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The impact of EPA and DHA on blood lipids and lipoprotein metabolism: influence of apoE genotype

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Fish and fish oil-rich sources of long-chain *n*-3 fatty acids have been shown to be cardio-protective, through a multitude of different pathways including effects on arrythymias, endothelial function, inflammation and thrombosis, as well as modulation of both the fasting and postprandial blood lipid profile. To date the majority of studies have examined the impact of EPA and DHA fed simultaneously as fish or fish oil supplements. However, a number of recent studies have compared the relative biopotency of EPA *v*. DHA in relation to their effect on blood lipid levels. Although many beneficial effects of fish oils have been demonstrated, concern exists about the potential deleterious impact of EPA and DHA on LDL-cholesterol, with a highly-heterogenous response of this lipid fraction reported in the literature. Recent evidence suggests that apoE genotype may be in part responsible. In the present review the impact of EPA and DHA on cardiovascular risk and the blood lipoprotein profile will be considered, with a focus on the apoE gene locus as a possible determinant of lipid responsiveness to fish oil intervention.

Fish oils: Long-chain *n*-3 PUFA: TAG: ApoE genotype

Although some inconsistencies in the literature exist, over the last 20 years a large body of epidemiological data and evidence from randomised controlled trials has demonstrated the cardio-protective action of the fish oil fatty acids EPA and DHA (Burr *et al*. 1989; Hu *et al*. 2002; Kris-Etherton *et al*. 2003; Hooper *et al*. 2006). At intakes of EPA + DHA of 0·5–1·0 g/d the cardio-protective benefits have largely been attributed to an anti-arrhythmic effect, with increased EPA and DHA content of heart muscle tissue resulting in decreased ventricular fibrillation and increased survival post myocardial infarction (MI). Reductions in relative risk of 20–30 % in total mortality, cardiovascular mortality and sudden death are frequently reported (Burr *et al*. 1989; Marchioli, 2001). The benefits of higher levels of intake of >2 g EPA + DHA have been attributed to: (a) blood lipid modulatory effects, with fish oil fatty acids known to be potent hypotriacylglycerolaemic agents; (b) an anti-inflammatory action; (c) an anti-thrombotic effect; (d) a positive impact on endothelial function (Harris, 1997; Minihane *et al*. 2000; Nestel *et al*. 2002; Thies *et al*. 2003; Calder, 2004; Balk *et al*. 2006). The present review will consider the available literature on the impact of increased fish oil intake on CVD incidence and mortality. A particular subsequent focus will be the beneficial effects of fish oil supplementation on blood lipids and lipoprotein metabolism, examining the dose–response relationship. Furthermore, recent literature that has examined the differential impact of EPA and DHA on lipoprotein metabolism will be considered. The blood lipid and lipoprotein response to increased EPA and DHA intake is highly heterogenous, with inter-individual genetic variability thought to be largely responsible. Here, the importance of inter-individual variability in response to fish oil supplementation will be discussed with reference to apoE genotype as an example.

Fatty acid structure and nomenclature

There are two families of PUFA that derive their nomenclature from the position of the first double bond within the hydrocarbon chain from the methyl end (Buttriss, 1999). The *n*-6 PUFA are derived from linoleic acid (18:2) and the *n*-3 PUFA are derived from α-linolenic acid (18:3), a precursor that gives rise to the bioactive fatty acids EPA (20:5), docosapentaenoic acid (22:5) and DHA (22:6) that

Abbreviations: LC, long-chain; MI, myocardial infarction.
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are commonly found in oily fish. These precursor PUFA cannot be synthesised by the human body and as they must be obtained entirely from the diet they are termed 'essential fatty acids'. However, the majority of the UK population consume very little fish. For example, in the National Diet and Nutrition Survey (Henderson *et al*. 2002) it was found that 74% of the participants did not consume oily fish (excluding canned tuna), 65% did not consume coated and/or fried white fish and 82% did not consume other white fish and fish dishes. In the UK the current average intake of oily fish amongst adults is a one-third portion per week (53 g *per capita* per week; one portion is considered to be about 140 g). Thus, it is recommended that the population should eat at least two portions of fish per week, of which one should be oily fish (Scientific Advisory Committee on Nutrition, 2004). Two portions of fish per week, one white and one oily, would provide approximately 0·45 g long-chain (LC) *n*-3 PUFA/d.

Studies examining the health benefits of oily fish intake

The possible benefits of LC *n*-3 PUFA in relation to CVD incidence and death were first highlighted by investigations into the diet and health of Greenland Eskimos, who were known to consume diets containing large amounts of these fatty acids. In this population EPA, docosapentaenoic acid and DHA were shown to constitute 13·1% total fatty acids compared with 0·8% total fatty acids in a control Danish population (Bang *et al*. 1980). The remarkably low incidence of CVD disease in Eskimos (7·5% predicted incidence based on the high-fat diet that they consumed) was thought to be in part attributable to the dietary fat composition, and it was suggested that the anti-thrombotic effect of the marine LC PUFA may be responsible for the cardioprotection. Subsequent studies among other populations with a high intake of LC *n*-3 PUFA, such as Alaskans and the Japanese, have also observed a low incidence of CVD (Hamazaki *et al*. 1988; Newman *et al*. 1993). From this starting point many subsequent studies have provided evidence that more modest EPA and DHA intakes have cardio-protective properties, although epidemiological studies in Western populations have at times demonstrated mixed outcomes. The Nurses' Health Study (Hu *et al*. 2000, 2002, 2003), which was conducted over a period of 16 years in >80 000 females, has observed a higher risk of total CHD events in volunteers whose total fish consumption is less than once monthly compared with those that consume fish more regularly, with a relative risk of 0·71 and 0·66 for those who consume fish once weekly and up to four times weekly respectively (Hu *et al*. 2000, 2002). In a subset of women with type 2 diabetes within the same study fish consumption was also shown to be associated with reduced risk of fatal CHD and non-fatal MI (Hu *et al*. 2003). The US Physicians' Health Study (Albert *et al*. 2002), which was conducted in >20 000 males, has demonstrated that baseline levels of LC *n*-3 PUFA are inversely associated with risk of sudden death; those in the highest quartile having an 81% lower risk of sudden death compared with men with levels in the lowest quartile. In the Health Professionals Follow-up Study (Ascherio *et al*. 1995), which was conducted in >43 000 volunteers, median *n*-3 PUFA intake (ranging from 0·07 g/d in the lowest quintile to 0·58 g/d in the highest quintile) was not found to affect relative risk of CHD (1·12 in the lowest quintile of intake compared with 0·98 in the highest quintile). In a Danish study (Osler *et al*. 2003) of 7000 participants the frequency of total fish consumption was found to have no significant effect on CHD mortality or morbidity, with hazard rate ratios of 1·09 and 0·98 in participants who consumed fish once monthly or less compared with twice weekly or more respectively. The diverse findings in observational studies have been subject to a meta-analysis (He *et al*. 2004) that has investigated fish consumption and CHD mortality in >22 000 volunteers. It was concluded that for every 20 g/d increase in fish consumption, there is a 7% decrease in CHD mortality risk and that eating fish once weekly may markedly reduce CHD mortality rates.

In addition to prospective studies there have been several intervention studies that have investigated the effects of LC *n*-3 PUFA on cardiovascular events and mortality. In the Diet and Reinfarction Trial (Burr *et al*. 1989), a secondary prevention study that examined a population of 2033 men who had previously suffered a MI, volunteers were instructed to (a) increase their consumption of oily fish to about 300 g/week through dietary means or to a similar extent via fish oil capsules (a supplement of 0·8 g EPA + DHA/d), (b) increase dietary fibre intake, (c) reduce SFA : PUFA, or they were given no dietary advice to follow. Cardiovascular health as well as overall mortality was followed up for 2 years. Volunteers who were instructed to increase either their oily fish consumption or their fish oil consumption were reported to exhibit a 29% reduction in overall mortality compared with controls, with a 16% reduction in cardiovascular death. However, a 10-year follow-up study (Ness *et al*. 2002) has demonstrated that the effects of the initial dietary changes that were imposed did not result in continued survival benefit, although this follow up has been criticised for the lack of assessment of dietary habits during the follow-up period. The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico Prevenzione Trial (Marchioli, 2001), which involved >11 000 volunteers who had recently suffered an MI, has also investigated the effects of LC *n*-3 PUFA (1 g/d) and vitamin E supplementation (300 mg/d) over a period of 3·5 years. No significant effect of vitamin E supplementation was observed. However, LC *n*-3 PUFA supplementation was found to result in reductions in many primary and secondary outcomes, the most striking of which was a 44% decrease in sudden death in those volunteers taking the EPA and DHA supplement; the benefits being attributed in large part to the anti-arrhythmic effects of the LC *n*-3 PUFA. In contrast, in Diet and Reinfarction Trial 2 (Burr *et al*. 2003), a trial involving >3000 patients suffering from angina pectoris who were advised to eat oily fish twice weekly over a period of 3–9 years, coronary deaths were found to increase by 26%. These unexpected findings were explained by the investigators (see Burr, 2007) as chance, or perhaps risk compensation by participants. In addition, other possible explanations put forward by the authors (see Burr, 2007) are drug interaction, differing effects of

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gradual *v*. bolus consumption of fish oil (dietary *v*. supplement) or the possibility that fish oil may have anti-arrhythmic effects post MI but may be pro-arrhythmic in patients suffering from angina.

In order to bring together the data emerging from intervention trials Bucher *et al*. (2002) have conducted a meta-analysis of eleven trials with approximately 8000 patients with CHD. They have concluded that patients who follow LC *n*-3 PUFA-enriched diets or supplements have reduced overall mortality, with relative risks of 0·8 and 0·7 for MI-induced mortality and incidence of sudden death respectively compared with the control group (Bucher *et al*. 2002). However, these findings are not replicated by a second more-recent meta-analysis of forty-eight randomised control studies (Hooper *et al*. 2006) in which *n*-3 PUFA do not exhibit clear protective effects on total CHD mortality or other cardiovascular events. The possible reason for this lack of consistency with the Bucher *et al*. (2002) meta-analysis may be the inclusion of the angina study (Diet and Reinfarction Trial 2 (Burr *et al*. 2003); see earlier discussion) in the Hooper *et al*. (2006) meta-analysis. When this study is removed risk of death is comparable between the two meta-analyses (relative risk 0·83 and 0·80 respectively; Hooper *et al*. 2006).

The impact of long-chain *n*-3 PUFA on fasting and postprandial lipids

The cardio-protective effects of LC *n*-3 PUFA are thought to be in part a result of the hypotriacylglycerolaemic effects of EPA and DHA. Although traditionally it has been thought that intakes of >2 g EPA + DHA/d are needed to bring about TAG-lowering effects (as discussed earlier), recent unpublished data (BM Kofler, MJ Caslake, EA Miles, P Curtis, CK Armah, L Farrell, AC Kimber, G Lietz, JC Mathers, PC Calder, CJ Packard, CM Williams and AM Minihane, unpublished results) indicate that TAG-lowering effects may be evident at much lower intakes. Fasting TAG levels have emerged as independent risk factors for CHD and elevated TAG are thought to be the metabolic 'driver' of the characteristic dyslipidaemia found in type 2 diabetes and the metabolic syndrome. An abundance of data now illustrates the potent TAG-lowering effect of LC *n*-3 PUFA in both individuals who are normolipidaemic and those who are hyperlipidaemic. A meta-analysis conducted by Harris (1997) that has compiled data from thirty-six cross-over and thirty-two parallel studies has examined the impact of fish oil supplementation on plasma TAG, total cholesterol, LDL-cholesterol and HDL-cholesterol. In both experimental designs volunteers were grouped according to their baseline TAG levels as either normolipidaemic (TAG <2 mmol/l) or hyperlipidaemic (TAG ≥2 mmol/l). The duration of supplementation ranged from 2 to 52 weeks, while the level of supplementation ranged from 1·1 to 7 g *n*-3 fatty acids/d from a variety of sources, although the majority used MaxEPA (a fish oil-derived fatty acid complex; Seven Seas Ltd, Hull, UK). According to this meta-analysis the consumption of an average of 3–4 g EPA + DHA/d for intervention periods of ≥2 weeks is followed by a decrease in TAG levels of approximately 25 % in subjects who are normolipidaemic and 25–34 % in subjects who are hyperlipidaemic. A second meta-analysis conducted approximately 10 years later by Balk *et al*. (2006) corroborates these findings. The most recent meta-analysis combines the lipid outcomes of twenty-five recent trials investigating the effects of fish oil, fish diets and *n*-3 plant oils. Earlier studies included in the Harris (1997) review had predominately used doses of EPA + DHA of >3 g/d. However, the Balk *et al*. (2006) review includes a number of more recent studies that had administered lower, more dietary achievable, intakes to a range of study volunteers who were healthy, diabetic, hypertensive, dyslipidaemic or diagnosed with CVD. The doses of EPA + DHA ranged from 0·045 to 5·9 g/d with intervention periods of 6–104 weeks. Results indicate a 0·22–0·33 mmol/l reduction (≥15–25 %) in fasting TAG levels on average. This reduction is dependent on the dose of fish oil given; there is a greater reduction following a higher dose. In addition, there is a greater reduction in fasting TAG concentration if baseline levels are elevated. The decrease in fasting TAG is in agreement with the meta-analysis conducted by Harris (1997).

In addition to TAG analysis, studies have also investigated the effects of fish oil supplementation on fasting levels of total cholesterol, LDL-cholesterol and HDL-cholesterol. Harris (1997) and Balk *et al*. (2006) have demonstrated that there are no significant effects of supplementation on HDL-cholesterol or total cholesterol levels. However, there is a 5 and 10 % increase in LDL-cholesterol in individuals who are normolipidaemic and hyperlipidaemic respectively, although both authors comment that this finding is inconsistently reported by individual studies. The impact of fish oil supplementation on subclass distribution of HDL and LDL has been investigated but less frequently. On the whole, the effect of fish oil on HDL levels is considered to be positive, increasing HDL 2 : HDL 3, although the overall concentration of HDL does not change (Abbey *et al*. 1990). The percentage distribution of LDL subclass and LDL size are associated with increased risk of CHD, since they are more susceptible to oxidation and so more atherogenic than LDL particles of a larger size (Campos *et al*. 1992). Enrichment of sunflower oil margarine with fish oil does not affect LDL particle size in volunteers after consumption of 30 g/d as compared with sunflower oil margarine alone (Sorensen *et al*. 1998). A similar finding has been reported following supplementation with high-dose fish oil or maize oil for 4 months (Nenseter *et al*. 1992). However, LDL particle size has been observed to be larger after a 6-week intervention with 4 g fish oil/d as compared with a maize oil intervention (Suzukawa *et al*. 1995), and after a 6-week intervention with 5·2 g fish oil/d as compared with a placebo oil (Tinker *et al*. 1999).

These studies illustrate the clear beneficial effects of fish oil supplementation on fasting plasma TAG levels, which would be particularly important for individuals who are hypertriacylglycerolaemic. However, this decrease in TAG is accompanied by a rise in LDL-cholesterol, which could have a detrimental effect on cardiovascular risk in susceptible subgroups.

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The impact of EPA and DHA on postprandial TAG levels (postprandial lipaemia) has been investigated in a number of studies, which can be categorised into acute and chronic depending on whether the fish oil was administered as a single test meal or as a chronic supplementation before investigating postprandial lipaemia. The acute studies that have investigated the inclusion of EPA and DHA in a test meal on the subsequent postprandial lipaemic response have generated mixed findings. No significant effect of fish oil on the duration or magnitude of the TAG response was observed in volunteers who were normolipidaemic (Harris *et al*. 1988). Similarly, no differences were observed between test oils that included SFA, *n*-6 PUFA and LC *n*-3 PUFA (50:50 mixture of highly-purified fish oil and safflower oil) as well as MUFA when examining peak TAG response in post-menopausal women (Jackson *et al*. 2002; Robertson *et al*. 2002). However, Zampelas *et al*. (1994) have observed a reduction in the postprandial TAG concentration with a LC *n*-3 PUFA meal. Thus, the effects of acute fish oil supplementation on postprandial lipaemia are unclear from these studies, and may depend on the status of the study participants, the dose of EPA + DHA administered and the overall macronutrient composition of the test meal. Further investigations are needed to clarify the potential of inclusion of EPA and DHA in a meal as an important determinant of the subsequent lipaemia.

Studies examining the effect of chronic fish oil supplementation on postprandial lipaemia tend to be more consistent in their outcomes. Those studies in which encapsulated fish oil was given to subjects who were normolipidaemic have consistently reported a decrease in the postprandial TAG response following 2·7–5 g EPA + DHA/d (Brown & Roberts, 1991; Williams *et al*. 1992; Minihane *et al*. 2000). The effects of incorporation of EPA and DHA through fish or EPA + DHA-fortified foods have also been investigated. Agren *et al*. (1996) have demonstrated lower postprandial TAG responses following a fish diet providing 0·4–0·7 g EPA + DHA/d compared with a control diet (Agren *et al*. 1996). Lovegrove *et al*. (1997) have observed a tendency towards a lower postprandial TAG response following consumption of EPA + DHA-enriched foods, although the effects were not significant, probably because of the relatively low dose of EPA and DHA administered (0·5–0·9 g/d) and the small group size. The impact of fish oil on postprandial lipaemia in subjects having hypertriacylglycerolaemia or the atherogenic lipoprotein phenotype (which represents the dyslipidaemia evident in the metabolic syndrome and diabetes) has also been investigated. A 26% reduction in the postprandial response was found in males with atherogenic lipoprotein phenotype following supplementation with 6 g fish oil/d (28% EPA, 23% DHA; Tinker *et al*. 1999; Minihane *et al*. 2000).

Although numerous studies on the lipid-modulating effect of fish oils have included males and females of all ages, little attempt has been made to date to establish whether age or gender impact on responsiveness. From a public health perspective this omission represents an important question in relation to EPA and DHA recommendations that is worthy of investigation. Studies examining the impact of LC *n*-3 PUFA in women and the impact of menopausal status on response to treatment are distinctly lacking. In a study that included thirty-six post-menopausal women supplementation with 2·4 g EPA and 1·6 g DHA/d for 4 weeks was found to be associated with a 20–26% reduction in fasting TAG levels, a 8% higher HDL-cholesterol concentration and a 28% lower overall HDL:TAG (Stark *et al*. 2000; Stark & Holub, 2004), suggesting comparable responsiveness in females *v*. males. However, further studies investigating the impact of EPA + DHA in female populations or comparing responsiveness in men *v*. women are needed.

Effects of EPA *v*. DHA on plasma lipid profile

Although the majority of literature focuses on the impact of co-feeding EPA + DHA as fish oils on the plasma lipid profile, a number of studies have also compared the differential effects of EPA *v*. DHA on the blood lipid response. Elucidation of the individual biopotency of each fatty acid in relation to risk of disease is important in an era in which the fatty acids may be purchased separately and plant sources are being developed through the application of metabolic engineering techniques. Such knowledge could lead to the development of plant products enriched with a maximised EPA and DHA content. Although a number of studies have examined the impact of either relatively pure EPA or DHA *v*. a placebo, only those placebo-controlled studies that have directly compared equivalent doses of EPA and DHA are included here.

Buckley *et al*. (2004) have demonstrated a 22% decrease in TAG with a DHA-rich oil (4·9 g DHA/d) but a 15% decrease (NS) with an EPA-rich oil (4·8 g EPA/d). However, such differential effects of EPA and DHA are not supported by other studies. Using supplements of 3·8 g EPA/d and 3·6 g DHA/d it has been demonstrated (Childs *et al*. 1990; Grimsgaard *et al*. 1997) that both EPA and DHA reduce TAG and VLDL-TAG concentrations by approximately 26–35%. On the other hand, it has been concluded (Bonaa *et al*. 1992; Rambjor *et al*. 1996) that supplementation with 3–3·3 g EPA/d is responsible for a 16–21% reduction in TAG. In studies investigating volunteers with dyslipidaemia there is a more consistent 20–35% reduction in TAG concentration with both EPA and DHA (Mori *et al*. 2000*a*; Leigh-Firbank *et al*. 2002; Nestel *et al*. 2002; Woodman *et al*. 2002). Interestingly, Woodman *et al*. (2002) have observed a greater TAG-lowering effect with 4 g EPA/d (19%) compared with 4 g DHA/d (15%), although both PUFA were found to significantly lower TAG. The overall findings of these studies seem to indicate that with a high dose of either EPA or DHA plasma TAG levels can be beneficially reduced. However, it still remains unclear whether EPA and DHA have similar TAG-lowering capabilities. In addition, the use of mixed-gender volunteer groups in some studies (Bonaa *et al*. 1992; Rambjor *et al*. 1996; Grimsgaard *et al*. 1997; Tinker *et al*. 1999; Nestel *et al*. 2002; Woodman *et al*. 2002; Buckley *et al*. 2004; Theobald *et al*. 2004) but men only in other studies (Childs *et al*. 1990; Mori *et al*. 2000*a*,*b*; Leigh-Firbank *et al*. 2002) may have had an impact on the findings, but a comparison between men and women has not been carried out.

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Studies that have examined the effect of fish oil supplementation on HDL-cholesterol or HDL_2:HDL_3 in volunteers who are normolipidaemic have shown an increase following DHA treatment in some studies (Rambjor *et al*. 1996; Grimsgaard *et al*. 1997), but in other studies (Bonaa *et al*. 1992) an inverse relationship has been observed. Similarly, with fish oil plasma EPA has been shown to be positively associated with total HDL-cholesterol (Bonaa *et al*. 1992), but an EPA-rich fish oil (pollock oil) has been reported to induce a 16% decrease in HDL-cholesterol (Childs *et al*. 1990). In subjects who are dyslipidaemic the proportion of HDL-cholesterol after treatment with EPA and DHA is increased; however, few studies have examined HDL_2 and HDL_3 separately. On the whole, EPA seems to lower the concentration of HDL_3, whereas DHA increases the concentration of HDL_2 (Mori *et al*. 2000*a*; Woodman *et al*. 2002), but this finding is not in agreement with those of Childs *et al*. (1990), who have observed a decrease in both HDL subclasses with EPA. The variability of results has also been reflected in the effect on apoB concentration, which has been shown to be both increased (3·4%; Theobald *et al*. 2004) and decreased (13%; Childs *et al*. 1990) by DHA treatment. It has also been reported (Theobald *et al*. 2004) that apoB:LDL-cholesterol, a measure of particle size, is increased after treatment with DHA. In two separate studies of a population who were overweight and hyperlipidaemic (Mori *et al*. 2000*a*,*b*) the diameter of LDL particles has been reported to be increased after treatment with 4 g DHA/d.

Overall, high levels (>2 g/d) of EPA and DHA supplementation seem to beneficially lower plasma TAG levels, but can have adverse effects on levels of LDL-cholesterol. However, the data demonstrate large inter-study variability in response to treatment that is likely to be attributable to the dose administered, intervention time, health status of the participants, background diet and many other confounding factors. Even within studies large heterogeneity in response is observed that is likely to be attributable to genetic variability within the study population.

The influence of apoE genotype on blood lipids

One possible genetic variant that may impact on the response to treatment is apoE polymorphism. The gene is located on chromosome 19, and human apoE is composed of 317 amino acids, with an eighteen amino acid single peptide sequence that is cleaved to yield a mature 299 amino acid peptide chain (Fig. 1; Siest *et al*. 1995; Hagberg *et al*. 2000; Bocksch *et al*. 2001). Although predominantly produced by the liver and brain, apoE is synthesised in many tissues of the body, including the kidney, adrenal glands and monocyte derived macrophages (Eichner *et al*. 2002), and it is the structural characteristics of apoE that indicate its major function in lipid metabolism (Mahley & Rall, 2000). Two distinct but interacting functional domains have been identified on human apoE. The carboxyl terminal domain (216–299 amino acids) contains the major lipid-binding sites, while the amino terminal domain (1–191 amino acids), which is composed of the four amphipathic helices (typical of apo) that

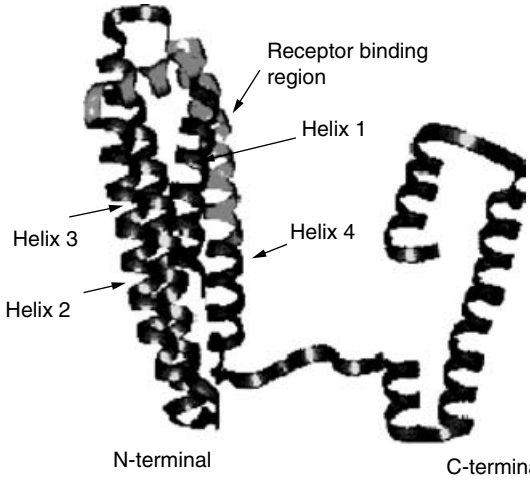

Fig. 1. Ribbon diagram of apoE illustrating a distinct binding region. (Image adapted from Garner, 2006.)

contain the receptor recognition region, binds to heparin sites and receptors such as the LDL receptor and LDL receptor-related protein (Siest *et al*. 1995).

Forty-seven polymorphisms of apoE have been characterised to date but the most widely studied have been the apoE2, apoE3 and apoE4 protein isoforms, which differ in the amino acid present at position 112 and 158 of the mature protein. In the most common isoform, apoE3, there is a single cysteine at position 112 and an arginine at 158. In the apoE2 polymorphism cysteine resides at both positions 112 and 158, whereas apoE4 displays arginine at both these positions. These polymorphisms lead to six different phenotypes in the human population, three homozygous (apoE3/3, apoE2/2 and apoE4/4) and three heterozygous (apoE2/3, apoE2/4, apoE3/4). The allelic variations in apoE are thought to have a profound effect on many aspects of protein structure, function and metabolism. In apoE4 the amino acid substitutions result in a changed structure, with the formation of a salt bridge between an arginine at position 61 and a glutamic acid at position 255, which causes the isoform to bind preferentially to VLDL (Mahley & Rall, 2000). In addition to their effect on the lipid-binding domain, the various apoE genotypes also impact on receptor-binding activity. Carriers of the apoE2 allelic variation are thought to have impaired binding capacity to hepatic LDL receptors as a result of changes within the salt bridge of helix four that results in the lowering of the positive ion potential of receptor-binding regions (Davignon *et al*. 1988). Individuals who are homozygous apoE2/E2 typically present with exaggerated fasting and postprandial lipaemia, and apoE2 homozygosity is a common cause of type 3 hyperlipidameia.

The distributions of the apoE phenotypes are detailed in Table 1 (adapted from a comprehensive review by Eichner *et al*. 2002). There are population differences in genotype distribution, with African populations tending to have a higher frequency of apoE4 compared with European countries (Eichner *et al*. 2002). Furthermore, there is a gradient in apoE4 frequency across Europe, with the

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Table 1. Frequency distribution (%) of apoE genotype in some populations (adapted from Eichner *et al.* 2002)

Population	Sample size	ApoE2	ApoE3	ApoE4
African	176	2·8	66·2	31
German	1557	8·2	78·2	13·6
Finnish	1577	3·9	76·7	19·4
French	504	8·1	80·2	11·7
Italian	260	7·3	82·7	10
Japanese	576	3·7	84·6	11·7

frequency of the allele being approximately 2-fold higher in Scandinavia compared with Mediterranean countries.

The impact of apoE genotypes on risk of developing CHD has been widely investigated and has been the subject of a recent meta-analysis (Song *et al*. 2004). This meta-analysis has demonstrated that the apoE4 allele confers an approximately 40% increase in the risk of CHD as compared with the common apoE3/E3 genotype and apoE2 carriers. The manipulation of dietary saturated fat:carbohydrate is often used as a way of decreasing total cholesterol, LDL-cholesterol and TAG. However, there has always been heterogeneity in the response among individuals. The reasons for this variability are numerous and include differences in age, gender, BMI and the dietary intervention used. The additional influence of genetic variability has also been the source of heterogeneity, with apoE polymorphisms being the most widely investigated. Numerous studies (Martin *et al*. 1993; Lopez-Miranda *et al*. 1994; Dreon *et al*. 1995; Schaefer *et al*. 1995) have also shown that the apoE genotype can influence the magnitude of total cholesterol and LDL-cholesterol responses to changes in dietary cholesterol alone (Martin *et al*. 1993), changes in dietary fat composition alone (Dreon *et al*. 1995) or combined changes in dietary cholesterol and dietary fat composition (Lopez-Miranda *et al*. 1994; Schaefer *et al*. 1995). In these studies carriers of the apoE4 allele have been shown to have a greater lipid response to dietary changes than individuals who do not have the apoE4 allele, indicating that individuals with the apoE4 allele are often sensitive to the manipulation of dietary fat, although this finding is not supported universally (Lefevre *et al*. 1997).

The gene–nutrient interaction between apoE polymorphisms and fish oil supplementation and their subsequent effect on lipid metabolism has not been extensively studied, despite apoE being one of the most-widely-explored genotypes in relation to lipid and apo metabolism. In addition, the individual effects of EPA and DHA and their relationship to apoE genotype has not been examined to date. However, a recent double-blind placebo-controlled cross-over trial (Minihane *et al*. 2000) has examined the effect of apoE polymorphisms and fish oil supplementation in volunteers with an atherogenic lipoprotein phenotype, which is characterised by moderate hypertriacylglycerolaemia, low levels of HDL-cholesterol and a predominance of small dense LDL3 particles (collective characteristics that can occur in $\leq$25% of middle-aged men and can lead to a 3-fold increase in CHD risk; Griffin, 1995). The volunteers were supplemented with 6 g fish oil (27·9% EPA and 22·3% DHA)/d or 6 g olive oil placebo/d for 6 weeks, with a 12-week wash-out period between treatments, and genotyping for apoE polymorphisms was carried out retrospectively. Fish oil was found to lower fasting and postprandial TAG responses, with a tendency towards greater responsiveness in individuals with the apoE2 allele. In the group as a whole there was a non-significant 7% rise in LDL-cholesterol following fish oil supplementation. However, in the subgroups based on apoE genotype the greatest responsiveness was observed in the apoE4 carriers, with a more atherogenic shift in the plasma lipid profile, including a 7·4% (non-significant) decrease in HDL-cholesterol and 3·5% increase in total cholesterol, with a 16% increase in LDL-cholesterol. On the other hand, there was also a 26% reduction in the percentage of small dense LDL in this subgroup, which could in part counteract this pro-atherogenic shift in the LDL-cholesterol–HDL-cholesterol profile.

The study conducted by Minihane *et al*. (2000) demonstrates that the apoE genotype may in part determine the blood lipid response to fish oil intervention, and that the LDL-cholesterol increases may be largely evident in apoE4 carriers. However, only one such study is available in the literature and further studies are currently ongoing to examine the impact of EPA *v*. DHA and the apoE genotype on the blood lipid response and also to determine whether the increases in LDL-cholesterol evident in individuals who have the apoE4 allele are observed at lower, more dietary achievable, EPA + DHA intakes.

Concluding remarks

Long-term supplementation with fish oils is an effective method of reducing the levels of both fasting and postprandial TAG, which are recognised risk factors contributing to the initiation and progression of atherosclerosis. Responsiveness is highly variable, which is likely to be attributable to the dose of fish oils used, the EPA:DHA of the supplement, the gender and health status of the study participants and their genetic profile. Currently, in the UK 'blanket' dietary EPA and DHA recommendations are provided to the non-pregnant adult population. Further research may provide a better understanding of the relative impact of EPA *v*. DHA in various genotype and gender subgroups, which may in the future allow the provision of more individualised dietary advice with a view to maximising the benefit gained by the individual.

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