but staff from CTSU would contact these individuals to encourage the continuation of study treatment(s) when appropriate. Finally, unblinding of the study treatment allocations was possible via the 24-hour Freefone service; however, maintaining the blind was prioritised unless knowing the treatment allocation would influence the clinical management of a participant. During the in-trial period, 63 participants were unblinded to their aspirin allocation: 39 were assigned to active treatment, and 24 were assigned the matching placebo. Meanwhile, 13 participants were unblinded in the omega-3 FAs arm: four were assigned to active treatment, and nine were assigned the matching placebo [unpublished analyses conducted by Dr William Stevens of the ASCEND Data

[*] Serious bleeds were defined as any intracranial haemorrhage, sight-threatening eye bleeds (defined in Section 3.2.1.1.1), bleeding that required hospitalisation or transfusion, or fatal or disabling bleeds.

Analysis Team at the Nuffield Department of Population Health, University of Oxford in 2022]. The scheduled treatment period in ASCEND ended on 31st July 2017.

3.1.2 Event Ascertainment, Outcome Definitions and Results

Every returned postal follow-up questionnaire was scanned and coded using optical character recognition (OCR) software. All adverse event data were coded using the UK Read coding thesaurus of medical terms.[380] On occasion, it was necessary to clarify a diagnosis and dates or to check that a participant was not inadvertently taking anti-thrombotic therapy at the same time as their aspirin allocation. Once these data were instantiated, supporting documents (e.g. hospital discharge letters) were sought from participants' GPs to confirm or refute the trial's cardiovascular and bleeding endpoints. Reports of death and cancer were verified using linkage data obtained from NHS Digital in England, Digital Health and Care Wales (previously called the NHS Wales Informatics Service), and Public Health Scotland. All adjudication was performed using pre-specified criteria by CTSU clinicians who were blind to the study treatment allocations.

The primary efficacy endpoint in ASCEND was any serious vascular event, a composite of non-fatal myocardial infarction, non-fatal stroke (excluding intracranial haemorrhage), transient ischaemic attack, or cardiovascular death (excluding intracranial haemorrhage). The primary safety endpoint was any major bleed, a composite of confirmed intracranial haemorrhage, sight-threatening eye bleeding or any other serious bleeding (those requiring hospitalisation, transfusion or which were fatal or disabling). The results of these analyses were presented at the European Society of Cardiology Conference in Munich, Germany, on Sunday 26th August 2018, with simultaneous publications in the New England Journal of Medicine.[12,13] Over the mean follow-up of 7.4 years, allocation to active aspirin was associated with a 12% proportional reduction in the cumulative incidence of serious vascular events (658 [8.5%] *vs.* 743 [9.6%]; rate ratio, 0.88; 95% CI, 0.79 to 0.97; P = 0.01), but this was largely counterbalanced by a higher risk of major bleeding (314 [4.1%] *vs.* 245 [3.2%]; rate ratio 1.29; 95% CI 1.09-1.52; P = 0.003).[12] Meanwhile, there were no significant differences in the primary, secondary or safety outcomes between those assigned omega-3 FAs *vs.* the placebo.[13]

3.2 ASCEND-Eye

3.2.1 Methods of Data Collection and Event Ascertainment

ASCEND-Eye will derive information on eye health from three discrete data sources:

i) eye events reported by participants during ASCEND;

ii) linkage to the NHS Diabetic Eye Screening Programme records; and a

iii) Visual Function Questionnaire (VFQ).

Sections 3.2.1.1 to 3.2.1.3 describe the nature of the data, methods of data collection and event ascertainment for each of these three sources. Sections 3.2.2 to 3.2.3 review the legal basis and regulatory and ethics applications for ASCEND-Eye. Section 3.2.4 describes the approach taken to clean the raw linkage data. Finally, Section 3.2.5 summarises some preliminary blinded assessments of the linkage quality.

3.2.1.1 Participant-Reported Eye Events

As introduced in Section 3.1, ASCEND participants could report new serious illnesses, hospital admissions and bleeding from any site for which they saw a doctor via their in-trial, six-monthly follow-up questionnaires (Appendix 6). Although there were no specific questions about new diagnoses of eye conditions in the same way there were for the primary cardiovascular outcomes, the questionnaires allowed participants to report chronic eye diseases associated with a significant or persistent visual disability and major bleeding in the eye as important medical events, under the Internal Council for Harmonisation Requirements for Pharmaceuticals for Human Use (ICH) E2A clinical safety data definition.[381]

Whilst ASCEND was ongoing, ASCEND-Eye sought supporting evidence from GPs to confirm or refute participant reports of eye-bleeding, AMD, DMO, and other ocular event terms which could have been related to an underlying eye-bleed, including blindness, visual deterioration, and retinal

detachment (Appendix 7). After the documentation was received, eye-bleeds were adjudicated according to the procedures defined in a sub-study Standard Operating Procedure (SOP; Appendix 8), prepared by the author, whilst blind to any knowledge of the study treatment effects. Other eye events were adjudicated according to the procedures defined in SOP number 8 for ASCEND (archived in an electronic document management system at CTSU and not appended to this thesis). The author completed all adjudication whilst unaware of the treatment group assignments. Eye events were coded using the same IT systems developed for ASCEND.

Another opportunity to report incident eye events was on a Visual Function Questionnaire (see Section 3.2.1.3) sent to a subset of participants after ASCEND had been completed. However, events originating from this source were not adjudicated because of practical difficulties in obtaining supporting evidence from GPs long after participants were first recruited into the parent study and competing priorities at the central coordinating centre. Allowance for this difference in the event verification is made in the definitions of outcomes specified in the Data Analysis Plan (DAP) in Section 3.4. Definitions for the eye events adjudicated during ASCEND are described in Sections 3.2.1.1.1 and 3.2.1.1.2.

3.2.1.1.1 Confirmation and Coding of Eye-Bleeding Events

The composite primary safety endpoint of "Major haemorrhage" in ASCEND included confirmed sight-threatening eye bleeds.[12] With no gold-standard way of grading the severity of eye bleeds, an important contribution of ASCEND-Eye to the parent trial was the development of a new way to distinguish non-serious bleeds, such as subconjunctival haemorrhages, from those with more serious prognoses, such as vitreous haemorrhages. Previous studies had reported the occurrence of a retinal haemorrhage, pre-retinal haemorrhage, or both as a binary present/absent outcome or as a qualitative endpoint, but this risks losing useful prognostic information.[382] The first objective method of grading vitreous haemorrhages was based on the number of retinal clock hours obscured by the blood on fundoscopy examination.[382] However, this approach excludes retinal or anterior chamber haemorrhages and does not consider subsequent visual loss or use of healthcare resources.[382] Without access to retinal images at the time of each reported eye bleed, their severity needed to be characterised from information that

could be obtained from clinic letters sent between hospital services and primary care. The adjudication rules were intended to be clinically meaningful; hence, they were developed in collaboration with Mr Praveen Patel, a Consultant Ophthalmologist at Moorfield's Eye Hospital, London.

Sight-threatening bleeds were defined as clinically significant bleeding in the eye that resulted in unresolved visual loss or required an urgent intervention such as laser photocoagulation, vitreoretinal surgery, intraocular injection(s) of anti-angiogenesis therapies, or both. Participants were considered to have visual loss if there was documented evidence of reduced visual acuity from a previously higher level or if the totality of the evidence available was consistent with significant visual loss. Examples included but were not limited to the following scenarios: Certificate of Vision Impairment registration documents dated after the bleed or clinic letters describing a fundus view obscured by a dense vitreous haemorrhage which made PRP infeasible. It was considered to be permanent if it had yet to resolve based on the latest information available at the time of adjudication.

According to Mr Patel, Ophthalmologists typically expect to intervene for an eye bleed within 3-6 months from the onset. Hence, interventions were to be considered "urgent" when they fell within this time window and were:

- evidence of referrals for retinal laser therapy or surgical interventions for posterior chamber eye bleeds; or
- evidence of referrals for intravitreal injections[*], retinal laser therapy, or surgical interventions for anterior chamber eye bleeds.

The definition included those for whom the totality of the available evidence was consistent with a significant bleed that would ordinarily warrant an urgent intervention, but a clinical decision was made to manage the patient conservatively because their visual potential was poor or when other comorbidities precluded surgery. Confirmed eye bleeds that did not meet these criteria were considered non-serious.

[*] According to Mr Patel, intravitreal injections are not usually indicated for retinal or vitreous haemorrhages associated with PDR. Therefore, if an injection is given around the time of a retinal or vitreous bleed, it is more likely to have been indicated for an unrelated condition. An exception to this rule is using intravitreal injections for an anterior chamber bleed secondary to neovascular glaucoma. Such individuals are likely to receive prophylactic intravitreal injections to prevent vitreous haemorrhages secondary to proliferative disease. Hence, a distinction was made between the interventions indicated for anterior *vs.* posterior chamber eye bleeds.

After applying these definitions, participant-reported eye-bleeding events were given a new code to reflect their location, interventions received, and any resultant visual loss. All adjudication was completed within the in-house software developed for ASCEND.

3.2.1.1.2 Confirmation and Coding of Other Eye Events

The fact and start date of AMD, blindness, visual deterioration, retinal detachment and DMO events were verified according to the criteria defined in ASCEND SOP 8. Briefly, this required documented evidence that a specialist had confirmed the diagnosis on a date closely matching that given by the participant. Sufficiency flags were used to indicate the certainty around the coded data for every adjudicated event[*]. All adjudication decisions were recorded within the software developed in-house for ASCEND. The IT systems prevented events from being adjudicated to a date later than the follow-up form was received and gave a warning if the user changed the date by more than 30 days. In such instances, a new event was reported based on the supporting evidence, and the original event was adjudicated with a sufficiency = N flag. The # symbol was used to denote missing day, month or year information. Methods used to handle partial date information are described in Section 3.4.5.14.1.

3.2.1.2 Linkage to NHS Diabetic Eye Screening Programme Records

3.2.1.2.1 The Data Specification

Longitudinal information about ASCEND participants' diabetic eye disease was sought from electronic linkage to their NHS DESP records in England and Wales, including:

i) the date of each retinal assessment from 1st January 2003 onwards;

ii) retinopathy (R) and maculopathy (M) grades, and their photocoagulation (P) status, based on the standardised, quality-controlled, NSC grading system[59-61] (see Table 2, Section 1.2.2); and

[*] The use of sufficiency flags is also described in Appendix 8: SOP: Confirmation and Coding of Eye Bleeding Events.

iii) best-corrected visual acuity score and the name of the visual acuity chart used.

Data were eligible irrespective of whether mydriasis was used during the examination, the frequency of assessments, and regardless of subsequent treatment decisions. If there was missing image capture for one eye because the participant was blind or enucleated, data for the other eye was still eligible for inclusion. However, the following individuals were excluded:

- those who withdrew their consent or were lost to follow-up in ASCEND;
- those who wished to opt out of the DESP linkage exercise;
- those who resided in Scotland or Northern Ireland;[*]
- those without an NHS number; and
- those who attended a GP practice in England that did not register their data with Public Health England (PHE) (see Section 3.2.1.2.2).

3.2.1.2.2 Linkage Process

Without a single central data repository for NHS DESPs in England, a two-stage process of linking participants to one of 58 programmes[†] was necessary. First, participants were linked to every GP practice they had ever been registered with during ASCEND, based on information they provided at enrolment or from updated correspondence during the trial. Information was also sought from PHE (which became the UK Health Security Agency in September 2021) to link GP practice codes to their serving DESPs. Finally, VLOOKUP functions in Microsoft Excel were used to connect all three pieces of information: participants were linked to their GP practices and then to their DESP(s). In Wales, a single registry existed; therefore, participants who resided in Wales could be linked directly to the Diabetic Eye Screening Wales (DESW) programme records.

[*] During the planning stage of ASCEND-Eye, obtaining linkage data from England and Wales was prioritised over navigating other regulatory systems for the much smaller number of participants who resided in Scotland (n=323) and Northern Ireland (n=183). In hindsight, given the time it took to gain regulatory approvals in England and Wales, it may have been possible to pursue these.

[†] When linkage applications were made for ASCEND-Eye, there were 58 DESPs in England. Documents produced by PHE now refer to 57 programmes because the Staffordshire and Shropshire DESPs merged in January 2020. Subsequent sections of the thesis continue to refer to 58 programmes rather than 57, based on the organisation of the service when ASCEND-Eye started.

A detailed description of the regulatory and ethics applications made to access the linkage data is provided in Section 3.2.3. With approvals in place, all 58 DESPs in England, and DESW, were separately invited to collaborate on the study. Those who responded favourably were sent the relevant regulatory authorisations and given the option of allowing their software provider to do the linkage on their behalf. Two companies hold national contracts to provide the software used by programmes in England: Northgate Ltd and Health Intelligence Ltd. When applying, Northgate Ltd was the data controller for personal data processed by 7 of the 47 DESPs they served, whilst Health Intelligence Ltd controlled the data for all 11 programmes they supported. Local hospital Trusts controlled the data for the remaining 40 programmes using Northgate's software. The working models used to obtain approval for the data linkage differed as follows:

i) Trust-controlled English DESPs

Regulatory submission packages were sent to Trust-controlled DESPs in England for review by their local Research and Development (R&D) team. All were asked to sign a Data Sharing Agreement (DSA) and to provide Confirmation of the Capacity and Capability (CCC) to collaborate on the project.

ii) Company-controlled English DESPs

The data governance credentials of Northgate Ltd and Health Intelligence Ltd were verified in separate Third Party Security Assessments (TPSA). Next, the regulatory submission package was sent to the Data Protection Officer in each company. They were then asked to sign bespoke DSAs, which clearly defined the data processor-controller relationships at each stage of the data handling. Both DSAs were prepared in collaboration with the University's Legal Officers. Finally, a separate Financial Service Contract was used to authorise payments to Northgate Ltd for their work on the project; Health Intelligence Ltd provided their collaboration for free.

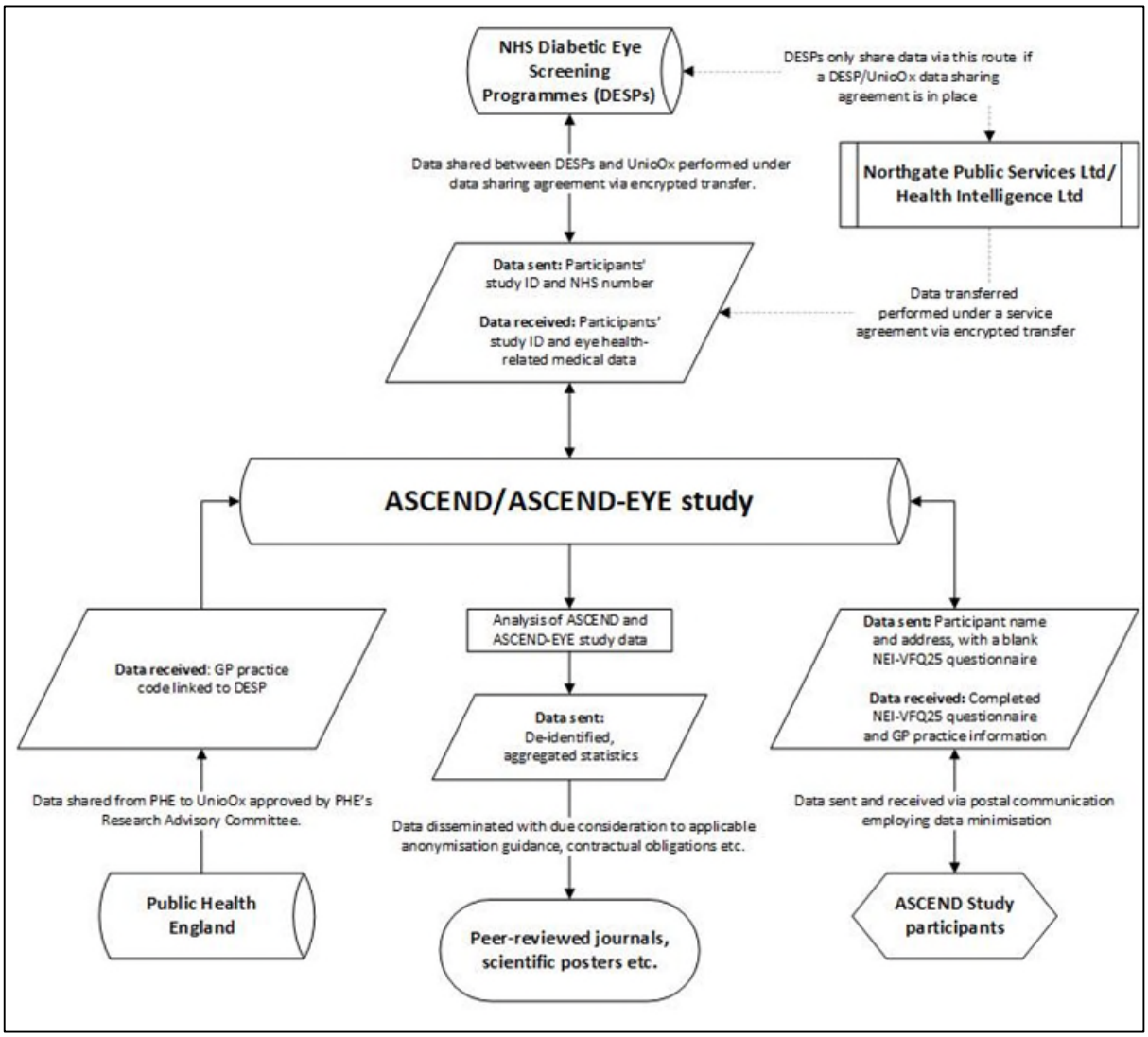
iii) Wales

A separate approval was needed from the Research and Evaluation Division of PHW to permit linkage to their single registry for participants who resided in Wales. Personnel from PHW were identified who could write a script and interrogate their local database, and a bespoke DSA was used to define the roles and responsibilities of those involved in this process.

In order to identify and mitigate the risks involved with processing personal data by these third parties, a Data Protection Impact Assessment (DPIA) was prepared in conjunction with the University of Oxford's Information Security and Information Governance teams. The DPIA also described the data processing involved with the VFQ. A summary of all the data processing for ASCEND-Eye data is shown in Figure 10.

After contracts relevant to the three working models were fully executed, a minimal dataset, which included the participants' unique ASCEND Trial ID number and NHS number, was encrypted before being sent to a nominated person at the relevant Trust, software company or DESW. NHS numbers were chosen as the sole linkage identifier because they are a unique pseudonym that remains the same throughout an individual's lifetime and have previously been shown to be valid and complete for the majority of primary and secondary care records in England.[383] After linkage, the data were encrypted again before being returned. In England, all data transfers were between nhs.net email accounts; in Wales, a file-sharing portal of equivalent security was used. Passwords to decrypt each file were sent via a separate communication channel for both directions of data transfer.

Figure 10 Summary of ASCEND-Eye Data Flows



UnioOx = University of Oxford

3.2.1.3 Visual Function Questionnaire

All surviving participants of ASCEND, who were on web- or mail-based follow-up at the end of the trial (31st July 2017), were sent a double-sided A3 VFQ via the post (Appendix 9). The VFQ consisted of a bespoke page of questions which explicitly sought incident diagnoses of serious eye conditions, including AMD, cataracts, glaucoma and retinal vein thrombosis, followed by the National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ-25)* standard instrument.[2] The latter was intended to gather information from participants about the effect(s) of visual impairment on activities of daily living

* I am grateful to Mr Kevin Murphy, ASCEND Senior Trial Manager, who formatted the VFQ on my behalf.

and emotional well-being. From this, a composite score and 11 sub-scale scores are calculated: general vision, ocular pain, near vision, distance vision, social function, mental health, role limitations, dependency, driving, colour vision and peripheral vision.[2] Each score ranges from 0 to 100, with higher scores representing better vision-specific quality of life.[2] This multi-domain instrument has been reported to be a reliable and valid questionnaire across various chronic eye conditions.[2]

Composite and subdomain scores derived from the NEI-VFQ-25 for near-vision and distance-vision are strongly and independently correlated with visual acuity.[2] Although the latter is an objective measure of macular function, it may not reflect the extent to which retinal diseases such as DR and AMD affect other dimensions of visual function, such as contrast sensitivity, colour perception and stereoscopic vision.[384] Therefore, the NEI-VFQ-25 has been recommended by regulators as a more holistic endpoint in ophthalmology trials, and for vision-targeted cost-utility analyses, and hence it was used in ASCEND-Eye.[385]

Each returned VFQ was scanned and coded by the author using the same OCR and quality control software developed and used in ASCEND[*]. The participant's signature and date of birth on every form were checked for consistency with those recorded on the ASCEND randomisation form. Occasionally, it was also necessary to clarify a diagnosis and dates or to resolve multiple or contradictory responses by telephoning the participant directly. Data were ready to be analysed after every form was instantiated in this way.

3.2.2 The Legal Basis and Data Protection

Participants gave their consent in ASCEND, knowing their data would be used for medical research. Individuals were informed of how their data would be processed for ASCEND-Eye via a Participant Information Sheet (Appendix 10) and Privacy Notice[†]. Both were produced by the author, in line with the shared Information Commissioner's Office and NHS Digital transparency checklists, and after

[*] I am grateful to Ola Murawska, ASCEND Trial Developer, who programmed this software specifically for the VFQ.

[†] Available from: https://ascend.medsci.ox.ac.uk/about/privacy-notice-ascend-eye (Last accessed 07/06/2023).

consulting with the University of Oxford's Information Security and Information Governance teams.[386] The lawful basis for processing personal data in ASCEND-Eye was that it was research carried out in the public interest (Article 6(1)(e) General Data Protection Regulation (GDPR)).[386] The additional condition met for the processing of the special category data was that it is necessary for the purposes of research (Article 9(2)(j) GDPR).[386] Personal user accounts and permission controls restricted access to all data collected by the coordinating centre. The data store was kept secure behind a default deny-all firewall.

3.2.3 Regulatory and Ethics Submissions

Gaining regulatory and ethics approval for different aspects of the ASCEND-Eye study was an iterative, time-consuming, and often demoralising process. It necessitated a familiarity with complex legal terminology and governance structures and the coordination of multiple applications to different authorities and data controllers, each with specific requirements. Some of the difficulties were due to ASCEND predating the current legal framework of GDPR and its implementation alongside the Common Law Duty of Confidentiality, but also because its registration lies outside the contemporary Integrated Research Application System (IRAS). Another complication was the lack of a single central repository of eye-screening data in England and the unprecedented nature of what ASCEND-Eye set out to achieve; no previous studies had successfully obtained linked data for research purposes on a national scale in England before.

The following paragraphs describe each regulatory and ethics application made to permit the linkage and VFQ components of ASCEND-Eye. Figure 11 is a Gantt chart which summarises these activities. The author prepared every submission package and managed all correspondence herself; however, a debt of gratitude is owed to the following people for their expert guidance and support: Dr Karen Melham (Senior Clinical Research Support Manager), Dr Gavin Brown (Research Contracts Manager), John Latham-Mollart (Information Security Coordinator), and Anna Brewer (Deputy Director of Administration), all from the University of Oxford, who advised on the legal basis of processing sensitive personal data, and helped with the preparation of DSAs and Financial Service contracts.

Securing Multi-centre Research Ethics Committee (MREC) approval for ASCEND-Eye began in September 2016. First, a proposal was submitted to the University of Oxford's Clinical Trials and Research Governance department to make additional assessments of eye health in the form of the VFQ and to notify participants about the wider objectives of the project (i.e. the DESP-linkage) in the form of a Participant Information Sheet and Privacy Notice. Once institutional approval was given, a substantial amendment application was submitted to the Northwest-Haydock MREC in October 2016. Another substantial amendment was submitted in December 2016, which sought approval to extend the main ASCEND trial ethics approval to 2037 to permit long-term follow-up of ASCEND participants and allow for sub-studies such as ASCEND-Eye.

Next, two separate applications were prepared for Diabetic Eye Screening Research Advisory Committees in PHE and separately, PHW, who have been responsible for commissioning the NHS screening programmes in England and Wales, respectively, since 2013. Approval to use the screening data for research purposes, as opposed to service planning, was given by PHE in July 2018 and PHW in September 2019. Annual progress reports have been submitted to both committees ever since. With these approvals secured, a letter endorsed by Professor Peter Scanlon, Clinical Director of the NHS Diabetic Eye Screening Programme in both nations, was sent to the Clinical Lead(s) and Programme Manager(s) of all 58 DESPs in England and to DESW, to invite their collaboration on the linkage exercise. After interest had been expressed, another substantial amendment was submitted to the MREC in January 2019 to add DESP registries under the legal definition of a research "site" that would perform the data linkage. Although some NHS Trusts were previously defined as Participant Identification Centres (PICs) for ASCEND, others were not formally involved with the parent study. By changing their role to a research "site" or opening ASCEND-Eye as a new study, it was necessary to bring the project under the approval of the Health Research Authority (HRA). Along with the MREC documentation, this also required a Schedule of Events and a Statement of Activities. The HRA intends for both documents to be used in prospective clinical trials. Therefore, they were difficult to adapt and largely irrelevant in the context of ASCEND-Eye. Nevertheless, with MREC, HRA, and PHE approvals in place, and local support from the Clinical Leads and Programme Managers of some DESPs, it became

possible to liaise with Trust R&D personnel to secure local CCCs and execute DSAs. In response to the specific requirements of one R&D, a fourth substantial amendment was submitted to the MREC in December 2019 to better integrate ASCEND-Eye and its documentation into the ASCEND trial as a whole.

Out of 59 programmes (58 English DESPs and DESW), 22 responded to either the first invitation to collaborate or a second reminder. By late 2019, one R&D had denied approval for the data linkage, but CCC approvals were obtained for nine others. Despite having centralised approvals, the average time for R&D reviews in those nine Trusts was 9.5 months (range of 1-15 months). The remaining twelve R&Ds suspended all non-coronavirus research at the start of the Covid-19 pandemic in March 2020, but this was nearly 16 months after they were first sent the submission package. The author suspended her DPhil studies in late May 2020 to begin maternity leave. Upon returning in August 2021, progress had yet to be made by those 12 R&Ds, and in the interests of progressing the DPhil, the author and her supervisors decided not to pursue them any further.

Concurrent with these activities, it became clear that most DESPs lacked somebody with enough capacity or expertise to write a script and interrogate their local database. Therefore, collaborations were sought with Northgate Ltd and Health Intelligence Ltd. As the data controllers for some of the DESPs they supported, they could legally provide data for participants who attended those DESPs, without involving Trust R&Ds. DSAs were fully executed with these companies in January 2020 and August 2021, respectively. In parallel, a DPIA and two TPSAs for Northgate Ltd and Health Intelligence Ltd were submitted to the Information Governance Lead at the Nuffield Department of Population Health, University of Oxford, in March 2020.

3.2.3.1 Lessons Learnt

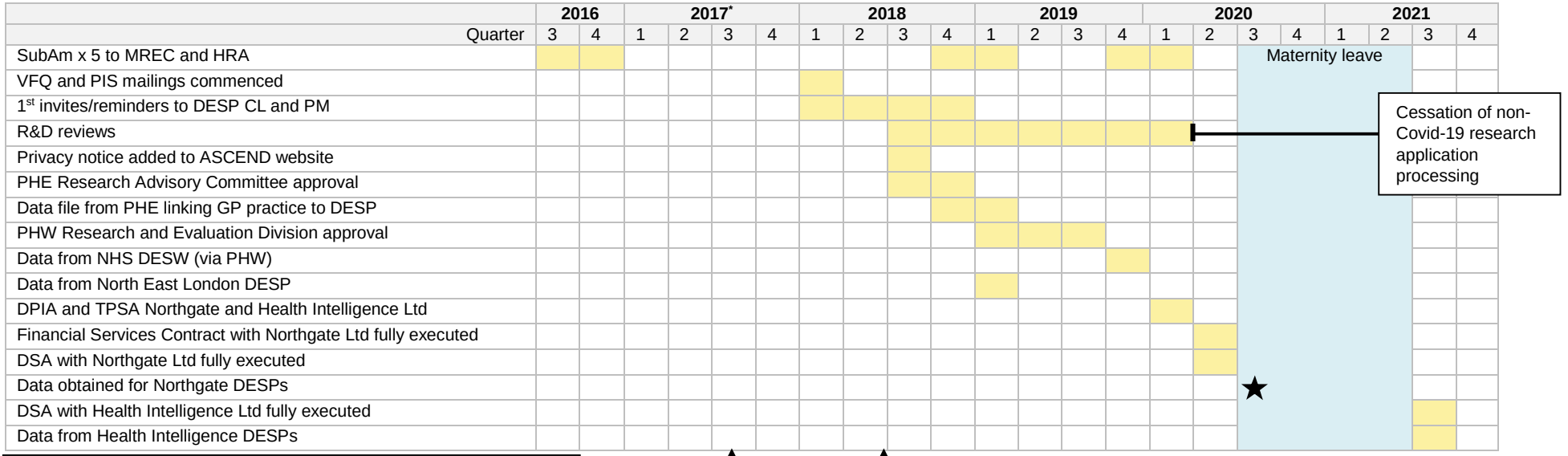
The UK has a wealth of high-quality registry data, which makes it an invaluable resource for studies like ASCEND-Eye. My first mistake was not defining the data governance specific to the national eye screening service early in the project. This was not helped by a lack of documentation identifying who

owned the data and needed to approve its use, the limitations on using the data and, therefore, the separate applications needed in addition to overarching authorisations from the MREC and HRA. The next challenge I faced was the innate complexity of liaising with multiple individuals in different agencies. Although similar information was included in each R&D application, all required additional forms, which differed in format and detail. Most then requested further information, assurances or alterations of these documents, the DSA, or both, between 3 to 42 times. Specific factors which delayed the processing of the submission package, or caused confusion, included the age of the original ASCEND registrations and the ongoing validity of participant consents, the frequent turnover of R&D staff, infrequent Research Advisory Committee meetings in PHE and PHW, fears of a legal misstep in the event of a data breach after GDPR was ratified, and justifying the need for pseudonymised participant identifiers. Allowing for my maternity leave, it took over four years to obtain the final linked data set for ASCEND-Eye. The main difficulty associated with these delays was maintaining enthusiasm for the project with third parties such as the Macular Society and the British Heart Foundation, from whom I secured grants to fund my University fees and Northgate's services, respectively (see Appendices 11 and 12). The lessons I will take away from this experience include the following:

i) identify data-governance issues specific to the dataset being linked to at an early stage;
ii) involve institutional Information Governance, Finance and Legal teams from the outset of a project;
iii) request a single point of contact within Trust R&D teams; and
iv) ask data controllers to identify themselves in the subject line of each email, which helps to organise the correspondence.

At a higher level, regulatory application forms need to be tailored to the retrospective collection of data for linkage studies like ASCEND-Eye, and central authorisations from an MREC or the HRA must replace the need for lengthy governance reviews by individual Trust R&Ds.

Figure 11 Gantt Chart Summarising the Process of Gaining Regulatory Approvals



[*] Work undertaken in 2017 for ASCEND-Eye included 1) consultations with Mr Praveen Patel to define the severity of eye bleeding events, 2) the adjudication of participant-reported eye events before the database lock in ASCEND, 3) consultations with Professor Peter Scanlon whilst planning for the linkage data applications, and 4) regular meetings with administrative and programming teams at CTSU ahead of the VFQ mailings.

3.2.4 Cleaning the Raw Linkage Data

After each controller had transferred their datasets, the raw linkage data were combined and cleaned to ensure consistent formatting and remove duplicate information. First, columns with the randomisation and censoring dates for each linked participant were added from an existing ASCEND analysis dataset, and the date of each eye screening record was configured in the same way. Next, retinopathy, maculopathy, photocoagulation and visual acuity grades were recoded into a standard format. For example, R, M and P prefixes were removed where this was relevant, and "Null", "ungraded", "U", or "not assessed" were given the same code to indicate that those fields were missing. In May 2015, DESW revised its grading criteria to align with the English NSC system shown in Table 2 (see Section 1.2.2).[387] Before then, the Welsh protocol included more subdivisions of grade 1 retinopathy, maculopathy and photocoagulation. Therefore, data from DESW dated prior to 1st May 2015 were converted to their equivalent English NSC grades. Appendix 13 shows the conversions that were applied.[387] Finally, before the linkage data were received, it was conceivable that individual DESPs were using one of several standardised charts to measure visual acuity. Thus, grades were given a new code called the "ASCEND-Eye Recode Score" to facilitate analyses of visual acuity measurements derived from different charts. Appendix 14 is the table used for this purpose; each row represents a complete line of letters of equivalent size on each chart. Incremental scores between rows indicated that a participant could read some, but not all, of the smaller letters on the next row. In such cases, scores were rounded down to the nearest complete line.[388]

3.2.5 Linkage Quality Assessments

Both incomplete and unmatched data records, and the linkage methodology, can introduce bias into the results of studies using electronic healthcare data.[389] Using NHS numbers as the sole identifier reduced the potential for inaccuracies due to missed matches, false matches or both; the linkage specification sent to each third-party data provider was also the same.[383] During the development of scripts by each data controller, a validation study to ensure the accuracy of the linkage was not requested; however, to identify potential sources of bias, tests for heterogeneity and standardised differences between the

baseline characteristics of those who were matched (n=7535) and not matched (n=573) from the total who were thought to attend a collaborating DESP (n=8108[*]) were assessed whilst still blind to the randomised treatment allocations (Table 7). Although mainly used in propensity score matching studies, which attempt to infer causal effect from observational data, several authors have recommended using standardised differences to evaluate linkage quality because unlike inferential tests, they are unaffected by sample size.[389-391] The index, also known as Cohen's *d*, is calculated as the difference between the population means or proportions divided by their pooled standard deviation[†].[389,391] The values 0.2, 0.5 and 0.8 represent small, moderate and large differences, respectively[‡].[391] Whilst there was statistically significant imbalance of some baseline covariates between those who were linked and those who were not, the standardised differences were small to moderate.

Other quality assurance measures included: 1) checking for eye screening records dated after a participant was known to have died and 2) checking the provision of records for participants who moved to or from general practices served by different DESPs. There were no screening records dated after a participant was known to have died. Among the entire randomised cohort of 15,480 participants, 4004 (25.9%) moved general practice one or more times during ASCEND. A smaller proportion (9.3%; n= 697) transferred their primary care among the 7535 who were linked. Having relocated, 381 individuals went from one collaborating DESP to another, and 316 moved from a collaborating DESP to one that did not participate. The timing of these moves was consistent with the data received. Overall, these assessments support the validity of the linkage methodology.

[*] A detailed description of the participant flow for the linkage exercise is presented in Section 4.1.2.

[†] Cohen's *d* may overestimate the standardised difference when the sample size is small (n<20). Under these circumstances, a correction factor may be applied to the denominator, producing an index called Hedges' *g*. Alternatively, the mean difference may be divided by the standard deviation of the control population, known as Glass' delta. Glass' delta may also be preferred when the standard deviation of two populations is considerably different.[384]

[‡] Appendix 15 includes a table which summarises the degree of overlap between two populations based on their standardised difference score. There is also an illustrative representation of the distributions of a covariate in two populations with a standardised difference of 0.8, which helps to visualise the proportions described in the table.

Table 7 Differences Between the Baseline Characteristics of Matched and Not-Matched Individuals

Baseline Characteristic	Matched (n=7535)	Not matched (n=573)	Standardised Difference (95% CI)*	P-values†
Age at randomisation (years)				
Mean (SD)	63.5 ±8.9	64.9±10.4	0.14 (0.06-0.23)	P = 0.002
<60	2643 (35.1%)	188 (32.8%)		
≥60<70	3111 (41.3%)	197 (34.4%)		
≥70	1781 (23.6%)	188 (32.8%)	0.21 (0.13-0.29)	P<0.001
Sex				
Male	4717 (62.6%)	375 (65.4%)		
Female	2818 (37.4%)	198 (34.6%)	0.06 (-0.02-0.15)	P = 0.18
Type of diabetes‡				
Type 1	392 (5.2%)	71 (12.4%)		
Type 2	7143 (94.8%)	502 (87.6%)	0.36 (0.28-0.45)	P<0.001
Duration of diabetes (years)				
Median (IQR)	7 (3-12)	11 (6-20)	0.49 (0.40-0.58)	P<0.001
≥0<5 years	2412 (32.0%)	99 (17.3%)		
≥5<10 years	2136 (28.3%)	134 (23.4%)		
≥10<20 years	1741 (23.1%)	159 (27.7%)		
≥20 years	825 (10.9%)	149 (26.0%)	0.51 (0.42-0.60)	P<0.001
Unknown	421 (5.6%)	32 (5.6%)		
Diabetes management				
Diet only	1225 (16.3%)	66 (11.5%)		
Oral hypoglycaemic agent(s) only	4561 (60.5%)	247 (43.1%)		
Insulin +/- oral hypoglycaemic agent(s)	1749 (23.2%)	260 (45.4%)	0.48 (0.39-0.56)	P<0.001
Participant-reported diabetic retinopathy				
Yes	1402 (18.6%)	238 (41.5%)		
No	6069 (80.5%)	329 (57.4%)	0.52 (0.43-0.63)	P<0.001
Unknown	64 (0.8%)	6 (1.0%)		
Participant-reported treatment for hypertension				
Yes	4616 (61.3%)	371 (64.7%)		
No	2859 (37.9%)	198 (34.6%)	0.07 (-0.02-0.16)	P = 0.12
Unknown	60 (0.8%)	4 (0.7%)		
Systolic blood pressure (mmHg) §				
Mean (SD)	136.2±15.3	137.2±17.3	0.06 (-0.04-0.16)	P = 0.25
<130	1681 (22.3%)	131 (22.9%)		
≥130<140	1534 (20.4%)	94 (16.4%)		
≥140	2290 (30.4%)	183 (31.9%)	0.11 (0.01-0.21)	P = 0.02
Unknown	2030 (26.9%)	165 (28.8%)		
Diastolic blood pressure (mmHg) §				
Mean (SD)	77.1±9.4	75.2±9.8	0.19 (0.09-0.29)	P<0.01
<75	2083 (27.6%)	185 (32.3%)		
≥75 <85	2349 (31.2%)	159 (27.7%)		
≥85	1069 (14.2%)	64 (11.2%)	0.16 (0.06-0.26)	P<0.01
Unknown	2034 (27.0%)	165 (28.8%)		
Body mass index (kg/m^2) ¶				
Mean (SD)	31.1±6.6	30.4±6.6	0.09 (0.01-0.18)	P = 0.03
<25	1011 (13.4%)	113 (19.7%)		
≥25, <30	2665 (35.4%)	203 (35.4%)		
≥30<35	2214 (29.4%)	146 (25.5%)		
≥35	1588 (21.1%)	107 (18.7%)	0.18 (0.10-0.27)	P <0.001
Not available	57 (0.8%)	4 (0.7%)		

Baseline Characteristic	Matched (n=7535)	Not matched (n=573)	Standardised Difference (95% CI)*	P-values†
Cigarette smoking				
Current	587 (7.8%)	51 (8.9%)		
Former	3501 (46.5%)	267 (46.6%)		
Never	3357 (44.6%)	252 (44.0%)	0.04 (-0.05-0.12)	P = 0.66
Unknown	90 (1.2%)	3 (0.5%)		
Non-study medication				
ACE inhibitor or ARB	4384 (58.2%)	360 (62.8%)	0.10 (0.01-0.18)	P = 0.04
Aspirin use before screening	2590 (34.4%)	224 (39.1%)	0.10 (0.01-0.18)	P = 0.03
Thiazide or related diuretic	1396 (18.5%)	121 (21.1%)	0.07 (-0.02-0.15)	P = 0.14
Calcium channel blocker	1852 (24.6%)	146 (25.5%)	0.02 (-0.06-0.11)	P = 0.97
Statin	5723 (76.0%)	425 (74.2%)	0.04 (-0.04-0.13)	P = 0.32
Total cholesterol (mmol/L) (mean (SD))				
Mean (SD)	4.1±0.9	4.1±0.9	0.09 (-0.02-0.19)	P = 0.11
<4	2292 (30.4%)	163 (28.4%)		
≥4 <5	1923 (25.5%)	141 (24.6%)		
≥5	664 (8.8%)	64 (11.2%)	0.11 (0.001-0.21)	P = 0.13
Not available	2656 (35.2%)	205 (35.8%)		
HDL cholesterol (mmol/L) (mean (SD))				
Mean (SD)	1.3±0.4	1.3 ±0.4	0.05 (-0.06-0.15)	P = 0.40
<1	1036 (13.7%)	86 (15.0%)		
≥1<1.5	2786 (37.0%)	182 (31.8%)		
≥1.5	1047 (13.9%)	88 (15.4%)	0.09 (-0.01-0.20)	P = 0.23
Not available	2666 (35.4%)	207 (36.1%)		
Non-HDL cholesterol (mmol/L)				
Mean (SD)	2.9±0.8	2.9±0.8	0.08 (-0.03-0.19)	P = 0.16
<2.5	1712 (22.7%)	117 (20.4%)		
≥2.5 <3.5	2211 (29.3%)	170 (29.7%)		
≥3.5	945 (12.5%)	79 (13.8%)	0.08 (-00.03-0.18)	P = 0.38
Not available	2667 (35.4%)	207 (36.1%)		
Glycosylated haemoglobin - HbA1c				
IFCC (mmol/mol) mean (SD)	54.4±12.7	58.1±14.9	0.27 (0.17-0.38)	P<0.001
DCCT (%) mean (SD)	7.1±1.2	7.5±1.4	0.27 (0.17-0.38)	P<0.001
<48 (6.5)	1666 (22.1%)	99 (17.3%)		
≥48 (6.5), <64 (8.0)	2329 (30.9%)	173 (30.2%)		
≥64 (8.0)	882 (11.7%)	94 (16.4%)	0.21 (0.10-0.31)	P<0.001
Not available	2658 (35.3%)	207 (36.1%)		
CKD-EPI estimated GFR (ml/min/1.73m^2) **				
Mean (SD)	84.6±20.6	79.9±24.9	0.20 (0.09-0.31)	P<0.001
≥90	2172 (28.8%)	149 (26.0%)		
≥60<90	2065 (27.4%)	132 (23.0%)		
<60	639 (8.5%)	87 (15.2%)	0.28 (0.17-0.38)	P<0.01
Not available	2659 (35.3%)	205 (35.8%)		
Urinary albumin:creatinine ratio (mg/mmol)				
Median (IQR)	0.55 (0.16-1.29)	0.82 (0.28-2.47)	0.23 (0.12-0.34)	P<0.001
<3	4272 (56.7%)	282 (49.2%)		
≥3	598 (7.9%)	80 (14.0%)	0.26 (0.16-0.37)	P<0.01
Not available	2665 (35.4%)	211 (36.8%)		
Townsend Deprivation Index††				
<-3	2562 (34.0%)	191 (33.3%)		
≥-3<0	2980 (39.5%)	220 (38.4%)		
≥0<2	999 (13.3%)	69 (12.0%)		
≥2<4	585 (7.8%)	56 (9.8%)		
≥4<6	289 (3.8%)	25 (4.4%)		
≥6	106 (1.4%)	12 (2.1%)	0.10 (0.01-0.18)	P = 0.36
Unknown	14 (0.2%)	0 (0.0%)		

Baseline Characteristic	Matched (n=7535)	Not matched (n=573)	Standardised Difference (95% CI)*	P-values†
Ethnic origin				
White	7255 (96.3%)	557 (97.2%)		
Indian/Pakistani/Bangladeshi	100 (1.3%)	3 (0.5%)		
African/Caribbean	62 (0.8%)	5 (0.9%)	0.09 (0.001-0.17)	P = 0.41
Other/unknown	118 (1.6%)	8 (1.4%)		

DCCT = Diabetes Control and Complications Trial; GFR = Glomerular Filtration Rate; HDL=High-density lipoprotein; IQR = Interquartile range; IFCC =International Federation of Clinical Chemistry; SD = Standard Deviation
Figures presented are counts with percentages unless otherwise stated. Percentages may not total 100 because of rounding.
*Standardised differences were calculated using specific functions of the "stddiff" software package developed for R for continuous or categorical (ordinal or nominal) baseline variables.[390,392] The values 0.2, 0.5 and 0.8 represent small, moderate and large differences, respectively.[391] A negative score indicates that those who were not matched had a lower mean score than those who were. The standardised difference is not statistically significant if the 95% confidence interval includes zero.[391]
†Formal testing for differences between the baseline characteristics of those who were matched and not matched used the Chi-squared test for heterogeneity for categorical variables and grouped continuous variables (including age, duration of diabetes, systolic blood pressure, diastolic blood pressure, BMI, total cholesterol, HDL-cholesterol, non-HDL cholesterol, HbA1c, eGFR and urinary albumin:creatinine ratio), the Mantel-Haenszel Chi-square test for trend for ordinal variables (i.e. Townsend Deprivation Index), and the t-test or Wilcoxon-Mann-Whitney U test for continuous variables with a normal (summarised by mean±SD) or non-normal (summarised by median and interquartile range) distribution, respectively.
Categories that were "unknown" or "not available" were excluded from the balance diagnostic tests.
‡The presence of type 2 diabetes was based on a broad clinical definition involving the participant's age at the diagnosis of diabetes, the use of insulin within one year after diagnosis, and the body-mass index.
§ From blood and urine consent forms, generally before randomisation.
¶ The body-mass index (the weight in kilograms divided by the square of the height in metres) was based on values for height and weight the participants reported on their randomisation questionnaires.
**Calculated from blood cystatin c concentration using the CKD-EPI formula.[393]
†Townsend scores of 0 represent an area with a UK-average level of deprivation, positive values indicate areas of above-average deprivation, and negative values indicate areas of below-average deprivation.

3.3 Preliminary Blinded Analyses of the Linkage Data

Diabetic eye screening data is collected for administrative and clinical purposes without a specific *a priori* research question in mind. Whilst processing the raw data, it became apparent that there was incomplete longitudinal coverage of screening results per participant, year, eye or component of the screening assessment (i.e. R, M, and P grades and visual acuity score). There were several possible explanations for this, including[*]:

i) expansion of the national screening service after its inception in 2003 and the close proximity of starting recruitment for ASCEND in 2005;
ii) changes in the geographical boundaries, software providers and data-controller relationships for some English DESPs during ASCEND and the lengthy process of obtaining the regulatory approvals for ASCEND-Eye;
iii) clinical reasons for not grading an eye, such as dense cataracts obscuring clear retinal views; and

[*] Based on personal communication with Professor Peter Scanlon.

iv) participant preferences regarding the frequency of their NHS screening *vs.* independent optometry assessments.

Therefore, given the complexity of the data, some blinded preliminary analyses were conducted to determine the most appropriate trial endpoints and eligibility for each type of analysis from first principles. The following sections describe work carried out over several months between late 2021 and early 2022, which was discussed in regular meetings involving the author, her supervisors and Professor Sarah Parish (Lead Trial Statistician for ASCEND). The DAP in Section 3.4 reflects decisions made after this initial data exploration.

3.3.1 Distributions of the Linked Data

The analyses described in this section reflect an iterative process in which the extent of missing information was assessed in parallel with plotting distributions of the linkage data according to the following three parameters:

- laterality of data;
- retinopathy *vs.* maculopathy components of the NSC grade; and
- time.

Having found that maculopathy data nearly always accompanied retinopathy results, Sections 3.3.1.1 to 3.3.3 primarily focus on the availability of retinopathy data.

3.3.1.1 In-Trial Eye Screening Records

Section 4.1.2 describes participation in the linkage exercise in detail. Overall, the full linked dataset included 7535 successfully-matched ASCEND participants. Figure 12 shows the distribution of every available eye screening record among those individuals relative to their date of randomisation. Of these, 7360 had at least one record, with retinopathy data in at least one eye, within the scheduled treatment period of ASCEND. This was defined as day one post-randomisation up to and including the person-

specific censoring dates applied in the parent study.[11] The mean number of in-trial records per participant was 4.5 (range = 1 to 14; Figure 13), and the mean service use was 0.63 screenings per year (median = 0.63; Figure 14). This number is lower than the screening rate recommended by the NHS of at least one assessment per year.[8]

Figure 12 Histogram of Eye Screening Records Before and After the Date of Randomisation among the Full Linked Dataset for 7535 Participants

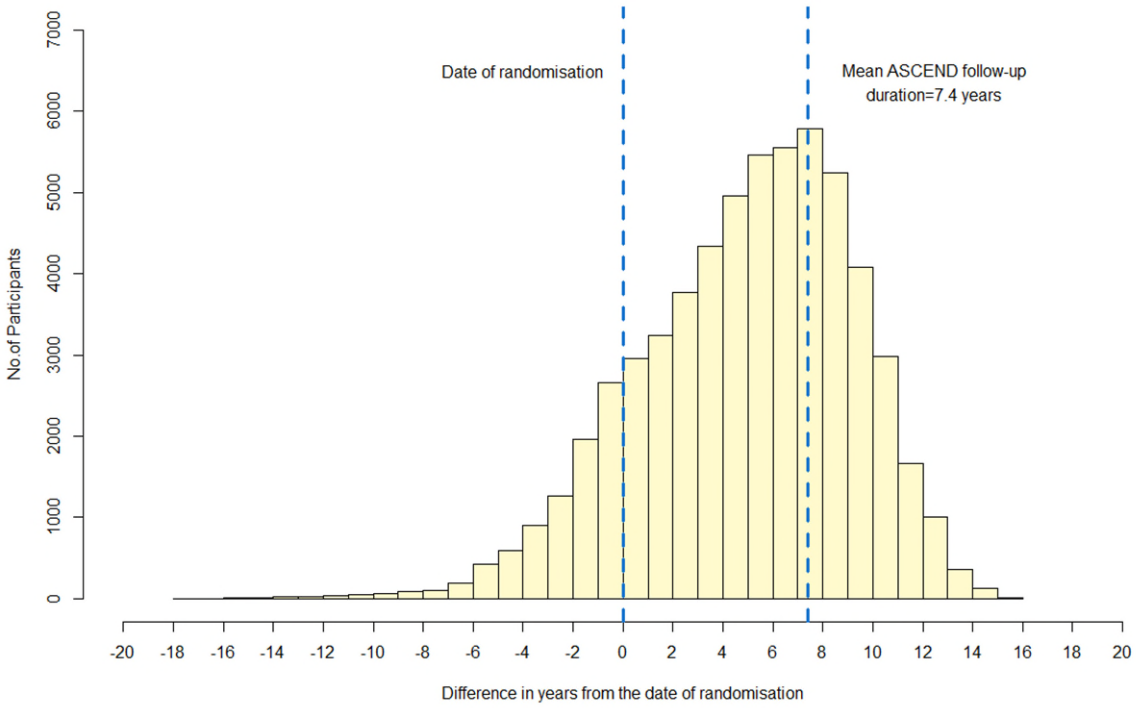


Figure 13 Histogram Showing the Total Number of Eye Screening Records with Retinopathy Data in at Least One Eye during the In-Trial Period, among 7360 Participants

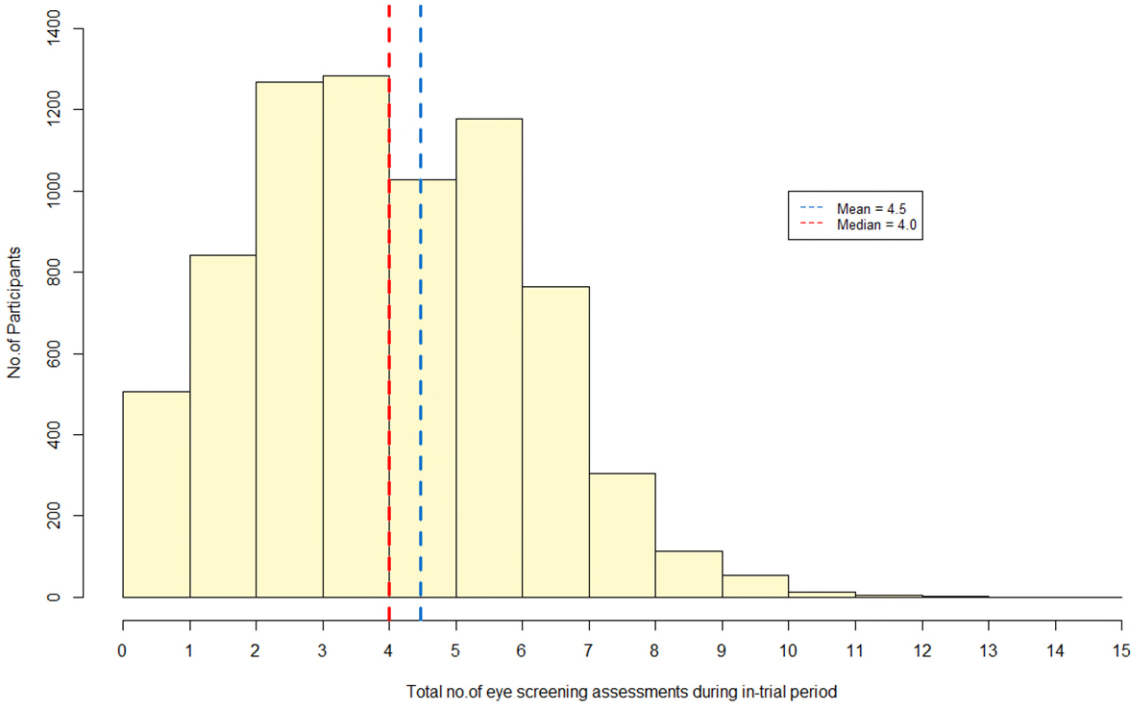
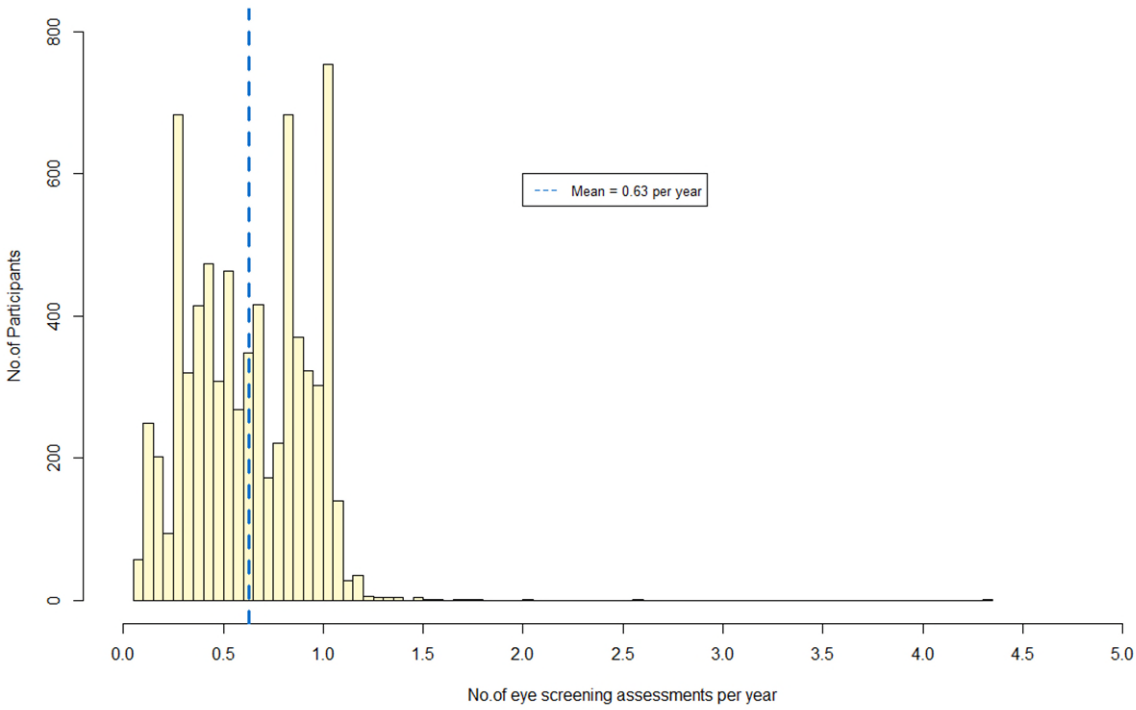


Figure 14 Histogram of In-Trial Eye Screening Assessments with Retinopathy Data in One or Both Eyes per Year, among 7360 Participants with In-Trial Data



3.3.1.1.1 Implications for the Data Analysis Plan

Survival analyses are typically used in clinical trials to compare the cumulative incidence or risk of an outcome of interest by treatment allocation over the trial follow-up period.[394] An important consideration in survival analyses is the issue of censoring.[395] During a trial, recruitment is staggered, and some participants would be expected to have the event of interest, whereas others do not because of death, losses to follow-up or the finite duration of the study.[395] Thus, the length of observation for each participant differs. For most analytic approaches, censoring is assumed to be non-informative, i.e. unrelated to the study design, and non-prognostic in terms of the probability of having future events.[395] When the irregularity of eye screening records was identified, there were concerns that a lack of precision around the timing of incident retinal lesions might deviate too far from the assumptions for the most commonly-used logrank or Cox proportional hazards regression methods of survival analysis. An alternative approach in which the survival analysis was weighted by the number of eye screening visits each participant had was dismissed for being too complicated statistically and potentially biased because the frequency of assessments offered by the NHS is driven by DR severity. Another suggestion was to perform survival analyses with year rather than day intervals, but the data were not regular enough. Finally, using interval-censored data, in which the exact timing of events would be narrowed to a period between two screening records, was considered; however, mid or late-point imputation can be a source of bias in some studies.[395]

The issue of accurately timed events can be avoided by comparing the between-group occurrences of an outcome at a single time point relative to the date of randomisation.[395] Dichotomising the data in this way allows for simpler analyses such as contingency tables or logistic regression methods.[395] However, it risks wasting information because this approach cannot identify variability in the timing of events.[395] Therefore, to ensure the statistical validity of ASCEND-Eye, the effects of each study treatment will be compared both ways: longitudinally, using survival methods, and with cross-sectional ordinal analyses of retinopathy grades at the final record during the scheduled follow-up period. The latter will entail proportional odds modelling.

Proportional odds analysis is implemented using ordinal logistic regression, with the outcome variable having more than two ordered levels.[396] The method is done under the assumption that the treatment

has a similar effect on each level of the outcome variable (i.e. the proportional odds assumption).[396] Thus, the analysis estimates a common odds ratio which measures the likelihood that the active treatment would lead to a higher or lower score on the ordinal scale than the placebo.[396] This approach was chosen because there is simulated[397,398] and empirical evidence[396,399] that the method has greater power to detect differences in the treatment effect than if the data is dichotomised into a binary favourable *versus* unfavourable outcome.

3.3.1.2 Final Eye Screening Records

Figure 15 is a histogram of the last available record before the end of scheduled treatment, with retinopathy data in at least one eye, among the 7360 participants with in-trial data. The median time from randomisation to this final eye screening record was 6.4 years (range = 3 days to 11 years). Most participants attended an eye screening assessment within a year before they stopped the study medications; median=225 days (range 0 to 9.5 years; Figure 16). These findings helped to define the censoring rules in Section 3.4.5.1.

Figure 15 Distribution of the Latest Available Eye Screening Records, with Retinopathy Data in at Least One Eye, among 7360 Participants with In-Trial Data

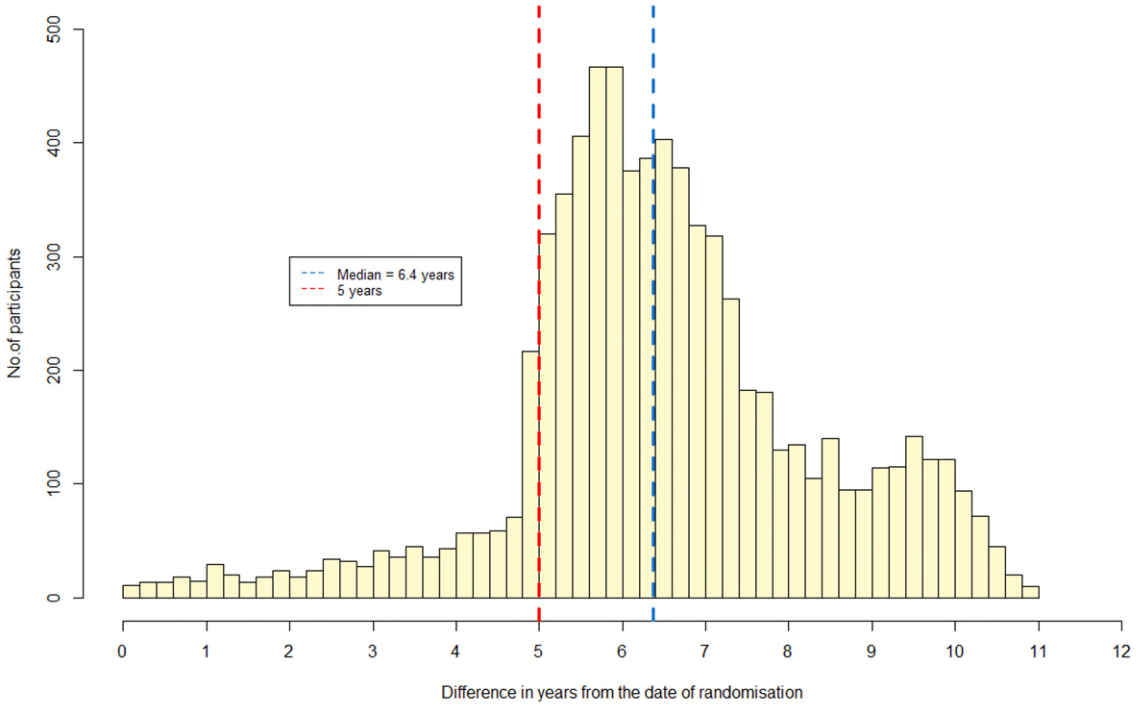
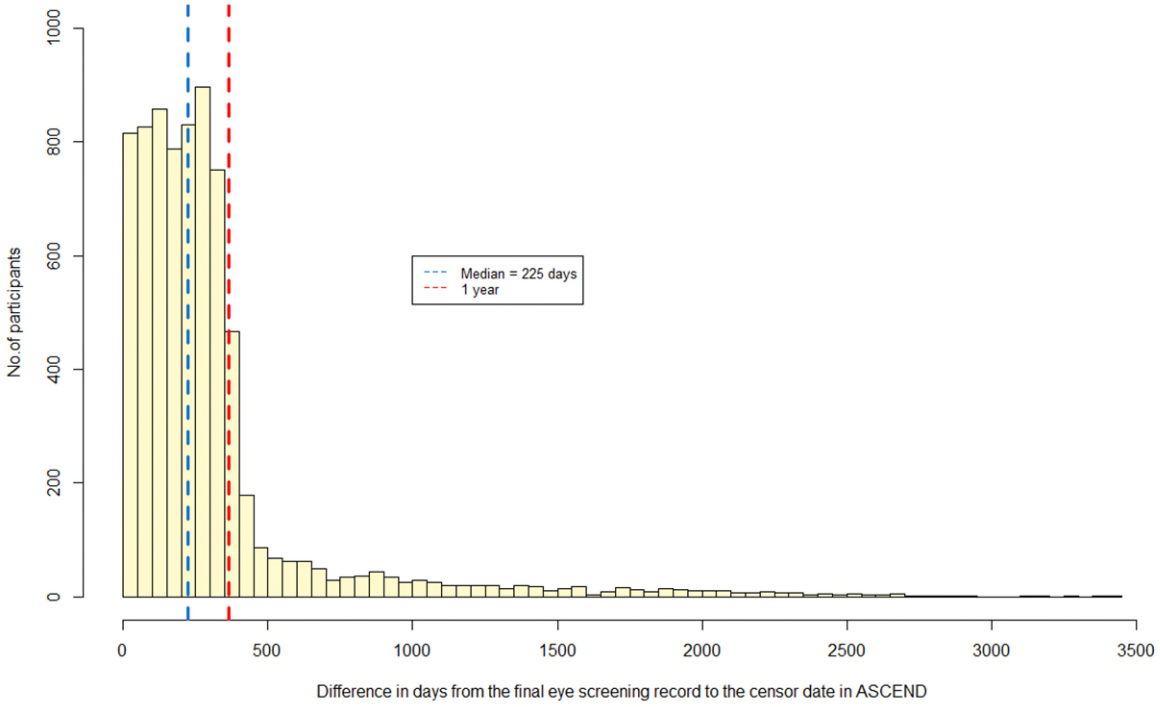


Figure 16 Days Difference from the Last Available Eye Screening Record to the Censoring Date in ASCEND, among 7360 Participants with In-Trial Data



3.3.1.3 Pre-Randomisation Eye Screening Records

Figure 17 is a histogram of eye screening records with retinopathy data in at least one eye, closest to the date of randomisation, among the 7360 participants with in-trial data. The majority were distributed after randomisation; hence, it became apparent that any analyses restricted to those with a baseline DESP record and by pre-randomisation disease status would involve a much smaller cohort and therefore have less power to detect differences between each treatment group. Hence, the primary efficacy outcome will be of time to the first post-randomisation occurrence of referable disease, defined in Section 3.4.3.3 (cohort B1), among the largest cohort with in-trial eye screening data; secondary efficacy outcomes involving the linkage data, will compare incident events among those with a baseline DESP record (cohorts B2 and B3; see Section 3.4.3.5).

Figure 18 shows the distribution of pre-randomisation records closest to the date of randomisation among participants with in-trial retinopathy data in one or both eyes (n=7360). Of those who had pre-randomisation records, most attended an eye screening assessment within a year before they were randomised (median = 207 days difference; interquartile range (IQR) = 103 to 316 days). However, to be as inclusive as possible, a two-year window was chosen for the baseline-restricted analyses defined in the DAP in Section 3.4.5.2. After applying this condition, baseline records were available for 39% of those with in-trial retinopathy data (n=2884 out of 7360). The records of those 2884 participants were further explored in Sections 3.3.2, 3.3.3 and 3.4.4.1; however, the number of participants included in the final baseline-restricted analysis cohorts ended up being slightly less than this because there was further refinement of the analysis rules, which took into account the availability of bilateral retinopathy data, maculopathy data, or both.

Figure 17 Distribution of Eye Screening Records Closest to the Date of Randomisation among 7360 Participants with In-Trial Retinopathy Data in at Least One Eye

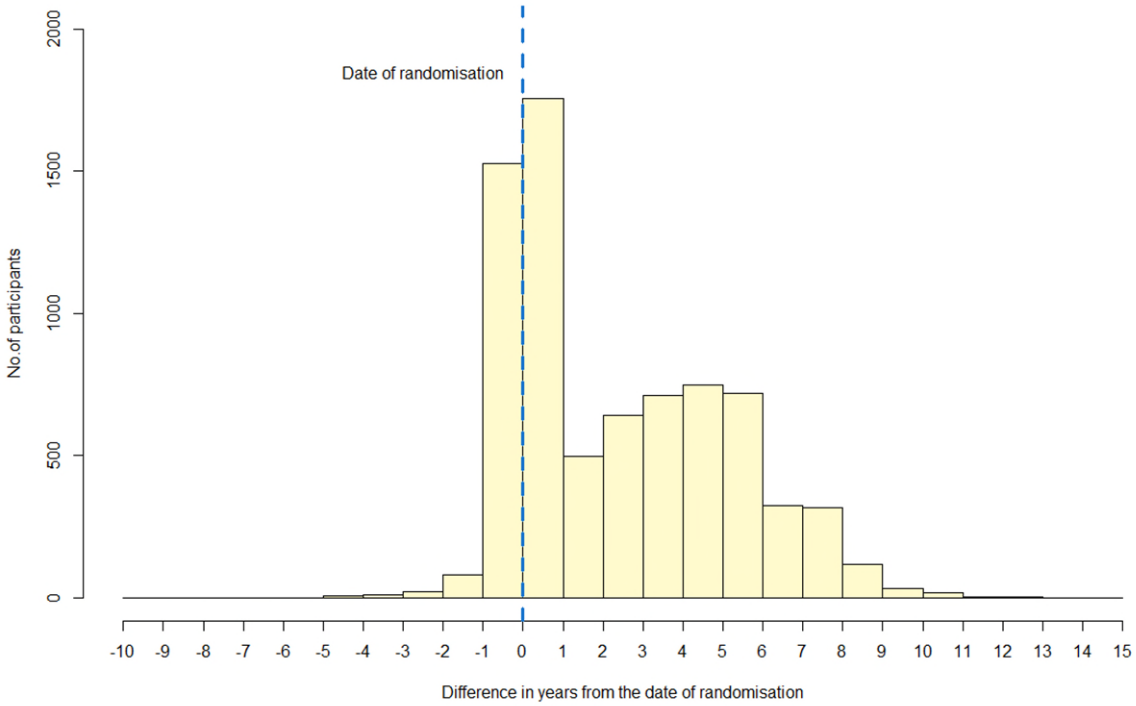
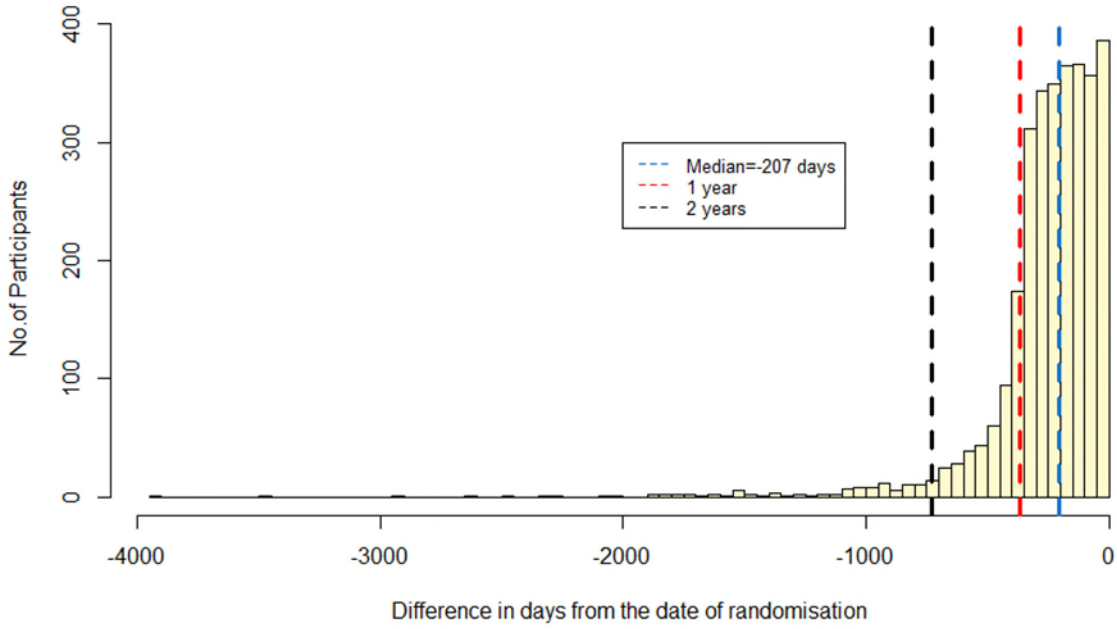


Figure 18 Distribution of the Closest Pre-Randomisation Eye Screening Records to the Date of Randomisation among 7360 Participants with In-Trial Retinopathy Data



3.3.2 Extent of Missing Data

The majority (97.0%) of 33,295 in-trial records for 7360 participants included retinopathy data in both eyes, and 96.0% had bilateral retinopathy and maculopathy data (Table 8). The extent of missing data in these individuals' baseline and final records were separately analysed and are now discussed.

Most final records included retinopathy data for both eyes on the same day (n=7207; 97.9%; Table 8). Of the remaining 153 (2.1%) with unilateral data, 102 were graded in the fellow eye at an earlier screening assessment; the remaining 51 had retinopathy data in just one eye throughout the entire in-trial period. There were no instances where maculopathy data alone was available after the final retinopathy record, which made the censoring rules for the chosen composite efficacy endpoints easier to define (cohorts B1 and B2; described in Sections 3.4.3.3 and 3.4.3.5, respectively).

Similarly, among 2884 participants with a baseline record, 2856 (99.0%) had bilateral retinopathy data in the record closest to randomisation, 22 (0.8%) had retinopathy data for just one eye, and 6 (0.2%) had retinopathy data for both eyes at different times within the two-year window before randomisation (Table 8). Of those with bilateral retinopathy data at baseline (n=2862), 93.4% also had bilateral maculopathy data[*], which helped to define eligible data for analyses of one of the composite secondary efficacy endpoints (cohort B2; described in Section 3.4.3.5).

Finally, the feasibility of following up those with unilateral data was considered. Table 9 is a contingency table showing the availability of retinopathy data in one or both eyes on the baseline and final eye screening records. Among 2862 participants with bilateral baseline records, 2854 (99.7%) also had retinopathy data in both eyes at their final record. Of those with unilateral baseline data (n=22), 15 had a bilateral final record (11 were recorded simultaneously, and four were at different times), and seven had a unilateral final record with data for the same eye as their baseline. There were no instances of a different eye being followed up in those with a unilateral baseline record. Following these comparisons, there was agreement that restricting analysis cohorts based on the presence of bilateral

[*]This figure derives from the 2672 participants with bilateral retinopathy and maculopathy data at baseline, divided by the total number of participants with bilateral retinopathy data at baseline shown in Table 8. The calculation is as follows: 2672/(2856+6) = 93.4%.

retinopathy, bilateral maculopathy data or both at either time point would not exclude too many participants. Hence, the data analysis rules could be made simpler.

Table 8 Extent of Missing Retinopathy and Maculopathy Data

	All in-trial eye screening records No. of records (%)	Final eye screening records No. of records (%)*	Baseline eye screening records No. of records (%)*
Retinopathy data			
Available for both eyes	**32,288 (97.0%)**	**7309 (99.3%)**	**2862 (99.2%)**
Available for both eyes on the same date	-	7207 (97.9%)	2856 (99.0%)
Available for both eyes but on different dates†	-	102 (1.4%)	6 (0.2%)
Available for one eye only	605 (1.8%)	51 (0.7%)	22 (0.8%)
Missing in both eyes	402 (1.2%)	-	-
Total	**33,295**	**7360**	**2884**
Maculopathy data			
Available for both eyes	**31,981 (96.1%)**	**7301 (99.2%)**	**2673 (92.7%)**
Available for one eye only	616 (1.9%)	50 (0.7%)	37 (1.3%)
Missing in both eyes	698 (2.1%)	9 (0.1%)	174 (6.0%)
Total	**33,295**	**7360**	**2884**
Retinopathy and maculopathy data			
Available for both eyes	**31,972 (96.0%)**	**7301 (99.2%)**	**2672 (92.6%)**
Available but incomplete	921 (2.8%)‡	59 (0.8%)§	212 (7.4%)¶
Missing in both eyes	402 (1.2%)	-	-
Total	**33,295**	**7360**	**2884**

*Equivalent to the number of participants.
†For final retinopathy records, this represented the second from last available eye screening assessment; For baseline retinopathy records, this included an earlier eye screening which occurred up to two years prior to randomisation in ASCEND.
‡Includes 587 participants with unilateral retinopathy data and maculopathy data, 287 participants with bilateral retinopathy data and no maculopathy data, 29 participants with bilateral retinopathy data and unilateral maculopathy data, nine participants with unilateral retinopathy data and no maculopathy data, and nine participants with unilateral retinopathy data and bilateral maculopathy data.
§Includes 50 participants with unilateral retinopathy and maculopathy data, eight participants with bilateral retinopathy data and no maculopathy data, and one participant with unilateral retinopathy data and no maculopathy data.
¶Includes 171 participants with bilateral retinopathy data and no maculopathy data, 19 participants with bilateral retinopathy data and unilateral maculopathy data, 18 participants with unilateral retinopathy and maculopathy data, three participants with unilateral retinopathy data and no maculopathy data, and one participant with unilateral retinopathy data and bilateral maculopathy data.

Table 9 Cross Tabulation of Participants with Retinopathy Data in One or Both Eyes at Baseline and their Final Eye Screening Records

	Final retinopathy record	
	Both eyes	**One eye only**
Baseline retinopathy record		
Both eyes	2854	8
One eye only, same eye as final record	15	7
One eye only, different eye to final record	0	0

3.3.3 Baseline Disease Severity and Trajectory over Time

Table 10 summarises the retinopathy and maculopathy grades of those with a baseline DESP record. Among those with retinopathy data in at least one eye (n=2884), 67.6% had no DR at baseline (R_0), 31.0% had background DR (R_1), 1.1% had moderate-severe NPDR (R_2), and 0.3% had proliferative disease (R_3). Of those with maculopathy data in at least one eye (n=2710), 97.6% had no maculopathy at baseline (M_0 or M_0/M_0), compared to 2.4% who did (M_1/any).

On the randomisation questionnaires for ASCEND, participants were asked if they were *"…known to have diabetes changes at the back of the eye"*, to which they could answer "Yes" or "No" (Appendix 5). Those who answered "Yes" were asked if they had received laser therapy; however, accurate phenotyping of pre-existing DR was not sought. The questions were repeated on ASCEND's Quality of Life questionnaire, which accompanied materials sent for the trial's final follow-up assessments. Among the participants with a baseline DESP record (n=2884), 32.4% (n=935) had pre-existing DR ($\geq R_1$ in either eye) based on their linked data, but only 18.4% (n=532) reported retinal changes on their ASCEND randomisation questionnaires (Table 11). Of those who denied having pre-existing DR (n=2352), 474 (20.2%) were graded $\geq R_1$ in either eye on a DESP record within two years before they were randomised (not tabulated). Conversely, among those who said they had pre-existing DR (n=532), 69 (13%) were graded R_0/R_0, i.e. no disease, on DESP records within the same time window (not tabulated). Given this discordance, responses to the randomisation questionnaire about pre-existing DR will be ignored from the pre-specified outcomes for ASCEND-Eye[*]; however, every data item measured at randomisation for ASCEND will be presented in the baseline characteristics tables for completeness.

[*] The proportion of participants from the full randomised cohort in ASCEND who reported having DR on their final follow-up questionnaires for ASCEND was assessed in a post-hoc exploratory analysis after the DAP for ASCEND-Eye was made publicly available and unblinded analyses were completed. Excluding those who reported pre-existing DR on their randomisation form, 1062 [13.7%] in the active group of the aspirin arm developed DR compared to 1007 [13.0%] in the placebo group ($\chi^2 = 1.69$; P = 0.19). In the omega-3 FAs arm, 1048 [13.5%] participants assigned to active treatment developed DR, compared to 1021 [13.2%] in the placebo group ($\chi^2 = 0.41$; P = 0.52).

Table 12 shows retinopathy grades averaged over both eyes at the final record in those with and without baseline data. Most participants had little or no DR; 95.9% of 7360 individuals were graded between R_0 to R_1 in either eye on their final record[*]. Only 61 out of 7360 participants (1.2%) received photocoagulation during the trial. Therefore, as a proxy indicator of higher R grades, a decision was made to omit photocoagulation status (P grades) from any of the endpoints described in the final DAP.

Among those with baseline records (n=2884), two-thirds had no change in their average retinopathy grade by the end of the scheduled treatment period (n=1908; Table 13). Similarly, there was minimal progression to non-proliferative (R_2) or proliferative disease ($R_{3a/s}$)[†], regardless of whether those comparisons were made for the best or worst eye at the final eye screening record (Tables 14 and 15, respectively), or in either eye at any time during the in-trial period (Table 16). This was in keeping with expectations; the baseline characteristics of ASCEND participants predicted a low risk for the development of retinopathic features, including short durations of diabetes (median 7 years; IQR 3-13 years), reasonable glycaemic and blood pressure control, and minimal microvascular disease at enrolment.[11] Therefore, given their low disease severity, previous interest in omega-3 FAs for diabetic maculopathy[70,400,401], and a desire to improve the study's power, it seemed reasonable to consider a composite endpoint involving both the retinopathy and maculopathy data.

Table 10 Distribution of Retinopathy and Maculopathy Grades at Baseline

No DR		Mild NPDR (Background DR)			Moderate-severe NPDR			Proliferative DR			Maculopathy Status			
R_0	R_0/R_0	R_1	R_1/R_0	R_1/R_1	R_2R_0	R_2R_1	R_2R_2	R_3/R_1	R_3/R_2	R_3/R_3	M_0	M_0/M_0	M_0/M_1	M_1/M_1
20	1929	8	440	447	1	18	13	3	3	2	37	2612	35	26

The total number of participants with a baseline record that included retinopathy data was 2884. The total number of participants with a baseline record that included maculopathy data was 2710. Unilateral data is denoted by X_y; bilateral data is denoted by X_y/X_y.

[*] This includes four people graded R_2 in one eye and R_0 in the other eye.

[†] Given their small numbers, a decision was made to combine R_{3a} and R_{3s} grades into a single R_3 grade for both the preliminary and final analyses.

Table 11 Proportion of Participants with a Baseline DESP Record who Reported having Diabetes Changes at the Back of the Eye on their Randomisation and Final Follow-up Questionnaires for ASCEND

	At randomisation (n=2884)	At the final follow-up (n=2281)
Participant-reported retinopathy	532 (18.4%)	580 (25.4%)
Participant-reported laser therapy*	59 (2.0%)	92 (4.0%)

*An additional five participants at randomisation and nine participants at the final follow-up answered "Yes" to having laser therapy without answering "Yes" to having diabetes changes at the back of the eye.

Table 12 Retinopathy Grade Averaged over Both Eyes at the Latest-Available In-Trial Eye Screening Record in those with and without Baseline Data

	Retinopathy grade averaged over both eyes at the final record							
Baseline data	**R_0**	**$R_{0.5}$**	**R_1**	**$R_{1.5}$**	**R_2**	**$R_{2.5}$**	**R_3**	**Total**
Total	4214 (57.3%)	1233 (16.8%)	1602 (21.8%)	118 (1.6%)	110 (1.5%)	23 (0.3%)	60 (0.8%)	**7360**
Available	1811 (62.8%)	454 (15.7%)	539 (18.7%)	40 (1.4%)	27 (0.9%)	4 (0.1%)	9 (0.3%)	**2884**
Not available	2403 (53.7%)	779 (17.4%)	1063 (23.7%)	78 (1.7%)	83 (1.9%)	19 (0.4%)	51 (1.1%)	**4476**

Where a retinopathy grade was missing for one eye, the grade for the fellow eye is presented as the average.

Table 13 Change in Average Retinopathy Grade from Baseline to the Latest Available In-trial Eye Screening Record among those with a Baseline Eye Screening Record

Change in average retinopathy grade among those with a baseline eye screening record	N (%)
-2	1 (<0.1%)
-1.5	2 (<0.1%)
-1.0	89 (3.1%)
-0.5	289 (10.0%)
0	1908 (66.2%)
+0.5	401 (13.9%)
+1.0	176 (6.1%)
+1.5	11 (0.4%)
+2.0	7 (0.2%)
Total	**2884**

Table 14 Cross Tabulation Showing Participants' Transition in Retinopathy Grades in the Best Eye

	Retinopathy grade in the best eye at the final in-trial eye screening record								
Retinopathy grade in the best eye at baseline	**R_0**		**R_1**		**R_2**		**R_3**		**Total**
No baseline data	3189	(71.2%)	1158	(25.9%)	78	(1.7%)	51	(1.1%)	4476
R_0	2113	(88.3%)	272	(11.4%)	7	(0.3%)	0	(0.0%)	2392
R_1	155	(32.7%)	299	(63.1%)	16	(3.4%)	4	(0.8%)	474
R_2	0	(0.0%)	6	(37.5%)	6	(37.5%)	4	(25.0%)	16
R_3	0	(0.0%)	1	(50.0%)	0	(0.0%)	1	(50.0%)	2
Total	5457	(74.1%)	1736	(23.6%)	107	(1.5%)	60	(<0.1%)	7360

In this table, "best eye" was defined as the one with the lowest retinopathy grade at the eye screening assessment chosen as the baseline record and separately at the latest available record. Where retinopathy grades were the same in both eyes, the right eye was arbitrarily chosen as the "best eye". Where retinopathy data was missing in one eye, the present eye was chosen as the "best eye". Therefore, for 42 participants, the same eye is represented in Tables 14 and 15. Row-wise percentages of the figures in the total column are shown in parentheses.

Table 15 Cross Tabulation Showing Participants' Transition in Retinopathy Grades in the Worst Eye

	Retinopathy grade in the worst eye at the final in-trial eye screening record								
Retinopathy grade in the worst eye at baseline	**R_0**		**R_1**		**R_2**		**R_3**		**Total**
No baseline data	2403	(53.7%)	1840	(41.1%)	134	(2.9%)	99	(2.2%)	4476
R_0	1510	(77.5%)	424	(21.8%)	14	(0.7%)	1	(<0.1%)	1949
R_1	299	(33.5%)	557	(62.3%)	35	(3.9%)	3	(0.3%)	894
R_2	2	(6.2%)	9	(28.1%)	17	(53.1%)	4	(12.5%)	32
R_3	0	(0.0%)	1	(11.1%)	0	(0.0%)	8	(88.9%)	9
Total	4214	(57.3%)	2831	(38.5%)	200	(2.7%)	115	(1.6%)	7360

In this table, "worst eye" was defined as the one with the highest retinopathy grade at the eye screening assessment chosen as the baseline record and separately at the latest available record. Where retinopathy grades were the same in both eyes, the left eye was arbitrarily chosen as the "worst eye". Where retinopathy data was missing in one eye, the present eye was chosen as the "worst eye". Therefore, for 42 participants, the same eye is represented in Tables 14 and 15. Row-wise percentages of the figures in the total column are shown in parentheses.

Table 16 Cross Tabulation Showing Participants' Worst Ever Retinopathy Grade in Either Eye at any time during the In-trial Period

	Worst in-trial retinopathy grade in either eye								
Retinopathy grade in the worst eye at baseline	**R_0**		**R_1**		**R_2**		**R_3**		**Total**
No baseline data	1688	(37.7%)	2481	(55.4%)	193	(4.3%)	114	(2.6%)	4476
R_0	1016	(52.1%)	914	(46.9%)	17	(0.9%)	2	(0.1%)	1949
R_1	97	(10.9%)	728	(81.4%)	63	(7.1%)	6	(0.7%)	894
R_2	0	(0.0%)	6	(18.8%)	21	(65.6%)	5	(15.6%)	32
R_3	0	(0.0%)	0	(0.0%)	1	(11.1%)	8	(88.9%)	9
Total	2801	(38.1%)	4129	(56.1%)	295	(4.0%)	135	(1.8%)	7360

Row-wise percentages of the figures in the total column are shown in parentheses.

3.3.4 Endpoint Eligibility and Power Calculations

Following the analyses described in Sections 3.3.1 to 3.3.3, further assessments of the linkage data were conducted to determine the eligibility for different analysis cohorts, to calculate the number of events contributing to some proposed endpoints, and to perform their associated power calculations[*]. This work is presented in Section 3.4.4 of the DAP to avoid repeating definitions of the study endpoints here.

[*] I am grateful to Ms Georgina Buck (Supervisor and ASCEND Trial Statistician), who analysed the number of endpoint events. Their associated power calculations were conducted by myself.

3.3.5 Summary of Preliminary Blinded Analyses

The preliminary analyses described in Section 3.3 helped to define each study endpoint in the DAP in Section 3.4. As clinically important treatment effects could be missed in analyses comparing the proportion of duplex retinopathy grades cross-sectionally, time-to-event analyses were to be prioritised as more informative analysis methods. The size of each analysis cohort also determined the hierarchy of endpoints; the primary efficacy endpoint was to be the biggest of these.

3.4 Data Analysis Plan

All analyses described in this DAP were pre-specified, and this was made publicly available on the ASCEND trial website before unblinding the ASCEND-Eye results, except for sight-threatening eye bleeding events, which were included with the parent trial's safety results*.[12] The latter were pre-specified in online materials included with ASCEND's baseline manuscript before the main results were unblinded.[11] The structure of this DAP conforms with guidelines for the contents of statistical analysis plans, which were developed in collaboration with the network of UK Clinical Research Collaboration-registered trial units.[402]

3.4.1 Analysis Populations

The analyses described henceforth refer to three discrete participant populations, reflecting the three different data collection sources that were described in Section 3.2:

- Those involving the full cohort of randomised ASCEND participants (Cohort A)
- Those involving participants with linked data from the NHS DESP records (Cohort B)

* https://ascend.medsci.ox.ac.uk. The online version of the DAP was intended to be read as a stand-alone document and to accompany a baseline characteristics manuscript. As such, it included a brief description of the three methods of data collection used in ASCEND-Eye. More detailed information about each of these was presented in Section 3.2.1 of the thesis. Therefore, those paragraphs were omitted from the DAP shown here. The thesis DAP also describes the literature supporting the chosen statistical methodology in more detail and has more information regarding the preliminary blinded analyses that contributed to the power calculations. However, the pre-specified definitions of each analysis population, outcome measure and analysis method are the same as the online version.

- Those involving participants who returned a VFQ (Cohort C)

3.4.1.1 Cohort A (Every ASCEND Participant)

This cohort includes the entire population of randomised participants from the ASCEND trial and shall be relevant to safety analyses, incident AMD events and other participant-reported eye outcomes.

3.4.1.2 Cohort B (DESP-Linked)

This cohort includes randomised ASCEND participants who met the eligibility criteria defined in Section 3.2.1.2 and attended diabetic eye screening appointments at a collaborating DESP. Elsewhere in the DAP, the linkage cohort is divided into cohorts B1-7 for specific analyses, as summarised in Appendix 16.

3.4.1.3 Cohort C (VFQ Responders)

This cohort includes randomised participants from ASCEND who were eligible to receive the VFQ and who returned it. Those sent the VFQ included anyone alive and on web-, mail- or telephone-based follow-up at the end of ASCEND (31st July 2017). It excludes the following individuals:

- those who were known to be dead prior to beginning mailings of the VFQ on 23rd February 2018;
- those on GP- or central registry-follow-up at the end of ASCEND; and
- those who withdrew their consent or were lost to follow-up at the end of ASCEND.

3.4.1.4 Consort Diagrams for each Analysis Cohort

The presentation of results for each of the three main analysis cohorts (A, B1, C) will begin with a consort diagram which summarises the number of participants who were:

- valid for inclusion in the dataset used to estimate the primary outcomes and, where relevant, the secondary and tertiary outcomes (by reason); and
- excluded from the dataset used to estimate the primary outcomes and, where relevant, the secondary and tertiary outcomes (by reason).

Truncated consort diagrams showing those who were excluded from cohort B1 in DESP subsets B2-7, and the reason, will also be produced.

3.4.2 Baseline Characteristics

3.4.2.1 Baseline Characteristics of Cohorts A, B1 and C

To assess the balance of important demographic and clinical variables between randomised treatment arms, the following baseline characteristics, recorded at the time of randomisation into ASCEND, will be presented for analysis cohorts A, B1 and C, overall and by randomised treatment allocation (Table 17):

Table 17 Baseline Characteristics to be Tabulated

Characteristic	Stratification	Units	Summary statistics
Age at randomisation	<60 years ≥60<70 years ≥70 years	Years	Mean ± SD Count (%)
Sex	Male Female	-	Count (%)
Type of diabetes*	Type 1 Type 2	-	Count (%)
Duration of diabetes	0 to <5 years ≥5 <10 years ≥10 to <20 years ≥20 years Unknown	Years	Median Interquartile range (IQR) Count (%)
Diabetes management	Diet-only Oral hypoglycaemic agent(s) only Insulin ± oral hypoglycaemic agent(s)	-	Count (%)
Participant-reported diabetic retinopathy	Yes No Unknown	-	Count (%)
Participant-reported hypertension	Yes No Unknown	-	Count (%)
Systolic blood pressure†	<130 ≥130<140 ≥140 Unknown	mmHg	Mean ± SD Count (%)

Characteristic	Stratification	Units	Summary statistics
Diastolic blood pressure†	<75 ≥75<85 ≥85 Unknown	mmHg	Mean ± SD Count (%)
Body Mass Index‡	<25 ≥25<30 ≥30<35 ≥35 Unknown	kg/m^2	Mean ± SD Count (%)
Cigarette smoking	Current Former Never Unknown	-	Count (%)
Non-study Medication§	ACE-inhibitor or ARB Aspirin use before screening Thiazide or related diuretic Calcium channel blocker Statin	-	Count (%)
Total cholesterol	<4.0 ≥4.0<5.0 ≥5.0 Not available	mmol/l	Mean ± SD Count (%)
HDL-cholesterol	<1.0 ≥1.0<1.5 ≥1.5 Not available	mmol/l	Mean ± SD Count (%)
Non-HDL cholesterol	<2.5 ≥2.5<3.5 ≥3.5 Not available	mmol/l	Mean ± SD Count (%)
Glycosylated haemoglobin (HbA1c)	<48 (6.5) ≥48 (6.5), <64 (8.0) ≥ 64 (8.0) Not available	IFCC (mmol/mol) DCCT (%)	Mean±SD Count (%)
CKD-EPI estimated GFR¶	≥90 ≥60<90 <60 Not available	$ml/min/1.73m^2$	Mean±SD Count (%)
Urinary albumin:creatinine ratio	<3 ≥3 Not available	mg/mmol	Median IQR Count (%)
Townsend Deprivation Index**	<-3 ≥-3<0 ≥0<2 ≥2<4 ≥4<6 ≥6 Unknown	-	Count (%)
Ethnic origin	White Indian/Pakistani/Bangladeshi African/Caribbean Other/unknown	-	Count (%)

DCCT = Diabetes Control and Complications Trial; GFR = Glomerular Filtration Rate; HDL=High-density lipoprotein; IQR = Interquartile range; IFCC =International Federation of Clinical Chemistry; SD = Standard Deviation

*The presence of type 2 diabetes was based on a broad clinical definition involving the age of the participant at the diagnosis of diabetes, the use of insulin within one year after diagnosis, and the body-mass index.

† From blood and urine consent forms, generally before randomisation.

‡ The body-mass index (the weight in kilograms divided by the square of the height in meters) was based on values for height and weight the participants reported on their randomisation questionnaires.

§ Concomitant medications will be coded and grouped by class the same way they were in the ASCEND trial.[11]

¶ Calculated from blood cystatin c concentration using the CKD-EPI formula.[393]

**The Townsend Deprivation Index is derived from four key variables of the UK census data: households without a car, overcrowded households, households not owner-occupied and unemployed persons. A score of 0 represents an area with a UK average value; positive values indicate relative deprivation, and negative values indicate relative affluence.[403]

3.4.2.2 Baseline Characteristics of Cohort B Analysis Subsets (B2-7)

To compare differences between the baseline characteristics of B1 and its analysis subsets (B2-7), a truncated set of only the key determinants of DR progression, recorded at the time of randomisation into ASCEND will be tabulated for cohorts B1-7 (Table 18). The results will be presented for each cohort without stratification for the randomised treatment allocations.

Table 18 Truncated Baseline Characteristics to be Tabulated for Cohort B Analysis Subsets

Characteristic	Stratification (where relevant)	Units	Summary statistics
Age at randomisation	<60 years ≥60<70 years ≥70 years	Years	Mean ± SD Count (%)
Sex	Male Female	-	Count (%)
Type of diabetes*	Type 1 Type 2	-	Count (%)
Duration of diabetes		Years	Median IQR
Systolic blood pressure†		mmHg	Mean±SD
Diastolic blood pressure†		mmHg	Mean±SD
Glycosylated haemoglobin (HbA1c)		IFCC (mmol/mol) DCCT (%)	Mean±SD
CKD-EPI estimated GFR‡		ml/min/1.73m^2	Mean±SD
Urinary albumin:creatinine ratio		mg/mmol	Median IQR
Ethnic origin	White	-	Count (%)

DCCT = Diabetes Control and Complications Trial; GFR = Glomerular Filtration Rate; IQR = Interquartile range; IFCC =International Federation of Clinical Chemistry; SD = Standard Deviation
*The presence of type 2 diabetes was based on a broad clinical definition involving the age of the participant at the diagnosis of diabetes, the use of insulin within one year after diagnosis, and the body-mass index.
† From blood and urine consent form, generally before randomisation.
‡ Calculated from blood cystatin c concentration using the CKD-EPI formula.[393]

3.4.2.3 Comparison of Participants Included and Excluded from Cohorts B1 and C

Analysis cohorts B1-7 (DESP-linkage) and C (VFQ) represent subsets of cohort A (the full ASCEND population). The baseline characteristics of those included and excluded from analysis cohorts B1 and C will be presented overall, without stratification for treatment allocation, in order to assess whether the results are generalisable to the full population. Formal testing for differences in baseline characteristics between those included in and excluded from analysis cohorts B1 and C will use the Chi-squared test for heterogeneity for categorical variables and grouped continuous variables (including age, duration of diabetes, systolic blood pressure, diastolic blood pressure, BMI, total cholesterol, HDL-cholesterol, non-HDL cholesterol, HbA1c, eGFR and urinary albumin:creatinine ratio), the Mantel-

Haenszel Chi-square test for trend for ordinal variables (i.e. Townsend Deprivation Index), and the t-test or Wilcoxon-Mann-Whitney U test for continuous variables with normal (summarised by mean±standard deviation (SD)) or non-normal (summarised by median and IQR) distributions, respectively. Categories that are "unknown" or "not available" will be excluded. Standardised differences, representing the difference between means or proportions divided by the data's combined standard deviation, will also be calculated using specific functions within the "stddiff" software package developed for R, for continuous or categorical (ordinal or nominal) baseline variables.[390,392]

3.4.3 Definitions for Efficacy and Safety Outcomes

3.4.3.1 General Principles

All comparisons of the main efficacy outcomes will be between those individuals allocated aspirin daily *versus* matching placebo, and separately, omega-3 FA supplement daily *versus* matching placebo, during the scheduled treatment period. Every randomised participant will be compared, regardless of whether they took all, some or none of their allocated treatment (i.e. intention-to-treat analyses). The factorial design of ASCEND does not affect the statistical sensitivity with which the effects of each treatment arm can be assessed.[394,404] Likewise, no clinically-significant interactions between the study treatments are anticipated.[157] Comparisons of the aspirin arm will therefore be made without stratification by omega-3 FA allocation (and vice versa for omega-3 FA analyses). All analyses will be of time from randomisation to the first occurrence of each outcome during the scheduled treatment period, except for cross-sectional comparisons of duplex retinopathy grades, and scores from the NEI-VFQ-25. Participants may contribute to more than one assessment if they have more than one type of event. All data will be reviewed, blind to treatment allocation, by coordinating centre clinical staff and, where relevant, coded in accordance with pre-specified criteria.

3.4.3.2 Hypotheses

For all statistical tests, the null hypothesis will be that allocation to aspirin and, separately, allocation to omega-3 FAs, on each outcome of interest, is the same as the effect of allocation to placebo. Therefore, the alternative hypothesis is that there is a difference in effect by treatment allocation.

Objectives, outcomes, and the relevant source of the following pre-specified data are summarised in Appendix 17.

3.4.3.3 Primary Efficacy Endpoint

The primary assessment will include a comparison of the effects of allocation to aspirin *versus* placebo, and separately, omega-3 FAs supplement *versus* placebo, on:

- **Referable disease** during the scheduled treatment period, where referable disease is defined as the composite of referable diabetic retinopathy (R_2 or $R_{3a/s}$) or referable diabetic maculopathy (M_1), in either eye, among all those with in-trial retinopathy data (*Analysis cohort B1*)

3.4.3.4 Primary Safety Endpoint

It is anticipated that aspirin may increase the risk of sight-threatening eye bleeding. Therefore, the primary safety assessment will include a comparison of the effects of allocation to aspirin *versus* placebo on:

- **Confirmed sight-threatening eye bleeds** in either eye (as defined in Section 3.2.1.1.1; *Analysis cohort A*)

3.4.3.5 Secondary Efficacy Endpoints

The secondary assessments will include a comparison of the effects of allocation to aspirin *versus* placebo, and separately, omega-3 FAs supplement *versus* placebo, on:

- **Confirmed or unrefuted incident diagnoses of AMD** (*Analysis cohorts A and C*)

- **Referable disease** (as defined in Section 3.4.3.3) during the scheduled treatment period, restricted to those with both retinopathy and maculopathy data, for both eyes at baseline and at the final eye screening record, who are either without disease (R_0) or with only background diabetic retinopathy (R_1) and no maculopathy (M_0), in both eyes at baseline *(Analysis cohort B2).*

  To be analysed within the following strata of baseline retinopathy, and as an overall stratified analysis:

  - R_0/R_0
  - R_0/R_1 or R_1/R_0
  - R_1/R_1

- **Progression in retinopathy grade** in either eye during the scheduled treatment period, where progression is defined as an increase by one step or more in R grade, based on the NSC scoring protocol for retinopathy, excluding those with proliferative disease in both eyes on their baseline record (i.e. $R_{3a/s}/R_{3a/s}$), and those with data in one eye only at baseline and/or the final eye screening record (*Analysis cohort B3).*

  To be analysed within the following strata of baseline retinopathy, and as an overall stratified analysis:

  - R_0/R_0
  - R_0/R_1 or R_1/R_0
  - R_1/R_1
  - R_2/R_1 or R_1/R_2, R_2/R_2, or $R_2/R_{3a/s}$ or $R_{3a/s}/R_2$

  No attempt shall be made to distinguish the eye which progresses in those with a different R grade in each eye on their baseline record.

- **Composite NEI-VFQ-25 score** (*Analysis cohort C*).

3.4.3.6 Secondary Safety Endpoint

The secondary safety assessment will include a comparison of the effects of allocation to aspirin *versus* placebo, on:

- **Any eye bleed**, overall and subdivided by severity: (*Analysis cohorts A and C*)
    - Sight-threatening eye bleeds (*Analysis cohort A*)
    - Non-serious eye bleeds (*Analysis cohort A*)
    - Unrefuted eye bleeds (*Analysis cohorts A and C*)

3.4.3.7 Tertiary Endpoints

Tertiary analyses are planned to assess the effects of allocation to aspirin *versus* placebo, and separately, omega-3 FAs supplement *versus* placebo, on:

- **Incident reports of diabetic maculopathy**, overall and subdivided by the source of this information[*]:
    - Those DMO events that were confirmed or unrefuted from the 6-monthly follow-up questionnaires in ASCEND (*Analysis cohort A)*
    - Those with referable maculopathy (M_1)[†] in either eye among those without maculopathy (M_0) in both eyes at baseline *(Analysis cohort B2[‡])*

[*] If events are identified from more than one source, they will be represented in every subgroup; however, the overall incidence will be based on the earliest occurrence across all three data sources.

[†] According to the NSC, M_1 is defined by the *"presence of hard exudates within 1DD of the fovea, retinal thickening within 1DD of the fovea, or a group of exudates that is greater than or equal to half the disc area, inside the macular field. The macular field includes the area of retina within a circle that originates from the fovea at its centre to the temporal edge of the optic disc"*.[61] Thus, maculopathy events derived from the linkage records include both DMO and exudative disease. In contrast, those derived from ASCEND follow-up questionnaires or the VFQ only include DMO, i.e. transudatory events. This distinction is shown in the Forest plots presented in Section 4.4.5.

[‡] 2884 participants had a baseline DESP record with retinopathy and maculopathy data. The B2 cohort excluded those without bilateral R and M grades at baseline (n=212) or their final record (n=8) and those with referable disease at baseline (n=106), giving a final cohort size of 2558 people (see Figure 23). The B2 cohort was chosen for these analyses instead of creating another B cohort restricted by the maculopathy components only because those with referable disease move onto a more frequent schedule of follow-up by the NHS. They therefore have more opportunity to detect incident maculopathy events. In a post-hoc analysis conducted after unblinded analyses had begun, 20 participants were identified who were excluded from cohort B2, who had bilateral follow-up of their maculopathy grades and no referable maculopathy at baseline. Of those, 8 had referable maculopathy during the scheduled treatment period (4 were in the active group *vs.* 4 in the placebo group of each treatment arm). Thus, having an extra B cohort would not have affected the risk estimates from these tertiary analyses.

- Those DMO events that were reported on the visual function questionnaire (*Analysis cohort C*)

- **Final retinopathy grade** on the final eye screening record, in the following four combinations: R_0/R_0, R_1/R_0 or R_o/R_1, R_1/R_1, $\geq R_2/\geq R_0$ or $\geq R_0/\geq R_2$[*]. This analysis will exclude those with data in neither eye or one eye only at their final eye screening record, and those whose final retinopathy data on each eye was more than one year apart (*Analysis cohort B4*).

- **Worsening of visual acuity** in either eye during the scheduled treatment period, compared to baseline, defined as an increase of an ASCEND-Eye Recode Score of at least 3 points[†405] (*Analysis cohort B5*)

- The primary efficacy assessment by baseline characteristic (as listed in Section 3.4.2.1) (*Analysis cohort B1*)

- 11 vision-targeted subdomain scores from the NEI-VFQ-25 (*Analysis cohort C*)

3.4.3.8 Exploratory Assessments

Summary figures for incident reports of other participant-reported eye diseases will be presented overall and by randomised treatment allocation. Specific event Read codes to be included in these analyses are listed in Appendix 18.

[*]In blinded preliminary analyses, only a small number of individuals had the highest retinopathy grades, and therefore the following combinations were grouped into the $\geq R_2/\geq R_0$ category: $\geq R_2/R_0$ or $R_0/\geq R_2$, $\geq R_2/R_1$ or $R_1/\geq R_2$ and $\geq R_2/\geq R_2$. After the DAP was made publicly available, and in subsequent sections of this thesis, the $\geq R_2/\geq R_0$ category is referred to as $>R_1$/any.

[†] An increase of three or more points in the ASCEND-Eye Recode scoring system corresponds to a loss of 15 or more ETDRS letters, which the US Food and Drug Administration has recommended as a clinically-relevant outcome measure in ophthalmology trials.[398] A 15-letter change represents a doubling of the visual angle (or a doubling of the size of letters on a standard ETDRS chart).[398]

Other analyses may be performed in addition to the pre-specified comparisons, such as tabulations demonstrating the completeness of longitudinal DESP data and correlations between this and the number of endpoints. Given their data-dependent nature, the results from these analyses will be interpreted with due caution.

3.4.4 Power Calculations

Power analyses are central to the design of prospective clinical trials to ensure an adequate sample size to detect a target difference between the effects of two treatments.[406] Because ASCEND-Eye was a sub-study of a larger trial, the parameters for these calculations could not be changed. Nevertheless, some calculations were performed to understand the power of the study to detect a clinically-meaningful treatment effect at a two-sided 5% significance level. The formula used was based on formula 4.42 on page 141 of P. Armitage *et al.* "Statistical Methods in Medical Research" fourth edition[407], with a continuity correction from Fleiss *et al.* 1980.[408] Calculations were automated using Microsoft Excel, but a worked example is shown in Appendix 19. The calculations do not account for interactions between treatments in the factorial design nor the effect of non-adherence or drop-ins to treatment; therefore, the powers may be overestimated.[409] Power calculations were performed for the primary and secondary DESP endpoints (Section 3.4.4.1), eye bleeds (Section 3.4.4.2), and AMD events (Section 3.4.4.3). No power calculations were performed for the DESP tertiary endpoints or sensitivity analyses, because they were not to be main study outcomes for the reasons discussed in Section 3.3.1.1.1. Finally, power analyses conducted by developers of the NEI-VFQ-25 are considered in Section 3.4.4.4.

3.4.4.1 Power Calculations and Eligibility for the Primary and Secondary DESP Endpoints

The power calculations shown in this section were based on the non-significant 23% risk reduction for the development of proliferative DR (R_3), reported for those assigned to aspirin in a pre-specified subset of those with the least severe retinopathy (Level ≤35; n=609) in the ETDRS trial.[140] The power is shown in 5% increments up to this risk reduction. There is no precedent for omega-3 FAs on which separate

power calculations could be based; however, it seemed reasonable to assume that a proportional effect of similar magnitude would be important to patient and professional stakeholders.[406]

3.3.4.1.1 Primary Efficacy Endpoint Referable Disease (Cohort B1)

For this analysis, the full dataset of 7535 participants was restricted to those with retinopathy data in at least one eye during the scheduled treatment period (n=7360). Blinded preliminary analyses identified 1061 participants in cohort B1 who experienced a primary efficacy event (Table 19). Based on this, there is 81% to 97% power to detect 15% to 20% proportional reductions in the incidence of referable diabetic eye disease at 2P<0.05 (Table 20).

Table 19 Number of Referable Disease Outcomes during the Scheduled Treatment Period

No. of participants with in-trial records	No. of participants with post-randomisation referable disease (% of in-trial record no.)		
	R_2 or R_3	M_1	R_2, R_3 or M_1
7360	430 (5.8%)	899 (12.2%)	1061 (14.4%)

Table 20 Power of ASCEND-Eye to Detect Different Effects of the Interventions on Referable Disease among the Cohort of 7360 Participants with In-Trial Retinopathy Data

Proportional reduction of risk in the active group compared to the placebo group	N with event		Proportion of events in the active group	Proportion of events in the placebo group	Difference in proportions (higher - lower proportion)	Power at 2P = 0.05	Power at 2P = 0.01
	Active (N approx. 3680)	Placebo (N approx. 3680)					
25%	455	606	0.124	0.165	0.041	100%	99%
20%	472	589	0.128	0.160	0.032	97%	90%
15%	487	574	0.132	0.156	0.024	81%	60%
10%	503	558	0.137	0.152	0.015	44%	22%

3.4.4.1.2 Secondary Efficacy Endpoint – Referable Disease (Cohort B2)

In blinded preliminary analyses, the in-trial dataset of 7360 participants was restricted to those with bilateral retinopathy and maculopathy data at their baseline and final records and, separately, to those with these data in the same single eye throughout the follow-up period (Table 21). Out of 2664 participants with bilateral data, 2497 had no referable disease at any time before they were randomised, 61 participants had no referable disease on their baseline record but had referable disease prior to this,

and 106 were excluded for meeting the endpoint definition at baseline. An additional 45 individuals had unilateral follow-up of their retinopathy and maculopathy status, of whom nine had referable disease at baseline. The remaining 36 contributed very few additional endpoints. Therefore, to avoid over-complicating the eligibility criteria of this endpoint, a pragmatic decision was made to exclude those with unilateral baseline and final record data. Likewise, the pre-baseline status will be ignored.

In total, 240 of the 2558 participants in cohort B2 experienced the defined endpoint event (Table 22). Based on this, there is 24% to 41% power to detect 15% to 20% proportional reductions in the incidence of referable diabetic eye disease at 2P<0.05 (Table 23).

Table 21 Number of Referable Disease Outcomes during the Scheduled Treatment Period among those with Retinopathy and Maculopathy Data at Baseline and the Final Record

	No. of participants with referable disease during the in-trial period (% of those with in-trial records)							
	No. of participants with in-trial records	**R_2 or R_3**		**M_1**		**R_2, R_3 or M_1**	
Referable disease status before enrolment into ASCEND								
Total with maculopathy and retinopathy data in both eyes at baseline and final follow-up	**2664**	**108**	**(4.1%)**	**287**	**(10.8%)**	**319**	**(11.9%)**
No referable disease at any time pre-randomisation	2497	56	(2.2%)	195	(7.8%)	213	(8.5%)
No referable disease at baseline but had referable disease prior to this	61	13	(21.3%)	24	(39.3%)	27	(44.3%)
Referable disease at baseline	106	39	(36.8%)	68	(64.2%)	79	(74.5%)
Additional people with maculopathy and retinopathy data in the same one eye at baseline and final follow-up	**45**	**4**	**(8.8%)**	**5**	**(11.1%)**	**8**	**(17.8%)**
No referable disease at any time pre-randomisation	35	1	(2.9%)	0	(0.0%)	1	(2.9%)
No referable disease at baseline but had referable disease prior to this	1	0	(0.0%)	1	(100%)	1	(100%)
Referable disease at baseline	9	3	(33.3%)	4	(44.4%)	6	(66.7%)

Table 22 Number of Referable Disease Outcomes during the Scheduled Treatment Period among those with Maculopathy and Retinopathy Data at their Baseline and Final Record by their Baseline Retinopathy Grade, after Excluding those with Referable Disease at Baseline

	No. of participants with referable disease during the in-trial period (% of those with in-trial records)							
	No. of participants with in-trial records	R_2 or R_3		M_1		R_2, R_3 or M_1	
Total no. without referable disease at baseline (data in both eyes at baseline and the final record)	**2558**	**69**	**(2.7%)**	**219**	**(8.6%)**	**240**	**(9.4%)**
Baseline retinopathy grade							
R_0/R_0	1831	16	(0.9%)	72	(3.9%)	78	(4.3%)
R_1/R_0	386	11	(2.9%)	44	(11.4%)	50	(12.9%)
R_1/R_1	341	42	(12.3%)	103	(30.2%)	112	(32.8%)

Table 23 Power to Detect Different Effects of the Interventions on Referable Disease among the Cohort of 2558 Participants with Maculopathy and Retinopathy Data at their Baseline and Final Record, after Excluding those with Referable Disease at Baseline

Proportional reduction of risk in the active group compared to the placebo group	N with event		Proportion of events in the active group	Proportion of events in the placebo group	Difference in proportions (higher - lower proportion)	Power at 2P = 0.05	Power at 2P = 0.01
	Active (N approx. 1279)	Placebo N approx. 1279)					
25%	103	137	0.080	0.107	0.027	62%	37%
20%	107	133	0.083	0.104	0.021	41%	20%
15%	110	130	0.086	0.101	0.015	24%	9%
10%	114	126	0.089	0.099	0.010	12%	4%

3.4.4.1.3 Secondary Efficacy Endpoint – Retinopathy Progression (Cohort B3)

In blinded preliminary analyses, the baseline dataset of 2884 participants was restricted as follows: 22 participants were excluded because they had retinopathy data in only one eye at baseline, eight were excluded because they had retinopathy data in both eyes at baseline, but only one eye post-randomisation, and two had bilateral R_3 disease at baseline, meaning that they could not progress to a higher grade. After the dataset was reduced this way, 1249 out of 2852 (43.8%) progressed to a retinopathy grade higher than their baseline level (Table 24). The statistical power to detect a 15% proportional reduction in progression events at $2P<0.05$ is 97% (Table 25).

Table 24 First Progression in Retinopathy Grade during the Scheduled Treatment Period among those with Bilateral Retinopathy Data <$R_{3a/s}$/$R_{3a/s}$ at Baseline

	No. of participants with in-trial records	Post-randomisation progression of retinopathy (% of in-trial record no.)					
		Progression					
		Any eye		One eye		Both eyes	
Total with retinopathy data on both eyes at baseline and during the scheduled treatment period	**2852**	**1249**	**(43.8%)**	**838**	**(29.4%)**	**411**	**(14.4%)**
Baseline retinopathy grade							
R_0/R_0	1928	922	(47.8%)	539	(58.0%)	383	(19.9%)
R_1/R_0 or R_0/R_1	441	257	(58.3%)	251	(56.9%)	6	(1.4%)
R_1/R_1	446	55	(12.3%)	35	(7.8%)	20	(4.5%)
R_2/R_0 or R_0/R_2	1	1	(100%)	1	(100%)	0	(0.0%)
R_2/R_1 or R_1/R_2	17	5	(29.4%)	5	(29.4%)	0	(0.0%)
R_2/R_2	13	5	(38.5%)	3	(23.1%)	2	(15.4%)
R_3/R_0 or R_0/R_3	0	-	-	-	-	NA	NA
R_3/R_1 or R_1/R_3	3	2	(66.7%)	2	(66.7%)	NA	NA
R_3/R_2	3	2	(66.7%)	2	(66.7%)	NA	NA

NA = not applicable

Table 25 Power to Detect Different Effects of the Interventions on Retinopathy Progression among the Cohort of 2852 Participants

Proportional reduction of risk in the active group compared to the placebo group	N with event		Proportion of events in the active group	Proportion of events in the placebo group	Difference in proportions (higher - lower proportion)	Power at 2P = 0.05	Power at 2P = 0.01
	Active (N approx. 1426)	Placebo (N approx. 1426)					
25%	535	714	0.375	0.501	0.125	100%	100%
20%	555	694	0.389	0.487	0.097	100%	100%
15%	574	675	0.402	0.473	0.071	97%	89%
10%	592	657	0.415	0.461	0.046	69%	45%

3.4.4.2 Power Calculations for the Secondary Safety Endpoint - Any Eye Bleed

Based on a blinded preliminary analysis in which there were 395 incident eye bleeding events in total, there is 35% to 59% power to detect a 15% to 20% proportional increase in the incidence of eye bleeds in those assigned to aspirin at 2P<0.05 (Table 26). These analyses were conducted before the severity of eye bleeding events was characterised, and therefore, power calculations for the primary safety endpoint of sight-threatening eye bleeds are not shown.

Table 26 Power to Detect Different Effects of Aspirin on Eye Bleeds of Any Severity among all 15,480 Randomised ASCEND Participants

Proportional difference in risk in the active group compared to the placebo group	N with event		Proportion of events in the active group	Proportion of events in the placebo group	Difference in proportions (higher - lower proportion)	Power at 2P = 0.05	Power at 2P = 0.01
	Active (N approx. 7740)	Placebo (N approx. 7740)					
30%	163	232	0.021	0.030	0.009	94%	82%
25%	169	226	0.022	0.029	0.007	81%	60%
20%	176	219	0.023	0.028	0.006	59%	35%
15%	181	214	0.023	0.028	0.004	35%	16%
10%	187	208	0.024	0.027	0.003	17%	6%

3.4.4.3 Power Calculations for Secondary Efficacy Endpoint - AMD

From blinded preliminary analysis there were 260 AMD events in total, providing only 24% to 41% power to detect 15% to 20% proportional reductions in the incidence of AMD at 2P<0.05 (Table 27).

Table 27 Power to Detect Different Effects of the Interventions on AMD Incidence among all 15,480 Randomised ASCEND Participants

Proportional reduction of risk in the active group compared to the placebo group	N with event		Proportion of events in the active group	Proportion of events in the placebo group	Difference in proportions (higher - lower proportion)	Power at 2P = 0.05	Power at 2P = 0.01
	Active (N approx. 7740)	Placebo (N approx. 7740)					
30%	107	153	0.014	0.020	0.006	80%	59%
25%	111	149	0.014	0.019	0.005	62%	38%
20%	116	144	0.015	0.019	0.004	41%	20%
15%	119	141	0.015	0.018	0.003	24%	9%
10%	123	137	0.016	0.018	0.002	12%	4%

3.4.4.4 Power Calculations Secondary Efficacy Endpoint - NEI-VFQ-25

Table 28 estimates the number of individuals needed per treatment group to attain 80% power (α =0.05, two-tailed) to detect a 2-, 5-, 10- and 20- points difference in mean composite and 11 subdomain scores of the NEI-VFQ-25 when used as a one-off, post-intervention measurement (as presented by the score developers in Table 7 of Mangione *et al.*, 2001).[2] However, the analyses defined in the DAP are based on categories of NEI-VFQ-25 scores as the outcome variable rather than differences in mean values. Equivalent power tables for this type of analysis are not available in the literature. Even so, the numbers presented in Table 28 provide some reassurance that ASCEND-Eye is sufficiently large to detect

differences between those randomised to aspirin *versus* placebo and, separately, those randomised to omega-3 FAs *versus* placebo.

Table 28 Sample Sizes Needed to Detect Differences between Two Treatment Arms when Using the NEI-VFQ-25

	Points difference between the mean scores			
Domain	**2**	**5**	**10**	**20**
General Health	2650	424	106	26
General Vision	1729	277	69	17
Ocular Pain	1133	181	45	11
Near Activities	3297	527	132	33
Distance Activities	3297	527	132	33
Social Functioning	2858	457	114	29
Mental Health	2858	457	114	29
Role Difficulties	3297	527	132	33
Dependency	3073	492	123	31
Driving	4802	768	192	48
Colour Vision	2074	332	83	21
Peripheral Vision	2858	457	114	29
Composite Score	**1568**	**251**	**63**	**16**

This table was reproduced from the data published in: Mangione CM, *et al.* Development of the 25-list-item National Eye Institute Visual Function Questionnaire. *Archives of Ophthalmology* 2001; 119(7): 1050-8.[2]

3.4.5 Statistical Methodology

3.4.5.1 Definitions of Treatment Duration and Censoring Dates

The duration of the scheduled treatment period will be defined as day one post-randomisation up to and including the censoring date in ASCEND. The latter was the earliest of:

- an unrefuted death report (i.e. an adjudicated fatal serious adverse event);
- a final follow-up conducted by mail-based or electronic questionnaire, telephone to the participant, their relative or carer, or GP follow-up; and
- for anyone without a final follow-up via one of the methods listed above, until the end of the final follow-up window: 31st March 2017*; or
- the date of withdrawal of consent to direct or third-party follow-up.

*The main follow-up method for these individuals was through a central registry. Participants were considered to be on central registry follow-up if:

- their last known postcode was in England or Wales on 31st March 2017;

- they were not known to have moved abroad before 31st March 2017; or
- they had not withdrawn consent to follow-up.

The censoring date for those analyses involving cohort A and C will be the same as that applied in the main ASCEND trial. For analyses involving cohort B, participants will be censored at the date of their final eye screening record with retinopathy data in at least one eye before the end of the scheduled treatment period.

3.4.5.2 Definitions of Baseline

For analysis cohorts A, B1 and C, the baseline will be defined as the date of randomisation in ASCEND.

Relevant to the secondary DESP efficacy analyses (cohorts B2 and B3), the baseline record will be defined as the eye screening assessment closest to randomisation in each eye that was on the date of randomisation or up to two years before randomisation. The majority of individuals had retinopathy data in both eyes on the same baseline record (see Section 3.3.2). If retinopathy data is missing for one eye in the baseline record closest to randomisation, an earlier record may be used for the missing eye, so long as both records occurred in the two years prior to randomisation. To simplify the narrative, both scenarios shall be referred to as the "baseline record", irrespective of whether the data derived from eye screening assessments on different dates for different eyes. Data from pre-baseline records will be ignored for all analyses involving the linkage data.

For every analysis cohort, the demographic and clinical information recorded by participants on their randomisation questionnaire or biochemical samples collected at enrolment (see Section 3.1) constituted their baseline characteristic data.

3.4.5.3 Definition of the Final Eye Screening Record

For cohort B, the final eye screening record will be defined as the last available post-randomisation eye screening record with retinopathy data in at least one eye before the end of the scheduled treatment

period. The majority of individuals had retinopathy data in both eyes on the same final record. If retinopathy is missing for one eye on the final record, an earlier post-randomisation record will be used as the final grade for the missing eye. To simplify the narrative, both scenarios shall be referred to as the "final record", irrespective of whether the last available post-randomisation retinopathy data came from screening assessments on different dates for different eyes. Data from post-trial records will be ignored for all analyses involving the linkage data.

3.4.5.4 Unit of Analysis for the DESP-Linkage Data

DR is a bilateral disease in which lesions presenting in the left and right eye are usually correlated, whereas most statistical methods assume independence of the sampling units.[410,411] Some ophthalmology trials mitigate this by measuring outcomes in only one eye, but this can be a cause of selection bias or result in a loss of statistical power.[410] Therefore, the unit of analysis for all endpoints that involved the DESP data was to be the participant; however, data from either eye could contribute once in the time-to-first event analyses (B1-3, B5); a summary measure of the participant-dependent retinopathy grade combination in both eyes is to be used for the cross-sectional analyses involving cohort B4, B6 and B7.

Within the endpoint descriptions and all results tables, R_x/R_y denotes the combination of retinopathy grades in both eyes per participant; however, the ordering of these scores does not attempt to distinguish between the right and left eye.

3.4.5.5 Study Average Adherence

Study average adherence will be considered when interpreting all pre-specified analyses. ASCEND-Eye will observe the same rules regarding analyses of study average adherence as those defined in the main ASCEND trial DAP.[11] Here, it was stated that *"participants at risk of the event in question are considered to be definitely or probably adherent if a follow-up form was received that indicates the participant took their treatment "every day" or "most days" during the follow-up period. Participants*

were also considered adherent if they had previously been adherent, were still receiving medication and had not reported stopping treatment, and information was received within the previous seven months."[11]

Study-average reported definite or probable adherence with study treatment shall be reported for each of the three main analysis populations (A, B1, C), in each treatment arm by randomised allocation, and by:

- Time post-randomisation in the following intervals and overall: <3, ≥3<5, ≥5<7, ≥7 years; and
- Baseline characteristics (see Section 3.4.2.1).

A summary study average reported definite or probable adherence shall be reported for cohorts B2-7, overall and by randomised treatment allocation, but without stratifying by 3-year intervals or the baseline characteristics.

In the main ASCEND analyses, average adherence weighted by person-years at risk of the primary outcome was estimated because there was an expectation that following a vascular event, participants would stop their allocation in the aspirin arm to take prescribed aspirin. In ASCEND-Eye, adherence estimates will only be based on person-years in the study, i.e. with censoring for death or the end of the scheduled treatment period, because neither study medication is likely to be prescribed by third parties following the eye events. Hence, the adherence estimates presented for cohort A may differ slightly from those included in the ASCEND Clinical Study Report and publications.

3.4.5.5.1 Use of Non-Study Anti-platelet or Anti-coagulant Therapy

The use of non-study anti-platelet or anti-coagulant therapy shall be reported for analysis populations A, B1 and C by randomised allocation to aspirin *versus* placebo, overall, and by the number of years post-randomisation in the following intervals: <3, ≥3<5, ≥5<7 and ≥7 years.

3.4.5.5.2 Reasons for Stopping Treatment

Reasons for stopping treatment shall be reported for analysis populations A, B1 and C, overall and by randomised allocation to aspirin *versus* placebo and, separately, omega-3 FAs *versus* placebo, and by:

- Reason type; and
- Total stopped for any reason.

3.4.5.6 Completeness and Duration of Follow-up

ASCEND-Eye will observe the same rules regarding analyses of the completeness of follow-up as those defined in the main ASCEND trial DAP.[11]

Completeness of follow-up shall be reported for analysis cohort A, in separate tables for each treatment arm, by randomised allocation, and by the proportion of those with:

- Complete follow-up information – overall, and in the following categories:
    - Final follow-up visit completed by the participant or a carer
    - Final follow-up completed by the participant's GP
    - Final follow-up completed by linkage to a central registry
    - Died (follow-up prior to morbidity complete)

- Incomplete follow-up information – overall, and in the following categories:
    - Consent withdrawn
    - Moved abroad
    - Died (follow-up prior to morbidity incomplete)
    - No final follow-up information

Duration of follow-up shall be reported for analysis population A in each treatment arm by randomised allocation, and by:

- The mean length of follow-up (SD; years)

- The median length of follow-up (IQR; years)
- Person-years of follow-up overall, and by follow-up method:
    - Participant
    - General practitioner
    - Registry linkage-only
    - Died with complete follow-up
    - Incomplete follow-up information

Only the mean (SD) and median (IQR) length of follow-up in each treatment arm and by randomised allocation will be presented for cohorts B1-7 and C.

3.4.5.7 Baseline Characteristics

Univariate analyses will be used to describe baseline characteristics for all three analysis cohorts. These will be presented as counts (percentage) for categorical variables, as mean (SD) for normally distributed continuous variables, and as median (IQR) for non-normally distributed continuous variables.

3.4.5.8 Analyses of Time to First Event

Efficacy Analyses Involving Cohorts B1-3 and B5, the Safety and AMD Analyses Involving Cohorts A and C, and the Diabetic Maculopathy Analyses Involving Cohorts A, B2 and C

Logrank[*394,412] and stratified logrank[†413] methods will be used to estimate the average event rate ratio, 95% confidence intervals and two-sided P values in those allocated active aspirin *vs.* placebo and, separately, omega-3 FAs *vs.* placebo[‡]. The results shall be represented graphically in the form of Kaplan-Meier and Forest plots, respectively. These methods of survival analysis were chosen because

[*] Relevant to analyses involving cohort B1, B5 and the AMD analyses involving cohorts A and C.
[†] Relevant to analyses involving cohort B2, B3, the safety analyses involving cohorts A and C, and the diabetic maculopathy analyses involving cohorts A, B2 and C.
[‡] Calculations were automated using SAS statistical software, but a worked example of the logrank test for referable disease events in the aspirin arm is shown in Appendix 20. The example was produced after the DAP was made publicly available, and unblinded analyses had already begun.

there was no need to adjust for other covariates on the outcomes of interest since they were balanced between treatment groups in each arm, and extreme treatment effects were not expected; both of which are reasons why Cox proportional hazards regression models may be used instead (according to Chapter 17 on pages 568-591 of P. Armitage, G. Berry and JNS. Matthews "Statistical Methods in Medical Research" Fourth Edition).[407]

For the primary safety assessments, all analyses will be based on confirmed incident sight-threatening eye bleed diagnoses, defined as any relevant participant-reported outcome, verified at the time of adjudication by medical record evidence of the diagnosis, and after the date of randomisation. It will exclude events for which no supporting documentation was received from the participants' GP and those that derive from the VFQ which were not eligible to be adjudicated (i.e. unrefuted events). The secondary safety analyses will include confirmed or unrefuted events derived from ASCEND's 6-monthly follow-up questionnaires and the VFQ.

3.4.5.9 Analyses of the Ordinal Data

3.4.5.9.1 Tertiary Analysis of Final Retinopathy Grade (Cohort B4)

To compare the net severity of retinopathy grades across both eyes between treatment arms, a proportional odds logistic regression model[396,398] will be fitted to duplex final retinopathy grades grouped into a 4-point ordinal scale as the outcome variable: R_0/R_0, R_1/R_0 or R_0/R_1, R_1/R_1, $>R_1$/any.[*] A common odds ratio with a 95% confidence interval will be used to estimate the average effect size over the total ordinal scale, caused by allocation to aspirin *versus* placebo and, separately, omega-3 FAs *versus* placebo. If the proportional odds assumption is violated, separate odds ratios between active and placebo groups at each response level will not be presented to avoid multiple comparisons of the same data.

[*] Calculations were automated using SAS statistical software, but a worked example of ordinal logistic regression for the effect of aspirin on final duplex retinopathy grades is shown in Appendix 21. The example was produced after the DAP was made publicly available, and unblinded analyses had already begun.

3.4.5.9.2 Secondary Efficacy and Tertiary Analyses of Dependent Variables from the VFQ (Cohort C)

Calculating the NEI-VFQ-25 composite and subdomain scores will follow the scoring algorithm prescribed by the instrument developers (see Appendix 22).[2] The composite and 11 subdomain scores deriving from the NEI-VFQ-25 will be summarised descriptively for each treatment arm. A proportional odds logistic regression model[396,398] will be fitted to the composite scores grouped into a 5-point ordinal scale as the outcome variable: ≥90, 80-89, 70-79, 60-69 and <60. These categories were chosen because blinded preliminary analyses confirmed that the distribution of mean composite scores was negatively skewed. A common odds ratio with a 95% confidence interval will be used to interpret the effect size of allocation to aspirin *versus* placebo and, separately, omega-3 FAs *versus* placebo. The same approach will be used to analyse the 11 subdomains. A proportional odds logistic regression model will be fitted to subdomain scores grouped into the ordinal scales shown in Table 29 as the outcome variable. Groupings were based on a blinded preliminary assessment of the subdomain score distributions. If the proportional odds assumption is violated, separate odds ratios between active and placebo groups at each response level will not be presented to avoid multiple comparisons of the same data.

Table 29 Composite and Vision-Targeted Subdomain Scoring Categories

NEI-VFQ-25 Component	Ordinal scale
Composite score	≥90, 80-89, 70-79, 60-69 and <60
Subdomain score	
General Vision	100,80-99,60-79 and <60
Ocular Pain	≥90,80-89, 70-79,60-69 and <60
Near Activities	100, 90-99, 80-89, 70-79 and <70
Distance Activities	100, 90-99, 80-89, 70-79 and <70
Social Functioning	≥90, 80-89, 70-79 and <70
Mental Health	≥90, 80-89, 70-79, 60-69 and <60
Role Dependency	≥90, 80-89, 70-79 and <70
Vision-Specific Dependency	100, <100
Driving	100, 80-99, 60-79 and <60
Colour Vision	100,75-99 and <75
Peripheral Vision	100,75-99 and <75

3.4.5.10 Exploratory Analyses of Other Eye Events

The first occurrences of other eye events listed in Appendix 18 will be summarised descriptively by group in each treatment arm. No inferential testing of these is planned.

3.4.5.11 Multiplicity

Testing multiple hypotheses simultaneously increases the likelihood of a result being due to chance alone.[394,404] A two-tailed P value (2P) of less than 0.05 will be considered to indicate statistical significance for the primary efficacy and safety outcomes. No adjustment for multiplicity shall be made for the secondary or tertiary analyses. The results from these analyses will be interpreted cautiously and in the context of existing studies. A true effect of treatment allocation will be considered to be more likely if the comparisons are based on a large number of events and if the upper and lower confidence intervals are further away from zero (which would be associated with a more extreme P value).[394,404]

3.4.5.12 Sensitivity Analyses

3.4.5.12.1 Ordinal Logistic Regression of Final Retinopathy Grades in those with Baseline Eye Screening Records (Cohort B6)

A sensitivity analysis is planned to assess whether restricting comparisons of final retinopathy grade to those with a baseline record impacts the results of this tertiary endpoint (cohort B6; a subset of cohort B4). As described in Section 3.4.5.9.1, a proportional odds logistic regression model[396,398], will be applied to duplex final retinopathy grades grouped into a 4-point ordinal scale. A common odds ratio with a 95% confidence interval will be used to estimate the average effect size over the total ordinal scale, caused by allocation to aspirin *versus* placebo and, separately, omega-3 FAs *versus* placebo. If the proportional odds assumption is violated, separate odds ratios between active and placebo groups at each response level will not be presented, to avoid multiple comparisons of the same data.

3.4.5.12.2 Ordinal Logistic Regression of the Highest Retinopathy Grade Reached (Cohort B7)

A sensitivity analysis is planned to assess whether analyses of the highest retinopathy grade reached impact the result of the tertiary endpoint analysis of final retinopathy grades. As described in Section 3.4.5.9.1, a proportional odds logistic regression model[396,398], will be applied to duplex final retinopathy grades grouped into a 4-point ordinal scale. A common odds ratio with a 95% confidence interval will be used to estimate the average effect size over the total ordinal scale, caused by allocation to aspirin

versus placebo and, separately, omega-3 FAs *versus* placebo. This analysis will be restricted to those with retinopathy data in both eyes post-randomisation (cohort B7). If the proportional odds assumption is violated, separate odds ratios between active and placebo groups at each response level will not be presented, to avoid multiple comparisons of the same data.

Tabulations demonstrating the completeness of longitudinal DESP data and correlations between this and the number of screening assessments and endpoint events will be produced in conjunction with these analyses.

3.4.5.12.3 Effect of Adjudication on Event Categorisation

To interpret the impact of adjudication on secondary efficacy, tertiary and safety outcomes, and to help understand the validity of including unadjudicated events derived from the VFQ in some analyses, a contingency table shall be produced, and both the percentage agreement and Cohen's κ tests of agreement shall be performed between the pre-adjudicated and post-adjudication outcome categorisation of the first reports of the following events:[414-416]

- Any eye bleed
- Retinal detachment
- Diabetic macular oedema
- Age-related macular degeneration
- Blindness
- Visual deterioration

Read codes relevant to the post-adjudication categorisation of each of these events are presented in Appendix 23.

The percentage agreement will be calculated as the number of agreed-upon diagnoses divided by the total number of adjudicated first events. Estimates of the kappa statistic and their 95% confidence intervals will be calculated to account for chance agreement.[417] These analyses shall be restricted to those events that were either fully or partially assessed based on the sufficiency flags recorded during

adjudication (see Section 3.2.1.1.2) and where the source of information is the ASCEND follow-up questionnaire[*]. These restrictions, along with the re-categorisation and identification of unreported events during the adjudication process, will mean that the number and order of first events by type change. Therefore, the figures presented for these analyses may differ from the main results.

3.4.5.13 Tests for Heterogeneity and Trends in Effect

A limited number of AMD events are expected in the ASCEND-Eye study due to the relatively young age of participants in ASCEND (mean baseline age = 63.3; SD 9.2).[11] It is known that chance alone may result in differences in the treatment effect size on particular outcomes if comparisons are made in smaller subgroups.[394,404,418] Therefore, heterogeneity of the proportional effect of the ASCEND medications on AMD events in subgroups of baseline characteristics will not be formally tested.[419] However, event rates will be summarised descriptively, by group in each treatment arm, in the following categories[†]: (*Analysis cohorts A and C)*

- Age: <60 years, ≥60<70 years and ≥70 years
- Sex: male, female
- Smoking status: current, former, never, unknown

3.4.5.14 Handling Missing Data

The following rules will be applied to categorise participants into subgroups of the baseline characteristics where the information about that variable is unknown.

- Where a substantial number of participants have missing or unknown information on the variable, a subgroup of "Unknown" should be analysed separately.

[*] Most first events were reported on ASCEND follow-up questionnaires. Participants could also report events by telephoning the central coordinating office, but a clinician recorded these after clarifying what happened to them during the call. Thus, events that were phoned in may have been more accurately recorded than events reported on in-trial follow-up questionnaires or the VFQ without the same assistance. In total, 22 additional events, including seven first event reports that were phoned in, are excluded from the analyses described in Section 4.10.
[†] These represent the strongest risk factors for the development of AMD, as described in Section 2.1.5.

- Where only a few participants have missing information, those with missing information should be counted in the median category for continuous variables and the modal group for categorical variables.

The same rules were applied in the ASCEND trial analyses.[11]

3.4.5.14.1 Partial Dates

The same rules regarding partial dates that were applied to ASCEND will apply to ASCEND-Eye.[11]

- Where there is incomplete information for the day, for both serious and non-serious adverse events, the day should be assigned as the 15th day of the month unless the month and year were the same as the month and year of randomisation, in which case, the mid-point between the date of randomisation and end of the month was to be used.

- Where there is incomplete information for the day and month, the missing day will be imputed as described above, and the missing month will be imputed as June. Next, replacement data will be used according to the worst-case scenario:

    - In the case of missing data from a safety variable (e.g. eye bleeding events), if the replaced start date is prior to randomisation, the start date will be set to the date of randomisation (i.e. it will be considered a post-randomisation event).
    - In the case of missing data for efficacy outcomes, if the replaced date is prior to randomisation, the start date will be set to the day before randomisation (i.e. it will be considered a pre-randomisation event).

- Where information is missing for the day, month and year, replacement data will be used according to the worst-case scenario described above.

Where the same type of event has been reported during the main ASCEND trial follow-up and on the VFQ form, the 'first event' used for the analyses shall be the earliest occurrence confirmed by adjudication. If an event reported during the main ASCEND trial did not undergo adjudication, the first event shall be regarded as the earliest occurrence reported by either source.

3.4.6 Presentation of the Results

Results from the descriptive analyses will be shown to one decimal place unless otherwise appropriate. Ratios derived from the inferential analyses will be shown to two decimal places. A conventional approach of rounding numbers ending in 0-4 down and numbers ending in 5-9 up will be used to present the results. Rounding will occur as the last step in producing tables and figures; it shall not be used in intermediate calculations.

The formatting of probability (P) values will follow the New England Journal of Medicine statistical reporting guidelines[420]:

- Where $P>0.01$, show two decimal places unless the rounded P value is 0.05, in which case also show the third decimal place.
- Where $P\leq0.01$ and $P\geq0.001$, show three decimal places.
- Where $P<0.001$, display "$P<0.001$".

3.4.7 Analysis Procedures

Analyses were conducted using SAS version 9.4 (SAS Institute) and R version 4.1.2 (R Core Team 2021-11-01) statistical software.

3.5 Chapter Summary

This chapter described the research methodologies chosen to meet ASCEND-Eye's research aims. The results of the applied analyses are described in the next chapter.

Chapter IV - Results

Chapter IV begins with a presentation of the baseline characteristics, completeness of follow-up, and adherence of participants in each of the three analysis cohorts. These are followed by results of the between-treatment comparisons and sensitivity analyses that were defined in Chapter III.

4.1 Baseline Characteristics

4.1.1 Cohort A – Full ASCEND Population

Between June 2005 through July 2011, 15,480 British and Northern Irish men and women were randomised into ASCEND (Figures 19 and 20). Table 30 summarises the baseline characteristics of this population. They had a mean age of 63.3 years (SD 9.2), 62.6% were male, 96.5% were White, and they had been diagnosed with diabetes (94.1% type 2) for a median of 7 years before joining the study. Overall, 16.3% managed their diabetes by diet alone, 58.3% used an oral hypoglycaemic agent, but not insulin, and 25.4% took insulin (with or without other hypoglycaemic therapies). At baseline, most (82.2%) were overweight or obese, 61.6% reported that they were on medication for hypertension, and just over half (53.8%) had ever smoked cigarettes. Among those for whom biochemical results were available, only one-third (33.3%) achieved an HbA1c target of <6.5% (48 mmol/mol); the rest had HbA1c results that were higher. Meanwhile, approximately half (46.4%) had total cholesterol levels less than 4 mmol/l, and 75.3% reported taking statin therapy. Overall, there was minimal retinal or renal microvascular disease, and the majority were relatively affluent, based on the distribution of their Townsend Deprivation Index scores. The baseline characteristics between the treatment groups in each arm were well-balanced for factors associated with an increased risk of AMD, including age, smoking status and sex.

Figure 19 Consort Diagram for the Full ASCEND Population (Cohort A) – Aspirin Arm

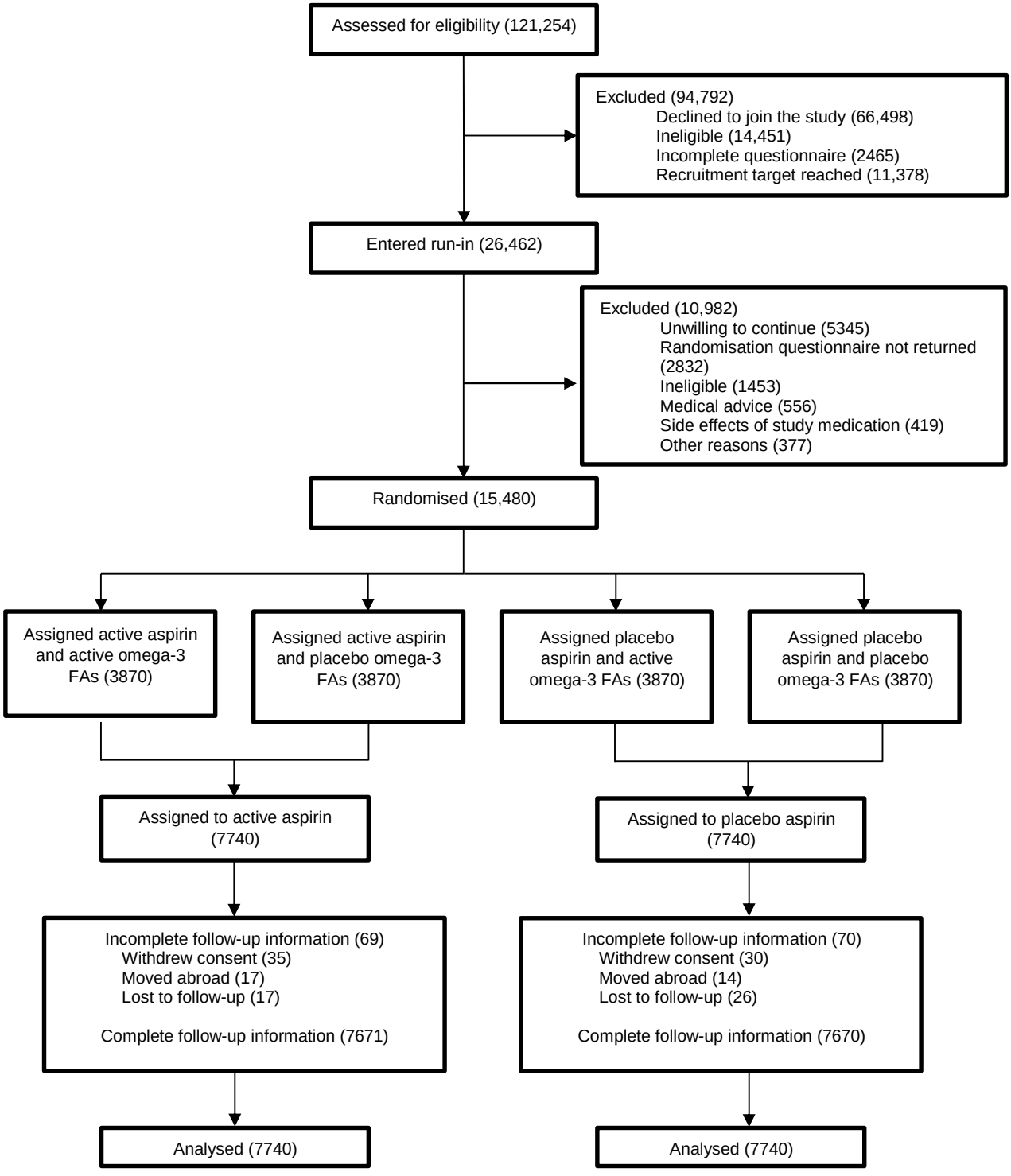


Figure 20 Consort Diagram for the Full ASCEND Population (Cohort A) - Omega-3 Fatty Acids Arm

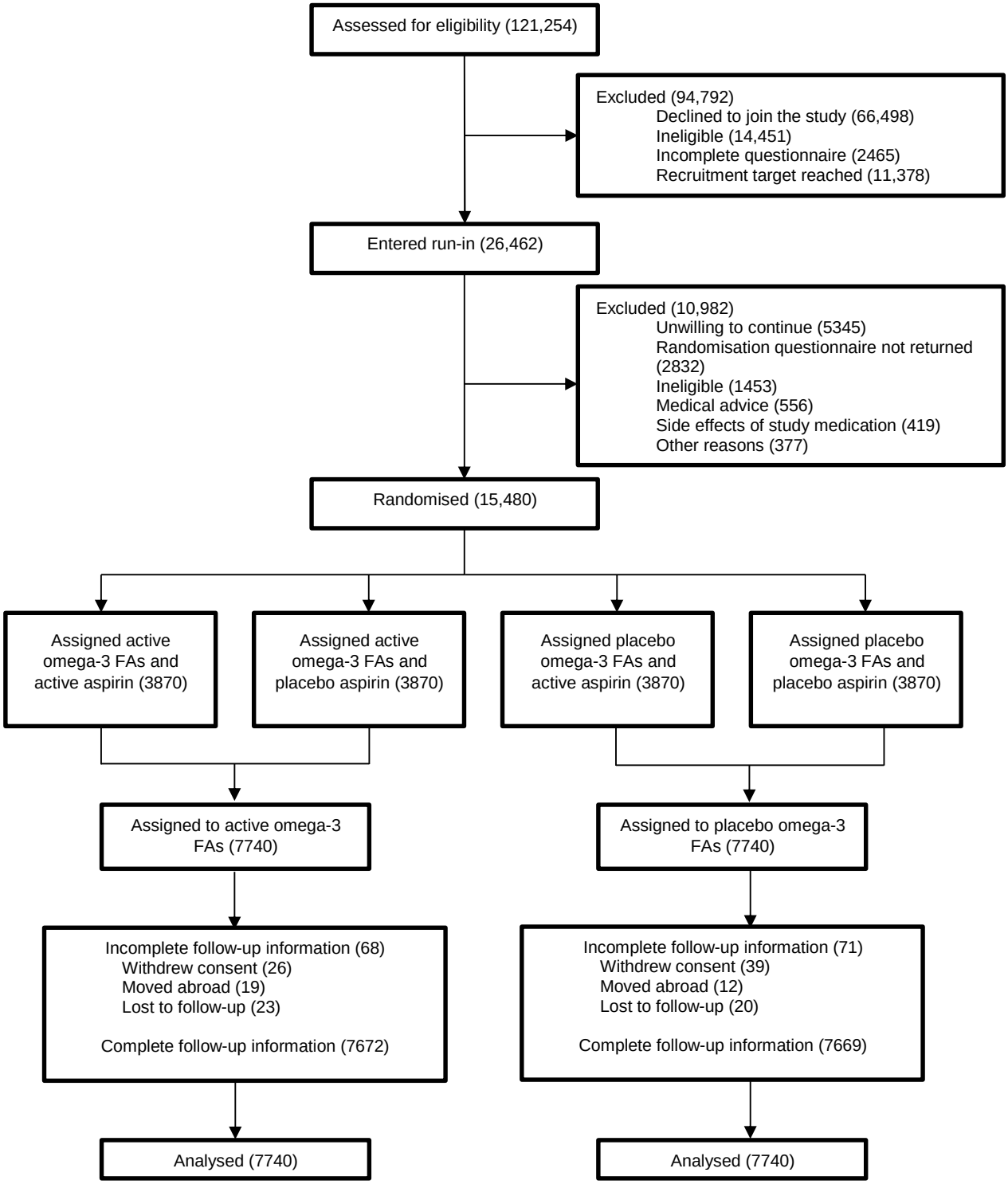


Table 30 Baseline Characteristics of the Full ASCEND Population (Cohort A)

Baseline Characteristic	Aspirin Arm		Omega-3 FAs Arm		Overall
	Active (n=7740)	Placebo (n=7740)	Active (n=7740)	Placebo (n=7740)	(n=15,480)
Age at randomisation (years)					
Mean (SD)	63.2±9.2	63.3±9.2	63.3±9.2	63.3±9.2	63.3±9.2
<60	2795 (36.1%)	2795 (36.1%)	2791 (36.1%)	2799 (36.2%)	5590 (36.1%)
≥60<70	3123 (40.3%)	3124 (40.4%)	3127 (40.4%)	3120 (40.3%)	6247 (40.4%)
≥70	1822 (23.5%)	1821 (23.5%)	1822 (23.5%)	1821 (23.5%)	3643 (23.5%)
Sex					
Male	4843 (62.6%)	4841 (62.5%)	4842 (62.6%)	4842 (62.6%)	9684 (62.6%)
Female	2897 (37.4%)	2899 (37.5%)	2898 (37.4%)	2898 (37.4%)	5796 (37.4%)
Type of diabetes*					
Type 1	458 (5.9%)	453 (5.9%)	460 (5.9%)	451 (5.8%)	911 (5.9%)
Type 2	7282 (94.1%)	7287 (94.1%)	7280 (94.1%)	7289 (94.2%)	14569 (94.1%)
Duration of diabetes (years)					
Median (IQR)	7(3-13)	7 (3-13)	7 (3-12)	7(3-13)	7(3-13)
≥0<5 years	2446 (31.6%)	2445 (31.6%)	2443 (31.6%)	2448 (31.6%)	4891 (31.6%)
≥5<10 years	2166 (28.0%)	2168 (28.0%)	2167 (28.0%)	2167 (28.0%)	4334 (28.0%)
≥10<20 years	1767 (22.8%)	1770 (22.9%)	1770 (22.9%)	1767 (22.8%)	3537 (22.8%)
≥20 years	934 (12.1%)	928 (12.0%)	932 (12.0%)	930 (12.0%)	1862 (12.0%)
Unknown	427 (5.5%)	429 (5.5%)	428 (5.5%)	428 (5.5%)	856 (5.5%)
Diabetes management					
Diet only	1271 (16.4%)	1258 (16.3%)	1229 (15.9%)	1300 (16.8%)	2529 (16.3%)
Oral hypoglycaemic agent(s) only	4510 (58.3%)	4510 (58.3%)	4533 (58.6%)	4487 (58.0%)	9020 (58.3%)
Insulin +/-oral hypoglycaemic agent(s)	1959 (25.3%)	1972 (25.5%)	1978 (25.6%)	1953 (25.2%)	3931 (25.4%)
Participant-reported diabetic retinopathy					
Yes	1526 (19.7%)	1497 (19.3%)	1549 (20.0%)	1474 (19.0%)	3023 (19.5%)
No	6144 (79.4%)	6169 (79.7%)	6121 (79.1%)	6192 (80.0%)	12313 (79.5%)
Unknown	70 (0.9%)	74 (1.0%)	70 (0.9%)	74 (1.0%)	144 (0.9%)
Participant-reported treatment for hypertension					
Yes	4766 (61.6%)	4767 (61.6%)	4768 (61.6%)	4765 (61.6%)	9533 (61.6%)
No	2919 (37.7%)	2916 (37.7%)	2917 (37.7%)	2918 (37.7%)	5835 (37.7%)
Unknown	55 (0.7%)	57 (0.7%)	55 (0.7%)	57 (0.7%)	112 (0.7%)
Systolic blood pressure (mmHg) †					
Mean (SD)	136.1±15.2	136.2±15.3	136.2±15.4	136.2±15.1	136.2±15.3
<130	1694 (21.9%)	1700 (22.0%)	1695 (21.9%)	1699 (22.0%)	3394 (21.9%)
≥130<140	1550 (20.0%)	1541 (19.9%)	1547 (20.0%)	1544 (19.9%)	3091 (20.0%)
≥140	2263 (29.2%)	2292 (29.6%)	2279 (29.4%)	2276 (29.4%)	4555 (29.4%)
Unknown	2233 (28.9%)	2207 (28.5%)	2219 (28.7%)	2221 (28.7%)	4440 (28.7%)
Diastolic blood pressure (mmHg) †					
Mean (SD)	77.0±9.4	77.2±9.5	77.1±9.5	77.1±9.5	77.1±9.5
<75	2113 (27.3%)	2110 (27.3%)	2097 (27.1%)	2126 (27.5%)	4223 (27.3%)
≥75 <85	2325 (30.0%)	2294 (29.6%)	2331 (30.1%)	2288 (29.6%)	4619 (29.8%)
≥85	1066 (13.8%)	1125 (14.5%)	1089 (14.1%)	1102 (14.2%)	2191 (14.2%)
Unknown	2236 (28.9%)	2211 (28.6%)	2223 (28.7%)	2224 (28.7%)	4447 (28.7%)
Body mass index (kg/m^2) ‡					
Mean (SD)	30.8±6.3	30.6±6.3	30.7±6.3	30.8±6.2	30.7±6.3
<25	1080 (14.0%)	1169 (15.1%)	1129 (14.6%)	1120 (14.5%)	2249 (14.5%)
≥25, <30	2753 (35.6%)	2776 (35.9%)	2771 (35.8%)	2758 (35.6%)	5529 (35.7%)
≥30<35	2150 (27.8%)	2090 (27.0%)	2131 (27.5%)	2109 (27.2%)	4240 (27.4%)
≥35	1515 (19.6%)	1446 (18.7%)	1453 (18.8%)	1508 (19.5%)	2961 (19.1%)
Unknown	242 (3.1%)	259 (3.3%)	256 (3.3%)	245 (3.2%)	501 (3.2%)

Baseline Characteristic	Aspirin Arm		Omega-3 FAs Arm		Overall (n=15,480)
	Active (n=7740)	Placebo (n=7740)	Active (n=7740)	Placebo (n=7740)	
Cigarette smoking					
Current	639 (8.3%)	640 (8.3%)	639 (8.3%)	640 (8.3%)	1279 (8.3%)
Former	3526 (45.6%)	3525 (45.5%)	3527 (45.6%)	3524 (45.5%)	7051 (45.5%)
Never	3489 (45.1%)	3488 (45.1%)	3489 (45.1%)	3488 (45.1%)	6977 (45.1%)
Unknown	86 (1.1%)	87 (1.1%)	85 (1.1%)	88 (1.1%)	173 (1.1%)
Non-study medication					
ACE-inhibitor or ARB	4520 (58.4%)	4535 (58.6%)	4569 (59.0%)	4486 (58.0%)	9055 (58.5%)
Aspirin use before screening	2740 (35.4%)	2768 (35.8%)	2744 (35.5%)	2764 (35.7%)	5508 (35.6%)
Thiazide or related diuretic	1480 (19.1%)	1477 (19.1%)	1448 (18.7%)	1509 (19.5%)	2957 (19.1%)
Calcium channel blocker	1926 (24.9%)	1847 (23.9%)	1912 (24.7%)	1861 (24.0%)	3773 (24.4%)
Statin	5854 (75.6%)	5799 (74.9%)	5791 (74.8%)	5862 (75.7%)	11653 (75.3%)
Total cholesterol (mmol/L)					
Mean (SD)	4.2±0.9	4.2±0.9	4.2±0.9	4.2±0.9	4.2±0.9
<4	2293 (29.6%)	2261 (29.2%)	2284 (29.5%)	2270 (29.3%)	4554 (29.4%)
≥4 <5	1875 (24.2%)	1920 (24.8%)	1900 (24.5%)	1895 (24.5%)	3795 (24.5%)
≥5	735 (9.5%)	735 (9.5%)	745 (9.6%)	725 (9.4%)	1470 (9.5%)
Not available	2837 (36.7%)	2824 (36.5%)	2811 (36.3%)	2850 (36.8%)	5661 (36.6%)
HDL cholesterol (mmol/L)					
Mean (SD)	1.3±0.4	1.3±0.4	1.3±0.4	1.3±0.4	1.3±0.4
<1	1116 (14.4%)	1054 (13.6%)	1104 (14.3%)	1066 (13.8%)	2170 (14.0%)
≥1<1.5	2708 (35.0%)	2815 (36.4%)	2775 (35.9%)	2748 (35.5%)	5523 (35.7%)
≥1.5	1072 (13.9%)	1035 (13.4%)	1036 (13.4%)	1071 (13.8%)	2107 (13.6%)
Not available	2844 (36.7%)	2836 (36.6%)	2825 (36.5%)	2855 (36.9%)	5680 (36.7%)
Non-HDL cholesterol (mmol/L)					
Mean (SD)	2.9±0.9	2.9±0.8	2.9±0.9	2.9±0.8	2.9±0.8
<2.5	1691 (21.8%)	1699 (22.0%)	1689 (21.8%)	1701 (22.0%)	3390 (21.9%)
≥2.5 <3.5	2213 (28.6%)	2180 (28.2%)	2200 (28.4%)	2193 (28.3%)	4393 (28.4%)
≥3.5	992 (12.8%)	1025 (13.2%)	1026 (13.3%)	991 (12.8%)	2017 (13.0%)
Not available	2844 (36.7%)	2836 (36.6%)	2825 (36.5%)	2855 (36.9%)	5680 (36.7%)
Glycosylated haemoglobin - HbA1c					
IFCC (mmol/mol) mean (SD)	54.7±12.9	54.9±12.9	54.9±13.0	54.7±12.8	54.8±12.9
DCCT (%) mean (SD)	7.2±1.2	7.2±1.2	7.2±1.2	7.2±1.2	7.2±1.2
<48 (6.5)	1661 (21.5%)	1611 (20.8%)	1631 (21.1%)	1641 (21.2%)	3272 (21.1%)
≥48 (6.5), <64 (8.0)	2314 (29.9%)	2350 (30.4%)	2333 (30.1%)	2331 (30.1%)	4664 (30.1%)
≥64 (8.0)	926 (12.0%)	951 (12.3%)	960 (12.4%)	917 (11.8%)	1877 (12.1%)
Not available	2839 (36.7%)	2828 (36.5%)	2816 (36.4%)	2851 (36.8%)	5667 (36.6%)
CKD-EPI estimated GFR (ml/min/1.73m^2) §					
Mean (SD)	85.2±21.1	85.2±21.1	85.2±21.3	85.2±20.8	85.2±21.1
≥90	2265 (29.3%)	2258 (29.2%)	2256 (29.1%)	2267 (29.3%)	4523 (29.2%)
≥60<90	1986 (25.7%)	2030 (26.2%)	2032 (26.3%)	1984 (25.6%)	4016 (25.9%)
<60	649 (8.4%)	627 (8.1%)	640 (8.3%)	636 (8.2%)	1276 (8.2%)
Not available	2840 (36.7%)	2825 (36.5%)	2812 (36.3%)	2853 (36.9%)	5665 (36.6%)
Urinary albumin:creatinine ratio (mg/mmol)¶					
Median (IQR)	0.56 (0.00-1.33)	0.55 (0.18-1.34)	0.56 (0.18-1.36)	0.55 (0.14-1.32)	0.55 (0.16-1.34)
<3	4267 (55.1%)	4259 (55.0%)	4253 (54.9%)	4273 (55.2%)	8526 (55.1%)
≥3	621 (8.0%)	627 (8.1%)	657 (8.5%)	591 (7.6%)	1248 (8.1%)
Not available	2852 (36.8%)	2854 (36.9%)	2830 (36.6%)	2876 (37.2%)	5706 (36.9%)
Townsend Deprivation Index**					
<-3	2502 (32.3%)	2602 (33.6%)	2573 (33.2%)	2531 (32.7%)	5104 (33.0%)
≥-3<0	3023 (39.1%)	2999 (38.7%)	2954 (38.2%)	3068 (39.6%)	6022 (38.9%)
≥0<2	1037 (13.4%)	1000 (12.9%)	1055 (13.6%)	982 (12.7%)	2037 (13.2%)
≥2<4	686 (8.9%)	629 (8.1%)	654 (8.4%)	661 (8.5%)	1315 (8.5%)
≥4<6	351 (4.5%)	352 (4.5%)	348 (4.5%)	355 (4.6%)	703 (4.5%)
≥6	121 (1.6%)	140 (1.8%)	137 (1.8%)	124 (1.6%)	261 (1.7%)
Unknown	20 (0.3%)	18 (0.2%)	19 (0.2%)	19 (0.2%)	38 (0.2%)

Baseline Characteristic	Aspirin Arm				Omega-3 FAs Arm				Overall (n=15,480)	
	Active (n=7740)		Placebo (n=7740)		Active (n=7740)		Placebo (n=7740)			
Ethnic origin										
White	7467	(96.5%)	7468	(96.5%)	7467	(96.5%)	7468	(96.5%)	14935	(96.5%)
Indian/Pakistani/Bangladeshi	91	(1.2%)	93	(1.2%)	92	(1.2%)	92	(1.2%)	184	(1.2%)
African/Caribbean	70	(0.9%)	70	(0.9%)	70	(0.9%)	70	(0.9%)	140	(0.9%)
Other/unknown	112	(1.4%)	109	(1.4%)	111	(1.4%)	110	(1.4%)	221	(1.4%)

DCCT = Diabetes Control and Complications Trial; GFR = Glomerular Filtration Rate; HDL=High-density lipoprotein; IFCC =International Federation of Clinical Chemistry; SD = Standard Deviation; IQR = Interquartile range
Figures presented are counts with percentages unless otherwise stated. Percentages may not total 100 because of rounding.
*The presence of type 2 diabetes was based on a broad clinical definition involving the participant's age when diagnosed with diabetes, the use of insulin within one year after diagnosis, and the body-mass index.
† From blood and urine consent forms, generally before randomisation.
‡ The body-mass index (the weight in kilograms divided by the square of the height in metres) was based on values for height and weight the participants reported on their randomisation questionnaires.
§ Calculated from blood cystatin c concentration using the CKD-EPI formula.[393]
¶There was an analysis rule in ASCEND which stated that those with a below detectable threshold albumin component of their urinary albumin:creatinine ratio would be recorded as zero. This applied to just over 25% of participants in the active group of the aspirin arm, and to just under 25% of participants in the placebo group. Hence the interquartile range included zero.
**Townsend scores of 0 represent an area with a UK-average level of deprivation, positive values indicate areas of above-average deprivation, and negative values indicate areas of below-average deprivation.

4.1.2 Cohort B1 and its Subsets – DESP-Linked Population

Figures 21 and 22 depict the flow of participants included in analyses involving the B1 cohort in the aspirin arm and omega-3 FAs arm, respectively. The first linked dataset, from the North East London Trust-controlled DESP, was received on 22nd March 2019. Linked datasets were then received from PHW on 25th October 2019, Northgate Ltd from 9th June 2020 through 2nd July 2020, and finally, from Health Intelligence Ltd on 8th October 2021. Out of 15,480 randomised ASCEND participants, 1252 (8.1%) attended a GP practice that did not register their data with PHE, 323 (2.1%) resided in Scotland, 183 (1.2%) lived in Northern Ireland, 60 (0.4%) withdrew their consent during the in-trial period, and six (0.04%) had no NHS number. Among the remaining 13,656 participants who were eligible to take part in the linkage activity, 2411 (17.7%) attended one of 18 Trust-controlled DESPs that never responded to several invitations to collaborate, 2516 (18.4%) attended one of 12 DESPs that had been willing to collaborate but suspended the processing of all non-Covid-19 research applications in March 2020, and 621 (4.5%) attended one Trust-controlled DESP that declined to collaborate.

Identifiers were sent to be linked for 8108 participants, who were thought to attend one of 28 collaborating DESPs. Table 31 summarises the DESP collaboration based on the location of their primary care provider. Of those whose identifiers were shared, 7535 were successfully matched (92.9% match rate), among whom, 175 (2.3%) were excluded because their records were outside the scheduled

treatment period of ASCEND. This left 7360 participants who were included in the primary efficacy endpoint analyses (cohort B1). Table 32 summarises the baseline characteristics of those individuals. Their mean age was 63.5 years (SD 8.9), 62.4% were male, and they were diagnosed with diabetes (94.9% type 2) a median of 6 years before randomisation. Overall, 16.5% managed their diabetes by diet alone, 60.8% used an oral hypoglycaemic agent, but not insulin, and 22.8% were insulin-treated (with or without other hypoglycaemic therapies). Of those for whom the data were available, 61.7% reported taking treatment for hypertension, 54.9% were former or current smokers, and 84.9% were overweight or obese. In total, 34.3% reported regular aspirin use before starting ASCEND, which they stopped to take part in the trial[*], 58.1% were prescribed ACE-inhibitor therapy, and 76.0% took a statin. Only 17.8% reported being told by a doctor that they had some form of DR. The baseline characteristics between the treatment groups in each arm were well-balanced for factors associated with an increased risk of DR, including the duration of diabetes, glycaemic control, blood pressure, and urinary albumin:creatinine ratio (where these data were available). Overall, the composition of cohort B1 was representative of the full ASCEND population; however, there was statistically significant heterogeneity between those who were included and excluded for some characteristics (Table 33). Those excluded were marginally younger (mean age 63.0 *vs*. 63.5 years), with a longer duration of diabetes, and a greater proportion had type 1 diabetes. More frequently, they reported having DR at baseline (21.1% *vs.* 17.8%), had less favourable lipid and renal parameters, were non-smokers, or had worse glycaemic control. There were no significant differences in sex, blood pressure, BMI or ethnicity. The standardised differences between every baseline covariate tested were minimal.

Figure 23 is a series of consort diagrams for cohorts B2 to B7, summarising the restrictions made to these analysis subsets. The 1:1 randomisation ratio was preserved in every cohort's aspirin and omega-3 FA arms (Table 34). The composition of clinical and demographic characteristics most associated with retinopathy progression was similar in each linkage subset, except for a slightly smaller proportion of individuals with type 1 diabetes in the cohorts restricted to those with baseline eye screening records (B2, B3, B5 and B6; Table 35).

[*] The dose, frequency and duration of pre-run-in aspirin use was not recorded for ASCEND.

Figure 21 Consort Diagram for the DESP-Linked Population (Cohort B1) - Aspirin Arm

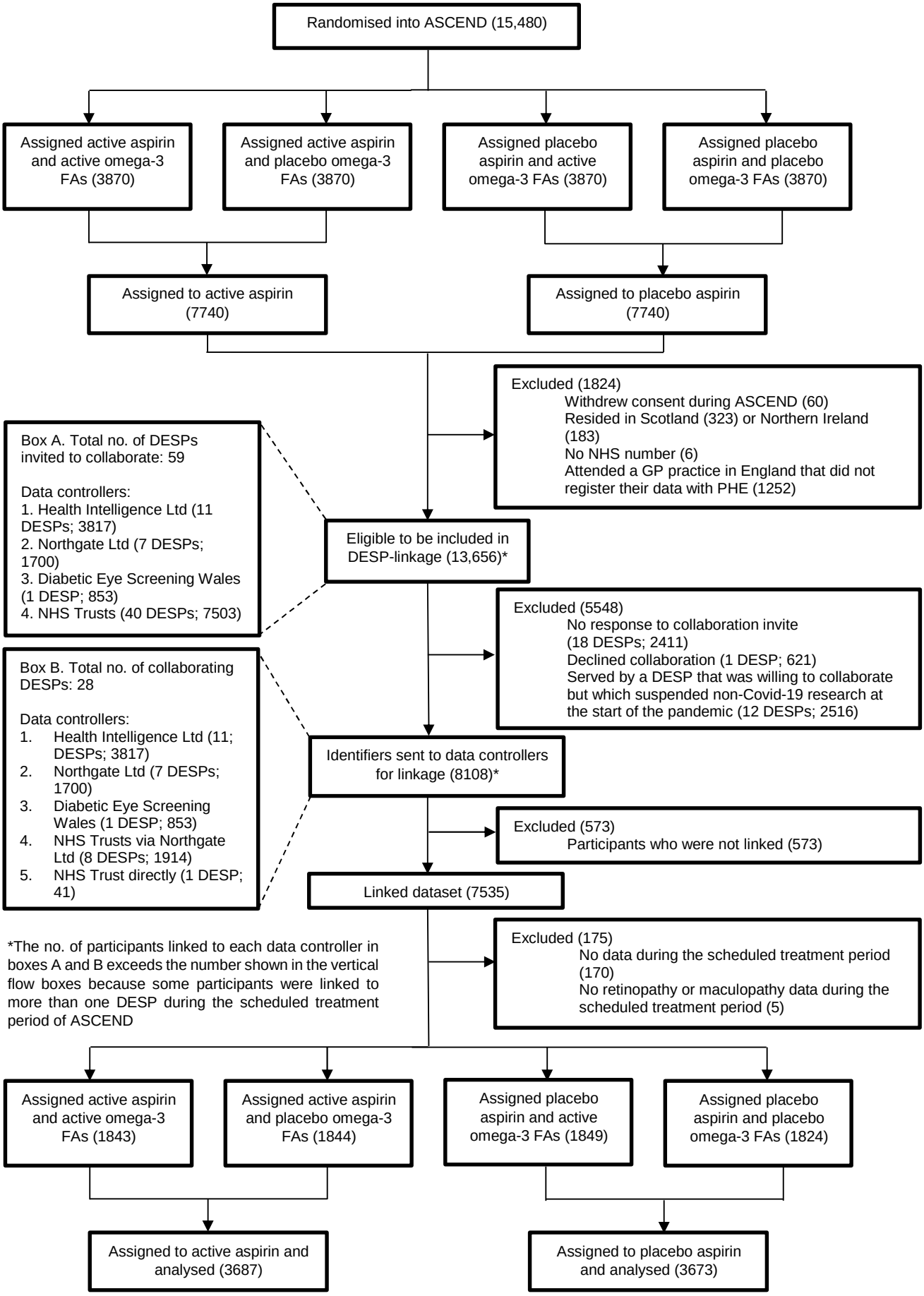


Figure 22 Consort Diagram for the DESP-Linked Population (Cohort B1) - Omega-3 Fatty Acids Arm

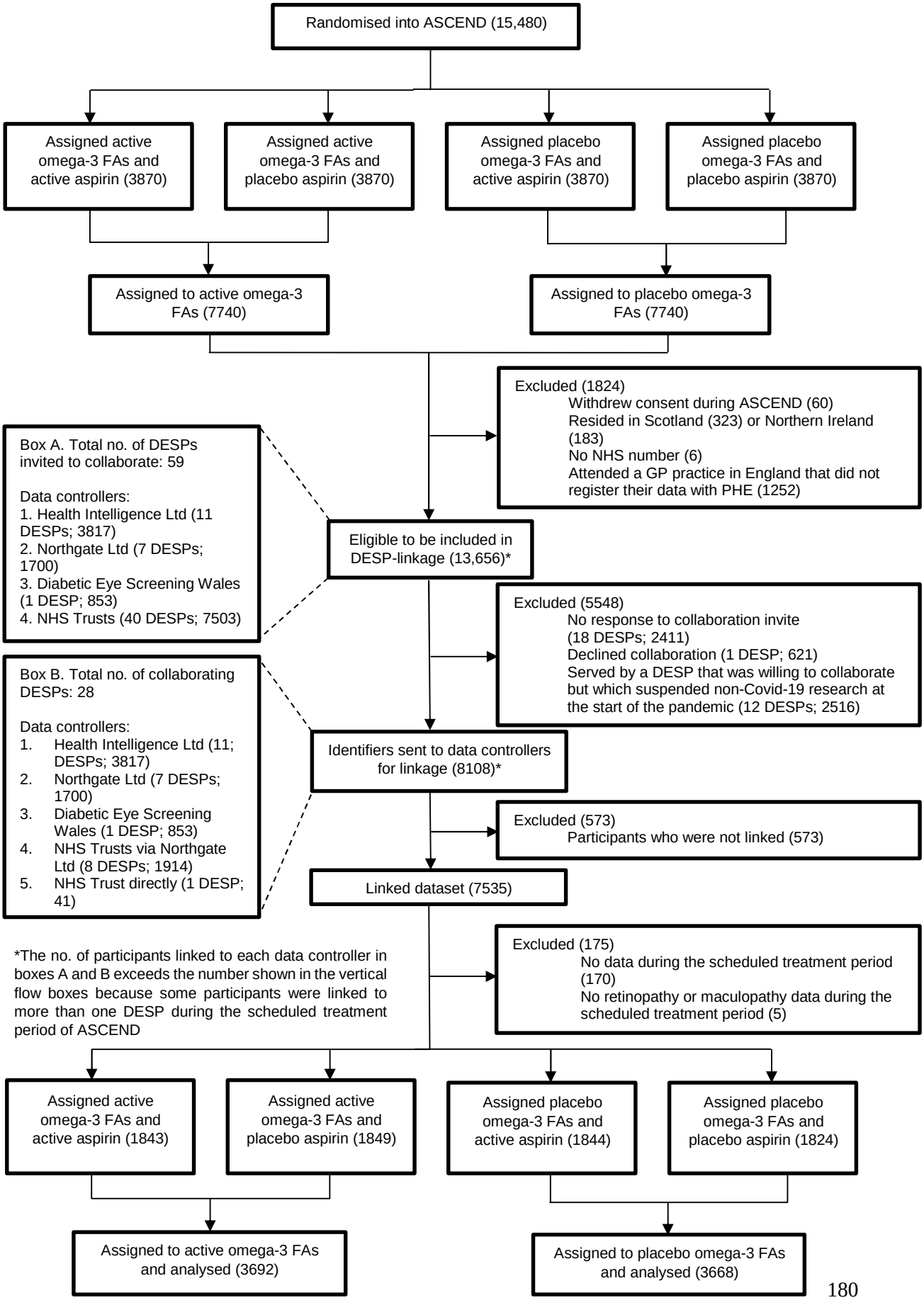


Table 31 Summary of DESP Collaboration

Collaborators				Non-Collaborators		
Trust-controlled DESPs	**Northgate-controlled DESPs**	**Health Intelligence-controlled DESPs**	**Wales**	**Trust-controlled DESPs that were willing to collaborate prior to the Covid-19 pandemic**	**R&D approval denied**	**Unresponsive DESPs**
Cheshire (203) East and North Hertfordshire (67) Greater Nottingham (369) Humber (370) North East London (41) North Nottinghamshire (260) North Yorkshire (320) Oxfordshire (275) South East Sussex (50)	Arden, Herefordshire and Worcestershire (273) Bath, Somerset, Swindon and Wiltshire (213) Central Mersey (25) North of Tyne and Gateshead (401) South West London (189) Surrey (469) West Riding and Craven (130)	Berkshire (136) Bristol and Weston (260) Devon (775) Dorset (117) East Anglia (460) Essex (360) Greater Manchester North (322) Greater Manchester South (128) Hampshire and Isle of Wight (761) Kent and Medway (318) North West London (180)	Diabetic Eye Screening Wales (853)	Birmingham, Solihull and Black Country (111) Brighton and Sussex (95) Derbyshire (747) Doncaster and Bassetlaw (76) Gloucestershire (184) Leicester, Leicestershire and Rutland (122) Norfolk and Norwich (176) Somerset (263) South East London (30) South Tees (148) Staffordshire (339) Shropshire (225)	Leeds and Mid Yorkshire (621)	Barnsley and Rotherham (38) Bedfordshire (44) Buckinghamshire (397) Cornwall (318) County Durham and Darlington (75) Cumbria (91) Lancashire (110) Lincolnshire (189) Liverpool (200) North Central London (37) North Mersey (10) North Tees (7) Northamptonshire (189) Sheffield (288) Sunderland and South Tyneside (146) West Hertfordshire (61) West Sussex (203) Wirral (8)
Total number of programmes						
9	**7**	**11**	**1**	**12**	**1**	**18**
Total number of participants thought to have ever attended each DESP service						
1955	**1700**	**3817**	**853**	**2516**	**621**	**2411**

Figures in parentheses represent the anticipated number of participants who were thought to have attended each DESP during the scheduled treatment period of ASCEND. Some participants were linked to more than one DESP due to changes in the geographical boundaries of each programme or because they moved their general practice. Therefore, the total number of participants across every DESP (n=13,873) exceeds the total number eligible (n=13,656) for the linkage exercise.

Table 32 Baseline Characteristics of the DESP-Linked Population (Cohort B1)

Baseline Characteristic	Aspirin Arm		Omega-3 FAs Arm		Overall
	Active (n=3687)	Placebo (n=3673)	Active (n=3692)	Placebo (n=3668)	(n=7360)
Age at randomisation (years)					
Mean (SD)	63.5±8.9	63.5±9.0	63.5±8.9	63.5±9.0	63.5±8.9
<60	1287 (34.9%)	1287 (35.0%)	1283 (34.8%)	1291 (35.2%)	2574 (35.0%)
≥60<70	1530 (41.5%)	1517 (41.3%)	1535 (41.6%)	1512 (41.2%)	3047 (41.4%)
≥70	870 (23.6%)	869 (23.7%)	874 (23.7%)	865 (23.6%)	1739 (23.6%)
Sex					
Male	2314 (62.8%)	2280 (62.1%)	2301 (62.3%)	2293 (62.5%)	4594 (62.4%)
Female	1373 (37.2%)	1393 (37.9%)	1391 (37.7%)	1375 (37.5%)	2766 (37.6%)
Type of diabetes*					
Type 1	186 (5.0%)	187 (5.1%)	181 (4.9%)	192 (5.2%)	373 (5.1%)
Type 2	3501 (95.0%)	3486 (94.9%)	3511 (95.1%)	3476 (94.8%)	6987 (94.9%)
Duration of diabetes (years)					
Median (IQR)	7 (3-12)	6 (3-12)	7 (3-12)	6 (3-12)	6 (3-12)
≥0<5 years	1185 (32.1%)	1202 (32.7%)	1198 (32.4%)	1189 (32.4%)	2387 (32.4%)
≥5<10 years	1062 (28.8%)	1040 (28.3%)	1048 (28.4%)	1054 (28.7%)	2102 (28.6%)
≥10<20 years	832 (22.6%)	849 (23.1%)	856 (23.2%)	825 (22.5%)	1681 (22.8%)
≥20 years	397 (10.8%)	381 (10.4%)	381 (10.3%)	397 (10.8%)	778 (10.6%)
Unknown	211 (5.7%)	201 (5.5%)	209 (5.7%)	203 (5.5%)	412 (5.6%)
Diabetes management					
Diet only	591 (16.0%)	621 (16.9%)	585 (15.8%)	627 (17.1%)	1212 (16.5%)
Oral hypoglycaemic agents) only	2253 (61.1%)	2219 (60.4%)	2265 (61.3%)	2207 (60.2%)	4472 (60.8%)
Insulin +/- oral hypoglycaemic agent(s)	843 (22.9%)	833 (22.7%)	842 (22.8%)	834 (22.7%)	1676 (22.8%)
Participant-reported diabetic retinopathy					
Yes	662 (18.0%)	646 (17.6%)	674 (18.3%)	634 (17.3%)	1308 (17.8%)
No	2984 (80.9%)	3007 (81.9%)	2988 (80.9%)	3003 (81.9%)	5991 (81.4%)
Unknown	41 (1.1%)	20 (0.5%)	30 (0.8%)	31 (0.8%)	61 (0.8%)
Participant-reported treatment for hypertension					
Yes	2252 (61.1%)	2250 (61.3%)	2265 (61.3%)	2237 (61.0%)	4502 (61.2%)
No	1408 (38.2%)	1390 (37.8%)	1395 (37.8%)	1403 (38.2%)	2798 (38.0%)
Unknown	27 (0.7%)	33 (0.9%)	32 (0.9%)	28 (0.8%)	60 (0.8%)
Systolic blood pressure (mmHg) †					
Mean (SD)	136.1±15.2	136.0±15.3	135.9±15.2	136.1±15.2	136.1±15.2
<130	823 (22.3%)	835 (22.7%)	835 (22.6%)	823 (22.4%)	1658 (22.5%)
≥130<140	744 (20.2%)	760 (20.7%)	744 (20.2%)	760 (20.7%)	1504 (20.4%)
≥140	1109 (30.1%)	1115 (30.4%)	1127 (30.5%)	1097 (29.9%)	2224 (30.2%)
Unknown	1011 (27.4%)	963 (26.2%)	986 (26.7%)	988 (26.9%)	1974 (26.8%)
Diastolic blood pressure (mmHg) †					
Mean (SD)	77.0±9.4	77.2±9.4	77.1±9.3	77.1±9.5	77.1±9.4
<75	1001 (27.1%)	1035 (28.2%)	1022 (27.7%)	1014 (27.6%)	2036 (27.7%)
≥75 <85	1156 (31.4%)	1141 (31.1%)	1162 (31.5%)	1135 (30.9%)	2297 (31.2%)
≥85	518 (14.0%)	531 (14.5%)	519 (14.1%)	530 (14.4%)	1049 (14.3%)
Unknown	1012 (27.4%)	966 (26.3%)	989 (26.8%)	989 (27.0%)	1978 (26.9%)
Body mass index (kg/m^2) ‡					
Mean (SD)	30.7±6.2	30.6±6.2	30.6±6.1	30.7±6.2	30.7±6.2
<25	520 (14.1%)	554 (15.1%)	535 (14.5%)	539 (14.7%)	1074 (14.6%)
≥25, <30	1306 (35.4%)	1327 (36.1%)	1339 (36.3%)	1294 (35.3%)	2633 (35.8%)
≥30<35	1037 (28.1%)	986 (26.8%)	1019 (27.6%)	1004 (27.4%)	2023 (27.5%)
≥35	698 (18.9%)	692 (18.8%)	675 (18.3%)	715 (19.5%)	1390 (18.9%)
Unknown	126 (3.4%)	114 (3.1%)	124 (3.4%)	116 (3.2%)	240 (3.3%)

Baseline Characteristic	Aspirin Arm		Omega-3 FAs Arm		Overall
	Active (n=3687)	Placebo (n=3673)	Active (n=3692)	Placebo (n=3668)	(n=7360)
Cigarette smoking					
Current	281 (7.6%)	296 (8.1%)	294 (8.0%)	283 (7.7%)	577 (7.8%)
Former	1703 (46.2%)	1714 (46.7%)	1728 (46.8%)	1689 (46.0%)	3417 (46.4%)
Never	1656 (44.9%)	1620 (44.1%)	1629 (44.1%)	1647 (44.9%)	3276 (44.5%)
Unknown	47 (1.3%)	43 (1.2%)	41 (1.1%)	49 (1.3%)	90 (1.2%)
Non-study medication					
ACE-inhibitor or ARB	2174 (59.0%)	2102 (57.2%)	2167 (58.7%)	2109 (57.5%)	4276 (58.1%)
Aspirin use before screening	1283 (34.8%)	1244 (33.9%)	1250 (33.9%)	1277 (34.8%)	2527 (34.3%)
Thiazide or related diuretic	676 (18.3%)	684 (18.6%)	673 (18.2%)	687 (18.7%)	1360 (18.5%)
Calcium channel blocker	924 (25.1%)	881 (24.0%)	923 (25.0%)	882 (24.0%)	1805 (24.5%)
Statin	2819 (76.5%)	2777 (75.6%)	2779 (75.3%)	2817 (76.8%)	5596 (76.0%)
Total cholesterol (mmol/L)					
Mean (SD)	4.1±0.9	4.1±0.8	4.1±0.8	4.1±0.9	4.1±0.9
<4	1123 (30.5%)	1124 (30.6%)	1120 (30.3%)	1127 (30.7%)	2247 (30.5%)
≥4 <5	941 (25.5%)	937 (25.5%)	969 (26.2%)	909 (24.8%)	1878 (25.5%)
≥5	318 (8.6%)	331 (9.0%)	314 (8.5%)	335 (9.1%)	649 (8.8%)
Not available	1305 (35.4%)	1281 (34.9%)	1289 (34.9%)	1297 (35.4%)	2586 (35.1%)
HDL cholesterol (mmol/L)					
Mean (SD)	1.3±0.4	1.3±0.3	1.3±0.4	1.3±0.4	1.3±0.4
<1	538 (14.6%)	474 (12.9%)	505 (13.7%)	507 (13.8%)	1012 (13.8%)
≥1<1.5	1318 (35.7%)	1415 (38.5%)	1373 (37.2%)	1360 (37.1%)	2733 (37.1%)
≥1.5	522 (14.2%)	496 (13.5%)	516 (14.0%)	502 (13.7%)	1018 (13.8%)
Not available	1309 (35.5%)	1288 (35.1%)	1298 (35.2%)	1299 (35.4%)	2597 (35.3%)
Non-HDL cholesterol (mmol/L)					
Mean (SD)	2.9±0.8	2.9±0.8	2.9±0.8	2.9±0.8	2.9±0.8
<2.5	827 (22.4%)	845 (23.0%)	831 (22.5%)	841 (22.9%)	1672 (22.7%)
≥2.5 <3.5	1098 (29.8%)	1072 (29.2%)	1106 (30.0%)	1064 (29.0%)	2170 (29.5%)
≥3.5	453 (12.3%)	468 (12.7%)	457 (12.4%)	464 (12.6%)	921 (12.5%)
Not available	1309 (35.5%)	1288 (35.1%)	1298 (35.2%)	1299 (35.4%)	2597 (35.3%)
Glycosylated haemoglobin - HbA1c					
IFCC (mmol/mol) mean (SD)	54.3±12.9	54.3±12.5	54.4±12.6	54.2±12.7	54.3±12.7
DCCT (%) mean (SD)	7.1±1.2	7.1±1.2	7.1±1.2	7.1±1.2	7.1±1.2
<48 (6.5)	841 (22.8%)	799 (21.8%)	807 (21.9%)	833 (22.7%)	1640 (22.3%)
≥48 (6.5), <64 (8.0)	1123 (30.5%)	1157 (31.5%)	1172 (31.7%)	1108 (30.2%)	2280 (31.0%)
≥64 (8.0)	418 (11.3%)	435 (11.8%)	424 (11.5%)	429 (11.7%)	853 (11.6%)
Not available	1305 (35.4%)	1282 (34.9%)	1289 (34.9%)	1298 (35.4%)	2587 (35.1%)
CKD-EPI estimated GFR (ml/min/1.73m^2) §					
Mean (SD)	85.0±20.6	84.3±20.7	84.7±20.7	84.7±20.6	84.7±20.6
≥90	1082 (29.3%)	1047 (28.5%)	1058 (28.7%)	1071 (29.2%)	2129 (28.9%)
≥60<90	994 (27.0%)	1024 (27.9%)	1042 (28.2%)	976 (26.6%)	2018 (27.4%)
<60	304 (8.2%)	320 (8.7%)	302 (8.2%)	322 (8.8%)	624 (8.5%)
Not available	1307 (35.4%)	1282 (34.9%)	1290 (34.9%)	1299 (35.4%)	2589 (35.2%)
Urinary albumin:creatinine ratio (mg/mmol)¶					
Median (IQR)	0.56 (0.00-1.29)	0.54 (0.18-1.27)	0.55 (0.17-1.29)	0.54 (0.00-1.26)	0.54 (0.16-1.28)
<3	2087 (56.6%)	2105 (57.3%)	2099 (56.9%)	2093 (57.1%)	4192 (57.0%)
≥3	290 (7.9%)	282 (7.7%)	300 (8.1%)	272 (7.4%)	572 (7.8%)
Not available	1310 (35.5%)	1286 (35.0%)	1293 (35.0%)	1303 (35.5%)	2596 (35.3%)
Townsend Deprivation Index**					
<-3	1217 (33.0%)	1289 (35.1%)	1262 (34.2%)	1244 (33.9%)	2506 (34.0%)
≥-3<0	1474 (40.0%)	1440 (39.2%)	1436 (38.9%)	1478 (40.3%)	2914 (39.6%)
≥0<2	502 (13.6%)	468 (12.7%)	497 (13.5%)	473 (12.9%)	970 (13.2%)
≥2<4	294 (8.0%)	275 (7.5%)	291 (7.9%)	278 (7.6%)	569 (7.7%)
≥4<6	141 (3.8%)	141 (3.8%)	140 (3.8%)	142 (3.9%)	282 (3.8%)
≥6	52 (1.4%)	54 (1.5%)	58 (1.6%)	48 (1.3%)	106 (1.4%)
Unknown	7 (0.2%)	6 (0.2%)	8 (0.2%)	5 (0.1%)	13 (0.2%)

Baseline Characteristic	Aspirin Arm				Omega-3 FAs Arm				Overall (n=7360)	
	Active (n=3687)		Placebo (n=3673)		Active (n=3692)		Placebo (n=3668)			
Ethnic origin										
White	3553	(96.4%)	3530	(96.1%)	3554	(96.3%)	3529	(96.2%)	7083	(96.2%)
Indian/Pakistani/Bangladeshi	50	(1.4%)	50	(1.4%)	51	(1.4%)	49	(1.3%)	100	(1.4%)
African/Caribbean	26	(0.7%)	35	(1.0%)	28	(0.8%)	33	(0.9%)	61	(0.8%)
Other/unknown	58	(1.6%)	58	(1.6%)	59	(1.6%)	57	(1.6%)	116	(1.6%)

DCCT = Diabetes Control and Complications Trial; GFR = Glomerular Filtration Rate; HDL=High-density lipoprotein; IQR = Interquartile range; IFCC =International Federation of Clinical Chemistry; SD = Standard Deviation
Figures presented are counts with percentages unless otherwise stated. Percentages may not total 100 because of rounding.
*The presence of type 2 diabetes was based on a broad clinical definition involving the participant's age when diagnosed with diabetes, the use of insulin within one year after diagnosis, and the body-mass index.
† From blood and urine consent forms, generally before randomisation.
‡ The body-mass index (the weight in kilograms divided by the square of the height in metres) was based on values for height and weight the participants reported on their randomisation questionnaires.
§ Calculated from blood cystatin c concentration using the CKD-EPI formula.[393]
¶There was an analysis rule in ASCEND which stated that those with a below detectable threshold albumin component of their urinary albumin:creatinine ratio would be recorded as zero. This applied to just over 25% of participants in the active group of the aspirin arm, and to just under 25% of participants in the placebo group. Hence the interquartile range included zero.
**Townsend scores of 0 represent an area with a UK-average level of deprivation, positive values indicate areas of above-average deprivation, and negative values indicate areas of below-average deprivation.

Table 33 Heterogeneity of Baseline Characteristics between those Included in and Excluded from Cohort B1

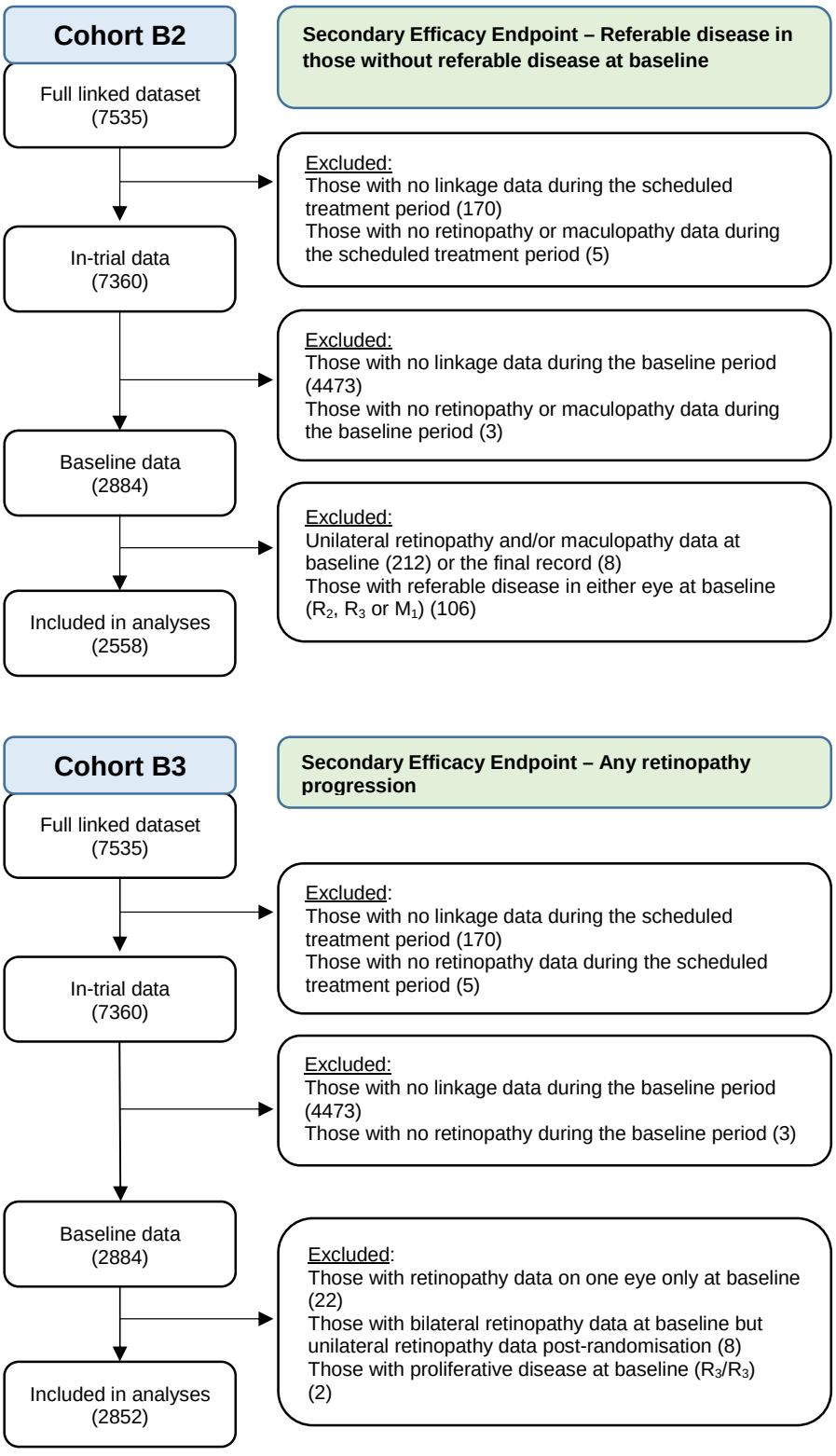
Baseline Characteristic	DESP-Linkage Exercise		Standardised difference (95% CI)*	P Values†
	Included (n=7360)	Excluded (n=8120)		
Age at randomisation (years)				
Mean (SD)	63.5±8.9	63.0±9.4	0.05 (0.02-0.08)	P = 0.002
<60	2574 (35.0%)	3016 (37.1%)		
≥60<70	3047 (41.4%)	3200 (39.4%)		
≥70	1739 (23.6%)	1904 (23.4%)	0.07 (0.04-0.1)	P = 0.01
Sex				
Male	4594 (62.4%)	5090 (62.7%)		
Female	2766 (37.6%)	3030 (37.3%)	0.01 (-0.03-0.04)	P = 0.75
Type of diabetes				
Type 1	373 (5.1%)	538 (6.6%)		
Type 2	6987 (94.9%)	7582 (93.4%)	0.07 (0.04-0.10)	P<0.001
Duration of diabetes (years)				
Median (IQR)	6 (3-12)	7 (3-12)	0.09 (0.06-0.12)	P<0.001
≥0<5 years	2387 (32.4%)	2504 (30.8%)		
≥5<10 years	2102 (28.6%)	2232 (27.5%)		
≥10<20 years	1681 (22.8%)	1856 (22.9%)		
≥20 years	778 (10.6%)	1084 (13.3%)	0.09 (0.06-0.12)	P<0.001
Unknown	412 (5.6%)	444 (5.5%)		
Diabetes management				
Diet only	1212 (16.5%)	1317 (16.2%)		
Oral hypoglycaemic agent(s) only	4472 (60.8%)	4548 (56.0%)		
Insulin +/- oral hypoglycaemic agent(s)	1676 (22.8%)	2255 (27.8%)	0.12 (0.09-0.15)	P<0.001
Participant-reported diabetic retinopathy				
Yes	1308 (17.8%)	1715 (21.1%)		
No	5991 (81.4%)	6322 (77.9%)	0.09 (0.05-0.12)	P<0.001
Unknown	61 (0.8%)	83 (1.0%)		
Participant-reported treatment for hypertension				
Yes	4502 (61.2%)	5031 (62.0%)		
No	2798 (38.0%)	3037 (37.4%)	0.01 (-0.02-0.05)	P = 0.39
Unknown	60 (0.8%)	52 (0.6%)		
Systolic blood pressure (mmHg)				
Mean (SD)	136.1±15.2	136.3±15.3	0.01 (-0.02-0.05)	P = 0.49
<130	1658 (22.5%)	1736 (21.4%)		
≥130<140	1504 (20.4%)	1587 (19.5%)		
≥140	2224 (30.2%)	2331 (28.7%)	<0.01 (-0.03-0.04)	P = 0.97
Unknown	1974 (26.8%)	2466 (30.4%)		
Diastolic blood pressure (mmHg)				
Mean (SD)	77.1±9.4	77.1±9.5	<0.01 (-0.04-0.04)	P = 0.93
<75	2036 (27.7%)	2187 (26.9%)		
≥75 <85	2297 (31.2%)	2322 (28.6%)		
≥85	1049 (14.3%)	1142 (14.1%)	0.03 (-0.01-0.07)	P = 0.23
Unknown	1978 (26.9%)	2469 (30.4%)		
Body mass index (kg/m^2)				
Mean (SD)	30.7±6.2	30.8±6.4	0.01 (-0.02-0.05)	P = 0.42
<25	1074 (14.6%)	1175 (14.5%)		
≥25, <30	2633 (35.8%)	2896 (35.7%)		
≥30<35	2023 (27.5%)	2217 (27.3%)		
≥35	1390 (18.9%)	1571 (19.3%)	0.01 (0.02-0.04)	P = 0.91
Unknown	240 (3.3%)	261 (3.2%)		

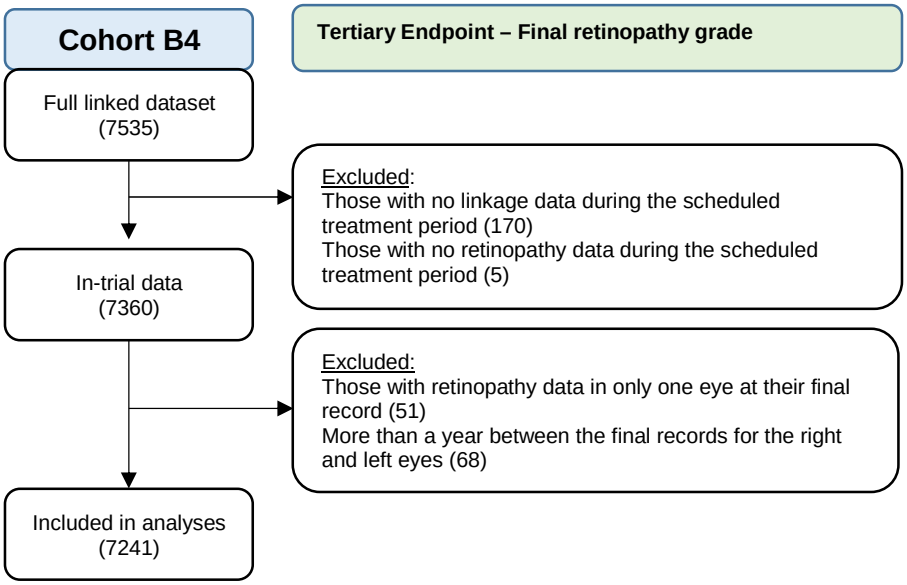
Baseline Characteristic	DESP-Linkage Exercise		Standardised difference (95% CI)*	P Values†
	Included (n=7360)	Excluded (n=8120)		
Cigarette smoking				
Current	577 (7.8%)	702 (8.6%)		
Former	3417 (46.4%)	3634 (44.8%)		
Never	3276 (44.5%)	3701 (45.6%)	0.04 (0.01-0.07)	P = 0.04
Unknown	90 (1.2%)	83 (1.0%)		
Non-study medication				
ACE-inhibitor or ARB	4276 (58.1%)	4779 (58.9%)	0.02 (-0.02-0.05)	P = 0.35
Aspirin use before screening	2527 (34.3%)	2981 (36.7%)	0.03 (<0.01-0.07)	P = 0.002
Thiazide or related diuretic	1360 (18.5%)	1597 (19.7%)	0.03 (0.00-0.06)	P = 0.06
Calcium channel blocker	1805 (24.5%)	1968 (24.2%)	0.01 (-0.03-0.04)	P = 0.69
Statin	5596 (76.0%)	6057 (74.6%)	0.05 (0.02-0.08)	P = 0.04
Total cholesterol (mmol/L)				
Mean (SD)	4.1±0.9	4.2±0.9	0.06 (0.02-0.10)	P = 0.005
<4	2247 (30.5%)	2307 (28.4%)		
≥4 <5	1878 (25.5%)	1917 (23.6%)		
≥5	649 (8.8%)	821 (10.1%)	0.08 (0.04-0.12)	P = 0.23
Not available	2586 (35.1%)	3075 (37.9%)		
HDL cholesterol (mmol/L)				
Mean (SD)	1.3±0.4	1.3±0.4	0.01 (-0.03-0.05)	P = 0.78
<1	1012 (13.8%)	1158 (14.3%)		
≥1<1.5	2733 (37.1%)	2790 (34.4%)		
≥1.5	1018 (13.8%)	1089 (13.4%)	0.05 (0.01-0.09)	P = 0.08
Not available	2597 (35.3%)	3083 (38.0%)		
Non-HDL cholesterol (mmol/L)				
Mean (SD)	2.9±0.8	2.9±0.9	0.06 (0.02-0.10)	P = 0.002
<2.5	1672 (22.7%)	1718 (21.2%)		
≥2.5 <3.5	2170 (29.5%)	2223 (27.4%)		
≥3.5	921 (12.5%)	1096 (13.5%)	0.06 (0.02-0.10)	P = 0.01
Not available	2597 (35.3%)	3083 (38.0%)		
Glycosylated haemoglobin - HbA1c				
IFCC (mmol/mol) mean (SD)	54.3±12.7	55.3±13.1	0.08 (0.04-0.12)	P<0.001
DCCT (%) mean (SD)	7.1±1.2	7.2±1.2	0.08 (0.04-0.12)	P<0.001
<48 (6.5)	1640 (22.3%)	1632 (20.1%)		
≥48 (6.5), <64 (8.0)	2280 (31.0%)	2384 (29.4%)		
≥64 (8.0)	853 (11.6%)	1024 (12.6%)	0.07 (0.03-0.11)	P = 0.005
Not available	2587 (35.1%)	3080 (37.9%)		
CKD-EPI estimated GFR (ml/min/1.73m^2)				
Mean (SD)	84.7±20.6	85.7±21.4	0.05 (0.01-0.09)	P = 0.02
≥90	2129 (28.9%)	2394 (29.5%)		
≥60<90	2018 (27.4%)	1998 (24.6%)		
<60	624 (8.5%)	652 (8.0%)	0.06 (0.02-0.10)	P = 0.01
Not available	2589 (35.2%)	3076 (37.9%)		
Urinary albumin:creatinine ratio (mg/mmol)				
Median (IQR)	0.54 (0.16-1.28)	0.56 (0.17-1.41)	0.05 (0.01-0.09)	P<0.001
<3	4192 (57.0%)	4334 (53.4%)		
≥3	572 (7.8%)	676 (8.3%)	0.05 (0.01-0.08)	P = 0.03
Not available	2596 (35.3%)	3110 (38.3%)		
Townsend Deprivation Index				
<-3	2506 (34.0%)	2598 (32.0%)		
≥-3<0	2914 (39.6%)	3108 (38.3%)		
≥0<2	970 (13.2%)	1067 (13.1%)		
≥2<4	569 (7.7%)	746 (9.2%)		
≥4<6	282 (3.8%)	421 (5.2%)		
≥6	106 (1.4%)	155 (1.9%)	0.10 (0.07-0.13)	P<0.001
Unknown	13 (0.2%)	25 (0.3%)		

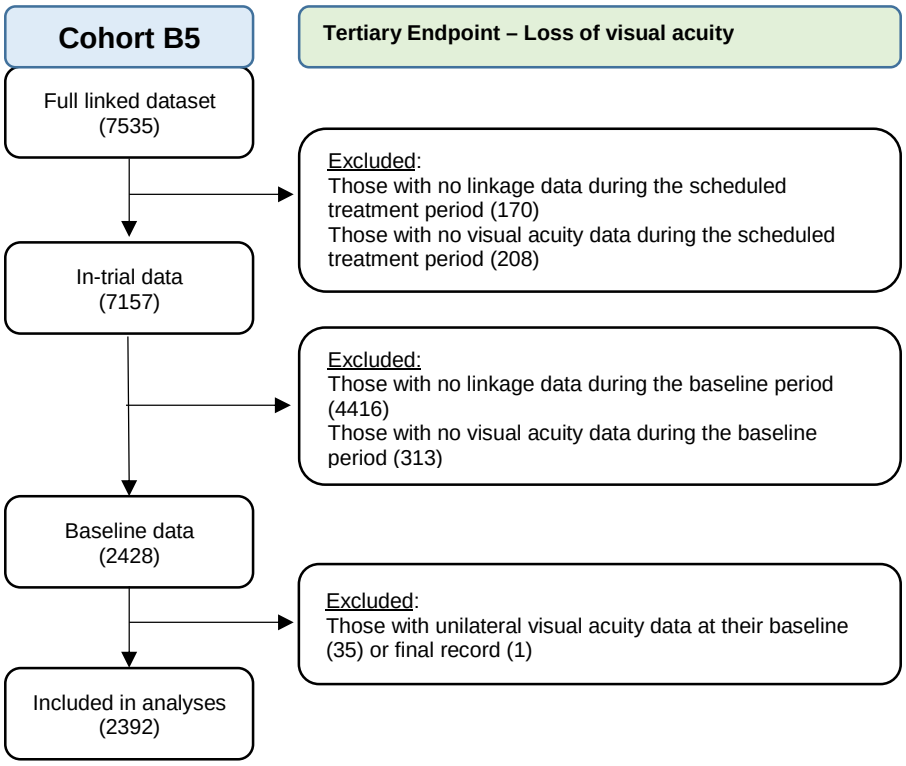
Baseline Characteristic	DESP-Linkage Exercise		Standardised difference (95% CI)*	P Values†
	Included (n=7360)	Excluded (n=8120)		
Ethnic origin				
White	7083 (96.2%)	7852 (96.7%)		
Indian/Pakistani/ Bangladeshi	100 (1.4%)	84 (1.0%)		
African/Caribbean	61 (0.8%)	79 (1.0%)	0.03 (<0.01-0.07)	P = 0.09
Other/unknown	116 (1.6%)	105 (1.3%)		

*Standardised differences were calculated using specific functions within the "stddiff" software package developed for R for continuous or categorical (ordinal or nominal) baseline variables.[390,392] The values 0.2, 0.5 and 0.8 represent small, moderate and large differences, respectively.[391] A negative score indicates that those who were not matched had a lower mean score than those who were. The standardised difference is not statistically significant if the 95% confidence interval includes zero.[391]
†Formal testing for differences between the baseline characteristics of those included in and excluded from the analysis cohort used the Chi-squared test for heterogeneity for categorical variables and grouped continuous variables (including age, duration of diabetes, systolic blood pressure, diastolic blood pressure, BMI, total cholesterol, HDL-cholesterol, non-HDL cholesterol, HbA1c, eGFR and urinary albumin:creatinine ratio), the Mantel-Haenszel Chi-square test for trend for ordinal variables (i.e. Townsend Deprivation Index), and the t-test or Wilcoxon-Mann-Whitney U test for continuous variables with a normal (summarised by mean±SD) or non-normal (summarised by median and interquartile range) distribution respectively.
Categories that were "unknown" or "not available" were excluded from balance diagnostic testing.
Percentages may not total 100 because of rounding.

Figure 23 Consort Diagrams for DESP Cohorts B2 to B7




Cohort B4
Tertiary Endpoint – Final retinopathy grade
Full linked dataset (7535)
Excluded:
Those with no linkage data during the scheduled treatment period (170)
Those with no retinopathy data during the scheduled treatment period (5)
In-trial data (7360)
Excluded:
Those with retinopathy data in only one eye at their final record (51)
More than a year between the final records for the right and left eyes (68)
Included in analyses (7241)


Cohort B5
Tertiary Endpoint – Loss of visual acuity
Full linked dataset (7535)
Excluded:
Those with no linkage data during the scheduled treatment period (170)
Those with no visual acuity data during the scheduled treatment period (208)
In-trial data (7157)
Excluded:
Those with no linkage data during the baseline period (4416)
Those with no visual acuity data during the baseline period (313)
Baseline data (2428)
Excluded:
Those with unilateral visual acuity data at their baseline (35) or final record (1)
Included in analyses (2392)

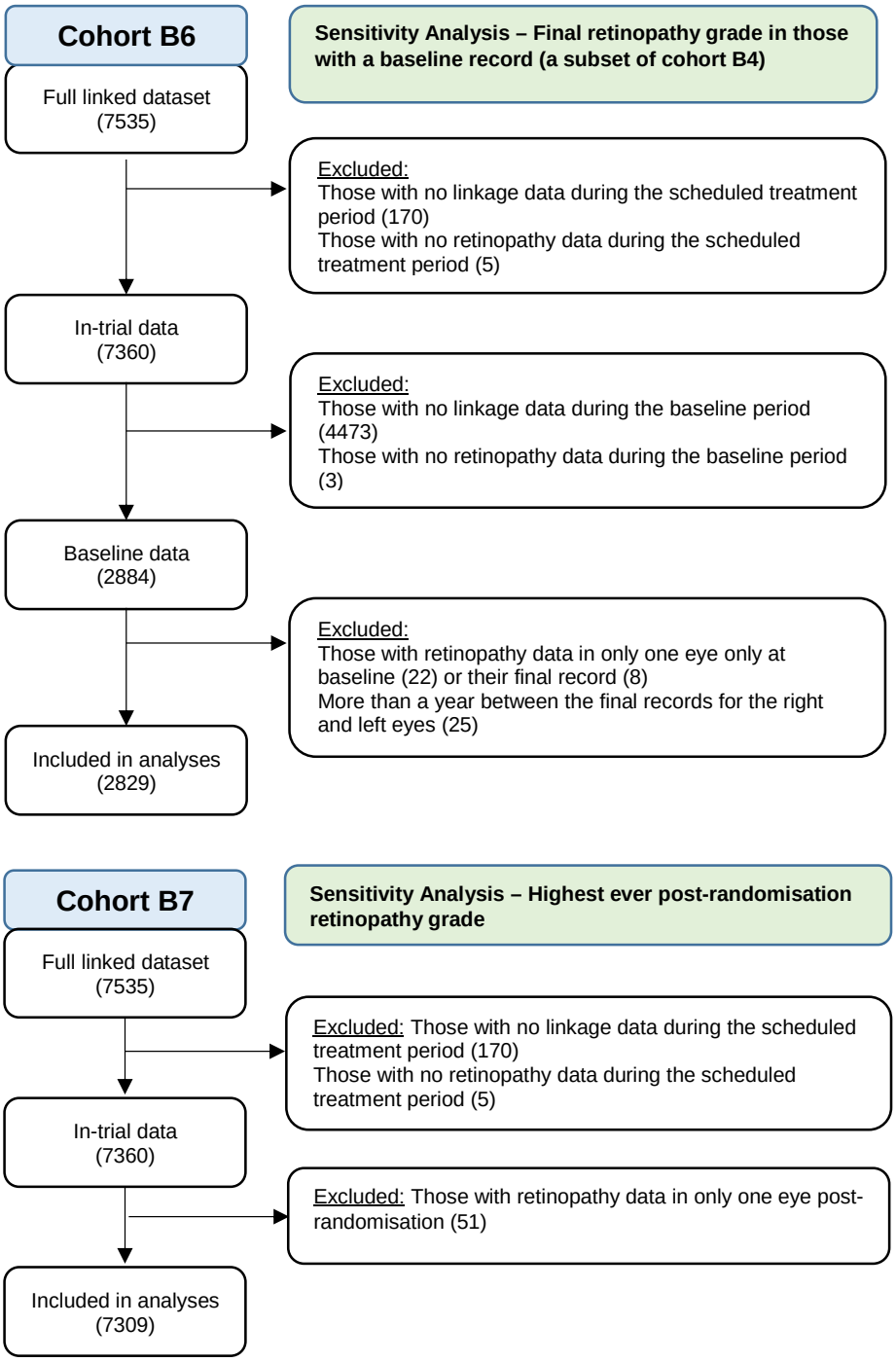


Table 34 Summary Tabulation showing the Number of Participants in each Treatment Arm in the B1-B7 cohorts

	No. of Participants: Aspirin Arm		No. of Participants: Omega-3 FAs Arm		Overall Cohort Size
	Active	Placebo	Active	Placebo	
B1 Cohort	3687	3673	3692	3668	7360
B2 Cohort	1268	1290	1287	1271	2558
B3 Cohort	1401	1451	1429	1423	2852
B4 Cohort	3627	3614	3634	3607	7241
B5 Cohort	1171	1221	1190	1202	2392
B6 Cohort	1393	1436	1418	1411	2829
B7 Cohort	3661	3648	3666	3643	7309

Table 35 Truncated Baseline Characteristics for DESP Linkage Cohorts B1-B7

Baseline characteristic	Cohort B1 (n=7360)	Cohort B2 (n=2558)	Cohort B3 (n=2852)	Cohort B4 (n=7241)	Cohort B5 (n=2392)	Cohort B6 (n=2829)	Cohort B7 (n=7309)
Age at randomisation (years)							
Mean (SD)	63.5±8.9	64.7±8.1	64.5±8.2	63.4±8.9	64.7±8.1	64.5±8.2	63.5±8.9
<60	2574 (35.0%)	750 (29.3%)	855 (30.0%)	2550 (35.2%)	696 (29.1%)	852 (30.1%)	2563 (35.1%)
≥60<70	3047 (41.4%)	1181 (46.2%)	1307 (45.8%)	3006 (41.5%)	1112 (46.5%)	1300 (46.0%)	3032 (41.5%)
≥70	1739 (23.6%)	627 (24.5%)	690 (24.2%)	1685 (23.3%)	584 (24.4%)	677 (23.9%)	1714 (23.5%)
Sex							
Male	4594 (62.4%)	1600 (62.5%)	1801 (63.1%)	4518 (62.4%)	1514 (63.3%)	1787 (63.2%)	4558 (62.4%)
Female	2766 (37.6%)	958 (37.5%)	1051 (36.9%)	2723 (37.6%)	878 (36.7%)	1042 (36.8%)	2751 (37.6%)
Type of diabetes*							
Type 1	373 (5.1%)	57 (2.2%)	84 (2.9%)	362 (5.0%)	71 (3.0%)	83 (2.9%)	368 (5.0%)
Type 2	6987 (94.9%)	2501 (97.8%)	2768 (97.1%)	6879 (95.0%)	2321 (97.0%)	2746 (97.1%)	6941 (95.0%)
Median (IQR) duration of diabetes (years)	6 (3-12)	6 (3-11)	6 (3-11)	6 (3-12)	6 (3-11)	6 (3-11)	6 (3-12)
Mean (SD) systolic blood pressure† (mmHg)	136.1±15.2	135.9±15.1	136.1±15.2	135.9±15.2	136.1±15.1	136.1±15.2	136.0±15.2
Mean (SD) diastolic blood pressure† (mmHg)	77.1±9.4	76.8±9.5	76.8±9.5	77.1±9.4	76.9±9.5	76.9±9.5	77.1±9.4
Mean (SD) glycosylated haemoglobin -HbA1c (IFCC (mmol/mol) (DCCT(%))	54.3±12.7 (7.1±1.2)	53.5±12.7 (7.0±1.2)	53.7±12.6 (7.1±1.2)	54.3±12.7 (7.1±1.2)	53.9±12.8 (7.1±1.2)	53.7±12.6 (7.1±1.2)	54.3±12.7 (7.1±1.2)
Mean (SD) CKD-EPI estimated GFR‡ (ml/min/1.73m^2)	84.7±20.6	81.5±20.3	81.8±20.5	84.8±20.6	80.9±20.6	81.9±20.5	84.7±20.6
Mean (IQR) urinary albumin: creatinine ratio (mg/mmol)	0.54 (0.16-1.28)	0.55 (0.24-1.32)	0.56 (0.25-1.32)	0.54 (0.16-1.27)	0.58 (0.26-1.36)	0.56 (0.24-1.32)	0.54 (0.16-1.28)
White ethnicity	7083 (96.2%)	2467 (96.4%)	2755 (96.6%)	6968 (96.2%)	2333 (97.5%)	2732 (96.6%)	7034 (96.2%)

DCCT = Diabetes Control and Complications Trial; GFR = Glomerular Filtration Rate; IQR = Interquartile range; IFCC =International Federation of Clinical Chemistry; SD = Standard Deviation
Figures presented are counts with percentages unless otherwise stated.
*The presence of type 2 diabetes was based on a broad clinical definition involving the age of the participant when diabetes was diagnosed, the use of insulin within one year after diagnosis, and the body-mass index.
† From blood and urine consent forms, generally before randomisation.
‡ Calculated from blood cystatin c concentration using the CKD-EPI formula.[393]

4.1.3 Cohort C – VFQ Responders

Figures 24 and 25 are consort diagrams of participants included in Cohort C in the aspirin arm and omega-3 FAs arm, respectively. Mailings of the VFQ to eligible participants commenced on 23rd February 2018. Every returned form was scanned and coded by 25th February 2020. By the end of the in-trial period of ASCEND, 1540 (9.9%) randomised participants had died, 2415 (15.6%) were on GP- or registry follow-up, and 60 (0.4%) had withdrawn consent. A further 164 (1.1%) participants died before mailings of the VFQ began. Out of the remaining 11,301 participants who were sent the VFQ, 2455 (21.7%) did not respond; all 8846 (78.3%) who returned a form are included in analysis cohort C. Among the respondents, 7 answered the first page of questions about incident eye diagnoses but did not complete the NEI-VFQ-25, and 8839 completed both parts of the form. Separate analyses of the baseline characteristics for the 8839 NEI-VFQ-25 respondents have not been performed because it is unlikely that excluding those 7 participants would make meaningful differences to the average results. Likewise, the completeness of follow-up and adherence analyses in Sections 4.2.3 and 4.3.3 also include 8846 participants.

The baseline characteristics of cohort C are shown in Table 36. They had a mean age of 62.5 years (SD 8.3), 62.5% were male, and they were diagnosed with diabetes (93.7% type 2) a median of 7 years before randomisation. Overall, 17.0% managed their diabetes by diet alone, 58.4% used an oral hypoglycaemic agent, but not insulin, and 24.6% were insulin-treated (with or without other hypoglycaemic therapies). Among those for whom the data were available, 61.3% reported taking treatment for hypertension, 51.5% were former or current smokers, and 84.7% were overweight or obese. The baseline characteristics between the treatment groups in each arm were well-balanced for risk factors associated with an increased risk of AMD and DMO, including age, sex and smoking status, and the duration of diabetes, glycaemic control, blood pressure, and urinary albumin:creatinine ratio, respectively.

Overall, the composition of VFQ respondents was representative of the full randomised ASCEND population; however, there was statistically significant heterogeneity between those included and excluded from the exercise for some characteristics (Table 37). Those excluded were slightly older,

which could reflect the eligibility criteria to receive the VFQ. They were also less affluent, with a longer duration of diabetes, and fewer had type 1 diabetes. More frequently, they reported having hypertension at baseline, had less favourable lipid and renal parameters, were current or former smokers, and had worse glycaemic control. There was no significant heterogeneity for sex or pre-trial use of aspirin therapy, which is relevant when interpreting the AMD analyses. The standardised differences between every baseline covariate tested were small or minimal, and, clinically the dissimilarities found with significance testing were unlikely to be important.

Figure 24 Consort Diagram for the VFQ Responders (Cohort C) - Aspirin Arm

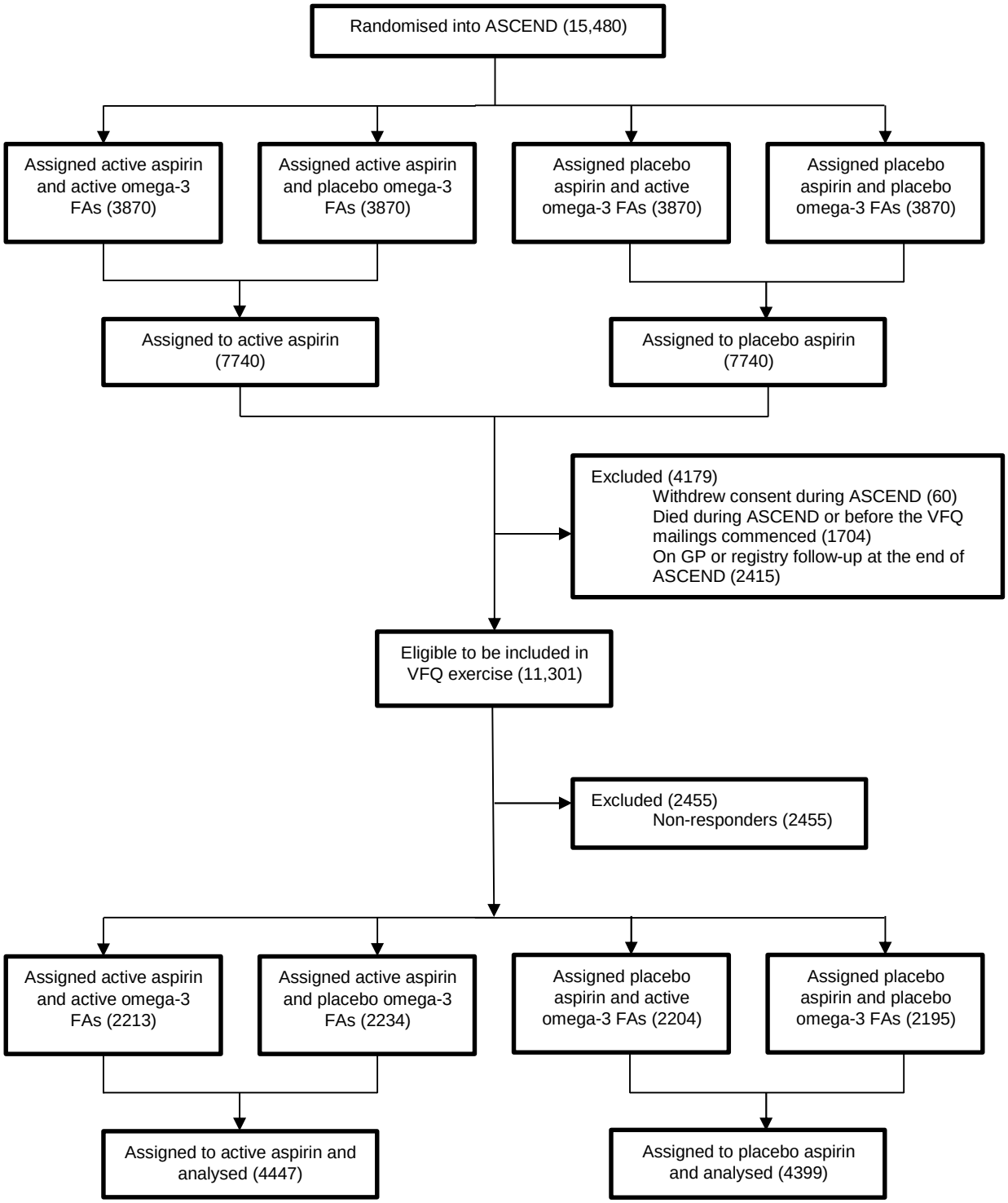


Figure 25 Consort Diagram for the VFQ Responders (Cohort C) - Omega-3 Fatty Acids Arm

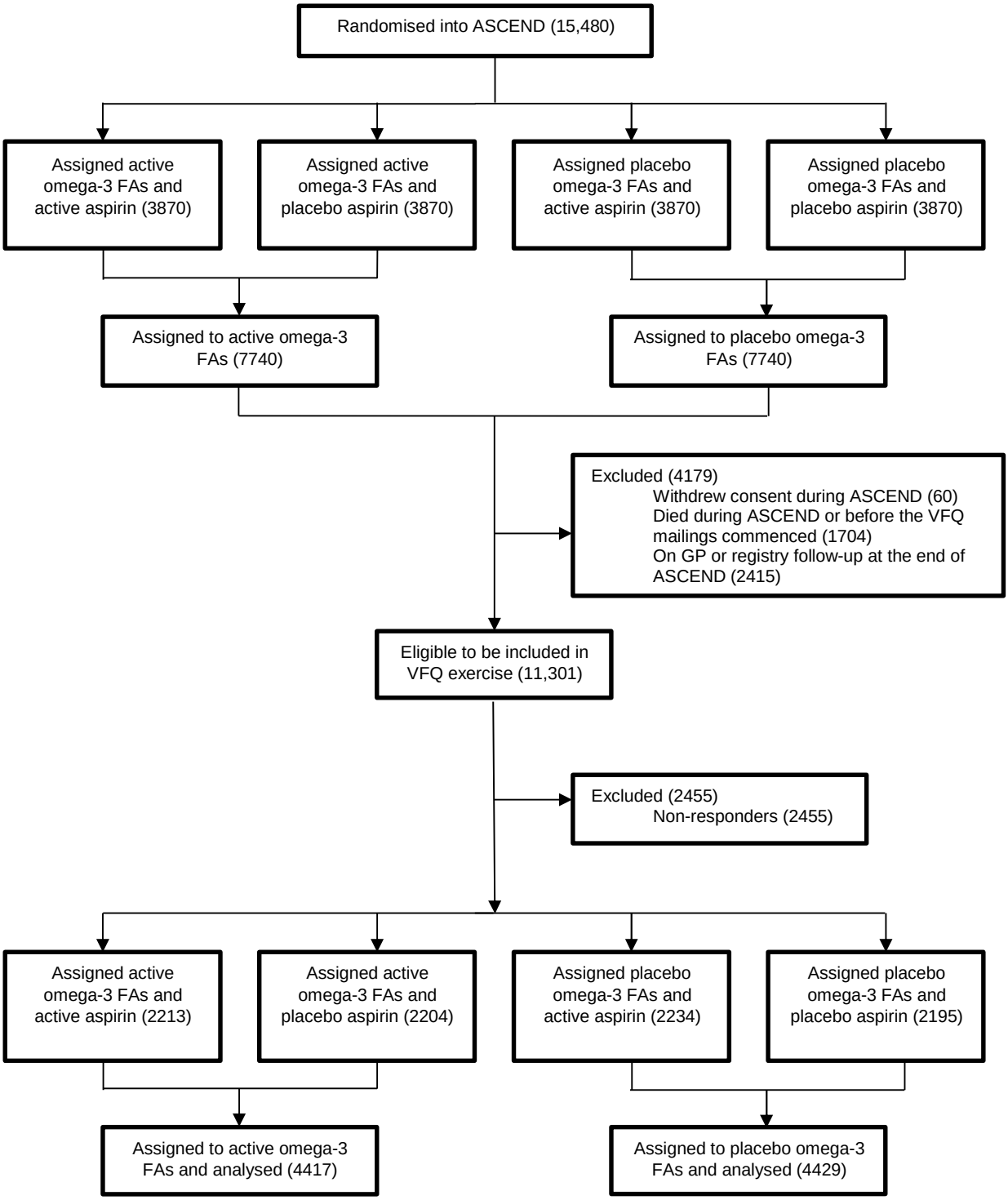


Table 36 Baseline Characteristics of the VFQ Responders (Cohort C)

Baseline Characteristic	Aspirin Arm		Omega-3 FAs Arm		Overall
	Active (n=4447)	Placebo (n=4399)	Active (n=4417)	Placebo (n=4429)	(n=8846)
Age at randomisation (years)					
Mean (SD)	62.4±8.3	62.5±8.3	62.4±8.3	62.5±8.3	62.5±8.3
<60	1679 (37.8%)	1660 (37.7%)	1673 (37.9%)	1666 (37.6%)	3339 (37.7%)
≥60<70	1938 (43.6%)	1922 (43.7%)	1916 (43.4%)	1944 (43.9%)	3860 (43.6%)
≥70	830 (18.7%)	817 (18.6%)	828 (18.7%)	819 (18.5%)	1647 (18.6%)
Sex					
Male	2785 (62.6%)	2744 (62.4%)	2758 (62.4%)	2771 (62.6%)	5529 (62.5%)
Female	1662 (37.4%)	1655 (37.6%)	1659 (37.6%)	1658 (37.4%)	3317 (37.5%)
Type of diabetes*					
Type 1	270 (6.1%)	289 (6.6%)	288 (6.5%)	271 (6.1%)	559 (6.3%)
Type 2	4177 (93.9%)	4110 (93.4%)	4129 (93.5%)	4158 (93.9%)	8287 (93.7%)
Duration of diabetes (years)					
Median (IQR)	7 (3-12)	6 (3-12)	7 (3-12)	6 (3-12)	7(3-12)
≥0<5 years	1459 (32.8%)	1459 (33.2%)	1436 (32.5%)	1482 (33.5%)	2918 (33.0%)
≥5<10 years	1305 (29.3%)	1239 (28.2%)	1273 (28.8%)	1271 (28.7%)	2544 (28.8%)
≥10<20 years	966 (21.7%)	986 (22.4%)	986 (22.3%)	966 (21.8%)	1952 (22.1%)
≥20 years	499 (11.2%)	521 (11.8%)	515 (11.7%)	505 (11.4%)	1020 (11.5%)
Unknown	218 (4.9%)	194 (4.4%)	207 (4.7%)	205 (4.6%)	412 (4.7%)
Diabetes management					
Diet only	766 (17.2%)	742 (16.9%)	728 (16.5%)	780 (17.6%)	1508 (17.0%)
Oral hypoglycaemic agent(s) only	2585 (58.1%)	2581 (58.7%)	2577 (58.3%)	2589 (58.5%)	5166 (58.4%)
Insulin +/- oral hypoglycaemic agent(s)	1096 (24.6%)	1076 (24.5%)	1112 (25.2%)	1060 (23.9%)	2172 (24.6%)
Participant-reported diabetic retinopathy					
Yes	824 (18.5%)	804 (18.3%)	851 (19.3%)	777 (17.5%)	1628 (18.4%)
No	3590 (80.7%)	3561 (81.0%)	3537 (80.1%)	3614 (81.6%)	7151 (80.8%)
Unknown	33 (0.7%)	34 (0.8%)	29 (0.7%)	38 (0.9%)	67 (0.8%)
Participant-reported treatment for hypertension					
Yes	2690 (60.5%)	2696 (61.3%)	2705 (61.2%)	2681 (60.5%)	5386 (60.9%)
No	1732 (38.9%)	1673 (38.0%)	1689 (38.2%)	1716 (38.7%)	3405 (38.5%)
Unknown	25 (0.6%)	30 (0.7%)	23 (0.5%)	32 (0.7%)	55 (0.6%)
Systolic blood pressure (mmHg) †					
Mean (SD)	135.7±15.1	135.9±14.9	135.9±15.1	135.7±14.9	135.8±14.9
<130	1022 (23.0%)	1001 (22.8%)	999 (22.6%)	1024 (23.1%)	2023 (22.9%)
≥130<140	899 (20.2%)	923 (21.0%)	908 (20.6%)	914 (20.6%)	1822 (20.6%)
≥140	1301 (29.3%)	1325 (30.1%)	1311 (29.7%)	1315 (29.7%)	2626 (29.7%)
Unknown	1225 (27.5%)	1150 (26.1%)	1199 (27.1%)	1176 (26.6%)	2375 (26.8%)
Diastolic blood pressure (mmHg) †					
Mean (SD)	77.3±9.3	77.6±9.1	77.5±9.2	77.4±9.1	77.4±9.2
<75	1192 (26.8%)	1179 (26.8%)	1161 (26.3%)	1210 (27.3%)	2371 (26.8%)
≥75 <85	1389 (31.2%)	1399 (31.8%)	1398 (31.7%)	1390 (31.4%)	2788 (31.5%)
≥85	639 (14.4%)	672 (15.3%)	658 (14.9%)	653 (14.7%)	1311 (14.8%)
Unknown	1227 (27.6%)	1149 (26.1%)	1200 (27.2%)	1176 (26.6%)	2376 (26.9%)
Body mass index (kg/m^2) ‡					
Mean (SD)	30.8±6.3	30.4±5.9	30.6±6.1	30.6±6.1	30.6±6.1
<25	636 (14.3%)	677 (15.4%)	638 (14.4%)	675 (15.2%)	1313 (14.8%)
≥25, <30	1604 (36.1%)	1626 (37.0%)	1616 (36.6%)	1614 (36.4%)	3230 (36.5%)
≥30<35	1223 (27.5%)	1206 (27.4%)	1222 (27.7%)	1207 (27.3%)	2429 (27.5%)
≥35	859 (19.3%)	760 (17.3%)	810 (18.3%)	809 (18.3%)	1619 (18.3%)
Unknown	125 (2.8%)	130 (3.0%)	131 (3.0%)	124 (2.8%)	255 (2.9%)

Baseline Characteristic	Aspirin Arm		Omega-3 FAs Arm		Overall
	Active (n=4447)	Placebo (n=4399)	Active (n=4417)	Placebo (n=4429)	(n=8846)
Cigarette smoking					
Current	264 (5.9%)	291 (6.6%)	278 (6.3%)	277 (6.3%)	555 (6.3%)
Former	1995 (44.9%)	1952 (44.4%)	1959 (44.4%)	1988 (44.9%)	3947 (44.6%)
Never	2139 (48.1%)	2104 (47.8%)	2131 (48.2%)	2112 (47.7%)	4243 (48.0%)
Unknown	49 (1.1%)	52 (1.2%)	49 (1.1%)	52 (1.2%)	101 (1.1%)
Non-study medication					
ACE-inhibitor or ARB	2559 (57.5%)	2556 (58.1%)	2597 (58.8%)	2518 (56.9%)	5115 (57.8%)
Aspirin use before screening	1598 (35.9%)	1559 (35.4%)	1582 (35.8%)	1575 (35.6%)	3157 (35.7%)
Thiazide or related diuretic	818 (18.4%)	821 (18.7%)	805 (18.2%)	834 (18.8%)	1639 (18.5%)
Calcium channel blocker	1074 (24.2%)	1014 (23.1%)	1040 (23.5%)	1048 (23.7%)	2088 (23.6%)
Statin	3424 (77.0%)	3345 (76.0%)	3356 (76.0%)	3413 (77.1%)	6769 (76.5%)
Total cholesterol (mmol/L)					
Mean (SD)	4.1±0.9	4.2±0.9	4.2±0.9	4.1±0.8	4.1±0.9
<4	1360 (30.6%)	1321 (30.0%)	1321 (29.9%)	1360 (30.7%)	2681 (30.3%)
≥4 <5	1115 (25.1%)	1131 (25.7%)	1112 (25.2%)	1134 (25.6%)	2246 (25.4%)
≥5	398 (8.9%)	419 (9.5%)	413 (9.4%)	404 (9.1%)	817 (9.2%)
Not available	1574 (35.4%)	1528 (34.7%)	1571 (35.6%)	1531 (34.6%)	3102 (35.1%)
HDL cholesterol (mmol/L)					
Mean (SD)	1.3±0.4	1.3±0.4	1.3±0.4	1.3±0.4	1.3±0.4
<1	632 (14.2%)	590 (13.4%)	612 (13.9%)	610 (13.8%)	1222 (13.8%)
≥1<1.5	1576 (35.4%)	1626 (37.0%)	1581 (35.8%)	1621 (36.6%)	3202 (36.2%)
≥1.5	662 (14.9%)	648 (14.7%)	646 (14.6%)	664 (15.0%)	1310 (14.8%)
Not available	1577 (35.5%)	1535 (34.9%)	1578 (35.7%)	1534 (34.6%)	3112 (35.2%)
Non-HDL cholesterol (mmol/L)					
Mean (SD)	2.9±0.8	2.9±0.8	2.9±0.9	2.9±0.8	2.9±0.8
<2.5	1032 (23.2%)	1021 (23.2%)	1006 (22.8%)	1047 (23.6%)	2053 (23.2%)
≥2.5 <3.5	1288 (29.0%)	1270 (28.9%)	1254 (28.4%)	1304 (29.4%)	2558 (28.9%)
≥3.5	550 (12.4%)	573 (13.0%)	579 (13.1%)	544 (12.3%)	1123 (12.7%)
Not available	1577 (35.5%)	1535 (34.9%)	1578 (35.7%)	1534 (34.6%)	3112 (35.2%)
Glycosylated haemoglobin - HbA1c					
IFCC (mmol/mol) mean (SD)	53.9±12.1	54.2±12.2	54.2±12.1	53.9±12.3	54.0±12.2
DCCT (%) mean (SD)	7.1±1.1	7.1±1.1	7.1±1.1	7.1±1.1	7.1±1.1
<48 (6.5)	1011 (22.7%)	946 (21.5%)	963 (21.8%)	994 (22.4%)	1957 (22.1%)
≥48 (6.5), <64 (8.0)	1363 (30.6%)	1429 (32.5%)	1376 (31.2%)	1416 (32.0%)	2792 (31.6%)
≥64 (8.0)	496 (11.2%)	494 (11.2%)	503 (11.4%)	487 (11.0%)	990 (11.2%)
Not available	1577 (35.5%)	1530 (34.8%)	1575 (35.7%)	1532 (34.6%)	3107 (35.1%)
CKD-EPI estimated GFR (ml/min/1.73m^2) §					
Mean (SD)	87.9±19.6	87.7±19.9	87.7±20.2	87.8±19.3	87.8±19.8
≥90	1459 (32.8%)	1443 (32.8%)	1429 (32.4%)	1473 (33.3%)	2902 (32.8%)
≥60<90	1139 (25.6%)	1160 (26.4%)	1148 (26.0%)	1151 (26.0%)	2299 (26.0%)
<60	273 (6.1%)	267 (6.1%)	268 (6.1%)	272 (6.1%)	540 (6.1%)
Not available	1576 (35.4%)	1529 (34.8%)	1572 (35.6%)	1533 (34.6%)	3105 (35.1%)
Urinary albumin:creatinine ratio (mg/mmol) ¶					
Median (IQR)	0.50 (0.00-1.16)	0.49 (0.00-1.12)	0.50 (0.00-1.17)	0.50 (0.00-1.12)	0.50 (0.00-1.14)
<3	2571 (57.8%)	2549 (57.9%)	2520 (57.1%)	2600 (58.7%)	5120 (57.9%)
≥3	303 (6.8%)	310 (7.0%)	323 (7.3%)	290 (6.5%)	613 (6.9%)
Not available	1573 (35.4%)	1540 (35.0%)	1574 (35.6%)	1539 (34.7%)	3113 (35.2%)
Townsend Deprivation Index**					
<-3	1537 (34.6%)	1564 (35.6%)	1574 (35.6%)	1527 (34.5%)	3101 (35.1%)
≥-3<0	1830 (41.2%)	1758 (40.0%)	1762 (39.9%)	1826 (41.2%)	3588 (40.6%)
≥0<2	537 (12.1%)	534 (12.1%)	555 (12.6%)	516 (11.7%)	1071 (12.1%)
≥2<4	322 (7.2%)	303 (6.9%)	292 (6.6%)	333 (7.5%)	625 (7.1%)
≥4<6	161 (3.6%)	162 (3.7%)	162 (3.7%)	161 (3.6%)	323 (3.7%)
≥6	52 (1.2%)	66 (1.5%)	64 (1.4%)	54 (1.2%)	118 (1.3%)
Unknown	8 (0.2%)	12 (0.3%)	8 (0.2%)	12 (0.3%)	20 (0.2%)

Baseline Characteristic	Aspirin Arm				Omega-3 FAs Arm				Overall (n=8846)	
	Active (n=4447)		Placebo (n=4399)		Active (n=4417)		Placebo (n=4429)			
Ethnic origin										
White	4309	(96.9%)	4255	(96.7%)	4279	(96.9%)	4285	(96.7%)	8564	(96.8%)
Indian/Pakistani/Bangladeshi	53	(1.2%)	54	(1.2%)	53	(1.2%)	54	(1.2%)	107	(1.2%)
African/Caribbean	26	(0.6%)	28	(0.6%)	22	(0.5%)	32	(0.7%)	54	(0.6%)
Other/unknown	59	(1.3%)	62	(1.4%)	63	(1.4%)	58	(1.3%)	121	(1.4%)

DCCT = Diabetes Control and Complications Trial; GFR = Glomerular Filtration Rate; HDL=High-density lipoprotein; IQR = Interquartile range; IFCC =International Federation of Clinical Chemistry; SD = Standard Deviation
Figures presented are counts with percentages unless otherwise stated. Percentages may not total 100 because of rounding.
*The presence of type 2 diabetes was based on a broad clinical definition involving the participant's age when diagnosed with diabetes, the use of insulin within one year after diagnosis, and the body-mass index.
† From blood and urine consent forms, generally before randomisation.
‡ The body-mass index (the weight in kilograms divided by the square of the height in metres) was based on values for height and weight the participants reported on their randomisation questionnaires.
§ Calculated from blood cystatin c concentration using the CKD-EPI formula.[393]
¶There was an analysis rule in ASCEND which stated that those with a below detectable threshold albumin component of their urinary albumin:creatinine ratio, would be recorded as zero. This applied to just over 25% of participants in the active group of the aspirin arm, and to just under 25% of participants in the placebo group. Hence the interquartile range included zero.
**Townsend scores of 0 represent an area with a UK-average level of deprivation, positive values indicate areas of above-average deprivation, and negative values indicate areas of below-average deprivation.

Table 37 Heterogeneity of the Baseline Characteristics in those who were Included in and Excluded from the VFQ Exercise (Cohort C)

Baseline Characteristic	VFQ Exercise		Standardised difference (95% CI)†	P Values‡
	Included (n=8846)	Excluded* (n=6634)		
Age at randomisation (years)				
Mean (SD)	62.5±8.3	64.3±10.2	0.20 (0.17-0.23)	P<0.001
<60	3339 (37.7%)	2251 (33.9%)		
≥60<70	3860 (43.6%)	2387 (36.0%)		
≥70	1647 (18.6%)	1996 (30.1%)	0.29 (0.26-0.32)	P<0.001
Sex				
Male	5529 (62.5%)	4155 (62.6%)		
Female	3317 (37.5%)	2479 (37.4%)	<0.01 (-0.03-0.04)	P = 0.88
Type of diabetes				
Type 1	559 (6.3%)	352 (5.3%)		
Type 2	8287 (93.7%)	6282 (94.7%)	0.04 (0.01-0.08)	P = 0.009
Duration of diabetes (years)				
Median (IQR)	7(3-12)	7 (4-13)	0.05 (0.02-0.09)	P<0.001
≥0<5 years	2918 (33.0%)	1973 (29.7%)		
≥5<10 years	2544 (28.8%)	1790 (27.0%)		
≥10<20 years	1952 (22.1%)	1585 (23.9%)		
≥20 years	1020 (11.5%)	842 (12.7%)	0.08 (0.05-0.12)	P<0.001
Unknown	412 (4.7%)	444 (6.7%)		
Diabetes management				
Diet only	1508 (17.0%)	1021 (15.4%)		
Oral hypoglycaemic agent(s) only	5166 (58.4%)	3854 (58.1%)		
Insulin+/- oral hypoglycaemic agent(s)	2172 (24.6%)	1759 (26.5%)	0.06 (0.03-0.09)	P = 0.002
Participant-reported diabetic retinopathy				
Yes	1628 (18.4%)	1395 (21.0%)		
No	7151 (80.8%)	5162 (77.8%)	0.07 (0.04-0.10)	P<0.001
Unknown	67 (0.8%)	77 (1.2%)		
Participant-reported treatment for hypertension				
Yes	5386 (60.9%)	4147 (62.5%)		
No	3405 (38.5%)	2430 (36.6%)	0.04 (0.01-0.07)	P = 0.03
Unknown	55 (0.6%)	57 (0.9%)		
Systolic blood pressure (mmHg)				
Mean (SD)	135.8±14.9	136.6±15.7	0.05 (0.02-0.09)	P = 0.005
<130	2023 (22.9%)	1371 (20.7%)		
≥130<140	1822 (20.6%)	1269 (19.1%)		
≥140	2626 (29.7%)	1929 (29.1%)	0.04 (<-0.01-0.07)	P = 0.14
Unknown	2375 (26.8%)	2065 (31.1%)		
Diastolic blood pressure (mmHg)				
Mean (SD)	77.4±9.2	76.6±9.8	0.08 (0.05-0.12)	P<0.001
<75	2371 (26.8%)	1852 (27.9%)		
≥75 <85	2788 (31.5%)	1831 (27.6%)		
≥85	1311 (14.8%)	880 (13.3%)	0.08 (0.04-0.12)	P<0.001
Unknown	2376 (26.9%)	2071 (31.2%)		
Body mass index (kg/m^2)				
Mean (SD)	30.6±6.1	30.9±6.4	0.05 (0.02-0.08)	P = 0.004
<25	1313 (14.8%)	936 (14.1%)		
≥25, <30	3230 (36.5%)	2299 (34.7%)		
≥30<35	2429 (27.5%)	1811 (27.3%)		
≥35	1619 (18.3%)	1342 (20.2%)	0.06 (0.03-0.09)	P = 0.007
Unknown	255 (2.9%)	246 (3.7%)		

Baseline Characteristic	VFQ Exercise		Standardised difference (95% CI)†	P Values‡
	Included (n=8846)	Excluded* (n=6634)		
Cigarette smoking				
Current	555 (6.3%)	724 (10.9%)		
Former	3947 (44.6%)	3104 (46.8%)		
Never	4243 (48.0%)	2734 (41.2%)	0.19 (0.16-0.23)	P<0.001
Unknown	101 (1.1%)	72 (1.1%)		
Non-study medication				
ACE-inhibitor or ARB	5115 (57.8%)	3940 (59.4%)	0.03 (0.00-0.06)	P = 0.051
Aspirin use before screening	3157 (35.7%)	2351 (35.4%)	0.07 (0.04-0.10)	P = 0.76
Thiazide or related diuretic	1639 (18.5%)	1318 (19.9%)	0.03 (<0.01-0.07)	P<0.001
Calcium channel blocker	2088 (23.6%)	1685 (25.4%)	0.04 (0.01-0.07)	P = 0.01
Statin	6769 (76.5%)	4884 (73.6%)	0.01 (-0.03-0.04)	P<0.001
Total cholesterol (mmol/L)				
Mean (SD)	4.1±0.9	4.2±0.9	0.04 (-0.01-0.08)	P = 0.07
<4	2681 (30.3%)	1873 (28.2%)		
≥4 <5	2246 (25.4%)	1549 (23.4%)		
≥5	817 (9.2%)	653 (9.8%)	0.05 (0.01-0.09)	P = 0.046
Not available	3102 (35.1%)	2559 (38.6%)		
HDL cholesterol (mmol/L)				
Mean (SD)	1.3±0.4	1.3±0.4	0.08 (0.04-0.12)	P<0.001
<1	1222 (13.8%)	948 (14.3%)		
≥1<1.5	3202 (36.2%)	2321 (35.0%)		
≥1.5	1310 (14.8%)	797 (12.0%)	0.08 (0.04-0.12)	P<0.001
Not available	3112 (35.2%)	2568 (38.7%)		
Non-HDL cholesterol (mmol/L)				
Mean (SD)	2.9±0.8	2.9±0.9	0.07 (0.03-0.11)	P<0.001
<2.5	2053 (23.2%)	1337 (20.2%)		
≥2.5 <3.5	2558 (28.9%)	1835 (27.7%)		
≥3.5	1123 (12.7%)	894 (13.5%)	0.08 (0.04-0.12)	P = 0.002
Not available	3112 (35.2%)	2568 (38.7%)		
Glycosylated haemoglobin - HbA1c				
IFCC (mmol/mol) mean (SD)	54.0±12.2	55.9±13.8	0.14 (0.10-0.18)	P<0.001
DCCT (%) mean (SD)	7.1±1.1	7.3±1.3	0.14 (0.10-0.18)	P<0.001
<48 (6.5)	1957 (22.1%)	1315 (19.8%)		
≥48 (6.5), <64 (8.0)	2792 (31.6%)	1872 (28.2%)		
≥64 (8.0)	990 (11.2%)	887 (13.4%)	0.11 (0.07-0.15)	P<0.001
Not available	3107 (35.1%)	2560 (38.6%)		
CKD-EPI estimated GFR (ml/min/1.73m^2)				
Mean (SD)	87.8±19.8	81.5±22.3	0.30 (0.26-0.34)	P<0.001
≥90	2902 (32.8%)	1621 (24.4%)		
≥60<90	2299 (26.0%)	1717 (25.9%)		
<60	540 (6.1%)	736 (11.1%)	0.29 (0.25-0.33)	P<0.001
Not available	3105 (35.1%)	2560 (38.6%)		
Urinary albumin:creatinine ratio (mg/mmol) §				
Median (IQR)	0.50 (0.00-1.14)	0.66 (0.24-1.66)	0.12 (0.08-0.16)	P<0.001
<3	5120 (57.9%)	3406 (51.3%)		
≥3	613 (6.9%)	635 (9.6%)	0.15 (0.11-0.19)	P<0.001
Not available	3113 (35.2%)	2593 (39.1%)		
Townsend Deprivation Index				
<-3	3101 (35.1%)	2003 (30.2%)		
≥-3<0	3588 (40.6%)	2434 (36.7%)		
≥0<2	1071 (12.1%)	966 (14.6%)		
≥2<4	625 (7.1%)	690 (10.4%)		
≥4<6	323 (3.7%)	380 (5.7%)		
≥6	118 (1.3%)	143 (2.2%)	0.21 (0.17-0.24)	P<0.001
Unknown	20 (0.2%)	18 (0.3%)		

Baseline Characteristic	VFQ Exercise		Standardised difference (95% CI)†	P Values‡
	Included (n=8846)	Excluded* (n=6634)		
Ethnic origin				
White	8564 (96.8%)	6371 (96.0%)		
Indian/Pakistani/ Bangladeshi	107 (1.2%)	77 (1.2%)		
African/Caribbean	54 (0.6%)	86 (1.3%)	0.07 (0.04-0.10)	P<0.001
Other/unknown	121 (1.4%)	100 (1.5%)		

*Excluded participants includes those who were not eligible to receive the VFQ and those who were eligible but did not respond.
†Standardised differences were calculated using specific functions within the "stddiff" software package developed for R for continuous or categorical (ordinal or nominal) baseline variables.[390,392] The values 0.2, 0.5 and 0.8 represent small, moderate and large differences, respectively.[391] A negative score indicates that those who were not matched had a lower mean score than those who were. The standardised difference is not statistically significant if the 95% confidence interval includes zero.[391]
‡Formal testing for differences between the baseline characteristics of those included in and excluded from the analysis cohort used the Chi-squared test for heterogeneity for categorical variables and grouped continuous variables (including age, duration of diabetes, systolic blood pressure, diastolic blood pressure, BMI, total cholesterol, HDL-cholesterol, non-HDL cholesterol, HbA1c, eGFR and urinary albumin:creatinine ratio), the Mantel-Haenszel Chi-square test for trend for ordinal variables (i.e. Townsend Deprivation Index), and the t-test or Wilcoxon-Mann-Whitney U test for continuous variables with a normal (summarised by mean±SD) or non-normal (summarised by median and interquartile range) distribution respectively.
§There was an analysis rule in ASCEND which stated that those with a below detectable threshold albumin component of their urinary albumin:creatinine ratio, would be recorded as zero. This applied to just over 25% of participants included in the VFQ analyses.
Categories that were "unknown" or "not available" were excluded from balance diagnostic testing.
Percentages may not total 100 because of rounding.

4.2 Completeness of Follow-Up

4.2.1 Cohort A – Full ASCEND Population

At the end of the scheduled treatment period in ASCEND, the mean duration of follow-up was 7.4 years (Table 38). This yielded 57,000 person-years (py) and 56,945 py of follow-up in the active and placebo groups of the aspirin arm, respectively, and 57,021 py and 56,923 py in the active and placebo groups of the omega-3 FAs arm, respectively (Table 39). Complete morbidity follow-up was available for 15,341 (99.1%) of those randomised in each treatment arm (Table 40).

4.2.2 Cohort B1 and its Subsets – DESP-Linked Population

After censoring by the date of the final eye screening record, the mean duration of follow-up in the linkage cohort was less than in cohorts A or C. The average for the largest cohorts (B1, B4 and B7) was 6.5 years (Table 41), whereas average follow-ups in the baseline-restricted cohorts B2, B3, B5 and B6 were shorter: 5.7, 5.7, 5.5 and 5.7 years respectively. Those with a baseline record were recruited into ASCEND later than those without a baseline record.

4.2.3 Cohort C – VFQ Responders

The mean duration of follow-up among those who returned the VFQ was 7.5 years (median = 7.3 years; Table 42). The mean time from randomisation to completion of the VFQ was 8.6 years (Table 42).

Table 38 Duration of Follow-Up- Full ASCEND Population (Cohort A)

	Aspirin Arm		Omega-3 FAs Arm		
	Active (n=7740)	Placebo (n=7740)	Active (n=7740)	Placebo (n=7740)	Overall (n=15,480)
Mean(SD) length of follow-up (years)	7.4 (1.8)	7.4 (1.8)	7.4 (1.8)	7.4 (1.8)	7.4 (1.8)
Median (IQR) length of follow-up (years)	7.3 (6.4-8.5)	7.3 (6.4-8.5)	7.3 (6.4-8.5)	7.3 (6.4-8.5)	7.3 (6.4-8.5)

Table 39 Person-Years of Follow-up, Overall and by Follow-Up Method (Cohort A)

	Aspirin Arm		Omega-3 FAs Arm		
	Active (n=7740)	Placebo (n=7740)	Active (n=7740)	Placebo (n=7740)	Overall (n=15,480)
Follow-up method					
Participant	43,627.0 (76.5%)	43,053.7 (75.6%)	43,416.6 (76.1%)	43,264.1 (76.0%)	86,680.7 (76.1%)
General practitioner	8018.3 (14.1%)	8423.6 (14.8%)	8274.2 (14.5%)	8167.7 (14.3%)	16,441.9 (14.4%)
Record linkage	1742.2 (3.1%)	1674.1 (2.9%)	1685.9 (3.0%)	1730.4 (3.0%)	3416.3 (3.0%)
Died with complete follow-up	3309.2 (5.8%)	3410.3 (6.0%)	3286.2 (5.8%)	3433.3 (6.0%)	6719.5 (5.9%)
Incomplete follow-up information	303.7 (0.5%)	383.4 (0.7%)	358.7 (0.6%)	328.4 (0.6%)	687.1 (0.6%)
Total	**57,000.4**	**56,945.0**	**57,021.5**	**56,923.9**	**113,945.4**

All figures represent person-years of follow-up. Percentages may not total 100 because of rounding.

Table 40 Completeness of Follow-Up (Cohort A)

	Aspirin Arm		Omega-3 FAs Arm		
Randomised	Active n=7740	Placebo n=7740	Active n=7740	Placebo n=7740	Overall n=15,480
Complete follow-up information	**7671 (99.1%)**	**7670 (99.1%)**	**7672 (99.1%)**	**7669 (99.1%)**	**15,341 (99.1%)**
Final FU completed by participant or carer	5763 (74.5%)	5682 (73.4%)	5730 (74.0%)	5715 (73.8%)	11,445 (73.9%)
Final FU completed by GP	999 (12.9%)	1045 (13.5%)	1032 (13.3%)	1012 (13.1%)	2044 (13.2%)
Final FU completed by registry linkage	224 (2.9%)	218 (2.8%)	221 (2.9%)	221 (2.9%)	442 (2.9%)
Died (FU for morbidity complete)	685 (8.9%)	725 (9.4%)	689 (8.9%)	721 (9.3%)	1410 (9.1%)
Incomplete follow-up information	**69 (0.9%)**	**70 (0.9%)**	**68 (0.9%)**	**71 (0.9%)**	**139 (0.9%)**
Consent withdrawn	35 (0.5%)	30 (0.4%)	26 (0.3%)	39 (0.5%)	65 (0.4%)
Moved abroad	17 (0.2%)	14 (0.2%)	19 (0.2%)	12 (0.2%)	31 (0.2%)
Died (FU for morbidity not complete)	9 (0.1%)	13 (0.2%)	11 (0.1%)	11 (0.1%)	22 (0.1%)
No final FU information	8 (0.1%)	13 (0.2%)	12 (0.2%)	9 (0.1%)	21 (0.1%)

Percentages represent the proportion each follow-up method contributed to the total number of person-years of follow-up. Percentages may not total 100 because of rounding.

Table 41 Duration of Follow-up in the Linkage Cohorts (B1-B7)

	Aspirin Arm		Omega-3 FAs Arm		Overall
	Active	Placebo	Active	Placebo	
B1 Cohort (n=7360)					
Mean(SD) length of follow-up (years)	6.5 (1.9)	6.5 (1.9)	6.5 (1.8)	6.5 (1.9)	6.5 (1.9)
Median (IQR) length of follow-up (years)	6.4 (5.5-7.5)	6.4 (5.5-7.5)	6.4 (5.5-7.5)	6.4 (5.5-7.5)	6.4 (5.5-7.5)
B2 Cohort (n=2558)					
Mean(SD) length of follow-up (years)	5.6 (1.5)	5.7 (1.5)	5.7 (1.5)	5.6 (1.5)	5.7 (1.5)
Median (IQR) length of follow-up (years)	5.7 (5.1-6.2)	5.7 (5.1-6.4)	5.6 (5.1-6.3)	5.7 (5.2-6.3)	5.7 (5.1-6.3)
B3 Cohort (n=2852)					
Mean(SD) length of follow-up (years)	5.6 (1.6)	5.7 (1.6)	5.7 (1.5)	5.7 (1.6)	5.7 (1.6)
Median (IQR) length of follow-up (years)	5.7 (5.1-6.3)	5.7 (5.1-6.5)	5.7 (5.1-6.4)	5.7 (5.1-6.4)	5.7 (5.1-6.4)
B4 Cohort (n=7241)					
Mean(SD) length of follow-up (years)	6.5 (1.9)	6.5 (1.8)	6.5 (1.8)	6.5 (1.9)	6.5 (1.8)
Median (IQR) length of follow-up (years)	6.4 (5.5-7.5)	6.4 (5.5-7.5)	6.4 (5.5-7.5)	6.4 (5.5-7.5)	6.4 (5.5-7.5)
B5 Cohort (n=2392)					
Mean(SD) length of follow-up (years)	5.5 (1.4)	5.5 (1.4)	5.5 (1.3)	5.4 (1.5)	5.5 (1.4)
Median (IQR) length of follow-up (years)	5.6 (5.1-6.1)	5.6 (5.1-6.2)	5.6 (5.1-6.1)	5.6 (5.1-6.2)	5.6 (5.1-6.1)
B6 Cohort (n=2829)					
Mean(SD) length of follow-up (years)	5.7 (1.6)	5.7 (1.6)	5.7 (1.5)	5.7 (1.6)	5.7 (1.6)
Median (IQR) length of follow-up (years)	5.7 (5.1-6.3)	5.7 (5.1-6.5)	5.7 (5.1-6.4)	5.7 (5.1-6.4)	5.7 (5.1-6.4)
B7 Cohort (n=7309)					
Mean(SD) length of follow-up (years)	6.5 (1.9)	6.5 (1.8)	6.5 (1.8)	6.5 (1.9)	6.5 (1.8)
Median (IQR) length of follow-up (years)	6.4 (5.5-7.5)	6.4 (5.5-7.5)	6.4 (5.5-7.5)	6.4 (5.5-7.5)	6.4 (5.5-7.5)

Table 42 Duration Follow-up in the VFQ Responders (Cohort C)

	Aspirin Arm		Omega-3 FAs Arm		Overall
	Active (n=4447)	Placebo (n=4399)	Active (n=4417)	Placebo (n=4429)	Overall (n=8846)
Mean(SD) length of follow-up (years)	7.5 (1.4)	7.5 (1.4)	7.5 (1.4)	7.5 (1.4)	7.5 (1.4)
Median (IQR) length of follow-up (years)	7.3 (6.4-8.3)	7.3 (6.4-8.3)	7.3 (6.4-8.7)	7.3 (6.4-8.2)	7.3 (6.4-8.3)
Mean (SD) time from randomisation to completing the VFQ (years)*	8.6 (1.4)	8.6 (1.4)	8.6 (1.4)	8.6 (1.4)	8.6 (1.4)
Median (IQR) time from randomisation to completing the VFQ (years)*	8.3 (7.5-9.5)	8.4 (7.5-9.6)	8.4 (7.5-9.6)	8.3 (7.5-9.5)	8.4 (7.5-9.5)

*These analyses were exploratory.

4.3 Adherence

4.3.1 Cohort A – Full ASCEND Population

The study average adherence was estimated to be 67.7% in the aspirin arm and 75.4% in the omega-3 FAs arm, with similar proportions in the active and placebo group assignments of each arm (Table 43). Adherence rates did not differ by baseline characteristic in either treatment arm (Table 44). Regarding the aspirin arm, adherence declined substantially in the later years of follow-up, and the use of non-study aspirin, alternative anti-platelet agents, or anti-coagulants increased (Table 45). In total, 7.9% of participants in the placebo group were prescribed a non-study anti-platelet drug, and 9.6% were given a non-study anti-platelet or anti-coagulant. Although adherence was higher in the omega-3 FAs arm, there was a similar trend of declining adherence towards the end of the scheduled treatment period (Table 43). At baseline, 10% of the participants took a non-study fish oil supplement, compared to 7% at the end of ASCEND, with no significant between-group differences.[13] The reasons for stopping treatment in each arm are shown in Table 46. In both regimens, "Participant wishes" represented the main reason for discontinuing. There were no significant differences in the number of participants who stopped taking active treatment as compared with the placebo, in either treatment arm, overall, or for specific reasons, except for those who stopped because of bleeding among those who were allocated active aspirin.

Table 43 Reported Definite or Probable Adherence with the Study Treatment by Follow-Up Period Stratified by Years Post-Randomisation (Cohort A)

	Aspirin Arm			Omega-3 FAs Arm		
	Active (%)	Placebo (%)	Overall (%)	Active (%)	Placebo (%)	Overall (%)
Years post-randomisation						
<3	82.0%	81.7%	81.9%	86.1%	85.7%	85.9%
≥3<5	66.0%	64.9%	65.5%	74.1%	73.6%	73.9%
≥5<7	56.2%	55.1%	55.6%	66.9%	66.2%	66.6%
≥7	46.2%	45.6%	46.0%	58.9%	58.1%	58.5%
All	**68.0%**	**67.3%**	**67.7%**	**75.6%**	**75.1%**	**75.4%**

Criteria used to define definite or probable adherence:

- Definitely adherent = participant reported taking treatment every or most days during the follow-up period.
- Probably adherent = participant was previously adherent, was still receiving medication and had not reported stopping treatment within the last seven months.

Table 44 Study Average Reported Definite or Probable Adherence in each Treatment Arm, Overall and by Baseline Characteristics (Cohort A)

	Aspirin Arm			Omega-3 FAs Arm		
Baseline Characteristic	**Active (n =7740)**	**Placebo (n=7740)**	**Overall (n=15,480)**	**Active (n=7740)**	**Placebo (n=7740)**	**Overall (n=15,480)**
Age at randomisation (years)						
<60	69.3%	68.5%	68.9%	74.9%	74.7%	74.8%
≥60<70	69.3%	68.9%	69.1%	77.3%	77.4%	77.4%
≥70	63.5%	62.3%	62.9%	73.6%	71.7%	72.6%
Sex						
Male	69.8%	68.9%	69.3%	77.8%	77.2%	77.5%
Female	65.2%	64.7%	64.9%	72.0%	71.6%	71.8%
Type of diabetes						
Type 1	68.7%	69.9%	69.3%	77.3%	74.5%	75.9%
Type 2	68.0%	67.1%	67.6%	75.5%	75.1%	75.3%
Duration of diabetes (years)						
≥0<5 years	69.7%	68.6%	69.2%	76.4%	76.5%	76.4%
≥5<10 years	68.2%	66.6%	67.4%	75.8%	74.3%	75.1%
≥10<20 years	67.2%	67.7%	67.5%	75.6%	75.3%	75.4%
≥20 years	66.9%	68.1%	67.5%	76.7%	74.9%	75.8%
Unknown	63.4%	60.2%	61.8%	68.0%	70.6%	69.3%
Diabetes management						
Diet only	67.1%	65.6%	66.3%	75.9%	73.2%	74.5%
Oral hypoglycaemic agent(s) only	68.4%	67.7%	68.0%	75.6%	75.9%	75.8%
Insulin +/- oral hypoglycaemic agent(s)	67.9%	67.5%	67.7%	75.6%	74.4%	75.0%
Participant-reported diabetic retinopathy						
Yes	66.4%	65.9%	66.2%	75.6%	74.9%	75.3%
No	68.5%	67.7%	68.1%	75.6%	75.1%	75.4%
Participant-reported treatment for hypertension						
Yes	67.6%	67.2%	67.4%	76.2%	74.8%	75.5%
No	68.8%	67.5%	68.2%	74.7%	75.5%	75.1%
Systolic blood pressure (mmHg)						
<130	69.8%	70.1%	69.9%	76.9%	76.9%	76.9%
≥130<140	69.9%	69.1%	69.6%	78.1%	76.8%	77.5%
≥140	68.5%	68.3%	68.4%	77.2%	77.4%	77.3%
Unknown	64.9%	62.9%	63.9%	71.4%	70.2%	70.8%
Diastolic blood pressure (mmHg)						
<75	79.9%	80.3%	80.1%	88.2%	87.7%	87.9%
≥75 <85	70.6%	69.9%	70.3%	78.1%	78.0%	78.1%
≥85	68.4%	69.4%	68.9%	76.6%	77.8%	77.2%
Unknown	65.0%	62.9%	63.9%	71.4%	70.2%	70.8%
Body mass index (kg/m2)						
<25	68.6%	69.2%	68.9%	77.1%	76.0%	76.6%
≥25, <30	68.6%	68.3%	68.5%	76.2%	76.5%	76.3%
≥30<35	68.2%	66.5%	67.3%	74.9%	74.9%	74.9%
≥35	66.9%	65.7%	66.3%	74.9%	72.9%	73.9%
Unknown	58.8%	63.8%	61.4%	69.5%	60.0%	64.8%
Cigarette smoking						
Current	60.7%	61.3%	60.9%	67.3%	67.9%	67.6%
Former	67.4%	67.3%	67.4%	76,2%	75.3%	75.8%
Never	69.9%	68.5%	69.2%	76.5%	76.1%	76.3%
Unknown	70.6%	64.1%	67.3%	74.2%	79.9%	77.1%
Non-study medication						
ACE-inhibitor or ARB	67.4%	67.6%	67.5%	76.5%	74.8%	75.7%
Aspirin use before screening	70.8%	69.5%	70.1%	79.3%	78.1%	78.7%
Thiazide or related diuretic	68.9%	67.7%	68.3%	76.3%	74.9%	75.7%
Calcium channel blocker	67.5%	67.1%	67.3%	76.8%	76.5%	76.7%
Statin	69.7%	68.2%	68.9%	76.9%	76.6%	76.7%

Baseline Characteristic	Aspirin Arm			Omega-3 FAs Arm		
	Active (n =7740)	Placebo (n=7740)	Overall (n=15,480)	Active (n=7740)	Placebo (n=7740)	Overall (n=15,480)
Total cholesterol (mmol/L)						
<4	71.1%	69.4%	70.3%	78.6%	78.4%	78.5%
≥4 <5	69.2%	68.3%	68.8%	77.1%	76.9%	76.9%
≥5	64.1%	65.2%	64.7%	72.3%	71.1%	72.2%
Not available	65.9%	65.4%	65.7%	73.1%	71.9%	72.5%
HDL cholesterol (mmol/L)						
<1	80.7%	80.6%	80.7%	89.5%	87.9%	88.7%
≥1<1.5	68.7%	68.1%	68.4%	78.9%	76.9%	77.9%
≥1.5	69.6%	70.1%	69.8%	77.7%	76.8%	77.2%
Not available	65.9%	65.5%	65.7%	73.1%	72.0%	72.6%
Non-HDL cholesterol (mmol/L)						
<2.5	70.8%	70.2%	70.5%	78.9%	78.3%	78.6%
≥2.5 <3.5	70.3%	68.1%	69.2%	77.0%	77.8%	77.4%
≥3.5	64.6%	65.5%	65.1%	74.1%	72.3%	73.2%
Not available	65.9%	65.5%	65.7%	73.1%	72.0%	72.6%
Glycosylated haemoglobin - HbA1c (IFCC mmol/mol (DCCT %))						
<48 (6.5)	69.3%	67.4%	68.4%	78.1%	76.1%	77.1%
≥48 (6.5), <64 (8.0)	69.9%	69.7%	69.8%	77.1%	78.3%	77.7%
≥64 (8.0)	67.9%	66.6%	67.3%	75.1%	74.8%	74.9%
Not available	65.9%	65.5%	65.7%	73.1%	71.9%	72.5%
CKD-EPI estimated GFR (ml/min/1.73m2)						
≥90	62.1%	61.5%	61.8%	71.8%	70.7%	71.2%
≥60<90	67.8%	67.4%	67.6%	76.9%	76.2%	76.6%
<60	72.4%	70.9%	71.6%	78.4%	79.0%	78.7%
Not available	65.7%	65.5%	65.7%	73.1%	71.9%	72.5%
Urinary albumin:creatinine ratio (mg/mmol)						
<3	69.9%	68.8%	69.4%	77.3%	77.3%	77.3%
≥3	64.6%	65.2%	64.9%	75.6%	75.11%	75.4%
Not available	65.8%	65.4%	65.6%	73.1%	71.7%	72.4%
Townsend Deprivation Index						
<-3	68.8%	67.7%	68.2%	77.7%	75.8%	76.7%
≥-3<0	68.9%	68.2%	68.5%	76.1%	75.7%	75.9%
≥0<2	68.6%	68.2%	68.4%	74.5%	75.9%	75.1%
≥2<4	64.3%	63.1%	63.7%	70.5%	72.5%	71.5%
≥4<6	64.3%	63.1%	63.7%	71.7%	70.9%	71.3%
≥6	59.9%	63.5%	61.9%	69.7%	66.9%	68.4%
Unknown	60.5%	82.5%	70.8%	70.9%	72.6%	71.7%
Ethnic origin						
White	68.1%	67.4%	67.8%	75.8%	75.2%	75.5%
Indian/Pakistani/Bangladeshi	71.9%	65.6%	68.8%	73.5%	78.3%	75.9%
African/Caribbean	55.0%	57.8%	56.4%	57.9%	68.0%	62.9%
Other/unknown	67.4%	67.6%	67.5%	73.2%	72.9%	73.1%

Criteria used to define definite or probable adherence:

- Definitely adherent = participant reported taking treatment every- or most days during the follow-up period.
- Probably adherent = participant was previously adherent, was still receiving medication and had not reported stopping treatment within the last seven months.

Table 45 Use of Anti-platelet or Anti-coagulant Therapy in the Aspirin Arm during Follow-Up (Cohort A)

	Aspirin Arm				
	Active (n=7740)			Placebo (n=7740)	
	Study aspirin	Study aspirin or non-study anti-platelet	Study aspirin or non-study anti-platelet agent or anti-coagulant	Non-study anti-platelet	Non-study anti-platelet agent or anti-coagulant
Years post-randomisation					
<3	82.0%	85.3%	85.8%	4.1%	4.6%
≥3<5	66.0%	73.4%	74.9%	8.8%	10.5%
≥5<7	56.2%	65.9%	68.3%	11.0%	13.9%
≥7	46.2%	59.1%	61.9%	12.9%	16.3%
All	**68.0%**	**74.9%**	**76.4%**	**7.9%**	**9.6%**

Table 46 Reasons for Stopping Treatment (Cohort A)

Reasons for Stopping Treatment	Aspirin Arm						Omega-3 FAs Arm					
	Active (n=7740)		Placebo (n =7740)		Overall (n=15,480)		Active (n=7740)		Placebo (n=7740)		Overall (n=15,480)	
Participant wishes	1372	(35.8%)	1424	(36.4%)	2796	(36.1%)	1755	(60.9%)	1778	(60.5%)	3533	(60.7%)
Minor bleeding/bruising	115	(3.0%)	72	(1.8%)	187	(2.4%)	8	(0.3%)	4	(0.1%)	12	(0.2%)
Major Bleeding	42	(1.1%)	24	(0.6%)	66	(0.9%)	8	(0.3%)	7	(0.2%)	15	(0.3%)
Aspirin or anti-platelet therapy prescribed by a doctor (clear indication)	630	(16.5%)	748	(19.1%)	1378	(17.8%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Aspirin or anti-platelet therapy prescribed by a doctor (no clear indication)	257	(6.7%)	241	(6.2%)	498	(6.4%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Other contraindicated drugs started	249	(6.5%)	267	(6.8%)	516	(6.7%)	15	(0.5%)	12	(0.4%)	27	(0.5%)
Upper GI symptoms/diagnoses/procedures	195	(5.1%)	205	(5.2%)	400	(5.2%)	88	(3.1%)	81	(2.8%)	169	(2.9%)
Lower GI symptoms/diagnoses/procedures	74	(1.9%)	50	(1.3%)	124	(1.6%)	55	(1.9%)	84	(2.9%)	139	(2.4%)
Respiratory or cardiovascular symptoms/diagnoses/procedures	16	(0.4%)	23	(0.6%)	39	(0.5%)	11	(0.4%)	18	(0.6%)	29	(0.5%)
GU symptoms/diagnoses/procedures	13	(0.3%)	13	(0.3%)	26	(0.3%)	11	(0.4%)	14	(0.5%)	25	(0.4%)
Skin symptoms/diagnoses/procedures	17	(0.4%)	14	(0.4%)	31	(0.4%)	14	(0.5%)	9	(0.3%)	23	(0.4%)
Other symptoms/diagnoses/procedures	312	(8.2%)	341	(8.7%)	653	(8.4%)	305	(10.6%)	333	(11.3%)	638	(10.9%)
Administrative reasons (e.g. moved abroad)	237	(6.2%)	257	(6.6%)	494	(6.4%)	279	(9.7%)	266	(9.1%)	545	(9.4%)
Other miscellaneous	32	(0.8%)	22	(0.6%)	54	(0.7%)	34	(1.2%)	33	(1.1%)	67	(1.2%)
Does not have diabetes	23	(0.6%)	29	(0.7%)	52	(0.7%)	23	(0.8%)	21	(0.7%)	44	(0.8%)
Medical advice other	192	(5.0%)	150	(3.8%)	342	(4.4%)	177	(6.1%)	188	(6.4%)	365	(6.3%)
Difficulties swallowing tablets	14	(0.4%)	14	(0.4%)	28	(0.4%)	73	(2.5%)	72	(2.5%)	145	(2.5%)
Difficulties getting tablets out of blister pack	38	(1.0%)	21	(0.5%)	59	(0.8%)	23	(0.8%)	18	(0.6%)	41	(0.7%)
Total stopped for any reason	**3828**	**(49.5%)**	**3915**	**(50.6%)**	**7743**	**(50.0%)**	**2879**	**(37.2%)**	**2938**	**(38.0%)**	**5817**	**(37.6%)**

Percentages represent the proportion of the total number of participants who stopped treatment for each reason in each column, except for the total row, which represents the proportion of the total population who stopped for any reason. Percentages may not total 100 because of rounding.

4.3.2 Cohort B1 and its Subsets – DESP-Linked Population

In the B1 cohort, the study average adherence with allocated study treatments was estimated to be 71.9% in the aspirin arm and 79.3% in the omega-3 FAs arm, with similar proportions in the active and placebo group assignments in each arm (Table 47). When stratified by baseline characteristics, adherence rates appeared to be slightly lower in older age groups, women, and those with a longer duration of diabetes (Table 48). As with cohort A, adherence with both treatments declined in the later years of follow-up. With respect to the aspirin arm, the use of non-study aspirin, alternative anti-platelet agents or anti-coagulants also increased with time. The proportion who dropped into treatment in the placebo group of the aspirin arm was 7.3% for non-study anti-platelet therapy and 9.1% for non-study anti-platelet or anti-coagulant therapy (Table 49). Like cohort A, the "Participant's wishes" category was the main reason for stopping either study treatment (Table 50). Finally, there was a similar study average adherence between the treatment groups of each arm in the B2-B7 subsets (Table 51).

Table 47 Reported Definite or Probable Adherence with Study Treatment Stratified by Years Post-Randomisation (Cohort B1)

	Aspirin Arm			Omega-3 FAs Arm		
	Active (%)	**Placebo (%)**	**Overall (%)**	**Active (%)**	**Placebo (%)**	**Overall (%)**
Years post-randomisation						
<3	83.2%	83.6%	83.4%	87.9%	88.5%	87.6%
≥3<5	68.1%	67.4%	67.8%	76.5%	76.3%	76.4%
≥5<7	59.9%	57.6%	58.8%	69.6%	70.1%	69.8%
≥7	53.0%	49.8%	51.4%	62.2%	65.2%	63.7%
All	**72.3%**	**71.6%**	**71.9%**	**79.3%**	**79.3%**	**79.3%**

Criteria used to define definite or probable adherence:

- Definitely adherent = participant reported taking treatment every or most days during the follow-up period.
- Probably adherent = participant was previously adherent, was still receiving medication and had not reported stopping treatment within the last seven months.

Table 48 Study Average Reported Definite or Probable Adherence in each Treatment Arm, Overall and by Baseline Characteristics (Cohort B1)

	Aspirin Arm			Omega-3 FAs Arm		
Baseline Characteristic	**Active (n =3687)**	**Placebo (n=3673)**	**Overall (n=7360)**	**Active (n=3692)**	**Placebo (n=3668)**	**Overall (n=7360)**
Age at randomisation (years)						
<60	74.0%	73.6%	73.8%	78.3%	79.4%	78.8%
≥60<70	73.4%	72.3%	72.9%	82.0%	80.7%	81.3%
≥70	67.2%	67.2%	67.2%	76.2%	76.5%	76.3%
Sex						
Male	73.5%	73.2%	73.4%	80.9%	80.9%	80.9%
Female	70.3%	69.0%	69.7%	76.6%	76.5%	76.6%
Type of diabetes						
Type 1	76.2%	75.1%	75.7%	79.3%	78.4%	78.9%
Type 2	72.0%	71.4%	71.7%	79.3%	79.3%	79.3%
Duration of diabetes (years)						
≥0<5 years	74.5%	73.0%	73.8%	81.2%	80.6%	80.9%
≥5<10 years	70.6%	72.5%	71.5%	79.4%	78.6%	78.9%
≥10<20 years	71.7%	71.5%	71.6%	78.7%	80.3%	79.5%
≥20 years	71.5%	71.5%	71.5%	78.6%	77.7%	78.1%
Unknown	71.9%	59.8%	65.9%	71.1%	73.9%	72.5%
Diabetes management						
Diet only	71.4%	69.9%	70.7%	79.7%	77.9%	78.8%
Oral hypoglycaemic agent(s) only	71.8%	71.7%	71.7%	79.4%	79.2%	79.3%
Insulin +/- oral hypoglycaemic agent(s)	74.1%	72.8%	73.5%	78.9%	80.4%	79.7%
Participant-reported diabetic retinopathy						
Yes	71.8%	71.2%	71.5%	79.5%	80.3%	79.9%
No	72.4%	71.7%	72.1%	79.3%	79.1%	79.2%
Participant-reported treatment for hypertension						
Yes	71.7%	71.4%	71.6%	80.0%	79.1%	79.6%
No	73.1%	71.9%	72.5%	78.2%	79.6%	78.9%
Systolic blood pressure (mmHg)						
<130	72.7%	74.3%	73.5%	80.7%	80.1%	80.4%
≥130<140	75.7%	72.5%	74.1%	81.7%	81.1%	81.4%
≥140	73.2%	72.8%	72.9%	81.3%	81.4%	81.4%
Unknown	68.3%	67.4%	67.9%	74.1%	74.7%	74.4%
Diastolic blood pressure (mmHg)						
<75	72.9%	72.6%	72.7%	80.4%	80.1%	80.2%
≥75 <85	74.5%	74.7%	74.6%	82.5%	81.5%	81.9%
≥85	73.7%	71.2%	72.5%	80.1%	81.1%	80.6%
Unknown	68.3%	67.3%	67.8%	74.2%	74.8%	74.5%
Body mass index (kg/m2)						
<25	73.3%	72.8%	73.0%	81.6%	79.5%	80.6%
≥25, <30	71.7%	72.2%	71.9%	79.4%	79.9%	79.7%
≥30<35	72.7%	71.9%	72.3%	78.9%	79.4%	79.2%
≥35	72.1%	69.6%	70.8%	78.4%	77.9%	78.2%
Unknown	67.1%	75.1%	71.0%	76.1%	69.9%	73.0%
Cigarette smoking						
Current	67.2%	66.3%	66.8%	72.8%	72.9%	72.8%
Former	70.9%	71.9%	71.4%	79.7%	79.5%	79.6%
Never	74.3%	72.2%	73.3%	80.1%	79.8%	79.9%
Unknown	75.6%	73.6%	74.6%	80.9%	85.9%	83.6%

Baseline Characteristic	Aspirin Arm			Omega-3 FAs Arm		
	Active (n =3687)	Placebo (n=3673)	Overall (n=7360)	Active (n=3692)	Placebo (n=3668)	Overall (n=7360)
Non-study medication						
ACE-inhibitor or ARB	71.5%	71.9%	71.7%	80.2%	78.7%	79.5%
Aspirin use before screening	75.1%	73.6%	74.4%	82.3%	83.1%	82.7%
Thiazide or related diuretic	71.0%	69.6%	70.2%	78.5%	77.8%	78.1%
Calcium channel blocker	69.4%	69.3%	69.3%	78.4%	78.7%	78.5%
Statin	73.7%	72.4%	73.1%	80.4%	80.4%	80.4%
Total cholesterol (mmol/L)						
<4	75.6%	74.3%	74.9%	82.5%	82.4%	82.5%
≥4 <5	73.7%	72.3%	73.0%	80.3%	81.1%	80.7%
≥5	67.1%	69.5%	68.3%	77.9%	73.9%	75.9%
Not available	69.5%	69.3%	69.4%	76.2%	76.4%	76.3%
HDL cholesterol (mmol/L)						
<1	73.6%	72.1%	72.9%	81.4%	80.3%	80.9%
≥1<1.5	73.5%	72.8%	73.1%	80.4%	81.1%	80.7%
≥1.5	74.6%	73.6%	74.1%	82.1%	80.2%	81.2%
Not available	69.4%	69.5%	69.5%	76.2%	76.5%	76.3%
Non-HDL cholesterol (mmol/L)						
<2.5	75.4%	74.9%	75.2%	83.6%	81.8%	82.7%
≥2.5 <3.5	74.8%	72.2%	73.5%	79.9%	82.2%	80.9%
≥3.5	68.3%	70.4%	69.4%	78.9%	75.5%	77.2%
Not available	69.4%	69.5%	69.5%	76.2%	76.5%	76.3%
Glycosylated haemoglobin - HbA1c (IFCC mmol/mol (DCCT %))						
<48 (6.5)	73.3%	72.6%	72.9%	82.4%	80.3%	81.3%
≥48 (6.5), <64 (8.0)	74.7%	72.7%	73.7%	79.9%	81.4%	80.7%
≥64 (6.5)	72.2%	73.6%	72.9%	81.3%	79.9%	80.6%
Not available	69.4%	69.3%	69.4%	76.2%	76.4%	76.3%
CKD-EPI estimated GFR (ml/min/1.73m2)						
≥90	66.6%	64.2%	65.4%	73.3%	75.8%	74.6%
≥60<90	72.4%	73.1%	72.7%	80.9%	80.7%	80.8%
<60	76.7%	75.0%	75.9%	83.1%	82.0%	82.5%
Not available	69.4%	69.3%	69.4%	76.2%	76.5%	76.3%
Urinary albumin:creatinine ratio (mg/mmol)						
<3	74.1%	73.1%	73.6%	80.9%	81.1%	81.0%
≥3	71.8%	69.2%	70.6%	80.6%	78.9%	79.8%
Not available	69.3%	69.7%	69.5%	76.4%	76.2%	76.3%
Townsend Deprivation Index						
<-3	72.3%	71.9%	72.1%	81.3%	78.8%	80.0%
≥-3<0	72.2%	72.6%	72.4%	79.1%	79.8%	79.5%
≥0<2	75.3%	71.5%	73.5%	79.2%	81.3%	80.2%
≥2<4	68.9%	67.4%	68.2%	74.9%	76.3%	75.6%
≥4<6	70.1%	68.9%	69.5%	77.4%	76.9%	77.2%
≥6	66.3%	66.4%	66.3%	70.8%	77.2%	73.7%
Unknown	74.9%	87.1%	80.3%	76.4%	86.6%	80.3%
Ethnic origin						
White	72.3%	71.8%	72.0%	79.6%	79.4%	79.5%
Indian/Pakistani/Bangladeshi	79.5%	69.1%	74.4%	79.2%	80.2%	79.7%
African/Caribbean	68.5%	60.9%	64.0%	64.2%	75.3%	70.0%
Other/unknown	67.5%	71.2%	69.4%	71.0%	73.8%	72.4%

Criteria used to define definite or probable adherence:

- Definitely adherent = participant reported taking treatment every or most days during the follow-up period.
- Probably adherent = participant was previously adherent, was still receiving medication and had not reported stopping treatment within the last seven months.

Table 49 Use of Anti-platelet or Anti-coagulant Therapy in the Aspirin Arm during Follow-Up (Cohort B1)

	Aspirin Arm				
	Active (n=3687)			Placebo (n=3673)	
	Study aspirin	Study aspirin or non-study anti-platelet	Study aspirin or non-study anti-platelet agent or anti-coagulant	Non-study anti-platelet	Non-study anti-platelet agent or anti-coagulant
Years post-randomisation					
<3	83.2%	86.3%	86.8%	3.8%	4.5%
≥3<5	68.1%	75.3%	77.0%	8.5%	10.7%
≥5<7	59.9%	69.4%	71.7%	11.3%	14.7%
≥7	53.0%	66.3%	68.4%	13.5%	17.5%
All	**72.3%**	**78.5%**	**79.8%**	**7.3%**	**9.1%**

Table 50 Reasons for Stopping Treatment (Cohort B1)

Reasons for Stopping Treatment	Aspirin Arm						Omega-3 FAs Arm					
	Active (n=3687)		Placebo (n =3673)		Overall (n=7360)		Active (n =3692)		Placebo (n =3668)		Overall (n=7360)	
Participant wishes	608	(35.3%)	619	(35.3%)	1227	(35.3%)	768	(60.2%)	786	(60.0%)	1554	(60.1%)
Minor bleeding/bruising	50	(2.9%)	32	(1.8%)	82	(2.4%)	29	(2.3%)	40	(3.1%)	69	(2.7%)
Major Bleeding	19	(1.1%	8	(0.5%)	27	(0.8%)	5	(0.4%)	4	(0.3%)	9	(0.3%)
Aspirin or anti-platelet therapy prescribed by a doctor (clear indication)	317	18.4%)	342	(19.5%)	659	(19.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Aspirin or anti-platelet therapy prescribed by a doctor (no clear indication	112	(6.5%)	123	(7.0%)	235	(6.8%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Other contraindicated drugs started	116	(6.7%)	134	(7.6%)	250	(7.2%)	5	(0.4%)	7	(0.5%)	12	(0.5%)
Upper GI symptoms/diagnoses/procedures	80	(4.7%)	97	(5.5%)	177	(5.1%)	39	(3.1%)	35	(2.7%)	74	(2.9%)
Lower GI symptoms/diagnoses/procedures	34	(1.9%)	24	(1.4%)	58	(1.7%)	28	(2.2%)	37	(2.8%)	65	(2.5%)
Respiratory or cardiovascular symptoms/diagnoses/procedures	4	(0.2%)	8	(0.5%)	12	(0.3%)	5	(0.4%)	6	(0.5%)	11	(0.4%)
GU symptoms/diagnoses/procedures	4	(0.2%)	9	(0.5%)	13	(0.4%)	5	(0.4%)	7	(0.5%)	12	(0.5%)
Skin symptoms/diagnoses/procedures	7	(0.4%)	7	(0.4%)	14	(0.4%)	7	(0.5%)	5	(0.4%)	12	(0.5%)
Other symptoms/diagnoses/procedures	138	(8.0%)	143	(8.2%)	281	(8.1%)	127	(10.0%)	135	(10.3%)	262	(10.1%)
Administrative reasons (e.g. moved abroad)	91	(5.3%)	90	(5.1%)	181	(5.2%)	105	(8.2%)	98	(7.5%)	203	(7.8%)
Other miscellaneous	15	(0.9%)	9	(0.5%)	24	(0.7%)	11	(0.9%)	13	(1.0%)	24	(0.9%)
Does not have diabetes	11	(0.6%)	16	(0.9%)	27	(0.8%)	11	(0.9%)	10	(0.8%)	21	(0.8%)
Medical advice other	90	(5.2%)	73	(4.2%)	163	(4.7%)	91	(7.1%)	79	(6.0%)	170	(6.6%)
Difficulties swallowing tablets	9	(0.5%)	6	(0.3%)	15	(0.4%)	29	(2.3%)	40	(3.1%)	69	(2.7%)
Difficulties getting tablets out of blister pack	15	(0.9%)	13	(0.7%)	28	(0.8%)	11	(0.9%)	8	(0.6%)	19	(0.7%)
Total stopped for any reason	**1720**	**(46.7%)**	**1753**	**(47.7%)**	**3473**	**(47.2%)**	**1276**	**(34.6%)**	**1310**	**(35.7%)**	**2586**	**(35.1%)**

Percentages represent the proportion of the total number of participants who stopped treatment for each reason in each column, except for the total row, which represents the proportion of the total population who stopped for any reason. Percentages may not total 100 because of rounding.

Table 51 Study Average Definite or Probable Adherence with Study Treatment in Cohorts B2-B7

	Aspirin Arm			Omega-3 FAs Arm		
	Active (%)	Placebo (%)	Overall (%)	Active (%)	Placebo (%)	Overall (%)
Analysis Cohort						
B2 (n=2558)	74.2%	73.3%	73.8%	81.2%	80.0%	80.6%
B3 (n=2852)	73.9%	73.2%	73.5%	80.7%	80.3%	80.5%
B4 (n=7241)	72.3%	71.6%	71.9%	79/5%	79.2%	79.3%
B5 (n=2392)	74.5%	72.9%	73.7%	80.5%	80.7%	80.6%
B6 (n=2829)	73.9%	72.9%	73.4%	80.7%	80.3%	80.5%
B7 (n=7309)	72.3%	71.7%	71.9%	79.5%	79.2%	79.3%

Criteria used to define definite or probable adherence:

- Definitely adherent = participant reported taking treatment every or most days during the follow-up period.
- Probably adherent = participant was previously adherent, was still receiving medication and had not reported stopping treatment within the last seven months.

4.3.3 Cohort C – VFQ Responders

Among the subset of ASCEND participants who took part in the VFQ exercise, the estimated study average adherence with both treatments was higher than in the full randomised cohort: 79.3% in the aspirin arm and 87.9% in the omega-3 FAs arm, with similar proportions in the active and placebo group assignments in each arm (Table 52). As with cohort A, adherence to the study medications declined in the later years of follow-up. When stratified by baseline characteristics, adherence appeared to be higher among younger age groups, men, those with type 1 diabetes, and those who had never smoked (Table 53). In the aspirin arm, the use of non-study aspirin, alternative anti-platelet agents or anti-coagulants increased over time; 6.9% of those assigned in the placebo group were taking a non-study anti-platelet drug, and 8.6% were given a non-study anti-platelet or anti-coagulant (Table 54). Reasons for discontinuing either study medication are shown in Table 55. Compared with cohort A, a slightly higher proportion stopped the study treatment for clinical reasons or a doctor's advice. However, the "Participant wishes" category was still the main reason for most of those who stopped.

Table 52 Reported Definite or Probable Adherence with Study Treatment Stratified by Years Post-Randomisation (Cohort C)

	Aspirin Arm			Omega-3 FAs Arm		
	Active (%)	Placebo (%)	Overall (%)	Active (%)	Placebo (%)	Overall (%)
Years post-randomisation						
<3	89.3%	90.3%	89.5%	93.8%	93.2%	93.5%
≥3<5	78.2%	78.3%	78.2%	87.8%	86.9%	87.4%
≥5<7	70.5%	70.6%	70.5%	83.8%	82.6%	83.2%
≥7	62.9%	64.0%	63.5%	79.6%	78.4%	79.0%
All	**79.3%**	**79.3%**	**79.3%**	**88.4%**	**87.5%**	**87.9%**

Criteria used to define definite or probable adherence:

- Definitely adherent = participant reported taking treatment every or most days during the follow-up period.
- Probably adherent = participant was previously adherent, was still receiving medication and had not reported stopping treatment within the last seven months.

Table 53 Study Average Reported Definite or Probable Adherence in each Treatment Arm, Overall and by Baseline Characteristic (Cohort C)

	Aspirin Arm			Omega-3 FAs Arm		
Baseline Characteristic	**Active (n =4447)**	**Placebo (n=4399)**	**Overall (n=8846)**	**Active (n=4417)**	**Placebo (n=4429)**	**Overall (n=8846)**
Age at randomisation (years)						
<60	82.7%	82.3%	82.5%	89.1%	88.8%	88.9%
≥60<70	77.9%	79.3%	78.6%	88.2%	87.4%	87.8%
≥70	74.9%	73.6%	74.3%	87.1%	84.9%	86.0%
Sex						
Male	80.3%	80.8%	80.5%	89.8%	89.4%	89.6%
Female	77.6%	77.2%	77.4%	86.1%	84.3%	85.2%
Type of diabetes						
Type 1	82.4%	81.3%	81.9%	89.7%	88.3%	89.1%
Type 2	79.0%	79.3%	79.2%	88.3%	87.4%	87.9%
Duration of diabetes (years)						
≥0<5 years	80.9%	80.5%	80.7%	89.4%	88.4%	88.9%
≥5<10 years	77.6%	78.8%	78.2%	87.7%	85.7%	86.7%
≥10<20 years	79.5%	79.4%	79.4%	88.6%	88.8%	88.7%
≥20 years	79.7%	79.2%	79.5%	88.3%	87.8%	88.0%
Unknown	76.3%	76.3$	76.3%	85.2%	85.5%	85.3%
Diabetes management						
Diet only	76.6%	78.3%	77.5%	88.8%	84.4%	86.5%
Oral hypoglycaemic agent(s) only	79.9%	79.3%	79.6%	88.2%	88.3%	88.3%
Insulin +/- oral hypoglycaemic agent(s)	79.4%	80.6%	79.9%	88.5%	87.8%	88.2%
Participant-reported diabetic retinopathy						
Yes	77.5%	79.1%	78.3%	88.5%	87.9%	88.2%
No	79.6%	79.6%	79.6%	88.4%	87.4%	87.9%
Participant-reported treatment for hypertension						
Yes	78.2%	79.1%	78.6%	88.3%	87.3%	87.8%
No	80.8%	79.9%	80.3%	88.5%	87.8%	88.2%
Systolic blood pressure (mmHg)						
<130	79.4%	80.8%	80.1%	88.5%	88.0%	88.2%
≥130<140	81.9%	79.0%	80.4%	89.7%	88.4%	89.1%
≥140	78.0%	79.2%	78.6%	88.5%	88.2%	88.3%
Unknown	78.6%	78.9%	78.8%	87.2%	85.6%	86.4%
Diastolic blood pressure (mmHg)						
<75	78.2%	78.1%	78.1%	87.5%	87.1%	87.3%
≥75 <85	80.8%	80.4%	80.6%	89.7%	88.5%	89.1%
≥85	79.4%	80.7%	80.1%	89.3%	89.7%	89.5%
Unknown	78.6%	78.9%	78.7%	87.3%	85.6%	86.4%
Body mass index (kg/m2)						
<25	80.5%	81.5%	80.9%	89.6%	88.0%	88.8%
≥25, <30	78.4%	79.9%	79.1%	88.3%	87.7%	88.0%
≥30<35	79.9%	79.3%	79.6%	88.1%	88.4%	88.2%
≥35	79.1%	77.4%	78.3%	88.2%	85.9%	87.1%
Unknown	76.5%	80.4%	78.6%	86.1%	73.6%	80.5%
Cigarette smoking						
Current	77.4%	78.2%	77.8%	85.3%	86.7%	86.1%
Former	78.5%	79.5%	78.9%	88.4%	88.2%	88.3%
Never	80.1%	79.8%	79.9%	88.8%	86.9%	87.9%
Unknown	83.4%	70.9%	76.9%	89.6%	87.9%	88.8%

Baseline Characteristic	Aspirin Arm			Omega-3 FAs Arm		
	Active (n =4447)	Placebo (n=4399)	Overall (n=8846)	Active (n=4417)	Placebo (n=4429)	Overall (n=8846)
Non-study medication						
ACE-inhibitor or ARB	78.3%	79.7%	79.0%	88.7%	87.7%	88.2%
Aspirin use before screening	80.0%	80.2%	80.1%	90.1%	88.9%	89.6%
Thiazide or related diuretic	78.6%	79.3%	78.9%	88.2%	86.9%	87.5%
Calcium channel blocker	77.7%	77.7%	77.7%	89.2%	87.9%	88.5%
Statin	80.3%	79.9%	80.1%	88.8%	88.2%	88.5%
Total cholesterol (mmol/L)						
<4	80.5%	78.9%	79.7%	88.6%	89.3%	88.9%
≥4 <5	79.6%	79.9%	79.8%	89.9%	87.5%	88.7%
≥5	77.0%	76.7%	76.9%	86.9%	85.3%	86.1%
Not available	78.5%	80.3%	79.4%	87.5%	86.5%	87.0%
HDL cholesterol (mmol/L)						
<1	80.8%	78.7%	79.8%	90.5%	89.8%	90.2%
≥1<1.5	78.8%	78.6%	78.8%	88.7%	87.4%	88.0%
≥1.5	80.5%	80.2%	80.4%	87.9%	87.9%	87.9%
Not available	78.5%	80.3%	79.4%	87.4%	86.6%	86.9%
Non-HDL cholesterol (mmol/L)						
<2.5	80.5%	80.2%	80.3%	89.3%	88.8%	89.1%
≥2.5 <3.5	80.2%	78.7%	79.5%	88.9%	88.4%	88.6%
≥3.5	77.1%	77.6%	77.3%	88.2%	85.6%	86.9%
Not available	78.5%	80.3%	79.4%	87.4%	86.6%	86.9%
Glycosylated haemoglobin - HbA1c (IFCC mmol/mol (DCCT %))						
<48 (6.5)	78.5%	78.5%	78.5%	89.5%	87.2%	88.3%
≥48 (6.5), <64 (8.0)	79.9%	79.4%	79.6%	88.4%	88.7%	88.5%
≥64 (8.0)	81.6%	79.1%	80.3%	88.9%	87.9%	88.5%
Not available	78.5%	80.2%	79.3%	87.5%	86.5%	87.0%
CKD-EPI estimated GFR (ml/min/1.73m2)						
≥90	73.1%	71.8%	72.5%	84.3%	83.6%	83.9%
≥60<90	77.4%	77.8%	77.6%	88.4%	87.1%	87.8%
<60	82.5%	81.2%	81.9%	90.0%	89.5%	89.7%
Not available	78.5%	80.3%	79.4%	87.5%	86.5%	87.0%
Urinary albumin:creatinine ratio (mg/mmol)						
<3	80.1%	79.6%	79.8%	88.7%	88.3%	88.5%
≥3	76.7%	74.2%	75.4%	89.7%	85.9%	87.9%
Not available	78.4%	80.3%	79.4%	87.5%	86.5%	87.0%
Townsend Deprivation Index						
<-3	78.8%	78.8%	78.8%	88.8%	87.7%	88.2%
≥-3<0	78.4%	79.4%	78.9%	87.8%	86.8%	87.3%
≥0<2	81.4%	80.8%	81.1%	88.7%	88.5%	88.6%
≥2<4	81.3%	79.3%	80.3%	87.1%	88.0%	87.6%
≥4<6	82.9%	80.09%	81.9%	92.1%	89.7%	90.9%
≥6	80.3%	78.8%	79.5%	87.6%	86.7%	87.2%
Unknown	74.2%	94.2%	86.1%	99.2%	86.9%	91.9%
Ethnic origin						
White	79.2%	79.5%	79.3%	88.5%	87.4%	87.9%
Indian/Pakistani/Bangladeshi	81.2%	78.6%	79.9%	83.2%	88.8%	86.0%
African/Caribbean	70.2%	80.1%	75.2%	88.3%	91.7%	90.3%
Other/unknown	88.5%	77.0%	82.6%	86.2%	91.4%	88.2%

Criteria used to define definite or probable adherence:

- Definitely adherent = participant reported taking treatment every or most days during the follow-up period.
- Probably adherent = participant was previously adherent, was still receiving medication and had not reported stopping treatment within the last seven months.

Table 54 Use of Anti-platelet or Anti-coagulant Therapy in the Aspirin Arm during Follow-Up (Cohort C)

	Aspirin Arm				
	Active (n=7740)			Placebo (n=7740)	
	Study aspirin	Study aspirin or non-study anti-platelet	Study aspirin or non-study anti-platelet agent or anti-coagulant	Non-study anti-platelet	Non-study anti-platelet agent or anti-coagulant
Years post-randomisation					
<3	89.5%	92.1%	92.5%	3.3%	3.8%
≥3<5	78.2%	84.6%	86.2%	7.3%	8.9%
≥5<7	70.5%	79.4%	81.9%	9.8%	12.9%
≥7	63.5%	75.9%	78.7%	12.6%	16.4%
All	**79.3%**	**85.5%**	**86.9%**	**6.9%**	**8.6%**

Table 55 Reasons for Stopping Treatment (Cohort C)

Reasons for stopping treatment	Aspirin Arm						Omega-3 FAs Arm					
	Active (n=4447)		Placebo (n =4399)		Overall (n=8846)		Active (n =4417)		Placebo (n =4429)		Overall (n=8846)	
Participant wishes	406	(27.6%)	429	(29.6%)	835	(28.6%)	486	(65.4%)	550	(67.0%)	1036	(66.2%)
Minor bleeding/bruising	59	(4.0%)	46	(3.2%)	105	(3.6%)	4	(0.5%)	3	(0.4%)	7	(0.4%)
Major Bleeding	22	(1.5%)	7	(0.5%)	29	(1.0%)	3	(0.4%)	2	(0.2%)	5	(0.3%)
Aspirin or anti-platelet therapy prescribed by a doctor (clear indication)	347	(23.6%)	381	(26.3%)	728	(24.9%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Aspirin or anti-platelet therapy prescribed by a doctor (no clear indication	138	(9.4%)	126	(8.7%)	264	(9.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Other contraindicated drugs started	140	(9.5%)	152	(10.5%)	292	(10.0%)	7	(0.9%)	10	(1.2%)	17	(1.1%)
Upper GI symptoms/diagnoses/procedures	107	(7.3%)	101	(7.0%)	208	(7.1%)	42	(5.7%)	23	(2.8%)	65	(4.2%)
Lower GI symptoms/diagnoses/procedures	37	(2.5%)	26	(1.8%)	63	(2.2%)	27	(3.6%)	40	(4.9%)	67	(4.3%)
Respiratory or cardiovascular symptoms/diagnoses/procedures	2	(0.1%)	9	(0.6%)	11	(0.4%)	2	(0.3%)	1	(0.1%)	3	(0.2%)
GU symptoms/diagnoses/procedures	5	(0.3%)	6	(0.4%)	11	(0.4%)	4	(0.5%)	6	(0.7%)	10	(0.6%)
Skin symptoms/diagnoses/procedures	8	(0.5%)	6	(0.4%)	14	(0.5%)	4	(0.5%)	3	(0.4%)	7	(0.4%)
Other symptoms/diagnoses/procedures	83	(5.6%)	73	(5.0%)	156	(5.3%)	49	(6.6%)	61	(7.4%)	110	(7.0%)
Administrative reasons (e.g. moved abroad)	3	(0.2%)	4	(0.3%)	7	(0.2%)	2	(0.3%)	5	(0.6%)	7	(0.4%)
Other miscellaneous	4	(0.3%)	7	(0.5%)	11	(0.4%)	6	(0.8%)	6	(0.7%)	12	(0.8%)
Does not have diabetes	13	(0.9%)	17	(1.2%)	30	(1.0%)	8	(1.1%)	14	(1.7%)	22	(1.4%)
Medical advice other	75	(5.1%)	42	(2.9%)	117	(4.0%)	58	(7.8%)	56	(6.8%)	114	(7.3%)
Difficulties swallowing tablets	6	(0.4%)	6	(0.4%)	12	(0.4%)	30	(4.0%)	35	(4.3%)	65	(4.2%)
Difficulties getting tablets out of blister pack	17	(1.2%)	9	(0.6%)	26	(0.9%)	11	(1.5%)	6	(0.7%)	17	(1.1%)
Total stopped for any reason	**1472**	**(33.1%)**	**1447**	**(32.9%)**	**2919**	**(33.0%)**	**743**	**(16.8%)**	**821**	**(18.5%)**	**1564**	**(17.7%)**

Percentages represent the proportion of the total number of participants who stopped treatment for each reason in each column, except for the total row, which represents the proportion of the total population who stopped for any reason. Percentages may not total 100 because of rounding.

4.4 Effects of the Study Treatments on Diabetic Retinopathy and Diabetic Maculopathy

4.4.1 Primary Efficacy Assessment: Referable Disease (Cohort B1)

In total, 899 participants in the B1 cohort developed referable diabetic maculopathy (M_1), and 430 developed referable diabetic retinopathy (R_2 or R_3) components of the primary efficacy outcome during the scheduled treatment period (Table 56). This included some participants with simultaneous M_1 and R_2 or R_3 grades (n=152) and those with one component event followed by the first incidence of the other (n=116); those with a longer duration of remaining follow-up and more DESP records after the first component event had more opportunities to develop its counterpart (Table 56). Therefore, the final number of participants with a composite outcome of referable disease was 1061.

Referable disease occurred in 539 participants [14.6%] in the active group of the aspirin arm compared to 522 participants [14.2%] in the placebo group (rate ratio 1.03; 95% CI: 0.91-1.16; P = 0.64; Figure 26). There was no sign of the rates levelling off over time; at 3, 5 and 7 years from randomisation, 6%, 10% and 14% had referable disease.

There was also no statistically significant between-group difference in the number of referable disease events in the omega-3 FAs arm, which occurred in 548 participants [14.8%] in the active group and in 513 [13.9%] of those assigned to placebo (rate ratio 1.07; 95% CI: 0.95-1.20; P = 0.29; Figure 27).

The proportional effects of each treatment on the primary efficacy outcome in pre-specified subgroups of baseline characteristics did not vary from the overall effect (Figures 28-31 and 32-35, respectively).

Table 56 Incident Referable Disease Events Stratified by Occurrence of Referable Retinopathy or Referable Maculopathy Components of the Primary Outcome

	Number of participants with incident referable disease events (n=1061)	Number of further records after an incident referable disease component event and before the end of the scheduled treatment period						Median number of DESP records after the incident referable disease component event and before the end of the scheduled treatment period (IQR)		Median duration of follow-up after the incident referable disease component event (Years; IQR)	
		0	1	2	3	4	≥5				
R_2 or R_3 only*	162	74	32	14	19	7	16	1.0	(0.0-3.0)	2.8	(1.6-5.1)
M_1 only†	631	227	118	91	87	54	54	1.0	(0.0-3.0)	3.1	(1.6-4.9)
R_2 or R_3 and M_1 at the same time‡	152	67	33	24	14	3	11	1.0	(0.0-2.0)	3.9	(2.1-5.6)
R_2 or R_3 and then M_1§	50	0	15	8	7	7	13	3.0	(1.0-4.8)	4.9	(3.5-5.9)
M_1 and then R_2 or R_3¶	66	0	22	14	12	6	12	2.0	(1.0-4.0)	5.2	(3.9-5.9)

*Number of participants who reached the R_2 or R_3 component of the composite referable disease endpoint and then, irrespective of fluctuations in the R grade, never developed M_1 during the remaining scheduled treatment period.

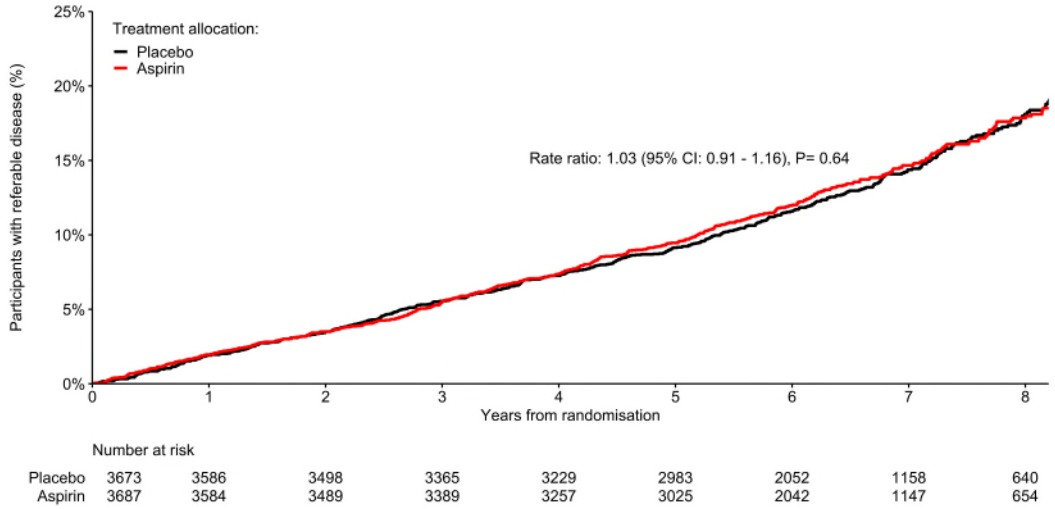
†Number of participants who reached the M_1 component of the composite referable disease endpoint and then, irrespective of fluctuations in their M grade, never developed R_2 or R_3 during the remaining scheduled treatment period.

‡Number of participants who had an incident referable disease event with both retinopathy (R_2 or R_3) and maculopathy (M_1) components simultaneously.

§ Number of participants who reached the R_2 or R_3 component of the composite referable disease endpoint and then, irrespective of fluctuations in their R grade, later developed M_1 at any time and irrespective of its stability during the remaining scheduled treatment period.

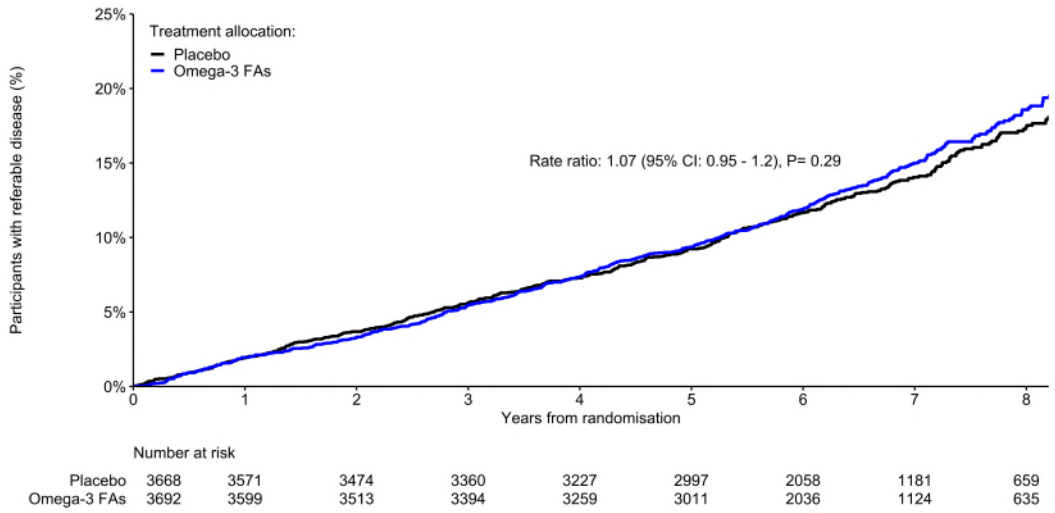
¶ Number of participants who reached the M_1 component of the composite referable disease endpoint and then, irrespective of fluctuations in their M grade, later developed R_2 or R_3 at any time and irrespective of its stability during the remaining scheduled treatment period.

Figure 26 Kaplan-Meier Plot of Time to the First Referable Disease by Aspirin Allocation



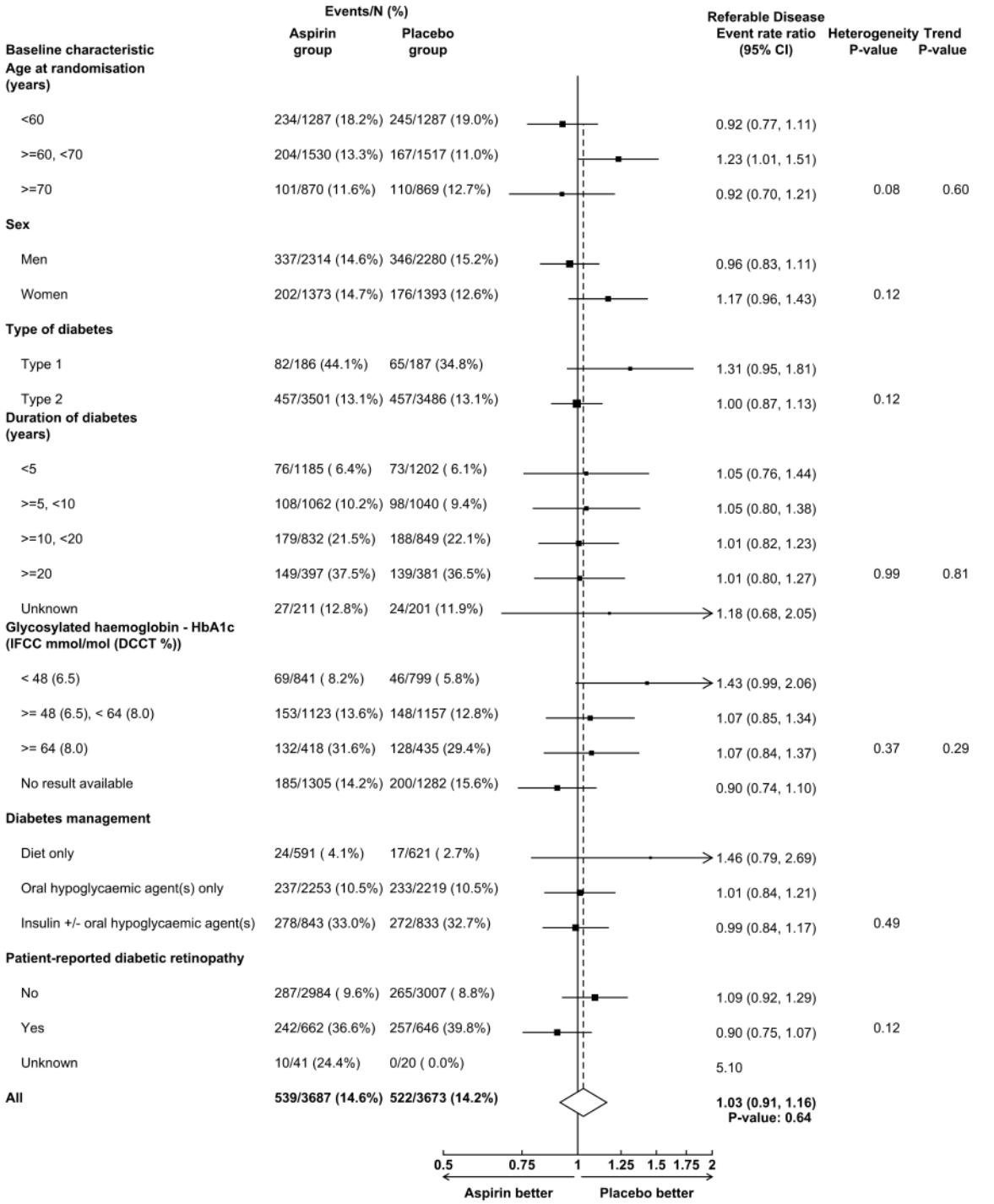
The number of participants at risk at the start of each year of follow-up is shown. The rate ratio is for referable disease among participants in the active aspirin group, compared to those in the placebo group.

Figure 27 Kaplan-Meier Plot of Time to the First Referable Disease by Omega-3 Fatty Acids Allocation



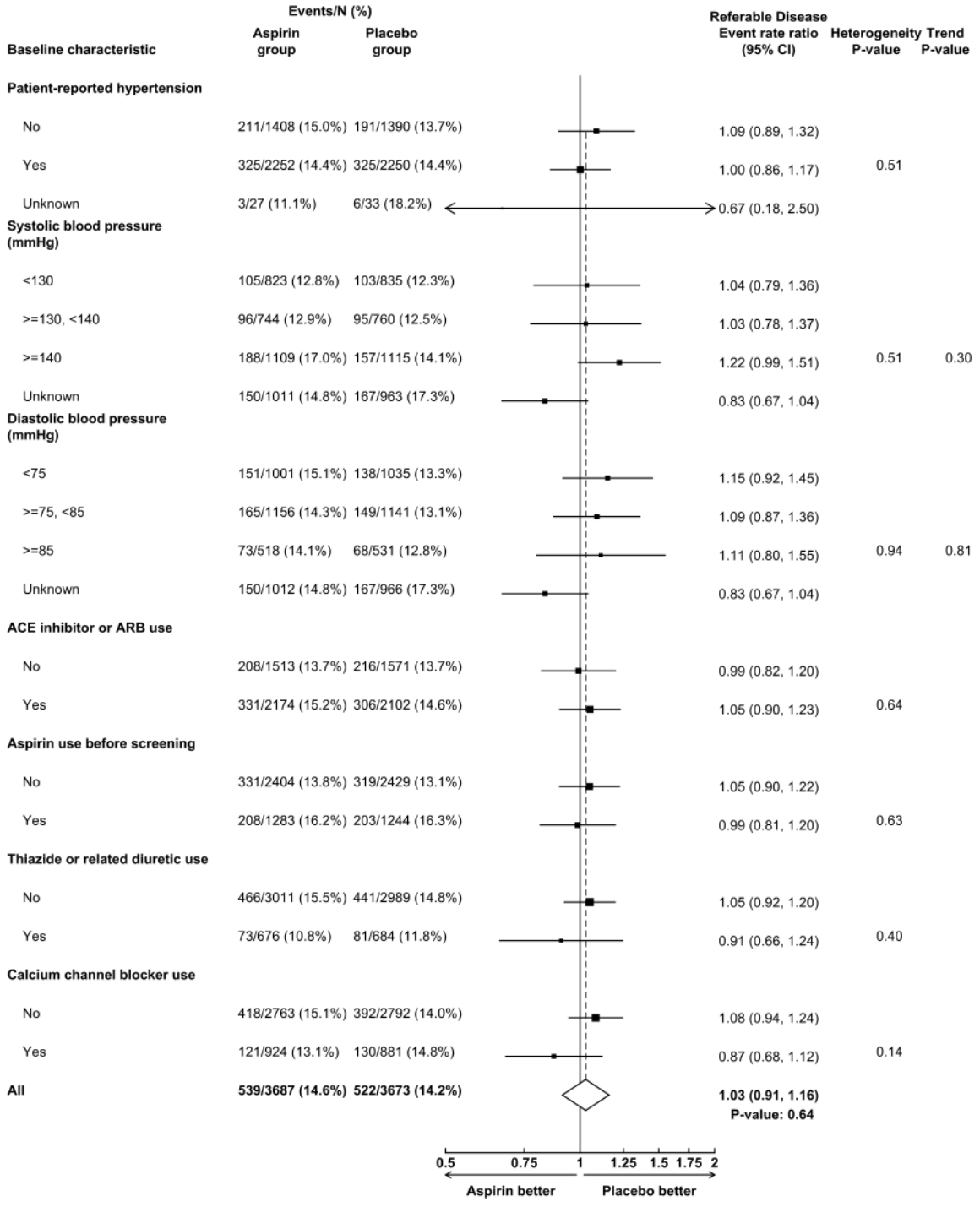
The number of participants at risk at the start of each year of follow-up is shown. The rate ratio is for referable disease among participants in the active omega-3 FAs group, compared to those in the placebo group.

Figure 28 Event Rate Ratios for Referable Disease in the Aspirin Arm by Baseline Characteristic Subgroups: Age, Sex and Diabetes Parameters



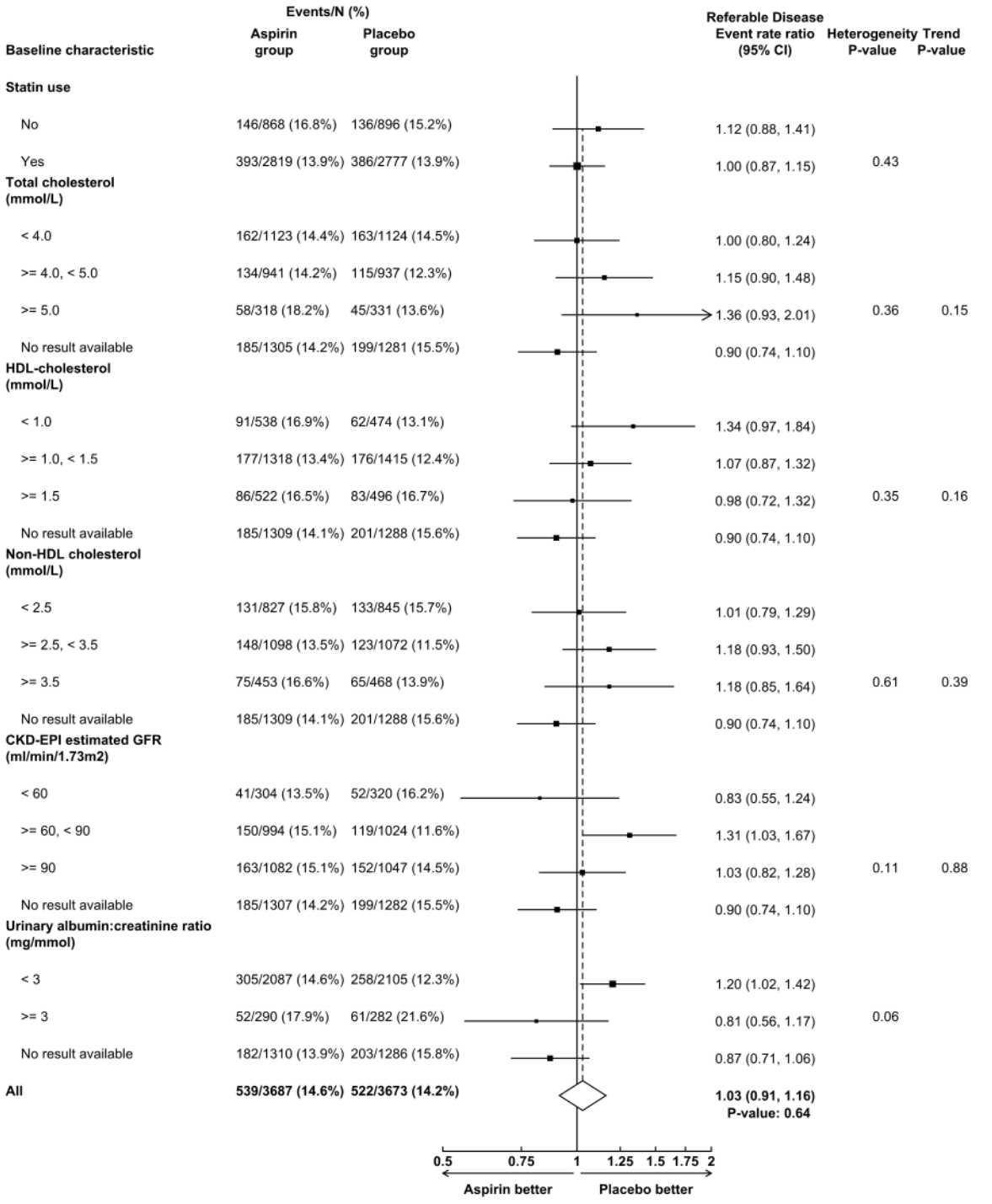
Event rate ratios are plotted as squares, with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to aspirin on the first referable disease. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with aspirin. However, the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line. 95% confidence intervals are not adjusted for multiplicity.

Figure 29 Event Rate Ratios for Referable Disease in the Aspirin Arm by Baseline Characteristic Subgroups: Blood Pressure Parameters



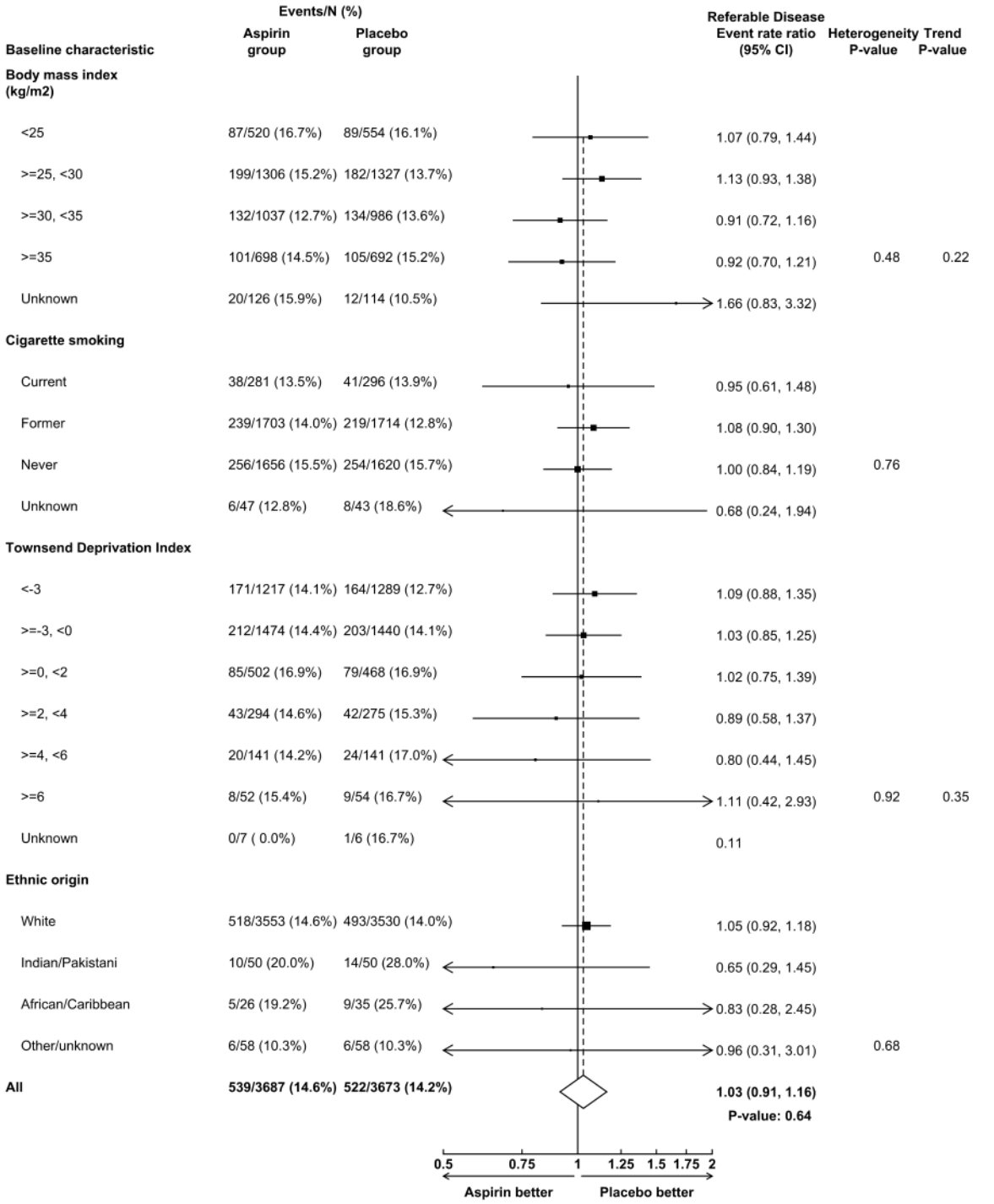
Event rate ratios are plotted as squares, with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to aspirin on the first referable disease. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with aspirin. However, the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line. 95% confidence intervals are not adjusted for multiplicity.

Figure 30 Event Rate Ratios for Referable Disease in the Aspirin Arm by Baseline Characteristic Subgroups: Lipid and Renal Parameters



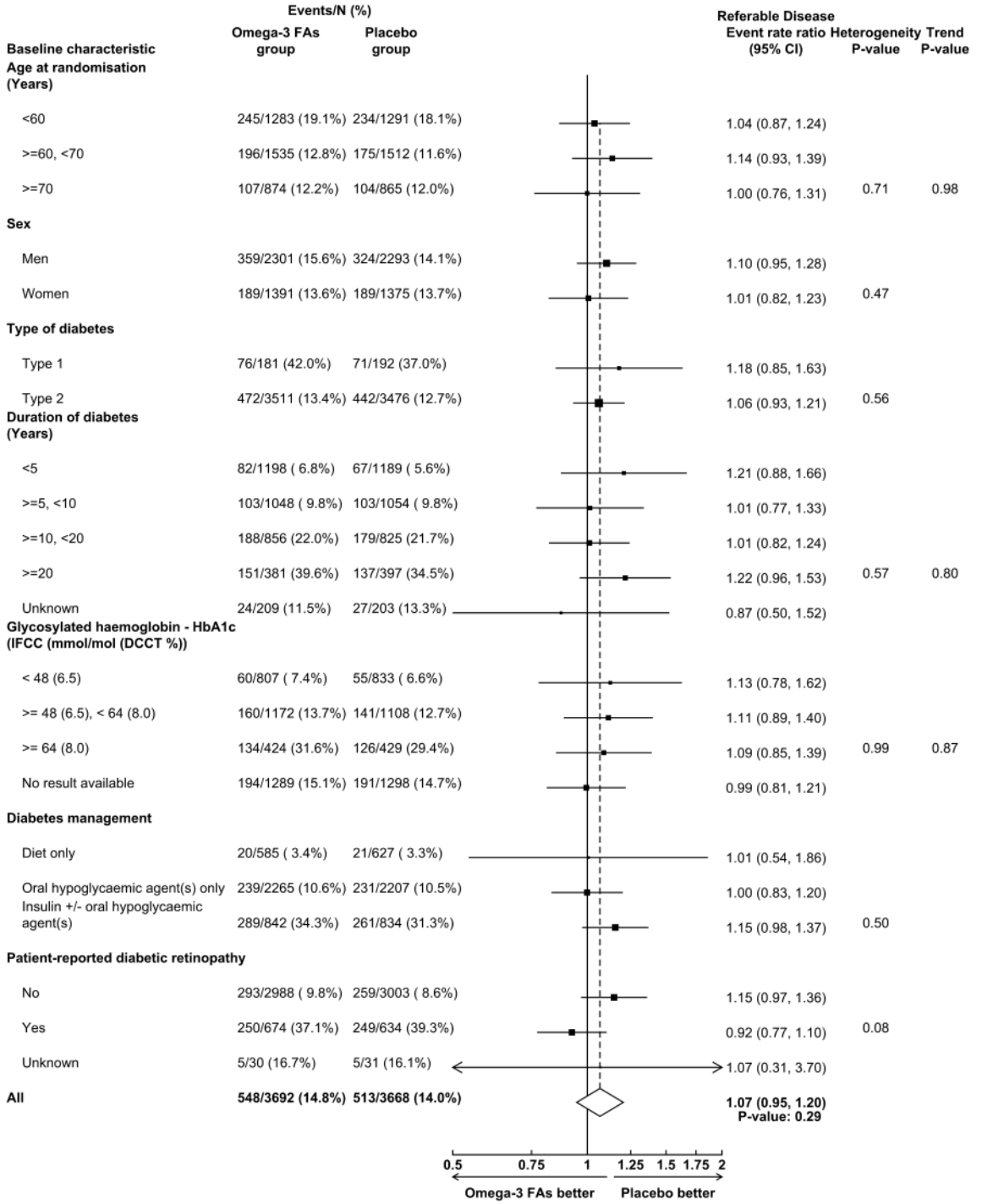
Event rate ratios are plotted as squares, with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to aspirin on the first referable disease. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with aspirin. However, the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line. 95% confidence intervals are not adjusted for multiplicity.

Figure 31 Event Rate Ratios for Referable Disease in the Aspirin Arm by Baseline Characteristic Subgroups: Body Mass Index, Smoking, Deprivation and Ethnicity Parameters



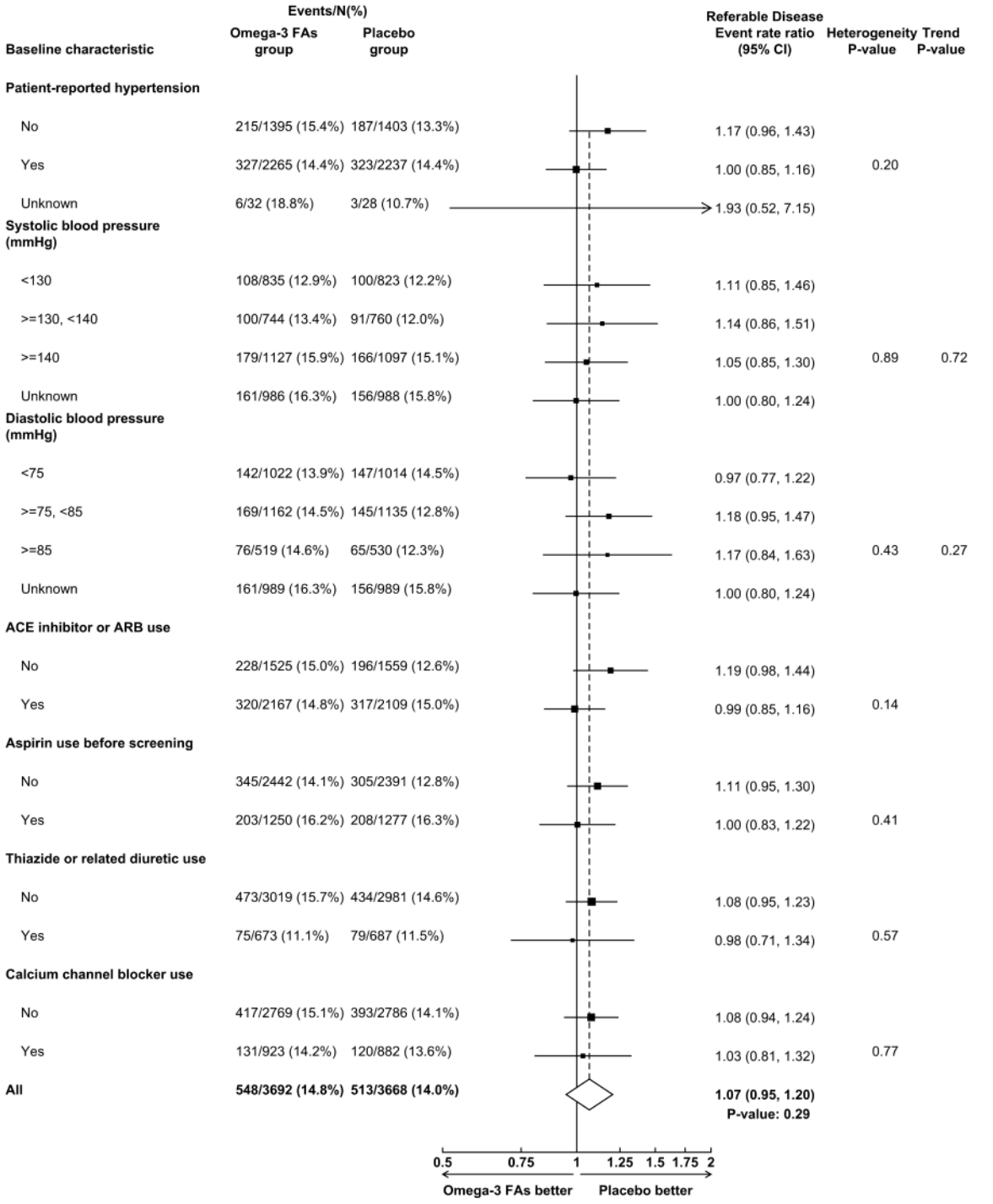
Event rate ratios are plotted as squares, with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to aspirin on the first referable disease. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with aspirin. However, the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line. 95% confidence intervals are not adjusted for multiplicity.

Figure 32 Event Rate Rations for Referable Disease in the Omega-3 Fatty Acids Arm by Baseline Characteristic Subgroups: Age, Sex and Diabetes Parameters



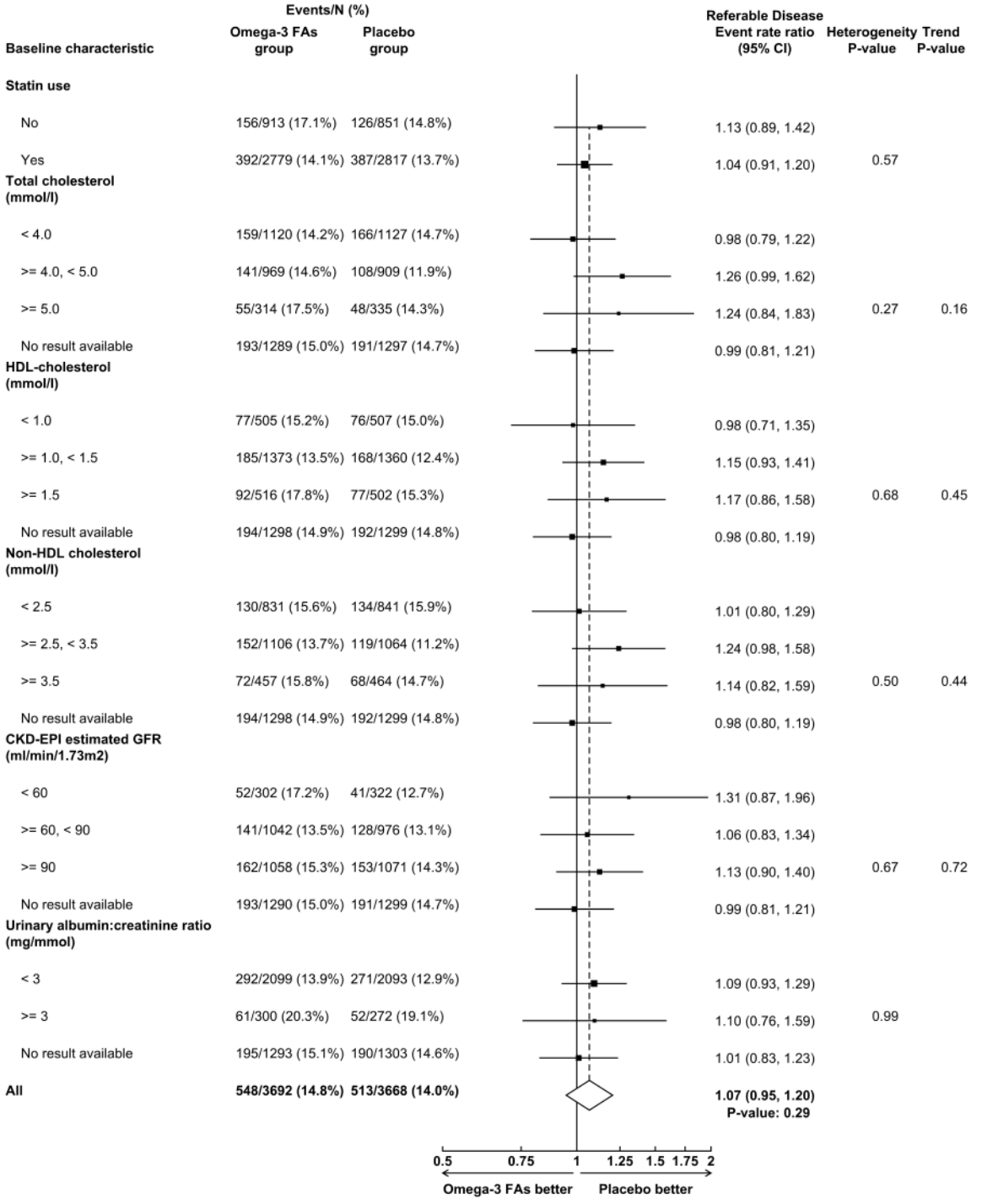
Event rate ratios are plotted as squares, with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to omega-3 FAs on the first referable disease. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with omega-3 FAs. However, the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line. 95% confidence intervals are not adjusted for multiplicity.

Figure 33 Event Rate Ratios for Referable Disease in the Omega-3 Fatty Acids Arm by Baseline Characteristic Subgroups: Blood Pressure Parameters



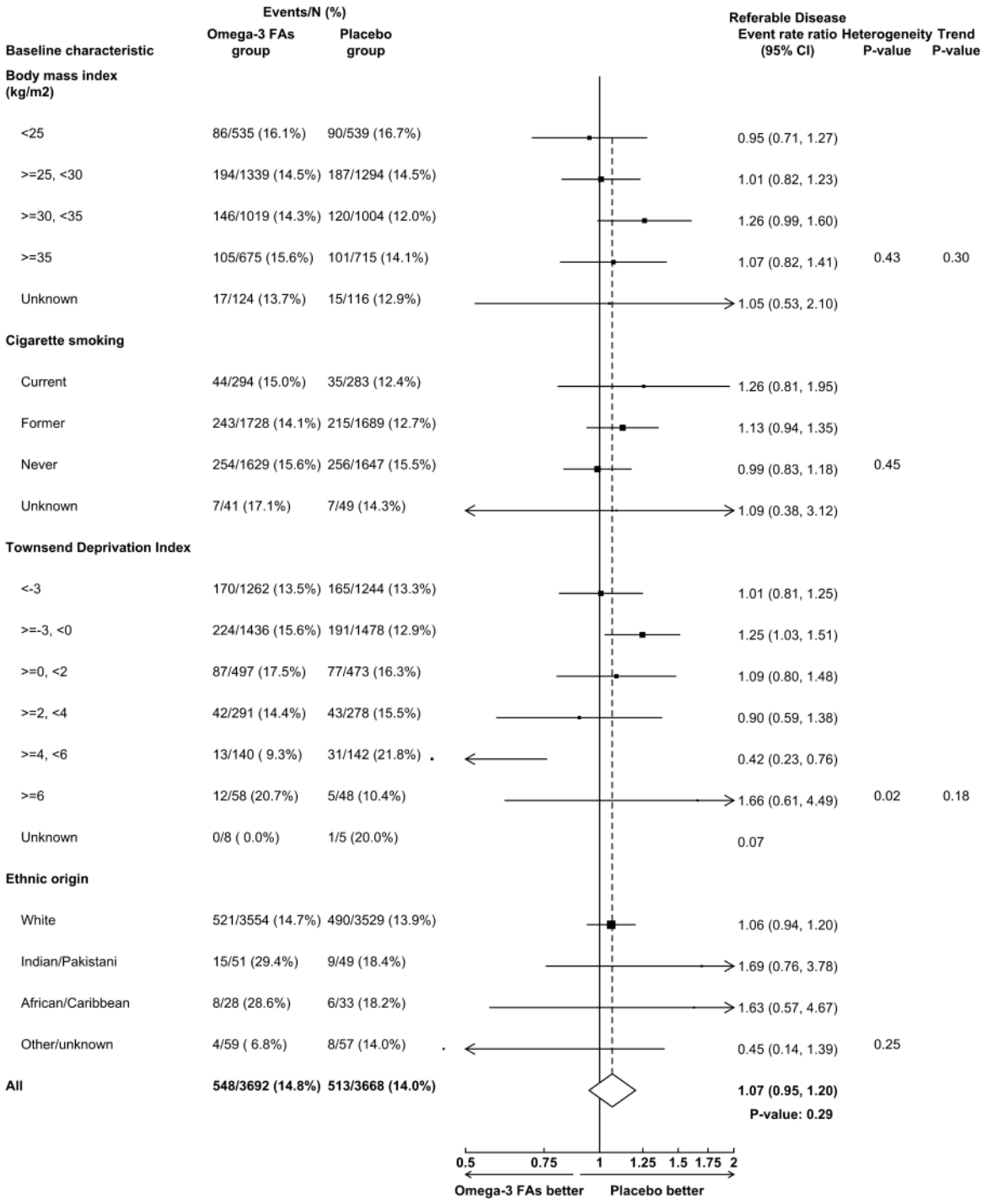
Event rate ratios are plotted as squares, with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to omega-3 FAs on the first referable disease. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with omega-3 FAs. However, the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line. 95% confidence intervals are not adjusted for multiplicity.

Figure 34 Event Rate Ratios for Referable Disease in the Omega-3 Fatty Acids Arm by Baseline Characteristic Subgroups: Lipid and Renal Parameters



Event rate ratios are plotted as squares, with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to omega-3 FAs on the first referable disease. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with omega-3 FAs. However, the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line. 95% confidence intervals are not adjusted for multiplicity.

Figure 35 Event Rate Ratios for Referable Disease in the Omega-3 Fatty Acids Arm by Baseline Characteristic Subgroups: Body Mass Index, Smoking, Deprivation and Ethnicity Parameters



Event rate ratios are plotted as squares, with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to omega-3 FAs on the first referable disease. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with omega-3 FAs. However, the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line. 95% confidence intervals are not adjusted for multiplicity.

4.4.1.1 Post-hoc Exploratory Analyses: Stability of the Referable Disease Events

During the blinded preliminary analyses of the linkage data, the trajectories of some participants' M and R grades were noticed to fluctuate during the scheduled treatment period. The prognostic significance of those with an untreated disease regression is not clear.[8] Therefore, fluctuations in M or R grades were not factored into any of the pre-specified endpoints. After the DAP was made publicly available and the unblinded analyses had already begun, some post-hoc exploratory analyses were undertaken to assess the stability of the composite primary efficacy outcome. Table 57 shows the proportion of participants who developed referable disease and continued to meet the definition until the end of the study ("Progressors"[*]), those with referable disease who sustained a regression until the end of the study ("Regressors"), and those who fluctuated in and out of the primary endpoint for the duration of the study ("Reprogressors"). The table's footnotes describe how each of these was defined in more detail. Data are shown for the composite outcome and by separate R_2 or R_3 and M_1 components.

Out of 430 participants who progressed to $R_2/_3$, 179 (42%) had no further records, 94 (22%) had only one further record (of which a quarter (n=23) regressed back to $R_0/_1$) and 157 (37%) had at least two further records. Of the 157 with at least two further records, 50 (32%) remained at $R_2/_3$, 85 (54%) regressed back to $R_0/_1$ and 22 (14%) regressed and then re-progressed at least once.

Out of 899 participants who progressed to M_1, 315 (35%) had no further records, 188 (21%) had only one further record (of which over a third (n=72) regressed back to M_0) and 396 (44%) had at least two further records. Of the 396 with at least two further records, 86 (22%) remained at M_1, 216 (55%) regressed back to M_0 and 94 (24%) regressed and then re-progressed at least once.

Out of 1061 participants with the composite primary efficacy outcome of referable disease, 368 (35%) had no further records, 220 (21%) had only one further record (of which over a quarter (n=61) regressed) and 473 (45%) had at least two further records. Of the 473 with further records, 134 (28%) continued

[*] Use of the term 'progressors' in this section is unrelated to use of the term 'progression' in the outcome definitions used elsewhere.

to have referable disease, 228 (48%) regressed and 111 (23%) regressed and then re-progressed at least once.

Overall, the stability of each subgroup appeared to be influenced by the number of DESP records after an outcome event and their proximity to the end of the scheduled treatment period; progressors had fewer assessments and a shorter duration of remaining follow-up than the regressors or reprogressors.

Table 57 Number of Further Records and Duration of Follow-up after an Incident Component Event of the Referable Disease Endpoint among Progressors, Regressors and Reprogressors

	Number with incident progressions	Number of further records after progression and before the end of the scheduled treatment period						Median number of DESP records after the incident event and before the end of the scheduled treatment period (IQR)		Median duration of follow-up after the incident event (Years; IQR)	
		0	1	2	3	4	≥5				
R_2 or R_3*											
Progressors	300	179	71	21	14	5	10	0	(0-1)	3.1	(1.6-5.1)
Regressors	108	0	23	28	29	17	11	3	(2-5)	5.2	(3.9-6.6)
Reprogressors	22	0	0	4	6	3	9	4	(3-6)	5.7	(4.3-6.5)
M_1†											
Progressors	517	315	116	47	19	9	11	0	(0-1)	2.7	(1.3-4.8)
Regressors	288	0	72	88	98	21	9	3	(2-4)	4.3	(3.0-5.7)
Reprogressors	94	0	0	19	26	15	34	4	(3-5)	5.3	(3.9-6.6)
M_1, R_2 or R_3											
Progressors	661	368	159	66	34	13	21	0	(0-1)	2.6	(1.2-4.8)
Regressors	289	0	61	85	105	24	14	3	(2-4)	4.2	(3.1-5.7)
Reprogressors	111	0	0	22	31	19	39	4	(3-5)	5.2	(3.9-6.4)

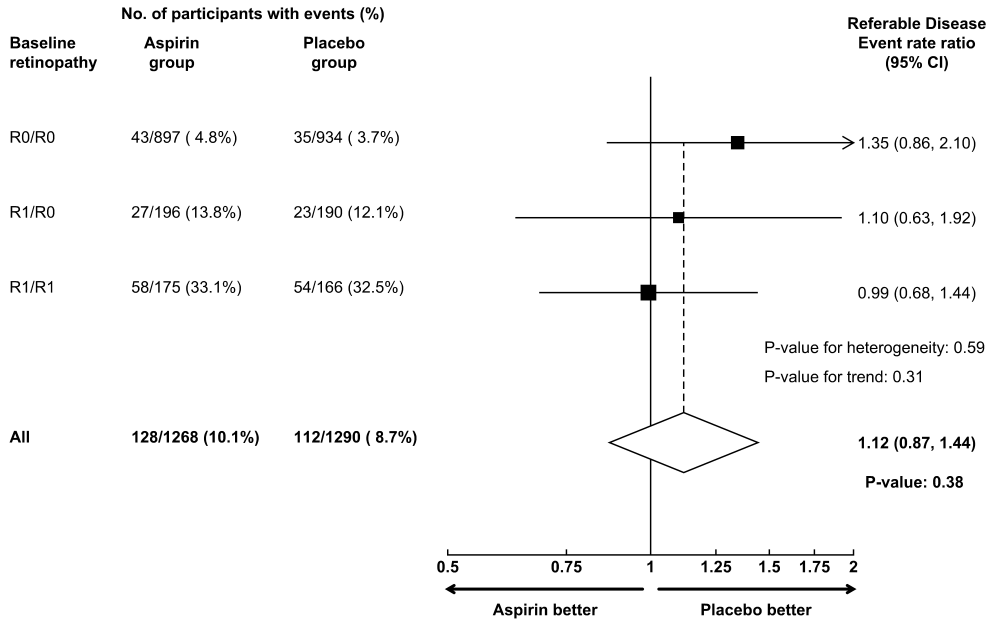
*Progressors were defined as anyone who reached R_2 or R_3 in any eye and remained at this level in subsequent eye screening records until the end of the study. Regressors included those who reached R_2 or R_3 in any eye and returned to R_1 or R_0 in both eyes in subsequent eye screening records until the end of the study (i.e. neither eye met the endpoint definition again). Reprogressors reached R_2 or R_3 in any eye, regressed to R_1 or R_0 in both eyes, and then progressed at least one more time to R_2 or R_3 in any eye on subsequent eye screening records before the end of the study.
†Progressors were defined as anyone who reached M_1 in any eye and remained at this level in subsequent eye screening records until the end of the study. Regressors included those who reached M_1 in any eye and went back to M_0 in both eyes in subsequent eye screening records until the end of the study (i.e. neither eye met the endpoint definition again). Reprogressors were defined as those who reached M_1 in any eye, regressed to M_0 in both eyes, and then progressed at least one more time to M_1 in any eye on subsequent eye screening records before the end of the study.

4.4.2 Secondary Efficacy Assessment: Referable Disease in those with a Baseline Record (Cohort B2)

In analyses of time to referable disease restricted to those with a baseline eye screening record, the overall proportion who developed the composite outcome was lower than in the B1 cohort (240 out of 2558 [9.4%] *vs.* 1061 out of 7360 [14.4%], respectively); consistent with the B1 cohort including some

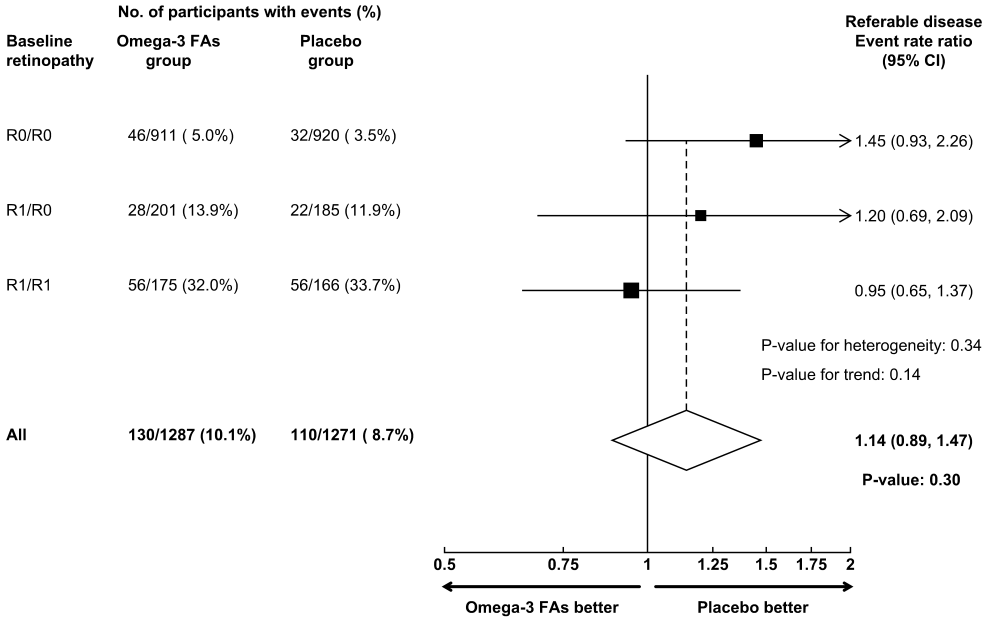
people with unrecorded referable disease at randomisation. This outcome occurred in 128 out of 1268 participants [10.1%] in the active group of the aspirin arm compared to 112 out of 1290 participants [8.7%] in the placebo group (rate ratio 1.12; 95% CI: 0.87-1.44; P = 0.38; Figure 36). The number of events increased with advancing severity of baseline retinopathy from 4.3% in those who were R_0/R_0 to 32.8% in those with R_1/R_1 disease at baseline. Although visually there may appear to be a gradient of decreasing risk of harm from allocation to active aspirin, from the least to most severe baseline retinopathy grade, there was considerable uncertainty around the true effect size given the small number of events in each stratum and, overall, there was no significant linear effect across the subgroups (P for trend = 0.31). Likewise, there was no significant effect heterogeneity between the subgroups (P for heterogeneity = 0.59). There was also no significant difference in this secondary efficacy outcome in the omega-3 FAs arm, which occurred in 130 participants [10.1%] in the active group, and in 110 participants [8.7%] in the placebo group (rate ratio 1.14; 95% CI: 0.89-1.47; P = 0.30; Figure 37). No statistically significant trend or heterogeneity of the effect of allocation to omega-3 FAs was apparent across subgroups of baseline retinopathy grade (P for trend = 0.14; P for heterogeneity = 0.34). Again, there was considerable uncertainty around the true effect size, given the small number of events in each stratum.

Figure 36 Event Rate Ratios for Referable Disease by Aspirin Allocation, in those with Baseline Retinopathy Data in both Eyes and Excluding those with Referable Disease at Baseline



Event rate ratios are plotted as squares with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to aspirin on first referable disease. For totals, the event ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with aspirin, but the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line.

Figure 37 Event Rate Ratios for Referable Disease by Omega-3 Fatty Acids Allocation, in those with Baseline Retinopathy Data in both Eyes and Excluding those with Referable Disease at Baseline

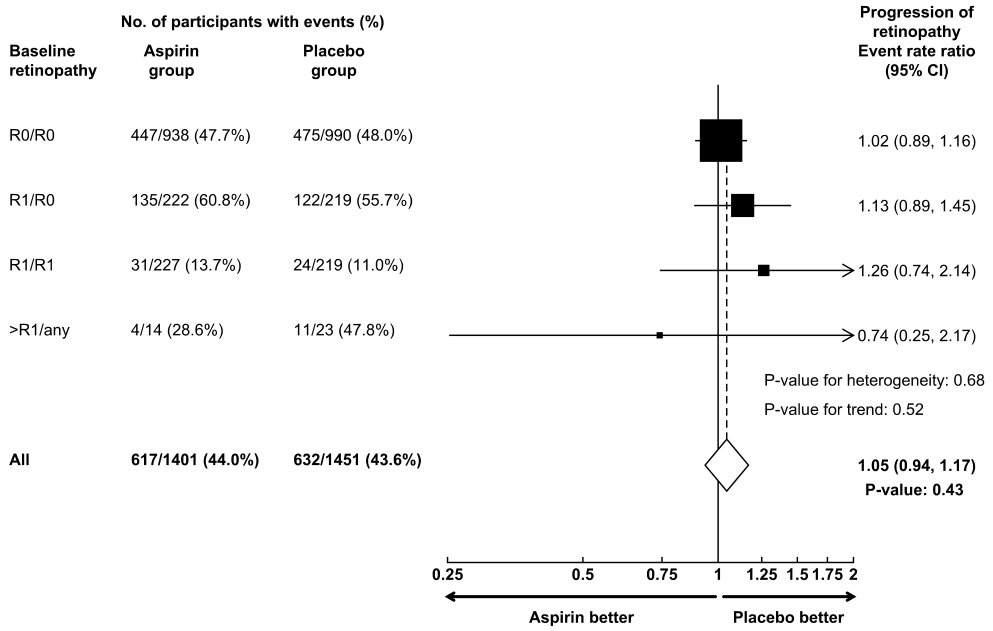


Event rate ratios are plotted as squares with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to omega-3 FAs on first referable disease. For totals, the event ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with omega-3 FAs, but the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line.

4.4.3 Secondary Efficacy Assessment: Progression in Retinopathy Grade (Cohort B3)

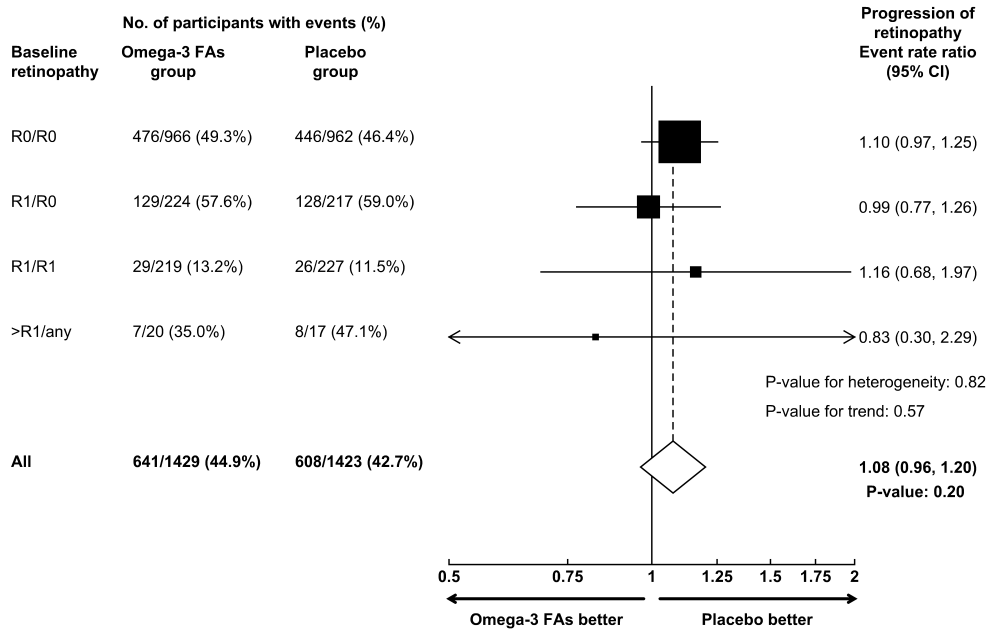
The secondary efficacy outcome of the first progression in retinopathy grade occurred in 617 out of 1401 participants [44.0%] in the active group of the aspirin arm and 632 out of 1451 participants [43.6%] in the placebo group (rate ratio 1.05; 95% CI: 0.94-1.17; P = 0.43; Figure 38). There was no statistically significant linear effect (P for trend = 0.52) or heterogeneity in the effect of allocation to aspirin over subgroups of increasing baseline retinopathy grade (P for heterogeneity = 0.68). There were also no statistically significant between-group differences in the omega-3 FAs arm overall or by subgroups of baseline retinal disease severity. In total, 641 out of 1429 participants [44.9%] in the active group had a progression of their retinopathy grade, compared to 608 out of 1423 participants [42.7%] in the placebo group (rate ratio 1.08; 95% 0.96-1.20; P = 0.20; P for trend = 0.57; P for heterogeneity = 0.82; Figure 39).

Figure 38 Event Rate Ratios for Progression of Retinopathy by Aspirin Allocation in those with Baseline Retinopathy Data in both Eyes, Excluding those who were R_3/R_3 at Baseline



Event rate ratios are plotted as squares with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to aspirin on the first progression of retinopathy. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with aspirin, but the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line.

Figure 39 Event Rate Ratios for Progression of Retinopathy by Omega-3 Fatty Acids Allocation in those with Baseline Retinopathy Data in both Eyes, Excluding those who were R_3/R_3 at Baseline



Event rate ratios are plotted as squares with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to omega-3 FAs on the first progression of retinopathy. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with omega-3 FAs, but the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line.

4.4.4 Tertiary Efficacy Assessment: Final Retinopathy Grade (Cohort B4)

Table 58 shows duplex retinopathy grades at the final record of the scheduled treatment period for 7309 participants, cross-tabulated against a subset of 2854 with bilateral baseline retinopathy grades[*]. The majority of participants had minimal retinopathy at the final eye screening record. Overall, 57.2% of participants in the B4 cohort were graded R_0/R_0, 16.9% R_0/R_1, 21.7% R_1/R_1 and 4.2% $>R_1$/any. There was no statistically significant effect of aspirin allocation on the net severity of final duplex retinopathy grades (common odds ratio for the likelihood of having a higher retinopathy grade with aspirin 0.99; 95% CI: 0.90-1.08; P value = 0.76; Figure 40). Similarly, receiving active omega-3 FAs made no

[*] Table 58 was produced during the blinded preliminary analyses to understand the distribution of disease severity at each time point. The baseline retinopathy grades are not relevant to this tertiary analysis, but they are shown for interest. I am grateful to Ms. George Buck (DPhil supervisor and ASCEND Trial Statistician) who conducted the analyses shown in Table 58.

difference to the likelihood of having a higher duplex retinopathy grade at the final follow-up (common odds ratio 1.05; 95% CI: 0.96-1.15; P = 0.27; Figure 41).

Table 58 Final Retinopathy Grades during the Scheduled Treatment Period

Retinopathy grade at final record (in those with data for both eyes)	No. of participants (%)	Baseline retinopathy grade (in those with data for both eyes)									
		Any baseline	R_0/R_0	R_1/R_0 or R_0/R_1	R_1/R_1	R_2/R_0 or R_0/R_2	R_2/R_1 or R_1/R_2	R_2/R_2	R_3/R_1 or R_1/R_3	R_3/R_2 or R_2/R_3	R_3/R_3
Any	**7309**	**2854**	**1928**	**441**	**446**	**1**	**17**	**13**	**3**	**3**	**2**
R_0/R_0	4184 (57.2%)	1794	1494	213	85	0	2	0	0	0	0
R_1/R_0 or R_0/R_1	1233 (16.9%)	449	266	119	63	0	1	0	0	0	0
R_1/R_1	1584 (21.7%)	532	153	103	267	0	6	2	0	0	1
R_2/R_0 or R_0/R_2	4 (0.1%)	2	2	0	0	0	0	0	0	0	0
R_2/R_1 or R_1/R_2	112 (1.5%)	39	8	4	19	0	5	3	0	0	0
R_2/R_2	81 (1.1%)	23	4	2	9	1	3	4	0	0	0
R_3/R_0 or R_0/R_3	6 (0.1%)	1	0	0	0	0	0	0	1	0	0
R_3/R_1 or R_1/R_3	26 (0.4%)	2	1	0	0	0	0	0	0	1	0
R_3/R_2 or R_2/R_3	23 (0.3%)	4	0	0	1	0	0	2	1	0	0
R_3/R_3	56 (0.8%)	8	0	0	2	0	0	2	1	2	1

Figure 40 Final Retinopathy Grade by Aspirin Allocation

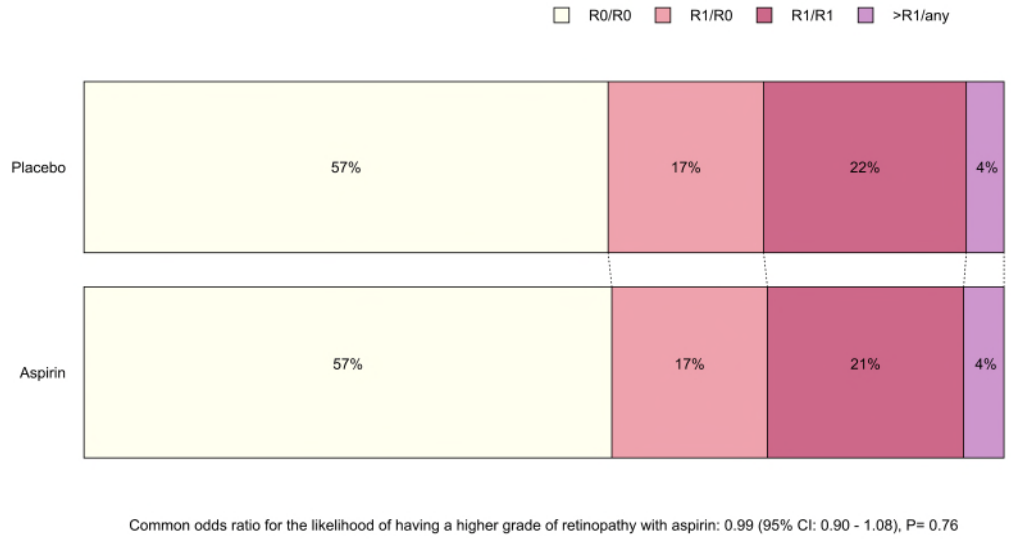
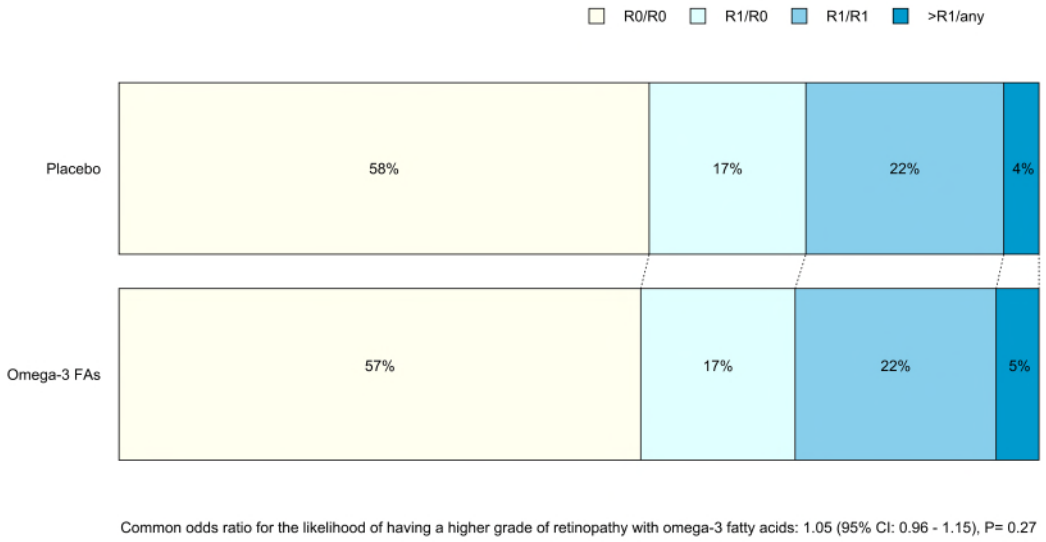


Figure 41 Final Retinopathy Grade by Omega-3 Fatty Acids Allocation



4.4.4.1 Sensitivity Analysis: Final Retinopathy Grade (Cohort B6)

Restricting comparisons of the final retinopathy grade in both eyes to those with a baseline eye screening record reduced the proportion with more severe disease compared to the B4 cohort. Overall, 62.8% of participants in the B6 cohort were graded R_0/R_0, 15.7% R_0/R_1, 18.7% R_1/R_1 and 2.8% $>R_1$/any (not tabulated). In this subgroup, the common odds ratio for the likelihood of having a higher final duplex grade with aspirin was 1.00 (95% CI: 0.87-1.16; P = 0.95; Figure 42), and for omega-3 FAs, it was 1.05 (95% CI: 0.91-1.22; P = 0.51; Figure 43).

Figure 42 Final Retinopathy Grade by Aspirin Allocation Restricted to those with Baseline Retinopathy Data for both Eyes

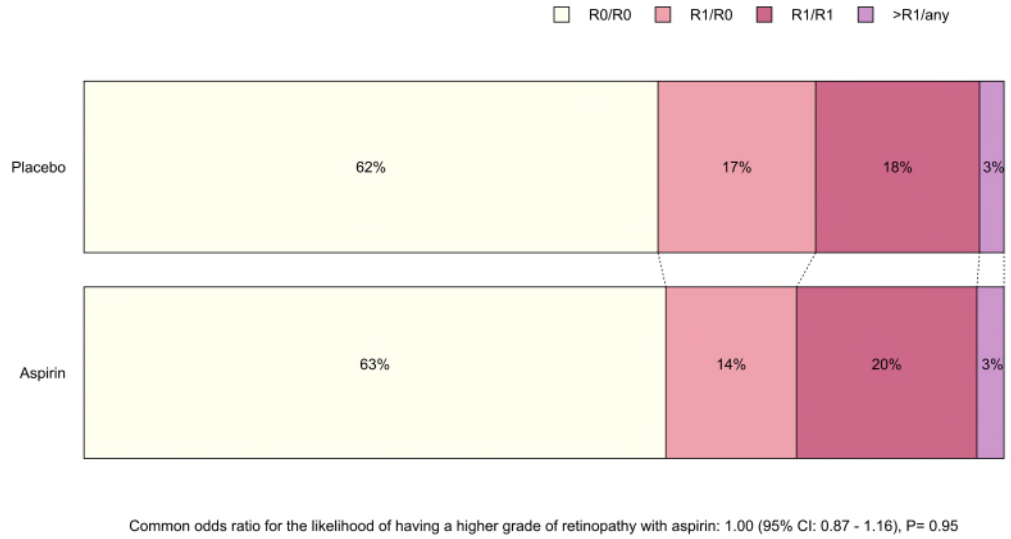
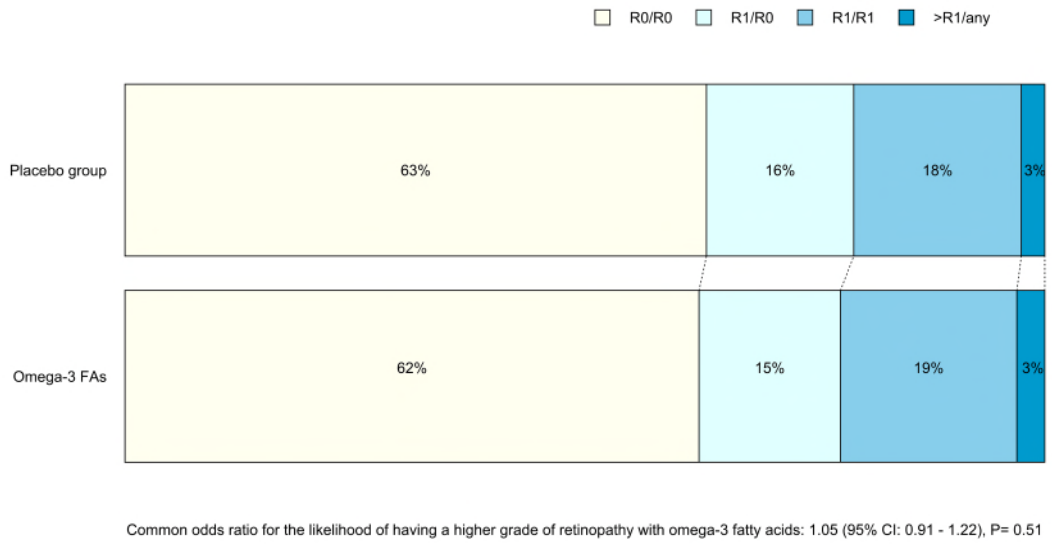


Figure 43 Final Retinopathy Grade by Omega-3 Fatty Acids Allocation Restricted to those with Baseline Retinopathy Data for both Eyes



4.4.4.2 Sensitivity Analysis: Highest Retinopathy Grade Ever Reached (Cohort B7)

The distribution of the highest retinopathy grades ever reached over the scheduled treatment period varied more than the final retinopathy grades, because there was instability of the R grades between screening visits. Overall, 38% of participants in the B7 cohort were graded R_0/R_0, 22.8% R_0/R_1, 33.5%

R_1/R_1 and 5.8% >R_1/any (Table 59)*. In accordance with the design of the NHS service, the frequency of eye screening assessments correlated to the worst retinal disease (Table 60). The common odds ratio for the likelihood of having a higher duplex grade with aspirin was 1.01 (95% CI: 0.93-1.10; P = 0.74; Figure 44), and for omega-3 FAs, it was 1.03 (95% CI: 0.95-1.13; P = 0.43; Figure 45).

* I am grateful to Ms. Georgina Buck (DPhil supervisor and ASCEND Trial Statistician) who conducted the analyses shown in Tables 59 and 60.

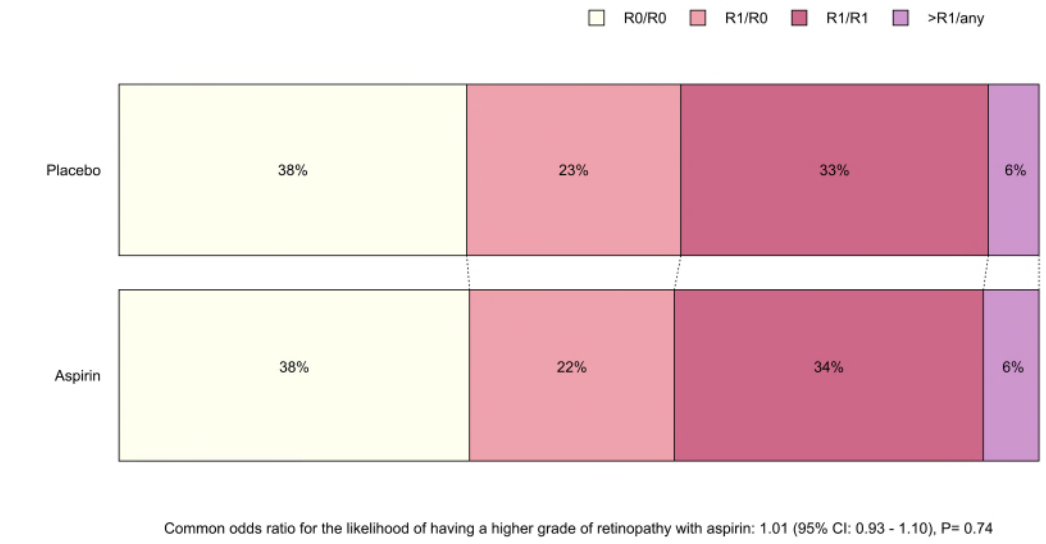
Table 59 Highest Retinopathy Grade Reached during the In-Trial Period

	No. of participants with retinopathy data in both eyes during the in-trial period		Number of records in the scheduled treatment period with retinopathy data in at least one eye					
			1-2	3	4	5	6	7+
Highest retinopathy grade reached during the in-trial period	**7309**		**1315**	**1264**	**1278**	**1024**	**1176**	**1252**
R_0/R_0	2774	(38.0%)	563	513	504	429	436	329
R_1/R_0 or R_0/R_1	1664	(22.8%)	203	277	286	247	334	317
R_1/R_1	2449	(33.5%)	383	417	423	304	370	552
R_2/R_0 or R_0/R_2	9	(0.1%)	3	0	0	2	2	2
R_2/R_1 or R_1/R_2	165	(2.3%)	49	26	21	24	17	28
R_2/R_2	117	(1.6%)	40	16	18	13	11	19
R_3/R_0 or R_0/R_3	5	(0.1%)	2	1	2	0	0	0
R_3/R_1 or R_1/R_3	37	(0.5%)	23	3	7	1	3	0
R_3/R_2 or R_2/R_3	30	(0.4%)	15	4	5	3	1	2
R_3/R_3	59	(0.8%)	34	7	12	1	2	3

Table 60 Correlation between the Worst Retinopathy Grade and Number of Records during the Scheduled Treatment Period (Overall and Per Year) and Length of Scheduled Treatment

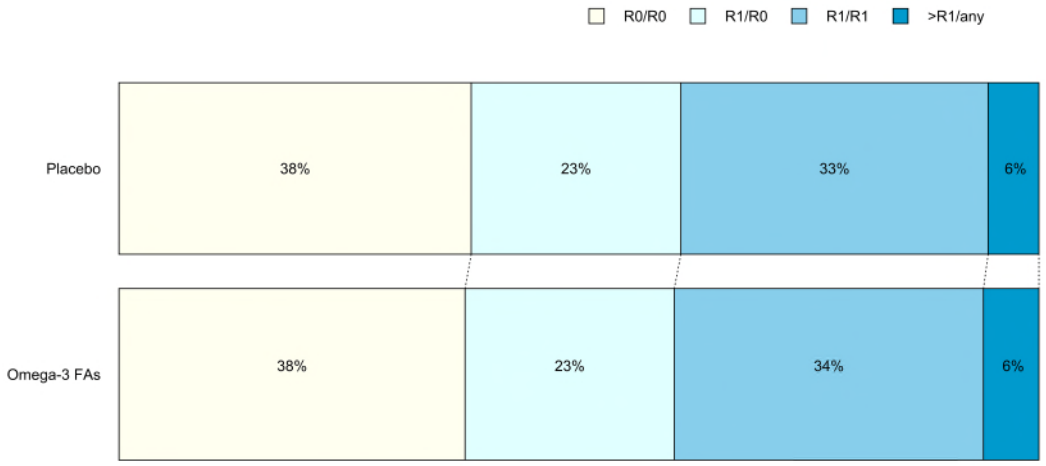
	Number of participants	No. of records in scheduled treatment period		No. of records per year		Length of scheduled treatment	
		Median (range)	Spearman correlation coefficient (P value)	Median (range)	Spearman correlation coefficient (P value)	Median (range)	Spearman correlation coefficient (P value)
Worst retinopathy grade (restricted to records with data in both eyes)	7309	4 (1-14)	0.044 (P = 0.0002)	0.63 (0.09-4.35)	-0.026 (P = 0.02)	6.9 years (84 days-11.2 years)	0.15 (P<0.0001)

Figure 44 Highest Retinopathy Grade by Aspirin Allocation



Common odds ratio for the likelihood of having a higher grade of retinopathy with aspirin: 1.01 (95% CI: 0.93 - 1.10), P= 0.74

Figure 45 Highest Retinopathy Grade by Omega-3 Fatty Acids Allocation

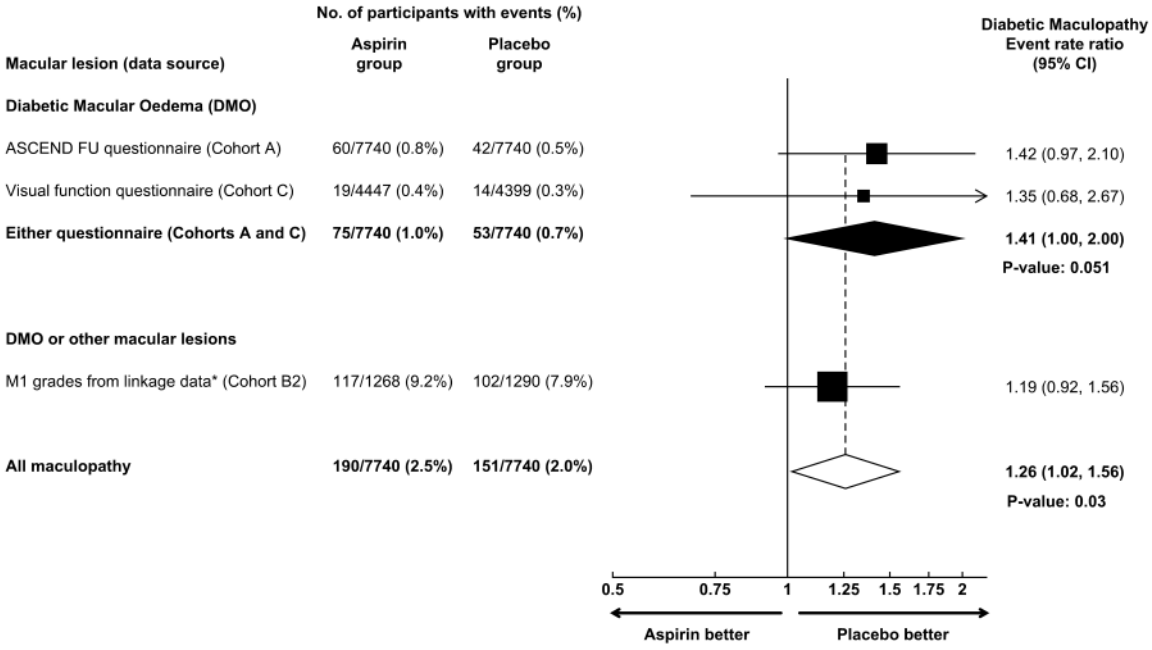


Common odds ratio for the likelihood of having a higher grade of retinopathy with omega-3 fatty acids: 1.03 (95% CI: 0.95 - 1.13), P= 0.43

4.4.5 Tertiary Efficacy Assessment: Incident Diabetic Maculopathy

As defined in the DAP in Section 3.4.3.7, M_1 events derived from the linkage records (cohort B2) include both DMO and exudative macular disease. In contrast, those derived from ASCEND follow-up questionnaires (cohort A) or the VFQ (cohort C) included DMO events only. There was a marginally significant trend towards harm from allocation to aspirin on incident diabetic maculopathy (Figure 46). In total, 190 out of 7740 participants [2.5%] had this tertiary outcome in the active group of the aspirin arm, compared with 151 out of 7740 participants [2.0%] in the placebo group (rate ratio 1.26; 95% CI: 1.02-1.56; P = 0.03; Figure 46). The individual data sources were consistent with this, but numbers with diabetic maculopathy within each source were too small to reach statistical significance. In addition, the overall result may be a chance finding given the multiple comparisons. Finally, there was no proportional difference in the effect of omega-3 FAs allocation (181 [2.3%] *vs.* 160 [2.1%]; rate ratio 1.13; 95% CI: 0.91-1.40; P = 0.26; Figure 47).

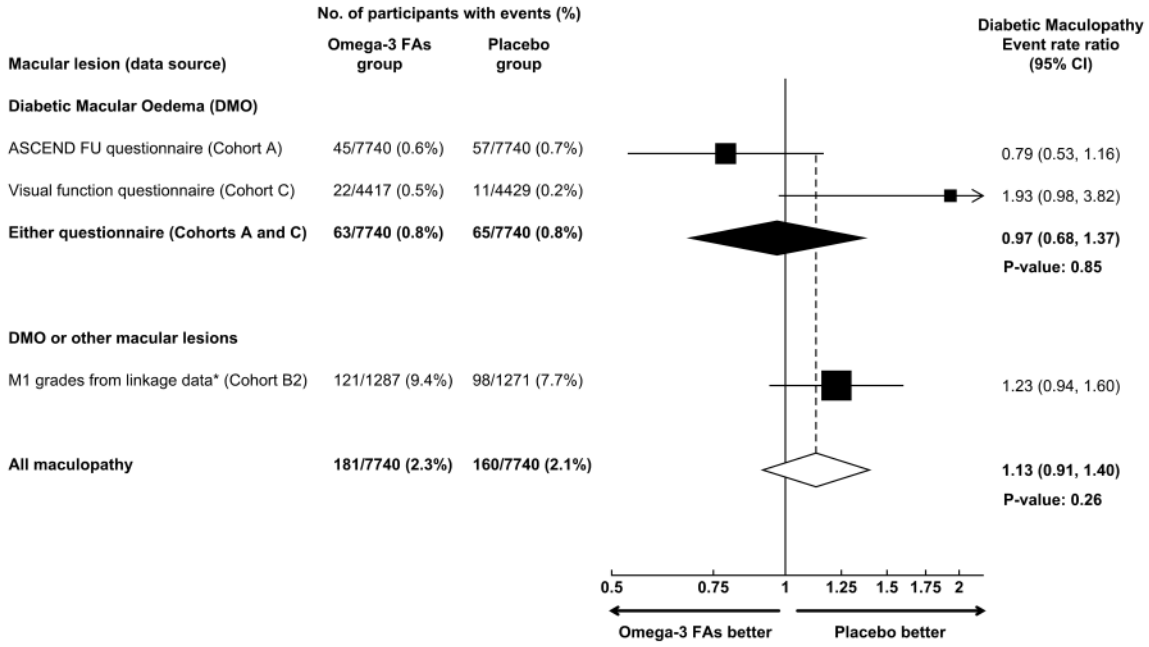
Figure 46 Event Rate Ratios for Incident Diabetic Maculopathy by Aspirin Allocation



*According to the NSC, M_1 is defined by the "presence of hard exudates within 1DD of the fovea, retinal thickening within 1DD of the fovea, or a group of exudates that is greater than or equal to half the disc area, inside the macular field. The macular field includes the area of retina within a circle that originates from the fovea at its centre, to the temporal edge of the optic disc."[61]

Event rate ratios are plotted as squares with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to aspirin on incident diabetic maculopathy. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with aspirin, but the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line.

Figure 47 Event Rate Ratios for Incident Diabetic Maculopathy by Omega-3 Fatty Acids Allocation



*According to the NSC, M1 is defined by the "presence of hard exudates within one 1DD of the fovea, retinal thickening within 1DD of the fovea, or a group of exudates that is greater than or equal to half the disc area, inside the macular field. The macular field includes the area of retina within a circle that originates from the fovea at its centre, to the temporal edge of the optic disc."[61]

Event rate ratios are plotted as squares with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to omega-3 FAs on incident diabetic maculopathy. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with omega-3 FAs, but the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line.

4.5 Effects of Aspirin on Eye Bleeding

4.5.1 Primary and Secondary Safety Assessments

The majority of eye bleeding events were confirmed with adjudication. Those unrefuted did not undergo adjudication either because the participants' GP did not provide supporting evidence or because they were not eligible to be adjudicated, having been reported on the VFQ. Of those confirmed, most were considered non-serious in severity, such as subconjunctival bleeds, whereas sight-threatening bleeds were less frequent.

Table 61 summarises the location, intervention, and visual loss associated with incident eye bleeding events. Among the sight-threatening eye bleeds, 95 out of 121 events occurred in the vitreous body (78.5%), 21 were retinal (17.4%), 3 were hyphaemas (2.5%), and two bled in an unspecified location

within the eye. The majority were managed with laser photocoagulation (68 out of 121; 56.2%), 20 had a vitrectomy (16.5%), 4 received an intravitreal injection (3.3%), 11 were managed conservatively (9.1%), and the intervention received by 18 participants was unknown (14.9%). Finally, 51 out of 121 were associated with visual loss (42.1%), 48 had no visual loss (39.7%), and visual function could not be ascertained for 22 participants (18.2%). Of the non-serious eye bleeds, 70 out of 245 events were subconjunctival haemorrhages (28.6%), 81 were vitreoretinal bleeds that did not result in visual loss or require any intervention (33.1%), and the rest bled in an unspecified location within the eye (94 out of 245; 38.4%).

Figure 48 shows the event rate ratios for eye bleeding events in the aspirin arm. There were no significant adverse effects of assignment to active aspirin, compared with placebo, on the primary safety outcome of incident sight-threatening eye bleeding, which occurred in 57 [0.7%] participants in the active group and 64 [0.8%] participants in the placebo group (rate ratio 0.89; 95% CI: 0.62-1.27; P = 0.52). Although there was a marginally significant adverse effect of allocation to aspirin on non-serious eye bleeds (138 [1.8%] *vs.* 107 [1.4%]; rate ratio 1.29; 95% CI: 1.00-1.66; absolute difference +0.4%; P = 0.046), this is likely to be a chance finding given the multiple comparisons. The incidence of bleeding events among those that were not adjudicated (i.e. unrefuted eye bleeds) was similar between the active and placebo group assignments (40 [0.5%] *vs.* 38 [0.5%]; rate ratio 1.05; 95% CI: 0.67-1.65). Overall, allocation to aspirin did not confer a statistically significantly higher risk of having any eye bleed (212 [2.7%] *vs.* 183 [2.4%]; rate ratio 1.16; 95% 0.95-1.41; P = 0.14).

Table 61 Location, Intervention and Visual Losses Associated with Incident Eye Bleeding Events

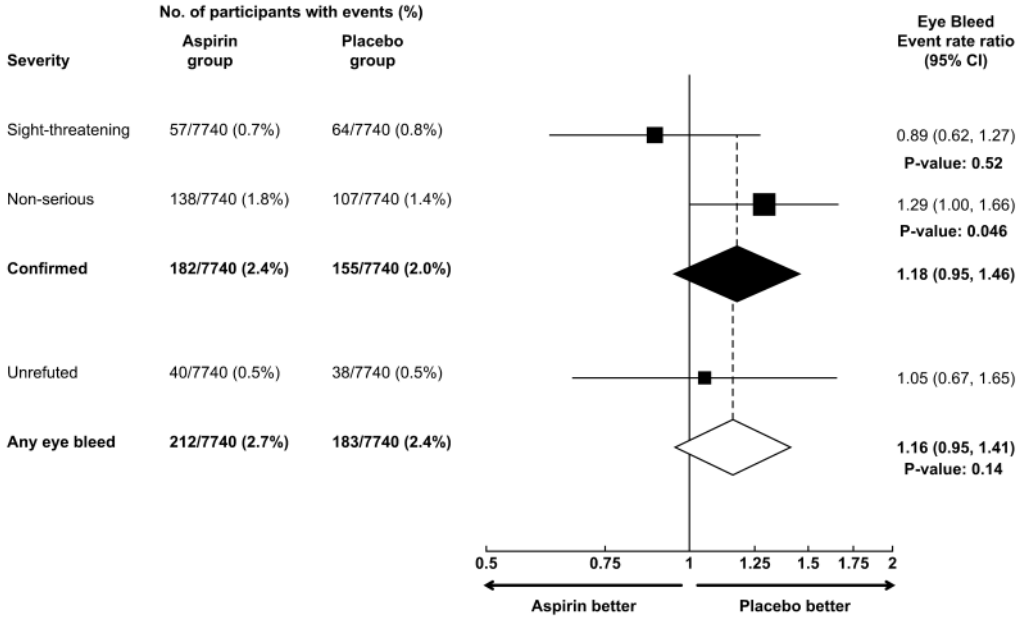
	Unrefuted*	Non-serious eye bleeds	Sight-threatening eye bleeds										
			No visual loss			Visual loss					Unknown if there was any visual loss		
			Injection	Laser	Vitrectomy	Watch & Wait	Injection	Laser	Vitrectomy	Unknown†	Injection	Laser	Vitrectomy
Location													
Subconjunctival haemorrhage	-	70	0	0	0	0	0	0	0	0	0	0	0
Anterior chamber haemorrhage (hyphaema)	-	0	0	0	0	1	0	1	0	1	0	0	0
Retinal haemorrhage‡	-	39	0	8	0	2	4	2	1	0	0	4	0
Vitreous haemorrhage	-	42	0	32	8	8	0	14	0	17	0	5	11
Site unknown	-	94	0	0	0	0	0	0	0	0	0	2	0
Total	78	245	0	40	8	11	4	17	1	18	0	11	11

*Participants were not asked to provide information about the location, interventions or visual loss associated with their eye bleeding event at the time they were reported. Hence, location information for the unadjudicated eye bleeds is unavailable.

†Includes those for whom it was unknown if any intervention was received.

‡Retinal haemorrhage included sub-hyaloid, pre-retinal, retinal, sub-retinal and sub-retinal pigment epithelial haemorrhages.

Figure 48 Eye Bleeding Event Rate Ratios in the Aspirin Arm



Event rate ratios are plotted as squares with the size of each square proportional to the amount of statistical information available. The horizontal lines represent 95% confidence intervals, and the dashed vertical line indicates the overall event rate ratio for the effect of randomisation to aspirin on eye bleeding events. For totals, the event rate ratio and corresponding 95% confidence interval are represented by a diamond. Squares or diamonds to the left of the solid vertical line indicate a benefit with aspirin, but the comparison was significant (P<0.05) only if the horizontal line or diamond does not overlap with the solid vertical line.

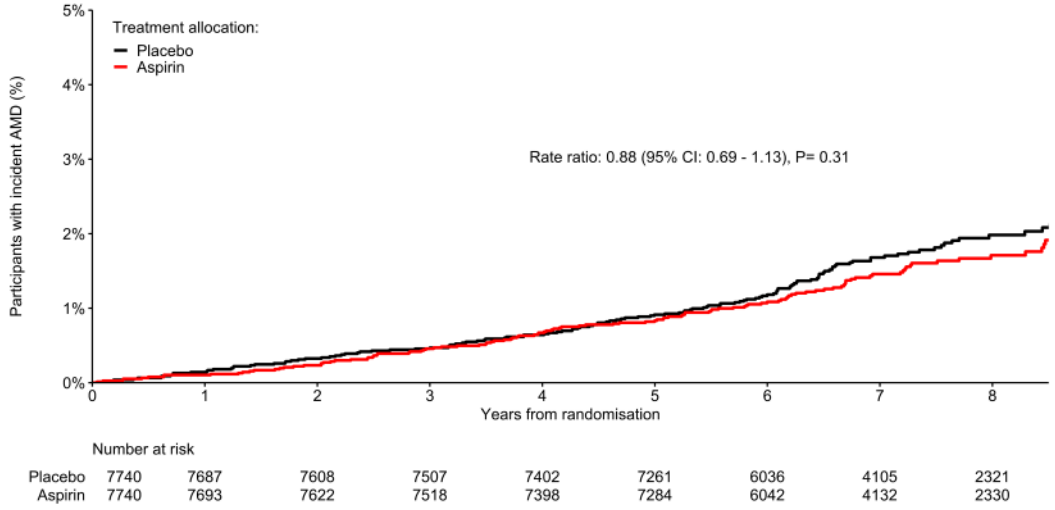
4.6 Effects of the Study Treatments on Age-Related Macular Degeneration

4.6.1 Secondary Efficacy Assessment: Incident AMD Diagnoses

Out of 260 incident AMD events, 73 were confirmed during the adjudication process, seven were unrefuted because supporting evidence was not received from the participants' GP to adjudicate them, and 180 were unrefuted, having derived from the VFQ. There were no significant effects of aspirin allocation compared with placebo on confirmed or unrefuted incident diagnoses of AMD, which occurred in 122 [1.6%] participants in the active group, compared to 138 [1.8%] in the placebo group (rate ratio 0.88; 95% CI: 0.69-1.13; P = 0.31; Figure 49). The survival curves appear to separate in favour of aspirin after approximately six years of follow-up but never attained statistical significance. Similarly, there were no proportional differences in the omega-3 FAs arm. Incident AMD occurred in 130 [1.7%] participants allocated to active treatment and in 130 [1.7%] allocated to placebo (rate ratio 1.00; 95% CI: 0.78-1.27; P = 0.99; Figure 50). A higher proportion of AMD events were in women and those aged 70 years or older, but no clear association with smoking status emerged (Table 62). The

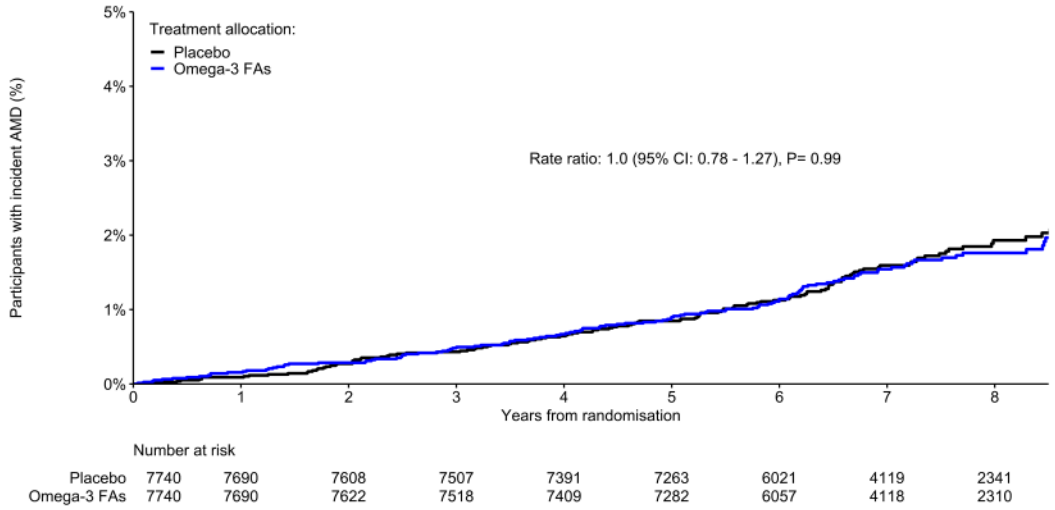
heterogeneity of these effects was not formally tested, given the small number of events in each stratum and to avoid multiple comparisons.

Figure 49 Kaplan-Meier Plot of Time to Confirmed or Unrefuted Incident Diagnoses of Age-Related Macular Degeneration by Aspirin Allocation



The number of participants at risk at the start of each year of follow-up is shown. The rate ratio is for incident confirmed or unrefuted age-related macular degeneration among participants in the aspirin group, as compared with those in the placebo group.

Figure 50 Kaplan-Meier Plot of Time to Confirmed or Unrefuted Incident Diagnoses of Age-Related Macular Degeneration by Omega-3 Fatty Acids Allocation



The number of participants at risk at the start of each year of follow-up is shown. The rate ratio is for incident confirmed or unrefuted age-related macular degeneration among participants in the omega-3 FAs group, as compared with those in the placebo group.

Table 62 Age-Related Macular Degeneration Events by Age, Sex and Smoking Status at Baseline

Baseline characteristic	Aspirin Arm		Omega-3 FAs Arm		Overall (n=15,480)
	Active (n=7740)	Placebo (n=7740)	Active (n=7740)	Placebo (n=7740)	
Age (yr)					
<60	21/2795 (0.8%)	31/2795 (1.1%)	29/2791 (1.0%)	23/2799 (0.8%)	52/5590 (0.9%)
≥60<70	53/3123 (1.7%)	49/3124 (1.6%)	44/3127 (1.4%)	58/3120 (1.9%)	102/6247 (1.6%)
≥70	48/1822 (2.6%)	58/1821 (3.2%)	57/1822 (3.1%)	49/1821 (2.7%)	106/3643 (2.9%)
Sex					
Male	71/4843 (1.5%)	66/4841 (1.4%)	71/4842 (1.5%)	66/4842 (1.4%)	137/9684 (1.4%)
Female	51/2897 (1.8%)	72/2899 (2.5%)	59/2898 (0.8%)	64/2898 (2.2%)	123/5796 (2.1%)
Smoking status					
Current	8/639 (1.3%)	8/640 (1.3%)	12/639 (1.9%)	4/640 (0.6%)	16/1279 (1.3%)
Former	48/3526 (1.4%)	58/3525 (1.6%)	52/3527 (1.5%)	54/3524 (1.6%)	106/7051 (1.5%)
Never	63/3489 (1.8%)	69/3488 (2.0%)	65/3489 (1.9%)	67/3488 (1.9%)	132/6977 (1.9%)
Unknown	3/86 (3.5%)	3/87 (3.4%)	1/85 (1.2%)	5/88 (5.7%)	6/173 (3.5%)
Total no. of AMD events	**122**	**138**	**130**	**130**	**260**

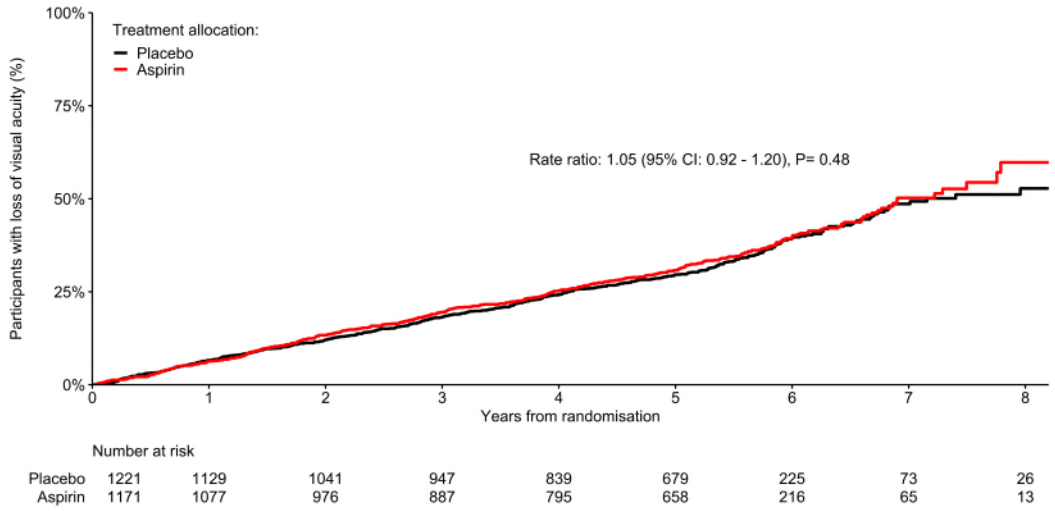
Figures in parentheses represent the proportion of the total number of participants in each category who suffered an AMD event.

4.7 Effects of the Study Treatments on Visual Acuity

4.7.1 Tertiary Efficacy Assessment: Worsening Visual Acuity (Cohort B5)

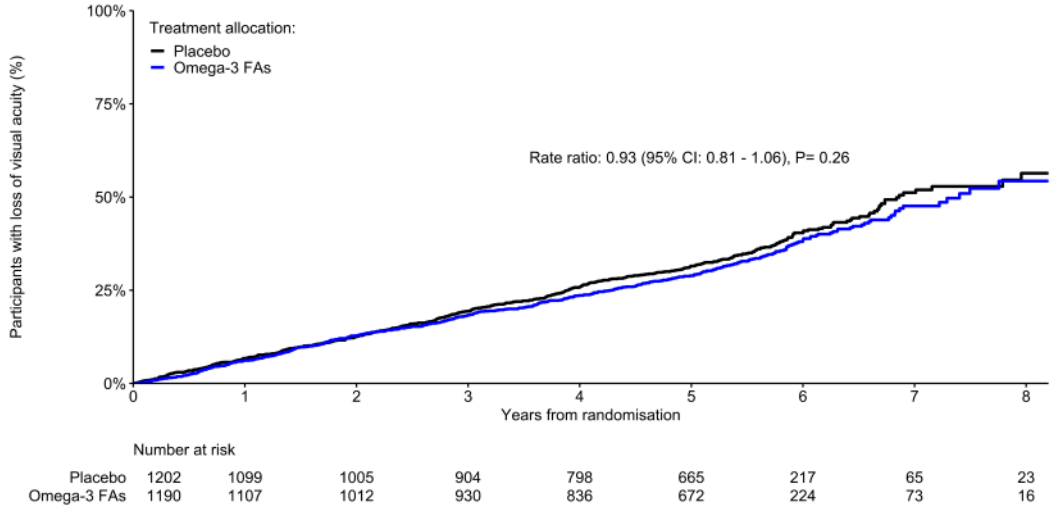
The mean visual acuity averaged over left and right eyes in cohort B5 at baseline was ASCEND-Eye recode score = 5.5 (SD 2.3), approximately equivalent to LogMar ≤0 or Snellen 6/6. Therefore, most participants in cohort B5 had normal visual acuity at the start of ASCEND. There were no significant effects of assignment to active aspirin compared with the placebo on time to worsening visual acuity, which was equivalent to a loss of 15 or more ETDRS letters (427 [36.5%] *vs.* 433 [35.5%]; rate ratio 1.05; 95% CI: 0.92-1.20; P = 0.48; Figure 51). At 3, 5 and 7 years, 20%, 30% and 50% of participants had a deterioration of their visual acuity. Likewise, no significant proportional differences were found in the omega-3 FAs arm (417 [35.0%] active group *vs.* 443 [36.9%] placebo group; rate ratio 0.93; 95% CI: 0.81-1.06; P = 0.26; Figure 52).

Figure 51 Kaplan-Meier Plot of Time to Loss of Visual Acuity by 3 Points or More on the ASCEND-Eye Scale by Aspirin Allocation



The number of participants at risk at the start of each year of follow-up is shown. The rate ratio is for loss of visual acuity among participants in the aspirin group, as compared with those in the placebo group.

Figure 52 Kaplan-Meier Plot of Time to Loss of Visual Acuity by 3 Points or More on the ASCEND-Eye Scale by Omega-3 Fatty Acids Allocation



The number of participants at risk at the start of each year of follow-up is shown. The rate ratio is for loss of visual acuity among participants in the omega-3 FAs group, as compared with those in the placebo group.

4.8 Effects of the Study Treatments on NEI-VFQ-25 Scores

4.8.1 Secondary and Tertiary Efficacy Assessments: Composite and Subdomain Scores from the NEI-VFQ-25

Mean composite and subdomain scores from the NEI-VFQ-25 overall and by the randomised treatment allocation in each arm are shown in Table 63. There was no statistically significant effect of either

aspirin or omega-3 FAs on the mean composite scores derived from this instrument. The common odds ratio for the likelihood of having a lower composite score from assignment to active aspirin *versus* placebo was 1.04 (95% CI 0.96-1.13; P = 0.36; Figure 53), and for active omega-3 FAs *versus* placebo, it was 1.01 (95% CI 0.93-1.09; P = 0.87; Figure 54). The odds of having lower scores in the active groups of each arm were also non-significant for most of the 11 subdomains (Figures 55-76). Although there was a statistically significant greater likelihood of having a less-than-perfect score for the vision-specific dependency subdomain in those assigned to active omega-3 FAs (odds ratio 1.16; 95% CI 1.01-1.33; P = 0.04; Figure 70), this is likely to be a chance finding given the multiple comparisons. The assumption of proportional odds was upheld in every model except for the driving subdomain scores by omega-3 FAs allocation (P = 0.01). Separate analyses to calculate an odds ratio at each data cutpoint of this subdomain have not been performed, as per the DAP in Section 3.4.

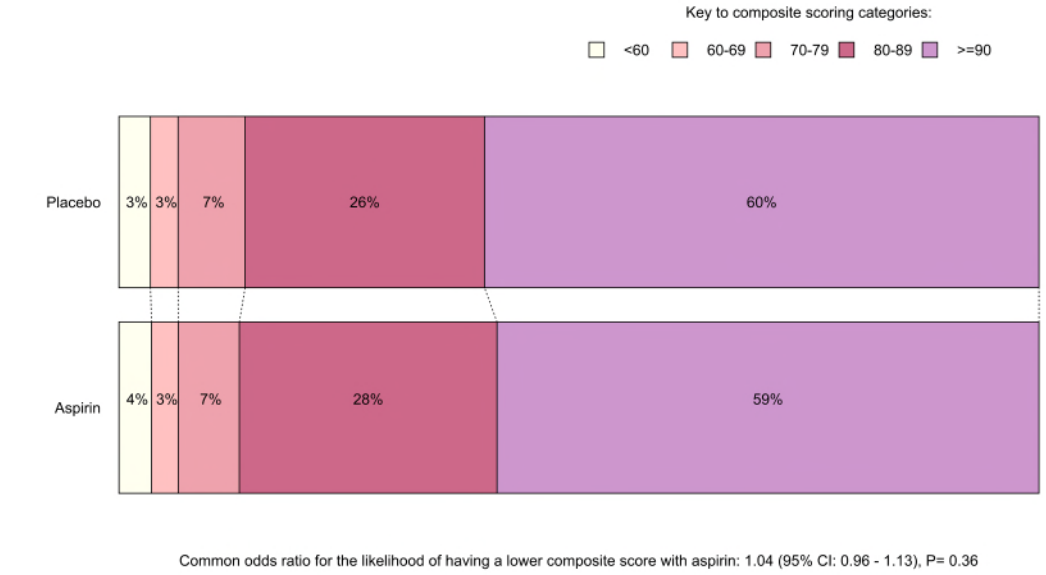
Table 63 Average Composite NEI-VFQ-25 Scores - Overall, by Subdomain and by Randomised Treatment Allocation

	Aspirin Arm		Omega-3 FAs Arm		
	Active	Placebo	Active	Placebo	Overall
Composite score	**n=4444** 87.9 (11.1)	**n=4395** 88.2 (11.0)	**n=4415** 87.9 (11.3)	**n=4424** 88.2 (10.8)	**n=8839** 88.1 (11.1)
Subdomain score					
General Health*	**n=4305** 53.9 (21.2)	**n=4242** 54.2 (21.7)	**n=4288** 54.1 (21.1)	**n=4259** 54.1 (21.8)	**n=8547** 54.1 (21.4)
General Vision	**n=4431** 78.4 (13.7)	**n=4381** 78.4 (13.4)	**n=4403** 78.4 (13.7)	**n=4409** 78.3 (13.7)	**n=8812** 78.4 (13.5)
Ocular Pain	**n=4444** 91.3 (14.1)	**n=4394** 91.7 (13.8)	**n=4414** 91.4 (14.1)	**n=4424** 91.6 (13.8)	**n=8838** 91.5 (13.9)
Near Activities	**n=4433** 88.0 (15.9)	**n=4382** 88.1 (15.9)	**n=4402** 87.8 (16.3)	**n=4413** 88.3 (15.5)	**n=8815** 88.1 (15.9)
Distance Activities	**n=4433** 91.9 (13.9)	**n=4381** 92.1 (13.6)	**n=4404** 91.9 (14.0)	**n=4410** 92.2 (13.5)	**n=8814** 92.1 (13.8)
Social Functioning	**n=4429** 96.5 (11.0)	**n=4381** 96.5 (10.9)	**n=4401** 96.3 (11.4)	**n=4409** 96.7 (10.5)	**n=8810** 96.5 (10.9)
Mental Health	**n=4441** 90.4 (14.8)	**n=4392** 90.7 (14.5)	**n=4413** 90.2 (15.0)	**n=4420** 90.9 (14.3)	**n=8833** 90.5 (14.7)
Role Dependency	**n=4389** 91.2 (17.6)	**n=4345** 91.2 (17.7)	**n=4361** 91.0 (17.9)	**n=4373** 91.5 (17.3)	**n=8734** 91.2 (17.6)
Vision-Specific Dependency	**n=4421** 96.8 (12.1)	**n=4372** 97.0 (11.5)	**n=4390** 96.8 (12.1)	**n=4403** 97.1 (11.6)	**n=8793** 96.9 (11.8)
Driving	**n=3215** 87.9 (20.7)	**n=3201** 88.9 (19.1)	**n=3221** 87.9 (20.8)	**n=3195** 88.8 (18.9)	**n=6416** 88.4 (19.9)
Colour Vision	**n=4392** 97.2 (10.7)	**n=4350** 97.3 (10.3)	**n=4365** 97.3 (10.5)	**n=4377** 97.2 (10.6)	**n=8742** 97.3 (10.5)
Peripheral Vision	**n=4411** 93.3 (15.2)	**n=4356** 93.3 (15.2)	**n=4382** 93.3 (15.2)	**n=4315** 93.3 (15.2)	**n=8767** 93.3 (15.2)

Figures are presented as mean (standard deviation). The number of participants who gave non-missing answers and contributed to the composite and subdomain score analyses is shown in the row above the mean and standard deviation results.

*The general health subdomain is not included in the calculation of composite scores from the NEI-VFQ-25. The instrument developers intend the composite and subdomain scores to be interpreted alongside the general health score and hence, they are displayed in the table. However, comparisons of the responses to this question by randomised treatment allocation have not been performed.

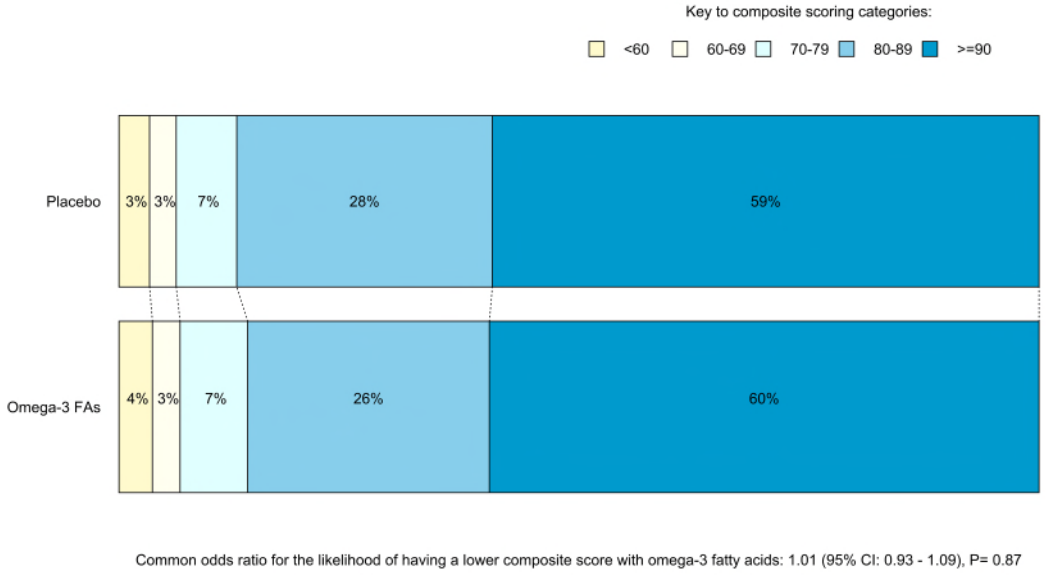
Figure 53 Composite Scores from the NEI-VFQ-25 by Aspirin Allocation



Common odds ratio for the likelihood of having a lower composite score with aspirin: 1.04 (95% CI: 0.96 - 1.13), P= 0.36

The number of participants contributing to this analysis was 8839

Figure 54 Composite Scores from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Common odds ratio for the likelihood of having a lower composite score with omega-3 fatty acids: 1.01 (95% CI: 0.93 - 1.09), P= 0.87

The number of participants contributing to this analysis was 8839

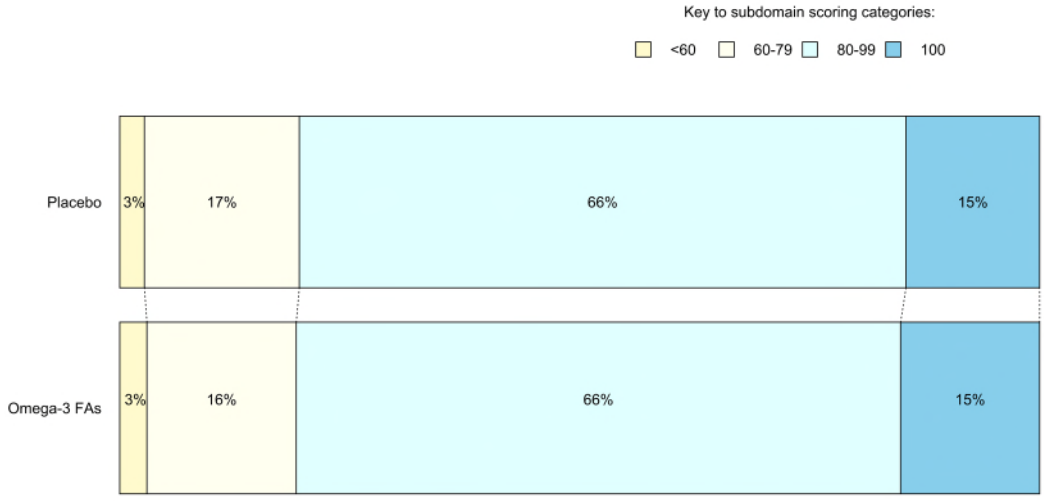
Figure 55 General Vision Subdomain Score from the NEI-VFQ-25 by Aspirin Allocation



Common odds ratio for the likelihood of having a lower score for the general vision subdomain with aspirin: 1.00 (95% CI: 0.92 - 1.09), P= 1.00

The number of participants who gave non-missing answers and contributed to this analysis was 8812

Figure 56 General Vision Subdomain Score from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Common odds ratio for the likelihood of having a lower score for the general vision subdomain with omega-3 fatty acids: 0.97 (95% CI: 0.89 - 1.06), P= 0.50

The number of participants who gave non-missing answers and contributed to this analysis was 8812

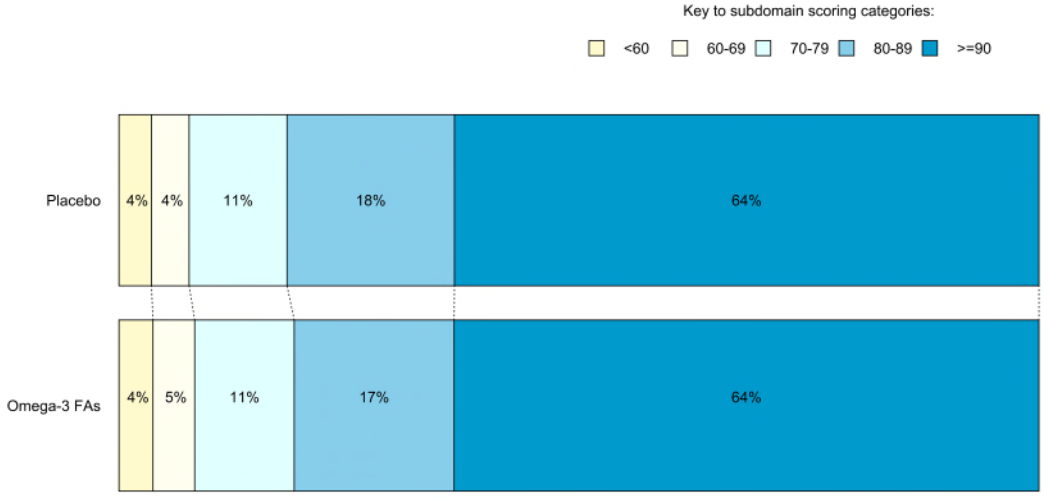
Figure 57 Ocular Pain Subdomain Score from the NEI-VFQ-25 by Aspirin Allocation



Common odds ratio for the likelihood of having a lower score for the ocular pain subdomain with aspirin: 1.02 (95% CI: 0.94 - 1.11), P= 0.59

The number of participants who gave non-missing answers and contributed to this analysis was 8838

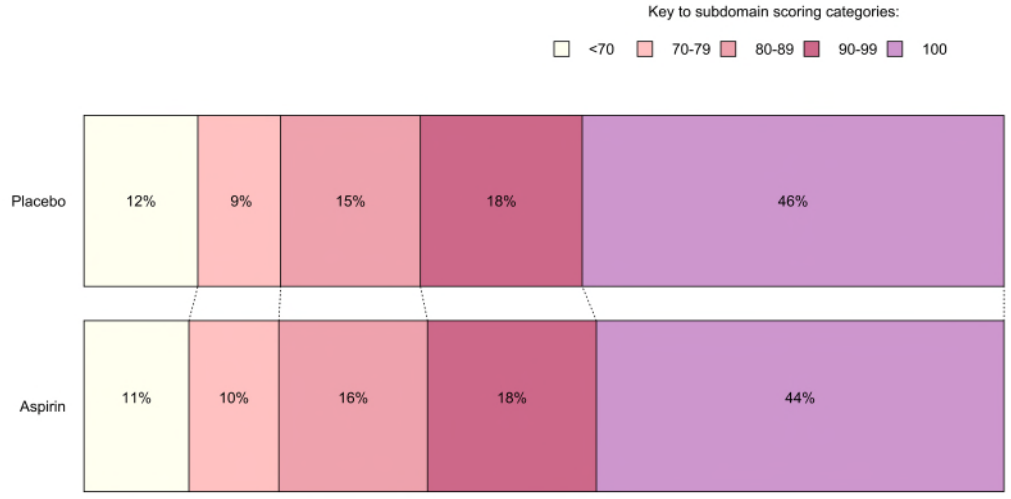
Figure 58 Ocular Pain Subdomain Score from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Common odds ratio for the likelihood of having a lower score for the ocular pain subdomain with omega-3 fatty acids: 1.01 (95% CI: 0.93 - 1.10), P= 0.78

The number of participants who gave non-missing answers and contributed to this analysis was 8838

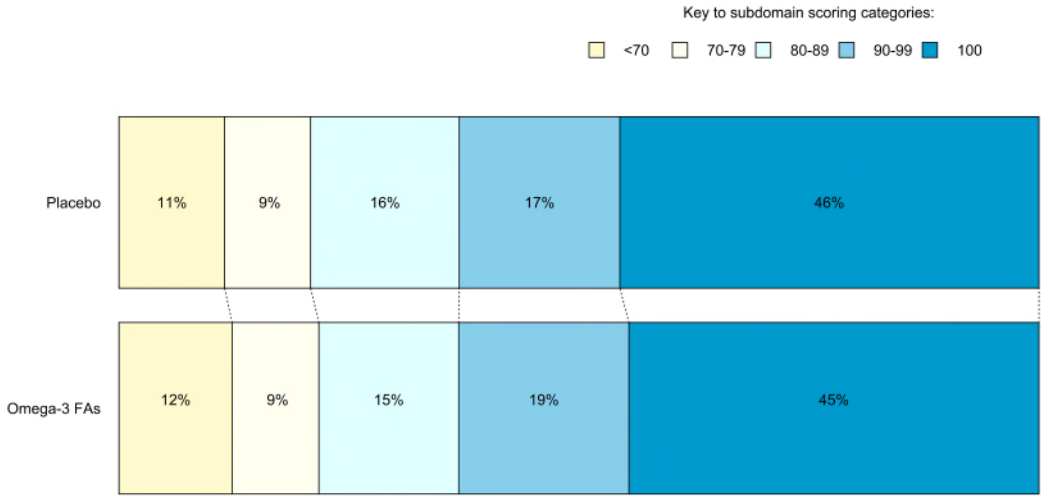
Figure 59 Near Activities Subdomain Score from the NEI-VFQ-25 by Aspirin Allocation



Common odds ratio for the likelihood of having a lower score for the near activities subdomain with aspirin: 1.03 (95% CI: 0.96 - 1.12), P= 0.40

The number of participants who gave non-missing answers and contributed to this analysis was 8815

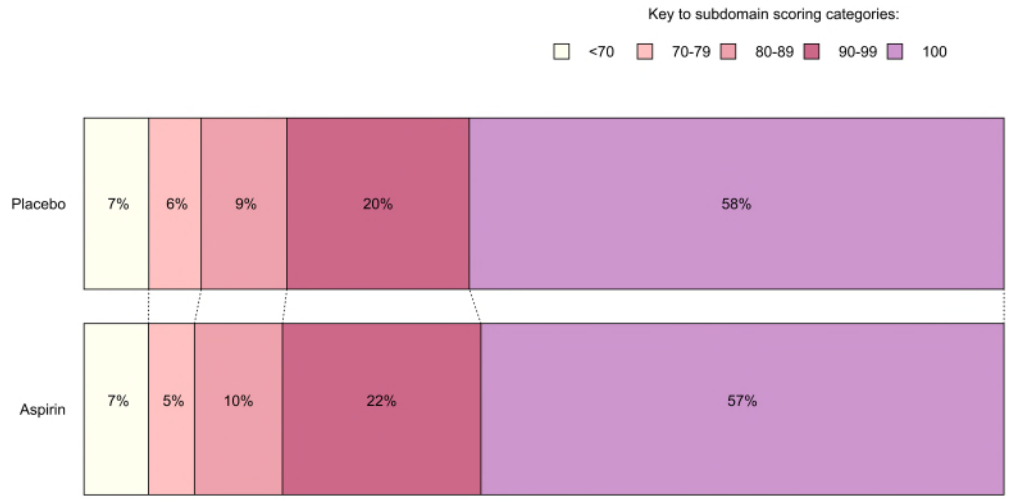
Figure 60 Near Activities Subdomain Score from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Common odds ratio for the likelihood of having a lower score for the near activities subdomain with omega-3 fatty acids: 1.03 (95% CI: 0.96 - 1.12), P= 0.39

The number of participants who gave non-missing answers and contributed to this analysis was 8815

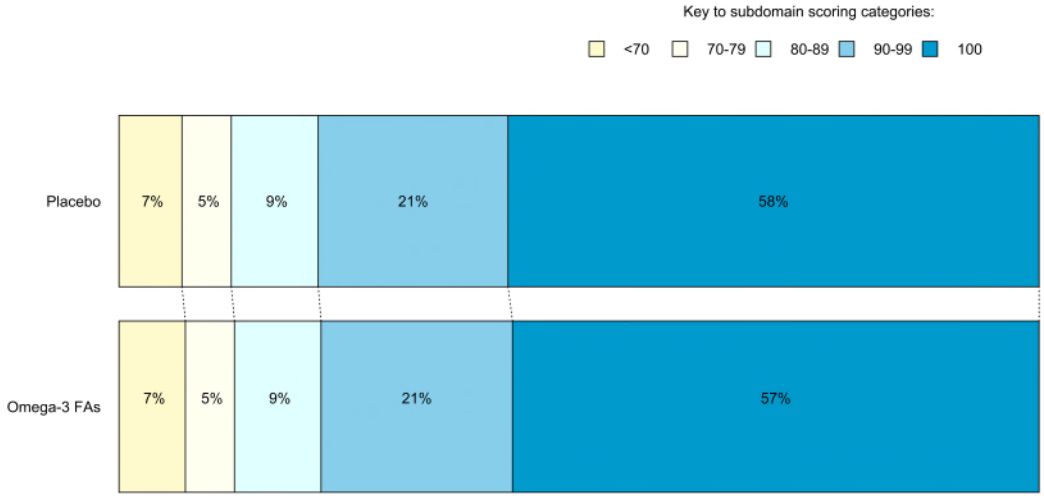
Figure 61 Distance Activities Subdomain Score from the NEI-VFQ-25 by Aspirin Allocation



Common odds ratio for the likelihood of having a lower score for the distance activities subdomain with aspirin: 1.03 (95% CI: 0.95 - 1.11), P= 0.51

The number of participants who gave non-missing answers and contributed to this analysis was 8814

Figure 62 Distance Activities Subdomain Score from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Common odds ratio for the likelihood of having a lower score for the distance activities subdomain with omega-3 fatty acids: 1.02 (95% CI: 0.94 - 1.11), P= 0.60

The number of participants who gave non-missing answers and contributed to this analysis was 8814

Figure 63 Social Functioning Subdomain Score from the NEI-VFQ-25 by Aspirin Allocation



Common odds ratio for the likelihood of having a lower score for the social functioning subdomain with aspirin: 1.03 (95% CI: 0.91 - 1.16), P= 0.63

The number of participants who gave non-missing answers and contributed to this analysis was 8810

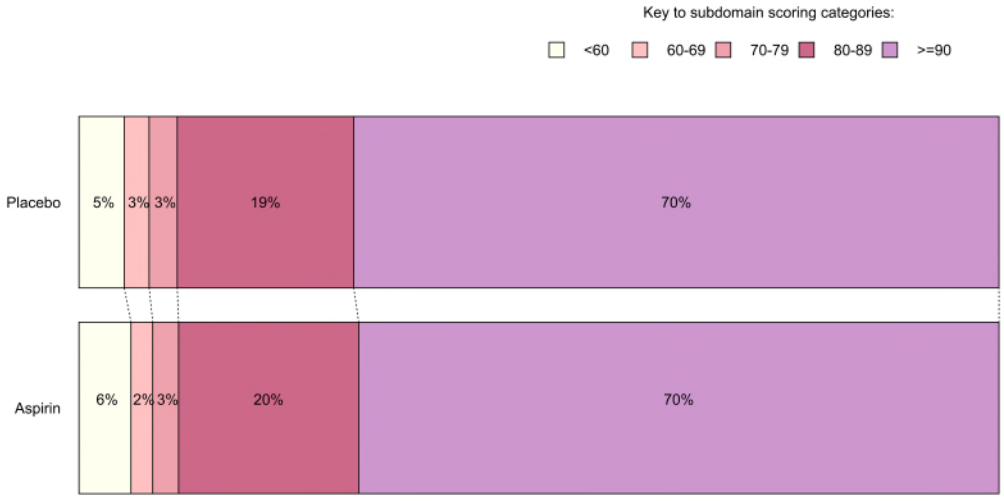
Figure 64 Social Functioning Subdomain Score from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Common odds ratio for the likelihood of having a lower score for the social functioning subdomain with omega-3 fatty acids: 1.11 (95% CI: 0.98 - 1.25), P= 0.10

The number of participants who gave non-missing answers and contributed to this analysis was 8810

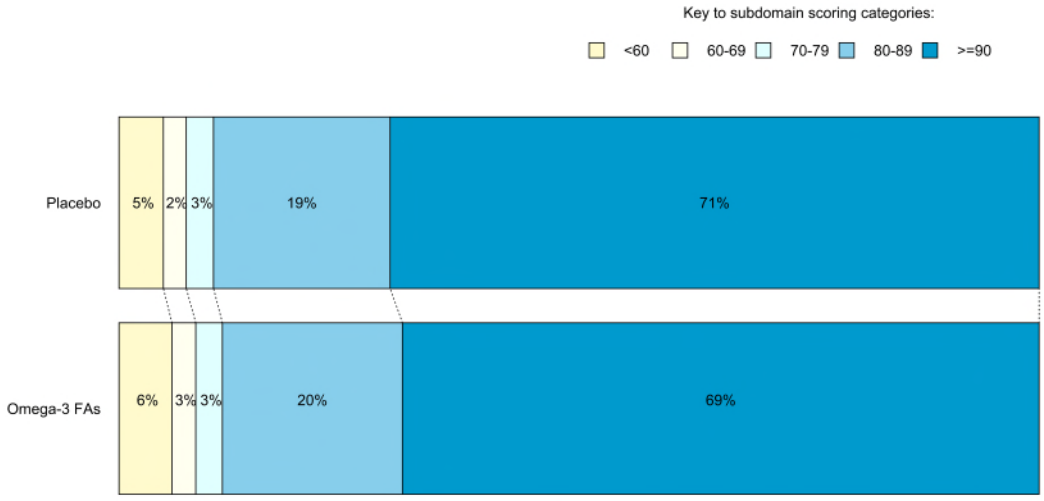
Figure 65 Mental Health Subdomain Score from the NEI-VFQ-25 by Aspirin Allocation



Common odds ratio for the likelihood of having a lower score for the mental health subdomain with aspirin: 1.03 (95% CI: 0.94 - 1.12), P= 0.55

The number of participants who gave non-missing answers and contributed to this analysis was 8833

Figure 66 Mental Health Subdomain Score from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Common odds ratio for the likelihood of having a lower score for the mental health subdomain with omega-3 fatty acids: 1.07 (95% CI: 0.98 - 1.17), P= 0.12

The number of participants who gave non-missing answers and contributed to this analysis was 8833

Figure 67 Role Dependency Subdomain Score from the NEI-VFQ-25 by Aspirin Allocation



Common odds ratio for the likelihood of having a lower score for the role dependency subdomain with aspirin: 1.02 (95% CI: 0.93 - 1.11), P= 0.71

The number of participants who gave non-missing answers and contributed to this analysis was 8734

Figure 68 Role Dependency Subdomain Score from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Common odds ratio for the likelihood of having a lower score for the role dependency subdomain with omega-3 fatty acids: 1.03 (95% CI: 0.94 - 1.12), P= 0.58

The number of participants who gave non-missing answers and contributed to this analysis was 8734

Figure 69 Vision-Specific Dependency Subdomain Score from the NEI-VFQ-25 by Aspirin Allocation



Odds ratio for the likelihood of having a lower score for the vision-specific dependency subdomain with aspirin: 1.06 (95% CI: 0.92 - 1.22), P= 0.41

The number of participants who gave non-missing answers and contributed to this analysis was 8793

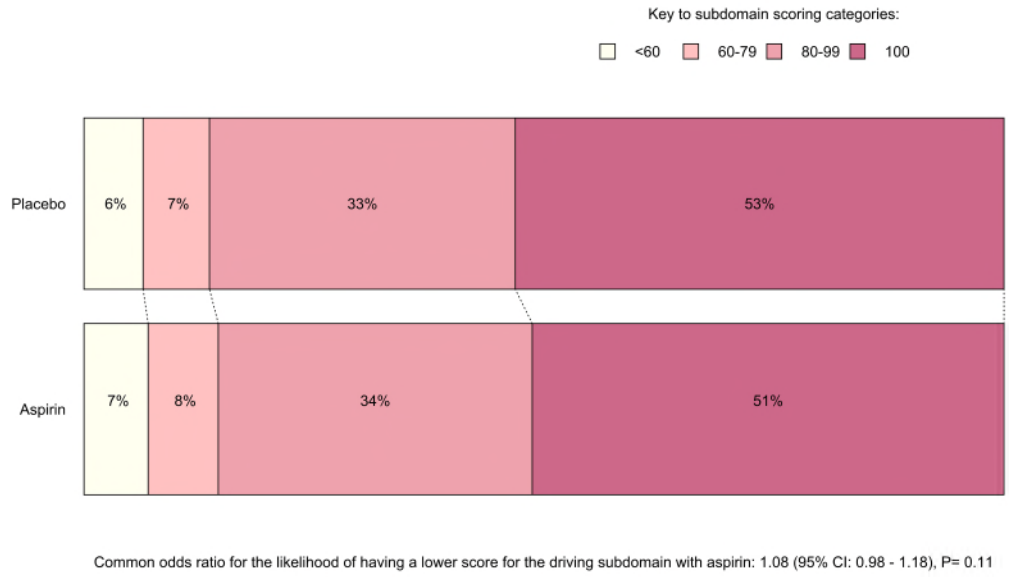
Figure 70 Vision-Specific Dependency Subdomain Score from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Odds ratio for the likelihood of having a lower score for the vision-specific dependency subdomain with omega-3 fatty acids: 1.16 (95% CI: 1.01 - 1.33), P= 0.04

The number of participants who gave non-missing answers and contributed to this analysis was 8793

Figure 71 Driving Subdomain Score from the NEI-VFQ-25 by Aspirin Allocation



Common odds ratio for the likelihood of having a lower score for the driving subdomain with aspirin: 1.08 (95% CI: 0.98 - 1.18), P= 0.11

The number of participants who gave non-missing answers and contributed to this analysis was 6416

Figure 72 Driving Subdomain Score from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Common odds ratio for the likelihood of having a lower score for the driving subdomain with omega-3 fatty acids: 1.02 (95% CI: 0.93 - 1.12), P= 0.60

The number of participants who gave non-missing answers and contributed to this analysis was 6416

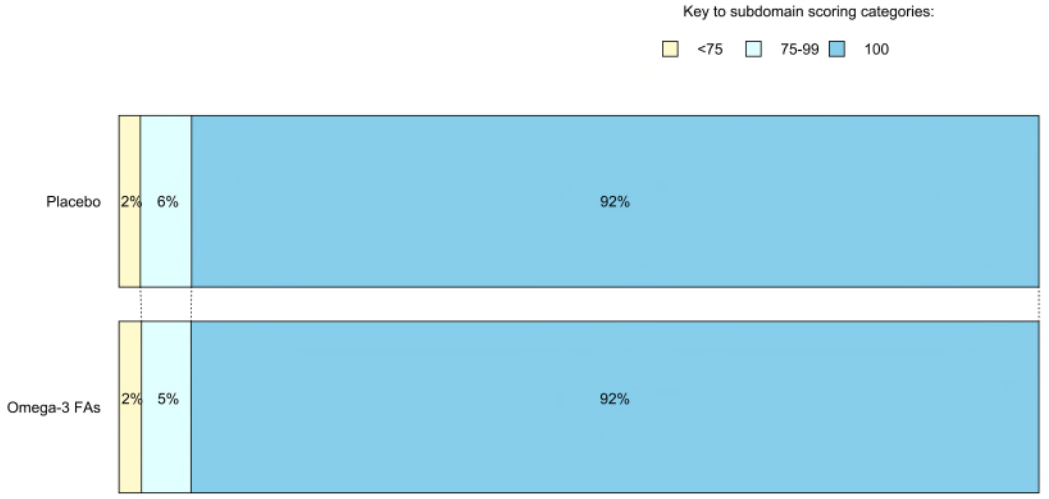
Figure 73 Colour Vision Subdomain Score from the NEI-VFQ-25 by Aspirin Allocation



Common odds ratio for the likelihood of having a lower score for the colour vision subdomain with aspirin: 1.04 (95% CI: 0.89 - 1.21), P= 0.64

The number of participants who gave non-missing answers and contributed to this analysis was 8742

Figure 74 Colour Vision Subdomain Score from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Common odds ratio for the likelihood of having a lower score for the colour vision subdomain with omega-3 fatty acids: 0.99 (95% CI: 0.85 - 1.16), P= 0.93

The number of participants who gave non-missing answers and contributed to this analysis was 8742

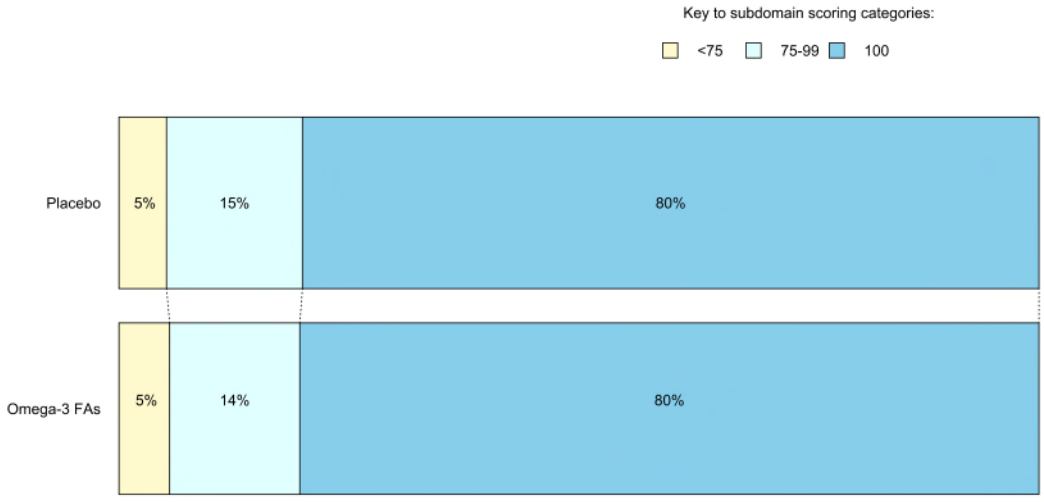
Figure 75 Peripheral Vision Subdomain Score from the NEI-VFQ-25 by Aspirin Allocation



Common odds ratio for the likelihood of having a lower score for the peripheral vision subdomain with aspirin: 1.00 (95% CI: 0.90 - 1.11), P= 0.94

The number of participants who gave non-missing answers and contributed to this analysis was 8767

Figure 76 Peripheral Vision Subdomain Score from the NEI-VFQ-25 by Omega-3 Fatty Acids Allocation



Common odds ratio for the likelihood of having a lower score for the peripheral vision subdomain with omega-3 fatty acids: 0.99 (95% CI: 0.89 - 1.10), P= 0.81

The number of participants who gave non-missing answers and contributed to this analysis was 8767

4.9 Effects of the Study Treatments on Other Eye Diseases

Summary figures for incident reports of other eye diseases in cohorts A and C are shown in Table 64. Formal hypothesis testing of these results has not been performed to avoid multiple comparisons of the same data.

Table 64 Counts of Participant-Reported Other Eye Diseases - First Events Only

	Aspirin Arm		Omega-3 FAs Arm		
	Active (n =7740)	Placebo (n=7740)	Active (n=7740)	Placebo (n=7740)	Overall (n=15,480)
Other ocular event categories					
Eye infection	12	11	10	13	23
Other ophthalmitis	0	0	0	0	0
Retinal detachment/surgery	44	56	50	50	100
Amaurosis fugax/transient visual loss*	3	2	2	3	5
Eye inflammation unspecified	12	8	13	7	20
Iritis/iridocyclitis/uveitis	11	1	5	7	12
Glaucoma/surgery for/trabeculectomy	112	105	110	107	217
Visual disturbances	17	22	16	23	39
Blindness	4	3	4	3	7
Corneal disorder	19	7	14	12	26
Conjunctivitis	5	7	8	4	12
Eyelid disorder	13	6	10	9	19
Retinal artery occlusion/retinal thrombosis/visual loss thought to be ischaemic in nature*	4	1	3	2	5
Giant cell arteritis/temporal arteritis	5	4	5	4	9
Retinal vein occlusion/blood clot in the eye	54	46	47	53	100
Cranial nerve palsy	33	32	32	33	65
Cataract/cataract extraction/lens implant	990	960	971	979	1950

*Events in these categories were included in the primary efficacy assessment ("Serious Vascular Event") in the main ASCEND trial.[11]

4.10 Sensitivity Analyses: Effect of Adjudication on Event Categorisation

Sensitivity analyses were undertaken to support the conclusion of randomised comparisons of participant-reported eye events. Tables 65 and 66 show the completeness of adjudication for all eligible eye events and first-reported events, respectively. Results are shown for events derived from any source (i.e. in-trial follow-up questionnaires and those reported directly to the central coordinating centre by the participant, a relative or carer, or a non-study healthcare professional via a telephone call or email) and those derived from in-trial follow-up questionnaires-only.

Adjudication was completed for 93.7% of all eligible eye events (Table 65). The majority (75.7%) were classified as "fully assessed" based on the sufficiency of supporting documents to confirm or refute the diagnostic and date criteria specified in the adjudication SOPs (see Section 3.2.1.1); meanwhile, 18.0% were classified as "partially assessed" to reflect a lower quality of the evidence received, whilst 6.3%

were not adjudicated. A marginally smaller proportion (92.8%) of eligible first events were adjudicated (Table 66). Of those, 74.6% were "fully assessed", 18.1% were "partially assessed", and 7.2% were not adjudicated (Table 66). There were no meaningful differences in the sufficiency of supporting evidence received for events derived from follow-up questionnaires compared with phoned-in or emailed-in sources.

After the database was finalized for ASCEND, the effect of adjudication on the categorisation of eye events was evaluated (Table 67). Analyses were restricted to the first reports of each event type that were fully or partially assessed, and where the source of information was the participant's ASCEND follow-up questionnaire[*]. The adjudication process resulted in significant re-categorisation of incident eye bleeding events. Since participants were not asked to judge the severity of their eye bleeds on either of the mailed questionnaires, the percentage agreement was based on "any eye bleed", which included both sight-threatening and non-serious eye bleeds. Applying this definition, the clinical adjudicator agreed with the participant that an eye bleed had or had not occurred in 67.6% of cases (Table 68). A kappa statistic of 0.4 indicates fair inter-rater reliability (95% CI 0.34-0.47; Table 68). Participants more reliably recorded the first reports of other eye diagnoses. The percentage agreement for AMD, DMO, and retinal detachment events was 94.9% (κ=0.71), 90.6% (κ=0.41) and 96.0 (κ=0.77), respectively (Table 68). Visual disturbance was selected to undergo adjudication in case it was related to an underlying eye-bleeding event. The kappa statistic of 0.29 for this code was lower than the other event types, which is not surprising as this symptom was likely to be adjudicated to a diagnostic term, in line with SOP 8 for ASCEND. There was 100% agreement for participant-reported blindness, but there were (thankfully) too few of these events to draw robust conclusions. Therefore, the adjudication process significantly affected analyses of incident eye bleeds but had less effect on incident AMD or DMO events. These high confirmation rates supported the decision not to adjudicate events derived from the VFQ.

[*] For completeness, Appendix 24 is a contingency table showing the effect of adjudication on categorising every event report that was eligible to be adjudicated in ASCEND-Eye. The agreement of pre-adjudicated *versus* adjudicated categories for these data is shown in Appendix 25.

Table 65 Completeness of Adjudication – All Events

	Fully assessed (%)	Partially assessed (%)	Not adjudicated (%)	Total no. of events
Any event source*				
Any eye bleed	595 (71.3%)	186 (22.3%)	53 (6.4%)	**834**
Retinal detachment	67 (88.2%)	6 (7.9%)	3 (3.9%)	**76**
Age-related macular degeneration	119 (83.2%)	11 (7.7%)	13 (9.1%)	**143**
Diabetic macular oedema	55 (96.5%)	1 (1.8%)	1 (1.8%)	**57**
Blindness	3 (100.0%)	0 (0.0%)	0 (0.0%)	**3**
Visual deterioration	28 (87.5%)	2 (6.3%)	2 (6.3%)	**32**
Total	**867 (75.7%)**	**206 (18.0%)**	**72 (6.3%)**	**1145**
ASCEND FU questionnaires only†				
Any eye bleed	570 (71.3%)	178 (22.3%)	51 (6.4%)	**799**
Retinal detachment	58 (86.6%)	6 (9.0%)	3 (4.5%)	**67**
Age-related macular degeneration	101 (80.8%)	11 (8.8%)	13 (10.4%)	**125**
Diabetic macular oedema	47 (95.9%)	1 (2.0%)	1 (2.0%)	**49**
Blindness	2 (100.0%)	0 (0.0%)	0 (0.0%)	**2**
Visual deterioration	28 (90.3%)	2 (6.5%)	1 (3.2%)	**31**
Total	**806 (75.1%)**	**198 (18.5%)**	**69 (6.4%)**	**1073**

FU=Follow-up
Row-wise percentages of the figures in the total no. of events column are shown in parentheses; Percentages may not add up to 100 due to rounding.
*Includes events that the participant reported on their in-trial follow-up questionnaires, as well as those reported directly to CTSU via a telephone call with the participant, a relative or carer, or a managing healthcare professional.
†Restricted to events reported by participants on their in-trial follow-up questionnaires in ASCEND-only.

Table 66 Completeness of Adjudication - First-Reported Events Only

	Fully assessed (%)	Partially assessed (%)	Not adjudicated (%)	Total no. of first events
Any event source*				
Any eye bleed	375 (69.1%)	125 (23.0%)	43 (7.9%)	**543**
Retinal detachment	56 (86.2%)	6 (9.2%)	3 (4.6%)	**65**
Age-related macular degeneration	81 (86.2%)	6 (6.4%)	7 (7.4%)	**94**
Diabetic macular oedema	35 (94.6%)	1 (2.7%)	1 (2.7%)	**37**
Blindness	3 (100.0%)	0 (0.0%)	0 (0.0%)	**3**
Visual deterioration	27 (87.1%)	2 (6.5%)	2 (6.5%)	**31**
Total	**577 (74.6%)**	**140 (18.1%)**	**56 (7.2%)**	**773**
ASCEND FU questionnaires only†				
Any eye bleed	366 (69.8%)	125 (23.9%)	33 (6.3%)	**524**
Retinal detachment	49 (84.5%)	6 (10.3%)	3 (5.2%)	**58**
Age-related macular degeneration	67 (83.8%)	6 (7.5%)	7 (8.8%)	**80**
Diabetic macular oedema	28 (90.3%)	2 (6.5%)	1 (3.2%)	**31**
Blindness	2 (100.0%)	0 (0.0%)	0 (0.0%)	**2**
Visual deterioration	27 (90.0%)	2 (6.7%)	1 (3.3%)	**30**
Total	**539 (74.3%)**	**141 (19.4%)**	**45 (6.2%)**	**725**

FU=Follow-up
Row-wise percentages of the figures in the total no. of events column are shown in parentheses. Percentages may not add up to 100 due to rounding.
*Includes events that the participant reported on their in-trial follow-up questionnaires, as well as those reported directly to CTSU via a telephone call with the participant, a relative or carer, or a managing healthcare professional.
†Restricted to events reported by participants on their in-trial follow-up questionnaires in ASCEND-only.

Table 67 Effect of Adjudication on the Categorisation of First Reports of each Eligible Event Type

		Post-adjudication categorisation																											
		Sight-threatening eye bleed	Non-serious eye bleed	Retinal or posterior vitreous detachment	Age-related macular degeneration	Diabetic macular oedema	Diabetic retinopathy or laser therapy for	iritis/iridocyclitis/uveitis	Eye inflammation unspecified	Eye infection unspecified	Glaucoma	Retinal vein thrombosis	Retinal artery occlusion or TIA	Giant cell arteritis	Retinitis pigmentosa	Blindness	Visual disturbance	Cataract diagnosis or operation	Laser therapy	Eye injection	Vitrectomy	Eye investigation	Orbital injury	Cranial nerve palsy	Anterior chamber/external eye conditions or surgery	Other non-serious eye events (e.g. conjunctivitis)	Other serious medical events	Other non-serious adverse events	**Total**
Pre-adjudicated categories	Any eye bleed	81	193	18	10	45	68	1	3	2	4	25	3	1	1		4	7		1	1	5	1	4	4	4		5	**491**
	Retinal detachment	1		51			1										1	1											**55**
	Age-related macular degeneration	1		1	50	11	1				1						4			2		1			1				**73**
	Diabetic macular oedema				1	26						1						1		1									**30**
	Blindness															2													**2**
	Visual disturbance		1	3	1	4	1	1						1			7	2	1		1			4	1		1		**29**
	Total	**83**	**194**	**73**	**62**	**86**	**71**	**2**	**3**	**2**	**5**	**26**	**3**	**2**	**1**	**2**	**16**	**11**	**1**	**4**	**2**	**6**	**1**	**8**	**6**	**4**	**1**	**5**	**680**

Analyses were restricted to events that were fully or partially assessed, where the source of information was the participant's follow-up questionnaire.

Table 68 Agreement of Pre-Adjudicated *vs.* Adjudicated First Reports of each Eligible Event Type

	Percentage agreement	Cohen's kappa	Interpretation of κ value	95% Confidence Interval	P value
Any eye bleed	67.7%	0.40	Fair	0.34-0.47	$P = 2.2 \times 10^{-16}$
Retinal detachment	96.0%	0.77	Substantial	0.68-0.85	$P = 2.2 \times 10^{-16}$
Age-related macular degeneration	94.9%	0.71	Substantial	0.62-0.81	$P = 2.2 \times 10^{-16}$
Diabetic macular oedema	90.6%	0.41	Moderate	0.27-0.55	$P = 1.6 \times 10^{-6}$
Blindness	100%	1.0	Perfect	-	-
Visual disturbance	95.4%	0.29	Fair	0.05-0.53	P = 0.02

κ values ≤ 0 indicate there was no agreement, 0.01–0.20 none to slight agreement, 0.21–0.40 fair agreement, 0.41– 0.60 moderate agreement, 0.61–0.80 substantia agreement, and 0.81–1.00 almost perfect agreement.
Analyses were restricted to events that were fully or partially assessed, where the source of information was the participant's follow-up questionnaire.

4.10.1 Post-hoc Exploratory Analysis: Agreement between Events Reported on the VFQ and ASCEND Follow-up Questionnaires

A post-hoc exploratory analysis was undertaken to evaluate the agreement between events recorded by cohort C participants on their VFQ *versus* the ASCEND follow-up questionnaires (Table 69). Although primarily conducted to help interpret the AMD results, the analyses were extended to include the other eye events listed in Appendix 23. Comparisons were restricted to the first events of each type, which occurred during the in-trial period of ASCEND.

VFQ respondents constituted 69.4% (n=2105) of the total number of randomised participants who reported at least one eye event during ASCEND. Of those, 580 (27.6%) reported an event on an in-trial follow-up questionnaire only, 884 (42.0%) reported an event on the VFQ only, and 641 (30.5%) reported an event in both places, suggesting there were some differences in the psychometric properties of the two questionnaires.

Among the full randomised population in ASCEND, the majority of incident AMD events (69.2%) and glaucoma (63.6%) events were derived from the VFQ-only, whereas in-trial forms captured most of the other eye events, including the first eye bleeds (392 out of 401; 97.8%) and DMO (102 out of 128; 79.7%) events.

Table 69 Agreement between Events Reported by Cohort C Participants on the VFQ and their ASCEND Follow-up Questionnaires

	Number of participants with at least one event of this type										
				VFQ Respondents							
	Total no. of participants with an event of this type*	In those with no VFQ (% of total no. of people with events of this type)†		Overall (% of total no. of participants with events of this type)‡		Reported on both ASCEND trial forms and on the VFQ (% of total no. of events of this type) §		Reported on ASCEND trial forms only (% of total no. of events of this type) ¶		Reported on the VFQ form only (% of total no. of events of this type)**	
Events specifically sought on the VFQ											
Age-related macular degeneration	260	34	(13.1%)	226	(86.9%)	33	(12.7%)	13	(5.0%)	180	(69.2%)
Cataract	1925	447	(23.2%)	1478	(76.8%)	531	(27.6%)	218	(11.3%)	729	(37.9%)
Glaucoma	217	24	(11.1%)	193	(88.9%)	23	(10.6%)	32	(14.7%)	138	(63.6%)
Retinal vein occlusion	100	21	(21.0%)	79	(79.0%)	12	(12.0%)	25	(25.0%)	42	(42.0%)
Other eye events as listed in Appendix 23											
Any eye bleed	401	169	(42.1%)	232	(57.9%)	3	(0.7%)	220	(54.9%)	9	(2.2%)
Retinal/posterior vitreous detachment	100	28	(28.0%)	72	(72.0%)	21	(21.0%)	35	(35.0%)	16	(16.0%)
Diabetic retinopathy/laser therapy for diabetic retinopathy	719	270	(37.6%)	449	(62.4%)	25	(3.5%)	318	(44.2%)	106	(14.7%)
Diabetic macular oedema	128	40	(31.3%)	88	(68.8%)	7	5.5%)	55	(43.0%)	26	(20.3%)
Iritis/iridocyclitis/uveitis	12	1	(8.3%)	11	(91.7%)	3	(25.0%)	7	(58.3%)	1	(8.3%)
Retinitis pigmentosa	1	1	(100.0%)	0	-	0	-	0	-	0	-
Eye investigation	70	22	(31.4%)	48	(68.6%)	0	-	40	(57.1%)	8	(11.4%)
Eye injection	139	50	(36.0%)	89	(64.0%)	1	(0.7%)	84	(60.4%)	4	(2.9%)
Eyelid/eyebrow operation	54	19	(35.2%)	35	(64.8%)	0	-	30	(55.6%)	5	(9.3%)
Other eye operation	73	27	(37.0%)	46	(63.0%)	0	-	46	(63.0%)	0	-
Operation on the vitreous body/vitrectomy	60	23	(38.3%)	37	(61.7%)	4	(6.7%)	31	(51.7%)	2	(3.3%)
Eye injury/trauma	11	2	(18.2%)	9	(81.8%)	0	-	5	(60.4%)	4	(36.4%)
Cranial nerve palsy	65	24	(36.9%)	41	(63.1%)	4	(6.2%)	32	(55.6%)	5	(7.7%)
Amaurosis fugax	5	2	(40.0%)	3	(60.0%)	0	-	2	(40.0%)	1	(20.0%)
Retinal artery occlusion	5	2	(40.0%)	3	(60.0%)	0	-	0	-	3	(60.0%)
Stroke codes	43	27	(62.8%)	16	(37.2%)	0	-	14	(32.6%)	2	(4.7%)
Giant cell arteritis/temporal arteritis	9	4	(44.4%)	5	(55.6%)	2	(22.2%)	2	(22.2%)	1	(11.1%)
Conjunctivitis	12	0	-	12	(100.0%)	1	(8.3%)	8	(66.7%)	3	(25.0%)
Eyelid disorder	19	9	(47.4%)	10	(52.6%)	0	-	10	(52.6%)	0	-
Eye infection	23	9	(39.1%)	14	(60.9%)	1	(4.3%)	10	(43.5%)	3	(13.0%)
Visual loss/eyesight deterioration	73	20	(27.4%)	53	(72.6%)	2	(2.7%)	23	(31.5%)	28	(38.4%)
Eye inflammation	20	4	(20.0%)	16	(80.0%)	0	-	8	(40.0%)	8	(40.0%)
Corneal disorder	26	4	(15.4%)	22	(84.6%)	2	(7.7%)	9	(34.6%)	11	(42.3%)
Blindness	7	3	(42.9%)	4	(57.1%)	0	-	1	(14.3%)	3	(42.9%)
Total	**3033**	**928**	**(30.6%)**	**2105**	**(69.4%)**	**641**	**(21.1%)**	**580**	**(19.1%)**	**884**	**(29.1%)**

*Total number of participants who reported at least one eye event during ASCEND out of the full randomised cohort of 15,480 participants, from either the in-trial follow-up questionnaires or the VFQ. Overall, 3033 participants reported 4577 first eye events.

†Number of participants who reported at least one eye event during ASCEND among those who were ineligible to receive or did not return a VFQ (n=6634). Overall, 928 participants reported 1286 first eye events.
‡Number of VFQ respondents who reported at least one eye event on either their VFQ or during ASCEND on an in-trial follow-up form among those who returned a VFQ (n=8846). Overall, 2105 participants reported 3291 first eye events.
§Number of VFQ respondents who reported at least one eye event on both their VFQ and during ASCEND on an in-trial follow-up form. Overall, 641 participants reported 675 first eye events.
¶Number of VFQ respondents who reported at least one eye event on their ASCEND in-trial follow-up forms but not on the VFQ. Overall, 580 participants reported 1278 first eye events.
**Number of VFQ respondents who reported at least one eye event on the VFQ but not on their ASCEND in-trial follow-up forms. Overall, 884 participants reported 1338 first eye events.

4.11 Chapter Summary

This chapter presented the results of pre-specified and exploratory analyses for ASCEND-Eye. The data contribute to a clearer understanding of the effects of aspirin and, separately, omega-3 FAs on diabetic eye disease and AMD; an interpretation of these results in the context of existing studies is given in the next chapter.

Chapter V - Discussion

This chapter summarises the main findings of ASCEND-Eye and how they relate to previous studies. A discussion regarding the implications of these results for theory development, practice, public policy and future research follows this. Finally, the strengths and limitations of the study as a whole are considered.

5.1 Effects of Aspirin on Diabetic Retinopathy and Diabetic Maculopathy

In the ASCEND-Eye sub-study of a large randomised placebo-controlled trial, allocation to enteric-coated aspirin at a dose of 100mg daily for 6.5 years was associated with a statistically non-significant 3% increase in the risk of referable disease; a composite of referable DR ($\geq R_2$) or referable diabetic maculopathy (M_1), based on the NSC's grading criteria for diabetic eye disease. However, a small benefit could not be ruled out; the 95% confidence interval around the risk estimate included the possibility of protective effects as large as 9% and potential harms of up to 16%. Similarly, aspirin had no clinically-meaningful beneficial effects on the secondary or tertiary outcomes of referable disease stratified by baseline DR severity, progression in retinopathy grade, or duplex retinopathy grades at the final eye screening record, respectively. Although there was a marginally significant trend towards harm from allocation to aspirin on incident diabetic maculopathy, it should not be over-interpreted, given the multiple comparisons.

These results build on the evidence from ETDRS[140], in which 650mg of aspirin daily taken over seven years did not prevent the development of high-risk proliferative features in 3711 people with established DR. However, some important methodological differences between the two trials should be recognised. First, the primary efficacy endpoints were dissimilar. In ETDRS, the main comparison was a composite of neovascularisation of the optic disc or neovascularisation accompanied by a vitreous or pre-retinal

haemorrhage, where either component exceeded the defined areas of a standard fundus photograph*. The equivalent retinopathy component in ASCEND-Eye would include anyone who progressed to R_3 disease, of whom there were very few. ASCEND-Eye also characterised the severity of eye bleeding events in more detail in separate safety analyses. Next, there was no maculopathy component of the primary endpoint in ETDRS, whereas M_1 screening grades contributed the majority of referable disease events in ASCEND-Eye. This difference probably reflects the factorial design in ETDRS in which, in addition to the aspirin assignment, one eye of each participant was separately randomised to early photocoagulation and the other to deferred photocoagulation for CSMO, defined as retinal thickening or hard exudates at or within 500μm of the central fovea. However, in contrast to ASCEND-Eye, albeit in a subgroup analysis that was not adjusted for the multiple comparisons, aspirin had no significant beneficial or harmful effects on incident CSMO events in eyes assigned to either schedule.[140] In eyes assigned to deferred photocoagulation, there were 523 [56.5%] CSMO events in the aspirin group, compared to 516 [55.3%] in the placebo group (RR 1.02; 99% CI 0.87-1.19).[140] The null effects of aspirin in eyes assigned to early photocoagulation were narratively described, but summary statistics were not shown.[140]

Other differences between ASCEND-Eye and ETDRS included the study population and aspirin dose. As described in Chapter I, preclinical studies have previously identified retinal capillary occlusion caused by platelet thrombi[124,125], or inflammatory changes involving the vascular endothelium[128-131], as triggers for the chain of events causing diabetic microangiopathy in the eye. At baseline, the participants in ETDRS already had pathological changes beyond either of these cascading incidents, whereas the study population in ASCEND-Eye had minimal disease. Therefore, ASCEND-Eye was a good test of aspirin at an earlier stage of DR, whereas it may have been too late for it to have an effect in ETDRS.

At the dose tested in ASCEND-Eye (100mg daily), aspirin irreversibly inhibits platelet COX-1, resulting in the reduced formation of thromboxane A2 and, subsequently, a lower risk of

* The primary endpoint in ETDRS was "high-risk proliferative retinopathy", a composite of:[136]

- Neovascularisation of the optic disc: new vessels covering 1/4th to1/3rd of the disc area, within 1 disc diameter of the optic disc on a standard 10A stereoscopic fundus photographic field; or
- A pre-retinal or vitreous haemorrhage accompanied by neovascularisation of the disc, neovascularisation of the disc less than 1/4th of the disc area, or new vessels elsewhere covering an area ≥1/4 of the disc area.

vasoconstriction and platelet thrombosis.[119] However, this may not have suppressed an inflammatory mechanism of disease. At higher doses (≥300mg daily), aspirin inhibits the COX-2 isoform, suppressing the production of prostaglandins associated with inflammation in animal models of DR.[421] Although ETDRS did test an anti-inflammatory dose of aspirin, the control of participants' cardiovascular risk factors was relatively poor compared to contemporary standards. Table 70 compares the baseline characteristics of participants in the B1 cohort to those in ETDRS based on strata used in the older study. Experimental research has shown that dyslipidaemia and hypertension independently cause or exacerbate inflammatory processes induced by diabetes, including the dysregulation of nitric oxide synthase, leukostasis, oxidative stress, and the formation of advanced glycation end products within retinal tissues.[421] Whilst the randomisation in ETDRS ensured that these risks were balanced between the treatment arms, it is possible that a small beneficial effect of the anti-inflammatory dose of aspirin was outweighed by larger harmful effects of the study population's longer diabetes durations and poor glycaemic, lipid and blood pressure control. Evidence to support this hypothesis is limited but is now discussed.

The DAMAD trial, which also tested a higher dose of aspirin (330mg taken three times a day), detected fewer microaneurysms on annual fluorescein angiograms in those assigned active treatment.[143] However, this endpoint is too dissimilar, and the study was underpowered to be compared with either ASCEND-Eye or ETDRS.[143] Microaneurysms have also been observed to regress due to remodelling and reperfusion.[146,147] Therefore, the marginal differences reported in the small number of DAMAD participants (n=475) may have been due to chance. Surrogate evidence from studies of other anti-inflammatory drugs to treat DR is also lacking. Randomised trials of oral steroids or selective COX-2 inhibitors for DR are contraindicated due to their adverse systemic effects on glycaemic control and risk of ischaemic cardiovascular events, respectively.[422] Although topical non-steroidal anti-inflammatory therapies, such as bromfenac, nepafenac and ketorolac, have been used successfully to reduce inflammation after cataract surgery or to treat DMO in the short-term, their longer-term use to treat DR has not been studied in randomised trials of sufficient size or quality.[423] Therefore, there is

insufficient evidence to support the speculation that ASCEND-Eye studied the correct population at the wrong dose of aspirin, whilst ETDRS studied the wrong population at the correct dose of aspirin.

Overall, given both studies' large size and long follow-up durations, it seems more plausible that aspirin does not confer large beneficial effects in DR or diabetic maculopathy, irrespective of the disease severity, when taken for at least 6.5 years.

Table 70 Comparison of the Baseline Characteristics between ASCEND-Eye and ETDRS

Baseline characteristic	Participants with Linkage Data in ASCEND-Eye	ETDRS	
		Aspirin (n=1856)	Placebo (n=1855)
Age (years)	**n=7360**		
≤49	391 (6%)	896 (48%)	898 (48%)
≥50	6903 (94%)	960 (52%)	957 (52%)
Sex	**n=7360**		
Male	4594 (62%)	1031 (56%)	1065 (57%)
Female	2766 (38%)	825 (45%)	790 (43%)
Type of diabetes	**n=7360**		
Type 1	373 (5%)	559 (30%)	571 (31%)
Type 2	6987 (95%)	1297 (70%)	1284 (69%)
Duration of diabetes (years)	**n=6948**		
<10	4489 (65%)	306 (16%)	304 (16%)
10-19	1618 (23%)	1084 (58%)	1035 (56%)
≥20	778 (11%)	456 (25%)	516 (28%)
Systolic blood pressure (mmHg)	**n=5386**		
≥130<159	3295 (61%)	1228 (66%)	1220 (66%
≥160	405 (8%)	385 (21%)	364 (20%)
Diastolic blood pressure (mmHg)	**n=5382**		
≥85<90	506 (9%)	735 (40%)	716 (39%)
≥90	543 (10%)	552 (30%)	509 (27%)
Glycosylated haemoglobin (HbA1c)	**n=4773**		
≥10% (DCCT)	133 (3%)	538 (41%)	584 (43%)
Non-HDL cholesterol	**n=4763**		
≥160mg/dl (≥4.14 mmol/l)	295 (6%)	336 (26%)	328 (25%)
Severity of retinopathy*	**n=2854**		
R_0 (ETDRS level 10,14, 15)	2370 (83%)	0 (0%)	0 (0%)
R_1 (ETDRS level 20, 35, 43)	466 (16%)	725 (39%)	790 (43%)
R_2 (ETDRS level 47, 53)	16 (1%)	807 (44%)	723 (39%)
R_3 (ETDRS level 60, 61, 65, 71, 75, 81)	2 (<0.1%)	324 (18%)	342 (18%)
Visual acuity†	**n=2392**		
ASCEND-Eye score ≤5 (≥85 ETDRS letters)	1852 (77%)	938 (50%)	901 (49%)
ASCEND-Eye score ≥6≤8 (84-70 ETDRS letters)	502 (21%)	721 (39%)	734 (39%)
ASCEND-Eye score ≥9 (<70 ETDRS letters)	38 (2%)	197 (11%)	220 (12%)
Ethnicity	**n=7360**		
White	7083 (96%)	1420 (76%)	1414 (76%)
Smoking status	**n=7270**		
Current smokers	577 (8%)	819 (44%)	822 (44%)

Percentages, shown in parentheses next to each parameter, were based on the total number of participants with linkage data for whom these data were known.

*These analyses were restricted to the best eye in those with bilateral retinopathy data at baseline and/or the final record. The "best eye" was defined as the one with the lowest retinopathy grade at the baseline eye screening record. When retinopathy grades were the same in both eyes, the right eye was arbitrarily chosen as the "best eye".

†These analyses were restricted to the best eye in those with bilateral visual acuity data at baseline and/or the final record. The "best eye" was defined as the one with the lowest ASCEND-Eye visual acuity score at the baseline eye screening record. When the visual acuity scores were the same in both eyes, the right eye was arbitrarily chosen as the "best eye".

5.2 Effects of Omega-3 Fatty Acids on Diabetic Retinopathy and Diabetic Maculopathy

The ASCEND-Eye study showed that, compared to placebo, there were no clinically-meaningful favourable effects of randomisation to active omega-3 FAs for 6.5 years on the primary efficacy outcome of referable disease. There were also no pre-specified subgroups that appeared to benefit proportionally more or less. Moreover, there were no significant between-group differences in the secondary or tertiary outcomes, including referable disease stratified by baseline DR severity, DR progression, incident diabetic maculopathy, or duplex retinopathy grades at the final eye screening record.

The potential of omega-3 FAs has mainly been demonstrated in experimental animal models, in which the protective effects against DR and diabetic maculopathy were mediated through antioxidant and anti-angiogenesis pathways.[51] In particular, DHA is thought to competitively displace retinal stores of omega-6 FAs, thereby blocking the production of eicosanoid-derived cytokines, such as VEGF, which cause abnormal angiogenesis and increased vascular permeability.[149-155,157,424-426] ASCEND-Eye is the first large, well-conducted, and prospective test of omega-3 FAs on DR and diabetic maculopathy. As such, there are no studies of equivalent quality with which it is comparable. Existing observational studies have lacked detail regarding omega-3 FAs exposures and may be confounded by variables not included in the analysis models.[162,164] Meanwhile, a single previous randomised trial of adjunct ALA supplementation in a high-risk population receiving intravitreal therapies for DMO was too small to draw a robust conclusion.[179] Whilst allocation to active omega-3 FAs in ASCEND-Eye was associated with a statistically non-significant 7% increase in the risk of referable disease, it did not exclude the possibility of a more minor, but potentially important, benefit; the 95% confidence intervals around the risk estimate included the possibility of protective effects as large as 5% and potential harms of up to 20%.

DHA supplementation has been associated with the down-regulation of tyrosine kinase nuclear factor-kappa B, a transcription factor that increases COX-1 and -2 receptor expression.[51] The randomised factorial comparison with aspirin, with its inhibitory effects on these enzymes, was therefore expected

to complement the effect of omega-3 FAs on the retina. Although the null results reported are probably attributable to a true lack of efficacy, adequacy of the DHA or EPA dose, lower bioavailability of the ethyl ester compound as opposed to triglyceride form, treatment duration, or both, may also be relevant. However, overall, these data provide insufficient strength of evidence to recommend omega-3 FAs for DR or diabetic maculopathy and should be taken into account when designing other trials in the future.

5.3 Effects of Aspirin on Eye Bleeding

In the ASCEND-Eye population of diabetic adults over 40 years of age, randomisation to aspirin for 7.4 years did not increase the risk of sight-threatening bleeding in the eye, defined as clinically significant bleeding in the eye which resulted in unresolved visual loss, or required an urgent intervention such as laser photocoagulation, vitreoretinal surgery or intraocular injection, or both. Likewise, there was no excess hazard for separate components of the secondary safety outcome, including non-serious or unrefuted eye bleeds, nor for confirmed or unrefuted incident eye bleeds of any severity combined.

Table 71 summarises the results of eye safety analyses from other large clinical trials of aspirin for the primary prevention of major cardiovascular events, similar to ASCEND. Safety data from DR and AMD trials of aspirin have also been tabulated for comparison. Although their outcome definitions, dosage, and methods of event ascertainment differed, the findings in ASCEND-Eye were broadly consistent with the ETDRS trial, in which the use of 650mg aspirin daily for seven years did not increase the occurrence of pre-retinal or vitreous haemorrhages, nor the extent or duration of unresorbed blood, either in those who developed high-risk proliferative features or those who did not.[140,142]

Neither DAMAD[143] nor the ancillary AMD analyses from the Physicians' Health Study (1982-1988)[323] or the Women's Health Study (1992-2005)[301] reported on the risk of eye bleeding with aspirin. Similarly, very few cardiovascular primary prevention trials included ocular bleeds as one of their main safety endpoints and those that did give scant detail regarding the severity or location of only a tiny number of events.[427-429] Finally, the Anti-Thrombotic Treatment Trialists' Collaboration does not have

more detailed individual patient data regarding eye bleeds from any of these trials [personal communication with the Principal Investigators of the Collaboration].

Overall, the findings of ASCEND-Eye provide further reassurance regarding the ophthalmological safety of aspirin for those with DR, the elderly, or both when it is required to treat cardiovascular disease or other clinical indications. The project also introduces a new, clinically-relevant way of defining the severity of eye-bleeding events, which future studies could adopt.

Table 71 Eye Bleeding Events in Other Large Clinical Trials of Aspirin for the Primary Prevention of Major Cardiovascular Events and, Separately, for Diabetic Retinopathy and Age-Related Macular Degeneration

Trial, participating countries, year of main publication (No. of participants; Follow-up duration)	Treatments	Participants	Trial design and methods	Eye bleeds
Cardiovascular Primary Prevention Trials				
Aspirin *vs*. no treatment				
British Doctors Trial, UK 1988[430] (n=5139; 5.6yr)	Aspirin 500mg daily *vs.* no aspirin	Healthy male doctors	Parallel groups Open-label	Not reported
Primary Prevention Project, Italy 2001[428] (n=4495; 3.6yr)	Aspirin 100mg daily *vs.* no aspirin (Randomised factorial comparison: vitamin E *vs.* open control)	Men and women with diabetes, aged >50 years and ≥1 CVD risk factor	Factorial design Open-label	1 participant in the active group *vs.* 1 participant in the placebo group had an "ocular bleed"
Japanese Primary Prevention of Atherosclerosis with Aspirin for Diabetes Trial (JPAD), Japan 2008[427] (n=2539; 4.4yr)	Aspirin 81mg or 100mg daily *vs.* no aspirin	Men and women with diabetes	Parallel groups Open-label	8 participants in the active group *vs.* 5 participants in the placebo group had a "retinal bleed"
Aspirin and Simvastatin Combination for Cardiovascular Events Prevention Trial in Diabetes (ACCEPT-D), Italy[431] (Target n=5170; Target 5yr)	Aspirin 100mg daily plus simvastatin *vs.* no aspirin daily plus simvastatin	Men and women with diabetes	Parallel groups Open-label	The main results paper has yet to be published. A baseline paper was published in 2007
Placebo-controlled trials				
Physicians' Health Study, US 1989[432] (n=22,071; 5yr)	Aspirin 325mg on alternate days *vs.* placebo (Randomised factorial comparison: beta-carotene *vs.* placebo)	Healthy male doctors	Factorial design Double-blind	Not reported
Thrombosis Prevention Trial, UK 1998[429] (n=5085; 6.7yr)	Aspirin 75mg daily (controlled release) *vs.* placebo (Randomised factorial comparison: warfarin *vs.* placebo)	Men at high risk of CVD	Factorial design Double-blind	6 participants in the active group *vs.* 5 participants in the placebo group had an "ocular bleed"
Prevention of Peripheral Arterial Disease and Diabetes Trial (POPADAD), Scotland 2008[433] (n=1276; 6.7yr)	Aspirin 100mg daily *vs.* placebo (Randomised factorial comparison: antioxidant vitamin and mineral supplement *vs.* placebo)	Men and women with diabetes and asymptomatic peripheral arterial disease	Factorial design Double-blind	Not reported
Aspirin to Reduce Risk of Initial Vascular Events Trial (ARRIVE), Europe and US, 2018[434] (n=12,546; 5.1yr)	Aspirin 100mg daily *vs.* placebo	Men aged ≥55 years with at least 2 CVD risk factors; Women aged ≥60 years with at least 3 CVD risk factors*	Parallel groups Double-blind	Not reported
Hypertension Optimal Treatment Trial, Europe, Asia and US 1998[435] (n=18,790; 3.8yr)	Aspirin 75mg daily *vs.* placebo (Randomised factorial comparison: 3 different BP control regimens)	Hypertensive men and women aged 50-80 years	Factorial design Double-blind	Not reported
Women's Health Study, US 2005[436] (n=39,876; 10yr)	Aspirin 100mg on alternate days *vs.* placebo (Randomised factorial comparison: vitamin E *vs.* placebo)	Female healthcare professionals	Factorial design Double-blind	Not reported
ASPirin in Reducing Events in the Elderly (ASPREE) trial, Australia, 2018[437,438] (n=19,114; 5yr)	Aspirin 100mg daily *vs.* placebo	Healthy men and women aged ≥70 years	Parallel groups Double-blind	Not reported

Trial, participating countries, year of main publication (No. of participants; Follow-up duration)	Treatments	Participants	Trial design and methods	Eye bleeds
Diabetic Retinopathy or Age-Related Macular Degeneration Trials				
Placebo-controlled trials				
DAMAD, Europe, 1989 (n=475; 3yr)[143]	Aspirin alone (300mg 3 x daily), Aspirin plus 75mg dipyridamole daily *vs.* placebo	Men and women with early DR	Parallel groups Double-blind	Not reported
ETDRS, US 1989[142] (n=3711;7yr)	Aspirin 650mg daily *vs.* placebo (Randomised factorial comparison: early *vs.* deferred photocoagulation)	Men and women with diabetes and non-proliferative to early proliferative DR	Factorial design Double-blind	Incident pre-retinal or vitreous haemorrhage: 190 [10.2%] *vs.* 203 [10.9%]; RR 1.05;99% CI 0.81-1.36
Physicians' Health Study, US, 2001[323] (n=22,071; 5yr) (AMD sub-study)	Aspirin 325mg on alternate days *vs.* placebo (Randomised factorial comparison: beta-carotene *vs.* placebo)	Healthy male doctors	Factorial design Double-blind	Not reported
Women's Health Study, US, 2009[301] (n=39,421; 10yr) (AMD sub-study)	Aspirin 100mg daily *vs.* placebo (Randomised factorial comparison: vitamin E *vs.* placebo)	Female healthcare professionals	Factorial design Double-blind	Not reported
ASPREE-AMD, Australia, 2017[332,372] (n=4993; 5yr)	Aspirin 100mg daily *vs.* placebo	Healthy men and women aged ≥70 years	Factorial design Double-blind	The results of this sub-study have not been published

CVD = cardiovascular disease

*Risk factors included high total cholesterol, high LDL-cholesterol, low HDL-cholesterol, current smoking, high systolic blood pressure, taking anti-hypertensive medication(s), or a family history of cardiovascular disease.

5.4 Effects of Aspirin on Age-Related Macular Degeneration

Participants of the ASCEND-Eye study who were randomised to a daily regimen of 100mg of aspirin daily, had a statistically non-significant 12% reduced risk of incident AMD, during 7.4 years of follow-up, compared to those assigned the placebo. The 95% confidence interval around the risk estimate included the possibility of a protective effect as large as 31% and a harmful effect of up to 13%.

These findings complement two other large, double-blind, randomised controlled trials of aspirin for the primary prevention of cardiovascular events.[323,439] The Physicians' Health Study randomised male US Physicians aged 40-84 years to 325mg of aspirin on alternate days or a matching placebo.[323] The trial, which was stopped early because of overwhelming reductions in myocardial infarction events, included 22,071 men who did not report having AMD at baseline.[323] After five years of treatment, there were fewer AMD diagnoses in those assigned to aspirin compared to those in the placebo group (51 [0.5%] *vs.* 66 [0.6%]; rate ratio 0.77; 95% CI: 0.54-1.11).[323] Similar results were reported in the Women's Health Study, which randomised female healthcare professionals in the US who were 45 years of age or older to 100mg of aspirin daily on alternate days or a matching placebo.[301] After ten years of follow-up, among 39,421 women without AMD at baseline, there was a non-significant trend towards a beneficial effect with the active treatment (111 [0.5%] *vs.* 134 [0.7%]; HR 0.82; 95% CI: 0.64 –1.06).[301] Like ASCEND-Eye, in which the survival curves appear to separate in favour of aspirin after six years of follow-up, there was a separation of the curves after three years of follow-up in the Women's Health Study, but overall, neither result attained statistical significance. Nevertheless, it may be important to obtain further data regarding the effects of aspirin on AMD over a more extended follow-up period.

AMD is a complex multifactorial disease, which makes disentangling the effects of aspirin challenging. In histological studies of ageing retinae, there have been consistent reports of fewer and more narrowed choriocapillaris vessels in the macular areas of those with senior maculopathy compared to disease-free controls.[440-442] However, whether these changes represent a cause or response to an underlying degeneration of the RPE and neural tissue layers is unclear.[440-442] Platelets are thought to play a key role in initiating and sustaining atherosclerosis by inducing the secretion of pro-inflammatory cytokines

responsible for changing the vascular endothelium's chemotactic and adhesive properties, and causing smooth muscle proliferation.[443-446] Therefore, if localised or systemic atherosclerosis is involved in the pathogenesis of AMD, chronic suppression of platelet function by aspirin might be one of the mechanisms through which the drug confers a small beneficial effect.[443,444] In preclinical studies, aspirin has also been reported to prevent AMD development through pathways not dependent on platelet inhibition, such as increased production of the anti-inflammatory mediator 15-epi-lipoxin A_4[447,448], and lipid peroxidation[449] and antioxidant pathways.[450-452] Conversely, hypoxia caused by the vasoconstrictive effects of COX-2 suppression of prostacyclin synthesis may increase the likelihood of neovascular AMD developing when aspirin is administered over a longer period.[303]

The benefits of aspirin for the secondary prevention of cardiovascular disease are well-established[139], but the risks posed by aspirin to those with early or undiagnosed ARM have previously been raised as a concern.[293] The findings of ASCEND-Eye mitigate some of this apprehension but should nevertheless be interpreted in the context of the wider literature and the study's limitations. ASCEND-Eye shares the same weaknesses as the two previous trials:

i) There were too few events in this relatively young population for there to be precision around the risk estimate. For the same reason, heterogeneity between subgroups of important prognostic risk factors, such as age, sex or smoking, could not be formally tested. Therefore, the possibility of a differential effect of aspirin in certain subgroups in ASCEND-Eye cannot be excluded.
ii) Participant-reported events were confirmed by medical record review instead of retinal imaging. From the supporting documents received from participants' GPs, it was not always possible to ascertain the AMD subtype nor to discern the effects of aspirin on the progression from early to late disease.
iii) The duration of follow-up may have needed to be longer to answer the research question, given the slow rate at which new macular lesions accumulate.

These issues could be relevant given the findings of previous prospective cohort studies. Those with a longer follow-up (15-20 years), which ascertained the two AMD subtypes, have reported harmful associations of aspirin with wet AMD.[303,316] Conversely, those with a shorter follow-up (≤10 years) that

did not phenotype the study population found no statistically significant association.[315,320,321] However, there was a possibility of misclassification in all of these observational studies because participants' exposure to aspirin was only obtained once at baseline, most lacked detail regarding the aspirin exposure, and all were vulnerable to confounding from unmeasured or unknown characteristics. By contrast, the baseline characteristics in ASCEND-Eye were equally balanced between the active and placebo treatment groups, leaving less chance of residual confounding, and the aspirin dose, duration and frequency were clearly defined. Nevertheless, if it is confirmed that harmful effects take longer to emerge, it might be appropriate to offer older people prescribed aspirin more frequent eye examinations, given the potential to prevent rapid visual loss associated with the wet form of AMD by administering anti-VEGF therapies.

In summary, these findings alone neither confirm nor refute the relationship between low-dose aspirin and senior maculopathy. Thus, longer-term follow-up of ASCEND participants, plus data from the sufficiently large ASPREE-AMD trial, is likely to be of public health importance.[332,372]

5.5 Effects of Omega-3 Fatty Acids on Age-Related Macular Degeneration

In the ASCEND-Eye study, individuals assigned 1g omega-3 FAs daily and followed up for an average of 7.4 years had no significant proportional reduction in their risk of incident AMD compared to those assigned the placebo. Like the aspirin comparison, the study was underpowered to detect significant differences between the active and placebo omega-3 FA treatment groups; however, the findings are still of value given the limitations and conflicting results of previous studies.

Most observational studies of omega-3 FAs and AMD have reported that individuals affected by AMD recall a statistically significantly lower intake of fish or omega-3 FAs.[334,339-341,343,345-347,349-351,353,356,358,360-362,364] In contrast, the ASCEND-Eye results were consistent with three previous randomised trials of omega-3 FAs for AMD, which also had null findings.[367,368,370] However, some methodological differences exist between ASCEND-Eye and its forbearers. First, because AMD is comparatively rare and slow to progress, all three previous trials recruited people known to have bilateral ARM or late

AMD in just one eye to ensure that a sufficient number developed the primary endpoint during the scheduled treatment period. The AMD status of participants when they were recruited in ASCEND is unknown. Therefore, it is not possible to say whether ASCEND-Eye was predominantly a test of omega-3 FAs for the primary prevention of early lesions, the secondary prevention of late disease, or both, a limitation that also applies to the aspirin comparison for AMD. All four trials were underpowered to detect small effects of treatment, which may nevertheless be important given the absence of effective therapies for the dry disease. In a subgroup analysis of the NAT-2 trial, which used a DHA-enriched supplement, those thought to be more adherent, based on the composition of their red blood cell membranes, had a marginally significantly lower risk of developing the wet form of AMD.[368] Similarly, the prospective observational studies which reported statistically significant benefits also isolated the effect of DHA.[334,343,347,348,350,353,356,358,362] Thus, the relatively lower dose of DHA in ASCEND-Eye, reduced bioavailability of the ethyl ester formulation compared to triglyceride-based compounds, and poor adherence in the later years of follow-up, may have shifted the risk estimate towards the null. However, overall, the collective weight of evidence suggests that the relationship between omega-3 FAs and senior maculopathy is modest at best; omega-3 FAs are unlikely to contribute significantly to the burden of AMD or modify against the disease development or progression. Having been comprehensively tested in four randomised clinical trials, there is insufficient data to support omega-3 FAs for AMD, nor are future studies into the relationship warranted.

5.6 Effects of the Study Treatments on Visual Acuity

Visual acuity depends on how well light is refracted by the cornea and lens, the retinal tissues' health, and the brain's interpretative faculties.[384] Neither randomisation to aspirin nor omega-3 FAs were found to be statistically significantly beneficial in preserving visual acuity, an endpoint chosen because it has been recommended by the US Food and Drug Administration as a standard objective outcome measure in Ophthalmology trials, which could have been relevant to health economic analyses, had any of the primary or secondary efficacy comparisons been statistically significant.[405] Individuals assigned to aspirin had a non-significant 5% higher risk of worsening visual acuity equivalent to a loss of 15 or

more ETDRS letters. The 95% confidence interval around the risk estimate included the possibility of a protective effect as large as 8% and a harmful effect of up to 20%. Meanwhile, there was a non-significant 7% beneficial effect of randomisation to omega-3 FAs. The 95% confidence interval around this risk estimate included the possibility of a protective effect as large as 19% and a harmful effect of up to 6%. These findings were consistent with the ETDRS and AREDS2 randomised placebo-controlled trials of aspirin for DR and omega-3 FAs for AMD, respectively.[140,367] Both reported no significant differences in their between-group comparisons of the proportion of study participants who lost three or more lines (15 or more letters) on a standard ETDRS visual acuity chart.[140,367] Other trials described in the systematic literature reviews in Chapters I and II did not have a visual acuity outcome.

5.7 Effects of the Study Treatments on NEI-VFQ-25 Scores

In the ASCEND-Eye sub-study of a large randomised placebo-controlled trial, there was no apparent effect of aspirin or, separately, omega-3 FAs allocation on mean composite scores derived from the NEI-VFQ-25, nor for any of its subdomains. Scores obtained from the NEI-VFQ-25 are strongly and independently correlated to visual acuity.[2] The latter serves as an objective indicator of macular function, but it may not reflect how retinal diseases such as DR and AMD affect other dimensions of vision, including contrast sensitivity, colour perception and stereoscopic vision.[384] Therefore, the NEI-VFQ-25 has been recommended by regulators as a more holistic endpoint, and for vision-targeted cost-utility analyses, in ophthalmology trials such as ASCEND-Eye.[385]

The distribution of scores from the NEI-VFQ-25 was very negatively skewed towards the highest possible values, both overall and in the subdomains. Therefore, showing between-group differences with this instrument would have required both a large treatment effect and a potential for the more rapid deterioration of retinal disease during the follow-up period. In ASCEND-Eye, very few participants had severe retinal disease, and both conditions that were of interest, DR and AMD, are known to progress slowly compared to the duration of follow-up.[8,9] By excluding those on third-party follow-up at the end of ASCEND, the eligibility to receive the questionnaire selected those who were slightly younger (see

Table 37), probably less frail and with better eyesight. These reasons, and the absence of a large treatment effect for the primary or secondary outcome comparisons are likely to explain why there were no significant treatment effects on the NEI-VFQ-25 composite or subdomain scores. There are no equivalent studies of aspirin or omega-3 FAs and the NEI-VFQ-25 for comparison.

Overall, the application of the NEI-VFQ-25 in ASCEND-Eye represents the largest survey of vision-targeted health-related quality of life in people with diabetes to date. Some post-doctoral observational analyses are planned to make further use of these data, which may identify the clinical and demographic characteristics associated with lower composite and subdomain scores in a diabetic population.

5.8 Effects of the Study Treatments on Other Eye Diagnoses

The effects of the study treatments on incident diagnoses of other eye conditions were not formally compared with inferential testing given the small numbers of most events, a desire to avoid multiple comparisons, and because, with the exception of aspirin and retinal artery or vein occlusions, neither study medication was expected to have any effect on them based on the existing literature.[8] The most frequent diagnoses were cataracts, glaucoma, retinal detachment and retinal vein thrombosis, consistent with the most common eye conditions among adults in the general population of the UK.[4] This observation, therefore, supports the generalisability of the ASCEND-Eye results.

5.9 Strengths and Limitations of the Methodology

5.9.1 Strengths

ASCEND-Eye has several strengths that facilitated a robust assessment of the effects of aspirin and, separately, omega-3 FAs on its main DR and AMD outcomes, including a large number of participants and a long duration of near-complete follow-up. The randomised design ensured a balance of the baseline characteristics between the active and placebo groups of each treatment arm, thereby avoiding residual confounding from unmeasured or unknown variables, which limited the findings of previous observational studies described in the four systematic literature reviews in Chapters I and II. In addition,

events were reported and confirmed whilst the participants and investigators were blind to the treatment allocations, which avoids the differential misclassification of outcomes between treatment groups.

5.9.2 Follow-up, Adherence and Generalisability

The limitations of ASCEND-Eye include the shorter mean duration of follow-up in the linkage cohorts (6.5 years) compared to the full ASCEND population (7.4 years; cohort A) or VFQ respondents (7.5 years; cohort C). Although this reduced the opportunity to have a referable disease, progressive retinopathy or diabetic maculopathy event, it should not introduce bias in the randomised comparisons of the linkage data.

Next, whilst the study average adherence was relatively good, poor adherence in the later years of follow-up, along with drop-ins to treatment in the placebo groups of both treatment arms, by those who were prescribed non-study anti-platelet therapy or took over-the-counter fish oil supplements, may have shifted risk estimates towards the null. More reassuringly, the largest contributing cause of treatment discontinuation in both arms ("Participant wishes") was unrelated to any ASCEND-Eye outcome variables.

As a sub-study of a larger trial, ASCEND-Eye had no control over its adherence figures. Several efforts were made to maintain adherence in ASCEND, including a placebo run-in phase to identify and exclude non-adherent individuals before they were randomised, regular participant newsletters to sustain enthusiasm and reiterate the importance of following trial procedures, and directly contacting those who could safely restart either treatment by a coordinating centre clinician. Although per-protocol approaches to analysis can be used in the context of low adherence, they invalidate the assumptions underlying randomisation, and therefore, intention-to-treat comparisons are the gold standard for clinical trials.[369,453] Thus, sensitivity analyses of the efficacy and safety outcomes, excluding those who were non-adherent, have not been performed.

Finally, although the findings of ASCEND-Eye are internally valid, their generalisability in a real-world setting may be limited by a lack of ethnic diversity and the relative under-representation of women in the ASCEND study population, an issue that has been described in other cardiovascular trials.[454]

5.9.3 Participant-Reported Events and Adjudication

Another limitation of ASCEND-Eye includes its reliance on participant-reported AMD, DMO and eye bleeding events without the benefit of multimodal imaging, such as fundus photography or OCT, which would facilitate more accurate phenotyping. This issue is especially relevant to identifying early ARM and distinguishing the wet and dry subtypes of late AMD, which may respond differently to aspirin (as discussed in Section 5.4). The UK National Ophthalmology Database (NOD) AMD Audit[455] began collecting this level of detail in June 2021, but they currently lack Section 251 exemption to access patient identifiers.[456] If Section 251 exemption is awarded, the first data extracted will cover the period from 1st April 2010 to 31st March 2022. Although this timeframe would not cover the entire in-trial period of ASCEND, it would still be of value and could be used to explore the longer-term effects of aspirin on AMD as a post-doctoral research project; however, the audit will not begin accepting data access applications before August 2023 [personal communication with Ms Lynne Sander, Project Manager for the NOD AMD Audit].

There is a possibility of some under-reporting of events by participants on their in-trial follow-up questionnaires, which did not explicitly ask about new eye diagnoses in the same way as cardiovascular and cancer events were sought. This information was specifically requested on the VFQ, but only some were eligible to receive it and of those, just under a quarter did not respond. The need to have good vision to complete a mailed questionnaire might also have self-selected respondents with better eye health. The post-hoc analyses described in Section 4.10.1 provide some insight into the ability of the ASCEND follow-up questionnaires and VFQ to discover eye events (i.e. their validity) and consistently measure the same thing (i.e. their reliability). There were no meaningful differences in the proportions of each eye event type reported during ASCEND between those included in and excluded from cohort C, which would suggest that cohort C participants responded to their in-trial questionnaires in a similar way to those who were ineligible to receive or did not respond to the VFQ. Conversely, there were differences in how cohort C participants responded to the two questionnaires for certain event types. For example, AMD and glaucoma events were more likely to be recorded on the VFQ than during ASCEND. Therefore, the possibility that AMD events were under-reported by those who did not receive the VFQ cannot be excluded. Although these influences on the reporting behaviours of ASCEND

participants would reduce the study's power, they would not be expected to influence the results in favour of a particular statistical finding.

A further constraint of some comparisons is the combined inclusion of participant-reported events confirmed at the time of adjudication by medical record evidence of the diagnosis, and those unrefuted having derived from the VFQ or because no supporting evidence was received from the participant's GP. With the exception of AMD, the total number of incident eye events contributed by the VFQ was small, and therefore, most of the outcomes included in the safety and diabetic maculopathy analyses were based on adjudicated data. Adjudication was completed for the majority (92.8%) of incident eye events that were eligible to be assessed. Most of which had good quality supporting evidence to verify both the date and diagnosis given by the participant (74.6% were "fully assessed"). Although the adjudication process resulted in very little re-categorisation of AMD and DMO events, eye-bleeding events were recoded to a different, non-bleeding diagnosis more frequently. A high confirmation rate of AMD and DMO events supported the decision not to adjudicate events originating from the VFQ. However, ascertaining the severity of eye bleeding events by clinical adjudicators is still necessary.

5.9.3.1 Use of Other Routinely-Collected Healthcare Data

Data from routinely-collected electronic healthcare records, including Hospital Episode Statistics and the OPCS* Classification of Interventions and Procedures, were obtained for most randomised participants in ASCEND, and covered the full scheduled treatment period. However, these were not used in ASCEND-Eye to look for unreported eye events or to confirm unadjudicated events because the definition of some eye conditions in the International Classification of Diseases 10th revision (ICD-10) is too broad. For example, the H35.3 diagnosis code "degeneration of macula and posterior pole" is applied to AMD and several other age-related conditions, including cystoid macular oedema or macula holes, but they have different underlying pathogeneses. The ICD-10 Clinical Modification, developed by the Centers for Disease Control and Prevention in the US, uses additional digits to

* Operating Procedure Codes Supplement.

subclassify conditions more specifically[457]; however, it has not been adopted for coding purposes in the UK.

5.9.4 Incomplete Linkage Coverage

The reliability of the primary efficacy analyses may be limited because usable linkage data could only be obtained for a suboptimal proportion (47.5%) of the randomised participants. There were several reasons for this, including:

i) changes in the regulatory environment over time and outdated registrations of the parent ASCEND study;
ii) the close proximity of ASCEND's recruitment to the initiation and expansion of the eye screening service in England and Wales;
iii) the eye screening records being held in multiple local DESP provider management systems rather than a single central repository; and
iv) no use of the linkage data for research purposes on a national scale in England before.

These issues prevented access to the records of participants whose general practice did not register their data with PHE, and those who attended DESPs that never responded to several invitations to collaborate, declined participation or were hampered by a combination of bureaucratic delay, the Covid-19 pandemic or both.

The methodological choices were further constrained by incomplete longitudinal coverage of the data that was received. This was due, in part, to changes in the geographical boundaries, software suppliers and data controller-processor arrangements for some English DESPs over the course of ASCEND. To mitigate these problems, participants were linked to every known general practice they attended during the scheduled treatment period rather than just their last known health centre registration. The quality assurance analyses described in Section 3.2.5 also endorse the validity of the methodology used to obtain linkage data when it could be accessed. Finally, the conclusions were supported by more than one type of statistical analysis, selected after a comprehensive blinded preliminary evaluation of the raw linkage data (see Section 3.3). Although there might have been a lack of precision regarding the

timing of retinal lesions, logrank methods were chosen to analyse the primary efficacy endpoint because they made the best use of the longitudinal data. There was no reason to suspect this was biased between groups in either treatment arm. Moreover, there were no significant effects of either study treatment on retinopathy outcomes in the cross-sectional tertiary and sensitivity analyses of the final and worst duplex retinopathy grades, which supports results derived from the logrank methods.

Despite these challenges, with 7360 participants included in the primary efficacy comparisons, ASCEND-Eye is the largest randomised trial of aspirin, and the first prospective test of omega-3 FAs on DR to date. It is also the first to obtain linked data from 27 DESPs in England and DESW for research purposes. In September 2021, it was announced that the eye screening service would be required to submit data to the National Diabetes Audit (NDA) each year.[458] Although there are currently no details regarding the administrative and clinical scope of these data, it might one day be possible to make a single application to NHS Digital, the data controllers for the NDA, which could make future linkage studies involving eye screening records more straightforward.

5.9.5 Diabetic Maculopathy

The NHS Diabetic Eye Screening service currently refers anyone graded M_1 to Digital Surveillance clinics or Hospital Eye Services (see Figure 4, Section 1.2.6).[59,61] M_1 is defined by the presence of transudative and exudative disease within the macular field, as described in Table 2, Section 1.2.2.[59,61] The service uses mydriatic, flash colour, and red-free fundus photography to screen for diabetic maculopathy.[59,61] However, OCT is preferred for diagnosing and monitoring responses to treatment for the disease, because agreement between the two imaging modalities is relatively poor.[93,459,460] For example, a small observational study published in 2021 sought to measure the inter-observer reliability, sensitivity and specificity of the technologies in 187 individuals referred for further assessment after being graded R_1M_1 during routine screening.[180] Two-thirds of the participants, referred based on the service's definition of central exudative lesions on fundoscopy, had no retinal thickening on OCT.[180] Conversely, a fifth of those with oedema on OCT had no signs of maculopathy when their eyes were re-imaged with fundus photography.[180] Moreover, only three individuals (1.4%) were eligible for anti-VEGF therapy based on the criteria set by NICE.[180] Therefore, including those who screened positive

for maculopathy in ASCEND-Eye's primary efficacy endpoint might have been less reliable than diagnoses made using tomography. Metrics derived from OCT were not sought in ASCEND-Eye because it was an emerging technology that had not been widely adopted by the NHS when ASCEND began its recruitment [personal communication with Professor Peter Scanlon]. In addition, there is currently no requirement to commission OCT imaging in the national service specification for Digital Surveillance clinics in England or Wales.[92] Hence, the longitudinal coverage of any OCT data that could be obtained may have been poor. More recently, new imaging modalities have emerged based on confocal scanning laser ophthalmoscopy, which uses different wavelengths of light to delineate particular retinal lesions at high resolution.[461] An advantage of these technologies is that they can be integrated with spectral domain OCT in a single instrument.[461] Therefore, future studies may be able to use data derived from these. Finally, although there was some fluctuation in the trajectory of M (and R) grades during the scheduled treatment period, their stability appeared to depend on the length of remaining follow-up, based on the post hoc descriptive analyses described in Section 4.4.1.1. There is no reason to think this was biased between groups in either treatment arm.

5.9.6 Surrogate Indicators of Advanced Diabetic Retinopathy

It was not possible to conduct sensitivity analyses for the entire randomised cohort, comparing the effects of each study treatment on a composite outcome of surrogate indicators of advanced DR, such as photocoagulation, intravitreal injections or vitrectomy. This was due to a limitation of the Read coding system used to record adverse event information in ASCEND, in which the clinical indication for certain procedures was sometimes indeterminable. For example, the non-specific code of "laser therapy" was used to record treatments for post-cataract operation complications, some types of AMD or refractive errors, as well as DR. There were also several indications for events given the "eye injection" code, including DR, AMD or retinal vein occlusions. For the same reasons, the proportion of those with the referable disease outcome who subsequently had an intervention could not be reliably assessed. However, the later use of healthcare resources by those referred was only likely to become an important consideration if either randomised comparison of the primary endpoint had shown a statistically significant beneficial effect. Using routinely collected healthcare data to supplement the

data required for these analyses was considered, but marrying the timings of diagnosis and procedure codes was dismissed because it would require an extensive programme of methodological research beyond the scope of this DPhil.

5.9.7 Power of the Age-Related Macular Degeneration Analyses

As a comparatively rare endpoint, a single study was always unlikely to be large enough to confirm or refute the relationships between aspirin and omega-3 FAs on AMD on its own. ASCEND-Eye was underpowered to detect smaller effects of the study treatments on AMD due to the young age of the cohort and there being too few events. For example, with 260 AMD events in the study population in total, there was 80% power to detect a 30% reduction in incident AMD events at 2P<0.05 (see Table 27) and only 16% power to detect the 12% risk reduction that was found for the aspirin comparison of the trial. However, ASCEND-Eye could contribute to future meta-analyses of aspirin and AMD after the ASPREE-AMD results have been published. If long-term follow-up of ASCEND participants via linkage to the NOD AMD Audit became possible, more events would boost the statistical power to explore the relationship between aspirin and AMD further. Given the late separation of survival curves in ASCEND-Eye and Women's Health Study[301], which might support an anti-atherogenic mechanism of protection against AMD, such research could also consider excluding events reported during the first few years of aspirin treatment.

5.9.8 Age-Related Macular Degeneration and Diabetes

Whilst diabetes is not considered a strong risk factor for AMD[182-185], the possibility cannot be excluded that the pharmacodynamic effects of aspirin and of omega-3 FAs differ in those with diabetes compared to those without it.

5.9.9 Visual Acuity Assessments

Upon receiving the linkage data, it was apparent that the NHS screening service in England and Wales used only two types of visual acuity charts during the scheduled treatment period of ASCEND: Snellen and LogMar. Among those who were eligible for the B5 cohort (n = 2392), 1785 (74.6%) were assessed

using only a Snellen chart, 116 (4.8%) were assessed using only a LogMar chart, and 491 (20.5%) were assessed with different charts at separate screenings. Whilst it is possible to convert Snellen scores to LogMar, by taking the log to the base 10 of the reciprocal of the Snellen fraction*, there are limitations of assuming equivalency of the visual acuity measured by the different chart types, as done for the ASCEND-Eye analyses.[462,463] Previous studies have described differences in the number, size progression and shape of optotypes and the inter-line or inter-letter spacing ratios of Snellen charts, reducing their test-retest reliability compared to LogMar charts.[462] Therefore, the International Council of Ophthalmology has recommended charts with a LogMar design, such as the ETDRS chart, as the gold standard for measuring visual acuity in clinical trials.[463] The ETDRS chart displays the ten Sloan letters (C, D, H, K, N, O, R, S, V and Z) in 5x5 rows at a distance of 4 meters.[463] However, both charts are affected by the clinical conditions in which the test is being carried out, including the illuminance of the room, refractive corrections used, the enthusiasm of patients or healthcare professionals to pursue the next letter, and the termination rules applied (i.e. how many mistakes the patient is allowed to make on each line of letters before they are said to have missed that line).[462] Analyses of the visual acuity outcome stratified by chart type were not performed because of the time constraints of the DPhil. However, whilst the discriminative performance of each chart may differ, it is unlikely that the methods used in ASCEND-Eye were a source of differential bias between the treatment groups in either arm.

5.10 Conclusions and Recommendations

With its large number of participants, long follow-up, well-defined exposures and outcomes, and randomised design, ASCEND-Eye comprehensively extends the work of previous aspirin and omega-3 FA studies for DR and AMD separately. In conclusion, these data indicate that neither aspirin nor omega-3 FAs have a significantly large protective or harmful effect on either condition. However, smaller but potentially important effects of aspirin on AMD may emerge over a more extended follow-

* LogMar scores approximately convert to Snellen scores as follows. The Snellen numerator represents the distance from which the patient reads the chart. Next, the antilog of the LogMar score is multiplied by the distance from which the patient reads the chart to produce the Snellen denominator.[456,457]

up period. Avenues for future research include linkage to the NOD AMD Audit to consolidate the in-trial data and allow post-trial follow-up. These data could also contribute to a meta-analysis of aspirin and AMD after the ASPREE-AMD results have been published. Some post-doctoral observational analyses of the NEI-VFQ-25 are also planned.

Although a greater understanding of their pathogenesis has led to effective interventions to stabilise DR and AMD in some people, such as intravitreal anti-angiogenesis medication, many continue to lose their sight. Future research is needed to understand the ocular risks and benefits of drugs commonly taken by people with diabetes, the elderly, or both, and finding new treatments remains an important goal. Even a modest treatment effect would be of value given the large number of people anticipated to be affected by these conditions globally in the future, including 160 million with DR by 2045[464] and 288 million with AMD by 2040.[198]

Appendices

Appendix 1: NEI-VFQ-25 and Diabetes

Previous studies of the NEI-VFQ-25 by those with diabetes have used multivariate regression methods to compare composite and subdomain scores with demographic characteristics, best-corrected visual acuity, and the severity of retinopathic lesions.[384,465-475] Some have found deficits in subdomain scores for vision-specific mental health, near-vision and distance-vision[384,466,469,470], and peripheral vision in those treated with PRP.[384,468] After controlling for visual acuity, lower composite scores have been associated with being older, having a longer duration of diabetes or co-existing sensory neuropathy.[384,470] However, the relationship with the severity of DR or diabetic maculopathy is relatively unstudied. Previous studies are limited by their small size ($n \leq 200$)[465,467,471-475], and those that were larger ($n \sim 450\text{-}1000$)[384] included people with either type 1[384] or type 2[468-470] diabetes, but rarely both[466]. Therefore, in addition to the between-treatment comparisons of the questionnaire, there is an opportunity to model characteristics associated with lower composite scoring and to look for decrements in subdomains of the questionnaire that may not have been identified in previous studies due to their small size. Applying the NEI-VFQ-25 in ASCEND-Eye represents the largest survey of vision-related quality of life in people with diabetes to date; however, as described in the Introduction, these observational analyses will be a post-doctoral research project.

Appendix 2: Systematic Literature Search Proformas

Aspirin/Omega-3 FAs and Diabetic Retinopathy	
Inclusion and exclusion Criteria	**Studies:** RCTs, meta-analyses and observational studies **Participants:** type 1 or type 2 DM (no limitations on age, sex, ethnicity, duration of DM, type of DM) **Interventions:** platelet aggregation inhibitors (**search 1**) and omega-3 fatty acids (**search 2**) **Outcome measures:** including but not limited to • Incidence DR – defined as mild NPDR (ETDRS Score 35 or greater) – based on static imaging (e.g. fundus photography or slit lamp biomicroscopy) • Visual acuity scores • Clinically significant macular oedema • Quality of life – if NEI-VFQ25 or other eye-specific PROM available • Change in size/count/morphology of specific pathological lesion associated with DR • Safety outcomes **Exclusions:** • Studies comparing the efficacy or safety of aspirin or omega-3 fatty acids in combination with another anti-thrombotic or multivitamin, respectively, meaning that the effects of aspirin or omega-3 fatty acids could not be isolated • Studies which only included comparisons of the omega-6 fatty acid components of the PUFA class • Editorials/commentaries or letters to the editor • Feasibility studies
Databases searched	• OVID Medline In Process and Other Non-Indexed Citations and Ovid Medline (R) • PubMed • ClinicalTrials.gov • Cochrane Library → Subsequently search references of identified articles for other studies of interest (including grey literature such as conference abstracts)
Restrictions	• No early date limit; up to 1st July 2022 • Both humans and animals* • English language *Animal models and systematic reviews were included in this search to broaden my understanding of the topic. However these have not been tabulated
Selection of studies	**Plan:** • Review titles +/- abstracts of seemingly relevant studies • Obtain full text of those considered relevant • Read full text of relevant studies In conjunction with this, I will compile lists of excluded studies, included studies, ongoing studies and studies with insufficiently available evidence
Data extraction	**Information to be extracted:** • Design • Year and country of publication • Population and sample size • Follow-up duration • Methods • Demographic characteristics of participants: mean age or age range, % male • Intervention • Main outcome measure(s) • Summary Statistic(s)
Search terms	**Search 1:** (randomised controlled trial OR controlled trial OR randomised OR placebo OR drug therapy OR randomly OR trial OR groups OR clinical trial OR comparative study OR experimental study OR control group OR observational stud* OR case control study OR cross sectional study OR population-based study OR cohort study) AND

	(diabetic retinopathy OR retinopathy OR maculopathy) AND (Aspirin OR salicylic acid OR anti-platelet OR acetylsalicyclic acid OR salicylic acid acetate)* **Search 2:** This was the same as search 1, with substitution of aspirin search terms for omega-3 fatty acid search terms: (Fatty acids, omega-3 OR docosahexanoic acid ethyl ester OR ethyl docosahexaenoate OR eicosapentanoic acid OR eicosapentanoic acid ethyl ester OR omega 3 ethyl esters OR linolenic acid OR alpha linolenic acid OR ALA OR omega 3 marine triglycerides OR omega 3 OR polyunsaturated OR LCPUFA OR PUFA OR docosahexaenoic OR DHA OR eicosapentaenoic OR EPA)* *Includes Martindale synonyms. However I excluded brand names and multi-drug preparations that were included in Martindale search
Missing data	If there is no information on ocular outcomes, describe the paper but exclude
Quality Assessment	Application of the Downs and Black Checklist: The following cut-points were used to categorize studies by quality: excellent (25–27), good (20–24), fair (15–19) and poor (⩽14)
Data synthesis	By including trials and observational studies, I anticipate that studies will be methodologically and clinically heterogeneous; therefore narrative summaries may be more appropriate
Limitations of this review	It is beyond the scope of these reviews to compare cost differences between interventions that yield similar effects with respect to DR outcomes

Aspirin/Omega-3 FAs and AMD	
Inclusion and exclusion Criteria	**Studies:** RCTs, meta-analyses and observational studies **Participants:** early ARM, wet AMD, dry AMD (no limitations on age, sex, ethnicity, duration of disease, type of AMD) **Interventions:** platelet aggregation inhibitors (**search 3**) and omega-3 fatty acids (**search 4**) **Outcome measures:** including but not limited to • Incident late AMD of either type, or early ARM - based on static imaging (e.g. fundus photography or slit lamp biomicroscopy) and using a standardised grading system e.g. WARMGS or IARMGS • Progression from early ARM to late AMD • Visual acuity scores • Quality of life – if NEI-VFQ25 or other eye-specific PROM available • Change in size/count/morphology of specific pathological lesion associated with early ARM or late AMD • Safety outcomes **Exclusions:** • Studies comparing the efficacy or safety of aspirin or omega-3 fatty acids in combination with another anti-thrombotic or multivitamin, respectively, meaning that the effects of aspirin or omega-3 fatty acids could not be isolated • Studies which only included comparisons of the omega-6 fatty acid components of the PUFA class • Editorials/commentaries or letters to the editor • Feasibility studies
Databases searched	• OVID Medline In Process and Other Non-Indexed Citations and Ovid Medline (R) • PubMed • ClinicalTrials.gov • Cochrane Library → Subsequently search references of identified articles for other studies of interest (including grey literature such as conference abstracts)
Restrictions	• No early date limits; up to 1st July 2022 • Both humans and animals* • English language

	*Animal models and systematic reviews were included in this search to broaden my understanding of the topic. However they have not been tabulated
Selection of studies	**Plan:** • Review titles +/- abstracts of seemingly relevant studies • Obtain full text of those considered relevant • Read full text of relevant studies In conjunction with this, I will compile lists of excluded studies, included studies, ongoing studies and studies with insufficiently available evidence
Data extraction	**Information to be extracted:** • Design • Year and country of publication • Population and sample size • Follow-up duration • Methods • Demographic characteristics of participants: mean age or age range, % male • Intervention • Main outcome measure(s) • Summary Statistic(s)
Search terms	**Search 3:** (randomised controlled trial OR controlled trial OR randomised OR placebo OR drug therapy OR randomly OR trial OR groups OR clinical trial OR comparative study OR experimental study OR control group OR observational stud* OR case control study OR cross sectional study OR population-based study OR cohort study) AND (Macular Degeneration OR Retinal Degeneration OR Retinal Neovascularization OR choroidal neovascularization OR Macula Lutea OR Age-related macular degeneration OR ARM OR age-related maculopathy OR ARMD OR AMD OR CNV OR geographic atrophy OR drusen OR Macula* near degenerate* OR retina* near degenerate* OR choroid* near neovascul* OR macula* near neovascul* OR retina* near neovascul* OR choroid* near neovascul*) AND (Aspirin OR salicylic acid OR anti-platelet OR acetylsalicyclic acid OR salicylic acid acetate)* **Search 4:** This was the same as **search 3**, with substitution of aspirin search terms for omega-3 fatty acid search terms: (Fatty acids, omega-3 OR docosahexanoic acid ethyl ester OR ethyl docosahexaenoate OR eicosapentanoic acid OR eicosapentanoic acid ethyl ester OR omega 3 ethyl esters OR linolenic acid OR alpha linolenic acid OR ALA OR omega 3 marine triglycerides OR omega 3 OR polyunsaturated OR LCPUFA OR PUFA OR docosahexaenoic OR DHA OR eicosapentaenoic OR EPA)* *Includes Martindale synonyms. However I have excluded brand names and multi-drug preparations that were included in Martindale search
Missing data	If there is no information on ocular outcomes, describe the paper but exclude
Quality Assessment	Application of the Downs and Black Checklist: The following cut-points were used to categorize studies by quality: excellent (25–27), good (20–24), fair (15–19) and poor (⩽14)
Data synthesis	By including trials and observational studies, I anticipate that studies will be methodologically and clinically heterogeneous; therefore narrative summaries may be more appropriate
Limitations of this review	It is beyond the scope of these reviews to compare cost differences between interventions that yield similar effects with respect to DR outcomes

Appendix 3: Systematic Literature Searches – Excluded or Untabulated Studies

Aspirin and Diabetic Retinopathy Review:

- **Animal studies (5):**
  - Kern and Engerman. *Diabetes* 2001; 50(7): 1636-42
  - Joussen *et al. Federation of American Societies for Experimental Biology Journal* 2002; 16(3): 438-40
  - Bucolo *et al. Investigative Ophthalmology & Visual Science* 2009; 50(8): 3846-52
  - Wright *et al. Experimental Eye Research* 2009; 88(1): 106-12
  - Sun *et al. Diabetes* 2005; 54 (12):3418 –3426
- **Editorial/commentaries (2):**
  - Kohner. *British Medical Journal* 2003;327:1060–1
  - Kershenovich and Alarcon-Segovia. *Lancet* 1967;10:833
- **Experimental studies (3):**
  - Boeri *et al. Diabetes* 2001; 50(6): 1432-9
  - Pope. *Diabetes* 1960; 9: 9-13
  - Powel and Field. *Lancet* 1964; 2(7349): 17-8
- **Non-English language (1):**
  - Carmago *et al. Arquivos Brasileiros de Endocrinologia & Metabologia* 2007;51(3):457-65
- **Studies reporting on aspirin and cataract incidence or extraction in people with diabetes (4):**
  - ETDRS Research Group Report No. 16 *Archives of Ophthalmology* 1992; 110 (3): 339-42
  - Christen *et al. Archives of Ophthalmology* 2001; 119 (3): 405-12
  - Seigel *et al. Ophthalmology* 1982; 89 (4): 47A-9A
  - Klein *et al. Diabetes Care* 1987; 10 (4): 495-9
- **Systematic review which described DAMAD and ETDRS (1):**
  - Bergerhoff *et al. Endocrinology and Metabolism Clinics of North America* 2002; 31 (3): 779-93
- **RCT of an alternative anti-platelet therapy (1):**
  - TIMAD Study Group. *Archives of Ophthalmology* 1990; 108 (11): 1577-83
- **RCT assessing effects of aspirin on retinal blood flow in people with diabetes (1):**
  - Feke *et al. Ophthalmic Research* 1996; 28 (2): 108-16
- **Observational studies where it was not possible to isolate the effect of aspirin alone (4):**
  - Salinero-Fort *et al. PLoS One* 2013;8(10):e76417
  - Mandas *et al. Frontiers in Aging Neuroscience* 2014;6:309
  - Wang *et al. International Journal of Ophthalmology* 2017; 10(3): 461-6
  - Fabinyi *et al. Clinical & Experimental Ophthalmology* 2011; **39**(9): 878-84
  - Jeng *et al. Ophthalmology* 2022; 22(1): 127

Omega-3 Fatty Acids and Diabetic Retinopathy Review:

- **Animal studies (7):**
  - Fu *et al. American Journal of Clinical Nutrition* 2015;101(4):879-88
  - Sapieha *et al. Science Translational Medicine* 2011; 3(69): 69ra12-69ra12
  - Connor *et al. Nature medicine* 2007; 13(7): 868-73
  - Stahl *et al. Circulation Research* 2010;107:495-500
  - Tikhonenko *et al. PLoS One* 2013; 8(1): e55177
  - Shen *et al. Archives of Medical Research* 2013; 44: 514-520
  - Hammes *et al. Diabetologia* 1996; 39:251-255
- **Experimental (4):**
  - Johansson *et al. Autophagy* 2015;11(9):1636-51
  - Domanico *et al. Indian Journal of Ophthalmology* 2015;63(1):9-14
  - Viteri *et al. Antioxidants* 2020 Sep 4;9(9):828 [doi: 10.3390/antiox9090828]
  - Zhao *et al. E Clinical Medicine* 2021;39:101089 [doi: 10.1016/j.eclinm.2021.101089]
- **Narrative reviews (9):**
  - Keppel Hesselink *et al. Journal of Ophthalmology* 2015;2015:430596 [doi: 10.1155/2015/430596]
  - Chang *et al. Investigative Ophthalmology and Visual Science* 2015;56(4):2367-80
  - Shen *et al. Archives of Medical Science* 2014;10(6):1167-74
  - Yong *et al. Ophthalmology* 2015;122(3):595-9.
  - Chew. *New England Journal of Medicine* 2011; 364(20): 1970-1
  - Bazan. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 2009; 81: 205-11
  - Mukherjee *et al. Proceedings of the National Academy of Sciences of the United States of America* 2004; 101(22): 8491-6
  - Eynard and Repossi. *Lipids in Health and Disease* 2019; 18:114
  - Behl and Kotwani. *Journal of Pharmacology and Pharmacotherapeutics* 2017 69 (8): 946-954

- **Editorial/commentary (1):**
  - Kohn. *Journal of the Academy of Nutrition and Dietetics* 2015;115(9):1548
- **Post-hoc analysis superseded by a later publication by the same research group (1):**
  - Díaz-López *et al. Diabetes Care* 2015;38(11):2134-41
- **Observational study describing red cell membrane PUFA concentration and risk of diabetic retinopathy (1):**
  - Koehrer *et al. PLoS One* 2014;9(9):e106912
- **Studies comparing the efficacy or safety of aspirin or omega-3 fatty acids in combination with another anti-thrombotic or multivitamin, respectively, meaning that the effects of aspirin or omega-3 fatty acids could not be isolated (4)**
  - Chous *et al. British Journal of Ophthalmology* 2016; 100 (2): 227-34
  - Roig-Revert *et al. BioMed Research International* 2015; 4080-1
  - Lafuente *et al. Retina* 2019; 39 (6): 1083-90
  - Lafuente *et al. Retina* 2017; 37 (7): 1277-86
- **Studies which only included comparisons of the omega-6 fatty acid family of the PUFA class (2)**
  - Houtsmuller *et al. Documenta Ophthalmologica* 1980; 48(2): 363-71
  - Howard-Williams *et al. British Journal of Ophthalmology* 1985; 69: 15-18

Aspirin and Age-Related Macular Degeneration Review:

- **Irrelevant when full text was reviewed (4):**
  - Mason *et al. Retina* 2010;30(9):1386-9
  - Blumenkranz *et al. Ophthalmology* 1986; 93(5):552-558
  - Williams *et al. American Journal of Preventive Medicine* 2015; 48 (5):501-508
  - Chakravarthy *et al. BMC Ophthalmology* 2010; 10(31) [doi: 10.1186/1471-2415-10-31]
- **Narrative reviews (6):**
  - Wu *et al. Medical Hypothesis Discovery and Innovation in Ophthalmology* 2013;2(3):59-68
  - Klein. *Journal of the American Medical Association* 1991;266(16):2279
  - Christen and Chew. *Expert Opinion on Drug Safety* 2019; 13:4, 421-429
  - Chong *et al. Expert Opinion on Drug Safety*. 2014; 13(6): 691-693
  - Nowak. *Expert Opinion on Drug Safety*. 2014; 13(6): 687-690
  - Snow and Seddon. *Ophthalmic Epidemiology* 1999; 6(2): 125-43
- **Unable to isolate ocular bleed from bleeding elsewhere (1):**
  - Ying *et al. Ophthalmology* 2015; 123 (2): 352-60
- **Letters to the editor (2):**
  - Nowroozadeh. *Journal of the American Medical Association* 2013;309(12):1226
  - Wang and Zhang. *Investigative Ophthalmology and Visual Science* 2014; 55: 3954
- **Reprinted meta-analyses (1):**
  - Kahawita and Casson. *Canadian Journal of Ophthalmology* 2014; 49 (1): 35-9
- **Editorial/commentary (2):**
  - Sobrin and Seddon. *American Journal of Ophthalmology* 2013; 156(2):213-217
  - Lad. *Ophthalmology* 2019; 126 (12): 1657-1658
- **Unable to isolate the effect of aspirin from other NSAIDs, anti-platelets or anti-coagulants (4):**
  - Motjahedi *et al. American Journal of Ophthalmology* 2018;188:111-122
  - Kiernan *et al. Retina* 2010; 30(10): 1573-8
  - Tialanus *et al. Graefe's Archive for Clinical and Experimental Ophthalmology* 2000; 238(6): 482-5
  - Kuhli-Hattenbach *et al. American journal of ophthalmology* 2010; 149(2): 316-21
  - Moon *et al. Journal of Korean Medical Science* 2012; 27(5): 537-41

Omega-3 Fatty Acids and Age-Related Macular Degeneration Review:

- **Bioavailability study (1):**
  - Huang *et al. Investigative Ophthalmology and Visual Science* 2008; 49 (9): 3664-9
- **RCT of a multi-vitamin supplement where the effect of omega-3 FAs could not be isolated (6):**
  - Arnold *et al. Journal of the American Medical Association Ophthalmology* 2013; 131 (5): 564-72
  - Feher *et al. International Journal of Ophthalmology* 2005; 219 (3): 154-66
  - García-Layana *et al. Nutrients* 2013; 5 (2): 543-51
  - Cangemi *et al. BMC Ophthalmology* 2007; 7: 3
  - Garcia-Layana *et al. Nutrients* 2021; 13(4):1253
  - Piatti *et al. European Journal of Ophthalmology* 2020; 30(2): 376-381
- **Observational studies with an endpoint not comparable with ASCEND-Eye (4):**
  - Ho *et al. Archives of Ophthalmology* 2011; 129 (6): 758-66
  - Reynolds *et al. Ophthalmology* 2013; 120 (5): 1020-8
  - Delyfer *et al. Investigative Ophthalmology and Visual Science* 2012; 53 (3): 1204-10

    - Mance *et al. Collegium Anthropologicum* 2011; 35 (suppl 2): 307-10
- **Post-hoc analysis of RCT with endpoint not comparable with ASCEND-Eye (2):**
    - Merle *et al. PLoS One* 2015; 10 (7): e0130816
    - Querques *et al. PLoS One* 2016; 11 (2): e0149219
- **Superseded Cochrane Reviews (2):**
    - Evans and Lawrenson. *Cochrane Database of Systematic Reviews* 2012, Issue 6. [DOI: 10.1002/14651858.CD000253.pub3]
    - Evans and Lawrenson. *Cochrane Database of Systematic Reviews* 2012, Issue 11. [DOI: 10.1002/14651858.CD000254.pub3]
- **Narrative reviews (16):**
    - Hodge *et al. Retina* 2007;27(2):216-21
    - Kishan *et al. Survey of Ophthalmology* 2011;56(3):195-213
    - Lim *et al. Lancet* 2012;379(9827):1728-38
    - Sangiovanni. *Progress in Retinal and Eye Research* 2005;24(1):87-138
    - Matias-Perez and Garcia-Montalvo. *Journal of Oleo Science* 2021; 70 (4): 453-458
    - Valero-Vello *et al. Foods* 2021; 10(6): 1231
    - Di Carlo and Augustin. *Journal of Clinical Medicine* 2021; 10(15): 3297
    - Chapman *et al. Clinical and Experimental Ophthalmology* 2019; 47(1): 106-127
    - Walchuk and Suh. *Advances in Food and Nutrition Research* 2020; 93; 293-332
    - Mukhtar and Ambati. *International Ophthalmology* 2019; 39(12): 2975-2983
    - Cao *et al. Current Medicinal Chemistry* 2022 [doi: 10.2174/0929867329666220511142817]
    - Meng *et al. Nutricion Hospitalaria* 2022 [doi:10.20960/nh.03932]
    - van Leeuwen *et al. Progress in Retinal and Eye Research* 2018; 67:56-86
    - Pameijer *et al. Acta Ophthalmologica* 2022 [doi: 10.1111/aos.15191]
    - Jurek *et al. International Journal of Food Science and Nutrition* 2022 [doi: 10.1080/09637486.2022.2090520]
    - Krueger *et al. Critical Reviews in Food Science and Nutrition* 2021 [doi: 10.1080/10408398.2021.1915242]
- **Intervention studies with no data on AMD outcomes (5):**
    - Johnson *et al. American Journal of Clinical Nutrition* 2008;87(5):1521-9
    - Gerstenblith *et al. Investigative Ophthalmolmology and Visual Science* 2012;53(14):2936
    - Dangour *et al. Nutrition Journal* 2006;5:20
    - Scorolli *et al. Canadian Journal of Ophthalmology* 2002;37(7):399-404
    - Ziegler *et al. Medizinische Monatsschrift fur Pharmazeuten* 2013;36(9):348-9
- **Animal studies (4):**
    - Koto *et al. Investigative Ophthalmology and Visual Science* 2007;48(9):4328-34
    - Tuo *et al. American Journal of Pathology* 2009;175(2):799-807
    - Rotstein *et al. Investigative Ophthalmology and Visual Science* 2003;44:2252-2259
    - Yanai *et al. PLoS One* 2018; 13(4):e0196037
- **Experimental study (2):**
    - Valldeperas *et al. Investigative Ophthalmology and Visual Science* 2015;56:5157
    - Leung *et al. Neurochemical Research* 2020; 45(5):1007-1019
- **Meta-analysis or systematic review with heterogeneity of the included studies (1):**
    - Hodge *et al. Ophthalmology* 2006; 113 (7): 1165-72
- **Irrelevant after full-text was reviewed (9):**
    - Burdge *et al. British Journal of Nutrition* 2002;88(4):355-63
    - Calder. *American Journal of Clinical Nutrition* 2006;83:1505S–1519S
    - Lister. *Integrative Medicine* 2019; 18(6): 30-36
    - Elmore *et al. European Journal of Nutrition* 2022; 61(3):1585-1594
    - Rutledge *et al. BMC Ophthalmology* 2020; 20(1): 473
    - Abreu-Gonzalez *et al. Achivos de la Sociedad Espanola de Oftalmologia* 2018; 93(12):592-597
    - Zhang and Downie. *Nutrients* 2019; 11 (4): 817
    - Schnebelen-Berthier *et al. Nutrients* 2021; 13(10): 3347
    - Keenan *et al. Ophthalmology* 2018; 125(12): 1913-1928
- **Letters to the editor (2):**
    - Acar *et al. Clinical and Translational Medicine* 2021; 11(7): e404
    - Zanke. *Ophthalmology* 2020; e19 [doi: 10.1016/j.ophtha.2019.10.032]
- **Post-hoc analysis of genotype and the response to AREDS2 supplements (1):**
    - van Asten *et al. Ophthalmology* 2019; 126(11): 1541-1548
- **Systematic review protocol (1):**
    - Meng *et al. Medicine* 2019; 98(13):e14516
- **Feasibility study (1):**
    - Zanon-Moreno *et al. Ophthalmic Research* 2021; 64(6): 1068-1076
- **Identical to another manuscript by Leung *et al* 2019 (1):**
    - Ng *et al. Nutrients* 2019; 11(8): 1720

Appendix 4: Downs and Black Quality Assessments of the Existing Literature

The original Downs and Black checklist is scored out of 32. I have applied a modified version for the scoring of item 27, regarding the power of the study. This meant that the maximum score for item 27 was 1 (a power calculation was conducted), instead of 5. Hence the highest possible score with the modified instrument was 27. Score ranges were given the following corresponding levels of quality: excellent (25-27); good (20-24); fair (15-19); and poor (≤14).[476] I did not formally assess the meta-analyses identified by my systematic search using this checklist.

Aspirin and Diabetic Retinopathy

Reference: ETDRS Research Group Report No. 8 1991&ETDRS Research Group Report No.20 1995[140,142]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section? If the main outcomes are first mentioned in the Results section, the question should be answered no	x			
3. Are the characteristics of the patients included in the study clearly described? In cohort studies and trials, inclusion and/or exclusion criteria should be given. In case-control studies, a case definition and the source for controls should be given.	x			
4. Are the interventions of interest clearly described? Treatments and placebo (where relevant) that are to be compared should be clearly described.	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described? A list of principal confounders is provided.	x			
6. Are the main findings of the study clearly described? Simple outcome data (including denominators and numerators) should be reported for all major findings so that the reader can check the major analyses and conclusions.	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes? In non-normally distributed data, the interquartile range of results should be reported. In normally distributed data, the standard error, standard deviation or confidence intervals should be reported. If the distribution of the data is not described, it must e assumed that the estimates used were appropriate and the question should be answered yes.	x			
8. Have all important adverse events that may be a consequence of the intervention been reported? This should be answered yes if the study demonstrates that there was a comprehensive attempt to measure adverse events. (A list of possible adverse events is provided).	x			
9. Have the characteristics of patients lost to follow-up been described? This should be answered yes where there were no losses to follow-up or where losses to follow-up were so small that findings would be unaffected by their inclusion. This should be answered no where a study does not report the number of patients lost to follow-up.	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity All the following criteria attempt to address the representativeness of the findings of the study and whether they may be generalised to the population from which the study subjects were derived				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited? The study must identify the source population for patients and describe how the patients were selected. Patients would be representative if they comprised the entire source population, an unselected sample of consecutive patients, or a random sample. Random sampling is only feasible where a list of all members of the relevant population exists. Where a study does not report the proportion of the population from which the patients are derived, the question should be answered as unable to determine.	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited? The proportion of those asked who agreed should be stated. Validation that the sample was representative would include demonstrating that the distribution of the main confounding factors was the same in the study sample and the source population.			x	

13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive? For the question to be answered yes the study should demonstrate that the intervention was representative of that in use in the source population. The question should be answered no if, for example, the intervention was undertaken in a specialist centre unrepresentative of the hospitals most of the source population would attend.	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received? For studies where the patients would have no way of knowing which intervention they received, this should be answered yes.	x			
15. Was an attempt made to blind those measuring the main outcomes of the intervention?	x			
16. If any of the results of the study were based on "data dredging", was this made clear? Any analyses that had not been planned at the outset of the study should be clearly indicated. If no retrospective unplanned subgroup analyses were reported, then answer yes	x			
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls? Where follow-up was the same for all study patients, the answer should be yes. If different lengths of follow-up were adjusted for by, for example, survival analysis, the answer should be yes. Studies where differences in follow-up are ignored should be answered no.	x			
18. Were the statistical tests used to assess the main outcomes appropriate? The statistical techniques used must be appropriate to the data. For example non-parametric methods should be used for small sample sizes. Where little statistical analysis has been undertaken but where there is no evidence of bias, the question should be answered yes. If the distribution of the data (normal or not) is not described it must be assumed that the estimates used were appropriate and the question should be answered yes.	x			
19. Was adherence with the intervention/s reliable? Where there was non-adherence with the allocated treatment or where there was contamination of one group, the question should be answered no. For studies where the effect of any misclassification was likely to bias any association to the null, the question should be answered no	x			
20. Were the main outcome measures used accurate (valid and reliable)? For studies where the outcome measures are clearly described, the question should be answered yes. For studies which refer to other work or that demonstrates the outcome measures are accurate, the question should be answered as yes.	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population? For example, patients for all comparison groups should be selected from the same hospital. The question should be answered unable to determine for cohort and case-control studies where there is no information concerning the source of patients included in the study	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time? For a study which does not specify the time period over which patients were recruited, the question should be answered as unable to determine.	x			
23. Were study subjects randomised to intervention groups? Studies which state that subjects were randomised should be answered yes except where method of randomisation would not ensure random allocation. For example alternate allocation would score no because it is predictable.	x			
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable? All non-randomised studies should be answered no. If assignment was concealed from patients but not from staff, it should be answered no.	x			
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn? This question should be answered no for trials if: the main conclusions of the study were based on analyses of treatment rather than intention-to-treat; the distribution of known confounders in the different treatment groups was not described; or the distribution of known confounders differed between the treatment groups but was not taken into account in the analyses. In non-randomised studies if the effect of the main confounders was not investigated or confounding was demonstrated but no adjustment was made in the final analyses the question should be answered as no.	x			
26. Were losses of patients to follow-up taken into account? If the numbers of patients lost to follow-up are not reported, the question should be answered as unable to determine. If the proportion lost to follow- up was too small to affect the main findings, the question should be answered yes.	x			
Power				

27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%? Sample sizes have been calculated to detect a difference of x% and y%.	x			
Total 26/27 (Excellent)				

Yes (1 point), No (0 points), U = unable to determine (0 points), N/A = not applicable (0)

Reference: DAMAD Study Group 1989[143]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?	x			
15. Was an attempt made to blind those measuring the main outcomes of the intervention?	x			
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?	x			
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?	x			
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	x			
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?			x	
Total 24/27 (Good)				

Reference: Salinero-Fort *et al* 2013[136]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			

2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?		x		
4. Are the interventions of interest clearly described?				x
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?		x		
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?				x
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?			x	
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?				x
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?		x		
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 11/27 (Poor)				

Reference: Shi *et al* 2017[138]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		

9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?				x
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?				x
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?				x
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 12/27 (Poor)				

Reference: Pafundi *et al* 2020[137]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?				x
Internal Validity - Bias				

14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?				x
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?				x
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 11/27 (Poor)				

Omega-3 Fatty Acids and Diabetic Retinopathy

Reference: Haritoglou *et al* 2011[179]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?			x	
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?	x			
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	

17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?			x	
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?			x	
23. Were study subjects randomised to intervention groups?	x			
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	x			
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?			x	
Total 16/27 (Fair)				

Reference: Sala-Vila *et al* 2016[164]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?	x			
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			

22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?	x			
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?			x	
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?			x	
Total 18/27 (Fair)				

Reference: Sasaki *et al* 2015[162]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?		x		
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x

Total 13/27 (Poor)

Aspirin and Age-Related Macular Degeneration

Reference: Klein *et al* 2001[314]	Score			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?		x		
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?	x		x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 17/27 (Fair)				

Reference: Klein *et al* 2012[303]	Score			
Reporting	**Yes**	**No**	**U**	**NA**

1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?		x		
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?	x			
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?	x			
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 20/27 (Good)				

Reference: Christen *et al* 2001[323]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		

9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity – Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?	x			
15. Was an attempt made to blind those measuring the main outcomes of the intervention?	x			
16. If any of the results of the study were based on "data dredging", was this made clear?	x			
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?	x			
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?	x			
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	x			
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?		x		
Total 26/27 (Excellent)				

Reference: Christen *et al* 2009[301]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				

14. Was an attempt made to blind study subjects to the intervention they have received?	x			
15. Was an attempt made to blind those measuring the main outcomes of the intervention?	x			
16. If any of the results of the study were based on “data dredging”, was this made clear?	x			
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?	x			
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?	x			
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	x			
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?		x		
Total 26/27 (Excellent)				

Reference: Liew *et al* 2013[316]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?		x		
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?	x			

Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 19/27 (Fair)				

Reference: Wang *et al* 2003[321]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?		x		
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?	x			
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	

26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 17/27 (Fair)				

Reference: Aronow *et al* 2014[315]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 17/27 (Fair)				

Reference: Modjthahedi *et al* 2018[317]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**

1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?			x	
Total 17/27 (Fair)				

Reference: Keenan *et al* 2019[320]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		

9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?	x			
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 18/27 (Fair)				

Reference: Lee *et al* 2021[322]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				

14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 17/27 (Fair)				

Reference: Xu *et al* 2021[319]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			

Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 16/27 (Fair)				

Reference: De Jong *et al* 2012[307]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			

26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 17/27 (Fair)				

Reference: Hirvela *et al* 1996[309]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?		xx		
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 13/27 (Poor)				

Reference: Blumenkranz *et al* 1986[311]	**Score**

Reporting	Yes	No	U	NA
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?		x		
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?			x	
Total 12/27 (Poor)				

Reference: Rudnicka *et al* 2010[312]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			

8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?		x		
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on "data dredging", was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 13/27 (Poor)				

Reference: Swanson and McGwin 2008[304]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			

Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 18/27 (Fair)				

Reference: Wilson *et al* 2004[305]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?			x	
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x

20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?		x		
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 12/27 (Poor)				

Reference: Cheung *et al* 2013[310]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?		x		
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x

25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 15/27 (Fair)				

Reference: DeAngelis *et al* 2004[313]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?			x	
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?			x	
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?		x		
26. Were losses of patients to follow-up taken into account?		x		
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 12/27 (Poor)				

Reference: Douglas *et al* 2007[308]	**Score**

Reporting	Yes	No	U	NA
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 17/27 (Fair)				

Reference: Buitendijk *et al* 2018[306]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			

8. Have all important adverse events that may be a consequence of the intervention been reported?		x		
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?			x	
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?			x	
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on "data dredging", was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 11/27 (Poor)				

Reference: Rim *et al* 2019[318]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			

Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 19/27 (Fair)				

Reference: Kingham *et al* 1988[293]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?				x
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?		x		
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?		x		
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?				x
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?				x
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?				x
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?				x
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x

20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?				x
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?				x
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?				x
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 7/27 (Poor)				

Omega-3 Fatty Acids and Age-Related Macular Degeneration

Reference: AREDS2 Research group 2013[367]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?	x			
15. Was an attempt made to blind those measuring the main outcomes of the intervention?	x			
16. If any of the results of the study were based on “data dredging”, was this made clear?	x			
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable? **(11.1% in the placebo arm took over the counter omega-3 FA supplements)**		x		
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			

22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?	x			
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	x			
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?		x		
Total 26/27 (Excellent)				

Reference: Souied *et al* 2013[368]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?	x			
15. Was an attempt made to blind those measuring the main outcomes of the intervention?	x			
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?			x	
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?	x			
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?			x	
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?		x		
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?			x	

Total 20/27 (Good)

Reference: Christen *et al* 2020[370]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?	x			
15. Was an attempt made to blind those measuring the main outcomes of the intervention?	x			
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?	x			
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?	x			
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	x			
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?	x			
Total 27/27 (Excellent)				

Reference: Cho *et al* 2001[362]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			

4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?			x	
15. Was an attempt made to blind those measuring the main outcomes of the intervention?	x			
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?	x			
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?			x	
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?			x	
Total 21/27 (Good)				

Reference: Arnarsson *et al* 2006[363]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			

External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?			x	
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on "data dredging", was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 19/27 (Fair)				

Reference: Seddon *et al* 2003[361]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on "data dredging", was this made clear?		x		

17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?			x	
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 19/27 (Fair)				

Reference: Chong *et al* 2009[366]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?		x		
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			

22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?			x	
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 18/27 (Fair)				

Reference: Tan *et al* 2009[360]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x

Total 20/27 (Good)

Reference: Chua *et al* 2006[359]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 20/27 (Good)				

Reference: Christen *et al* 2011[358]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			

4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 17/27 (Fair)				

Reference: Parekh *et al* 2009[357]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			

External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?			x	
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on "data dredging", was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?		x		
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 14/27 (Poor)				

Reference: SanGiovanni *et al* 2009[356]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?		x		
15. Was an attempt made to blind those measuring the main outcomes of the intervention?		x		
16. If any of the results of the study were based on "data dredging", was this made clear?		x		

17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 18/27 (Fair)				

Reference: Merle *et al* 2011[364]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?		x		
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?			x	
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?			x	
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			

22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 11/27 (Poor)				

Reference: Merle *et al* 2013[365]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?		x		
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?			x	
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?			x	
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x

Total 11/27 (Poor)

Reference: Robman *et al* 2007[355]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?			x	
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?			x	
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?			x	
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?				x
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 15/27 (Fair)				

Reference: Wu *et al* 2017a[354]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			

4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?		x		
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 18/27 (Fair)				

Reference: Wu *et al* 2017b[353]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			

External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?		x		
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 18/27 (Fair)				

Reference: Chew *et al* 2022[352]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?	x			
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?			x	
15. Was an attempt made to blind those measuring the main outcomes of the intervention?	x			
16. If any of the results of the study were based on “data dredging”, was this made clear?	x			

17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?			x	
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?			x	
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?			x	
Total 22/27 (Good)				

Reference: Joachim *et al* 2015[351]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			

22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 18/27 (Fair)				

Reference: Karger *et al* 2022[350]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x

Total 18/27 (Fair)

Reference: Roh *et al* 2020[335]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 15/27 (Fair)				

Reference: Agron *et al* 2021[348]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			

4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 17/27 (Fair)				

Reference: Smith *et al* 2000[336]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			

External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?		x		
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?				x
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 15/27 (Fair)				

Reference: Heuberger *et al* 2001[337]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?		x		
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?		x		
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?				x

17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 14/27 (Poor)				

Reference: Seddon *et al* 2001[342]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?				x
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			

22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?		x		
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 16/27 (Fair)				

Reference: Mares-Perlman *et al* 1995[338]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?		x		
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?			x	
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x

Total 14/27 (Poor)

Reference: Seddon *et al* 2006[341]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?			x	
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 17/27 (Fair)				

Reference: San-Giovanni *et al* 2007[343]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			

4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 18/27 (Fair)				

Reference: San-Giovanni *et al* 2008[349]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?	x			
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			

External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on "data dredging", was this made clear?			x	
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	x			
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?	x			
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 18/27 (Fair)				

Reference: Delcourt *et al* 2007[339]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on "data dredging", was this made clear?				x

17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?				x
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?			x	
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 14/27 (Poor)				

Reference: Sanders *et al* 1993[344]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?				x
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			

22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 14/27 (Poor)				

Reference: Swenor *et al* 2010[340]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?				x
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x

Total 14/27 (Poor)

Reference: Augood *et al* 2008[207]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?	x			
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
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20. Were the main outcome measures used accurate (valid and reliable)?	x			
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21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
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23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	x			
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 17/27 (Fair)				

Reference: Merle *et al* 2014[345]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			

4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?		x		
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on “data dredging”, was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
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21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
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24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 14/27 (Poor)				

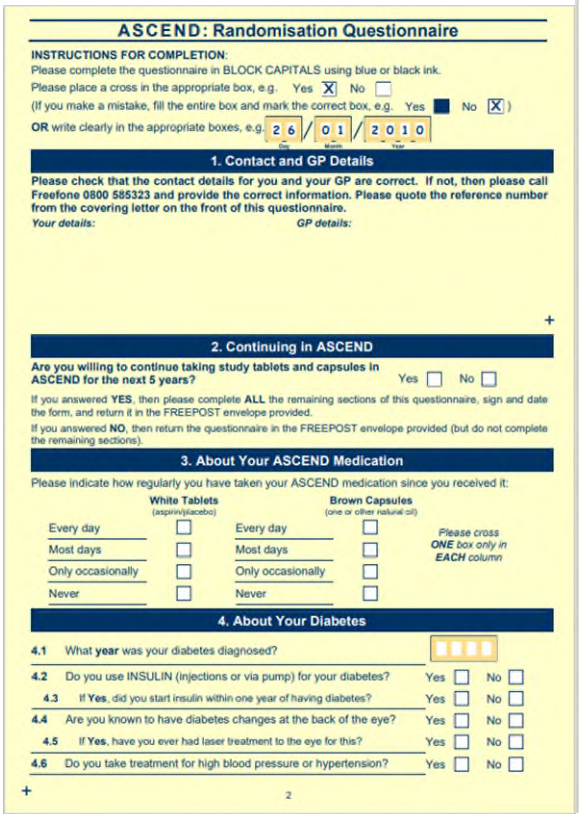
Reference: Aoki *et al* 2016[346]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
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5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			

External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on "data dredging", was this made clear?		x		
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
20. Were the main outcome measures used accurate (valid and reliable)?	x			
Internal Validity – Confounding (Selection Bias)				
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 15/27 (Fair)				

Reference: Leung *et al* 2019[347]	**Score**			
Reporting	**Yes**	**No**	**U**	**NA**
1. Is the hypothesis/aim/objective of the study clearly described?	x			
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	x			
3. Are the characteristics of the patients included in the study clearly described?	x			
4. Are the interventions of interest clearly described?		x		
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	x			
6. Are the main findings of the study clearly described?	x			
7. Does the study provide estimates of the random variability in the data for the main outcomes?	x			
8. Have all important adverse events that may be a consequence of the intervention been reported?	x			
9. Have the characteristics of patients lost to follow-up been described?				x
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	x			
External Validity				
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	x			
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	x			
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	x			
Internal Validity - Bias				
14. Was an attempt made to blind study subjects to the intervention they have received?				x
15. Was an attempt made to blind those measuring the main outcomes of the intervention?				x
16. If any of the results of the study were based on "data dredging", was this made clear?		x		

17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?				x
18. Were the statistical tests used to assess the main outcomes appropriate?	x			
19. Was adherence with the intervention/s reliable?				x
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21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	x			
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	x			
23. Were study subjects randomised to intervention groups?		x		
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?				x
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?			x	
26. Were losses of patients to follow-up taken into account?				x
Power				
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?				x
Total 15/27 (Fair)				

Appendix 5: ASCEND Randomisation Questionnaire



ASCEND: Randomisation Questionnaire

INSTRUCTIONS FOR COMPLETION:
Please complete the questionnaire in BLOCK CAPITALS using blue or black ink.
Please place a cross in the appropriate box, e.g. Yes [X] No [ ]
(If you make a mistake, fill the entire box and mark the correct box, e.g. Yes [■] No [X])
OR write clearly in the appropriate boxes, e.g. 26 / 01 / 2010 (Day / Month / Year)

1. Contact and GP Details

Please check that the contact details for you and your GP are correct. If not, then please call Freefone 0800 585323 and provide the correct information. Please quote the reference number from the covering letter on the front of this questionnaire.

Your details: ***GP details:***

2. Continuing in ASCEND

Are you willing to continue taking study tablets and capsules in ASCEND for the next 5 years? Yes [ ] No [ ]

If you answered **YES**, then please complete **ALL** the remaining sections of this questionnaire, sign and date the form, and return it in the FREEPOST envelope provided.

If you answered **NO**, then return the questionnaire in the FREEPOST envelope provided (but do not complete the remaining sections).

3. About Your ASCEND Medication

Please indicate how regularly you have taken your ASCEND medication since you received it:

	White Tablets (aspirin/placebo)	Brown Capsules (one or other natural oil)
Every day	[ ]	[ ]
Most days	[ ]	[ ]
Only occasionally	[ ]	[ ]
Never	[ ]	[ ]

*Please cross **ONE** box only in **EACH** column*

4. About Your Diabetes

4.1 What **year** was your diabetes diagnosed? [ ][ ][ ][ ]

4.2 Do you use INSULIN (injections or via pump) for your diabetes? Yes [ ] No [ ]

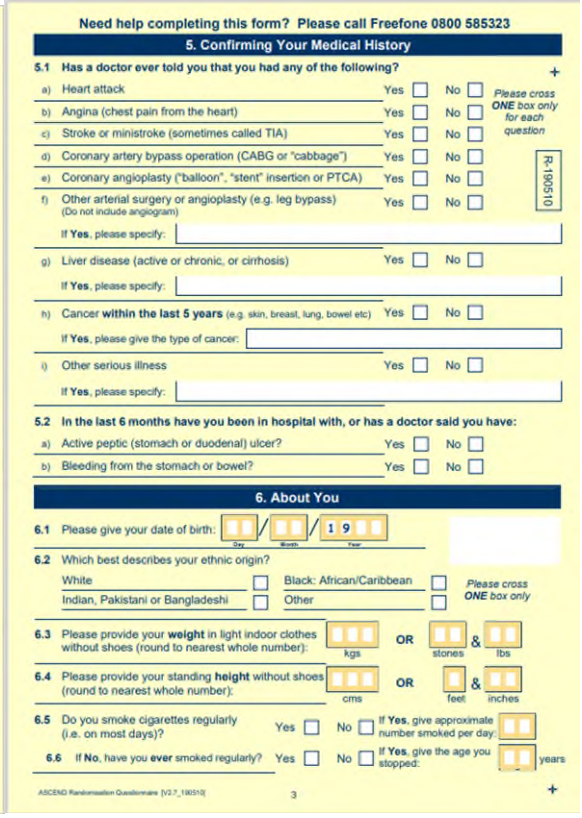
4.3 If **Yes**, did you start insulin within one year of having diabetes? Yes [ ] No [ ]

4.4 Are you known to have diabetes changes at the back of the eye? Yes [ ] No [ ]

4.5 If **Yes**, have you ever had laser treatment to the eye for this? Yes [ ] No [ ]

4.6 Do you take treatment for high blood pressure or hypertension? Yes [ ] No [ ]

2



Need help completing this form? Please call Freefone 0800 585323

5. Confirming Your Medical History

5.1 Has a doctor ever told you that you had any of the following?

*Please cross **ONE** box only for each question*

a)	Heart attack	Yes [ ] No [ ]
b)	Angina (chest pain from the heart)	Yes [ ] No [ ]
c)	Stroke or ministroke (sometimes called TIA)	Yes [ ] No [ ]
d)	Coronary artery bypass operation (CABG or "cabbage")	Yes [ ] No [ ]
e)	Coronary angioplasty ("balloon", "stent" insertion or PTCA)	Yes [ ] No [ ]
f)	Other arterial surgery or angioplasty (e.g. leg bypass) (Do not include angiogram)	Yes [ ] No [ ]
	If **Yes**, please specify:	
g)	Liver disease (active or chronic, or cirrhosis)	Yes [ ] No [ ]
	If **Yes**, please specify:	
h)	Cancer **within the last 5 years** (e.g. skin, breast, lung, bowel etc)	Yes [ ] No [ ]
	If **Yes**, please give the type of cancer:	
i)	Other serious illness	Yes [ ] No [ ]
	If **Yes**, please specify:	

R-190510

5.2 In the last 6 months have you been in hospital with, or has a doctor said you have:

a)	Active peptic (stomach or duodenal) ulcer?	Yes [ ] No [ ]
b)	Bleeding from the stomach or bowel?	Yes [ ] No [ ]

6. About You

6.1 Please give your date of birth: [ ][ ] / [ ][ ] / 1 9 [ ][ ] (Day / Month / Year)

6.2 Which best describes your ethnic origin?

*Please cross **ONE** box only*

White	[ ]	Black: African/Caribbean	[ ]
Indian, Pakistani or Bangladeshi	[ ]	Other	[ ]

6.3 Please provide your **weight** in light indoor clothes without shoes (round to nearest whole number): [ ][ ] kgs **OR** [ ][ ] stones & [ ][ ] lbs

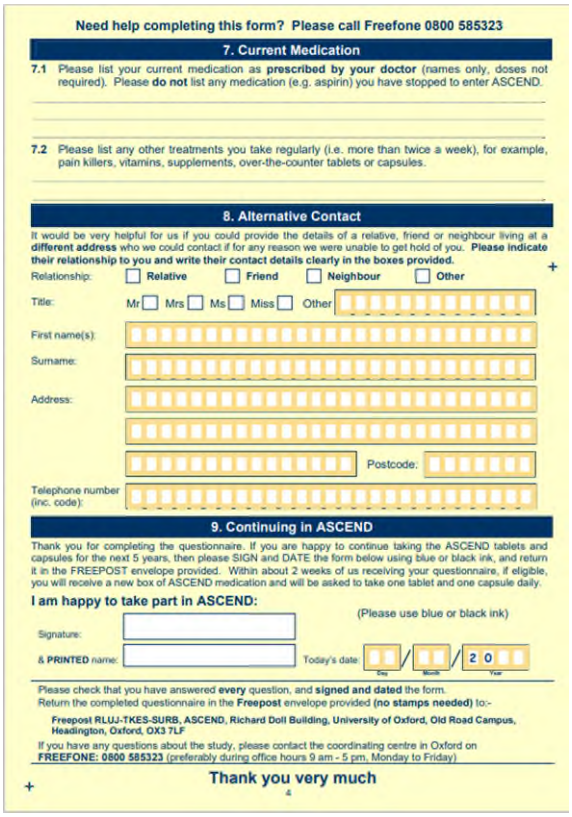
6.4 Please provide your standing **height** without shoes (round to nearest whole number): [ ][ ][ ] cms **OR** [ ] feet & [ ][ ] inches

6.5 Do you smoke cigarettes regularly (i.e. on most days)? Yes [ ] No [ ] If **Yes**, give approximate number smoked per day: [ ][ ]

6.6 If **No**, have you **ever** smoked regularly? Yes [ ] No [ ] If **Yes**, give the age you stopped: [ ][ ] years

ASCEND Randomisation Questionnaire [V2.7_190510]

3



Need help completing this form? Please call Freefone 0800 585323

7. Current Medication

7.1 Please list your current medication as **prescribed by your doctor** (names only, doses not required). Please **do not** list any medication (e.g. aspirin) you have stopped to enter ASCEND.

7.2 Please list any other treatments you take regularly (i.e. more than twice a week), for example, pain killers, vitamins, supplements, over-the-counter tablets or capsules.

8. Alternative Contact

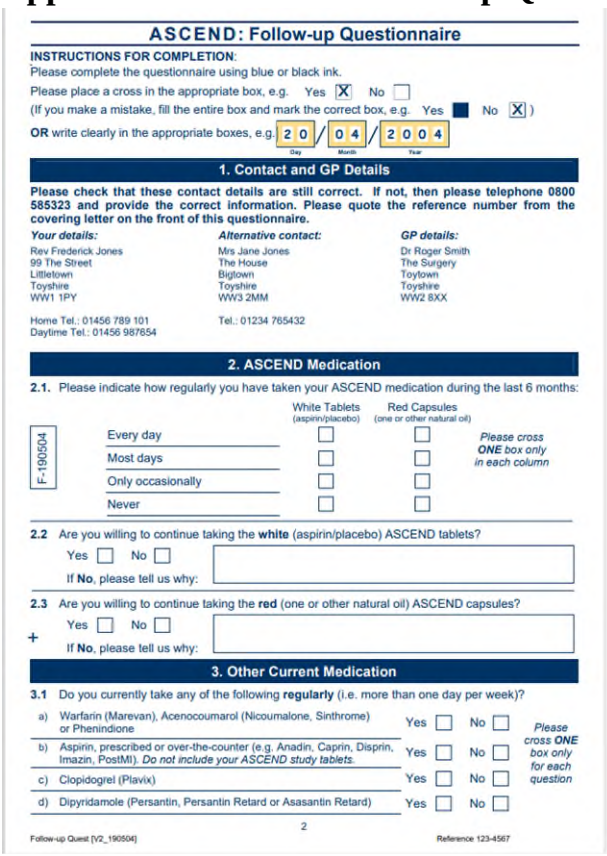
It would be very helpful for us if you could provide the details of a relative, friend or neighbour living at a **different address** who we could contact if for any reason we were unable to get hold of you. **Please indicate their relationship to you and write their contact details clearly in the boxes provided.**

Relationship: [ ] **Relative** [ ] **Friend** [ ] **Neighbour** [ ] **Other**

Title: Mr [ ] Mrs [ ] Ms [ ] Miss [ ] Other [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

First name(s): [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][

Appendix 6: ASCEND Follow-Up Questionnaire



ASCEND: Follow-up Questionnaire

INSTRUCTIONS FOR COMPLETION:
Please complete the questionnaire using blue or black ink.
Please place a cross in the appropriate box, e.g. Yes [X] No [ ]
(If you make a mistake, fill the entire box and mark the correct box, e.g. Yes [■] No [X])
OR write clearly in the appropriate boxes, e.g. 20 / 04 / 2004 (Day / Month / Year)

1. Contact and GP Details

Please check that these contact details are still correct. If not, then please telephone 0800 585323 and provide the correct information. Please quote the reference number from the covering letter on the front of this questionnaire.

Your details:	*Alternative contact:*	*GP details:*
Rev Frederick Jones 99 The Street Littletown Toyshire WW1 1PY	Mrs Jane Jones The House Biglown Toyshire WW3 2MM	Dr Roger Smith The Surgery Toytown Toyshire WW2 8XX
Home Tel.: 01456 789 101 Daytime Tel.: 01456 987654	Tel.: 01234 765432	

2. ASCEND Medication

2.1. Please indicate how regularly you have taken your ASCEND medication during the last 6 months:

F-190504

	White Tablets (aspirin/placebo)	Red Capsules (one or other natural oil)
Every day	☐	☐
Most days	☐	☐
Only occasionally	☐	☐
Never	☐	☐

*Please cross **ONE** box only in each column*

2.2 Are you willing to continue taking the **white** (aspirin/placebo) ASCEND tablets?
Yes ☐ No ☐
If **No**, please tell us why:

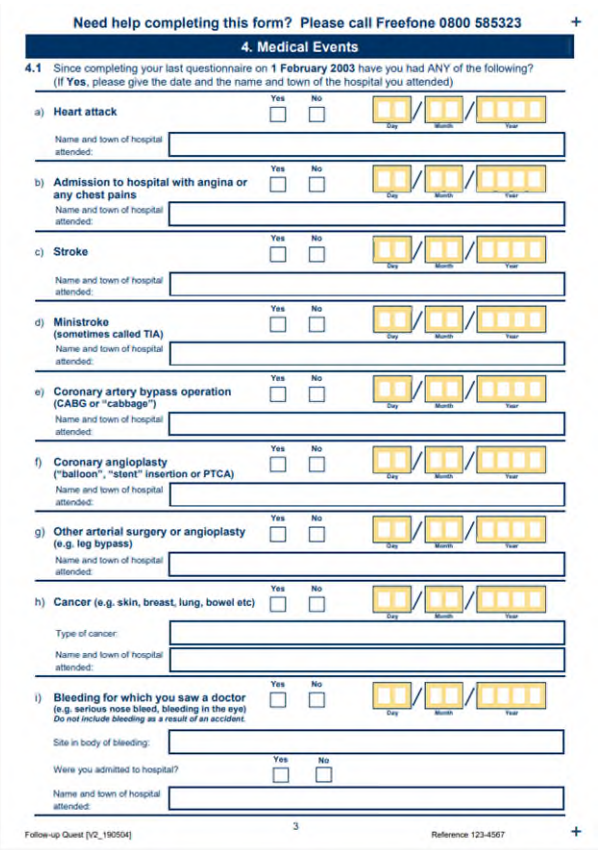
2.3 Are you willing to continue taking the **red** (one or other natural oil) ASCEND capsules?
Yes ☐ No ☐
If **No**, please tell us why:

3. Other Current Medication

3.1 Do you currently take any of the following **regularly** (i.e. more than one day per week)?

a)	Warfarin (Marevan), Acenocoumarol (Nicoumalone, Sinthrome) or Phenindione	Yes ☐ No ☐
b)	Aspirin, prescribed or over-the-counter (e.g. Anadin, Caprin, Disprin, Imazin, PostMI). *Do not include your ASCEND study tablets.*	Yes ☐ No ☐
c)	Clopidogrel (Plavix)	Yes ☐ No ☐
d)	Dipyridamole (Persantin, Persantin Retard or Asasantin Retard)	Yes ☐ No ☐

*Please cross **ONE** box only for each question*

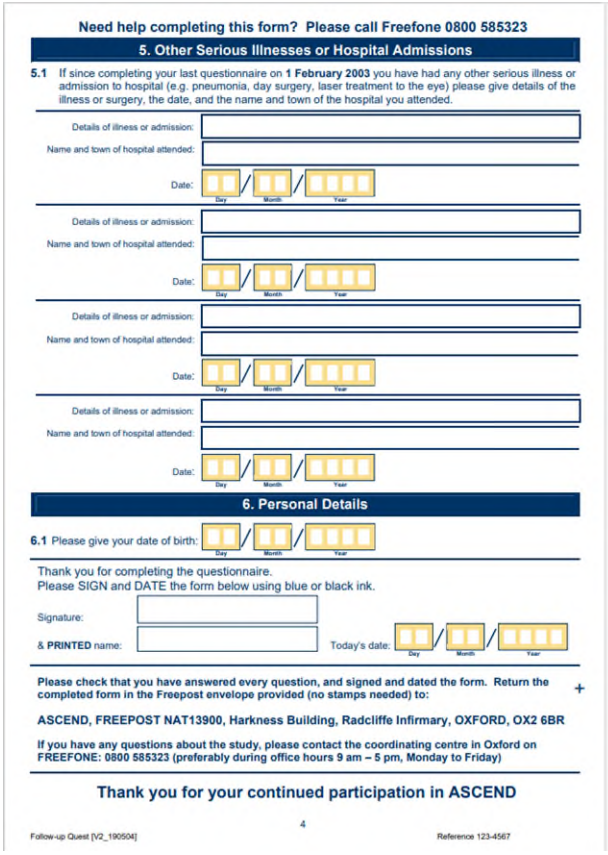


Need help completing this form? Please call Freefone 0800 585323

4. Medical Events

4.1 Since completing your last questionnaire on **1 February 2003** have you had ANY of the following?
(If **Yes**, please give the date and the name and town of the hospital you attended)

		Yes	No	Date (Day / Month / Year)
a)	**Heart attack** Name and town of hospital attended:	☐	☐	/ /
b)	**Admission to hospital with angina or any chest pains** Name and town of hospital attended:	☐	☐	/ /
c)	**Stroke** Name and town of hospital attended:	☐	☐	/ /
d)	**Ministroke (sometimes called TIA)** Name and town of hospital attended:	☐	☐	/ /
e)	**Coronary artery bypass operation (CABG or "cabbage")** Name and town of hospital attended:	☐	☐	/ /
f)	**Coronary angioplasty ("balloon", "stent" insertion or PTCA)** Name and town of hospital attended:	☐	☐	/ /
g)	**Other arterial surgery or angioplasty (e.g. leg bypass)** Name and town of hospital attended:	☐	☐	/ /
h)	**Cancer (e.g. skin, breast, lung, bowel etc)** Type of cancer: Name and town of hospital attended:	☐	☐	/ /
i)	**Bleeding for which you saw a doctor** (e.g. serious nose bleed, bleeding in the eye) *Do not include bleeding as a result of an accident.* Site in body of bleeding: Were you admitted to hospital? (Yes ☐ No ☐) Name and town of hospital attended:	☐	☐	/ /



Need help completing this form? Please call Freefone 0800 585323

5. Other Serious Illnesses or Hospital Admissions

5.1 If since completing your last questionnaire on **1 February 2003** you have had any other serious illness or admission to hospital (e.g. pneumonia, day surgery, laser treatment to the eye) please give details of the illness or surgery, the date, and the name and town of the hospital you attended.

Details of illness or admission:
Name and town of hospital attended:
Date: / / (Day / Month / Year)

Details of illness or admission:
Name and town of hospital attended:
Date: / / (Day / Month / Year)

Details of illness or admission:
Name and town of hospital attended:
Date: / / (Day / Month / Year)

Details of illness or admission:
Name and town of hospital attended:
Date: / / (Day / Month / Year)

6. Personal Details

6.1 Please give your date of birth: / / (Day / Month / Year)

Thank you for completing the questionnaire.
Please SIGN and DATE the form below using blue or black ink.

Signature:
& **PRINTED** name:
Today's date: / / (Day / Month / Year)

Please check that you have answered every question, and signed and dated the form. Return the completed form in the Freepost envelope provided (no stamps needed) to:

ASCEND, FREEPOST NAT13900, Harkness Building, Radcliffe Infirmary, OXFORD, OX2 6BR

If you have any questions about the study, please contact the coordinating centre in Oxford on FREEFONE: 0800 585323 (preferably during office hours 9 am – 5 pm, Monday to Friday)

Thank you for your continued participation in ASCEND



ASCEND

A Study of Cardiovascular Events iN Diabetes

ASCEND
Clinical Trial Service Unit
Harkness Building
Radcliffe Infirmary
Oxford
OX2 6HE

Office telephone: 01865 404888
Office fax: 01865 404871
Freefone: 0800 585323
e-mail: ascend@ctsu.ox.ac.uk
Website: www.ctsu.ox.ac.uk/ascend

Attachment 7: Amendments to B22-2
Participant Follow-up letter and Questionnaire

7 August 2003

Rev Frederick Jones
99 The Street
Littletown
Toyshire
WW1 PY1

Ref: 123-4567

Dear Rev Jones

ASCEND: A Study of Cardiovascular Events iN Diabetes

Thank you for your continued participation in ASCEND and your commitment to research into diabetes. On the back of this letter is your regular questionnaire. We would be grateful if you would complete it and return it promptly in the Freepost envelope enclosed. We cannot emphasise enough how important it is that we receive this information regularly from each of the 10,000 participants in ASCEND. We shall be sending you a new supply of study treatment automatically when it is required.

Please let us know if you have any questions or need help completing the questionnaire, by telephoning Freefone: 0800 585323.

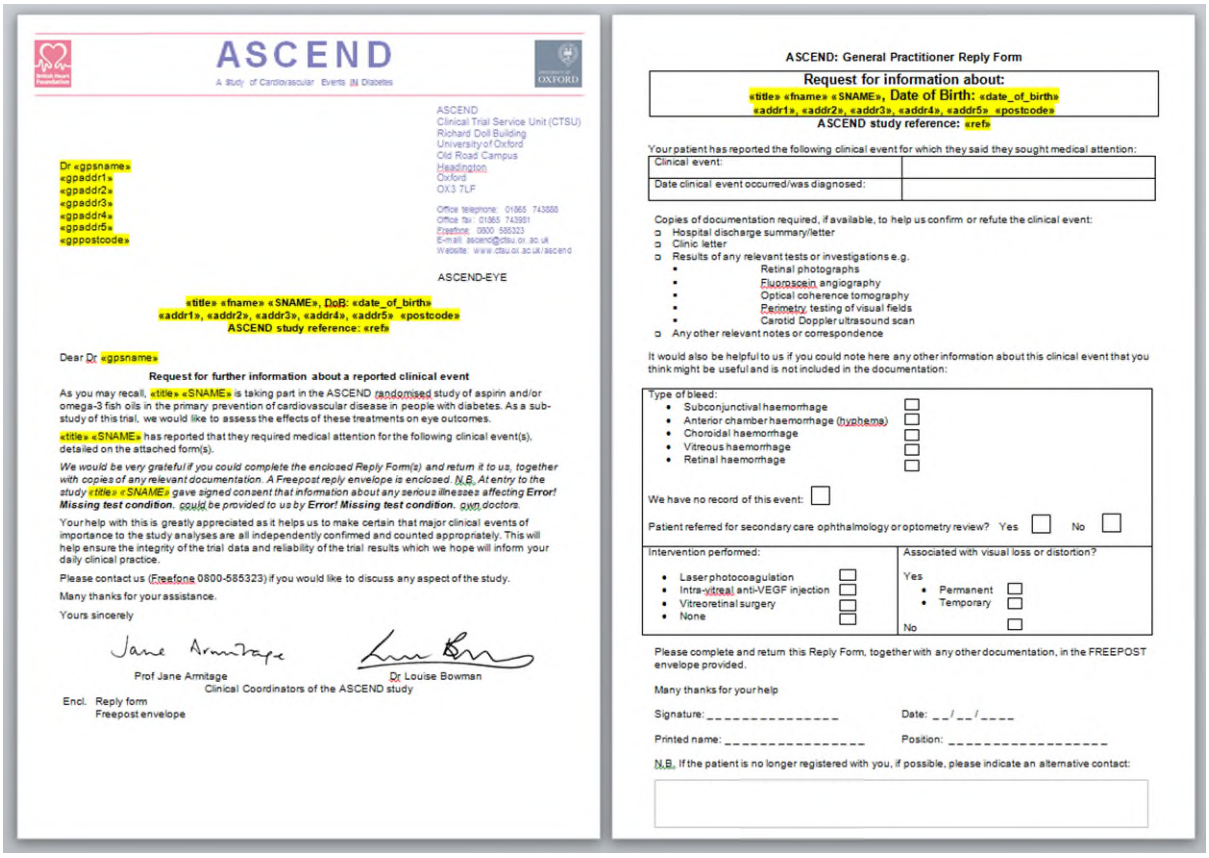
Thank you for your continued support of ASCEND and for completing the regular questionnaires. We are grateful for your help with finding ways of preventing the complications of diabetes.

Yours sincerely

Dr Jane Armitage Dr Louise Bowman
Study Coordinators

Enc: Freepost envelope

Appendix 7: Letter Requesting Supporting Evidence of Participant-Reported Eye Bleeds



ASCEND
A study of Cardiovascular Events IN Diabetes

ASCEND
Clinical Trial Service Unit (CTSU)
Richard Doll Building
University of Oxford
Old Road Campus
Headington
Oxford
OX3 7LF

Office telephone: 01865 743888
Office fax: 01865 743891
Freefone: 0800 585323
E-mail: ascend@ctsu.ox.ac.uk
Website: www.ctsu.ox.ac.uk/ascend

Dr «gpsname»
«gpaddr1»
«gpaddr2»
«gpaddr3»
«gpaddr4»
«gpaddr5»
«gppostcode»

ASCEND-EYE

«title» «fname» «SNAME», DoB: «date_of_birth»
«addr1», «addr2», «addr3», «addr4», «addr5» «postcode»
ASCEND study reference: «ref»

Dear Dr «gpsname»

Request for further information about a reported clinical event

As you may recall, «title» «SNAME» is taking part in the ASCEND randomised study of aspirin and/or omega-3 fish oils in the primary prevention of cardiovascular disease in people with diabetes. As a sub-study of this trial, we would like to assess the effects of these treatments on eye outcomes.

«title» «SNAME» has reported that they required medical attention for the following clinical event(s), detailed on the attached form(s).

We would be very grateful if you could complete the enclosed Reply Form(s) and return it to us, together with copies of any relevant documentation. A Freepost reply envelope is enclosed. N.B. At entry to the study «title» «SNAME» gave signed consent that information about any serious illnesses affecting ***Error! Missing test condition.*** *could be provided to us by* ***Error! Missing test condition.*** *own doctors.*

Your help with this is greatly appreciated as it helps us to make certain that major clinical events of importance to the study analyses are all independently confirmed and counted appropriately. This will help ensure the integrity of the trial data and reliability of the trial results which we hope will inform your daily clinical practice.

Please contact us (Freefone 0800-585323) if you would like to discuss any aspect of the study.

Many thanks for your assistance.

Yours sincerely

Jane Armitage

Prof Jane Armitage

Dr Louise Bowman

Clinical Coordinators of the ASCEND study

Encl. Reply form
Freepost envelope

ASCEND: General Practitioner Reply Form

Request for information about:
«title» «fname» «SNAME», Date of Birth: «date_of_birth»
«addr1», «addr2», «addr3», «addr4», «addr5» «postcode»
ASCEND study reference: «ref»

Your patient has reported the following clinical event for which they said they sought medical attention:

Clinical event:	
Date clinical event occurred/was diagnosed:	

Copies of documentation required, if available, to help us confirm or refute the clinical event:
- ☐ Hospital discharge summary/letter
- ☐ Clinic letter
- ☐ Results of any relevant tests or investigations e.g.
  - Retinal photographs
  - Fluoroscein angiography
  - Optical coherence tomography
  - Perimetry testing of visual fields
  - Carotid Doppler ultrasound scan
- ☐ Any other relevant notes or correspondence

It would also be helpful to us if you could note here any other information about this clinical event that you think might be useful and is not included in the documentation:

Type of bleed:
- Subconjunctival haemorrhage ☐
- Anterior chamber haemorrhage (hyphema) ☐
- Choroidal haemorrhage ☐
- Vitreous haemorrhage ☐
- Retinal haemorrhage ☐

We have no record of this event: ☐

Patient referred for secondary care ophthalmology or optometry review? Yes ☐ No ☐

Intervention performed:
- Laser photocoagulation ☐
- Intra-vitreal anti-VEGF injection ☐
- Vitreoretinal surgery ☐
- None ☐

Associated with visual loss or distortion?
Yes
- Permanent ☐
- Temporary ☐

No ☐

Please complete and return this Reply Form, together with any other documentation, in the FREEPOST envelope provided.

Many thanks for your help

Signature: _____ Date: __/__/____

Printed name: _____ Position: _____

N.B. If the patient is no longer registered with you, if possible, please indicate an alternative contact:

Appendix 8: Standard Operating Procedure: Confirmation and Coding of Eye Bleeding Events

ASCEND-Eye

Standard Operating Procedure*

SOP: Confirmation and Coding of Eye Bleeding Events

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1. Version history

Version history	Author
V1.0_28062017	E Sammons

2. Purpose

Unrefuted sight-threatening eye bleeds will be included in the secondary endpoint of "Major haemorrhage" for the purpose of the main ASCEND trial. Characterising the severity of eye-bleeding events will also be an integral area of investigation into the safety of aspirin and omega-3 fatty acids as part of the ASCEND-Eye sub-study. The purpose of this document is to describe the procedures for the confirmation and coding (adjudication) of eye bleeding events in both ASCEND and the ASCEND-Eye sub-study.

* References to different sections within Appendix 8 relate solely to the SOP: Confirmation and Coding of Eye Bleeding Events, and not to sections of the main thesis.

The definitions and adjudication rules outlined in this document were created in collaboration with Mr Praveen Patel, Consultant Ophthalmologist at Moorfield's Eye Hospital.

3. Definition of terms and abbreviations

The following terms and abbreviations are used in this SOP:

Term/abbreviation	Definition
ASCEND	A Study of Cardiovascular Events iN Diabetes
CTSU	Clinical Trial Service Unit
SOP	Standard Operating Procedure

4. Data flow and guidelines for the adjudication of eye bleeding events

The Clinical Trial Service Unit (CTSU) at Oxford University is the sponsor of the ASCEND-Eye sub-study and will have overall responsibility for the administration and coordination of the study. Clinicians from the ASCEND coordinating centre will be responsible for the adjudication of eye bleeding events in ASCEND-Eye.

This SOP is intended to be used in conjunction with ASCEND SOP 8: Confirmation and coding of serious adverse events (EDMS #5569)[*]. The author will refer back to relevant aspects of SOP 8 in this document.

4.1 Sources of information

ASCEND-Eye will compare the incidence of sight-threatening and non-serious/non-sight-threatening eye bleeding events between treatment arms for all participants randomised to the ASCEND trial. Data from this adjudication will contribute to secondary and tertiary endpoints of the parent study (**please refer to sections 2.4.2 and 2.4.3 SOP 8**).

Eye bleeds may be reported by one of the following ways:

- Self-reporting by participants on follow-up forms or via impromptu telephone or email communication with coordinating centre staff
- Impromptu telephone or email communication between friends, relatives, carers or healthcare professionals and coordinating centre staff.
- By GPs on specific GP follow-up forms

4.1.1 Telephone-reported eye bleeds

1. Eye bleeding events that are reported by telephone are first described in a telephone summary form in 'VISTA'.
2. A coordinating centre clinician will then capture information about the event in 'Anubis'.
3. Supporting information about events with the following Category E eye bleeding codes will be sought from the participant's general practitioner (GP):

[*] This document has not been included in this thesis. It is archived in the Electronic Document Management System at CTSU.

Table 1: Category E Codes

Read Code	Description
F421D	Retinal/vitreous haemorrhage
F4C	Bleeding in the eye (site unknown)
F4C7	Other eye bleeding (site known, not retinal/vitreous)
F421N	Retinal/vitreous haemorrhage (not known to have diabetic retinopathy)

4.1.2 Follow-up form-reported eye bleeds

1. Eye bleeding events that are reported in answer to question 4.1 (i) on a paper- or web-based follow-up form are first given a category E code in 'Eyes and Hands'*
2. Data written into 'Anubis' for event mailing purposes
3. Supporting information about events with the following Category E eye bleeding codes will be sought from the participant's GP

Supporting information provided by the participant's GP as a result of mailings generated through either of these reporting methods will be used to confirm or refute the event based on the guidelines to follow.

4.2 Event dates

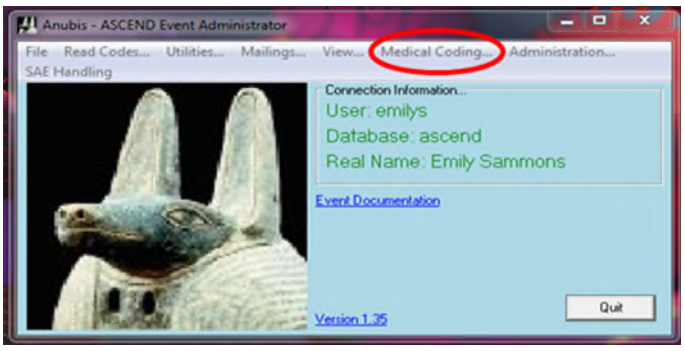
Please refer to **SOP 8 Section 3.3** for general guidelines about reporting and validity checking of event dates in 'Anubis'.

4.3 Adjudication

Adjudicated data will be recorded in the "Medical Coding Form" in 'Anubis' as follows:

1. From the home screen (Figure 1), select "Medical Coding…" and "Code Event(s)" from the drop-down list

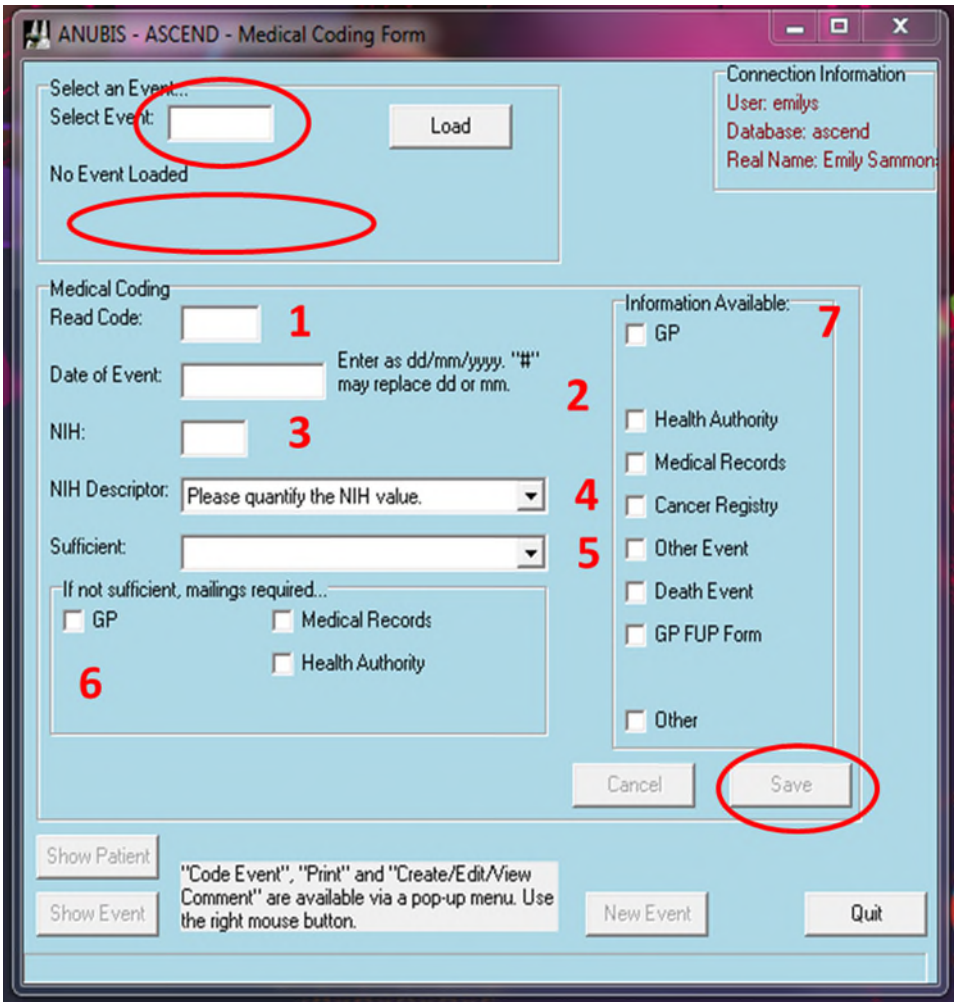
Figure 1: Anubis: ASCEND Event Administrator Home Screen



2. Type a relevant eye bleeding event ID into the "Select Event" box and click "Load". Participant identifiers will be displayed beneath this field, as well as pre-adjudicated Read code information.

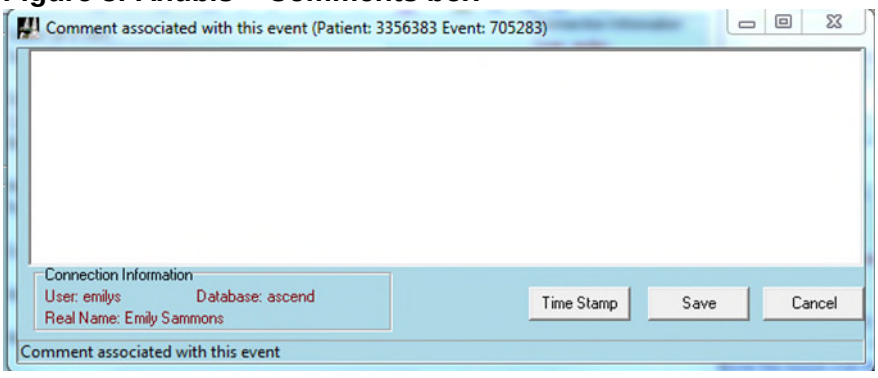
* Please refer to **section 5.2.4 and 5.2.5 SOP 8** regarding the recording of eye bleeding events and laser treatment through Eyes and Hands before adjudication.

Figure 2: Anubis – ASCEND Medical Coding Form



3. Complete data fields 1-7 which are defined in table 2.
4. Right mouse-click on the Medical Coding Form to “Edit/Create Comment”. A new “Comment” window will pop up (Figure 3) in which free text information associated with the event can be recorded. Enter aetiology and affected eye information (see section 4.3.1 of this SOP) and “Save”. You will then return to the ‘Medical Coding Form’ once again.

Figure 3: Anubis – Comments box



5. “Save” adjudicated data on the ‘Medical Coding Form’

Table 2: Medical Coding Form Data Fields 1-7

Data Field	Description
1. Read Code	Codes used to define the type of eye bleed are described in section of this SOP
2. Date of event	Wherever possible the use of partial date information is to be avoided. Please refer to **section 3.3 SOP 8** for more information.
3. NIH	If relevant a numeric value can be added to this field to represent the number of nights in hospital
4. NIH Descriptor	The following options are available from this drop-down menu. For the majority of eye bleeding events, it is expected that the participant will be treated on an outpatient basis. • Hospitalised (One or more night) • Hospitalised (But not kept in overnight) • Hospitalised (unknown duration) • Not Hospitalised • Outpatient • Unknown
5. Sufficient	Information about sufficiency codes can be found in section 4.4 of this SOP
6. Mailings	Where more information is needed in order to sufficiently adjudicate an event, adjudicators can indicate where automatic mailings should be sent to.
7. Information available	This field will be used to indicate the source of the information events were adjudicated from

4.3.1 Coding location, intervention and other relevant data

1. All eye bleeds will be adjudicated to one parent Read code stem: F6
2. Read code suffixes will be used to capture location, intervention and visual loss information as follows:

Table 3: Read code suffixes for intervention and location

Location	**Suffix**	**Definition**
Subconjunctival	S	
Anterior chamber	A	
Retinal	R	Including sub-hyaloid, pre-retinal, retinal, sub-retinal and sub-retinal pigment epithelial haemorrhage.
Vitreous	V	
Unknown	U	
Intervention	**Suffix**	**Definition**
Watch and Wait	W	
Intravitreal injection	J	Either anti-VEGF or steroid injection
Laser	L	Pan-retinal photocoagulation or focal
Surgery	S	Vitrectomy
Unknown	U	

Table 4 lists the resulting number of codes an eye bleeding event might be adjudicated to.

Table 4: Post-adjudication eye bleed codes

Subconjunctival*	Anterior chamber	Retinal	Vitreous	Unknown
F6SWN	F6AWN	F6RWN	F6VWN	F6UWN
~~F6SWY~~	F6AWY	F6RWY	F6VWY	F6UWY
~~F6SWU~~	F6AWU	F6RWU	F6VWU	F6UWU
~~F6SJN~~	F6AJN	F6RJN	F6VJN	F6UJN
~~F6SJY~~	F6AJY	F6RJY	F6VJY	F6UJY
~~F6SJU~~	F6AJU	F6RJU	F6VJU	F6UJU
~~F6SLN~~	F6ALN	F6RLN	F6VLN	F6ULN
~~F6SLY~~	F6ALY	F6RLY	F6VLY	F6ULY
~~F6SLU~~	F6ALU	F6RLU	F6VLU	F6ULU
~~F6SSY~~	F6ASN	F6RSN	F6VSN	F6USN
~~F6SSN~~	F6ASY	F6RSY	F6VSY	F6USY
~~F6SSU~~	F6ASU	F6RSU	F6VSU	F6USU
~~F6SUN~~	F6AUN	F6RUN	F6VUN	F6UUN
~~F6SUY~~	F6AUY	F6RUY	F6VUY	F6UUY
~~F6SUU~~	F6AUU	F6RUU	F6VUU	F6UUU

*If the location of an eye bleeding event is confirmed as subconjunctival, it is reasonable to assume that no intervention was performed and that there was no resulting visual loss. Hence we considered that only the F6SWN code is needed for bleeds confirmed to be in the subconjunctival location.

Key:

- 1st Suffix = location
- 2nd Suffix = intervention
- 3rd Suffix = visual loss

3. Use the “Comments” section to record additional information about aetiology (AET) and eye affected (Side). This information shall be recorded in a standardised way as described in table 5.

Table 5: Additional information about eye bleeding event

		Description
AET	**PDR**	Proliferative diabetic retinopathy
	AMD	Age-related macular degeneration
	SD8	Trauma
	Other	Other (excluding traumatic)
Side	**RE**	Right eye
	LE	Left eye

Example:

Documents support a vitreous haemorrhage of the right eye in a participant known to have proliferative diabetic retinopathy, which requires laser therapy and does not cause permanent visual loss would be adjudicated as follows:

- F6VLN
- Comments: AET = PDR, RE

4.4 Sufficiency flags

All relevant events are to be adjudicated according to the guidelines below. Furthermore, flags are to be added to each event depending on how certain the coder is about the code they have attributed to a given event. These “sufficiency flags” are to be used as follows.

4.4.1 Sufficiency = A

All events trawled from Follow-up forms are initially entered into the events database with a sufficiency flag A. This means they are awaiting adjudication (i.e. as initially reported).

4.4.2 Sufficiency = Y

If the coder has sufficient information in the supporting documentation to be confident that they can confirm or refute a given event according to the guidelines below, then the event should be coded and the sufficiency flag changed from A to Y.

4.4.3 Sufficiency = N

This flag is to be used for coding all relevant adverse events for which there is insufficient supporting information.

4.4.4 Sufficiency = V

This flag is to be used where a new event is entered as a result of a telephone conversation with a medical professional (e.g. local hospital nurse/doctor/GP) and a confirmatory verbal report of the event has been recorded in a telephone summary, a SUFF = V (sufficient but need written information) flag may be used.

4.4.5 Sufficiency = P

This flag is to be used when there is reason to believe that the event did occur, but no further information is going to become available.

5. Classifying the severity of eye bleeding events

[Definitions are given in Section 3.2.1.1 of the thesis]

5.1 Things considered in the preparation of this classification system/a few notes for adjudicators:

- It is important to distinguish the **type of laser** therapy participants have. Only retinal laser therapy (i.e. pan retinal or focal photocoagulation) is included in the definition of a sight-threatening bleed. Where the evidence supports YAG capsulotomies, adjudicators should follow-up whether cataract extractions have been recorded separately. YAG capsulotomies may be indicated for cataracts diagnosed and treated in the years prior to randomisation. Likewise laser therapy to correct refractive errors should not be related to eye bleeding events.

- It is unlikely to be possible to make a distinction between focal *vs.* pan retinal photocoagulation laser therapy and use this to further categorise bleed severity.

- It is accepted that an individual's past ocular history will influence the urgency of an intervention. For example, a lower threshold to intervene at an earlier stage is likely to be applied to pseudophakic individuals, those who are blind in the fellow eye and those who have less prior laser. Sometimes this level of detail may not be provided, however, the risk of bias from this is low because past ocular histories should be equally balanced in both treatment arms and because adjudication will be done blinded to treatment allocations.

- Late sequelae of eye bleeding events and any interventions associated with them are not included in the definition of a sight-threatening bleed. This is because linking complications to a discrete eye-bleeding event may not be reliable. For example, tractional retinal detachments often occur idiosyncratically and in myopic individuals. Likewise, epiretinal membranes may develop for reasons besides a previous vitreous haemorrhage. Finally, timescales evidenced in the documents sent from GPs may be variable and therefore it is important to look for urgent interventions-only. As a general rule of thumb, ophthalmologists would typically attempt retinal laser treatment within the first 1-3 months after a bleed. Depending on blood resorption, participant preferences and the extent of visual impairment, vitrectomies are typically performed, vitrectomy 3-6 months after a dense vitreous haemorrhage. **Please note that any interventions or complications that were not reported previously should be recorded separately by the adjudicator at the time of the adjudication.**

- Haemorrhages in the context of retinal vein occlusion should be viewed in the same way as haemorrhagic transformation of an ischaemic stroke and should be excluded as an eye bleeding event. **During adjudication, a diagnosis of retinal vein occlusion should be coded as a separate event i.e. it should not be given a F6 eye bleeding code.**

- Mode of presentation (e.g. acute presentation with symptomatic floaters *vs.* referral from the screening service) is not included in the categorisation of severity. The pathophysiology and management of acute bleeds *vs.* those found incidentally at screening are not different, and therefore only unresolved visual loss and/or the need for an urgent intervention are the only criterion we will apply to the definition of a sight-threatening bleed.

- Recurrent bleeds in the same eye should be recorded as a separate event in Anubis. A combination of first and recurrent bleeds in the same eye will be considered separately during analyses.

- It is accepted that macula-centred bleeds may be more detrimental to sight than more peripheral bleeds. However it is not possible to be too specific about the location of bleeds during our adjudication, and therefore a functional indicator of severity (i.e. visual loss) was chosen as a criterion of seriousness rather than location.

- Where the information provided for adjudication is limited, the use of NHS Diabetic Eye Screening grades before and after the dates of a bleeding event should be considered. If these “bookend” grades suggest no deterioration in visual acuity or retinopathy over time, the event should be regarded as a non-serious event.

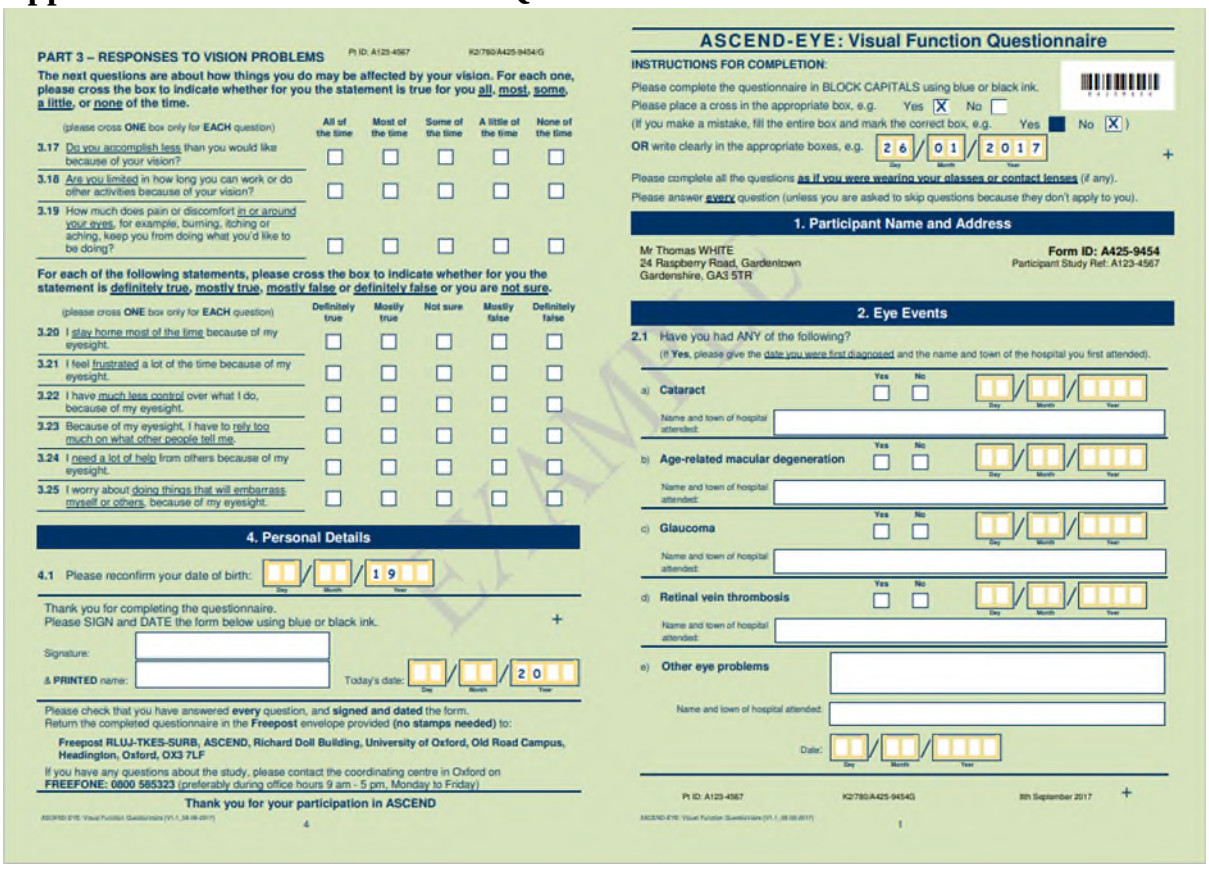
6. Eye Bleed Severity Analyses

After applying this definition, the following table shows sight-threatening eye bleed events that will contribute to the secondary endpoint of “major haemorrhage” (**see section 2.4.2 SOP 8**), and non-serious/non-sight-threatening bleeds that will be included as a tertiary endpoint of the main ASCEND trial (**see section 2.4.3 SOP 8**).

Subconjunctival*	Anterior chamber	Retinal	Vitreous	Unknown
F6SWN	F6AWN	F6RWN	F6VWN	F6UWN
~~F6SWY~~	F6AWY	F6RWY	F6VWY	F6UWY
~~F6SWU~~	F6AWU	F6RWU	F6VWU	F6UWU
~~F6SJN~~	F6AJN	F6RJN	F6VJN	F6UJN
~~F6SJY~~	F6AJY	F6RJY	F6VJY	F6UJY
~~F6SJU~~	F6AJU	F6RJU	F6VJU	F6UJU
~~F6SLN~~	F6ALN	F6RLN	F6VLN	F6ULN
~~F6SLY~~	F6ALY	F6RLY	F6VLY	F6ULY
~~F6SLU~~	F6ALU	F6RLU	F6VLU	F6ULU
~~F6SSY~~	F6ASN	F6RSN	F6VSN	F6USN
~~F6SSN~~	F6ASY	F6RSY	F6VSY	F6USY
~~F6SSU~~	F6ASU	F6RSU	F6VSU	F6USU
~~F6SUN~~	F6AUN	F6RUN	F6VUN	F6UUN
~~F6SUY~~	F6AUY	F6RUY	F6VUY	F6UUY
~~F6SUU~~	F6AUU	F6RUU	F6VUU	F6UUU

Green = non-serious/non-sight-threatening; Red = serious/sight-threatening

Appendix 9: The Visual Function Questionnaire



ASCEND-EYE: Visual Function Questionnaire

INSTRUCTIONS FOR COMPLETION:

Please complete the questionnaire in BLOCK CAPITALS using blue or black ink.
Please place a cross in the appropriate box, e.g. Yes [X] No [ ]
(If you make a mistake, fill the entire box and mark the correct box, e.g. Yes [ ] No [X])
OR write clearly in the appropriate boxes, e.g. 26 / 01 / 2017 (Day / Month / Year)

Please complete all the questions as if you were wearing your glasses or contact lenses (if any).
Please answer every question (unless you are asked to skip questions because they don't apply to you).

1. Participant Name and Address

Mr Thomas WHITE
24 Raspberry Road, Gardentown
Gardenshire, GA3 5TR

Form ID: A425-9454
Participant Study Ref: A123-4567

2. Eye Events

2.1 Have you had ANY of the following?
(If Yes, please give the date you were first diagnosed and the name and town of the hospital you first attended).

a) Cataract — Yes [ ] No [ ] — Day / Month / Year — Name and town of hospital attended:
b) Age-related macular degeneration — Yes [ ] No [ ] — Day / Month / Year — Name and town of hospital attended:
c) Glaucoma — Yes [ ] No [ ] — Day / Month / Year — Name and town of hospital attended:
d) Retinal vein thrombosis — Yes [ ] No [ ] — Day / Month / Year — Name and town of hospital attended:
e) Other eye problems — Name and town of hospital attended:

Date: Day / Month / Year

Pt ID: A123-4567 K2/780/A425-9454G 8th September 2017

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PART 3 – RESPONSES TO VISION PROBLEMS

Pt ID: A123-4567 K2/780/A425-9454G

The next questions are about how things you do may be affected by your vision. For each one, please cross the box to indicate whether for you the statement is true for you all, most, some, a little, or none of the time.

(please cross ONE box only for EACH question)

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
3.17 Do you accomplish less than you would like because of your vision?	☐	☐	☐	☐	☐
3.18 Are you limited in how long you can work or do other activities because of your vision?	☐	☐	☐	☐	☐
3.19 How much does pain or discomfort in or around your eyes, for example, burning, itching or aching, keep you from doing what you'd like to be doing?	☐	☐	☐	☐	☐

For each of the following statements, please cross the box to indicate whether for you the statement is definitely true, mostly true, mostly false or definitely false or you are not sure.

(please cross ONE box only for EACH question)

	Definitely true	Mostly true	Not sure	Mostly false	Definitely false
3.20 I stay home most of the time because of my eyesight.	☐	☐	☐	☐	☐
3.21 I feel frustrated a lot of the time because of my eyesight.	☐	☐	☐	☐	☐
3.22 I have much less control over what I do, because of my eyesight.	☐	☐	☐	☐	☐
3.23 Because of my eyesight, I have to rely too much on what other people tell me.	☐	☐	☐	☐	☐
3.24 I need a lot of help from others because of my eyesight.	☐	☐	☐	☐	☐
3.25 I worry about doing things that will embarrass myself or others, because of my eyesight.	☐	☐	☐	☐	☐

4. Personal Details

4.1 Please reconfirm your date of birth: Day / Month / 19

Thank you for completing the questionnaire.
Please SIGN and DATE the form below using blue or black ink.

Signature:
& PRINTED name:
Today's date: Day / Month / 20 Year

Please check that you have answered every question, and signed and dated the form.
Return the completed questionnaire in the Freepost envelope provided (no stamps needed) to:

Freepost RLUJ-TKES-SURB, ASCEND, Richard Doll Building, University of Oxford, Old Road Campus, Headington, Oxford, OX3 7LF

If you have any questions about the study, please contact the coordinating centre in Oxford on FREEFONE: 0800 585323 (preferably during office hours 9 am - 5 pm, Monday to Friday)

Thank you for your participation in ASCEND

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Need help completing this form? Please call Freefone 0800 585323

3. National Eye-Institute Visual Functioning Questionnaire - 25

PART 1 – GENERAL HEALTH AND VISION (please cross ONE box only for EACH question)

3.1 In general, would you say your overall health is:
Excellent: ☐ Very good: ☐ Good: ☐ Fair: ☐ Poor: ☐

3.2 At the present time, would you say your eyesight using both eyes (with glasses or contact lenses, if you wear them) is excellent, good, fair, poor, or very poor or are you completely blind?
Excellent: ☐ Good: ☐ Fair: ☐ Poor: ☐ Very poor: ☐ Completely blind: ☐

3.3 How much of the time do you worry about your eyesight?
None of the time: ☐ A little of the time: ☐ Some of the time: ☐ Most of the time: ☐ All of the time: ☐

3.4 How much pain or discomfort have you had in and around your eyes (for example, burning, itching, or aching)? Would you say it is:
None: ☐ Mild: ☐ Moderate: ☐ Severe: ☐ Very severe: ☐

PART 2 – DIFFICULTY WITH ACTIVITIES (please cross ONE box only for EACH question)

The next questions are about how much difficulty, if any, you have doing certain activities, wearing your glasses or contact lenses if you use them for that activity.

3.5 How much difficulty do you have reading ordinary print in newspapers? Would you say you have:
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

3.6 How much difficulty do you have doing work or hobbies that require you to see well up close, such as cooking, sewing, fixing things around the house or using hand tools? Would you say:
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

3.7 Because of your eyesight, how much difficulty do you have finding something on a crowded shelf?
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

3.8 How much difficulty do you have reading street signs or the names of shops?
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

3.9 Because of your eyesight, how much difficulty do you have going down steps, stairs, or kerbs in dim light or at night?
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

V-080817

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Pt ID: A123-4567 K2/780/A425-9454G 8th September 2017

3.10 Because of your eyesight, how much difficulty do you have noticing objects off to the side while you are walking along?
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

3.11 Because of your eyesight, how much difficulty do you have seeing how people react to things you say?
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

3.12 Because of your eyesight, how much difficulty do you have picking out and matching your own clothes?
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

3.13 Because of your eyesight, how much difficulty do you have visiting with people in their homes, at parties, or in restaurants?
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

3.14 Because of your eyesight, how much difficulty do you have going out to see films, plays, or sports events?
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

3.15 Are you currently driving, at least once in a while? Yes ☐ No ☐

3.15a IF NO: have you never driven a car or have you given up driving?
Never drove: ☐ Gave up: ☐ → Skip to Part 3, Q 3.17 on page 4

3.15b IF YOU GAVE UP DRIVING: Was that mainly because of your eyesight, mainly for some other reason, or because of both your eyesight and other reasons?
Mainly eyesight: ☐ Mainly other reason: ☐ Both eyesight and other reasons: ☐ → Skip to Part 3, Q 3.17 on page 4

3.15c IF CURRENTLY DRIVING: How much difficulty do you have driving during the daytime in familiar places? Would you say you have:
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐

3.16 How much difficulty do you have driving at night? Would you say you have:
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

3.16A How much difficulty do you have driving in difficult conditions, such as in bad weather, during rush hour, on the motorway, or in city traffic? Would you say you have:
No difficulty at all: ☐ A little difficulty: ☐ Moderate difficulty: ☐ Extreme difficulty: ☐ Stopped doing this because of your eyesight: ☐ Stopped doing this for other reasons or not interested in doing this: ☐

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Appendix 10: ASCEND-Eye Participant Information Sheet

ASCEND

A Study of Cardiovascular Events IN Diabetes

ASCEND
Clinical Trial Service Unit (CTSU)
Richard Doll Building
University of Oxford
Old Road Campus
Headington
Oxford
OX3 7LF

Office telephone: 01865 743888
Freefone: 0800 585323
E-mail: ascend@ctsu.ox.ac.uk
Website: www.ctsu.ox.ac.uk/ascend

ASCEND-EYE

An invitation to take part in the ASCEND-EYE sub-study

We are writing to tell you about a new voluntary part of ASCEND known as ASCEND-EYE. The purpose of ASCEND-EYE is to see whether aspirin and fish oils have any effect on various eye diseases.

Before you agree to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. You are free to decide whether or not to take part in this sub-study. If you choose not to take part, the standard of care you are given by your own doctors will not be affected.

Diabetes and the eye

Having diabetes can affect your eyes and your ability to see. The most common and serious problem is diabetic retinopathy which is caused by damage to the delicate network of blood vessels at the back of the eye known as the retina. In severe disease, disorganised growth of new blood vessels causes bleeding and scarring which may result in loss of sight. Fortunately diabetic retinopathy can be prevented and treated if it is caught at an early stage. This is why the NHS invites people with diabetes to attend regular eye screening examinations. As part of this examination photographs are taken of the back of the eyes.

Aspirin and omega-3 fatty acids on diabetic retinopathy

Studies from the 1960s first suggested that tiny blood clots blocking blood vessels in the retina could start a chain of events that results in diabetic retinopathy. Because aspirin is known to reduce the risk of blood clots elsewhere in the body, doctors thought that it might be a useful treatment for diabetic retinopathy, but there were also concerns that it might cause bleeding in the eye. Two well-conducted but small clinical trials sought to investigate this further. One found that aspirin neither harms nor protects against diabetic retinopathy, but they only included people who were already known to have moderate disease. The other found that aspirin prevented early changes in the back of the eye in those without existing disease. However, it wasn't clear whether aspirin would slow the progression of diabetic retinopathy in the long-term because the number of people who took part in the study was too small and the follow-up period was too short to assess this reliably. Given the large number of people taking part in the main ASCEND trial who have now been taking aspirin or placebo (dummy aspirin) for several years, we have the chance to assess whether aspirin might help to protect against diabetic retinopathy.

The effects of omega-3 fish oils on diabetic retinopathy have never been explored before. It is possible that they might also have a beneficial effect but it is not known. ASCEND-EYE is well placed to find this out.

Ageing and the Eye

People often notice they see less well as they get older. For most people, these changes are caused by weakening of the muscles that help us to focus on objects of interest. This is why older people often need reading glasses. However, a small proportion of people have a condition called age-related macular degeneration. This is a leading cause of poor sight and blindness among older adults in the UK. In macular degeneration damage occurs in the area of the back of the eye which is responsible for central vision and seeing fine detail and colour. This damage can be serious – affecting the ability to recognise faces, drive a car or read and write. Having diabetes **is not** thought to increase the risk of age-related macular degeneration, but research suggests that both aspirin and omega-3 fish oils could play a role.

Aspirin and omega-3 fish oils on age-related macular degeneration

Doctors think that taking regular aspirin may improve age-related macular degeneration because of its effects on the circulation. The use of aspirin by those with age-related macular degeneration has been investigated in previous studies, but with inconsistent results. Because aspirin is so widely used by older people it is important to understand whether it is having an effect.

Studies of fish oils have also produced inconsistent findings on age-related macular degeneration. Some studies suggest that people who eat oily fish regularly or take fish oil supplements have less eye problems but high quality studies have not shown benefit from taking fish oil supplements in people who already knew they had the disease.

What does the ASCEND-EYE sub-study hope to answer?

ASCEND-EYE will find out if there are any beneficial or harmful effects of taking aspirin and/or omega-3 fish oils on diabetic retinopathy and age-related macular degeneration. It will also collect information about other eye problems that are commonly seen in people with diabetes such as cataracts.

What will taking part in ASCEND-EYE involve?

Taking part in ASCEND-EYE will involve filling in a detailed questionnaire about your sight and how any problems affect your day-to-day activities like reading and driving. The questionnaire will take about 20 minutes to complete.

Coordinating centre activities in the ASCEND-EYE sub-study

The ASCEND coordinators will be writing to GPs to ask about any bleeding in the eye that you may have reported to us during ASCEND trial. At the beginning of ASCEND you gave consent to allow information about your health to be collected from national registries. As part of ASCEND-EYE we will be collecting information from the NHS Diabetic Eye Screening service about any diabetic retinopathy that has been recorded. If you would prefer us not to do this, please telephone the ASCEND Freefone number (0800 585323) and ask to speak to one of the study team.

What you have to do to join the study

If you are happy to take part, please complete and return the enclosed questionnaire in the FREEPOST envelope to the coordinating centre at Oxford University's Clinical Trial Service Unit. You should also keep this information sheet.

Will my taking part in the ASCEND-EYE sub-study be kept confidential?

Yes - all information which is collected about you during the course of ASCEND-EYE will be kept strictly confidential and used only for medical research purposes and for routine regulatory and audit purposes.

Study organisation

The ASCEND-EYE study has been designed and is coordinated by Oxford University's Clinical Trial Service Unit. The study design has been reviewed and agreed by an independent Research Ethics Committee, which includes people from outside the medical profession.

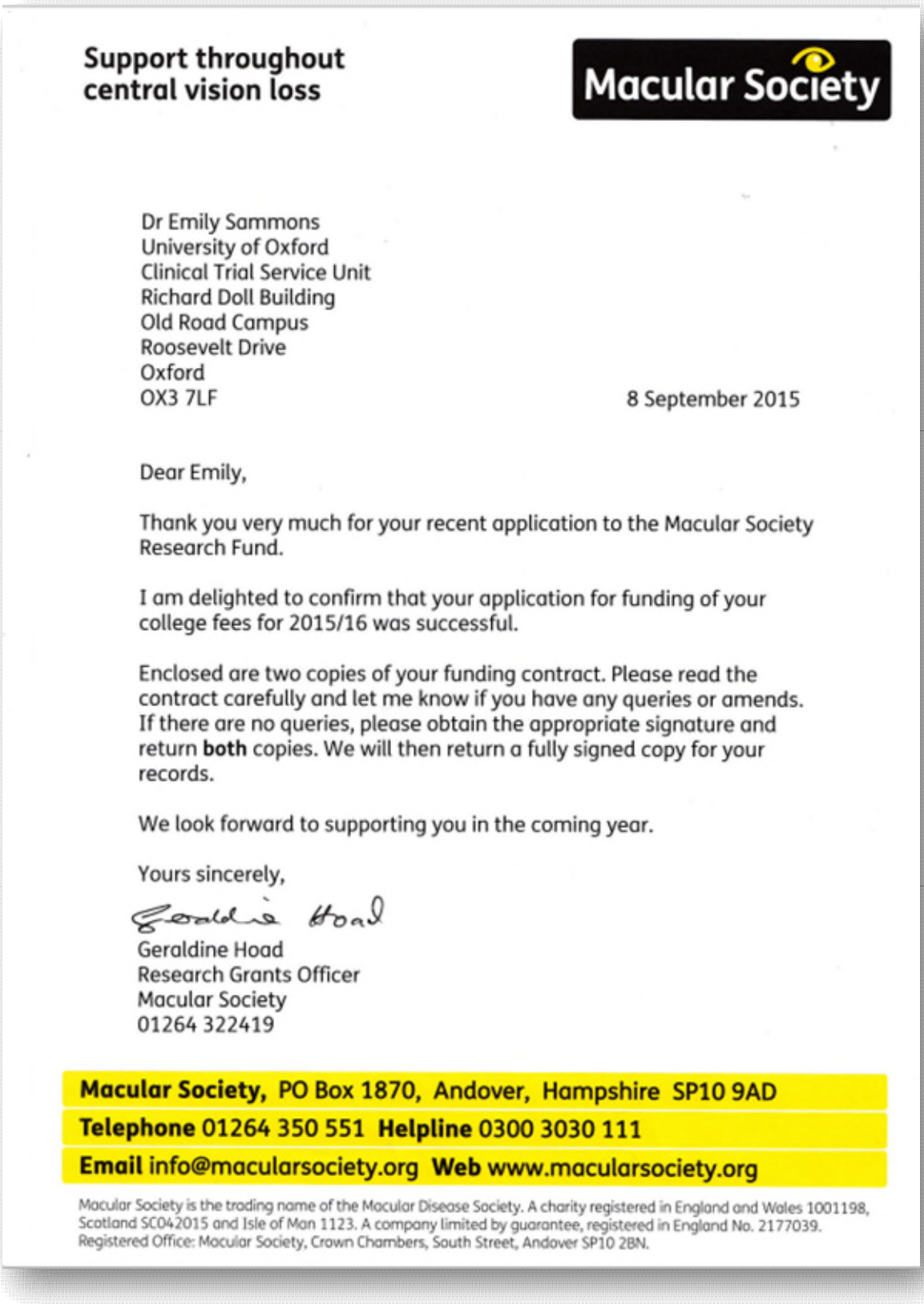
Contact Information

If there is anything that is not clear, or if you would like more information, please telephone the ASCEND Freefone number (0800 585323) and ask to speak to a study nurse or doctor. A larger font version of the questionnaire can also be viewed on our website: www.ctsu.ox.ac.uk/ascend

Thank you very much for taking the time to read this information sheet

Appendix 11: Funding Approval Letter from the Macular Society

Total awarded = £1424



Support throughout
central vision loss

Macular Society

Dr Emily Sammons
University of Oxford
Clinical Trial Service Unit
Richard Doll Building
Old Road Campus
Roosevelt Drive
Oxford
OX3 7LF

8 September 2015

Dear Emily,

Thank you very much for your recent application to the Macular Society Research Fund.

I am delighted to confirm that your application for funding of your college fees for 2015/16 was successful.

Enclosed are two copies of your funding contract. Please read the contract carefully and let me know if you have any queries or amends. If there are no queries, please obtain the appropriate signature and return **both** copies. We will then return a fully signed copy for your records.

We look forward to supporting you in the coming year.

Yours sincerely,

Geraldine Hoad

Geraldine Hoad
Research Grants Officer
Macular Society
01264 322419

Macular Society, PO Box 1870, Andover, Hampshire SP10 9AD
Telephone 01264 350 551 **Helpline** 0300 3030 111
Email info@macularsociety.org **Web** www.macularsociety.org

Macular Society is the trading name of the Macular Disease Society. A charity registered in England and Wales 1001198, Scotland SC042015 and Isle of Man 1123. A company limited by guarantee, registered in England No. 2177039. Registered Office: Macular Society, Crown Chambers, South Street, Andover SP10 2BN.

Appendix 12: Funding Approval Letter from the British Heart Foundation

BHF Centre of Research Excellence
Division of Cardiovascular Medicine,
Radcliffe Department of Medicine,
University of Oxford,
Level 6, West Wing,
John Radcliffe Hospital,
Oxford, OX3 9DU, UK

Tel: +44 (0)1865 234678 or 234657
Fax: +44 (0)1865 234658

25th April 2019

Dr Emily Sammons
Professors Jane Armitage & Louise Bowman
Nuffield Department of Public Health
Email: emily.sammons@ndph.ox.ac.uk

Dear Emily,

I am writing to confirm that your application for Infrastructure funding entitled "ASCEND-Eye Study Funding" has been approved for funding to the value of £15,000 by the Oxford BHF Centre of Research Excellence (BHF CRE).

Full details of the award are as follows:

Start date: 01/02/2019
End date: ~~31/03/2019~~ 31/08/2019

Funding breakdown:

Equipment	£15,000.00
Total	**£15,000.00**

The R12 Oracle Project number for your award is **HSR00030**, Task code ~~**H622.IN**~~ **H620.TR**
All costs associated with this project should be charged to this code.
Please note: The Principal Investigator for the Oracle sub-task is Associate Professor David Preiss

Please inform Philippa Major, Research Facilitator for the BHF CRE, of any publications, oral presentations or posters that arise from this funding. This will enable us to collate all the outputs from the CRE, for reporting back to the BHF.

In terms of journal publications, you should notify Philippa once a paper, arising from this award, has been accepted for publication by a journal. In addition, you must comply with the BHF's Policy on Open Access and deposit within EuropePubMed Central an electronic copy of each paper, funded wholly or in part by this BHF award that is accepted for publication in a peer reviewed journal, within six months of publication.

Acknowledging the Oxford BHF CRE in publications

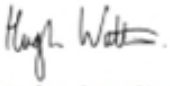
- Any publication arising from this work must contain the following acknowledgement:
  [PI name] acknowledges support from the BHF Centre of Research Excellence, Oxford.

- If you add the grant code (**RE/13/1/30181**) at the end of the above acknowledgment, then the BHF will reimburse fees associated with open access publishing (or a proportion of, when multiple funders are involved) via the Charity Open Access Fund (COAF) held in the University.

- Where possible, in any publication arising from this work please put "BHF Centre of Research Excellence" in your address, after your departmental affiliation.

I would like to take this opportunity to congratulate you and wish you every success with your research.

Kind regards,



Prof Hugh Watkins
Director, BHF Centre of Research Excellence

cc: NDPH pre-award: pre-award@ndph.ox.ac.uk
Emma Miller (emma.miller@ndph.ox.ac.uk)

Appendix 13: Conversion Table for the Welsh Screening Data Prior to 1st May 2015

Information in this table was taken directly from literature produced by the NHS DESW Programme.[387]

Previous Welsh grading protocol	Clinical Features	Equivalent NSC grade that the Welsh data were recoded to
Retinopathy Grade		
R_0	**No diabetic retinopathy**	R_0
$R_{1.1}$	**Minimal diabetic retinopathy:** ≥1 but <5 MA >1DD from fovea. ≥1 but <4 dot ±blot HM(s) >1DD from fovea Any exudates outside the macula ± <5 CWS	R_1
$R_{1.2}$	**Moderate background diabetic retinopathy:** ≥5 MAs >1DD from fovea ≥4-<8 dot±blot HMs >1DD from fovea with VA > 6/12 Circinate or grouped exudates outside the macula but within the temporal arcade Questionable IRMA only in the presence of microaneurysms or haemorrhage	
R_2	**Pre-proliferative diabetic retinopathy:** Multiple blot haemorrhages > 1DD from the fovea ≥8 blot haemorrhages per eye (superior and inferior hemifields) Venous irregularities, beading, reduplication, venous loops Definite IRMA ±CWS	R_2
R_{3s}	**Proliferative diabetic retinopathy – stable:** Stable pre-retinal fibrosis Stable fibrous proliferation (disc or elsewhere) Stable R_1 and R_2 features* *In the presence of photocoagulation	R_{3S}
R_{3a}	**Proliferative diabetic retinopathy – active:** NVD NVE New pre-retinal or vitreous haemorrhage New pre-retinal fibrosis New traction retinal detachment Reactivation in a previous stable R_{3s}	R_{3A}
Maculopathy Grade		
M_0	**No maculopathy**	M_0
M_1	**Possible maculopathy:** Any exudates or blot haemorrhages within the macula, i.e. < 2DD but > 1DD from the centre of the fovea 3 or more microaneurysms and/or any haemorrhage < 1DD of the centre of the fovea only if associated with unexplained VA of 6/12 or worse	M_1
M_2	**Definite maculopathy:** Any exudates ≤1DD of the fovea Retinal thickness ≤1DD of the fovea	
Photocoagulation Grade		
P_0	No evidence of previous photocoagulation	P_0
P_1	Focal/grid to the macula or peripheral scatter	P_1
P_{C2}	Focal laser	
P_{C3}	Pan retinal photocoagulation laser	
P_{C5}	Combined laser	

CWS = cotton wool spots; DD = disc diameters; HM = haemorrhage; IRMA = intraretinal microvascular abnormality; NVD = new vessels on the disc; NVE = new vessels elsewhere; MA = microaneurysms; VA = visual acuity

Appendix 14: Equivalence Table for the Visual Acuity Conversions

This table was compiled from several online sources.[388,477-482]

LogMar Chart	Snellen Chart (UK)	Snellen Chart (US)	ETDRS Letter Score	VAS Score (Visual acuity score)	ASCEND-Eye Recoded Score
-0.2	6/3.8	20/12.5	95	110	1
-0.18	6/4	20/13			2
-0.1	6/4.8	20/16	90	105	3
-0.08	6/5	20/17			4
0	6/6	20/20	85	100	5
0.1	6/7.5	20/25	80	95	6
0.2	6/9.5	20/32	75	90	7
0.3	6/12	20/40	70	85	8
0.4	6/15	20/50	65	80	9
0.48	6/18	20/60			10
0.5	6/19		60	75	11
0.6	6/24	20/80	55	70	12
0.7	6/30	20/100	50	65	13
0.78	6/36	20/120			14
0.8	6/38	20/125	45	60	15
0.9	6/48	20/160	40	55	16
1	6/60	20/200	35	50	17
1.1	6/76	20/250	30	45	18
1.2	6/95	20/320	25	40	19
1.3	3/60	20/400	20	35	20
1.4	3/75	20/500	15	30	21
1.48	2/60				22
1.5	2/63	20/630	10	25	23
1.6	2/80	20/800	5	20	24
1.7	2/100	20/1000		15	25
1.78	1/60				26
1.8	1/63	20/1250		10	27
1.9	1/79	20/1600		5	28
2.0	1/100	20/2000	2	0	29
(CF) Counts fingers at 1m	CF				30
(HM) Hand movements	HM				31
(PL) Perceives light	PL				32
(NPL) No perception of light	NPL				33
Enucleated eye	Enucleated eye				34

Appendix 15: Balance Diagnostic Tests

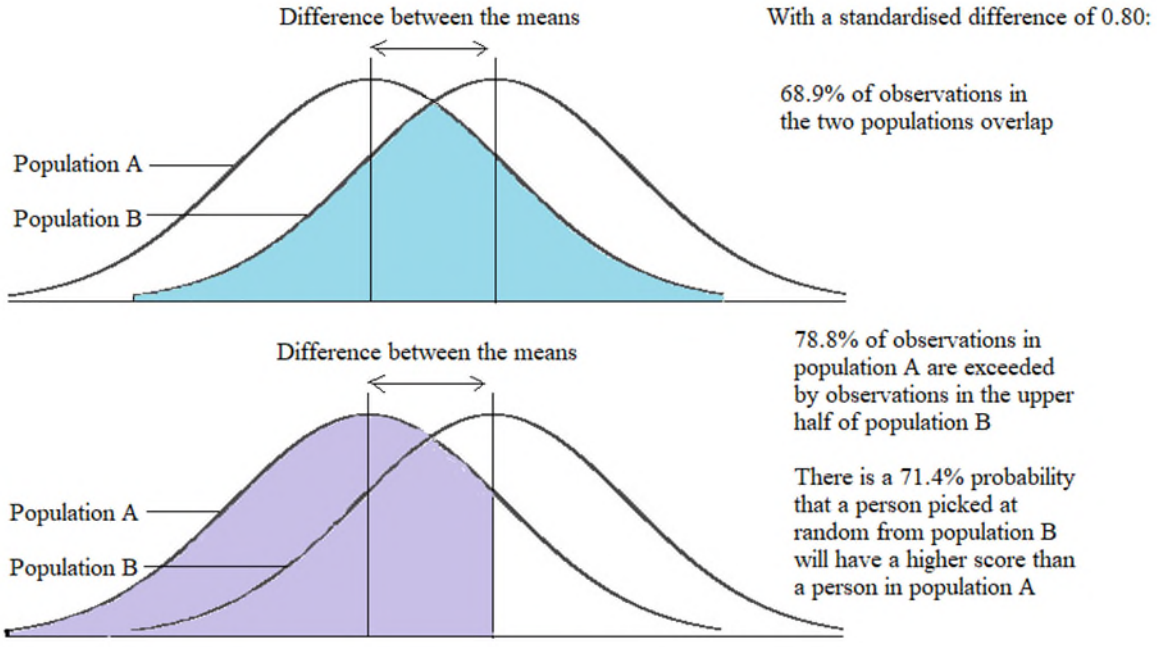
Interpretation of Standardised Difference Scores

Standardised difference (Cohen's *d*)	Interpretation of the standardised difference	Proportion of observations in the two populations that overlap	Proportion of the first population that the upper half of the second population exceeds	Probability that a person picked at random from the second population will have a higher score than a person in the first population ("probability of superiority)
0.0	None or minimal	100.0%	50.0%	50.0%
0.1		96.0%	54.0%	52.8%
0.2	Small	92.0%	57.9%	55.6%
0.3		88.1%	61.8%	58.4%
0.4		84.1%	65.5%	61.1%
0.5	Moderate	80.3%	69.1%	63.8%
0.6		76.4%	72.6%	66.4%
0.7		72.6%	75.8%	69.0%
0.8	Large	68.9%	78.8%	71.4%
0.9		65.3%	81.6%	73.8%
1.0		61.7%	84.1%	76.0%

Data shown in this table were compiled from the following sources:

- Magnusson, K. (2022). *Interpreting Cohen's d effect size: An interactive visualization* (Version 2.6.0) [Web App]. R Psychologist. https://rpsychologist.com/cohend/[483]
- https://support.sas.com/resources/papers/proceedings12/335-2012.pdf[390]

Visual Example of Two Distributions with a Standardised Difference of 0.8



This illustration was inspired by the interactive visualisation developed by Magnusson, K. (2022) for the interpretation of Cohen's *d* effect size. Available at: https://rpsychologist.com/cohend/ (accessed 10/05/2023)[483]

Appendix 16: Summary of DESP Analysis Cohort Restrictions

	Restrictions		
	Final visit*	**Baseline†**	**Summary of exclusions‡**
Primary Efficacy Endpoint Analysis – Any referable disease (cohort B1)	Not relevant	Not relevant	Those without any retinopathy data during the scheduled treatment period
Secondary Efficacy Endpoint Analysis – Referable disease in those without referable disease at baseline and Tertiary Analysis – Referable maculopathy (cohort B2)	Those with both retinopathy and maculopathy data in both eyes	Those with both retinopathy and maculopathy data in both eyes	Those with retinopathy data in neither eye or one eye only at baseline and/or the final record Those with maculopathy data in neither eye or one eye only at baseline and/or the final record Those with referable disease at baseline in either eye (R_2, R_3 and/or M_1)
Secondary Efficacy Analysis – Any retinopathy progression (cohort B3)	Those with retinopathy data in both eyes	Those with retinopathy data in both eyes	Those with retinopathy data in neither eye or one eye only at baseline and/or the final record Those with proliferative disease in both eyes at baseline (i.e. $R_{3a/s}$/$R_{3\ a/s}$)
Tertiary Analysis – Final retinopathy grade (cohort B4)	Those with retinopathy data in both eyes (if on different dates, then within a year of each other)	Not relevant	Those with retinopathy data in neither eye or one eye only at the final record Those with final retinopathy data on each eye more than one year apart
Tertiary Analysis – loss of visual acuity (cohort B5)	Those with visual acuity data in both eyes	Those with visual acuity data in both eyes	Those with visual acuity data in neither eye or one eye only at baseline and/or the final record
Sensitivity Analysis – Final retinopathy grade in those with a baseline record (a subset of cohort B4) (cohort B6)	Those with retinopathy data in both eyes (if on different dates, then within a year of each other)	Those with retinopathy data in both eyes	Those with retinopathy data in neither eye or one eye only at baseline and/or the final record Those with final retinopathy data on each eye more than one year apart
Sensitivity Analysis – highest ever post-randomisation retinopathy grade (cohort B7)	Those with retinopathy data in both eyes	Not relevant	Those with retinopathy data in neither eye or one eye only post-randomisation

*The majority of individuals had retinopathy data in both eyes on the same final record. If retinopathy is missing for one eye on the final record, an earlier (post-randomisation) record will be used as the final grade in the missing eye.
†The majority of individuals had retinopathy data in both eyes on the same baseline record. If retinopathy data is missing for one eye in the baseline record closest to randomisation, an earlier record may be used for the missing eye, so long as both baseline records occurred in the two years prior to randomisation.
‡All analyses will ignore pre-baseline and post-trial retinopathy and maculopathy data.

Appendix 17: Summary of Objectives, Outcomes, Data Sources and Analysis Populations

Objective	Outcomes	Data source	Analysis cohort
i) To determine whether aspirin or omega-3 FAs alter the course of diabetic retinopathy or diabetic maculopathy	**1º efficacy:** Referable diabetic eye disease for those with in-trial retinopathy data (R_2, $R_{3a/s}$ or M_1)	DESP-linkage	B1
	2º efficacy: Referable diabetic eye disease restricted to those with a baseline record (R_0 or R_1) and $M_0 \rightarrow$(R_2, $R_{3a/s}$ or M_1)	DESP-linkage	B2
	2º efficacy: Any progression in retinopathy grade	DESP-linkage	B3
	3º efficacy: all incident reports of diabetic maculopathy (M_1 from the DESP-linkage dataset and F426 codes from either the VFQ form or ASCEND follow-up forms)	Pt-reported outcomes, VFQ and DESP-linkage	A, B2 and C
	3º efficacy: 1º efficacy by baseline characteristics	DESP-linkage	B1
	3º efficacy: Final retinopathy grade	DESP-linkage	B4
	Sensitivity: Final retinopathy grade in those with a baseline record	DESP-linkage	B6
	Sensitivity: Highest ever post-randomisation retinopathy grade	DESP-linkage	B7
ii) To characterise the occurrence and severity of participant-reported eye bleeding events by treatment allocation in the aspirin arm	**1º safety:** Confirmed sight-threatening eye bleeds	Pt-reported outcomes	A
	2º safety: Any eye bleed subdivided by severity	Pt-reported outcomes	A and C
iii) To determine the role of aspirin and separately, omega-3 FAs on incident diagnoses of age-related macular degeneration	**2º efficacy:** Incident diagnoses of AMD	Pt-reported outcomes and VFQ	A and C
iv) To compare differences between group assignments in each study treatment arm on composite visual function scores derived from the NEI-VFQ-25	**2º efficacy:** Composite NEI-VFQ-25 score	VFQ	**C**
	3º efficacy: Subdomain NEI-VFQ-25 scores	VFQ	**C**
v) To explore longitudinal between-group differences in visual acuity in each study treatment arm	**3º efficacy**: worsening of visual acuity by ≥3 points (≥15 ETDRS letters)	DESP-linkage	B5
vi) To describe incident reports of other eye diseases in each treatment arm, such as but not limited to cataracts, glaucoma, retinal vein thrombosis, infections and ocular nerve palsies	**Exploratory outcome:** descriptive data on incident reports of other eye diseases of interest	Pt-reported outcomes	A and C
vii) To identify the clinical and demographic characteristics that are associated with lower composite and subdomain scores on the NEI-VFQ-25, and to consider how concerns about eye health impact activities of daily living and emotional well-being	**Exploratory:** Multivariate analyses of clinical and demographic characteristics associated with lower composite and subdomain scores on the NEI-VFQ-25.*	VFQ	C

Pt-reported outcomes = participant-reported outcomes from in-trial follow-up questionnaires for ASCEND.
*To be completed as a post-doctoral research project.

Appendix 18: Other Eye Events of Interest

Read code	Description
F40	Eye infection
F401	Other ophthalmitis
F41Z	Retinal detachment/surgery
F423	Amaurosis fugax/transient visual loss
F430	Eye inflammation unspecified
F440	Iritis/iridocyclitis/uveitis
F45	Glaucoma/surgery for/trabeculectomy
F48	Visual disturbances
F490	Blindness
F4B	Corneal disorder
F4C0	Conjunctivitis
F4E	Eyelid disorder
G745	Retinal artery occlusion/retinal thrombosis/visual loss thought ischaemic in nature
G755	Giant cell arteritis/temporal arteritis
G825	Retinal vein occlusion/blood clot in the eye
F32Z, F310	Cranial nerve palsy
7263	Cataract/cataract extraction/lens implant

NB: the Read codes that are to be used for the relevant secondary and tertiary analyses include:

- F425 Macular/posterior pole degeneration
- F426 Diabetic macular oedema

Appendix 19: A Worked Example of the Power Calculations for ASCEND-Eye

Power calculations were based on formula 4.42 on page 141 of P. Armitage, G. Berry and JNS. Matthews "Statistical Methods in Medical Research" Fourth Edition, with a continuity correction from Fleiss *et al*, 1980.[407,408]

Estimates for the number of events in each treatment group were derived from the formulae:

Nplacebo=Ntotal/(2-p)

Nactive=Nplacebo*(1-p)

Where p is the proportional reduction of risk in the active group compared to the placebo group, Ntotal = the total number of participants who experienced an event of interest, Nplacebo = the number of events in the placebo group, and Nactive = the number of events in the active group.

Formula 4.42 in Armitage *et al.* 2002 is intended to be used to calculate the sample size required in each of the two groups in order to detect a statistically significant difference between two independent proportions, with a specified significance level and power, and when the standard deviation is not known in advance:

$$N = \left\{ \frac{\left(Z_{2\alpha}\sqrt{[2\pi(1-\pi)]} + Z_{2\beta}\sqrt{[\pi_1(1-\pi_1) + \pi_2(1-\pi_2)]} \right)}{(\pi_1 - \pi_2)} \right\}^2$$

This rearranges to the following formula to calculate the power based on a given sample size:

$$Z_{2\beta} = \frac{\left(\left[(\pi_1 - \pi_2)\sqrt{N} \right] - Z_{2\alpha}\sqrt{[2\pi(1-\pi)]} \right)}{\sqrt{[\pi_1(1-\pi_1) + \pi_2(1-\pi_2)]}}$$

Where N = the sample size, π=the total proportion of events, π_1 = the proportion of events anticipated in the active group, and π_2= the proportion of events anticipated in the placebo group, $Z_{2\alpha}$ = the standardised normal deviation exceeded in either direction with a probability of $2\alpha = 0.05$ (i.e. the type I error rate for a two-tailed test), and $Z_{2\beta}$ = the Z value corresponding level of power (1-β, where 1-β = power, and β= the type II error rate).

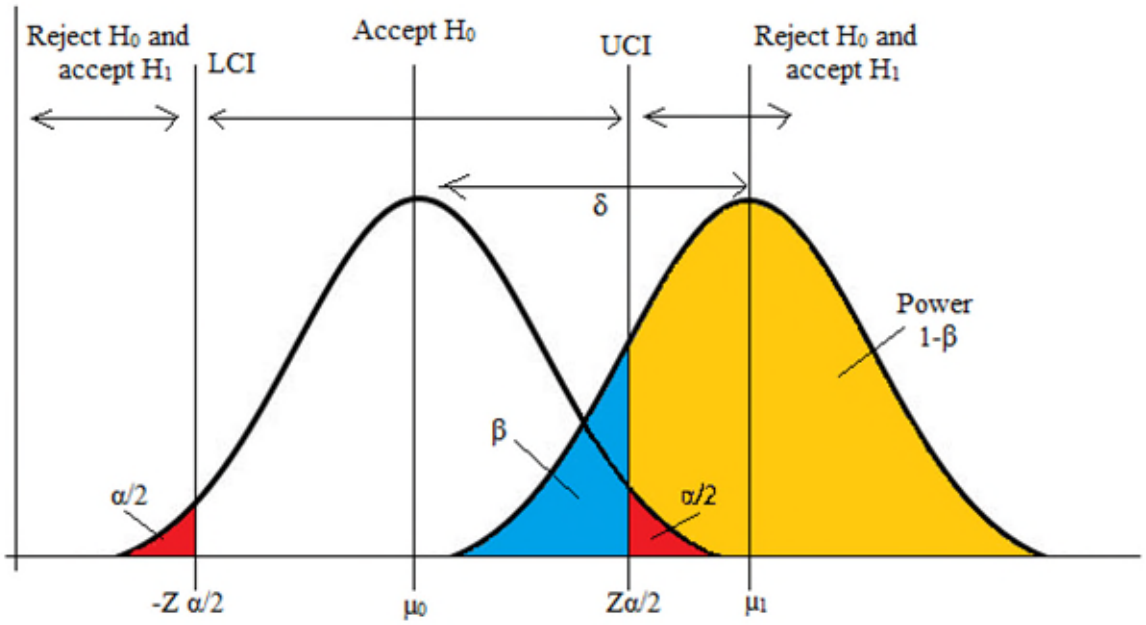
Look-up tables available in P. Armitage, G. Berry and JNS. Matthews "Statistical Methods in Medical Research", or online, can then be used to convert the $Z_{2\beta}$ value into power.

The work of Fleiss *et al.* 1980 showed that power estimates from the rearranged formula 4.42 more closely approximate to a normal distribution when a continuity correction factor ($2/|\pi_1 - \pi_2|$) is added, giving the formula:

$$Z_{2\beta} = \frac{\left(\left[(\pi_1 - \pi_2)\sqrt{(N - 2/(\pi_1 - \pi_2))}\right] - Z_{2\alpha}\sqrt{[2\pi(1-\pi)]}\right)}{\sqrt{[\pi_1(1-\pi_1) + \pi_2(1-\pi_2)]}}$$

These formulae may be easier to understand when they are represented visually in the form of two curves showing the distributions of the observed differences (δ) between two independent sample means (μ_0 and μ_1; Figure A). The curve on the left represents the control group (or the null hypothesis, H_0). The curve on the right represents the active group (or the alternative hypothesis, H_1). The red shaded area represents the type I error rate (α), or the probability of incorrectly rejecting the null hypothesis (i.e. drawing a false positive conclusion). For a two-tailed test at a 0.05 significance level, the area under the curve corresponding to α is halved between the left-hand and right-hand tails (α/2 = 0.025). Lower confidence intervals (LCI) and upper confidence intervals (LCI) are drawn at these points. The blue shaded area represents the type II error rate (β), or the probability of failing to detect a real difference (i.e. drawing a false negative conclusion). The power of the study to detect a true difference between two sample means is shaded in yellow (1-β).

Figure A: Distributions of the Observed Difference between Two Independent Samples



Worked example:

Blinded preliminary analyses identified 1061 out of a total of 7360 participants in cohort B1 who experienced the primary efficacy event of referable disease. The example is a power calculation for a 20% proportional risk reduction for this endpoint in the active group compared to the placebo group for a two-tailed test at the 0.05 significance level.

p=0.2

Nplacebo=Ntotal/ (2-p) =1061/ (2-0.2) =589 participants

Nactive=Nplacebo*(1-p) =589*(1-0.2) =472 participants

Power formula:

$$Z_{2\beta} = \frac{\left(\left[(\pi_1 - \pi_2)\sqrt{(N - 2/(\pi_1 - \pi_2))}\right] - Z_{2\alpha}\sqrt{[2\pi(1-\pi)]}\right)}{\sqrt{[\pi_1(1-\pi_1) + \pi_2(1-\pi_2)]}}$$

Sample size in each of the two groups: N = 3680 (i.e. 7360/2)

Proportion of events in the active group: π_1= 472/3680 = 0.128260870

Proportion of events in the placebo group: π_2 = 589/3680 = 0.160054348

Difference in proportions: π_1- π_2 = 589/3680 – 472/3680 = 117/3680 = 0.160054348 - 0.128260870 = 0.031793478

Overall proportion of participants with an event: π = (472+589)/7360 = 0.144157609

Using the power formula with the exact values calculated above for a 2-tailed significance level of 0.05 ($Z_{2\alpha}$ = 1.96) gives:

$Z_{2\beta}$ = ([0.031793478 x √3680-2/(0.031793478] - 1.96 x √[2 x 0.144157609 x (1-0.144157609)/ √ [0.128260870 x (1-0.128260870) +0.160054348 x (1-0.160054348)

=1.912132302-0.973613867/0.496232781

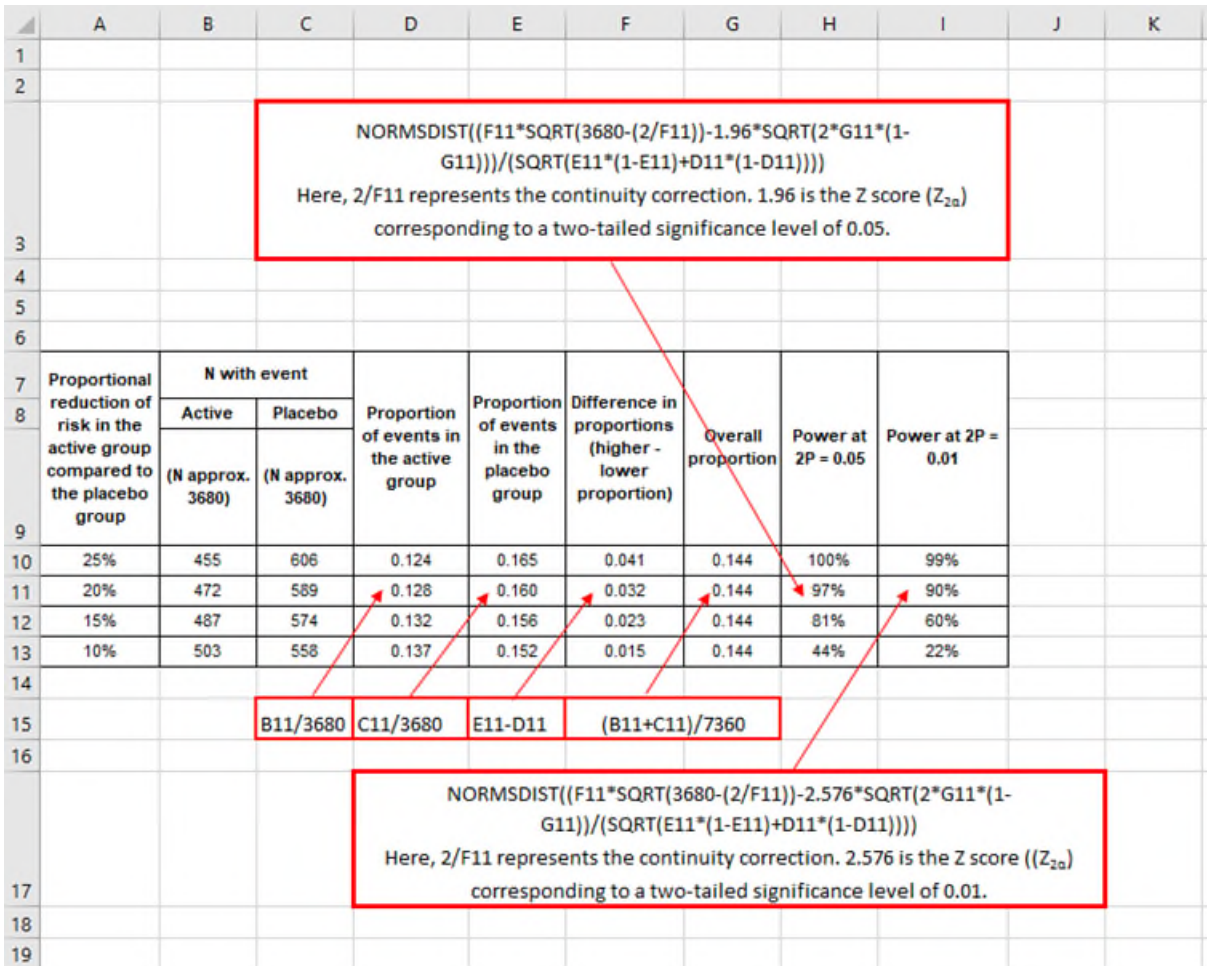
=0.938518155/0.496232781

=1.891286652

Power (based on look-up table) = 97%

Figure B is a screenshot of an annotated Microsoft Excel spreadsheet showing the coding used to automate the remaining power calculations. I am grateful to Ms Georgina Buck, who provided me with this code. Here, the NORMSDIST function converts the Z score into the percentage power.

Figure B: Screenshot of Microsoft Excel Spreadsheet Showing Coding for the Power Calculations



Appendix 20: A Worked Example of the Logrank Test for Referable Disease Events in the Aspirin Arm

Logrank methods are used to test the null hypothesis that the probability of having an event at any time point is distributed equally between two groups of survival data in proportion to the numbers at risk.[394,404,407] The test compares differences between the observed number of events at each time point with the numbers that would be expected under the null hypothesis.[394,404,407] The combination of these differences over the duration of a study generates a test statistic, which can be used to calculate an approximation to the hazard ratio and its 95% confidence interval when the effect size is modest.[394,404,407]

The worked example of the logrank test shown in this appendix was based on the lecture notes produced by Associate Professor Natalie Staplin, a Senior Statistician within CTSU, for the MSc in Global Health Sciences course*. Her approach was applied here to approximate the rate ratio and 95% CI for the referable disease outcome in the aspirin arm. It was prepared after the DAP was made publicly available and unblinded analyses had already begun.

Table A is a life table showing the number of participants at risk of referable disease at each time point by group assignment in the aspirin arm, and in both groups combined (columns 2, 3 and 4, respectively). For brevity, the data are compressed into year intervals instead of the day intervals used for the main analysis. Therefore, the resultant summary statistics are marginally different to those reported in Section 4.4.1. Columns 5, 6 and 7 show the observed number of referable disease events at each time point in the active and placebo groups of the aspirin arm, and in both groups combined, respectively. Additional columns were added to the life table to facilitate subsequent calculations of the event rate ratio.

Table A: Life-table of Referable Disease Outcomes in the Aspirin Arm

Time (days)	N_{1t}	N_{2t}	N_t	O_{1t}	O_{2t}	O_t	$\frac{O_t}{N_t}$	E_{1t}	E_{2t}	V_{1t}
0	3687	3673	7360	0	0	0	0	0	0	0
365	3686	3671	7357	73	71	144	0.019573	72.1468	71.8532	35.30002
730	3583	3585	7168	56	57	113	0.015765	56.48424	56.51576	27.80853
1095	3488	3497	6985	74	75	149	0.021331	74.40401	74.59599	36.46056
1460	3387	3360	6747	64	60	124	0.018379	62.24811	61.75189	30.43429
1825	3256	3227	6483	74	64	138	0.021286	69.30865	68.69135	33.77015
2190	3011	2976	5987	72	69	141	0.023551	70.91214	70.08786	34.4244
2568	2041	2047	4088	50	51	101	0.024706	50.42588	50.57412	24.63213
4016	1121	1141	2262	76	75	151	0.066755	74.83245	76.16755	35.24282
Sum				539				530.7623		258.0729

N_t = No. at risk during each time period, t; O_t = The observed number of events during each time period, t; E_t = The expected number of events during each time period, t; V_t = The variance during time period, t; $_1$ denotes those who received active aspirin; $_2$ denotes those who were allocated placebo.

Column 8 was calculated using the formula:

$$\frac{O_t}{N_t} = \text{Risk of event at time, t}$$

* Access to these lecture notes was permitted by the MSc Course Leaders, but they are not publicly available.

Columns 9 and 10 were calculated using the formulae:

$$E_{1t} = N_{1t}\left(\frac{O_t}{Nt}\right) \; ; \; E_{2t} = N_{2t}\left(\frac{O_t}{Nt}\right)$$

Column 11, representing the variance at each time period, was calculated using formula 17.12 on page 577 of P. Armitage, G. Berry and JNS. Matthews "Statistical Methods in Medical Research", Fourth Edition:[407]

$$V_{1t} = \frac{O_t \times N_{1t} \times N_{2t} \times (N_t - O_t)}{(N_t)^2 \times (N_t - 1)}$$

The test statistic was calculated as follows:

$$Test\ statistic = \frac{(\sum O_{1t} - \sum E_{1t})^2}{\sum V_{1t}} = \frac{(O-E)^2}{V}$$

$$Test\ statistic = \frac{(539 - 530.7623)^2}{258.0729}$$

$$Test\ statistic = 0.262949$$

The log-rank 'observed minus expected' statistic and its variance can then be used to calculate the one-step estimate of the log of the event rate ratio and confidence interval for the aspirin group versus the placebo group, as follows:[412]

1) The rate ratio was calculated from the formulae:

$$Log\ RR = \frac{O-E}{V} = 0.03912$$

$$Rate\ Ratio = exp\left(\frac{O-E}{V}\right) = 1.032435$$

2) 95% confidence intervals were calculated using the formulae:

$$95\%\ CI = exp\left(\frac{O-E}{V} \pm \frac{1.96}{\sqrt{V}}\right)$$

$$\therefore Upper\ 95\%\ CI = exp\left(0.03912 + \frac{1.96}{\sqrt{258.0729}}\right) = exp(0.03192 + 0.122007) = 1.166405736$$

$$\therefore Lower\ 95\%\ CI = exp\left(0.03912 - \frac{1.96}{\sqrt{258.0729}}\right) = exp(0.03192 - 0.122007) = 0.913851676$$

Thus, the rate ratio from this worked example is 1.03 (95% CI 0.91-1.17). For comparison, the rate ratio measured using day intervals in SAS statistical software, presented in Section 4.4.1 of the Results chapter was 1.03 (95% CI 0.91-1.16). Kaplan-Meier estimates for the time to event were also plotted with their associated log-rank P-values in Section 4.4.1.

The stratified logrank test is an extension of the logrank test. First, a logrank test statistic is calculated for individual strata.[413] The overall stratified rate ratio is the exponential of the sum of the observed minus expected events in each strata divided by the sum of the variance in each strata. 95% confidence intervals are then calculated as shown on page 395.[413] However, it is beyond the scope of this thesis to describe the chi-square and test for trend used to produce the P-value statistics.

Appendix 21: A Worked Example of the Ordinal Logistic Regression for Final Duplex Retinopathy Grades in the Aspirin Arm

For the relevant analyses, ASCEND-Eye will use proportional odds logistic regression to model the relationship between duplex retinopathy grades or NEI-VFQ-25 scores (the "dependent", "outcome", or "ordinal response" variable) and the active or placebo groups of each treatment arm (the "independent" or "explanatory" variable).

Ordinal logistic regression estimates the probability of an outcome with two or more response levels occurring based on one or more independent variables.[484,485] A fundamental assumption underlying this method is that the effect of the independent variable(s) is the same for every level of the response variable.[484,485] The test produces a common odds ratio (or cumulative logit) for the likelihood of having a higher or lower response over the full ordinal scale depending on the independent variable(s).[484,485]

Readily-available statistical software may be used to fit ordinal logistic regression models.[484] However, the functions available in different packages set different parameters for the models they use.[484] In ASCEND-Eye, the SAS PROC LOGISTIC proportional odds test was used. The parameters considered by this PROC are shown in the following formula[484]:

$$log\left(\frac{P(Y \geq j)}{1 - P(Y \geq j)}\right) = \alpha_j + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p x_p, j = 1, \cdots, J - 1$$

Where:

Y = the ordinal response variable with J categories;

J = the number of levels in the ordinal response variable;

j = one of the categories in the ordinal response scale;

$log\left(\frac{P(Y \geq j)}{1 - P(Y \geq j)}\right)$ = The log odds (logit) of having a higher response on the ordinal scale*;

α_j = intercept parameters, one for each of the log odds calculated;

$j = 1, \cdots, J - 1$ denotes that a log odds ratio will be calculated for every level in the ordinal response range;

β = the regression coefficient which explains the effect of the independent variable, x, on the ordinal response variable; and

p = reflects the number of independent variables.

The worked example which follows is for the effect of aspirin on final duplex retinopathy grades. These comparisons were conducted after the DAP was made publicly available and unblinded analyses had already begun. For reference, the common odds ratio for the likelihood of having a higher retinopathy grade with aspirin, calculated using SAS and presented in Section 4.4.4 of the Results chapter is 0.99; 95% CI: 0.90-1.08.

Since there is only one independent variable in this model, i.e. allocation to treatment groups in the aspirin arm, the formula may be simplified to:

* Note that an alternative way of expressing $1 - P\ (Y \geq j)$ in some texts is $P\ (Y < j)$.

$$log\left(\frac{P(Y \geq j)}{1 - P(Y \geq j)}\right) = \alpha_j + \beta x \; for \; j = 1, \cdots, J - 1$$

Where:

$j_1 = R_0/R_0$, $j_2 = R_1/R_0$, $j_3 = R_1/R_1$ and $j_4 = >R_1/any$

β = the regression coefficient for the effect of randomised allocation to active or placebo group in the aspirin treatment arm (x=1 for the aspirin group, x=0 for the placebo group)

The model has J – 1 = 3 cutoffs (reflecting 4-1 response levels of duplex retinopathy grade)

The SAS-calculated regression coefficient for randomised treatment allocation in the aspirin arm, $\beta = -0.0142$

The exponent of this coefficient is the common odds ratio[484]:

$$\exp(\beta) = exp(-0.0142) = 0.985900344 = 0.99$$

The SAS-calculated standard error for the model = 0.0456

95% CI are calculated using the formula:

$$95\%\ CI = \ exp\big(\beta \pm \ (1.96 \ \times standard \ error)\big)$$

$$\therefore \ Upper\ 95\%\ CI = exp(-0.0142 \ + \ (1.96 \ \times 0.0456)) = 1.078073875 = 1.08$$

$$\therefore \ Lower\ 95\%\ CI = exp(-0.0142 \ - (1.96 \ \times 0.0456)) = 0.901607498 = 0.90$$

The SAS PROC LOGISTIC function uses the Score test to assess whether the assumption of proportional odds is upheld.[486] In this example, the Score test was non-significant (χ^2 = 0.7030 on two degrees of freedom, p = 0.70). Therefore, the common odds ratio is valid. However, the intercept (α_j) and the regression coefficient (β) can also be used to assess the likelihood of proportionality.[484,485] When the assumption of proportional odds is met, probabilities from the model will be similar to the observed proportions.[484,485]

The following table displays the observed and predicted proportions of final duplex retinopathy grades by group assignment in the aspirin arm. The data are organised into rows showing the cumulative probability of having a response variable greater than or equal to one category.

		Aspirin Arm					
		Active (n=3627)			**Placebo (n=3614)**		
	Intercept (α_j)*	**Observed no. and proportion**		**Predicted proportion**	**No. and observed proportion**		**Predicted proportion**
j = 1, 2, 3 or 4	-	159+774+613+2081 = 3627	(100.0%)	**(100.0%)**	148+796+611+2059 = 3614	(100.0%)	**(100.0%)**
j = 2, 3 or 4	-0.2819	159+774+613 = 1546	(42.6%)	**(42.7%)**	148+796+611 = 1555	(43.0%)	**(43.0%)**
j = 3 or 4	-1.0429	159+774 = 933	(25.7%)	**(25.8%)**	148+796 = 944	(26.1%)	**(26.1%)**
j = 4	-3.1103	159	(4.4%)	**(4.2%)**	148	(4.1%)	**(4.3%)**

Where $j_1 = R_0/R_0$, $j_2 = R_1/R_0$, $j_3 = R_1/R_1$ and $j_4 = >R_1/any$. β = -0.0142
* From SAS PROC LOGISTIC output;

Probabilities were calculated using the formula[484]:

$$P(Y \geq j) = \frac{e^{\alpha_j + \beta x}}{1 + e^{\alpha_j + \beta x}}$$

The predicted proportions in the active group (x = 1) were calculated as follows:

$$P(Y \geq j = 2) = \frac{e^{\alpha_2 + \beta x}}{1 + e^{\alpha_2 + \beta x}} = \frac{e^{-0.2819 + -0.0142}}{1 + e^{-0.2819 + -0.0142}} = \frac{0.743713053}{1.743713053} = 0.42651115 = 42.7\%$$

$$P(Y \geq j = 3) = \frac{e^{\alpha_3 + \beta x}}{1 + e^{\alpha_3 + \beta x}} = \frac{e^{-1.0429 + -0.0142}}{1 + e^{-1.0429 + -0.0142}} = \frac{0.34746199}{1.34746199} = 0.257864038 = 25.8\%$$

$$P(Y \geq j = 4) = \frac{e^{\alpha_4 + \beta x}}{1 + e^{\alpha_4 + \beta x}} = \frac{e^{-3.1103 + -0.0142}}{1 + e^{-3.1103 + -0.0142}} = \frac{0.043958907}{1.043958907} = 0.04210789 = 4.2\%$$

The predicted proportions in the placebo group (x = 0) were calculated as follows:

$$P(Y \geq j = 2) = \frac{e^{\alpha_2 + \beta x}}{1 + e^{\alpha_2 + \beta x}} = \frac{e^{-0.2819}}{1 + e^{-0.2819}} = \frac{0.754349115}{1.754349115} = 0.429988027 = 43.0\%$$

$$P(Y \geq j = 3) = \frac{e^{\alpha_3 + \beta x}}{1 + e^{\alpha_3 + \beta x}} = \frac{e^{-1.0429}}{1 + e^{-1.0429}} = \frac{0.352431148}{1.352431148} = 0.260590824 = 26.1\%$$

$$P(Y \geq j = 4) = \frac{e^{\alpha_4 + \beta x}}{1 + e^{\alpha_4 + \beta x}} = \frac{e^{-3.1103}}{1 + e^{-3.1103}} = \frac{0.044587577}{1.044587577} = 0.042684383 = 4.3\%$$

The predicted proportions were similar to those observed, which also supports the likelihood that the assumption of proportional odds was upheld by the regression model. If proportionality is not met, other statistical methods may be applied, but it is beyond the scope of this work to describe those.[485]

Appendix 22: Calculation of NEI-VFQ-25 Scores

The NEI-VFQ-25 subscales and the total score will be calculated using the "NEI-VFQ-25 Scoring Algorithm – August 2000".[2] Some of the text in this section was copied directly from this document.

The NEI-VFQ-25 consists of 25 questions representing 11 vision-targeted subscales and an additional single general health rating question. Scoring the NEI-VFQ-25 is a two-step process:

1. Step 1: Numeric values from the survey are recoded following the scoring rules shown in table A. All items are scored so that a high score represents better functioning. In this format, scores represent the achieved percentage of the possible total, e.g. a score of 25 represents 25% of the highest possible score.

2. Step 2: Items within each subscale are averaged together. Table B indicates which items contribute to each subdomain.

The subscale scores are then averaged to calculate an overall composite score, excluding the general health rating question (Q1). By averaging subscale scores rather than individual items, equal weight is given to each visual domain, whereas averaging the items would give more weight to scales with more items.

Table A: Recoding of responses (analysis step 1)

Question Number	Change response category	Recoded value
1,3,4,15c	1	100
	2	75
	3	50
	4	25
	5	0
2	1	100
	2	80
	3	60
	4	40
	5	20
	6	0
5,6,7,8,9,10,11, 12, 13, 14, 16, 16a,	1	100
	2	75
	3	50
	4	25
	5	0
	6	*
17, 18, 19, 20, 21, 22, 23, 24, 25	1	0
	2	25
	3	50
	4	75
	5	100

*Response choice "6" indicates that the person does not perform the activity because of non-vision-related problems. If this choice is selected, the item is coded as missing.

Data analysis rules to guide analyses of responses to the driving questions implemented in ASCEND-Eye:

Questions 15c, 16 and 16a are the only questions included in the driving subdomain score. However, participants navigate to Q15c via three screening questions to clarify whether they are currently driving. Where there is missing data or where a nonsensical combination of answers has been given for questions 15 to 16a, ASCEND-Eye will apply the following data analysis rules:

Analyses of Q15c will include those who gave positive responses to both Q15 and Q15c plus negative responses to both Q15a and Q15b. Q15c has four response levels, which are expanded to a 5-level response using Q15b. According to the NEI-VFQ-25 scoring algorithm:

- If 15b = 1, then 15c should be recoded to 0
- If 15b = 2, then 15c should be recoded as missing
- If 15b = 3, then 15c should be recoded as missing.

Questions 16 and 16a may be answered by anyone who has ever driven, including those who are currently driving (as in question 15) and those who have previously driven but gave up (question 15a). Questions 16 and 16a offer a second opportunity to indicate that an individual has stopped driving at night or in adverse weather conditions, respectively, due to visual impairment or other reasons. Therefore, individuals will be included in analyses of questions 16 and 16a if an answer has been given, irrespective of their responses to questions 15, 15b or 15c, but will exclude those who have never driven in response to question 15a.

Averaging of questions to generate NEI-VFQ-25 subscales

Table B: average scores within each subscale

Scale	Number of questions	Questions to be averaged (after recoding)
General health	1	1
General vision	1	2
Ocular pain	2	4, 19
Near activities	3	5, 6, 7
Distance activities	3	8, 9. 14
Vision-specific: social functioning	2	11,13
Vision-specific: mental health	4	3, 21, 22, 25
Vision-specific: role difficulties	2	17, 18
Vision-specific: dependency	3	20, 23, 24
Driving	3	15c, 16, 16a
Colour vision	1	12
Peripheral vision	1	10

Formula:

Mean=(score for each item with a non-missing answer)/total no. of items with non-missing answers

Note: 100 = best possible score, 0 = worst possible score.

Per the analysis algorithm provided by the NEI, items left blank (missing data) are not considered when calculating the scale scores. Subscales with at least one item answered can generate a subscale score. Hence, scores represent the average for all items in the subscale that the respondent answered.

Appendix 23: Post-Adjudication Read Code Categories

Category	Description	Read Code
Sight-threatening eye bleed	-	F6xJx, F6xLx (excluding F6ALx codes), F6xSx (excluding F6ASx codes)
Non-serious eye bleed	-	F6xWN, F6xWU, F6ALx, F6ASx, F6xUN
Other medical retinal or uveal tract diagnosis	Other ophthalmitis	F401
	Retinal/posterior vitreous detachment	F41Z
	Diabetic retinopathy/laser therapy for diabetic retinopathy	F420
	Macular/posterior pole degeneration	F425
	Diabetic macular oedema	F426
	Iritis/iridocyclitis/uveitis	F440
	Glaucoma/surgery for/trabeculectomy	F45
	Retinitis pigmentosa	F7
Eye procedure or investigation	Eye investigation	1B8X
	Eye injection	724
	Eyelid/eyebrow operation	721
	Other eye operation	723
	Cataract operation	7263
	Operation on the vitreous body/vitrectomy	7270
Orbital injury	Eye injury/trauma	SD8
Cranial nerve palsy	-	F32Z, F310
Cerebrovascular event	Amaurosis fugax	F423
	Retinal artery occlusion	G745
	Stroke codes	G60C, G640C,G640A
Other vascular	Retinal vein occlusion	G825
	Giant cell arteritis/temporal arteritis	G755
Other non-serious eye conditions	Conjunctivitis	F4C0
	Eyelid disorder	F4E
	Eye infection	F40
	Visual loss/eyesight deterioration	1B75
	Eye inflammation	F430
	Corneal disorder	F4B
Other	-	-

Appendix 24: Effect of Adjudication on the Categorisation of all Eligible Eye Events

		Post-adjudication categorisation																											
		Sight-threatening eye bleed	Non-serious eye bleed	Retinal or posterior vitreous detachment	Age-related macular degeneration	Diabetic macular oedema	Diabetic retinopathy or laser therapy for	iritis/iridocyclitis/uveitis	Eye inflammation unspecified	Eye infection unspecified	Glaucoma	Retinal vein thrombosis	Retinal artery occlusion or TIA	Giant cell arteritis	Retinitis pigmentosa	Blindness	Visual disturbance	Cataract diagnosis or operation	Laser therapy	Eye injection	Vitrectomy	Eye investigation	Orbital injury	Cranial nerve palsy	Anterior chamber/external eye conditions or surgery	Other non-serious eye events (e.g. conjunctivitis)	Other serious medical conditions	Other non-serious adverse events	**Total**
Pre-adjudicated categories	Any eye bleed	133	255	21	13	85	120	1	7	3	5	37	1	1	1		5	10		4	10	10	1	4	5	7	3	6	**748**
	Retinal detachment	1		58					1								1	1	1		1								**64**
	Age-related macular degeneration	2		1	62	13	2				1					1	4			23		1			1		1		**112**
	Diabetic macular oedema				1	32						1						1		13									**48**
	Blindness															2													**2**
	Visual disturbance		1	3	1	4	1	1						1			8	2	1		1			4	1		1		**30**
	Total	**136**	**256**	**83**	**77**	**134**	**123**	**2**	**8**	**3**	**6**	**38**	**1**	**2**	**1**	**3**	**18**	**14**	**2**	**40**	**12**	**11**	**1**	**8**	**7**	**7**	**5**	**6**	**1004**

Analyses were restricted to events that were fully or partially assessed, where the source of information was the participant's follow-up questionnaire.

Appendix 25: Agreement between Pre-Adjudicated and Adjudicated Reports of All Eligible Eye Events

	Percentage agreement	Cohen's kappa	Interpretation of κ value	95% Confidence Interval	P value
Any eye bleed	63.7%	0.35	Fair	0.29-0.40	$P = 2.2 \times 10^{-16}$
Retinal detachment	96.9%	0.77	Substantial	0.69-0.85	$P = 2.2 \times 10^{-16}$
Age-related macular degeneration	93.5%	0.62	Substantial	0.53-0.71	$P = 2.2 \times 10^{-16}$
Diabetic macular oedema	88.2%	0.30	Fair	0.18-0.42	$P = 7.95 \times 10^{-6}$
Blindness	99.9%	0.80	Substantial	0.41-1.19	P = 0.04
Visual disturbance	96.8%	0.32	Fair	0.09-0.55	P = 0.01

κ values ≤ 0 indicate there was no agreement, 0.01–0.20 none to slight agreement, 0.21–0.40 fair agreement, 0.41– 0.60 moderate agreement, 0.61–0.80 substantia agreement, and 0.81–1.00 almost perfect agreement.
Analyses were restricted to events that were fully or partially assessed, where the source of information was the participants' follow-up questionnaire.

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