

The effect of the magnitude of weight loss on non-alcoholic fatty liver disease: a systematic review and meta-analysis

Dimitrios A Koutoukidis^{1, 2}, Constantinos Koshiaris¹, John A Henry¹, Michaela Noreik^{1, 2}, Elizabeth Morris¹, Indrani Manoharan¹, Kate Tudor^{1, 2}, Emma Bodenham¹, Anna Dunnigan¹, Susan A Jebb^{1, 2}, Paul Aveyard^{1, 2}

¹Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, OX2 6GG UK

²NIHR Oxford Biomedical Research Centre, Oxford, OX2 6GG, UK

Corresponding author: Dr Dimitrios Koutoukidis

Nuffield Department of Primary Care Health Sciences, University of Oxford

Radcliffe Observatory Quarter, Woodstock Road, Oxford, OX2 6GG

E: dimitrios.koutoukidis@phc.ox.ac.uk T: +44 (0)1865 617767

Additional e-mail addresses: constantinos.koshiaris@phc.ox.ac.uk,

john.henry@some.ox.ac.uk, michaela.noreik@phc.ox.ac.uk, elizabeth.morris@phc.ox.ac.uk,

indrani.manoharan@npeu.ox.ac.uk, kate.tudor@psych.ox.ac.uk, e.bodenham1@gmail.com

annadunnigan@hotmail.co.uk, susan.jebb@phc.ox.ac.uk, paul.aveyard@phc.ox.ac.uk

Abbreviations

NAFLD: non-alcoholic fatty liver disease

NASH: non-alcoholic steatohepatitis

ALT: alanine aminotransferase

AST: aspartate transaminase

ALP: alkaline phosphatase

BMI: Body mass index

GGT: gamma-glutamyl transferase

BWLPs: Behavioral weight loss programs

CI: Confidence interval

Abstract

Background

Trials show that weight loss interventions improve biomarkers of non-alcoholic fatty liver disease (NAFLD), but it is unclear if a dose-response relationship exists.

Objective

We aimed to quantify the dose-response relationship between the magnitude of weight loss and improvements in NAFLD.

Methods

Nine databases and trial registries were searched until October 2020. Single-arm, non-randomized comparative, or randomized trials of weight loss interventions (behavioral weight loss programs [BWLPs], pharmacotherapy, or bariatric surgery) in people with NAFLD were eligible for inclusion if they reported an association between changes in weight and changes in blood, radiological, or histological biomarkers of liver disease. The review followed Cochrane methods and the risk of bias was assessed using the Newcastle-Ottawa scale. Pooled unstandardized b coefficients were calculated using random-effect meta-analyses.

Results

Forty-three studies (BWMPs: 26, pharmacotherapy: 9, surgery: 8) with 2,809 participants were included. The median follow-up was 6 (interquartile range: 6) months. The direction of effect was generally consistent but the estimates imprecise. Every 1kg of weight lost was associated with 0.83-unit (95% CI: 0.53 to 1.14, $p < 0.0001$, $I^2 = 92\%$, $n=18$) reduction in alanine aminotransferase (U/L), 0.56-unit (95% CI: 0.32 to 0.79, $p < 0.0001$, $I^2 = 68\%$, $n=11$) reduction in aspartate transaminase (U/L), and 0.77 percentage point (95% CI: 0.51 to 1.03, $p < 0.0001$, $I^2 = 72\%$, $n = 11$) reduction in steatosis assessed by radiology or histology. There was evidence of a dose-response relationship with liver inflammation, ballooning, and resolution of NAFLD or NASH, but limited evidence of a dose-response relationship with fibrosis or NAFLD activity score. On average, the risk of bias for selection and outcome was medium and low, respectively.

Conclusion

57 Clinically significant improvements in NAFLD are achieved even with modest weight loss,
58 but greater weight loss is associated with greater improvements. Embedding support for
59 formal weight loss programs as part of the care pathway for the treatment for NAFLD could
60 reduce the burden of disease.

61 PROSPERO: CRD42018093676

62

63 **Keywords**

64 non-alcoholic fatty liver disease; weight loss; dose-response; meta-analysis; non-alcoholic
65 steatohepatitis

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of diseases ranging from steatosis to steatohepatitis and advanced fibrosis. It affects about 25% of adults worldwide and it is positively associated with morbidity, mortality, and reduced quality of life for individuals, as well as increased economic burden for the society [1-3]. NAFLD is associated with metabolic dysfunction and the metabolic syndrome [4]. About half of the people with NAFLD also have obesity [5], which is a risk factor for both disease onset and progression [6, 7].

Currently, no pharmacological therapy is licensed for the management of NAFLD and finding effective treatment options remains the top research priority in the field [8, 9]. Clinical guidelines recommend weight loss for people with overweight, improvements in diet quality, and increases in physical activity as the cornerstone for the management of NAFLD based on 'moderate' quality evidence [10]. They suggest general advice but rarely and weakly recommend offering formal weight loss programs having judged the evidence as 'low/very low' quality. Aiming to address this gap, a previous meta-analysis of randomized controlled trials of weight loss interventions demonstrated that weight loss is associated with improvements in blood, radiological, and histological biomarkers of liver disease in people with NAFLD [11]. In that review, most interventions led to only small weight losses and the improvement in the biomarkers of liver disease was modest. However, observational evidence from a cohort study with much larger mean intentional weight loss suggested greater benefits [12]. Furthermore, bariatric surgery leads to large improvements in liver disease, but it is unclear whether these benefits are due to greater weight loss or some other effect [13, 14]. If greater weight loss leads to greater improvements, weight loss programs that are more effective should be considered as the first-line option for the treatment of NAFLD.

The aim of this systematic review was to use data from intervention studies in people with NAFLD reporting on the association between weight loss and changes in biomarkers of

liver disease to conduct a meta-analysis to quantify this relationship and explore any differential effects of various weight-loss interventions.

2. Material and methods

The review protocol was prospectively registered (CRD42018093676) and was followed with no changes. The review is in line with the PRISMA reporting guidelines [15] with the checklist available in the appendix.

2.1 Eligibility criteria

Studies investigating interventions aiming to reduce weight in adults with NAFLD were eligible, whether single-arm, non-randomized comparative, or randomized trials. The review was confined to intervention studies, because they were likely to lead to greater mean weight losses than evident in prospective cohorts and weight loss was more likely to be intentional, reducing the likelihood of confounding effects on the liver due to concomitant disease. There is no widely accepted definition for a diagnosis of NAFLD, so we used the diagnostic criteria specified in each study which included but was not limited to NASH. Eligible interventions were those aiming to reduce weight through behavioral weight loss programs (BWLPs), pharmacotherapy licensed for weight loss (or that share a class effect with licensed pharmacotherapy), and bariatric surgery, alone or in combination, irrespective of length or length of follow-up. Studies that tested combinations of any of the above interventions with other interventions (e.g. dietary supplements or other pharmacotherapy) were excluded to eliminate confounding. We included data from comparative trials, using data from more than one intervention group as well as the control group where relevant.

Studies were included if they reported on any biomarker of liver disease, such as blood biomarkers (alanine aminotransferase [ALT], aspartate transaminase [AST], gamma-glutamyltransferase [GGT], alkaline phosphatase [ALP]), radiological biomarkers (steatosis measured by ultrasound, magnetic resonance imaging, or computed tomography), or histological biomarkers (steatosis, inflammation, ballooning, fibrosis, and the NAFLD activity score (NAS score)). Additionally, they had to report on the correlation coefficient or other

analogous measure of association (regression coefficient, odds ratio, or hazard ratio) between the changes in weight and the changes in one or more biomarkers of liver disease in the group(s) that received the weight loss intervention. In order to maximize study inclusion and minimize selection bias, studies reporting on a correlation coefficient between changes in fat mass or BMI and changes in biomarkers were included and the correlation coefficient was adjusted by a factor of 0.619 for fat mass based on data from one study [16] or 0.930 for BMI based on simulated data (details in the appendix). Where trials had more than one intervention group, we included only those analyzing the aforementioned association for the weight loss intervention group(s) or, in the absence of that, using data on the strength of the association from the combined weight loss intervention, lower-intensity weight loss intervention, minimal intervention, or groups with routine care.

2.2 Process

MEDLINE, Embase, PsycINFO, CINAHL, Cochrane Library, Web of Science, and trial registers were searched until 6th October 2020. Systematic reviews and reference lists of the included studies were used as sources of reference. Authors were contacted for additional information where required. The full search strategy is available in the appendix.

An online systematic review management software was used for study selection [17]. Following titles and abstract screening, full texts of potentially eligible studies were screened, and the data from eligible articles as specified in the PROSPERO protocol were extracted using a piloted form. Each step was completed in duplicate by two reviewers and discrepancies were resolved through discussion or referral to a third reviewer.

2.3 Risk of bias (quality) assessment

Two reviewers independently evaluated the risk of bias using the Newcastle Ottawa scale, which was chosen because it is validated for observational studies and all studies were analyzed in an observational manner. The reviewers rated risk of bias as low, medium, or high based on the criteria of selection and outcome (details in the appendix). We

excluded the sub-scale questions on the risk of bias of comparability as not applicable.

Publication bias was assessed by funnel plots.

2.4 Synthesis of results

We estimated an unstandardized b coefficient (with 95% confidence interval) between changes in weight and changes in each biomarker of liver disease for each study assuming a linear relationship between them. Estimates were derived using standard functions [18] and were based on regression or correlation coefficients of change for weight and biomarkers that were heterogeneously reported across studies. We estimated mean baseline and follow-up weight for studies only reporting BMI by using the mean height by sex for the respective country (details in the Appendix) [19]. The unstandardized coefficients were meta-analyzed using random-effects models following the DerSimonian and Laird method [20]. We ran a sensitivity analysis for each model using the Hartung-Knapp method which is a method that produces more robust estimates when the number of studies is small or when substantial heterogeneity is present as well as using the restricted maximum likelihood method [21].

We used meta-regression to estimate the change in biomarkers of liver disease as a function of 1-unit (kg or percentage) change in weight [22]. To do so, we calculated the mean change in weight and mean change in each biomarker for each study. We then weighted these by the weight allocated in meta-regression for each study and calculated the respective weighted means. These two values indicated the coordinates in the axes. Together with the b coefficient as the slope, the coordinates were used to calculate the intercept.

We aimed to investigate the dose-response relationship between weight and each outcome using unstandardized coefficients. To do so, we conducted separate analyses for the studies that reported steatosis on a scale of 0-100 and for those that reported it on a scale of 0-3, 0-4 or 0-6. For the latter, we scaled the estimates from a scale of 0-4 or 0-6 to 0-3 and conducted a sensitivity analysis excluding the studies reporting steatosis on a 0-4

scale (n=2) or 0-6 (n=1). For the dichotomous variables ALT resolution and NAFLD/NASH resolution, data were summarized as odds ratios with confidence intervals. We ran a sensitivity analysis on ALT by excluding studies that had defined NAFLD only by elevated liver enzymes and without radiological or histological assessment. We also ran a sensitivity analysis by the type of the reported correlation (linear (Pearson's correlation coefficient or unstandardized beta) or non-linear (Spearman's rank correlation coefficient)]. We ran two exploratory analyses: (1) by type of intervention to investigate whether the association between change in weight and change in biomarker differed by type of intervention, namely BWLPs, pharmacotherapy, and surgery, and (2), whether it differed by length of follow-up to investigate whether the short-term improvements (up to 6 months) are maintained in the long-term (more than 6 months). We run further post-hoc analyses: (1) by geographic region (2) by mean baseline BMI values grouping the studies based on the WHO obesity classification (3) on ALT only by grouping studies based on the mean baseline ALT (less than or at least 2x upper limit normal, with the cut-off defined as 60U/L), Statistical heterogeneity was measured with the I²-statistic. The consistency and precision of the evidence was evaluated based on the confidence intervals around the point estimates. Publication bias was judged by visual inspection of the funnel plots for ALT and steatosis. Analyses were conducted in RStudio (v 1.1.463) using the R package 'meta' [23].

3. Results

3.1 Study selection and characteristics

The systematic search returned 4,440 entries for screening and 43 studies with a total of 2,809 participants were included in the review (PRISMA diagram in Supplementary Figure S1) [12, 16, 24-46] [additional references in the appendix]. Most studies were conducted in high-income countries with five studies in upper-middle income [12, 34, 41, 45, 46] and three in lower-middle income countries [38, 40, 42]. NAFLD was principally diagnosed by biopsy (n=16), MRI (n=8), computed tomography (n=1), ultrasound (n=16), or liver function tests (n=2).

205 Overall, 44% of participants were female and the average (\pm SD) age and BMI were
206 46.8 (\pm 11.6) years and 32.8 (\pm 8.3) kg/m², respectively. Twelve (28%) studies recruited
207 participants specifically with NASH [12, 24, 31-33, 39-41, 43, 47, 48]. Diabetes and
208 hypertension affected 24% and 35% of participants among 32 and 17 studies, respectively,
209 that reported on these characteristics. Nine studies tested a weight loss medication [31-38,
210 42] and eight a form of bariatric surgery [24-30, 43], with the remaining testing an energy-
211 restricted diet with or without an exercise program (Table 1 and Table S1). The median
212 follow-up was 6 months (IQR: 6, range: 0.5 to 48) with bariatric studies having generally
213 longer follow-up (median: 17 months) than other interventions (median: 6 months). All
214 interventions achieved significant weight loss compared with baseline with greatest weight
215 loss following the surgical interventions (Figure 1).

Table 1 Characteristics of included studies

Study, country	Disease	Total N	Duration, months	Weight loss intervention(s)	Outcome measures
Armstrong 2016 [31] UK	NASH	52	12	Liraglutide, diet, and exercise compared with placebo, diet and exercise	NASH resolution
Khoo 2017 [37] Singapore	NAFLD	30	6	Liraglutide compared with diet and exercise	ALT, steatosis
Cuthbertson 2012 [36] UK	NAFLD	25	6	Liraglutide or exenatide	Steatosis
Kuchay 2020 [42] India	NAFLD	64	6	Dulaglutide and usual care compared with usual care	ALT, AST, GGT, steatosis, liver stiffness
Harrison 2009 [32] USA	NASH	41	9	Orlistat and diet compared with diet	NAS, steatosis, inflammation, ballooning
Hussein 2007 [33] Israel	NASH	14	6	Orlistat and diet	Steatosis, inflammation, fibrosis
Ye 2019 [34] China	NAFLD	130	6	Orlistat compared with diet and exercise	Steatosis
Zelber-Sagi 2006 [35] Israel	NAFLD	52	6	Orlistat, diet, and exercise compared with placebo, diet, and exercise	Steatosis, fibrosis
AliKhan 2017 [38] India	NAFLD	77	4	Group 1: Orlistat and diet Group 2: Diet	Steatosis
Vilar-Gomez 2015 [12] Cuba	NASH	293	12	Diet and exercise	ALT resolution, NAS, steatosis, inflammation, ballooning, fibrosis, NASH resolution
Vilar-Gomez 2009 [41] Cuba	NASH	30	6	Diet and exercise	NAS
Promrat 2010 [39] USA	NASH	31	12	Diet and exercise compared with minimal intervention	ALT, NAS, steatosis
AbdEl-Kader 2016 [47] Saudi Arabia	NASH	100	3	Diet and exercise compared with usual care	ALT, AST
Alam 2019 [40] Bangladesh	NASH	31	12	Diet and exercise	ALT, AST, GGT, steatosis, inflammation, ballooning, fibrosis, NAS, NASH resolution
O'Donnell 2019 USA	NAFLD	24	3	Diet	Steatosis
Thomas 2006 UK	NAFLD	10	6	Diet and exercise	ALT, AST, steatosis
Bugianesi 2005 [49] Italy	NAFLD	27	12	Diet and exercise	ALT, AST
Catalano 2008 [50] Italy	NAFLD	50	6	Diet and exercise	Steatosis
Moscatiello 2011 Italy	NAFLD	150	6 (long-term follow-up: 48)	Group 1: Diet and exercise Group 2: Lower-intensity diet and exercise	ALT, ALT resolution
Abenavoli 2017 [51] Italy	NAFLD	30	6	Diet and exercise compared with usual care	ALT, AST, GGT, steatosis

Study, country	Disease	Total N	Duration, months	Weight loss intervention(s)	Outcome measures
Schweinlin 2018 [52] Germany	NAFLD	36	3	Diet	Steatosis
Deibert 2019 [48] Germany	NASH	22	6	Group 1: Diet Group 2: Diet and exercise	ALT, steatosis
Arslanow 2016 [16] Germany	NAFLD	60	0.5	Diet	Steatosis
Stachowska 2019 [53] Poland	NAFLD	52	6	Diet	Steatosis
Copaci 2015 [45] Romania	NAFLD	86	12	Diet and exercise	Steatosis
Hwang 2004 [54] South Korea	NAFLD	9	2 to 6	Diet and exercise	Steatosis
Park 1995 South Korea	NAFLD	13	12	Diet and exercise	ALT, AST
Jin 2012 South Korea	NAFLD	120	2.5	Diet and exercise	Steatosis
Ishibashi 2009 Japan	NAFLD	75	9	Diet and exercise	ALT, steatosis
Iwasa 2010 [55] Japan	NAFLD	37	6	Diet and exercise	ALT
Suzuki 2005 Japan	NAFLD	348	12	Diet and exercise	ALT, ALT resolution
Scragg 2020 [44] UK	NAFLD	30	9	Diet and exercise	ALT, AST, GGT
Okita 2001 Japan	NAFLD	14	6	Diet	ALT
Wong 2018 Hong Kong	NAFLD	154	12	Diet and exercise compared with minimal intervention	NASH resolution
Rodríguez-Hernández 2011 [46] Mexico	NAFLD	54	6	Group 1: Diet (low-carbohydrate) and exercise Group 2: Diet (low-fat) and exercise	ALT, AST
Bazerbach 2020 [43] USA	NASH	21	6	Intragastric balloon	NAS, fibrosis
Gastaldelli 2009 [29] Italy	NAFLD	70	12	Laparoscopic adjustable gastric band	ALT
Dixon 2006 [28] Australia	NAFLD	60	30 (7 to 76)	Laparoscopic adjustable gastric band	ALT, AST, GGT, steatosis, inflammation, fibrosis
Ooi 2017 [27] Australia	NAFLD	84	12	Laparoscopic adjustable gastric band	ALT resolution
Barker 2006 [24] USA	NASH	19	21	Roux-en-y gastric bypass	Steatosis, inflammation, fibrosis
Pooler 2019 [30] USA	NAFLD	50	6 to 10	Roux-en-y gastric bypass	Steatosis
Algooneh 2016 [26] Kuwait	NAFLD	84	40	Sleeve gastrectomy	NAFLD resolution
Aller 2015 [25] Spain	NAFLD	50	48	Biliopancreatic diversion	ALT, AST
Additional references in the appendix					

3.2 Weight loss and blood biomarkers of liver disease

Reporting of correlations between weight loss and biomarkers of liver disease was diverse across studies regarding both methodology and findings. Every 1kg of weight lost was associated with 0.83-unit (95% CI: 0.53 to 1.14, $p < 0.0001$, $I^2 = 92\%$, $n=18$) reduction in ALT (U/L) and 0.56-unit (95% CI: 0.32 to 0.79, $p < 0.0001$, $I^2 = 68\%$, $n=11$) reduction in AST (U/L) (Figure 2 and Figure 3) based on fairly consistent direction of effect and precise evidence. Three studies showed that a 5% weight loss was associated with a 3-fold increased likelihood of return to normal ALT levels (OR = 3.84, 95% CI: 2.15 to 6.85, $p < 0.0001$, $I^2 = 93\%$) and the evidence was judged as imprecise but of consistent direction (Supplementary Figure S2). The pooled estimate between changes in weight and GGT was not significant ($b = 0.55$, 95% CI: -0.14 to 1.25, $p = 0.12$, $I^2 = 7\%$, $n = 4$) (Supplementary Figure S3).

3.3 Weight loss and radiological or histological biomarkers of liver disease

Every 1kg of weight lost was associated with 0.77 percentage point (95% CI: 0.51 to 1.03, $p < 0.0001$, $I^2 = 72\%$, $n = 11$) reduction in steatosis assessed by histology or MRI, providing fairly consistent direction of effect but imprecise evidence (Figure 4). Precise evidence with consistent direction of effect indicated that 1kg of weight lost was associated with a 0.03-point (95% CI: 0.02 to 0.04, $p < 0.0001$, $I^2 = 77\%$, $n = 12$) reduction of steatosis assessed on a 0-3 scale assessed by histology or ultrasound (Figure 5). Three studies that assessed steatosis could not be included in the meta-analysis, because one study reporting a non-significant b coefficient, assessed steatosis on a scale of 100-400 and two further studies demonstrated significant relationships between weight loss and improvement in steatosis but reported data in the form of either an odds ratio or hazard ratio. Regression lines demonstrating the relationship between weight change and ALT, AST, or steatosis change are shown in Figure 6.

Weight loss was associated with other radiological or histological markers, including liver inflammation ($b = 0.02$, 95% CI: 0.01 to 0.04, $p = 0.0005$, $I^2 = 34\%$, $n = 5$), ballooning (b

= 0.03, 95% CI: 0.02 to 0.03, $p < 0.0001$, $I^2 = 0\%$, $n = 3$), and resolution of NAFLD or NASH (OR = 1.23, 95% CI: 1.03 to 1.47, $p = 0.022$, $I^2 = 93\%$, $n = 5$) based on consistent direction of effect but imprecise evidence. Only one study reported on liver stiffness measured with transient elastography indicating a significant positive correlation [42]. The evidence on improvements in histological liver fibrosis was inconsistent and imprecise ($b = 0.02$, 95% CI: 0.00 to 0.04, $p = 0.037$, $I^2 = 0\%$, $n = 4$). No evidence was found that weight loss was associated with histological reduction in the NAFLD activity score ($b = 0.11$, 95% CI: -0.01 to 0.22, $p = 0.077$, $I^2 = 89\%$, $n = 3$) (Supplementary Figures S4-S8).

3.4 Glucose regulation and biomarkers of liver disease

Eight studies examined the correlation between changes in glucose regulation markers and changes in biomarkers of liver disease. Each of them reported on the correlation between different variables, so a meta-analysis was not possible. Six studies reported no significant correlations [27, 29, 31, 36, 42, 45], one study reported a significant correlation between changes in HbA1c and ALT [37], and another a significant correlation between changes in insulin sensitivity and steatosis [50].

3.5 Adverse events

Six studies examining BWLPs programs reported no adverse events [12, 39, 41, 46, 49, 51, 54, 55] and seven studies examining pharmacotherapy (orlistat, liraglutide, dulaglutide, or exenatide) reported mainly gastrointestinal adverse events [31, 33, 35-38, 42]. One study reported constipation, dizziness, headache, and increased sensitivity to cold during a very low-calorie diet [44]. The remaining studies did not report any information on adverse events (Table S1).

3.6 Exploratory analysis

There was strong evidence of subgroup differences in steatosis by type of intervention, with BLWPs and pharmacotherapy showing larger effects than bariatric surgery

but no clear difference between BLWPs and pharmacotherapy. The magnitude of the association between changes in weight loss and ALT was strongest for pharmacotherapy based on one study and it was stronger for BWLPs than for bariatric surgery. There was evidence of a difference by type of intervention in AST with larger magnitude of effect for pharmacotherapy than BWLPs and bariatric surgery (Supplementary Figures S9-S12).

There was no evidence that the association between changes in weight and ALT was affected by the length of follow-up. The magnitude of the association between changes in weight and AST was stronger at long-term follow-up, whereas the magnitude of the association between changes in weight and steatosis was stronger at short-term follow-up compared with long-term follow-up (Supplementary Figures S13-S16). In a post-hoc analysis by region, results showed larger improvements in liver markers with weight loss for Asian populations, particularly for steatosis measured on the 0-3 scale and ALT (Supplementary Figures S17-S20). Significantly larger improvements in steatosis measured on the 0-100 scale and ALT among people with lower baseline BMI were observed (Supplementary Figures S21-S24). There was no evidence of differential response on ALT by mean baseline ALT level (Supplementary Figure S25).

3.7 Risk of bias within and across studies

All but four studies were ranked as at medium risk of bias for selection mostly because of lack of evidence that the exposed cohort was representative of the population. More than half of the studies were judged at low risk of bias regarding outcomes, as their outcome assessment was robust, the length of follow-up was generally sufficient, and the retention rates were high. No sensitivity analysis based on risk of bias was performed, as only one study was judged at low risk of bias for both criteria (Table S3).

On visual inspection of funnel plots, there was no evidence of asymmetry in the correlations between weight loss and reductions in AST or steatosis. There was evidence of small asymmetry on the funnel plot of ALT, but it was unclear whether this was due to publication bias, as the estimate for one of the two studies at the bottom of the funnel plot

was similar to the average point estimate and excluding the other study from the analysis did not materially change the results (Supplementary Figure S26).

3.8 Sensitivity analyses

A sensitivity analysis on steatosis measured with a 0-3 scale which excluded the three studies measuring steatosis on a 0-4 or 0-6 scale [28, 52, 53] yielded essentially identical results (0.04, 95% CI: 0.03 to 0.06). After excluding two studies that diagnosed NAFLD only based on abnormal liver function tests, the estimate on ALT slightly attenuated but the direction of effect and precision remained unaffected (0.66, 95% CI: 0.34 to 0.97, $p < 0.0001$). There was no evidence of a difference in the estimates by the type of dose-response relationship (linear or non-linear) as per Supplementary Figures S27-S30). After the Hartung-Knapp statistical adjustment, only the ones for ALT, ALT resolution, AST, steatosis, inflammation, and ballooning remained statistically significant, but the estimates remained similar. Using the restricted maximum likelihood method, the estimates did not materially differ from the main analysis (Supplementary Table S3).

4. Discussion

To our knowledge, this is the first systematic review and meta-analysis to investigate the dose-response relationship between weight change and biomarkers of liver disease in people with NAFLD or NASH. There was a dose-response relationship between weight loss and improvements in blood biomarkers, such as ALT and AST, as well as radiological and histological markers, such as steatosis, inflammation, ballooning, and resolution of NAFLD/NASH. There was limited evidence on changes in fibrosis and no evidence on changes in the NAS score.

Our results suggest a minimal clinically meaningful absolute improvement in steatosis of 5% on average could be achieved with an initial weight loss of about 5kg, with each additional 6kg of weight loss associated with 5% additional reduction in steatosis. Similarly, clinically meaningful improvements in ALT of 12 U/L and AST of 7 U/L, on

average, could initially be achieved with about 4kg and 2kg of weight loss, respectively. Typical weight loss programs offered in routine care, such as referral to community weight loss groups, show mean weight losses of this magnitude at one year [56]. Our results showed that bariatric surgery led to the greatest weight loss with proportionally larger benefits for the liver. This was in line with the literature [13, 14] but bariatric surgery appeared to show a weaker dose-response relationship between weight loss and liver outcomes than other types of treatment, which might be due to non-linearity, attenuation of effect with longer term follow-up, or other metabolic or nutritional changes consequent on the surgery. However, further data are needed to verify this as the number of studies examining bariatric surgery was limited and the range of weight loss across studies was small.

The observed dose-response relationship aligns with evidence from pre-clinical data showing that weight loss is associated with dose-response improvements in liver mitochondrial dynamics that cause and propagate NAFLD [57]. Intentional weight loss primarily consists of a loss of subcutaneous and visceral fat, which would be expected to be the principal mechanism for improvements in the liver. A meta-analysis of 89 studies of BWLPs, pharmacotherapy, and bariatric surgery found that loss in visceral and subcutaneous fat was proportional to BMI loss ($r^2 = 0.89$) [58]. It also found no differences in loss of BMI, visceral, or subcutaneous fat between BWLPs and pharmacotherapy. This is in line with our results showing no difference in weight change between pharmacotherapy and BWLPs as well as no clear evidence of differential changes in steatosis between the two types of interventions. Each of the different types of dietary interventions (e.g. low-carbohydrate, Mediterranean, etc.), types of pharmacotherapy, and bariatric procedures, may have some small unique effects on the liver beyond weight loss, albeit further evidence to quantify such effects is needed [59, 60].

Our post-hoc analyses indicated some larger benefits to people of Asian origin. Such differential responses to interventions might be explained by differences in the genetic background, with people of Asian origin having higher propensity to develop visceral

adiposity and NAFLD at lower BMI levels [61]. Higher mean baseline BMI was also associated with smaller improvements in liver markers. This might be because many of the studies in people at lower baseline BMI were also conducted in Asia or because higher BMI is associated with more advanced liver disease where the response to weight loss might be compromised due to more permanent metabolic damage [62]. However, these exploratory observations should be considered preliminary.

The review has several strengths. Our search was limited to interventions intended for weight loss to minimize the risk of confounding from the hepatic effects of unintentional weight loss secondary to onset of disease. Most data are from single-arm interventions, and we followed the Cochrane methods to minimize bias in observational data. By expanding the scope beyond randomized trials, we were able to include studies of bariatric surgery which typically lead to larger weight losses than other interventions and therefore increase the range of the exposure measure. Furthermore, given that there is no ideal biomarker of NAFLD/NASH, the concordance of a dose-response relationship between weight loss and various liver biomarkers minimizes the risk of false positive findings. The review has also some limitations. In order to examine the magnitude of the association, we assumed a linear relationship between changes in weight loss and biomarkers of liver disease. This may not be the case, because reductions in liver markers might plateau after a certain amount of weight loss yet to be defined and because some studies reported Spearman rank coefficients which assume a monotonic rather than a linear relationship. Nonetheless, our sensitivity analysis of linear vs. non-linear estimates showed no evidence of difference. The design of the study, examining within-person changes, eliminates between-person confounding in the association between weight change and biomarker change. Time-varying confounding cannot be eliminated as a cause of the association, but this seems an unlikely explanation. Although some improvements in liver biomarkers could be explained by improvements in glucose and lipid regulation, weight loss causes such improvements and it is plausible that these either directly mediate the association between weight loss and NAFLD activity or are strongly associated with the true mediators. Thus, adjusting for these

effects could over-adjust and bias downwards the association between weight loss and NAFLD activity. However, such a mediation model could not be tested given the nature of the study and no individual studies reported on such a model. We excluded weight loss trials in people with NAFLD that did not report a measure of an association between changes in weight and biomarkers of liver disease and where this could not be obtained from authors. It is possible that such exclusions may materially have altered the result, if these studies had examined for an association, found no association, and failed to report this, but there is no reason to suspect this occurred. We also excluded studies that reported on this association but included participants who did not have NAFLD at baseline. Heterogeneity was generally high but was partly explained in the exploratory analysis by the type of intervention and length of follow-up. Heterogeneity may also have been introduced because studies used a range of methods to report on the association between weight change and liver biomarkers and we converted these to a common metric for pooling. Likewise, steatosis was assessed in various ways and we pooled studies regardless of this, which may account for some heterogeneity. Nevertheless, high heterogeneity in most analyses suggests that the underlying strength of association may vary between populations and requires caution in the interpretation of these data.

These results could empower clinicians to engage in conversations about weight loss with confidence in the benefits that appear to arise from weight loss. Offering patients structured support, such as referring them to available weight loss programs, and encouraging ongoing adherence is more effective than simply advising weight loss [63, 64]. On a larger scale, achieving this provision of weight loss support as a first line treatment for suspected NAFLD based on blood or radiological markers within primary care could potentially reduce the healthcare costs associated with additional tests and referral to secondary care. Given the high prevalence of NAFLD, this would require wider recognition of NAFLD in primary care and adoption of cost-effective weight loss support within the clinical care pathway [56]. Bariatric surgery is likely to offer the largest benefit for individual patients, but surgery is only appropriate for, and acceptable to, a small proportion of patients

[65], and is unlikely to be available at the necessary scale to be a routine treatment for NAFLD. Our analysis showed that BWLPs delivering larger weight losses were associated with greater improvements in markers of NAFLD, so treatment for NAFLD should focus on providing weight loss programs that deliver weight losses >5kg to increase confidence in the likelihood of improvements for individual patients. Total diet replacement programs with behavioral support achieve around 10kg absolute mean weight loss at 1 year, about double the mean effect reported of the BWLPs and pharmacotherapy studies in this review [66, 67]. They have been shown to lead to large reductions in liver fat in people with type 2 diabetes [68] and warrant further investigation as a specific treatment for NAFLD, especially as one of the studies in this review showed this was feasible in NAFLD [44]. Finally, studies need to follow-up participants for longer. We observed that the association between weight loss and improvements in steatosis was weaker at longer follow-up. This may be due to weight regain worsening liver disease, but some data suggest lasting improvements in overall liver histology 5 years after bariatric surgery [14] and in liver fat even after weight regain following dietary weight loss [69]. This requires further investigation.

In conclusion, there is a dose-response relationship between weight loss and improvements in biomarkers of liver disease in people with NAFLD. Clinically significant improvements in liver disease are achieved even with modest weight loss. Embedding support for weight loss as part of the care pathway for the treatment for NAFLD could reduce the burden of disease.

Acknowledgements

We would like to thank Ms. Nia Roberts for creating and running the searches. We also thank all authors of the included studies that provided additional data for the purposes of the review: Dr Shira Zelber-Sagi, Dr Ludovico Abenavoli, Dr Joan Khoo, Dr Giulio Marchesini Reggiani, Dr Dan Cuthbertson, Dr Ayako Suzuki, and Dr Ewa Stachowska.

Funding

This study was funded by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre (grant number: IS-BRC-1215-20008). PA and SAJ are NIHR senior investigators and are also funded by the NIHR Oxford Applied Research Collaboration. EM is funded by a Wellcome Trust Clinical Doctoral Research Fellowship. The funder had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health and Social Care.

Conflicts of interest

DAK, PA, and SAJ report being investigators in a NIHR-funded trial where the weight loss intervention was donated by Nestle Health Sciences to the University of Oxford outside the submitted work. PA has done half a day's consultancy for WW and has also given a talk at an academic conference on weight management in a seminar supported by Novo Nordisk. Both led to payments to the University of Oxford. SAJ spoke at a meeting on digital health hosted by Oviva for which expenses and an honorarium were paid to the University of Oxford. These associations did not lead to payments to them personally. The other authors have declared no competing interests.

Author contributions

Conceptualization: DAK, PA, SAJ. Methodology: DAK, CK, PA, SAJ. Investigation: All authors. Formal analysis: DAK, CK. Writing – Original Draft: DAK. Writing – Review & Editing: All authors. Visualization: DAK, EM. Supervision and funding acquisition: PA, SAJ.

Data statement

All data are either available within the paper and its supporting material or by contacting the primary points of contact for the original data (see Acknowledgements).

Appendix

Appendix: Supplementary material

Figure legends

Figure 1 Weight change in each study and by type of intervention.

Figure 2 Unstandardized b coefficients for weight loss and ALT reduction. B not estimable for two studies. NS/NR: Not significant/not reported.

Figure 3 Unstandardized b coefficients for weight loss and AST reduction. NS/NR: Not significant/not reported, LC: low-carbohydrate diet, LF: low-fat diet

Figure 4 Unstandardized b coefficients for weight loss and steatosis reduction measured in a scale of 0-100. B not estimable in one study.

Figure 5 Unstandardized b coefficients for weight loss and steatosis reduction measured in a scale of 0-3. NS/NR: Not significant/not reported.

Figure 6 The change in (A) ALT (U/L), (B) AST (U/L), and (C) and (D) steatosis (C: scale 0-100, D: scale 0-3 or 0-4) as a function of weight loss (kg). Each circle represents a study and the size of each circle corresponds to the study's relative weight in the meta-analyses of unstandardized b coefficients.

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