

## **Observational analyses from ADVANCE and ADVANCE-ON**

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## **Abstract**

Although the Action in Diabetes and Vascular disease: preterAx and diamicroN-MR Controlled Evaluation (ADVANCE) study was designed as a multicentre randomised factorial placebo-controlled clinical trial, from the outset it was envisaged that the study would be extended to include observational analyses. Thus, a post-randomization follow-up of patients surviving past the end of the clinical trial was planned, to explore legacy effects, and epidemiological studies using the trial data, but treating the study population as an epidemiological cohort, were planned. This article will describe operations and findings from both types of observational analyses. Additionally, we briefly review meta-analyses which ADVANCE has contributed to, and discuss future plans.

### **ADVANCE: Randomised treatment phase**

The Action in Diabetes and Vascular disease: preterAx and diamicroN-MR Controlled Evaluation (ADVANCE) randomised controlled trial had a factorial design which assessed the effects of routine blood pressure lowering with a fixed combination (single pill) of perindopril and indapamide and of more intensive glucose control with a gliclazide (modified release)-based regimen. The blood pressure intervention was associated with a reduction in the primary composite endpoint of major macrovascular or microvascular events and with significant reductions in all-cause and cardiovascular mortality and in nephropathy<sup>1</sup>. The gliclazide-based glucose intervention was also associated with reduced risk of the composite primary outcome, primarily due to a reduction in new or worsening nephropathy<sup>2</sup>. This included a reduction in end-stage renal disease, but not in renal death.<sup>2,3</sup>

### **ADVANCE-ON: Post-trial observational phase**

The first reports of the emergence of lower risk of macrovascular events post-trial, in patients with diabetes, came from the Epidemiology of Diabetes Intervention and Complications (EDIC) study extension of the Diabetes Intervention and Complications Trial (DCCT) in young patients with type 1 diabetes in 2005, which also reported sustained benefit for microvascular complications of randomised treatment.<sup>4</sup> This was followed by reports from the United Kingdom Prospective Diabetes Study (UKPDS) in 2008, which found beneficial effects of more intensive glucose control on vascular events and mortality in patients with type 2 diabetes, but not of more intensive blood pressure control, some ten years after cessation of randomised treatment.<sup>5,6</sup>

Of 11140 randomised patients who participated in ADVANCE, 10,261 and 10,082 were alive at completion of the blood pressure arm and glucose arm respectively, and 8494 of these enrolled in the post-trial follow-up, deemed ADVANCE-ON (Figure 1).<sup>7</sup> The pre-randomisation characteristics of the whole trial cohort and of the subgroup contributing further data during post-trial follow up were similar, as reported in the definitive ADVANCE post-trial observational study report.<sup>7</sup> The median in-trial, post-trial and total follow-up periods were 4.4, 5.9 and 9.9 years respectively for the blood pressure arm and 5.0, 5.4 and 9.9 years for the glucose arm.<sup>7</sup>

The mean difference in blood pressure observed during randomised therapy (5.6/2.2mmHg) were lost within 6 months, as recorded at the final randomised visit for the glucose arm, and remained similar throughout the post-trial period. The mean difference in HbA1c (0.67%) observed during randomised treatment was lost by the first post-trial visit, an average of 2.9

years later (0.08%,  $p=0.29$ ) and remained similar at the end of post-trial follow-up (7.2% vs 7.4%).

#### Major outcomes for ADVANCE-ON

Our two pre-specified primary outcomes for ADVANCE-ON were death from any cause, and major macrovascular events, a composite of cardiovascular death, non-fatal myocardial infarction and non-fatal stroke.<sup>7</sup> Consistent with the in-trial reduction of 14% in all-cause mortality in the blood pressure intervention (HR 0.86;  $p=0.03$ ), there was an attenuated, but significant, cumulative benefit of 9% for all-cause mortality (HR 0.91;  $p=0.03$ ) to the end of overall follow-up (Figure 2). There was no significant cumulative benefit for major macrovascular events with perindopril-indapamide treatment at the end of overall follow-up, with similar, non-significant HR as at the end of the in-trial period.

There were no cumulative benefits of intensive glucose control for all-cause death nor for major macrovascular events, consistent with results at the end of the in-trial phase (Figure 3). Also, there was no evidence that cumulative effects on death from any cause varied between patient subgroups studied for either the blood pressure lowering or the glucose control intervention.<sup>7</sup>

The only significant reduction in a secondary outcome recorded at the end of overall follow-up for the blood pressure intervention was for cardiovascular death (Figure 2), with a cumulative reduction of 12% (HR 0.88;  $p=0.04$ ), compared to the in-trial reduction of 18%.<sup>7</sup> For the glucose control intervention there was no benefit for major clinical microvascular events, but there was a significant cumulative benefit for end-stage renal disease (HR 0.54;  $p=0.007$ ) (Figure 3), although few events were recorded and there was no definite cumulative benefit with respect to renal death.<sup>7</sup>

There was no interaction between the effects of glucose control and blood pressure lowering for any primary or secondary outcome (all P for interaction >0.10). When we examined the post-trial observational period alone, there were no reductions in risk by treatment with perindopril-indapamide or intensive glucose control for any outcome (Supplementary table S8). While the risk of severe hypoglycaemia was low overall, the increase in the intensive versus the standard glucose control group during the trial was no longer evident at the end of post-trial follow-up.

## Discussion

After a total of ten years of in-trial and post-trial follow-up of the ADVANCE cohort, there were significant, but attenuated, reductions in all-cause and cardiovascular mortality, arising from the 4.5 years of active treatment with perindopril-indapamide, generating an average difference in blood pressure of 5.6/2.2mmHg versus the placebo group. In contrast there were no significant benefits in mortality, macrovascular events or microvascular events at the end of overall follow-up, arising from the 5 year period of intensive glucose control with an average difference in HbA1c of 6.7% between the intensive and standard glucose control groups. However, when the pre-specified components of the microvascular outcome were examined, there was a persistent benefit of intensive glucose control for end-stage renal disease, but no new benefits for serious eye complications (Table 1).<sup>7</sup>

The UKPDS post-trial follow-up study reported no persistence of the benefits of the earlier period of tight blood pressure control on macrovascular events or death.<sup>5</sup> While our results appear to differ, the point estimates for the major mortality end-points are similar and consistent with other post-trial observational studies of blood pressure lowering therapy in patients at high

cardiovascular risk.<sup>8-10</sup> Further, comparison of in-trial and post-trial event numbers suggests that the cumulative reductions in mortality seen in the perindopril-indapamide group can largely be ascribed to carry forward of the effects observed during randomised treatment. It seems possible that, with even longer post-trial follow-up, these effects would have become further dissipated, as occurred in the UKPDS. This emphasises the importance of continuing blood pressure lowering medications if the benefits of treatment are to be fully realised.

The DCCT-EDIC and the UKPDS post-trial follow-up studies demonstrated the long term beneficial effects of earlier periods of intensive glucose control with respect to macrovascular events and death.<sup>4,5</sup> We did not observe any such long-term benefits after post-trial follow-up. The original benefits of intensive glucose control in ADVANCE were mainly due to reductions in new or worsening nephropathy, driven by reductions in progression of albuminuria and serious renal disease requiring renal replacement therapy.<sup>2,3</sup> However, we were unable to collect the biochemical measurements required to detect new or worsening nephropathy, except for a minority of the patients who entered post-trial follow-up, and so were limited to certain components such as renal replacement therapy, which did not require biochemical confirmation.

The divergent outcomes compared to some other glucose control studies may be partly ascribed to differences in responses to glucose lowering across trial populations. First, younger patients with type 1 diabetes (as in DCCT-EDIC)<sup>4</sup> or newly diagnosed patients with type 2 diabetes (UKPDS)<sup>5</sup> may have been more likely to have long-term benefits from glucose control than older patients with established disease such as those in ADVANCE-ON. Second, there were differences between studies in the in-trial exposure to HbA1c which differed by only 0.67% over 5 years in ADVANCE but by 2% over a mean of 6.5 years in the DCCT and 0.9% over a

median of 10 years in the UKPDS.<sup>2,4,5</sup> Third, the post-trial follow up of 5 years was shorter in our trial than the average of 10 years in both DCCT-EDIC and UKPDS, and may have been insufficient for benefits to emerge.

### Limitations

Our post-trial follow-up study had many limitations, as fully documented in the original publication in the New England Journal of Medicine.<sup>7</sup> The most important are the fact that only 83% of the original population enrolled in the ADVANCE-ON follow-up study and that we were only able to collect biochemical measurements on a minority of our patients

### Conclusions

In patients with long-standing type 2 diabetes, blood pressure lowering treatment with perindopril-indapamide for an average of 4.5 years provided attenuated but significant long-term benefits for all-cause, and cardiovascular, death, whereas intensive glucose control for an average of 5 years did not provide long term benefits for death or major macrovascular events (Figure 4). There was a persistent benefit of intensive glucose control on end-stage renal disease. These findings highlight the importance of continuing blood pressure lowering therapy and maintaining optimal glucose control in these patients.

### **Epidemiological investigations**

Diabetes has a number of potential causal factors, such as overweight and obesity, and a number of related factors, such as albuminuria. There is considerable interest into the relationships between such factors and adverse outcomes, such as CVD and death, in patients with diabetes. Likewise, although it is clear that diabetes acts as an amplifier of vascular risk, knowledge of exactly how elevated glucose levels lead to vascular pathology remains limited.

A number of haemodynamic and metabolic factors operate in diabetes, which are not being identified through existing methods of risk stratification or targeted by current therapies.

The ADVANCE database has thus been utilised to explore many such classical epidemiological exposure-outcomes associations, with no end in sight at the time of writing. Such analyses ignore the randomised treatments; that is they assume that the treatments received do not have a modifying effect on the association (such as between overweight/obesity and CVD) being analysed. Generally, this is tested in subgroup analyses according to randomized treatment group; that is by adding the treatment x exposure interaction term to the final statistical model relating the exposure to the outcome.

To some extent, these epidemiological studies have been instigated purely due to the specific interests of researchers at the study's centre at the George Institute for Global Health in Sydney. Over the years the team involved has varied, particularly because of some highly productive medium-term visitors. We have been extremely lucky to host several gifted and dedicated researchers, particularly from Japan.

Unsurprisingly, more epidemiological studies in ADVANCE have looked at the key modifiable risk factors for diabetes, including those acted upon by the trial's interventions, blood pressure and glycaemia, than anything else. We have compared different indices of blood pressure, such as pulse pressure and mean arterial blood pressure, as risk predictors, concluding that systolic blood pressure (SBP) was the most appropriate in our study population with diabetes.<sup>11</sup> Two papers<sup>12,13</sup> have looked at blood pressure variability, which appears to be an independent predictor of vascular complications and death. A different type of analysis showed the ill-effects of discontinuing anti-hypertensive medication.<sup>14</sup> As with SBP, visit-to-visit variability



of glucose and HbA1c was found to be an independent risk factor,<sup>15</sup> implying that stability of both SBP and glycaemia will improve outcomes. A novel measure of glycaemia – the haemoglobin glycation index – was found to predict diabetic complications, although with no apparent advantage over HbA1c.<sup>16</sup> Epidemiological analyses of HbA1c as a risk factor in diabetes suggested a nadir in the relationship with key outcomes: we concluded<sup>17</sup> that HbA1c levels: “were associated with lower risks of macrovascular events and death down to a threshold of 7.0% and microvascular events down to a threshold of 6.5%. There was no evidence of lower risks below these levels but neither was there clear evidence of harm.” Another paper showed that severe hypoglycemia was strongly associated with many adverse outcomes, although whether this effect was causal remained in question (Figure 4).<sup>18</sup> An associated project<sup>19</sup> looked at the triad of age, age at diagnosis of diabetes and duration of diabetes as risk factors for vascular diseases and death. After adjustments, all three had an independent significant relationship with macrovascular disease and death, but only duration of diabetes showed such an effect on microvascular events.

Excess adiposity is widely recognised as a leading cause of diabetes. Whether it is a risk factor for the sequelae of diabetes is less well established. Two ADVANCE papers have compared different measures of adiposity to the risk of CVD.<sup>20,21</sup> Waist-to-height was found to have a slight superiority compared to other anthropometric variables, with suggested cut-points for overweight and obesity of 0.55 and 0.6 (Figure 5).<sup>21</sup> A practical advantage of this metric, compared with waist circumference as a measure of central obesity, is that these optimal cutpoints appear to be consistent across sex and other key subgroups, at least amongst people with diabetes. Body mass index (BMI) was also found to be associated with the risk of a major renal event - development of new macroalbuminuria, doubling of creatinine, end stage renal disease, or renal death – above a BMI of 25 kg/m<sup>2</sup> there was an increasing risk with increasing

BMI.<sup>22</sup> We have also considered the hypothesis that intensive glucose control increases weight using the ADVANCE data.<sup>23</sup> Although patients in the control group decreased weight, those in the intensive group retained a stable weight, with a small mean increase of 0.16 kg during the five years of the trial. Insulin and thiazolidinediones were found to have the greatest adverse effects on weight.

The randomized results in ADVANCE were strongest for renal disease. Five ADVANCE papers, to date, have analysed the effects of increased urinary albumin:creatinine ratio (ACR) and/or estimated glomerular filtration rate (eGFR) on adverse outcomes. We found ACR and eGFR to each have an independent effect on CVD and renal events, without any evidence of interaction.<sup>24</sup> Furthermore, change in both these renal markers was associated with increased risk of clinical outcomes,<sup>25-28</sup> after adjustment for their baseline values and potential confounding variables, with some evidence of a synergistic effect<sup>28</sup> – allowance for regression dilution attenuated results, but did not completely remove the associations.

Other phenotypic variables that have been analysed within the ADVANCE data include history of microvascular and macrovascular disease,<sup>29</sup> history of oral disease,<sup>30,31</sup> alcohol,<sup>32,33</sup> erectile dysfunction,<sup>34</sup> heart rate,<sup>35,36</sup> HDL-cholesterol,<sup>37,38</sup> physical activity<sup>39</sup> and level of education.<sup>40</sup> These analyses have mostly related the risk factor to components of the primary outcome in ADVANCE, although some ADVANCE publications have addressed cancer,<sup>38,41</sup> cognition and dementia (Figure 6)<sup>31,42</sup> and peripheral vascular disease.<sup>43-45</sup>

Another theme within the ADVANCE publications is that of health economics. Cost of treatment has been assessed in two papers<sup>46,47</sup> and quality of life in another two.<sup>48,49</sup>

### *Risk prediction*

Although blood pressure and glucose control are key strategies in preventing the consequences of diabetes, these do not completely account for risk, suggesting that other processes are at work. While all adult patients with DM have an increased likelihood of vascular complications, and are sometimes regarded as having an equivalent risk to those with documented coronary heart disease, there remains a spectrum of risk. Likewise, while intensive multi-factorial interventions can reduce the complications of the condition, the costs of such strategies exceed the resources of even the most affluent healthcare systems: particularly in the light of ever increasing numbers of diabetic patients. There is, therefore, a need to identify those at highest risk. Unfortunately, existing methods of risk-prediction, although useful, are imperfect. Macrovascular complications, such as myocardial infarction (MI) and stroke, play a pivotal role in determining the prognosis of diabetic patients and should, therefore, be a major focus of any outcome study. However, methods of predicting microvascular complications of DM, such as nephropathy, have been much less extensively studied. Given the high risk of progression to end stage renal failure and premature death in patients who develop diabetic nephropathy, as well as the strong emerging evidence of a substantial interaction between cardiovascular risk and even minor renal dysfunction, evaluating new risk markers for this outcome is also critical.

As a means for exploring these additional risk factors, the ADVANCE data have been used to create four different, sometimes related, risk scores<sup>50-52</sup> for application to patients with diabetes. In each case, the basic aim was to find which risk factors, from a pre-defined set decided upon by members of the ADVANCE collaborative team, were independent predictors of poor outcomes. A risk score was then developed to find the best linear combination of these risk factors to predict the outcome, from fitting Cox proportional hazards models.<sup>53</sup> The resultant

score was evaluated according to its ability to discriminate between those who did and did not develop the outcome during the study, and its calibration, meaning how well observed (yes/no) and predicted risks agree.<sup>53</sup> Discrimination was measured by the concordance statistic (generally called the c-statistic), and calibration was explored through the calibration plot;<sup>53</sup> for an example, see Figure 7.

#### Cardiovascular risk score

The first of the risk scores was developed to predict major CVD events,<sup>50</sup> having first shown that existing risk scores, derived from the UKPDS and Framingham Heart Study, underperformed when applied to ADVANCE subjects.<sup>54</sup> The risk factors included in the score were age at diagnosis of diabetes, duration of diabetes, pulse pressure, use of anti-hypertensives, urinary ACR, HbA1c, non-HDL cholesterol, retinopathy and atrial fibrillation. The c-statistic, when the score was applied in an external population, was moderate, 0.685 (95% confidence interval (CI), 0.646–0.724), and calibration was good.

#### Renal risk scores

ADVANCE risk scores were developed for early and late events in diabetic nephropathy.<sup>51</sup> The former was defined as the development of new-onset albuminuria (urinary ACR  $\geq 30$   $\mu\text{g}/\text{mg}$ ), confirmed by two positive results, in participants with urinary ACR  $< 30$   $\mu\text{g}/\text{mg}$  at baseline. The risk factors included were eGFR, ACR, SBP, HbA1c, diabetic retinopathy, use of anti-hypertensives, Asian ethnicity and waist circumference. The c-statistic, internally validated, was 0.647 (95%CI: 0.637-0.658) and calibration was good. Late events were defined as doubling of serum creatinine level to  $\geq 200$   $\mu\text{mol}/\text{L}$ , requirement for renal replacement therapy or death due to kidney disease. Factors in the score for late-onset disease were eGFR, urinary ACR, SBP, HbA1c, diabetic retinopathy, sex, and educational attainment.

Discrimination was good, c-statistic = 0.847 (95%CI: 0.815-0.880), as was calibration, albeit without external validation.

### Vascular risk score

On the whole, the risk of CVD and renal disease depend on common risk factors, whilst it is confusing to clinicians and patients alike to have different risk scores applied to different diseases. Thus a risk score was developed for any vascular event, defined as macrovascular disease or a renal disease.<sup>52</sup> By the time this work was started, ADVANCE-ON was complete and so, unlike the other scores,<sup>50-51</sup> this score used the ADVANCE-ON 10-year follow-up. To reflect this, the resulting risk score was called the AD-ON risk score. Also, the issue of calibration was approached in a different way to the other risk scores,<sup>50,51</sup> with a view to enabling use of the score internationally. In essence, this meant developing the vascular risk score just as for the other risk scores, but then applying a multiplicative conversion factor depending on the geographical region from whence the patient came. This addresses the standard problem in calibration, that the background (overall) risk of vascular disease varies with the jurisdiction (geographical and health system), as well as over time in the same jurisdiction.<sup>55</sup> Even when using the ADVANCE vascular risk score in regions outside those represented by subjects in ADVANCE, the user can choose her or his own conversion factor –using their own judgement to decide on how close they believe background risk is to the regions in ADVANCE, for which conversion factors are published.<sup>63</sup> Risk factors included were age, sex, SBP with and without use of antihypertensives, duration of diabetes, HbA1c, urinary ACR, eGFR and its square, age at completion of formal education, exercise, diabetic retinopathy, atrial fibrillation, and interactions between sex and eGFR, its square and age at final education. Once again, internally-validated discrimination was modest, with a c-statistic of 0.668 (95% CI: 0.651, 0.685), but calibration was excellent (Figure 7).

### *Blood-based substudies*

The ADVANCE risk scores, although useful for clinical practice, are limited by the factors available for analysis from ADVANCE. This is reflected in their sub-optimal discrimination. There is, thus, a need to identify additional factors that might, in conjunction with existing models and clinical factors, improve risk-stratification. Study of novel biomarkers, particularly those which explore hitherto poorly defined but biologically plausible mechanisms of vascular damage, may not only improve our understanding of the pathogenesis of diabetic complications (and thereby suggest new means to ameliorate them) but also clarify whether these markers can improve our current risk prediction models.

All participants in ADVANCE provided consent to the collection and storage of blood samples and their use for research purposes. Besides the blood, collected at repeated clinic visits, used to measure traditional risk factors, such as HbA1c, glucose, standard lipids and creatinine, some blood was put aside for long-term storage with a view to future epidemiological analyses of novel biomarkers. In principle, 20ml of whole blood was put aside for storage from every patient at the date of registration. Additionally, the same amount was stored for a random sample of 1000 patients at the one-year clinic visit. This was in order to account for regression dilution when analysing associations,<sup>56</sup> although it is also useful for assessing changes in biomarkers according to the randomized intervention.<sup>57</sup>

The 20ml collected was split into two tubes, one with an EDTA additive and one with heparin. The specimens were spun for 10 minutes in a centrifuge and then the plasma and buffy coat layers were immediately pipetted into microtubes. Three of these were filled with plasma and

one with buffy coat, for each of EDTA and heparin additives. The four types of microtube were distinguished by differently coloured caps, and labelled with a patient identifier. Samples were boxed and stored locally in freezers, ideally at  $-70^{\circ}\text{C}$ , and at least  $-20^{\circ}\text{C}$ . Subsequently, the frozen samples were transported to the central coordinating centre in Sydney, Australia for long-term storage. Although it was originally planned that all bloods would be collated for analysis, of the 20 countries represented by ADVANCE, China and India did not allow their blood samples to leave the country. Hence, all the blood-based analyses use only the data from the remaining 18 countries. After several years in storage, parts of the plasma samples were sent to specialist laboratories, in Finland, Scotland, the USA and elsewhere in Australia, for quantification of novel biomarkers. Buffy coats were shipped to the central genomics laboratory at the University of Montreal, Canada.

Three separate blood sample substudies were designed. The first of these was a simple case-control study (with 281 cases of major renal end points and 562 Event matched controls) which found that circulating bone morphogenetic protein-7 and transforming growth factor- $\beta$ 1 were significant risk factors for renal disease.<sup>58</sup>

The second blood substudy was a more comprehensive case-cohort study, making more efficient use of the bloods, and with a stronger statistical design.<sup>53</sup> Three case series were included: deaths, major macrovascular disease and major microvascular disease. Cases were all the constituent events observed during follow-up in the ADVANCE trial. Major macrovascular events were cardiovascular death, non-fatal MI and non-fatal stroke. Major microvascular events were major renal events (death due to renal disease, need for renal replacement therapy, development of macroalbuminuria (ACR ratio  $>300\mu\text{g}/\text{mg}$ ) or doubling of serum creatinine to at least  $200\mu\text{mol}/\text{L}$  and retinopathy (development of proliferative

retinopathy, macular edema, diabetes-related blindness and retinal photocoagulation therapy). Events were adjudicated by an independent, blinded outcomes committee, using source documentation. Cause of death was ascribed using all available clinical information, including hospital records, correspondence, death certificates and autopsy reports. The case-cohort study population consisted of a random sample of 3500 plus any cases (of the three types) outside this subcohort, from amongst ADVANCE participants from the 18 participating countries. After excluding patients with incomplete or missing bloods, for initial analyses<sup>59-61</sup> there were 706 who died, 709 with major macrovascular (first) events, 439 with major microvascular (first) events and 2502 who had none of these three outcomes. As blood samples were used up, progressively fewer patients were included in subsequent analyses, although the attenuation was not dramatic.

Using traditional assay methods, measures of inflammation and cardiac stress<sup>59-61</sup> were derived from the plasma samples and analysed as risk factors for one, or more, of the three case outcomes, as well as heart failure.<sup>62</sup> Figure 8 shows that N-terminal pro-B-type natriuretic peptide and high-sensitivity cardiac troponin T were independent predictors of all three outcomes, as was interleukin-6 except for microvascular disease. Other assays have produced results for Advanced Glycation End-products (AGEs).<sup>63,64</sup> The case-cohort bloods have also been analysed using lipidomic<sup>65</sup> and proteomic<sup>66</sup> platforms.

The final blood substudy used a cohort study design, comprising all subjects from ADVANCE with remaining stored samples, and analyses of both baseline and 1-year blood samples. As this study started after ADVANCE-ON terminated, case numbers were enhanced compared to the earlier blood substudies. To date, only one paper has appeared from this substudy; this paper considered both the impact of the blood pressure and glucose interventions on 1,5-



anhydroglucitol and the effect of 1,5-anhydroglucitol on CVD outcomes.<sup>57</sup> Similar papers, with such dual aims, are planned.

## Genomics

Several ADVANCE analyses have addressed genotypic associations with raised blood pressure<sup>67-70</sup> or kidney disease.<sup>71,72</sup> We have also identified the PROX1 gene CC genotype to be related to early-onset of diabetes. Since, as discussed earlier, the discrimination of the existing ADVANCE risk scores is disappointing, except for the score predicting major kidney outcomes,<sup>51</sup> we are investigating the use of genetic factors, either alone or in combination with phenotypic information, in risk prediction. Genetic risk scores have the great advantage of being able to quantify risk at a very early stage, before risky activities become established.

## Meta-analyses

Due to the paucity of large-scale studies in diabetes and both the high-quality of data and the broad coverage of its study population, we frequently receive requests to collaborate with others. In some cases, this has led to ADVANCE being included in meta-analyses of both clinical trials<sup>73,74</sup> and observational analyses.<sup>75-77</sup> The most productive of these has been the Chronic Kidney Disease-Prognosis Consortium, based at Johns Hopkins University, USA. Data from ADVANCE, and our past experiences with their analyses, have made a key contribution to the Consortium's enormous success in producing reliable estimates of the effects of progressive kidney disease on CVD and death and establishing new criteria for kidney disease classification.<sup>78</sup>

## Summary

Although feted as a seminal randomised controlled trial in diabetes, ADVANCE has contributed much to observational research in the field of diabetes. ADVANCE-ON has established legacy benefits, post-trial, from the blood pressure intervention for mortality (particularly, cardiovascular mortality) and from intensive glucose control for end-stage renal disease. Some epidemiological analyses from ADVANCE have quantified the effect of some non-traditional risk factors, and longitudinal changes or variation in some established risk factors, on CVD and death. Others have established several biomarkers as independent risk factors for vascular and mortal outcomes, in patients with diabetes. Furthermore, ADVANCE has made important contributions to international large-scale meta-analyses, which have informed clinical guidelines.

This report has summarised our contributions to observational analyses to date, but much is yet to come, particularly when we make use of existing blood samples, and analyse the remaining results from assay, genomic, lipidomic and proteomic substudies. Another important area of on-going research is sex differences in the effects of risk factors in the diabetes population of ADVANCE.<sup>79</sup> With an enrolment of 43% women, ADVANCE has a greater proportion of women than in comparable clinical trials. Throughout its existence, ADVANCE has remained nimble and ready to respond to new developments in medicine, particularly relating to novel biomarkers, and to new collaborations. It remains a truly exciting project.

## **Funding**

National Health and Medical Research Council (NHMRC) Australia project grants 211086, APP1006367 and 632507; Program Grants 358395, 571281, APP1052555 and APP1149987; and Fellowships (MW) APP1020812 and APP1080206. National Institutes of Health (USA)

grant R01DK108784, and unrestricted educational grants from Servier for ADVANCE and ADVANCE-ON.

### **Conflict of Interest**

Mark Woodward reports consultancy fees from Amgen and Kirin. John Chalmers received research grants from the NHMRC and from Servier for the ADVANCE trial and the ADVANCE-ON post-trial follow-up study.

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## Figure Legends

1. Time chart showing study times (median years, where appropriate) and calendar dates of significant occasions in ADVANCE-ON.
2. Cumulative incidence of events in ADVANCE-ON for the blood pressure arm of the trial. Shown is the percentage of patients who had events at any time after the start of randomized treatment in ADVANCE, according to assignment to the active-drug (perindopril–indapamide) group or the placebo group. Cumulative hazard ratios (active-drug group vs. placebo group) and P values are shown. The insets in Panels A and C (which show outcomes that were reduced significantly with the active drug) display the same data on an enlarged y axis. Reproduced with permission from New England Journal of Medicine 2014;371:1392-406.<sup>7</sup> Copyright © 2014 Massachusetts Medical Society.
3. Cumulative incidence of events in ADVANCE-ON for the glucose arm of the trial. Shown is the percentage of patients who had events at any time after the start of randomized treatment in ADVANCE, according to assignment to the intense glucose control or standard control group. Cumulative hazard ratios (intensive vs. standard group) and P values are shown. The inset in Panel E (which shows an outcome that was reduced significantly with intensive control) displays the same data on an enlarged y axis. Figure from Zoungas et al (2014) Reproduced with permission from New England Journal of Medicine 2014;371:1392-406.<sup>7</sup> Copyright © 2014 Massachusetts Medical Society.
4. Association of severe hypoglycemia with the risk of an adverse clinical outcome or death in ADVANCE. The hazard ratio represents the risk of an adverse clinical outcome or death among patients reporting severe hypoglycaemia, compared with those not reporting severe hypoglycemia. The centres of the squares are placed at the point estimates, and the horizontal lines represent the corresponding 95% confidence

intervals. The area of each square is proportional to the inverse of the variance of each estimate. The estimates were adjusted for baseline values of age, sex, randomised treatment assignment, duration of diabetes, presence or absence of a history of macrovascular disease, presence or absence of a history of microvascular disease and smoking status, and for the time-dependent values of use of sulfonylurea, metformin, thiazolidinedione, insulin, and any diabetes drug and the use of antihypertensive therapy, glycated haemoglobin, body mass index, creatinine, urinary albumin to creatinine ratio and systolic blood pressure. Reproduced with permission from New England Journal of Medicine 2010;363:1410-8.<sup>17</sup> Copyright © 2010 Massachusetts Medical Society.

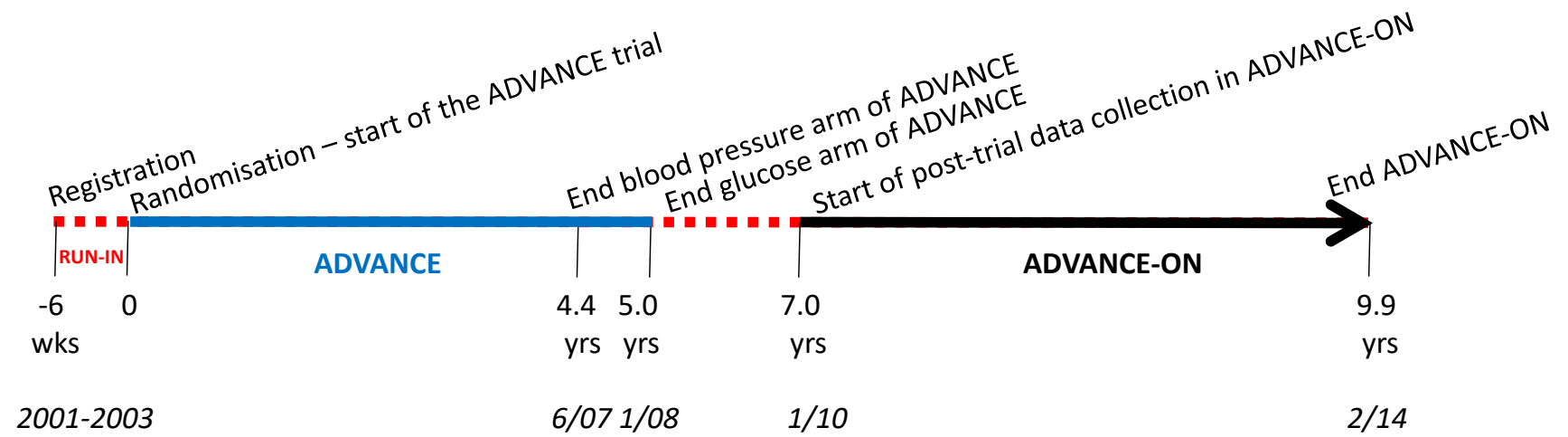
5. Hazard ratios (95% confidence intervals) per one standard deviation higher body mass index, waist hip ratio, waist circumference and waist to height ratio, respectively in ADVANCE-ON. Analyses are adjusted for age, sex, current smoking, Asian region and randomised treatment allocations. The Akaike Information Criterion (AIC) measures goodness-of-fit with smaller numbers suggesting better fit. Figure is as appears in Radholm et al (2018).<sup>21</sup>
6. Hazard ratios (95% confidence intervals) for dementia and cognitive decline by number of natural teeth. Analyses are adjusted for baseline comorbidities, diabetes duration, smoking, alcohol, vigorous physical activity, HbA1c, creatinine, body mass index, total cholesterol, HDL cholesterol, resting heart rate, blood pressure, quality of life (EQ-5d score), Mini Mental State Exam Score, age at completion of highest level of education, height, randomised treatment allocations and ethnicity. Data derived from Batty et al (2013).<sup>31</sup>

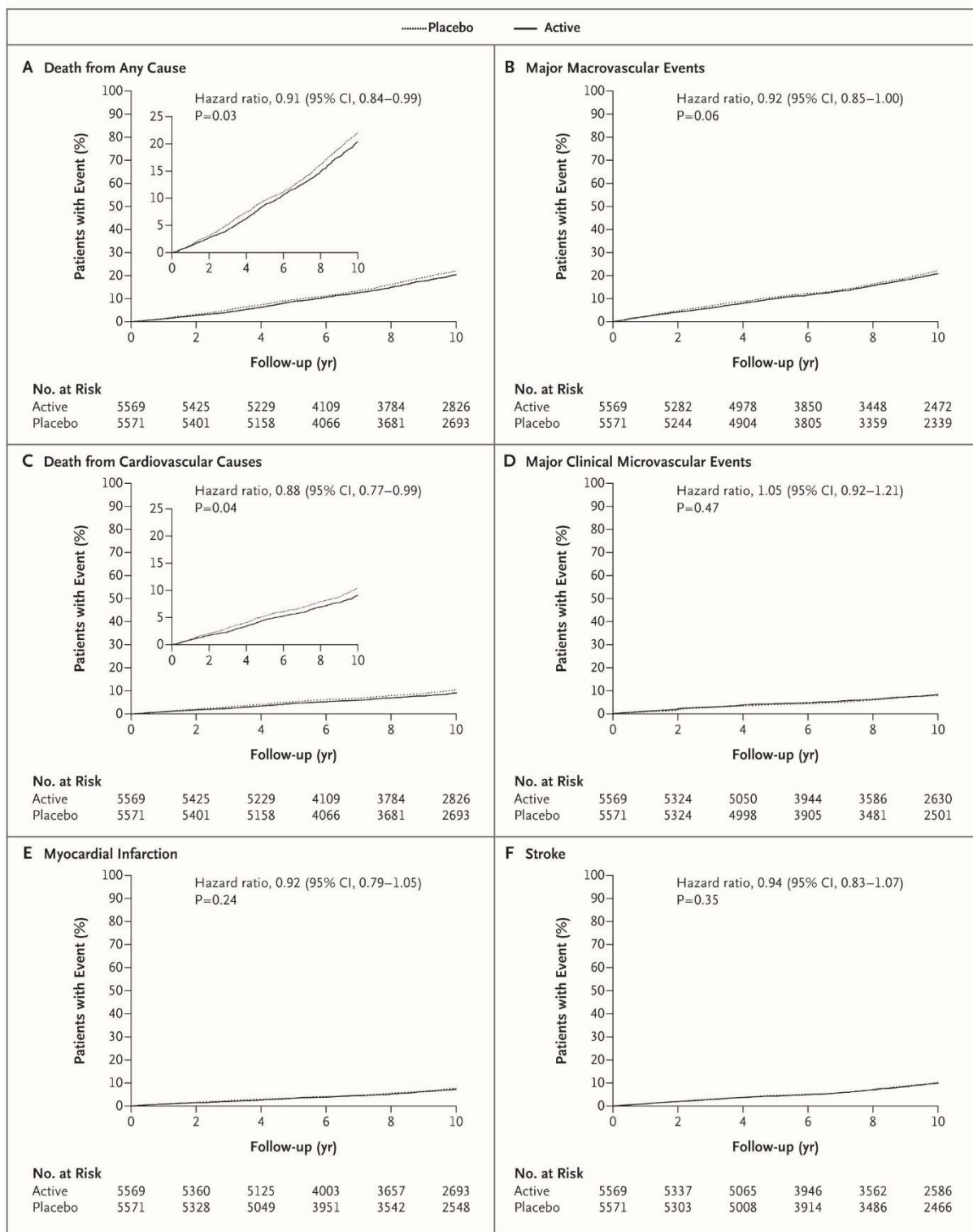
7. Region-specific calibration plot for the AD-ON vascular risk score. EMEs, established market economies; EE, Eastern Europe. Figure is as appears in Woodward et al (2016).<sup>52</sup>
8. Hazard ratios (95% confidence intervals) for N-terminal pro-B-type natriuretic peptide, high-sensitivity cardiac troponin T, C-reactive protein, fibrinogen and interleukin-6 related to major macrovascular disease, major microvascular disease and death in the ADVANCE case-cohort study. Data derived from Hillis et al (2014),<sup>59</sup> Lowe et al (2014)<sup>60</sup> and Welsh et al (2014).<sup>61</sup>
9. Mean (with standard error) of 1,5-anhydroglucitol (1,5-AG) at baseline and after one year, in A, glucose treatment arm and B, blood pressure treatment arm in ADVANCE. Figure is as appears in Selvin et al (2019).<sup>57</sup>

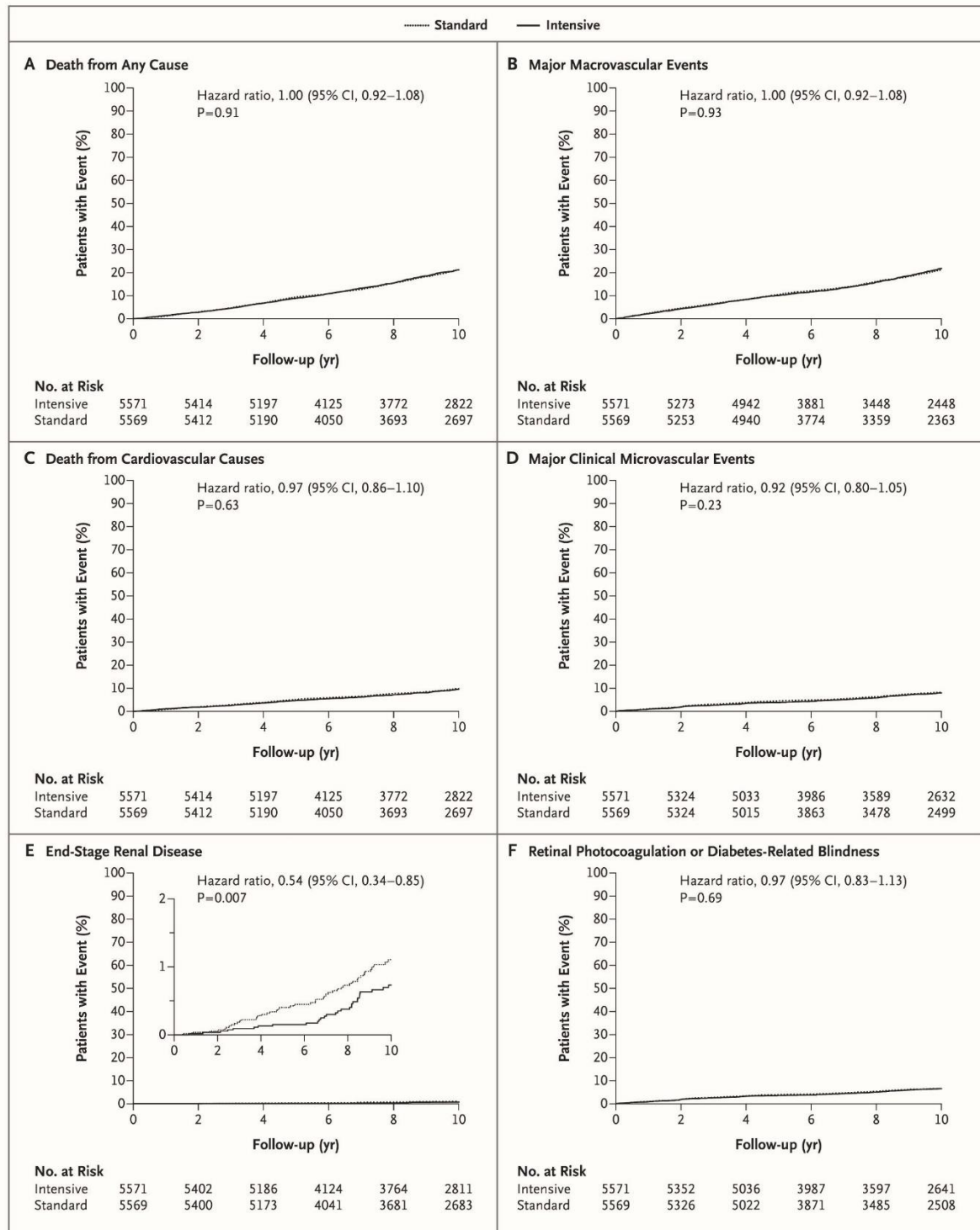
Table 1: Hazard ratios (95% confidence intervals) during the randomised trial (ADVANCE) and overall (ADVANCE plus ADVANCE-ON) for the blood pressure arm (active versus placebo) and the glucose arm (intensive versus control).

	Blood pressure arm		Glucose arm	
	In-Trial	Overall	In-Trial	Overall
All-cause mortality	0.86 (0.75, 0.98), p=0.03	0.91 (0.84, 0.99), p=0.03	0.93 (0.83, 1.06), p=0.28	1.00 (0.92, 1.08), p=0.91
Major macrovascular events	0.92 (0.81, 1.04), p=0.16	0.92 (0.85, 1.00), p=0.06	0.94 (0.84, 1.06), p=0.32	1.00 (0.92, 1.08), p=0.93
Cardiovascular death	0.82 (0.68, 0.98), p=0.03	0.88 (0.77, 0.99), p=0.04	0.88 (0.74, 1.04), p=0.12	0.97 (0.86, 1.10), p=0.63
Myocardial infarction	0.95 (0.77, 1.18), p=0.65	0.92 (0.79, 1.06), p=0.24	1.01 (0.83, 1.24), p=0.92	1.02 (0.89, 1.19), p=0.75
Stroke	0.97 (0.80, 1.17), p=0.73	0.94 (0.83, 1.07), p=0.35	0.96 (0.81, 1.15), p=0.68	1.01 (0.89, 1.15), p=0.82
Major microvascular events	1.12 (0.92, 1.36), p=0.26	1.05 (0.92, 1.21), p=0.47	0.86 (0.72, 1.03), p=0.11	0.92 (0.80, 1.05), p=0.23
End-stage renal disease	1.61 (0.67, 3.89), p=0.29	1.08 (0.70, 1.66), p=0.74	0.35 (0.15, 0.83), p=0.02	0.54 (0.34, 0.85), p<0.01
Renal death	1.14 (0.54, 2.40), p=0.72	1.04 (0.70, 1.53), p=0.86	0.85 (0.45, 1.62), p=0.62	0.89 (0.60, 1.31), p=0.56
Retinal photocoagulation or diabetes-related blindness	1.11 (0.91, 1.37), p=0.31	1.07 (0.92, 1.25), p=0.35	0.90 (0.74, 1.09), p=0.29	0.97 (0.83, 1.13), p=0.69

Fig 1









Events	Severe Hypoglycemia (N=231) <i>no. of patients with events (%)</i>	No Severe Hypoglycemia (N=10,909) <i>no. of patients with events (%)</i>	Hazard Ratio (95% CI)	
Major macrovascular events	33 (15.9)	1114 (10.2)		
Unadjusted model				4.05 (2.86–5.74)
Adjusted model				3.53 (2.41–5.17)
Major microvascular events	24 (11.5)	1107 (10.1)		
Unadjusted model				2.39 (1.60–3.59)
Adjusted model				2.19 (1.40–3.45)
Death from any cause	45 (19.5)	986 (9.0)		
Unadjusted model				4.86 (3.60–6.57)
Adjusted model				3.27 (2.29–4.65)
Cardiovascular disease	22 (9.5)	520 (4.8)		
Unadjusted model				4.87 (3.17–7.49)
Adjusted model				3.79 (2.36–6.08)
Noncardiovascular disease	23 (10.0)	466 (4.3)		
Unadjusted model				4.82 (3.16–7.35)
Adjusted model				2.80 (1.64–4.79)
Respiratory system events	18 (8.5)	656 (6.0)		
Unadjusted model				3.23 (2.02–5.17)
Adjusted model				2.46 (1.43–4.23)
Digestive system events	20 (9.6)	867 (7.9)		
Unadjusted model				2.97 (1.90–4.63)
Adjusted model				2.20 (1.31–3.72)
Diseases of the skin	6 (2.7)	146 (1.3)		
Unadjusted model				5.02 (2.20–11.40)
Adjusted model				4.73 (1.96–11.40)
Cancer	5 (2.2)	149 (1.4)		
Unadjusted model				3.44 (1.40–8.42)
Adjusted model				2.11 (0.65–6.82)

0.1 1.0 10.0

Fig 5

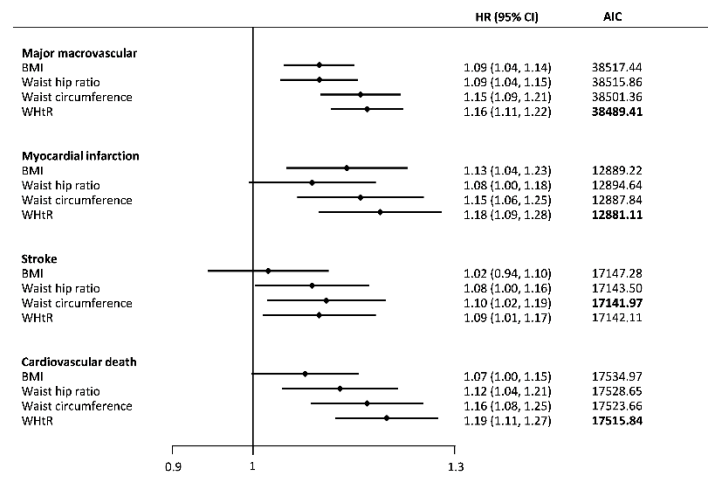


Fig 6

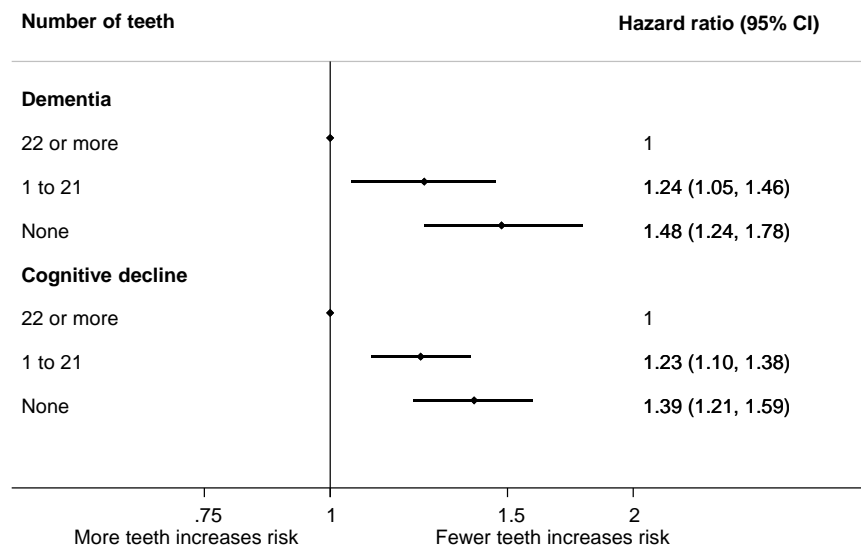


Fig 7

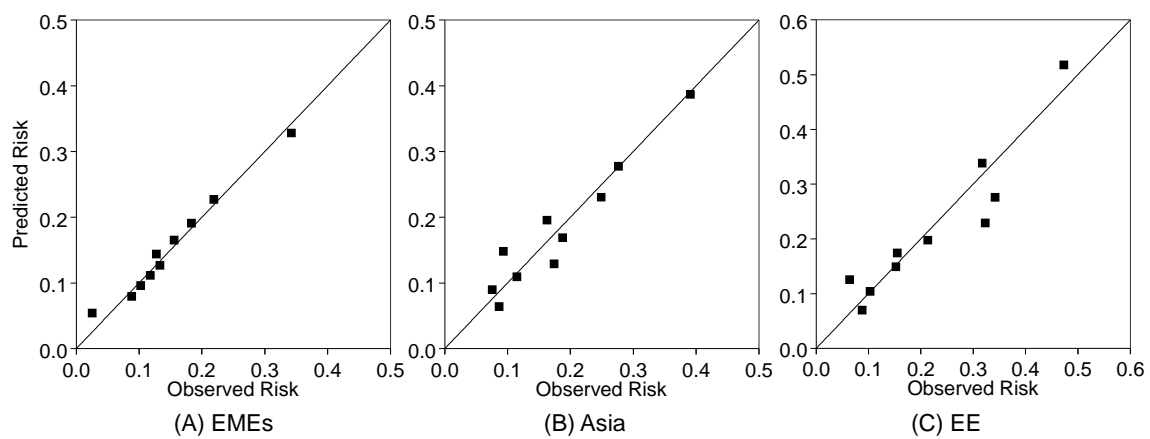


Fig 8

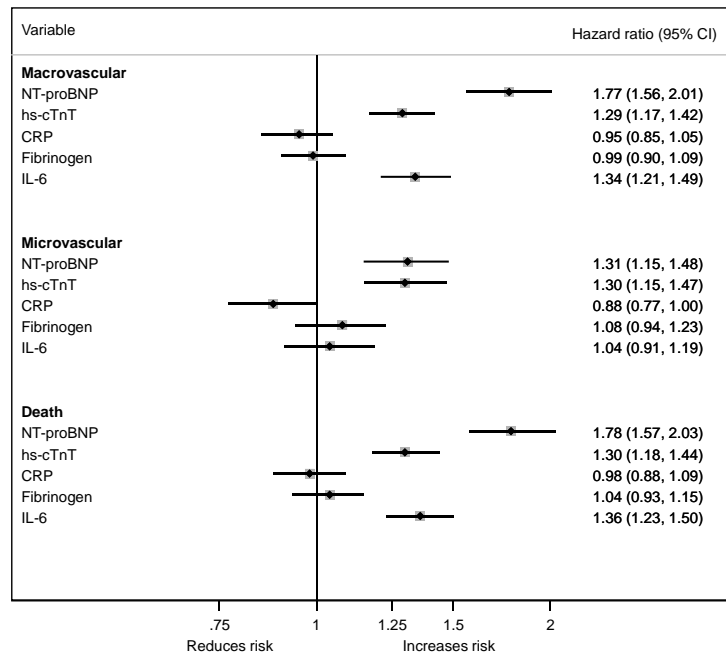


Fig 9

