ASCEND (A Study of Cardiovascular Events iN Diabetes):
A randomised 2x2 factorial design study of aspirin versus placebo, and of omega-3 fatty acid supplementation versus placebo, for the primary prevention of cardiovascular events in people with diabetes

Should aspirin be used routinely in people with diabetes but no vascular disease?

The role of antiplatelet therapy (chiefly aspirin) for the *secondary* prevention of cardiovascular disease is firmly established for many high-risk groups with diagnosed occlusive arterial disease, and the proportional reductions in heart attacks and strokes appear to be similar whether or not these patients have diabetes. But, most younger and middle-aged people with diabetes do not have manifest arterial disease – although they are still at significant cardiovascular risk – and yet the available randomised evidence for the use of antiplatelet therapy in such individuals is sparse. As a result, there is major uncertainty about the role of antiplatelet therapy for the *primary* prevention of cardiovascular events among people with diabetes, and only a small minority receives it.

ASCEND aims to demonstrate whether aspirin reduces the risk of cardiovascular events in individuals with diabetes who do not already have diagnosed occlusive arterial disease, and whether such benefits outweigh any potential hazards from bleeding. In order to do this reliably, at least 15,000 patients with diabetes and no clinical evidence of occlusive arterial disease will be randomly allocated to receive 100mg aspirin daily or matching placebo tablets for at least 7 years. A study of this size should have excellent power to detect a 12-15% proportional reduction in the cardiovascular event rate among such patients.

Do omega-3 fatty acids (fish oils) reduce cardiovascular risk in people with diabetes?

There is consistent evidence from observational studies of lower rates of cardiovascular disease (particularly cardiac and sudden death) in people with higher intakes, or higher blood levels, of omega-3 fatty acids (FA). Randomised evidence among people who have survived a heart attack suggests modest, but potentially worthwhile, reductions in coronary events of 15-20%. There is, however, no large-scale randomised evidence for the use of omega-3 fatty acids in the primary prevention of vascular events. People with diabetes are at increased cardiovascular risk, and may gain particular benefit from the effects of omega-3 fatty acid supplementation on platelet aggregation and dyslipidaemia. Hence, participants in ASCEND will also be randomly allocated in a 2x2 factorial design to receive 1g omega-3 FA daily or matching placebo capsules for at least 7 years. Such a study design allows all randomised patients to contribute fully to the assessment of the separate effects of aspirin therapy and of omega-3 fatty acids.

ASCEND: A streamlined, mail-based trial collecting only essential data

The reliable assessment of the important questions that ASCEND is addressing requires the randomisation of a very large number of people with diabetes, and their long-term treatment and follow-up. In order to be able to study 15,000 people with diabetes for at least 7 years at low cost, ASCEND is streamlined and being undertaken predominantly by mail (supplemented by central records). If it can reliably demonstrate that aspirin and/or omega-3 fatty acids safely reduces the risk of cardiovascular events and deaths in patients with diabetes who do not have pre-existing occlusive arterial disease, then this would be relevant to some tens of millions of people world-wide (who are currently not receiving such therapy) and could save tens of thousands of lives each year. Consequently the British Heart Foundation is supporting this large streamlined trial.

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1. BACKGROUND AND RATIONALE

1.1 Reliable assessment of the effects of aspirin for primary prevention of cardiovascular events in diabetes mellitus

1.1.1 Diabetes mellitus: An increasingly common cause of cardiovascular disease

Diabetes mellitus affects about 150 million individuals worldwide, with at least 40 million cases in the Established Market Economies and over one million diagnosed cases in the UK.[1] Moreover, the prevalence is increasing rapidly, and it is estimated that there will be 300 million people worldwide with type 2 diabetes mellitus by 2025, and a further 30 million with type 1 disease.[2] Patients with diabetes of either type are at substantially increased risk of cardiovascular events and death, and the majority (60-70%) of deaths in both types of diabetes are attributed to vascular causes.[3,4] However, among prevalent cases of diabetes in younger and middle age, the majority will not have a history of vascular disease.[5,6]

1.1.2 Lack of reliable evidence for benefit with antiplatelet therapy in patients with diabetes

In the "secondary" prevention of cardiovascular disease, there is reliable randomised evidence that antiplatelet therapy (chiefly aspirin) reduces the risk of further cardiovascular events by about one-quarter among a wide range of different high-risk groups with occlusive arterial disease,[7,8] and the benefits appear to be similar whether or not such patients also had diabetes (figure 1). As a consequence, most patients with diabetes who have diagnosed vascular disease are currently receiving antiplatelet therapy[9,10] and its use is widely included in guidelines for secondary prevention.[11,12]

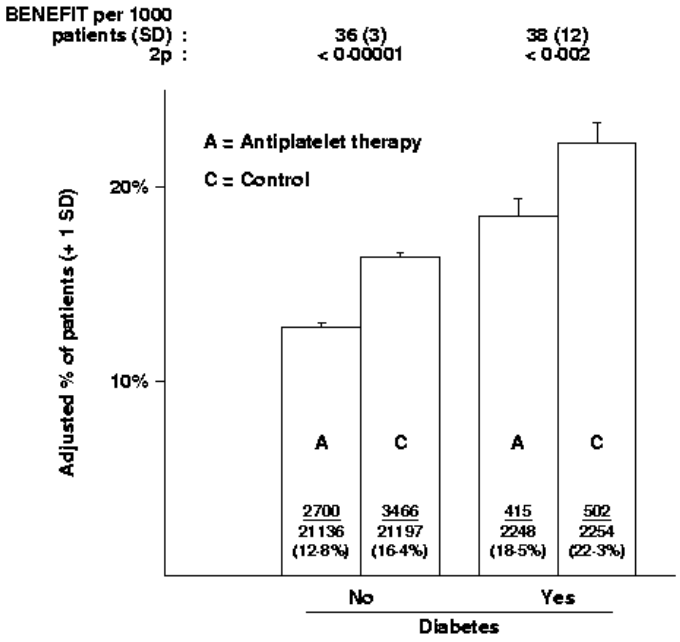

***Figure 1: Absolute effects of antiplatelet therapy on vascular events among patients with occlusive arterial disease in the absence and presence of diabetes*[7]**

However, the majority of people with diabetes do **not** have manifest occlusive arterial disease[5,6] (at least 0.5 million in Britain and several tens of million worldwide), and for them there is no direct evidence of benefit with aspirin or any other antiplatelet agent. The main randomised evidence currently available on the effects of antiplatelet therapy in such patients with diabetes comes from 9 trials involving a total of about 5000 patients, and a meta-analysis of their results indicates a

much smaller proportional reduction in cardiovascular events than has been found in the secondary prevention setting (just 7% compared with about 20-25%: figure 2).[8] Even in aggregate, however, those studies in diabetics involved relatively few events, and the confidence interval for the estimated effect is wide, ranging from a 23% risk reduction to an 8% hazard.

Given the consistency of the beneficial effect in other high-risk settings (including patients with diabetes with arterial disease: figure 1), it seems likely that the true effect of antiplatelet therapy in people with diabetes alone is similar to the reduction of about one-quarter seen overall in high-risk patients as, for example, has been shown with cholesterol-lowering[13] and anti-hypertensive therapies[14]).

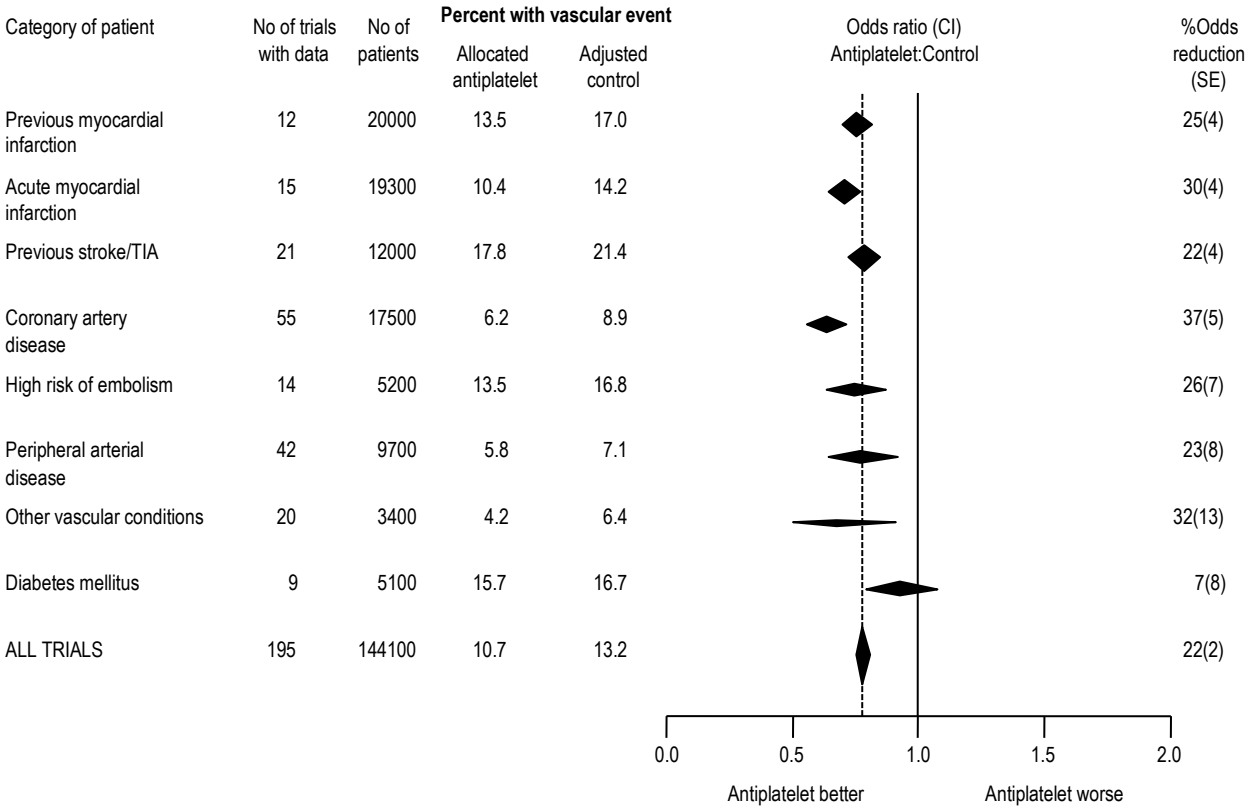

Figure 2: Proportional effects of antiplatelet therapy on vascular events in 195 trials among high-risk patients subdivided by disease category[8]

1.1.3 Aspirin increases the risk of major bleeding (but appears to be relatively safe in diabetes)

In the meta-analysis of previous trials among people with occlusive arterial disease, antiplatelet therapy was found to increase the risk of cerebral haemorrhage by about 25% and the risk of major extracranial bleeds by about 60%, with similar proportional increases in the different types of patient studied.[8] Among such high-risk patients, the absolute reductions in heart attacks and ischaemic strokes with antiplatelet therapy substantially outweighed the relatively small absolute risks of cerebral haemorrhage and major extracranial bleeds. There is also good evidence from the previous trials that antiplatelet therapy is not associated with any special risks in patients with diabetes. In particular, the Early Treatment Diabetic Retinopathy Study (ETDRS) of 650 mg aspirin daily versus placebo among 3700 people with diabetes indicated that aspirin did not increase the risk of retinal or vitreous haemorrhage.[15] Nevertheless, there is a lack of reliable direct evidence that the balance of benefits and risks of antiplatelet therapy among patients with diabetes alone is favourable.

1.1.4 Large-scale randomised evidence is required to demonstrate directly that the benefits of aspirin outweigh any risks in people with diabetes

The emergence of reliable evidence about the substantial net benefits produced by aspirin in people with occlusive arterial disease has rapidly lead to its widespread use in such patients (with, for example, over 80% of those with a history of previous heart attacks or strokes receiving some form of antiplatelet therapy).[10] Based on extrapolation from the evidence in these other high-risk settings, the American Diabetes Association (ADA) has recommended the use of aspirin in people with type 2 diabetes and at least one additional risk factor (e.g. hypertension or hypercholesterolaemia).[16] By contrast, UK and European guidelines are more circumspect in their recommendations about aspirin use for people with diabetes alone.[11,12] Presumably as a result of the current uncertainties about the net benefit of antiplatelet therapy in this setting, surveys in the US and UK indicate that only about 10-20% of patients with diabetes without diagnosed occlusive arterial disease are taking antiplatelet therapy regularly.[17,18] Similarly, less than 20% of diabetic patients without vascular disease were taking aspirin regularly in the United Kingdom Prospective Diabetes Study (UKPDS) and the MRC/BHF Heart Protection Study[19] (HPS) conducted in Britain, as well as in the ongoing FIELD trial conducted in Australia, New Zealand and continental Europe. Data from the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) also indicate that less than 20% of the hypertensive patients with diabetes and no occlusive vascular disease were taking aspirin in the last 6 months of the study in Sweden, Denmark and Norway (personal communication).

Currently, the only ongoing comparison of antiplatelet therapy versus no antiplatelet therapy in patients with diabetes without pre-existing occlusive arterial disease involves 2000 of the participants in the Women's Health Study (WHS), which is too few to assess the effects of treatment in such individuals reliably (see below). The Prevention of Progression of Asymptomatic Diabetic Arterial Disease (POPADAD)[20] study involves the assessment of aspirin among a further 1600 patients with diabetes, but all of the participants in that trial have diagnosed peripheral arterial disease. Further information about the effects of antiplatelet therapy among diabetic patients without pre-existing arterial disease will emerge from a collaborative meta-analysis of individual participant data from all of the previous "primary" prevention aspirin trials. But, preliminary results among the 3000 low-risk diabetic participants involved in that analysis indicate only a non-significant 25% (SD 16) reduction in coronary events (59 [3.9%] aspirin-allocated versus 71 [4.9%] placebo-allocated events; 2P=0.1) during median follow-up of 5 years (personal communication). Hence, there is a real need to initiate a much larger randomised trial of antiplatelet therapy in people with diabetes without occlusive arterial disease for whom there is not considered to be any clear indication for such treatment.

1.1.5 Aspirin 100mg (enteric coated) daily: an effective and well-tolerated antiplatelet regimen

The Anti-Thrombotic Trialists' (ATT) collaborative meta-analysis of previous trials found that high doses of 500-1500mg aspirin daily (which are more gastrotoxic[21]) are no more effective than lower doses of 75-100mg/day either in direct comparisons or in indirect comparisons (Figure 3).[7] As a consequence, daily doses of 75-150mg are generally preferred for long-term treatment as protection against serious vascular events in high-risk patients. The use of enteric-coating delays the dissolution of the contents of the tablet until the higher pH of the duodenum is reached, and so may reduce gastric injury and symptoms.[22] Hence, a regimen of 100mg daily enteric-coated aspirin is to be used in this study.

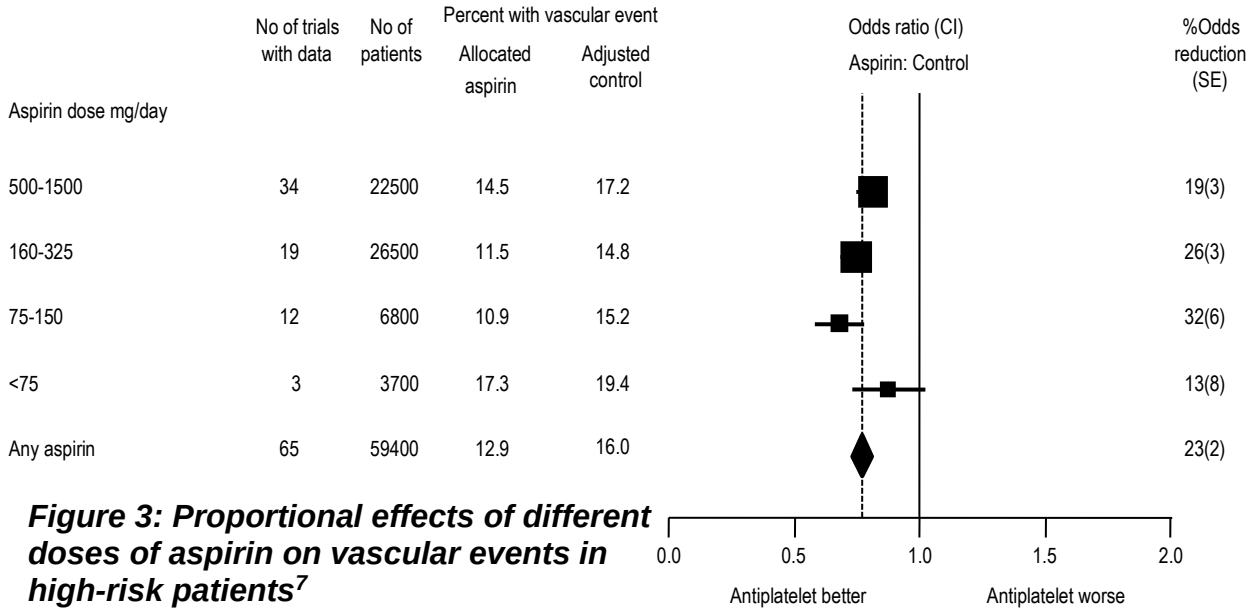

Figure 3: Proportional effects of different doses of aspirin on vascular events in high-risk patients[7]

Protocol addition December 2017

Aspirin and cancer

In the years since ASCEND was designed it has become apparent that aspirin therapy may also reduce the incidence of cancer. If this benefit is confirmed in prospective randomised trials, it has important implications for establishing the balance of benefits and risks of aspirin therapy.

Based on a series of post-hoc analyses of the long-term follow-up of certain randomised trials of aspirin, and of observational studies of cancer incidence in relation to aspirin use, it has been suggested that aspirin protects against various forms of cancer, but that this effect takes some years to become apparent. (Flossmann et al Lancet. 2007; 369: 1603-13, Rothwell et al. Lancet. 2010; 376: 1741-50; Lancet. 2011; 377: 31-41; Lancet. 2012; 379: 1602-12 and Lancet. 2012; 379: 1591-601; Algra et al Lancet Onc. 2012; 13: 518-27 Jacobs et al. J Nat Can I. 2012; 104: 1208-17 Downer et al Eur Urol. 2017 and Cook et al Ann Int Med. 2013; 159: 77-85). In one early analysis of data from 2 randomised trials, aspirin allocation scheduled for 5 or more years reduced the 20-year risk of colorectal cancer with a hazard ratio [HR] of 0.63 (95% CI 0.47-0.85), with no effect of aspirin seen for the first 10 years of follow-up. A later analysis by the same authors including 6 additional trials, indicated that aspirin allocation scheduled for 5 or more years was associated with a 20% lower risk of death from any cancer after 20 years; HR 0.78 (0.70-0.87). This effect was driven by reductions in colorectal and oesophageal cancer deaths leading to a 35% reduction in any gastrointestinal cancer deaths (HR 0.65 [0.53-0.78] based on 400 events) and a reduction in lung cancer deaths. The reduction was seen during the first 10 years, as well as between 10 and 20 years of follow up. The lag period before an effect on deaths was observed was around 5 years for oesophageal, pancreatic, brain, and lung cancer, but was longer for stomach, colorectal, and prostate cancer. In 2012, the same authors in further analyses of largely the same trials argued that aspirin reduced the risk of cancer incidence even during the scheduled treatment period of the trials; HR 0·88 (0·80–0·98), with no effect during the first 3 years of follow-up, but benefit increasing with duration of follow-up; 0–2·9 years, HR 1·00 (0·88–1·15); 3–4·9 years, HR 0·81 (0·67–0·98); ≥5 years HR 0·71 (0·57–0·89). Similar results for colorectal cancer have been observed in one large US trial of alternate day aspirin after 20 years; HR 0.80 (0.67–0.97) but with no impact on overall cancer incidence HR 0.98 (0.90-1.07).

The ASCEND trial provides one of the first opportunities to prospectively test the hypothesis that aspirin prevents gastrointestinal and overall cancer incidence and death, both during the trial and

during planned longer term post-trial follow-up (see below). For the in-trial analysis the primary cancer endpoint will be any gastrointestinal (GI) tract cancer. Little or no treatment effect is expected before about 3 years (based on the earlier trial data) therefore limiting the statistical power to detect plausible effects of aspirin during the scheduled treatment period. The expected ~430 GI tract cancers during this period provide ~86% power at 2p<0.05 to detect a 40% reduction in risk and 60% power at 2p<0.05 for a 30% reduction in risk. Analyses by time from randomisation will help assess whether effects are increasing with duration of aspirin use.

However, the main focus of the cancer analyses will be during planned longer term follow-up, when there will be much better power to detect plausible differences between the arms due to larger numbers of events. At about 5 years after the scheduled treatment period, there will be >90% at 2p<0.01 to detect a 30% or greater risk reduction and >90% at 2p<0.05 to detect a 25% reduction in any GI cancer risk (see Data Analysis Plan).

Long-term follow-up

Ethics approval was obtained during 2017 to continue to collect health-related information from ASCEND participants beyond the end of the scheduled treatment period for at least a further 20 years. Information about deaths, cancers and hospitalisation for any reason will continue to be collected from centrally held electronic records via NHS digital (formerly the Health and Social Care Information Centre). Planned analyses are described in the Data Analysis Plan.

1.2 Reliable assessment of the effects of dietary supplementation with omega-3 fatty acids

1.2.1 Higher intake of omega-3 fatty acids is associated with less coronary heart disease

Omega-3 fatty acids are long-chained polyunsaturated fatty acids (PUFA) with their first double-bond found at the third carbon atom from the methyl group (which is why they are referred to as n-3 or omega-3 fatty acids). Man is unable to manufacture these omega-3 fatty acids (FA) and is reliant upon intake from plants and animals. The richest dietary sources of the two principal omega-3 fatty acids, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), are marine animals.[23] Consumption of oily fish 2-3 times per week provides about 500mg daily of EPA and DHA combined, but consumption is less than about 50mg per day in people who do not eat fish regularly.[24]

The possible link between high intake of omega-3 FA and prevention of coronary heart disease was first noted in the 1940s when the diets of Greenland Eskimos, among whom coronary disease was rare, were compared with those of Danes living in Denmark where coronary heart disease (CHD) rates were about 10 times higher.[25] Despite similar total fat intake (about 40% of total calories), eskimo diets contained significantly greater proportions of omega-3 FA (>4%) compared with the Danes (<0.1%). These observations stimulated a large number of observational studies of omega-3 FA intake and heart disease risk in different populations. A 1999 systematic review of all of the observational data concluded that in high-risk populations consumption of the equivalent of 40-60 grams of fish per day (providing about 0.2-1g daily of omega-3 FA depending on the type of fish) is associated with 40-60% lower rate of cardiac death.[26] More recently, other observational studies have found similar protective associations of fish consumption and incidence of CHD,[27-29] (including among 5000 women with diabetes followed for about 9 years[30]) and stroke.[24]

1.2.2 Randomised trials of omega-3 FA supplementation in post-MI patients suggest 15-20% reductions in cardiovascular events but there is no information in diabetes

In the only large randomised trial of omega-3 FA supplementation that has been conducted to date, 11,000 heart attack survivors in Italy were allocated to receive 1g daily of n-3 PUFA (containing 0.46g of EPA and 0.38g of DHA) versus no PUFA treatment for 3.5 years.[31] Marginally significant reductions of 13% (95% CI 1-24%, p=0.04) in coronary events (i.e. non-fatal myocardial infarction [MI] or coronary death) and of 17% (95% CI 3-29%, p=0.02) in cardiovascular deaths, were observed among those allocated PUFA capsules in this GISSI-Prevenzione trial. This was despite 80-90% of patients in both groups eating fish at least once a week, and high use of cardioprotective drugs (including aspirin). In another randomised trial, 2000 men with a history of myocardial infarction in Wales were allocated to a recommended intake of at least 2 portions of fatty fish per week (or 1.5g Maxepa capsules daily, which contain about 0.5g EPA) versus no change in fish intake for 2 years.[32] There was a non-significant trend towards 17% fewer (95% CI 35% reduction to 8% excess) coronary events among patients allocated increased fish intake, and cardiac deaths were by 35% (95% CI 13-52%, p=0.004). Background intake of fish in that Welsh population was low, and only about 10% of the patients were taking aspirin. In a meta-analysis of all of the available unconfounded randomised evidence for increased omega-3 FA intake from these two trials and 9 much smaller trials[33,34] (which tested doses of EPA and DHA in the range 1-6g per day among a total of about 2000 patients), there was a highly significant reduction in coronary events of 18% (95% CI 8-27%, p=0.0008). Based on these studies – which were conducted chiefly among people with vascular disease – it would seem plausible that omega-3 FA supplementation might produce a 15-20% reduction in coronary and other occlusive vascular events among high or intermediate risk populations, including people with diabetes.

1.2.3 Cardioprotective effects of omega-3 fatty acids may be additional to those of aspirin

Aspirin irreversibly inhibits platelet cyclo-oxygenase, the enzyme that controls the conversion of arachidonic acid to prostaglandins and thromboxanes, which reduces the formation of thromboxane A2 in platelets and produces a potent anti-aggregatory effect.[21] But, aspirin also reduces the formation of prostacyclin, which is a potent vasodilator, and so may lead to vasoconstriction. Omega-3 FA (particularly EPA) compete with arachidonic acid for cyclo-oxygenase,[23] and so reduce thromboxane A2 production in platelets (albeit to a lesser extent than aspirin). Unlike aspirin, however, omega-3 FA enhance prostacyclin production in endothelial cells. Moreover, when aspirin and omega-3 FA are given together, there is a shift towards increased prostacyclin formation in endothelial cells and vasodilatation.[35] Consequently, any beneficial effects of aspirin and omega-3 FA on vascular disease that are mediated through these effects on prostaglandins and thromboxanes should be complementary.[35] Omega-3 FA might also have other cardioprotective effects, including: reducing myocardial susceptibility to ventricular arrhythmias;[36] increasing the stability of atherosclerotic plaques through anti-inflammatory effects that are mediated by prostaglandins and leukotrienes;[37] reducing blood pressure;[38] and reducing plasma concentrations of triglycerides (TG) and very-low-density lipoproteins, and inhibiting post-prandial lipaemia.[39-41] These effects of omega-3 FA on lipoproteins are seen both in the presence, and in the absence, of statin therapy.[34] As cardiovascular disease in diabetes derives both from platelet activation[42] and from disordered triglyceride metabolism,[3] omega-3 FA may be particularly worthwhile for people with diabetes.

1.2.4 Omega-3 fatty acids are considered safe and well tolerated

The Food and Drug Administration (FDA) consider omega-3 FA doses of up to at least 3g daily to be safe,[23] with no significant risk of bleeding. In the large GISSI Prevenzione trial,[31] 90% of participants were taking aspirin, but no excess of bleeding was observed with the addition of 1g omega-3 FA daily. The only side-effects reported in that open-label study were a slight fishy after-taste and some gastrointestinal disturbances, but only 3.8% of participants stopped their omega-3 FA supplements because of these side effects. Omega-3 FA have no effect on glycaemic control in diabetes[40,43] and their small, potentially adverse, effects on plasma concentrations of

LDL-cholesterol may be offset by beneficial changes in lipoprotein particle size.[39,41] For the present trial, a daily dose of approximately 1g capsules containing omega-3 FA (0.46g EPA and 0.38g DHA is to be used (as in GISSI)), which can be conveniently provided in 1 capsule of the concentrated preparation (with matching placebo capsules containing olive oil).

1.2.5 Need for a large-scale study of omega-3 FA supplementation in people with diabetes

As discussed above, diabetes is associated with a 2-4 fold increase in the risk of cardiovascular disease and the incidence of diabetes worldwide is increasing rapidly. Consequently, the demonstration that an inexpensive and readily available food supplement – such as omega-3 FA – reduces cardiovascular risk in patients with diabetes would have important public health consequences. By adopting a 2x2 factorial design within this large streamlined study, it will be possible to assess the separate and combined effects of both aspirin and omega-3 FA supplementation in a particularly cost-effective manner.

1.3 Mail-based studies for efficiency and cost-effectiveness

1.3.1 Previous successful experience of conducting cost-effective randomised trials by mail

Both aspirin and omega-3 FA are widely available and used, the hazards are low and well characterised, and neither requires biochemical monitoring. Several large randomised trials have been conducted using mailed drug supply and follow-up, including the CTSU-coordinated British Doctors' Study[44] and the (first) US Physicians Health Study[45] of aspirin for the prevention of myocardial infarction. Currently, there are 3 large studies[46-48] of either aspirin or various supplements being conducted entirely by mail in the US: the (second) US Physicians' Health Study II, the Women's Antioxidant Study (WACS) and the Women's Health Study (WHS). The latter study includes 40,000 American women from a wide range of educational and social backgrounds randomised to aspirin or matching placebo, and in a factorial design to different vitamin and mineral combinations. Experience from these studies shows that - with appropriate attention to the wording of information leaflets, consent forms and questionnaires, - good response rates and compliance can be achieved and reliable information about medical events gathered.[49] In addition, the 24-hour Freefone service established by CTSU for other large heart disease trials will allow study participants to discuss any aspects of the study with experienced clinical staff, and so help ensure good compliance and the early identification of serious problems.

2. PLAN OF INVESTIGATION

2.1 Study aims: assessment of outcomes

The aim of ASCEND is to determine whether 100mg daily aspirin and/or supplementation with 1 gram capsules containing 90% omega-3 fatty acids (0.46g EPA, 0.38g DHA) daily prevents cardiovascular events in patients with diabetes who do not already have clinically manifest arterial disease (without leading to significant bleeding or other adverse events).

2.1.1 Primary assessments

Aspirin therapy: The primary efficacy comparison will involve "logrank" analyses[54] of "serious vascular events" (defined as the combination of non-fatal myocardial infarction, non-fatal stroke or transient ischaemic attack, or vascular death excluding confirmed cerebral haemorrhage during the scheduled treatment period among all those allocated aspirin tablets versus all those allocated placebo tablets (i.e. "intention-to-treat" comparisons). (Vascular death includes ICD I00-I52 and I63-99 in the 10th International Classification of Diseases.)

The primary safety assessment will involve intention-to-treat comparisons among all randomised patients of allocation to aspirin versus placebo on the first occurrence of “any major bleed”, defined as: the incidence of any “major haemorrhage” (defined as any major intracranial haemorrhage, sight-threatening eye bleeding or any other bleeding episode that requires hospitalisation or transfusion, or is fatal or disabling)

Omega-3 fatty acid supplementation: The primary comparison will involve “logrank” analyses of “serious vascular events” during the scheduled treatment period among all those allocated omega-3 fatty acid capsules versus all those allocated placebo capsules.

2.1.2 Secondary assessments

The principal subsidiary comparisons will be of the effect of allocation to aspirin versus allocation to placebo tablets and, separately, of allocation to omega-3 FA versus allocation to placebo capsules on: the incidence of the combined endpoint of “serious vascular events (SVE) or revascularisations” (i.e. serious vascular event, or coronary or non-coronary revascularisation)

For the aspirin comparison only, secondary efficacy and safety assessments include:
Any incident gastrointestinal (GI) tract cancer (i.e any GI cancer excluding pancreas and hepatobiliary); and the first occurrence of:

i. haemorrhagic stroke (i.e. intracerebral or subarachnoid haemorrhage), overall and by level of disability (fatal; disabling; non-disabling; unknown disability);

ii. any major bleed by site:
- intracranial haemorrhage and separately its components (intracerebral, sub-arachnoid, subdural and other haemorrhage);
- sight-threatening eye bleed;
- serious gastrointestinal (GI) haemorrhage;
- other serious bleed (ie any extra-cranial, extra-ocular or non GI haemorrhage).

2.1.3 Tertiary and exploratory assessments

In addition, comparisons will be made of the effects during the scheduled treatment period of each of the study treatment allocations on: total and cause-specific mortality (coronary, other vascular and non-vascular death separately); other vascular outcomes (eg any coronary events (ie. non-fatal myocardial infarction, coronary death or coronary revascularisations [i.e. CABG and PTCA]); non-haemorrhagic strokes or transient ischaemic attacks); microvascular complications; venous thromboembolism; total and site-specific cancers; and hospitalisations for various other causes as indicated in the Data Analysis Plan. In addition, while it is not anticipated that the proportional effects of aspirin or omega-3 FA on particular outcomes will vary depending on particular baseline characteristics these will be explored in various prognostic subgroups (see Data Analysis Plan). Allowance for multiple hypothesis testing will be made in the interpretation of these analyses.

2.2 Sample size and predicted number of events

2.2.1 Random allocation of at least 15,000 patients with diabetes without arterial disease should provide good statistical power to detect plausible effects

One particular cohort of people with diabetes and no evident cardiovascular disease had a coronary event rate of around 3% per annum.[55] However, although that study is widely quoted, event rates may not be as high in diabetic populations with lower levels of other risk factors. For example, among the 5000 men and women with newly diagnosed type 2 diabetes (mean age 53 years) randomised in the UKPDS,[56] annual rates were 1.6% for coronary events and 1.1% for

death due to macrovascular disease (i.e. fatal MI, stroke or sudden death). Similarly, among about 3000 diabetics (mean age 63) without diagnosed occlusive arterial disease randomised in HPS,[13,57] the annual overall rate of cardiovascular events (fatal or non-fatal myocardial infarction or stroke) was 2.2%. Hence, it would seem prudent to base sample size calculations for any randomised trials in patients with diabetes and no arterial disease on serious vascular event rates of no more than about 2% per annum.

Aspirin has been shown to reduce cardiovascular event rates by about one quarter in a wide range of high-risk groups with arterial disease, with similar proportional reductions irrespective of whether or not diabetes is present (see Figure 1).[7] Hence, as in other high-risk populations, it seems plausible that aspirin might reduce the risk of serious vascular events by around one-quarter in patients with diabetes who do not have clinical evidence of arterial disease. Similarly omega-3 FA have reduced risk of cardiovascular events by 15-20% in high-risk populations. Proportional reductions of 15-20% among diabetics without diagnosed arterial disease would still correspond to substantial absolute benefits (see Table 1). But, even if such benefits do exist, at least 10,000 patients with diabetes would need to be randomised and followed for 5 years to detect these effects reliably. During the trial it is intended that blinded event rates (i.e. active and placebo groups combined) will be monitored and, if they are substantially lower than anticipated, the Steering Committee will have the option of increasing the sample size or prolonging the scheduled treatment period (see below)

Table 1: Statistical power to detect 15-20% proportional reductions in serious vascular events among 10,000 randomised patients (based on 10% 5 year control group event rate)

Proportional reduction	Control group 5000	Active group 5000	Power at 2P<0.01	Power at 2P<0.05	Events avoided/ 1000 over 5 years
25%	500	375	>95%	>95%	25
20%	500	400	80%	>90%	20
15%	500	425	60%	70%	15

Protocol addition January 2011: Accumulating evidence from within ASCEND, suggests that the overall (i.e. blinded) annual event rate (including transient ischaemic attacks) is likely to be about 1.2%, i.e. somewhat lower than the initial estimate of 2% pa in the control group. In addition, a recent meta-analysis of primary prevention trials of aspirin suggests that reductions in serious vascular events of 12-15% may be more likely than reductions of 20-25%.[58] With an annual event rate of 1.2% in the control group, randomisation of 15,000 patients with follow-up of 7.5 years would provide robust statistical power to detect plausible risk reductions of 12-15%. Hence, the Steering Committee agreed during 2010 to increase the sample size to 15,000 and the duration of follow-up to at least 7 years.

Protocol addition December 2017: A total of 15480 patients were randomised between 2005 and 2011 and have been followed for 7.5 years. Based on the blinded overall event rate for the primary outcome, the study has ~90% power at p<0.05 to detect a proportional reduction of 15% in risk of the primary endpoint.

2.2.2 Full efficiency of a 2 x 2 factorial design: separate assessment of both study questions without any material effect on non-drug cost or sample size requirements.

A factorial design will be used, with at least 7500 patients being randomly allocated to receive aspirin tablets versus 7500 patients allocated to receive matching placebo tablets (see figure 4).

Similarly, at least 7500 patients will be separately randomised to receive omega-3 FA capsules versus 7500 patients allocated to receive placebo capsules. The primary analyses will involve two-way comparisons of all those allocated aspirin versus all those allocated matching placebo tablets, irrespective of the omega-3 FA allocation (Figure 4: subtotal A versus subtotal B), and of all those allocated omega-3 FA versus all those allocated matching placebo capsules irrespective of the aspirin allocation (i.e. subtotal 1 versus subtotal 2). Hence, reliable assessment of the effects of aspirin will not interfere with reliable assessment of omega-3 FA (or vice versa), as outcomes among all those allocated active aspirin can still be compared with those among all those allocated placebo aspirin (even though half of each group will have received omega-3 FA). Use of such a factorial design instead of a simple 2-way design has little or no effect on the statistical sensitivity with which the overall effects can be assessed, or on the total number of patients required in the study.[53]

Figure 4: Factorial design of trial

	Aspirin Tablets	**Placebo Tablets**	
Omega-3 FA capsules	3750 Aspirin + Omega-3 FA	3750 Omega-3 FA	Subtotal 1: 7500 **Omega-3 FA**
Placebo capsules	3750 Aspirin	3750 Neither	Subtotal 2: 7500 **Placebo**
	Subtotal A: 7500 **Aspirin**	Subtotal B: 7500 **Placebo**	

2.3 Data and safety monitoring

2.3.1 Interim analyses: role of the Data Monitoring Committee and Steering Committee

During the study, the independent Data Monitoring Committee will review unblinded interim analyses, at least annually, of mortality, of cardiovascular events and of other serious adverse events, along with any other analyses requested. In the light of these analyses and the results of any other relevant trials or meta-analyses of trials, the Data Monitoring Committee will advise the Steering Committee if, in their view, the randomised comparisons in the study have provided **both** (a) "proof beyond reasonable doubt"* that for all patients, or for some specific types, aspirin therapy is clearly indicated or clearly contraindicated in terms of a net difference in mortality, **and** (b) evidence that might reasonably be expected to influence materially the patient management of many clinicians who are already aware of any other main trial results. The Steering Committee can then decide whether to end or modify the study (or to seek extra data). Unless this happens, the Steering Committee, the collaborators and the coordinating centre staff (except those who supply the confidential analyses) will remain ignorant of the interim results on mortality and

* Appropriate criteria of proof beyond reasonable doubt cannot be specified precisely, but in general a difference of **at least** 3 standard deviations in an interim analysis of a major endpoint would be needed to justify halting, or modifying, such a study prematurely, especially if the comparison was based on relatively few events (e.g. less than 100). If this criterion were to be adopted, it would have the practical advantage that the exact number of interim analyses would be of little importance, and so no fixed schedule is proposed [53].

morbidity until the study is terminated. Collaborators, and all others associated with the study, may write (preferably through the Oxford coordinating centre) to the chairman of the Data Monitoring Committee, drawing attention to any worries they may have about the possibility of particular side-effects, or about particular categories of patient requiring special consideration, or about any other matters that may be relevant.

2.3.2 Monitoring of any serious adverse events believed to be due to the study treatment

Throughout the trial, all serious adverse events believed with a reasonable probability to be due to study treatment are to be reported immediately by telephoning the 24-hour telephone service (see Section 3.6). A “serious” adverse event is defined as any untoward medical occurrence which results in death, is life-threatening, requires hospitalisation or the prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect. During this telephone call, standard information (i.e. identity of the patient and of the person reporting the event, nature and date of event, and reasons for attribution to study treatment) will be recorded directly on the coordinating centre computer. These reports will be reviewed immediately, blind to treatment allocation, by the clinical coordinators, and any further information required sought urgently. Confirmed reports will then be promptly forwarded “unblinded” to the chairman of the Data Monitoring Committee. and to Bayer Healthcare AG or to Abbott Products Operations AG, as appropriate and included in the Annual Safety Report sent to the Research Ethics Committee (REC). Any such serious adverse events that are also unexpected will be reported in an expedited fashion to the REC and relevant drug regulatory agencies.

2.4 Central Coordination

2.4.1 Central coordination and local collaboration

The Clinical Trial Service Unit (CTSU) at Oxford University is coordinating this study and will have overall responsibility for the administration and coordination of the study. There will be a Steering Committee to oversee the trial conduct (back page). CTSU is responsible for obtaining Multicentre Research Ethics Committee approval; for the training and monitoring of all staff directly involved in the study; for the supply of conveniently packaged study drugs and other study materials; for the identification, with the assistance of the local medical collaborators, of potentially eligible participants; for obtaining any relevant permissions to invite suitable patients to participate; for the initial invitation of participants and subsequent randomisation and follow-up by mail; for the provision of a 24-hour Freefone telephone service (for queries from participants or medical staff, for unblinding when medically necessary, and reporting of any serious adverse events believed to be due to study treatment); and for the collection and analysis of data, and blood samples. The medical collaborators around the UK are responsible, with the help of the Oxford coordinating centre, for obtaining local ethics committee approval and for assisting in the identification of potentially eligible individuals with diabetes (including liaison with hospital medical records staff). (This is summarised below, and is described in detail in the coordinating centre standard operating procedures [SOP].)

2.4.2 Training and monitoring

The administrative and nursing staff in the Oxford coordinating centre will be trained in correct study procedures (as summarised in Section 3 of the protocol and described in detail in the SOPs). The coordinating centre staff will also arrange regular meetings of all the collaborators to discuss the progress of the study and other general issues, and to provide an update on the results of any other relevant studies. Collaborators will be encouraged to contact the coordinating centre office (or 24-hour telephone service for urgent queries) if they wish to discuss some problem or other issue related to the study.

2.4.3 Supply of study materials

Aspirin and matching placebo tablets are to be manufactured and provided by Bayer Healthcare AG. Omega-3 FA capsules and matching placebo capsules are to be provided by Abbott Products Operations AG (formerly Solvay Pharmaceuticals). Both treatments are to be delivered in bulk to Brecon Pharmaceuticals for packaging and labeling prior to dispatch to participants. All study medication will be supplied in convenient treatment packs appropriate for mailing which contain the appropriate number of blister-strips for each period of the study. An inventory of study drug supply will be maintained on the coordinating centre computer, and any study drug not required by participants is to be returned to the coordinating centre for disposal.

2.4.4 Data handling

Lists of potentially eligible people with diabetes will be sought, preferably in computerized format, by the Oxford coordinating centre from medical collaborators who have access to diabetes registers, from trial databases and from general practitioners (GPs). This information will be used by the coordinating centre to generate invitations, in the name of the local medical collaborator, for patients to join the study (see Section 3.3). Hospital collaborators, general practitioners or practice nurses will also be able to offer a standard “invitation pack” (containing patient information leaflet, screening questionnaire and freepost envelope) to potentially eligible participants when they are seen for routine care in their clinic. In addition, randomised participants will have the option to recommend any friend or relative they think may be eligible and interested in participating in the study and potential participants can volunteer themselves if they hear about the study from any source. Responses from participants will be collected on questionnaires which are to be returned to the coordinating centre either on paper or electronically. Any coding that is required will be undertaken and data will then be entered into the coordinating centre computer (following an operating procedure for data handling). Any failure by participants to return Follow-up questionnaires will result in two mailed reminders being sent. Subsequently, when necessary, study coordinating staff will undertake a telephone follow-up.

Errors or omissions in the completion of study forms will result, if appropriate, in computer-generated correction requests being sent to participants for completion. All such corrections to the data will be entered on the central computer with an appropriate audit trail. The coordinating centre is also responsible for seeking confirmation and additional information about any relevant clinical events reported during follow-up, and for obtaining details from national registries of any deaths, non-fatal cancers or other relevant events available among study participants (see Section 3.7).

2.4.5 Laboratory measurements and sample storage

Blood and urine samples taken at GP practices from those patients who agree to start Run-in treatment (Section 3.3.2) and samples taken from a randomly selected group of patients during follow up (Section 3.3.1) will be mailed to the coordinating centre laboratory in Oxford. The central laboratory will use part of each blood sample for immediate assays, with the remainder being frozen for subsequent assays. The laboratory uses a number of internal and external quality control procedures and follows a standard operating procedure (in accordance with Good Laboratory Practice guidelines). Checked assay results will be transferred to the central computer and linked to the patients’ other data.

2.4.6 Source documents and archiving

The lists of potentially eligible patients provided to the Oxford coordinating centre by collaborators, the returned questionnaires from these patients, the additional information obtained on reported outcome measures and other relevant events, the death certificates, the blood assay results and the drug supply records constitute the “source documents” for the study. The coordinating centre will retain these data and records for at least 15 years. Regulatory agencies and the companies providing the study medications will have the right, in accordance

with Good Clinical Practice guidelines, to commission a confidential audit of such records kept in the coordinating centre, as long as this does not result in unblinding of the interim results while the study is still in progress.

2.4.7 Source of support and non-negligent liability cover

Funding has been obtained from the British Heart Foundation to cover the administrative and coordination costs of the trial. A supply of aspirin and matching placebo is to be provided by Bayer Healthcare AG, and a supply of omega-3 FA and matching placebo capsules by Abbott Products Operations AG (formerly Solvay Pharmaceuticals), with some funding from each company to cover drug packaging.

The trial is to be conducted, analysed and interpreted by CTSU entirely independently of the funding sources, which have no representation in its organisation and will, like the Steering Committee, remain blind to the main results as they accumulate. This arrangement is intended to ensure that no suggestions of lack of objectivity of the findings can be justified.

2.4.8 Publication in the names of all the collaborators

The success of this study depends on the wholehearted collaboration of a large number of doctors, nurses and patients. For this reason, chief credit for the main results will be given not to the central organizers, but to all those who have collaborated in the study. Draft copies of any manuscripts will be provided to all collaborators for review prior to their publication and will be published in the name of the collaboration.

3. SUMMARY OF PRACTICAL PROCEDURES

POTENTIALLY ELIGIBLE

- Diabetes mellitus (type 1 or 2)
- Male or female
- No diagnosed occlusive arterial disease
- Aged ≥ 40 years

IDENTIFICATION & INVITATION

- Potentially eligible patients identified from existing diabetes registers or databases and other sources
- Invited by GP, diabetologist or study coordinators, either in person or by mail. Invitation includes Information Leaflet, Consent Form and brief Screening Questionnaire
- Central Freefone number for any questions

SCREENING PROCESS (-2 months)

- Screening Questionnaire returned, which identifies eligible and consenting patients
- Run-in pack with 2-month supply of placebo treatment mailed to patient
- GP informed of patient's possible participation, and asked to return form if patient **not** to be randomised
- Blood and urine samples (optional) collected locally and mailed to central laboratory
- Freefone number (0800 585323) for medical advice and any questions

RANDOMISATION (0 months)

- Randomisation Questionnaire sent to re-confirm eligibility, and to characterize the patient more fully
- Randomisation Questionnaire returned, and eligible patient randomised by central computer
- Allocated treatment pack mailed to patient: 100 mg aspirin daily or matching placebo tablet, and 1g capsule containing omega-3 FA daily or matching placebo
- GP informed of patient's randomisation

FOLLOW-UP QUESTIONNAIRES (6-monthly)

- Follow-up Questionnaires and treatment packs sent 6-monthly
- Freefone number (0800 585323) for medical advice and any questions
- Further details sought from responsible clinicians about any relevant events reported on Follow-up questionnaires
- Flagging for mortality and cancer at central registries

3.1 Eligibility for ASCEND

Men or women aged at least 40 years at the time of invitation for Screening are eligible for the study, provided they fulfil **all** of the following criteria:

- **Clinical diagnosis of diabetes mellitus:** The participant's own doctor considers them to have type 1 or type 2 diabetes (based on standard WHO or ADA diagnostic criteria[50,51]);

- **No clear indication for aspirin:** The participant has no diagnosed occlusive arterial disease (i.e. a history of myocardial infarction, angina pectoris, coronary or non-coronary revascularisation procedure [i.e. peripheral arterial bypass surgery or angioplasty], stroke or transient ischaemic attack);

- **No clear contra-indication to aspirin:** The participant is not at high risk of bleeding due to: gastrointestinal haemorrhage or peptic ulcer within the previous 6 months; active hepatic disease such as cirrhosis or active hepatitis; use of warfarin, or other anti-coagulant therapy; or has a history of aspirin allergy;

- **Substantial uncertainty about whether antiplatelet or omega-3 FA therapy confers worthwhile benefit:** Neither the participant nor the participant's own doctor considers there to be a definite need for the patient to take aspirin or omega-3 FA supplements regularly (or a definite need not to do so);

- **No other predominant life-threatening medical problem:** The participant does not have some condition (other than diabetes) that might limit compliance with 5 years of study treatment, such as cancer (other than non-melanoma skin cancer).

3.2 Identification of participants

3.2.1 Large numbers of potentially eligible patients can be identified through diabetes registers, trial databases and general practice

Based on our previous experience, large numbers of potentially suitable individuals may need to be approached to randomise at least 15,000 eligible patients into this long-term trial. People with diabetes will be sought from 3 main sources: diabetes registers, trial databases and general practice. Diabetologists from around the UK will be invited to collaborate and allow invitation of potentially suitable individuals from locally held diabetes registers (such as those held for retinopathy screening or for service provision). Such registers vary in size from a few thousand to many thousands and at least one third of participants are expected to be recruited from these sources. Other people with diabetes will be identified from among the populations taking part in HPS and other diabetes trials. In order to streamline the invitation process, the contact details of potentially eligible people will be sought electronically whenever possible to allow central mailings in the name of the local doctor. This approach has allowed large numbers to be recruited by CTSU into the HPS and SEARCH trials, and is more efficient and cost-effective than mailings sent from individual centres or practices. It also allows over-selection of certain groups (e.g. older individuals) to ensure an appropriate balance of different types of participant. The third source will be directly from general practice. Diabetologists and other collaborators will be asked to identify 20-30 local general practices with computerized diabetes registers, and to seek their agreement to mailing a single batch of letters to potentially eligible individuals. Experience of screening notes in general practice indicates that ~3.5% of patients aged 50-65 have diabetes without diagnosed arterial disease. Hence, a typical group practice of about 10,000 registered patients may have 100-150 potentially eligible individuals. To complement these 3 main methods of recruitment, hospital collaborators, general practitioners or practice nurses will also be able to

offer a standard “invitation pack” (containing patient information leaflet, screening questionnaire and freepost envelope) to potentially eligible participants when they are seen for routine care in their clinic, or directly by mail if they have previously agreed to be approached for research. In addition, randomised participants will have the option to recommend any friend or relative they think may be eligible and interested in participating in the study and potential participants may volunteer themselves if they hear about the study from any source.

3.3 Screening (- 2 months)

3.3.1 Establishing eligibility

Patients with diabetes that are identified from any source as being possibly suitable will be invited by letter to take part. An invitation letter will be sent enclosing an information leaflet (Appendix 1) and a brief one-page Screening questionnaire to determine eligibility and to seek consent (Appendix 2), along with a Freepost envelope. Preliminary eligibility for the pre-randomisation Run-in phase will be based on information provided on the completed Screening questionnaire (i.e. diagnosis of diabetes, no history of diagnosed occlusive arterial disease, no contraindication to regular aspirin and signed consent to participate).

3.3.2 Pre-randomisation Run-in treatment and optional blood and urine sampling

Eligible patients will be sent a Run-in pack of medication (containing placebo tablets and placebo capsules) and asked to take one tablet and one capsule daily for 2 months. An information sheet about the medication will be provided and a copy of their signed agreement to participate will also be sent to them. About 2-4 weeks later, participants will be sent an optional blood and urine sampling kit, and asked to take this kit to their general practice for sample collection (and for measurement of blood pressure, height and weight), with this sample then mailed to the central laboratory in the containers provided. A supplementary information leaflet is to be provided and separate consent sought for this 5-10ml blood and urine collection which will allow baseline stratification by important biochemical prognostic variables (such as blood HbA_1C, lipids and markers of renal function, and urinary albumin/creatinine ratio).

During the Run-in period, the participant's general practitioner will be informed by letter of their patient's possible involvement in the study and asked to return a form if they consider there to be any reason **not** to randomise their patient (in which case the patient would be informed of their GP’s decision and withdrawn before randomisation). Patients are to be randomised only if, at the end of the Run-in period, they seem likely to comply with the study protocol for several more years. By this process, many potential drop-outs should be excluded before becoming part of the randomised comparison, with a consequent improvement in statistical sensitivity of the “intention-to-treat” analyses.[52] Patients who are not eligible will be thanked for completing the questionnaire, but will not proceed further.

3.4 Randomisation (0 months)

3.4.1 Final check of eligibility and compliance before randomisation

About 2 months after they have been sent their Run-in pack, participants will be sent a further more detailed Randomisation questionnaire asking about any significant problems (including any cardiovascular events) and their compliance with the study treatments during the Run-in period. Details of their diabetes history (in particular to allow classification as type 1 or 2), current medication, ethnic group, and smoking history will be sought to allow baseline risk stratification.[59] Participants will be asked to reiterate their commitment to a 7-year study and also, if willing, to provide details of a friend or relative living at a different address who may be contacted in the event of loss of contact with the participant.

3.4.2 Random allocation of aspirin 100mg daily versus placebo, and of 1g daily capsules containing omega-3 fatty acids versus placebo

Participants who indicate on the randomisation questionnaire that they remain eligible and willing to continue into the long-term part if the study will be randomised by the central computer in CTSU, using a minimisation algorithm to ensure balance by important baseline variables.[60] Eligible patients will be randomised in a 2 x 2 factorial blinded design between:

- Aspirin 100mg daily versus matching placebo
- Omega-3 fatty acid capsules 1 daily versus placebo

One aspirin tablet and one capsule are to be taken each day for about 7 years unless some clear reason to stop develops.

They will then be mailed a pack containing a 24-week supply of their allocated study treatment, along with relevant information about the medication and the CTSU Freefone number for any trial-related queries. The patient's general practitioner will be informed by letter of their patient's randomisation into the trial and the results of any relevant blood tests taken during Run-in (e.g. lipid profile and HbA_1C).

3.5 Post-randomisation Follow-up

3.5.1 6-Monthly follow-up questionnaires sent by mail (with telephone back-up)

Follow-up questionnaires asking about cardiovascular events, other serious adverse events (including bleeding episodes), compliance with study treatment and use of relevant non-study treatments will be sent 6-monthly with a further supply of the participant's allocated study treatment. All randomised patients - irrespective of whether or not they continue to take study treatments - are to be encouraged to return their questionnaire with up to 2 mailed reminders sent routinely. Failure to return a questionnaire will result in a study administrator telephoning the patient in order to complete the Follow-up questionnaire. Those who do not agree to being contacted in this way will be followed via their GPs and central registries.

3.5.2 Modifying study treatment

The aspirin component of the study treatment will be discontinued if a patient starts to use regular non-study aspirin or warfarin or is considered to have developed some other clear contraindication to the study aspirin (e.g. high risk of bleeding or aspirin allergy).
(N.B. Patients who stop the aspirin component of the study will be encouraged to continue the omega-3 FA component, unless this is thought to be clearly contraindicated.)

The study treatments will also be stopped if a serious adverse experience believed with a reasonable probability to be due to study treatment is reported (see Section 3.6). Patients may also stop either study treatment at their own request, or at the request of their own doctors. But, any patient who stops the study medications would still be encouraged to continue returning their Follow-up questionnaires and, if appropriate, to continue taking either study treatment alone if the other is to be stopped.

3.5.3 Follow-up of deaths and of non-fatal cancers through central registries

All randomised patients will be flagged through the Office for National Statistics and other central registries for death, cancer and other relevant events. Consequently, unbiased cause-specific mortality and site-specific cancer incidence data for all patients can be obtained, independent of whether they are still complying with study medication or responding to questionnaires.

3.6 Reporting serious adverse events

3.6.1 Immediate reporting of expected and unexpected serious adverse events believed with a reasonable probability to be due to study treatment

To fulfil regulatory authority requirements, serious adverse events believed with a reasonable probability to be due to study treatment are to be reported immediately by telephoning the 24-hour Freefone service, where a few brief details will be recorded. For the purposes of this study, the only adverse events that need to be reported in this way are those that are **both**:

- (i) serious (defined as any untoward medical occurrence which results in death is life-threatening, requires hospitalisation or the prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, congenital abnormality, or the result of an overdose); **and**
- (ii) believed with a reasonable probability to be due to study treatment.

All such serious drug related adverse events (whether expected or not) will be reported (unblinded) to the Chairman of the independent Data Monitoring Committee, and included in the Annual Safety Report for the Research Ethics Committee, to Bayer Healthcare AG and to Abbott Products Operations AG (formerly Solvay Pharmaceuticals). Any such serious drug related adverse events which are unexpected (SUSARs) will be reported, unblinded in an expedited fashion to the Medicines and Healthcare products Regulatory Agency (MHRA) and to the companies. Expected aspirin related serious adverse events might include those due to bleeding, gastro-intestinal perforation, broncho-spasm or other recognised side-effects of aspirin; there are no expected omega-3 fatty acid related serious adverse events.

3.6.2 Reporting of other serious adverse events on routine follow-up questionnaires

Any serious adverse events that are not thought to be due to study treatment, including study endpoints, should not be reported in this way. Such events are, however, to be routinely recorded on the Follow-up questionnaires (see Section 3.5) for central analysis and regular review by the Data Monitoring Committee (see Section 2.3).

3.6.3 Unblinding of study treatment allocation

Unblinding of study treatment allocation is available via the 24-hour Freefone service, where all such unblindings are logged. In general, unblinding of patients is only likely to be necessary if knowledge of treatment allocation will influence immediate patient management or for onward reporting of serious drug related adverse events (see Section 3.6.1).

3.7 Central ascertainment of biochemical effects and confirmation of reported vascular events, cancers and death

3.7.1 Assessing biochemical efficacy of study treatments by random sampling

As well as asking all participants routinely about their compliance with allocated study treatments, compliance will be assessed in a random sample of participants at intervals during the study. A randomly selected sub-set of randomised participants (5-10%) will be sent a kit for blood collection by their GP and mailing to the coordinating centre. Assays of serum or urine thromboxane levels to assess aspirin effects[61] and blood markers of omega-3 FA intake,[62] will be measured to estimate compliance with study treatments. At least once during follow-up assessments will be made in a random sample of participants of the effects of study treatments on blood HbA1c, lipids and markers of renal function, and on urinary albumin/creatinine ratio.

3.7.2 Confirmation of patient reported cardiovascular and other significant serious adverse events using mail-based systems

The coordinating centre will seek confirmation and additional information (including, if necessary, any relevant hospital discharge records) from the participant's GPs about each suspected myocardial infarction, stroke, coronary or non-coronary angioplasty, arterial surgery, cancer, or other relevant hospitalisation or serious adverse event recorded on Follow-up questionnaires or reported by participants during telephone calls or other contact. Similarly, further information will be sought from participant's GPs and other relevant sources about all cancers and deaths identified from national registries. All such information will then be reviewed, blind to treatment allocation, by coordinating centre clinical staff and coded in accordance with pre-specified criteria. The diagnosis of myocardial infarction (MI) requires information about either: (i) the presence of two or more of: (a) typical ischaemic chest pain, pulmonary oedema, syncope or shock; (b) development of pathological Q-waves and/or appearance or disappearance of localised ST-elevation followed by T-wave inversion in two or more of twelve standard electrocardiograph leads; and (c) increase in concentration of biochemical markers consistent with MI (e.g. CK >2xULN, or elevated troponins); or (ii) necropsy findings of MI of an age corresponding to time of onset of symptoms. (Silent myocardial infarctions are not to be included.) Stroke is defined as rapid (or uncertain) onset of focal or global neurological deficit lasting >24 hours or leading to death and transient ischaemic attack is defined by the same symptoms lasting <24 hours. Information (e.g. CT/MRI scan results) will be sought to ascertain the likely aetiology of the stroke (i.e. haemorrhagic or not). These procedures for reviewing reports from patients and other sources of possible study outcomes was developed by CTSU for the MRC/BHF Heart Protection Study, and allowed over 98% of such reports to be successfully confirmed or refuted.

Appendix 1: Information leaflet for potentially eligible patients

ASCEND: Patient Information Leaflet [V8.4_121010]

ASCEND: Invitation to join a large medical research project

A randomised study of aspirin and of natural oils for the primary prevention of cardiovascular events in diabetes

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what is involved. Please take time to read the following information carefully and discuss it with friends or relatives if you wish. You are entirely free to decide whether or not to take part in this trial. If you choose not to take part, the standard of care you are given by your own doctors will not be affected. If there is anything that is not clear, or if you would like more information, please telephone the ASCEND Freefone number (0800 585323) and speak to a study nurse or doctor. The study is to be conducted mainly by mail, so no extra clinic visits will be required.

Aspirin, heart disease and strokes

Patients with diabetes may be at increased risk of developing heart disease or suffering a stroke. Aspirin prevents heart attacks and strokes in people who have existing problems with their heart or blood circulation. But it is not known whether aspirin would be helpful in people with diabetes who have not yet been diagnosed with heart or circulatory problems. Serious (but uncommon) complications from the regular use of aspirin are bleeding in the stomach or intestinal tract. Typically this might happen in only about 1 per 1000 people taking aspirin regularly each year. Extremely rarely, aspirin may cause bleeding in the brain (about 1-2 per 10,000 people taking aspirin each year). Previous studies in people with known circulatory problems have shown that about 10 times as many people given aspirin have avoided a heart attack or stroke as have experienced a serious complication. However, in people with diabetes and no circulatory problems, it is not known whether the benefits of aspirin will outweigh the possible risks.

Omega-3 fatty acids and diabetes

Naturally occurring oils that are rich in omega-3 fatty acids (such as fish oils) may reduce the chances of a recurrent heart attack among people who have survived at least one heart attack. These oils have not been widely tested in people with diabetes, but there are reasons to hope that they may be helpful (although this is unproven). Taking regular supplements of such oils may have little or no beneficial effect among people living in a country (such as Britain) where most people eat a balanced diet. It is also possible that the long term use of these oils could, on balance, be slightly harmful – but this too is unknown.

What the study hopes to answer

The main purpose of the ASCEND study, is to find out whether long-term treatment with aspirin in people with diabetes who are not already known to have heart or circulatory problems, produces benefits by preventing heart attacks and strokes which outweigh the possible risks of bleeding. ASCEND will also help establish whether oils rich in omega-3 fatty acids are of any importance in reducing the chances of having a heart attack in people with diabetes who have not yet got circulatory problems.

Why have I been chosen?

ASCEND will involve at least ten thousand men and women from around Britain, who like you, are being invited to take part because they have diabetes. This invitation has come from either your own GP or a local Specialist because they think you might be suitable for the study. Alternatively you may have been recommended by a friend or relative who is already taking part in the study or volunteered yourself having read about the study. It is up to you to decide whether or not to take part in this study.

If you do decide to take part, you would, of course, be free to withdraw from the study treatment at any time without necessarily giving any reason (and without adversely affecting the medical care you can expect from your own doctors). In particular, at the end of the first 2 months, when you finish your first box of treatment, you will have the chance to withdraw if you have any second thoughts or problems with study treatment.

What taking part in ASCEND involves

Everyone taking part will have agreed to do so voluntarily, knowing that it may involve them in taking study treatment for at least 5 years. The daily study treatments (which would be sent to you by mail) will be a single white tablet and a single brown capsule taken from a blister pack. The white tablets will contain either active aspirin (100mg) or a similar looking inactive substance called a "placebo". Whether or not a participant receives active or placebo tablets will be determined randomly (like tossing a coin). Each participant will have a 50% chance of receiving active aspirin and a 50% chance of receiving placebo ("dummy") tablets. The brown capsules will each contain 1 gram of a naturally occurring oil, either mainly omega-3 fatty acids or mainly olive oil. Each participant will have a 50% chance of receiving the omega-3 containing capsules and a 50% chance of receiving olive oil capsules. The type of study treatment being taken will not generally be known by you or your doctor. This information will be known only by certain staff at the coordinating centre in Oxford, but it would be made available to your doctor if this were ever medically necessary. This design helps ensure that reliable information will be obtained about the effects of these potentially important treatments.

What you have to do to join the study

If you might like to participate in this study you should complete the brief Screening Questionnaire on the inside of the letter, sign the Agreement to Participate and return them both in the enclosed Freepost envelope. We will use your answers on the questionnaire to check that you are suitable for the study. If you are suitable, then we will send a box of conveniently packaged study treatments, and ask you to start taking one tablet and one capsule each day by mouth for the next 2 months. We shall also inform your general practitioner of your involvement in the study and check that they are happy for you to continue in the study.

Within a few weeks of receiving this first pack of study treatment, you will also be sent an **optional** blood and urine sampling kit. If it is convenient for you to do so, you would be asked to attend your local surgery to have a small blood sample taken (about 2 teaspoons full) and to provide a urine specimen. Measurements of your height, weight and blood pressure would also be recorded at the surgery and this information, along with the sample, would then be mailed to the ASCEND coordinating centre.

Long-term commitment to the study

Towards the end of the 2 months you will be sent a second study questionnaire. This will allow you to indicate whether or not you would be willing to continue taking the study treatments long-term. Participation in the study does require a commitment to take the study treatments regularly for at least 5 years and to complete questionnaires regularly. **If you do not think that you would be willing or able to do this then it would be better not to join in the first place.**

If you decide to continue you would then be sent further supplies of the study treatments and asked to take one tablet (which would be active or dummy aspirin) and one capsule (containing one or other naturally-occurring oil) every day for the next 5 years. Further questionnaires would be sent out at 6-monthly intervals. We would ask you to tell us about your current medication and any changes to your health since your last questionnaire. Additional supplies of study treatment would be sent to you 6-monthly if you were willing to continue taking it. If you do stop during the first 2 months then no further enquiries will be made of you. But, if you decide to continue, we would like to remain in contact with you for the next several years – even if you stop taking the study treatment during this period. **Throughout the study, your own doctors would remain fully responsible for all your other medical care as usual.** However, if you develop any unexpected symptoms which you believe may

be due to study treatment you should contact a study doctor on the 24-hour Freefone service: 0800 585323.

What are the side-effects and risks of taking part?

A low dose of aspirin is being used in this study in order to minimise any stomach upset or other gastrointestinal problems. Some minor bleeding (e.g. after having blood taken) and bruising may be experienced by some people, but serious bleeding is likely to be rare. We shall monitor whether aspirin causes an unacceptable level of bleeding during the study. Bleeding risks with aspirin may be somewhat greater among those who are taking warfarin (Marevan) or other blood thinning drugs (e.g. Acenocoumarol (Nicoumalone, Sinthrome) or Phenindione). So, if you are taking any of these blood thinning drugs you would not be suitable to join the study, and if you are prescribed them later we recommend stopping the study aspirin/placebo tablets. People who join the study would be asked to avoid taking aspirin-containing painkillers, and to take an alternative, (such as paracetamol), whenever pain relief is necessary. All other prescribed treatments can be taken as usual. There are no other lifestyle or dietary restrictions required. The doses of the naturally occurring oils being tested in ASCEND are not known to cause any particular problems, although some people may experience gastro-intestinal ("tummy") disturbances. If you did experience any symptoms that you thought were related to either of the study treatments, medical advice is available at all times through the 24-hour Freefone service: 0800 585323.

What are the possible benefits of taking part?

We hope that both the study treatments may help you. However, this cannot be guaranteed. The information we get from this study may help us to treat future patients with diabetes better and may help to prevent many thousands of heart attacks and strokes.

What if new information becomes available?

Sometimes during the course of a research project, relevant new information becomes available about the treatment that is being studied. If this happens we will tell you and your general practitioner about it and you can discuss whether you want to continue in the study. A study doctor is available through the 24-hour Freefone service if either you or your GP need to discuss any new information.

What happens at the end of the study?

When the research study finishes, we will inform you and your GP of the study results. You will then be able to decide whether or not you should take aspirin and/or omega-3 fatty acids. After the study finishes we will no longer continue to provide study medication for you. But, if the study results suggest possible benefit, you could discuss with your GP whether you should take either of these treatments routinely. We will also publish the study results in a professional medical journal as soon as possible after the study finishes. You would not be identified individually in any published report.

What if something goes wrong?

In the unlikely event of you being harmed as a result of taking part in the ASCEND study, the University of Oxford provides insurance cover and you would retain the same rights of care as any other patient treated in the National Health Service.

Will my taking part in this study be kept confidential?

The coordinating centre would seek information from participants' own doctors and from NHS and other central registries about any serious illnesses (such as heart attacks, strokes, cancers etc) that occur. All such information would be used, in confidence, only for medical research purposes and for routine regulatory and audit purposes.

Study organisation

The ASCEND study has been designed, and is coordinated, by Oxford University's Clinical Trial Service Unit. It involves the collaboration of many doctors and nurses around the country. The study design has been reviewed and agreed by independent Research Ethics Committees, which include people from outside the medical profession. The British Heart Foundation has provided a grant to

conduct this research study, and packaged study treatment has been provided free by Bayer (makers of the aspirin/placebo) and Abbott (who are providing the natural oils). An independent Data Monitoring Committee will review various outcomes among participants during the study, and will inform the organisers if any important new information has emerged that needs to be provided to participants and their doctors. Any questions about the study should be directed to the coordinating centre in Oxford either by telephone (24-hour Freefone service: 0800 585323) or by mail to: ASCEND Study, CTSU, Richard Doll Building, University of Oxford, Old Road Campus, Oxford, OX3 7LF. Alternatively you can e-mail us on ascend@ctsu.ox.ac.uk.

ASCEND: Summary of invitation to join a large medical research project

- Having diabetes may increase the risk of heart attacks and strokes
- Aspirin and omega-3 fatty acids benefit people who have survived a heart attack
- It is not clear whether people with diabetes who have not shown signs of circulatory problems should take aspirin or omega-3 supplements regularly
- Most people with diabetes and no circulatory problems do not take aspirin or omega-3 supplements regularly
- Low-dose aspirin is generally very safe, but does increase the risk of bleeding
- Omega-3 fatty acids at the doses being taken in ASCEND are also considered safe
- The purpose of ASCEND is to find out whether aspirin and/or omega-3 fatty acid supplementation prevents heart attacks and strokes in people with diabetes who have not shown signs of circulatory problems
- If these treatments are shown to be safe and effective for people with diabetes, then their widespread use could lead to the prevention of many thousands of heart attacks and strokes and the saving of many lives
- With your help we can answer these questions reliably with the ASCEND study

If you have any questions about the study then please feel free to contact the coordinating centre on Freefone: 0800 585323

If you think you might be interested in joining this research study please complete and return the attached questionnaire and agreement to participate. A copy of your signed agreement to participate will be returned to you when your first pack of study treatment is sent out.

Please keep this information sheet for your own records.

THANK YOU FOR YOUR HELP

Appendix 2: Consent form

Need help completing this form? Please call Freefone 0800 585323

Please read this **Agreement to Participate**, and if you are willing then please CROSS the boxes, SIGN and DATE the form using blue or black ink, and return it in the FREEPOST envelope provided.

7. Agreement to Participate

Please cross (☒) **EVERY box** to confirm that you have read and understood the following:

☐	I have read and understood the leaflet **"ASCEND: Invitation to join a large medical research project"** *[Version number of accompanying Patient Information Leaflet will be inserted here]*
☐	I have had an opportunity to telephone the Freefone number 0800 585323 and ask any relevant questions. All my questions have been answered to my satisfaction **OR** I decided that I did not need to ask any questions
☐	I understand that my participation in the ASCEND study is voluntary and that I am free to withdraw from the study at any time without my medical care or rights being affected
☐	I understand that information about my progress in the ASCEND study will be recorded on a computer database, and that these data will be stored securely and confidentially on a computer at Oxford University
☐	I agree that information about any serious illnesses (such as heart attacks, strokes or cancers) may be supplied in confidence to the study coordinators by my own doctors and by NHS and other central registries for use in the ASCEND study
☐	I agree that my hospital and other health records may be looked at in confidence by authorised individuals from the ASCEND study and by regulatory authorities to check the study is being carried out correctly
☐	I understand that my GP will be informed about this provisional agreement to participate in the ASCEND study, and that in about 2 months time I will have another opportunity to decide whether or not I want to join the long-term part of the study

I am happy to take part in ASCEND:

ASCEND Screening Questionnaire [V3.4_240407]

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(Please use blue or black ink)

Signature: _____

& **PRINTED** name: _____

Today's date: ☐☐ / ☐☐ / 2 0 ☐☐

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:-

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)

If this questionnaire indicates that you are suitable to enter the preliminary part of ASCEND, a box containing ASCEND tablets (aspirin or placebo) and capsules (one or other natural oil) will be mailed to you. A copy of this Agreement to Participate, for you to keep, will also be mailed.

If the questionnaire suggests that the study medications may not be suitable for you, then we shall write and tell you.

Thank you very much

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Appendix 3: ASCEND-Eye Sub-Study

Plan of Investigation

A sub-study of the ASCEND randomised placebo-controlled trial, exploring the effect(s) of aspirin and omega-3 fatty acids on eye disease.

Summary

Title of sub-study	Exploring the effect(s) of aspirin and/or omega-3 fatty acids on eye disease
Protocol short title/acronym	ASCEND-Eye
Medical condition under investigation	Diabetic retinopathy (DR) Age-Related Macular Degeneration (AMD)
Purpose of clinical trial	To determine whether either aspirin or omega-3 fatty acids alter the course of DR
Primary Assessments	The primary assessment will include a comparison of the effects of allocation to aspirin vs. placebo, and separately, omega-3 fatty acid supplementation vs. placebo on: • Longitudinal DR grade • Unrefuted incident AMD diagnosis
Secondary Assessments	Secondary assessments will include a comparison of the effects of allocation to aspirin vs. placebo, and separately, omega-3 fatty acid supplementation vs. placebo on: • Incident diabetic maculopathy • Initiation of laser photocoagulation • Best corrected visual acuity • Composite NEI-VFQ25 scores
Safety Assessment	Safety assessments will include a comparison of the effects of allocation to aspirin vs. placebo, and separately, omega-3 fatty acid supplementation vs. placebo on unrefuted sight-threatening eye bleeds.
Exploratory Assessment	Exploratory assessments will be made of other possible beneficial or adverse effects of aspirin and of omega-3 fatty acids during the scheduled treatment period on primary and secondary outcomes in a number of prognostic subgroups such as age, sex, duration of diabetes, systolic blood pressure, estimated glomerular filtration rate, urinary albumin: creatinine ratio, lipid profile, HbA1c and non-study medications. Subsidiary analyses will also compare incident reports of other ocular problems, for example cataracts, glaucoma, retinal vein thrombosis, infections and ocular nerve palsies between the treatment arms.
Trial design	A nested sub-study of the ASCEND 2 x 2 factorial design study of aspirin vs. placebo, and omega-3 fatty acids vs. placebo for the primary prevention of major cardiovascular events in people with diabetes.
Sample size	The 15,480 participants who were randomised to the parent ASCEND trial.
Summary of eligibility criteria	By necessity, the eligibility criteria of this sub-study is the same as the ASCEND main trial. **Inclusion criteria:** 1. Males or females with type 1 or type 2 diabetes mellitus. 2. Aged ≥ 40 years. 3. No previous history of vascular disease. 4. No clear contra-indication to aspirin. 5. No other predominant life-threatening medical problem. **Exclusion criteria:** 1. Definite history of myocardial infarction, stroke or arterial revascularisation procedure. 2. Currently prescribed aspirin, warfarin or any other blood thinning medication.
Intervention	100mg daily aspirin versus placebo and/or supplementation with 1 gram daily omega-3 fatty acids or placebo

Study Contacts

Project Lead of ASCEND-Eye	**Dr Emily Sammons*** Medical Research Council Population Health Research Unit, Clinical Trial Service Unit & Epidemiological Studies Unit, University of Oxford, Nuffield Department of Population Health, Richard Doll Building, Old Road Campus, Roosevelt Drive, Oxford, OX3 7LF Telephone: +44 (0) 1865 743526 Email: emily.sammons@ndph.ox.ac.uk *Dr Sammons shall project lead the ASCEND-Eye study in fulfilment of the academic requirement of the Doctor of Philosophy degree at the Nuffield Department of Population Health, University of Oxford.
ASCEND Principal Investigators	**Professor Jane Armitage** (jane.armitage@ndph.ox.ac.uk) **Professor Louise Bowman** (louise.bowman@ndph.ox.ac.uk)
ASCEND-Eye Collaborator	**Professor Peter Scanlon** Clinical Director for the English NHS Diabetic Eye Screening Programme and Consultant Ophthalmologist Gloucester Diabetic Retinopathy Research Group, Cheltenham General Hospital, Sandford Road, Cheltenham, GL53 7AN
ASCEND-Eye Collaborator	**Mr Praveen Patel** Consultant Ophthalmologist Moorfields Eye Hospital NHS Foundation Trust, 162 City Road London, EC1V 2PD
Sponsor	**University of Oxford** Nuffield Department of Population Health, Richard Doll Building, Old Road Campus, Roosevelt Drive, Oxford, OX3 7LF Telephone: +44 (0) 1865 743743 Fax: +44 (0) 1865 743985 Email: ascend@ndph.ox.ac.uk

Glossary of Abbreviations

ASCEND	A Study of Cardiovascular Events iN Diabetes
AMD	Age-related macular degeneration
CTSU	Clinical Trial Service Unit
DAMAD	Dipyridamole Aspirin Microangiopathy of Diabetes study
DESP	Diabetic Eye Screening Programme
DR	Diabetic retinopathy
ETDRS	Early Treatment Diabetic Retinopathy Study
GP	General Practitioner
NHS	National Health Service
RCT	Randomised controlled trial

1. Background and Rationale

In 2016 the Royal National Institute of Blind People published information on the state of eye health in the UK[1]. Utilising administrative, demographic and clinical data from official sources, it was estimated that 7.7 million people live with a sight-threatening condition, or visual loss that has a significant impact on their lives[1]. In the 2015/16 financial year, NHS expenditure on vision in England was £1.6 billion, representing 2.6% of the total Clinical Commissioning Group budget, but the indirect costs of visual loss to the UK economy were estimated to exceed £28 billion[1].

Ocular neovascularisation underlies two of the most common causes of blindness in adults in the UK: age-related macular degeneration (AMD) and diabetic retinopathy (DR)[1]. There is a conceptual role for aspirin and omega-3 fatty acids in the presentation of both conditions, but currently a lack of robust evidence to inform prescribing decisions. In this protocol we describe a sub-study of a large, double-blind, placebo-controlled trial that will reliably evaluate the safety and efficacy of aspirin and omega-3 fatty acids in DR and AMD.

1.1 Diabetic Retinopathy

Diabetic retinopathy (DR) and diabetic maculopathy are among the most common causes of registerable blindness among working-age adults in the UK[1]. The mainstay of DR management has included stringent glycaemic control, based on evidence from the historic Diabetes Control and Complications Trial[2] (1983-1993) and United Kingdom Prospective

Diabetes Study[3] (1977-1999). However in recent trials of more intensive blood glucose lowering, conducted in the setting of contemporary standards of care for other cardiovascular disease risk factors, strict glycaemic control does not appear to benefit microvascular outcomes[4]. Therefore there is a need to research other treatments that could be used alongside retinal screening to prevent new or worsening DR, such as aspirin or omega-3 fatty acids.

1.1.1 Aspirin and Diabetic Retinopathy

An early hallmark of DR is retinal capillary occlusion by platelet thrombi[5]. This finding engendered an idea that blocking platelet aggregation and the production of thromboxane with aspirin might protect individuals from developing the disease, but also raised concerns that it might increase the risk of bleeding in the eye. A number of observational studies in animals[6-11] and humans[12,13] have supported the supposition of a protective effect of aspirin; however randomised evidence has so far been inconclusive.

The Early Treatment Diabetic Retinopathy Study[14,15] (ETDRS) randomised 3711 people with moderate to severe non-proliferative DR or early proliferative DR to either 650mg aspirin OD or a matching placebo. Photographs of the fundus were compared at baseline and after 4 and 7 years of follow-up by a dedicated retinal image reading team. The study found that aspirin did not prevent the development of high-risk features, nor did it increase the risk of vitreous haemorrhage[16]. By contrast, the Dipyridamole Aspirin Microangiopathy of Diabetes[17] (DAMAD) study was a placebo-controlled clinical trial conducted in 475 participants with diabetes mellitus, to assess the effect of 990 mg aspirin daily, or 990 mg aspirin daily in combination with dipyridamole, on retinal microaneurysm count. After 3 years of follow-up with annual fluorescein angiograms, those allocated aspirin-alone or dual antiplatelet therapy, had significantly fewer microaneurysms than those in the placebo group[17]. Although these changes were unlikely to be noticeable to the participant, they are clinically important because microaneurysm formation has a high predictive value of sight-threatening maculopathy[18-21].

Differences in the design of these two trials may explain their conflicting findings. DAMAD[17] participants had minimal retinal changes at the study onset, whereas those who took part in ETDRS[15] had more advanced disease. DR tends to take an unremitting course after it has begun, and therefore the lack of any overall benefit from taking aspirin in ETDRS could reflect a greater severity of the baseline disease. Judged together, these trial and observational studies are consistent with a hypothesis that aspirin is more effective when initiated earlier rather than later. Unfortunately the DAMAD trial was underpowered to show whether aspirin or dual antiplatelet therapy slows the progression of DR 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 randomised trial with a well-defined and longer duration of aspirin exposure, alongside contemporary standards of glycaemic, blood pressure and lipid management, may more reliably assess whether aspirin does alter the course of DR.

1.1.2 Omega-3 Fatty Acids and Diabetic Retinopathy

It is biologically plausible that omega-3 fatty acids modulate the metabolic processes and physiological response to different environmental exposures that are implicated in the pathogenesis of vasoproliferative and neurodegenerative retinal diseases such as DR[22]. Observational studies have shown a protective effect of omega-3 fatty acids on DR[23], whilst two randomised trials of omega-3 fatty acids for diabetic macular oedema have been discordant[24,25]. One found that omega-3 supplementation alongside standard care anti-VEGF therapy, reduced the thickness of macular oedema on optical coherence tomography compared with controls, but was limited by its small size (n = 62) and single-blind design[24]. The other placebo-controlled, double-blind and larger (n = 467) trial found no effect of omega-3 supplementation[25].

1.2 Age-Related Macular Degeneration

AMD is the leading cause of legal blindness certifications in the UK[1]. Progressive loss of photoreceptors in the macula limits the ability to safely navigate surroundings, read, drive and recognise loved ones, and thereby compromises expectations of leading a fulfilling retirement. The advent of injectable anti-angiogenic agents and use of laser photocoagulation to limit overgrowth of fragile new blood vessels, offers some hope that visual acuity can be stabilised in people with the most common neovascular (or wet) form of the disease; however there is still no effective treatment for its atrophic (or dry) counterpart[26]. Therefore the attention of research groups and clinicians has moved to preventative treatments and addressing modifiable risk factors.

1.2.1 Age-Related Maculopathy and Aspirin

Atherosclerotic cardiovascular disease has been proposed as a potential risk factor for the development of senile maculopathy, through its deleterious effects on the choroidal circulation, including retinal arteriolar narrowing and deposition of lipids in Bruch's membrane[27-29]. By virtue of its indication to treat cardiovascular disease, aspirin could potentially modify the presentation of AMD; however existing studies of the association between aspirin and AMD have produced conflicting findings. Prospective cohort studies[30,31] have found a detrimental association with wet AMD, but not early maculopathy or dry AMD, and randomised trials have shown no hazard, and a non-significant trend towards a protective effect of aspirin[32,33]. Non-randomised studies are likely to have been confounded by treatment intention and recall bias, and many have lacked detail regarding the definition of aspirin exposure and adjustment for risk factors. Moreover, the published meta-analyses have included studies that used different definitions of early maculopathy and different grading criteria. Collectively, this

methodological heterogeneity and the incongruous results of former studies create a dilemma for clinicians with a competing need to prescribe aspirin for the primary or secondary prevention of cardiovascular disease or other indications.

1.2.2 Age-Related Maculopathy and Omega-3 Fatty Acids

Although humans are able to synthesise saturated fatty acids and some monounsaturated fatty acids from carbohydrate and protein, they lack Δ-15 and -12 desaturase enzymes that are required to insert more than one double bond near the methyl (or omega) terminal of a fatty acid hydrocarbon chain[22]. Thus omega-3 and omega-6 fatty acids are essential nutrients that can only be obtained from dietary sources such as vegetable oils, nuts, fatty fish and egg yolks[22]. The parent compounds of the omega-3 and omega-6 families are α-linolenic (ALA) and linoleic acid (LA) respectively[22]. Once consumed, humans can elongate ALA or LA chains into compounds with a structural role in cell membranes, and functional roles in immune regulation[22]. The main derivative of LA is arachidonic acid (AA), whereas eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are synthesised from ALA[22].

The retina has a uniquely high composition of long-chain polyunsaturated fatty acids. In contrast to other tissues which mainly use lipid for energy or structural roles, there is an amassing evidence base implicating omega-3- and omega-6 fatty acids in photoreceptor development, visual transduction pathways and protection against oxidative stress[22,34-39]. Our eyes are intermittently exposed to a range of adverse conditions such as high oxygen tensions, excessive light exposure, cigarette smoke and systemic inflammation. A ubiquitous response to such stressors is the activation of phospholipase A_2 (PLA_2), which releases free omega-6- and omega-3 fatty acids from retinal cell membranes[35]. The presence of these activates cyclooxygenase (COX), lipoxogenase (LOX) and cytochrome P450 oxidase enzymes, which catalyse the synthesis of vasoactive and immunogenic mediators[37]. Generally, AA-derived eicosanoids tend to be pro-inflammatory, pro-coagulant and potent vasoconstrictors, whereas docosanoids tend to be anti-inflammatory, vasodilating and anti-angiogenic[22]. Lipids may also be degraded via non-enzymatic peroxidation, where free radicals interact with the unsaturated double bonds of omega-3- and omega-6 fatty acids, generating reactive intermediaries that covalently bond with cell proteins and alter their stability and function[40]. Both lipid pathways and their products have been implicated in the pathogenesis of AMD[41,42]. The Age-Related Eye Disease Study[43] (AREDS) 1 randomised controlled trial provided proof of concept for the non-enzymatic oxidant mechanism of injury. In this study of 3640 men and women aged 55-80, who were followed up for 6.3 years, supplementing anti-oxidants vitamin C, vitamin E, beta-carotene, zinc and copper slowed progression from early to late-stage senile maculopathy (OR 0.72, 99% CI 0.52-0.98)[43]. It has also been hypothesised that supplementing omega-3 fatty acids might also be beneficial, by subverting lipid pathways towards the production of more protective docosanoid intermediaries[38,44]. However studies of omega-3 fatty acids on AMD have produced contradictory findings. Observational studies have calculated a relative risk or odds ratio by comparing the highest and lowest strata of lipid intake estimated from food frequency questionnaires. Using this method, a majority have reported benefits of taking omega-3 fatty acids so long as the level of omega-6 is low[45-47], and only by those who are homozygous for certain low risk single nucleotides polymorphisms[48,49]. As discussed previously, observational studies are limited by their lack of comparison group and ability to control for confounding factors. When evaluated prospectively in the AREDS2[50] and NAT2[51] randomised-controlled trials, supplementation with omega-3 fatty acids did not prevent progression of pre-existing AMD. To date there is no published clinical trial data on the effect of omega-3 fatty acid supplementation on incident AMD, but the ongoing VITAL-AMD trial (NCT01782352) that is running parallel with ASCEND will contribute data on this.

1.3 Aims of the ASCEND-Eye sub-study

The aim of this research is to evaluate the effect of aspirin and omega-3 fatty acids on eye disease as a sub-study of the ASCEND trial. ASCEND-Eye will answer the following questions:

1. Does either aspirin or omega-3 fatty acid supplementation alter the course of DR?
2. Does either aspirin or omega-3 fatty acid supplementation alter the course of AMD?
3. Subsidiary questions: what are the effects of aspirin and/or omega-3 fatty acids on other eye complications associated with diabetes, such as cataracts, retinal vein thrombosis, glaucoma, ocular nerve palsies and ocular infections?

2. Methods

ASCEND was a randomized 2 x 2 factorial design study of aspirin 100mg daily versus placebo, and separately of 1g omega-3 fatty acid supplementation daily versus placebo, for the primary prevention of major cardiovascular events in people with diabetes mellitus. The trial design has been described elsewhere[52,53]. Briefly, the trial used streamlined mail-based methods to follow-up 15,480 participants from the UK, who were recruited from DR screening registers (n = 6122), hospital diabetes clinics and general practices between 2005 and 2011. All randomised individuals were routinely sent a follow-up questionnaire once every 6-months, with a further supply of their allocated treatment via the post. Follow-up questionnaires specifically asked about the trial's primary endpoint, compliance with the study medications and other serious adverse events such as new diagnoses of cancer, major bleeding episodes and ocular complications. Participants were also able to report safety information directly to the central coordinating office at the University of Oxford using a dedicated telephone service. All data was captured and stored on programmes developed in-house at the University of Oxford. Results of the ASCEND trial were presented at the European Society of Cardiology in Munich on 26th August 2018[54,55].

ASCEND-Eye will supplement data collected as part of the main ASCEND trial via three discrete activities that are now described.

2.1 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), will be sent the National Eye Institute Visual Function Questionnaire-25 (VFQ). The latter seeks information from participants about the effect of visual impairment on activities of daily living and emotional well-being, and from this a composite score is derived[56]. This multi-domain instrument has been shown to be a reliable and valid questionnaire across a range of chronic eye conditions. Its composite scores are strongly and independently correlated with visual acuity and therefore it has regulatory-approval for use as an endpoint in ocular intervention trials[57].

2.2 Adjudication of Patient-Reported Outcomes

Unrefuted sight-threatening eye bleeds were included in the secondary endpoint of "Major haemorrhage" in the main ASCEND trial[52]. Characterising the severity of eye-bleeding events is therefore an integral part of the ASCEND-Eye study. Currently there is no standardised approach for grading the severity of eye-bleeding which makes inter-study comparisons, and monitoring of blood resorption in the clinical setting unreliable. Studies that have included the occurrence of retinal and/or pre-retinal haemorrhage have represented it as a binary present/absent outcome or as a qualitative endpoint. Lieberman *et al*[58] (2006) suggested the first objective method of grading vitreous haemorrhage based on the number of retinal clock hours obscured by blood on fundoscopy, however their scoring system did not include retinal or anterior chamber haemorrhages, nor did it assess the implications of bleeding on visual loss and use of healthcare resources. ASCEND-Eye does not have access fundus photographs at the time of reported bleeds, and therefore a pragmatic way of characterising their severity from clinic letters sought from participants' general practitioners was needed. Clinically-meaningful rules used to adjudicated patient-reported eye-bleeding events were developed in collaboration with Mr Praveen Patel, Consultant Ophthalmologist at Moorfield's Eye hospital.

Sight-threatening bleeds were defined as clinically significant bleeding in the eye which results in unresolved visual loss and/or requires an urgent intervention such as laser photocoagulation, vitreoretinal surgery or intraocular injection. Visual loss was considered to be permanent if it was not known to have resolved using the latest information available at the time of adjudication.

- Patients were considered to have visual loss if one or more of the following criteria were met:
    - Documented evidence of reduced visual acuity from a previously higher level.
    - 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 with dates subsequent to the bleed
        - Clinic letters describing a fundus view obscured by a dense vitreous haemorrhage which made pan-retinal photocoagulation infeasible

- Interventions subsequent to an eye-bleed typically happen within 3-6 months from the date of onset. These "urgent" interventions included*:
    - Reports of- or referrals for retinal laser therapy or surgical interventions for **posterior chamber** eye-bleeds
    - Reports of- or referrals for intravitreal injections, retinal laser therapy or surgical interventions for **anterior chamber** eye bleeds.

*If the totality of the evidence available is consistent with a significant bleed that would ordinarily warrant an urgent intervention, but a clinical decision was made to treat conservatively because the visual potential was poor, or when other comorbidities precluded surgery, these were also coded as sight-threatening bleeds.

Evidence was also sought from general practitioners to confirm patient reports of AMD and other ocular event terms which could have been related to an underlying bleed.

2.3 Linkage to the NHS Diabetic Eye Screening Programme

Longitudinal information about ASCEND participants' DR and maculopathy grades, their photocoagulation status and best corrected visual acuity will be sought by electronic linkage to the NHS Diabetic Eye Screening Programme (DESP) data.

2.4 Data Handling

All data in the study will be processed electronically using a set of custom-written programmes which include the clinical supervision of adverse events and event adjudication, and management of the VFQs. All research staff will be expected to comply with the requirements of the General Data Protection Regulation and Data Protection Act 2018 with regards to the collection, storage and processing of personal information. At CTSU, identifiable details are stored in a database, with unique usernames and password-controlled access for research staff, in line with departmental security policies, and trial

participants are identified by means of a unique ID number. Linkage to the DESP data will be dependent on the use of participant's NHS number.

2.5 Analysis

All comparisons of the primary and secondary outcomes will be between those individuals' allocated aspirin daily versus matching placebo, and separately, ω3-fatty acid supplement daily versus matching placebo, during the scheduled treatment period. The same censoring rules defining the scheduled treatment period in ASCEND will be applied to the ASCEND-Eye Study (see Data Analysis Plan which was included as a supplementary appendix with the ASCEND baseline paper[52]). For a typical patient, this will entail the period of time from their date of randomisation to the date of their final follow-up or death. 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). All data will be reviewed, blind to treatment allocation and coded in accordance with pre-specified criteria. Subjects will be classified by the findings in their worst affected eye and the statistical tests employed will vary according to the type of data under examination. Univariate analyses will be used to describe baseline characteristics, subdomain and composite VFQ scores, and mean DESP grades for the ASCEND-Eye Study population. 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. Incident diagnoses of AMD and macular oedema will be calculated from the number of participants with the condition divided by the number of person-years of follow-up. Individuals will contribute person-years to total follow-up beginning at the time of randomisation and continuing until the initial diagnosis or the end of the scheduled treatment duration, whichever comes first. Primary and secondary assessments will involve log rank analyses to estimate the average event rate ratio comparing those allocated aspirin versus placebo and separately, omega-3 fatty acids versus placebo. Estimates of the event rate ratio will be shown with 95% confidence intervals. In all analyses, two-sided p-values (2P) <0.05 will be considered statistically significant.

Primary Outcome

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

- Most severe DR grade during the scheduled treatment period
- Unrefuted incident AMD diagnosis

Secondary Outcomes

Secondary assessments will include a comparison of the effects of allocation to aspirin versus placebo, and omega-3 fatty acid supplement versus placebo on:

- Composite NEI-VFQ25 scores[56]
- Latest-available best corrected visual acuity
- Incident diabetic macular oedema
- Initiation of laser photocoagulation

Safety Outcome

Safety assessments will include a comparison of the effects of allocation to aspirin versus placebo, and ω3-fatty acid supplement versus placebo on:

- Sight-threatening eye bleeds

Exploratory Assessments

Exploratory assessments will be made of other possible beneficial or adverse effects of aspirin and of omega-3 fatty acids during the scheduled treatment period on primary and secondary outcomes in a number of prognostic subgroups such as age, sex, duration of diabetes, systolic blood pressure, estimated glomerular filtration rate, urinary albumin: creatinine ratio, lipid profile, HbA1c and non-study medications.

Subsidiary analyses will also compare incident reports of other ocular problems, for example cataracts, glaucoma, retinal vein thrombosis, infections and ocular nerve palsies between the treatment arms.

3. Study Coordination

The Clinical Trial Service Unit (CTSU) at the University of Oxford is coordinating ASCEND-Eye and will have overall responsibility for its administration and coordination. We are also consulting with Mike Olson, a Health Informaticist who has previous experience of extracting data from the NHS DESPs on behalf of Public Health England (mikeolson@hic-ltd.com). All research staff will be expected to comply with the requirements of the General Data Protection Regulation and Data Protection Act 2018 with regards to the collection, storage and processing of personal information. At CTSU, identifiable details are stored in a database, with password-controlled access for research staff in line with departmental security policies.

On completion of the ASCEND-Eye Study, the data will be analysed, tabulated and published in a peer-reviewed journal. Results will also be submitted for presentation at appropriate conferences. No published work will contain identifiable data. Participants will then be informed of the main results via a newsletter.

CTSU will retain data for at least 20 years after the end of the study. The ASCEND-Eye Study may be subject to inspection and audit by regulatory bodies to ensure adherence to recent legislation. In their remit as sponsor, Oxford University will permit REC review and regulatory audit, providing access to source data/documents.

4. Ethics and Regulatory Approvals

ASCEND-Eye will be conducted in accordance with the principles of the International Conference on Harmonisation Guidelines for Good Clinical Practice and relevant local, national and international regulations. Copies of a patient information sheet, the visual function questionnaire and the visual function questionnaire covering letter were submitted for review to the National Research Ethics Service Committee – North West, as a substantial amendment to the main ASCEND trial. Approval was given on 19th October 2016.

The main ethical consideration with regards to ASCEND-Eye is how accessible a mail-based questionnaire is for those with the most severe visual impairment, and the perceived insensitivity surrounding this. Participants in ASCEND-Eye will be familiar with the mail-based nature of ASCEND. For the latter we provided a telephone service to complete questionnaires on behalf of those with visual or motor impairment, and we intend to do the same for ASCEND-Eye. A video describing the ASCEND-Eye project and a larger-font version of the study materials is viewable on the ASCEND study website: https://ascend.medsci.ox.ac.uk.

At the start of the ASCEND trial, participants gave consent for information about any serious illnesses to be supplied in confidence to the study coordinators by their own doctors and by NHS and other central registries for use in the ASCEND trial.

5. Perceived impact of the ASCEND-Eye Study

The impact of visual loss extends well beyond the immediate financial costs to NHS ophthalmology services. Being unable to safely navigate surroundings by car or on foot, and difficulties reading food labels, prescriptions or blood glucose testing kits, create a fear of accidents, reduced mobility and dependency on others. Therefore the preservation of vision is extremely important. Unfortunately, there are currently few effective options to slow the progression of microvascular diseases in diabetes; hence there is a pressing need to research treatments that could be used alongside retinal screening, to prevent new or worsening DR. Both aspirin and omega-3 fatty acids have a conceptual role in the modification of DR, but there is a lack of randomised data to support prescribing decisions of these commonly-used and readily available treatments. By embedding within an existing trial, ASCEND-Eye can produce robust and reliable evidence at low cost, making use of existing sources of healthcare data.

6. Publication Policy

The success of the ASCEND-Eye sub-study depends on the collaboration of a large number of doctors, nurses and patients. For this reason, chief credit for the main results will be given not to the central organizers, but to all those who have collaborated in the study. Draft copies of any manuscripts will be provided to all collaborators for review prior to their publication and will be published in the name of the collaboration.

7. Financial Aspects

Funding was obtained from the British Heart Foundation to cover the administrative and coordination costs of the ASCEND trial. The supply of aspirin and matching placebo is provided by Bayer Healthcare AG, and the supply of omega-3 FA and matching placebo capsules by Abbott Products Operations AG (formerly Solvay Pharmaceuticals), with some funding from each company to cover drug packaging. Dr Emily Sammons also received a grant from the Macular Society towards the cost of her DPhil studies.

The ASCEND-Eye study is conducted, analysed and interpreted by CTSU entirely independently of the funding sources, which have no representation in its organisation and will, like the Steering Committee, remain blind to the main results as they accumulate. This arrangement is intended to ensure that no suggestions of lack of objectivity of the findings can be justified.

8. Indemnity

Indemnity for the ASCEND-EYE Study is provided by the study sponsors, the University of Oxford.

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Appendix 4: Heart failure outcomes in ASCEND (HF-ASCEND)
Plan of Investigation

An exploratory analysis of the ASCEND randomised, placebo-controlled trial, assessing the effect(s) of aspirin and omega-3 fatty acids on HF outcomes and validation of outcome ascertainment using routinely collected data.

Summary

Project title	Heart failure outcomes in ASCEND: an exploratory analysis and validation of routine data-based outcome ascertainment against clinical adjudication
Project short title/ acronym	HF-ASCEND
Medical condition under investigation	Heart failure (HF)
Purpose	• To determine the effect of aspirin or omega-3 fatty acids on the risk of HF using clinically adjudicated outcomes • To develop streamlined methods of heart failure outcome ascertainment using routinely collected data
Primary assessments	1. A comparison of the effects of allocation to aspirin vs. placebo, and separately, omega-3 fatty acid supplementation vs. placebo on: • hospitalisation for new or worsening HF and • death due to HF, using outcomes (identified through trial reporting and searching routine data) that have undergone gold standard clinical adjudication
Secondary assessments	1. Assessing the agreement between HF outcomes ascertained using: a) in-trial serious adverse event reporting, or b) searching for diagnostic codes in routine data, with or without clinician review of routine data, and gold standard clinical adjudication of events from both sources among participants with data linkage. 2. Assessing the likely relative power of the outcomes obtained using different methods to detect treatment effects.
Project design	A validation project and exploratory analysis of the ASCEND 2 x 2 factorial design study of aspirin vs. placebo, and omega-3 fatty acids vs. placebo for the primary prevention of major cardiovascular events in people with diabetes.
Sample size	The 15,480 participants who were randomised to the main ASCEND trial.
Summary of eligibility criteria	The eligibility criteria of this analysis are the same as the ASCEND main trial. **Eligibility criteria:** 1. Men or women aged at least 40 years 2. Clinical diagnosis of type 1 or type 2 diabetes mellitus. 3. No clear indication for aspirin (including occlusive vascular disease) 4. No clear contra-indication to aspirin (including high risk of bleeding, active hepatic disease, use of anticoagulant therapy and aspirin allergy) 5. Uncertainty about whether aspirin or omega-3 fatty acid therapy confers worthwhile benefit 6. No other predominant life-threatening medical problem. **Exclusion criteria:** 1. Withdrawal of consent during the scheduled treatment period 2. Withdrawal of consent for ongoing collection of health information after the scheduled treatment period
ASCEND Main Trial Intervention	100mg daily aspirin versus placebo and/or supplementation with 1-gram daily omega-3 fatty acids or placebo

Project Contacts

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Glossary of Abbreviations

ARIC	Atherosclerosis Risk In Communities
ASCEND	A Study of Cardiovascular Events iN Diabetes
BNP	B-type Natriuretic Peptide
CTSU	Clinical Trial Service Unit
DM	Diabetes mellitus
GP	General Practitioner
ICD-10	International Classification of Disease, 10th Revision
HF	Heart Failure
MI	Myocardial infarction
NHS	National Health Service
NT-proBNP	N-terminal-pro B-type Natriuretic Peptide
ONS	Office for National Statistics
PEDW	Patient Episode Database for Wales
RCT	Randomised controlled trial
REDUCE-IT	Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial
SMR	Scottish Morbidity Record

1 Background and Rationale

1.1 Potential role of aspirin and omega-3 fatty acids in heart failure

Heart failure (HF) is an important cause of morbidity and mortality in the general population. It affects 1-3% of the population and is a significant burden on health care, accounting for ~5% of all hospital admissions and ~2% of all healthcare expenditure.[1,2] A population-based study in the UK showed that the burden of HF is increasing over time.[3] Therefore, it is an important target for treatment and prevention, requiring large randomised, controlled trials (RCT) to assess new interventions.

Observational studies have shown that individuals with diabetes mellitus (DM) have a 2- to 4-fold increased risk of HF and are also at higher risk of developing coronary artery disease and in particular silent myocardial infarctions (MI).[4-6] About 1 in 6 patients with newly diagnosed type 2 DM had evidence of a silent MI in the UK Prospective Diabetes Study, and the presence of silent MI was independently associated with an increased risk of fatal MI and all-cause mortality.[7] Coronary artery disease is the commonest cause of HF. Therefore, it is possible that treatment of ASCEND participants with the anti-platelet agent aspirin could lead to a reduction in the risk of silent MI in addition to the 12% reduction in risk of serious vascular events demonstrated in the main ASCEND trial.[8] This could in turn translate to a significant reduction in the long-term risk of developing HF. This hypothesis is supported by the findings of the ARIC (Atherosclerosis Risk In Communities) study, where silent MI was associated with an increased risk of HF (hazard ratio [HR]: 1.35; 95% confidence interval [CI]:1.02-1.78) compared to no MI in patients free of cardiovascular disease at baseline.[9]

Although the effect on major vascular events of 1 g daily omega-3 fatty acids compared to placebo in ASCEND was non-significant, the recently published REDUCE-IT trial showed that among patients with established cardiovascular disease or diabetes and other risk factors on statins, with elevated triglyceride levels, icosapent ethyl 4 g daily led to a significant reduction in the risk of ischemic events including death compared to placebo.[10,11] HF was a pre-specified adjudicated outcome in this trial and the risk of HF did not differ significantly between the icosapent ethyl group and the placebo group (4.1% and 4.3% respectively).

To our knowledge, no large-scale RCTs have been conducted thus far to address the possibility that aspirin may contribute to a reduction in the risk of HF in individuals with diabetes and no prior cardiovascular disease. Therefore the ASCEND trial presents a unique opportunity to conduct an analysis of randomized trial data to explore this important hypothesis.[12] It may also help consolidate the available evidence on the effects of omega-3 fatty acids on HF.

1.2 Use of routine data for heart failure outcome ascertainment

Ascertainment of HF events in cardiovascular trials traditionally involves an arduous process of systematic questioning of participants or their doctors and gathering large numbers of clinical documents for reported events, followed by clinical adjudication of each event. This process could be streamlined to reduce the complexity and overall cost of trials and to facilitate the conduct of larger trials.[13,14] The use of routinely collected healthcare data to ascertain and adjudicate events may be a way to achieve this goal.[15,16] Hospital Episode Statistics (HES) is a dataset containing details of all admissions, outpatient appointments and emergency department attendances at National Health Service hospitals in England. The Welsh and Scottish equivalents are the Patient Episode Database for Wales (PEDW) and the Scottish Morbidity Record (SMR) respectively. Using coded data directly from these routine data sources is a potential alternative to time-consuming data collection and adjudication methods, and may allow low-cost long-term follow-up of HF events.[17] Hospitalisation for atherosclerotic vascular events (e.g. myocardial infarction, stroke or revascularization procedures) can be identified solely from the presence of a relevant diagnostic code in these datasets with a high degree of accuracy (ASCEND unpublished data), but the accuracy of these methods to asses hospitalization for HF has not been established. Most previous studies have considered either traditional adjudicated outcomes or outcomes identified solely from the presence of a single diagnostic code or a combination of codes (coding algorithms) in the routine dataset. However, review of individual routine data records by clinicians is straightforward and may increase the accuracy of these records to ascertain HF events.

There is currently little evidence on the validity of using these data directly for the ascertainment of HF outcomes.[18] Therefore, it is important to compare the outcomes ascertained using such routine data (either by using a code list on its own or with clinician review) to gold-standard adjudication using medical records. A better understanding of the way HF related morbidity is represented in health records is also required to inform outcome

selection for clinical trials and health economic analysis. The methods identified in this project will be used for the long-term analysis of the ASCEND trial and could potentially be used for other studies.

2 Aims:

1. Assess the effects of aspirin and omega-3 fatty acids on the risk of HF in people with diabetes and no prior atherosclerotic disease.
2. Develop streamlined methods to reliably ascertain HF outcomes in trials using routinely collected data.

3 Objectives:

1. Identify possible HF outcomes in routine data.
2. Undertake 'gold standard' clinical adjudication, seeking additional information from GPs, for HF outcomes in study reports and possible outcomes in routine data
3. Undertake routine data based adjudication, by clinician review of the routine data records, for the same HF outcomes.
4. Conduct an exploratory intention-to-treat analysis of the effects of randomised treatments on the gold standard HF outcome(s) in all randomised participants.
5. Among participants with linkage, compare the specificity and sensitivity of outcomes based on routine data only, trial data only and both, with and without different levels of adjudication.
6. Assess the likely relative power of the different outcomes to detect treatment effects.

4 Methods:

ASCEND was a randomised 2 x 2 factorial design study of aspirin 100mg daily versus placebo, and separately of 1g omega-3 fatty acid supplementation daily versus placebo, for the primary prevention of major cardiovascular events in people with diabetes mellitus. The trial design has been described elsewhere.[19,20] Briefly, the trial used streamlined mail-based methods to follow-up 15,480 participants from the UK, between 2005 and 2011. All randomised individuals were routinely sent a follow-up questionnaire once every 6-months, with a further supply of their allocated treatment via post. Follow-up questionnaires specifically asked about the trial's primary endpoint, other relevant vascular events, compliance with the study medications and other serious adverse events such as new diagnoses of cancer and bleeding episodes for which the participant sought medical advice. Participants were also able to report safety information directly to the central coordinating office at the University of Oxford using a dedicated telephone service. All data was captured and stored on programmes developed in-house at the University of Oxford. Data from HES, PEDW, ONS and other central registries were also linked for consenting participants to provide additional information on serious adverse events for participants lost to follow up. The main study outcomes and major bleeding outcomes underwent clinical adjudication in the main trial, but HF events were not clinically adjudicated. Results of the ASCEND trial were published in the New England Journal of Medicine in 2018.[8,11]

ASCEND-HF will use an extended list of *International Classification of Diseases, 10th Revision* (ICD-10) codes which will include a few codes not specific to HF such as J81 (pulmonary oedema) in addition to HF specific codes such as I500 (congestive heart failure) to identify ASCEND participants with potential HF events (see section 4.1.1 for details). All of these events will then undergo both clinical and routine data-based adjudication (see section 4.1.2) to confirm or refute hospitalization for new or worsening HF or death due to HF. Participants with pre-existing HF will be identified by searching their HES/PEDW records for the presence of HF specific codes prior to randomisation. A more limited list of HF specific codes will be used for this purpose. Any hospitalisation due to HF in participants with pre-existing HF will be considered worsening HF, whilst any HF hospitalisation in those with no prior HF will be considered a hospitalisation for new HF.

4.1 HF event ascertainment

4.1.1 Types of events

HF-ASCEND will identify ASCEND participants with potential hospitalisations or deaths due to HF occurring between June 2005 and March 2017, using two sources:

1. Events reported over the course of the original study (study reported events)

During the main ASCEND trial, participants were asked on their 6-monthly questionnaire if they had suffered any serious illnesses or admissions to hospital since their last follow-up questionnaire and asked to write a

short, free-text description of the event. These descriptions were reviewed by study clinicians blind to treatment allocation and coded, with any ambiguous responses resolved by discussion with the participants or their doctors. In addition, events could be reported from other sources such as relatives or other healthcare professionals involved in the participant's care and coded directly into the database by the study clinicians. This initial code was known as the pre-adjudication code. Events where the pre-adjudication code indicated HF will be included as 'study reported' events.

2. Events identified from the HES/PEDW databases (ICD code identified events)

These events will be obtained by directly searching the HES/PEDW database using a list of ICD-10 codes, suggesting the presence of HF. To develop this list of codes, a comprehensive literature review of studies validating the use of routine data for ascertainment of HF outcomes will be conducted. Once the codes are finalised the linked HES/PEDW-data for all ASCEND participants from England and Wales will be searched to identify potential events. (N.B. Linked registry data for Scotland are not available at present).

There will also be an extended code list which includes a few codes not specific to HF (e.g. pulmonary oedema and cardiogenic shock). Events captured using this extended code list will then undergo both routine data-based and clinical adjudication to explore their usefulness towards increasing the sensitivity of routine data-based adjudication. However, these additional events will not be counted towards ICD code identified events which will be limited to events identified using the limited list of HF specific codes.

Appearance of any HF specific code prior to the date of randomization will be counted as pre-existing HF. The first appearance of a HF specific code in participants with no HF codes prior to randomization will be considered a hospitalisation for new HF. For participants with codes indicating the presence of HF prior to randomization, the first appearance of a HF specific code will be considered a hospitalisation for worsening HF. In both groups the impact of the coding position (primary diagnostic position versus any position) on outcome ascertainment will be explored. Deaths due to HF will be identified from the ICD-10 coded underlying cause of death on the ONS record.

4.1.2 Adjudication methods

All potential events identified using the above two sources will be adjudicated using two different methods.

1. Clinical (gold-standard) adjudication

The project will seek confirmation and additional information from the GPs, or the treating hospitals, of participants for events identified from all sources, to conduct further clinical adjudication of events. The information requested from participant GPs will include hospital discharge letters, investigation reports (chest x-ray, echocardiography, BNP/NT-pro-BNP levels etc.) and any other clinical information the GP considers pertinent to the process of event adjudication. GPs will be asked if the participant is known to have HF and the date the original diagnosis was made. All information will be reviewed, blind to treatment allocation by a clinician and coded in accordance with pre-specified criteria.

2. Routine data-based adjudication

A standard operating procedure (SOP) will be developed to adjudicate HF events purely based on clinician review of linked HES/PEDW and ONS records. The SOP will specify methods to adjudicate hospitalisations and deaths due to HF. The SOP will then be used to conduct routine data based adjudication of all identified events for participants in England and Wales blind to treatment allocation.

This will lead to 9 main groups of HF outcomes ascertained using different methods or combinations of methods.

1. Study reported events alone
2. ICD-10 code identified events alone
3. Combined study reported and ICD-10 code identified events
4. Events from each of the above methods + clinical adjudication (3 groups)
5. Events from each of the above methods + routine data-based adjudication (3 groups)

4.2 Analysis

The HF-ASCEND analysis will have three main parts.

a) Assessing the effects of aspirin versus placebo, and omega-3 fatty acids versus placebo on the risk of HF in people with diabetes and no prior atherosclerotic disease based on combined study reported and ICD-10 code identified, clinically adjudicated HF outcomes.

b) Validation of different methods of HF outcome ascertainment against gold standard adjudicated data for participants with data linkage
c) Assessing the likely relative power of the different outcomes to detect treatment effects.

4.2.1 Effects of allocation to aspirin versus placebo, and omega-3 fatty acids versus placebo on HF

All comparisons of the HF outcomes will be between those individuals allocated aspirin daily versus matching placebo, and separately, omega-3 fatty acid supplement daily versus matching placebo, during the scheduled treatment period. Analyses will include only those events that occurred between randomisation and date of death or censoring. The same censoring rules defining the scheduled treatment period in ASCEND will be applied to the HF-ASCEND Study but individuals who have withdrawn consent for long-term follow up will be excluded from the analysis (see Data Analysis Plan which was included as a supplementary appendix with the ASCEND baseline paper).[8] 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). Individuals will contribute person-years to total follow-up beginning at the time of randomisation and continuing until the date of the first HF event or the end of the scheduled treatment duration, whichever comes first.

All randomised comparisons will use log rank analyses to estimate the average event rate ratio comparing those allocated aspirin versus placebo and separately, omega-3 fatty acids versus placebo, using combined study reported and ICD-10 code identified, clinically adjudicated events. The main outcome will be the composite of hospitalisation for new or worsening HF, and death due to HF. Estimates of the event rate ratio will be shown with 95% confidence intervals. In all analyses, two-sided p-values (2P) <0.05 will be considered statistically significant.

4.2.2 Validation of different methods of HF outcome ascertainment against gold standard adjudicated data

Sensitivity and specificity will be used to assess the level of agreement between events identified using the different methods of outcome ascertainment (i.e. study reporting, simple ICD code-based ascertainment and combined study reporting and ICD code based ascertainment; with or without routine data-based adjudication), and gold standard clinical adjudication (of both study reported and ICD code identified events). Overall levels of agreement will be established using Cohen's kappa (κ) co-efficient. This comparison will be restricted to participants with routine data linkage.

4.2.3 Assessing the likely relative power of the different outcomes to detect treatment effects.

The likely relative power of each outcome method to detect treatment effects will be assessed based on the sensitivity and specificity.

5 Study Coordination

The Clinical Trial Service Unit (CTSU) at the University of Oxford is coordinating HF-ASCEND and will have overall responsibility for its administration and coordination. All study procedures comply with the UK General Data Protection Regulation and Data Protection Act 2018 with regards to the collection, storage and processing of personal information. At CTSU, identifiable details are stored in a secure database within the University of Oxford, with password-controlled access for research staff in line with departmental security policies.

On completion of the HF-ASCEND analysis, the data will be tabulated and published in a peer-reviewed journal. Results will also be submitted for presentation at appropriate conferences. No published work will contain identifiable data.

CTSU will retain data for at least 25 years after the end of the study. The HF-ASCEND analysis may be subject to inspection and audit by regulatory bodies to ensure adherence to relevant legislation. In their remit as sponsor, Oxford University will permit Research Ethics Committee review and regulatory audit, providing access to source data/documents.

6 Ethics and regulatory approval

HF-ASCEND is an exploratory analysis of the original ASCEND study and will be conducted in accordance with the principles of the International Conference on Harmonization Guidelines for Good Clinical Practice and relevant local, national and international regulations. The approved main ASCEND trial protocol specified that all relevant serious adverse events would undergo clinical adjudication. At the start of the ASCEND study, all

participants gave consent for information about any serious illnesses to be supplied in confidence to the study coordinators by their own doctors and by NHS and other central registries for use in the ASCEND trial. At the end of the trial all participants were specifically informed through the final follow up questionnaire that the study would continue to collect information from NHS central registries to assess the long-term effects of aspirin and omega-3 fatty acids and given the choice of opting out of such follow up. Twenty-two participants opted out of ongoing follow up and will be excluded from this study. This analysis has been submitted as a non-notifiable amendment to the ASCEND trial as agreed by the Sponsor office (University of Oxford Clinical Trials and Research Governance).

7 Perceived impact of HF-ASCEND

Heart failure is an important disease that causes significant long term disease burden and reduced quality of life, any intervention that can prove effective at reducing the risk of HF or its complications will considerably reduce the burden on individuals with the disease and health care systems in general. This will also be the first large scale assessment of the effects of aspirin and omega-3 fatty acids on HF in people with diabetes.

This work will also contribute towards developing streamlined trial methodology to improve the efficiency and reduce the cost of large scale randomised controlled trials. The methods developed can be used as low-cost follow-up methods to assess long-term treatment effects in ASCEND and as well as future studies.

8 Publication policy

Draft copies of any manuscripts will be provided to the trial steering committee for review prior to publication.

9 Financial aspects

This analysis will be funded by CTSU, Nuffield Department of Population Health, University of Oxford.

10 Indemnity

Indemnity for the HF-ASCEND study is provided by the study sponsors, the University of Oxford.

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ASCEND: STUDY ORGANISATION

STEERING COMMITTEE
(Major organisational and policy decisions)

Study coordinator	J Armitage
Clinical coordinator	L Bowman
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Statisticians	S Parish
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Administrative coordinator	J Barton
Lay member	D Simpson
Other members	A Adler
	C Baigent
	J Bodansky
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	HAW Neil
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DATA MONITORING COMMITTEE
(Interim analyses and response to specific concerns)

Chairman	P Sandercock
Other members	R Gray, C Hennekens, H Gerstein

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