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ApoE genotype: from geographic distribution to function and responsiveness to dietary factors

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ApoE is a key protein in lipid metabolism with three major isoforms. *ApoE* allele frequencies show non-random global distribution especially in Europe with high *apoE* ε*3* frequency in the Mediterranean area, whereas the *apoE* ε*4* genotype is enriched in Northern Europe. The *apoE* ε*4* genotype is one of the most important genetic risk factors for age-dependent chronic diseases, including CVD and Alzheimer's disease (AD). The apoE polymorphism has been shown to impact on blood lipids, biomarkers of oxidative stress and chronic inflammation, which all may contribute to the isoform-dependent disease risk. Studies in mice and human subjects indicate that the *apoE* ε*3* but not the *apoE* ε*4* genotype may significantly benefit from dietary flavonoids (e.g. quercetin) and *n*-3 fatty acids. Metabolism of lipid soluble vitamins E and D is likewise differentially affected by the *apoE* genotype. Epidemiological and experimental evidence suggest a better vitamin D status in *apoE* ε*4* than ε*3* subjects indicating a certain advantage of ε*4* over ε*3*. The present review aims at evaluation of current data available on interactions between apoE polymorphism and dietary responsiveness to flavonoids, fat soluble vitamins and *n*-3 fatty acids. Likewise, distinct geographic distribution and chronic disease risk of the different apoE isoforms are addressed.

ApoE genotype: CVD: *n*-3 fatty acids: Vitamin D: Flavonoids

ApoE

ApoE is a prominent constituent of plasma and brain lipoproteins mediating cellular cholesterol uptake by interaction with cell surface receptors including LDL-receptor, LDL-receptor-related proteins and VLDL-receptor[1,2]. ApoE also binds to cell surface located glycosaminoglycans such as heparin sulphate proteoglycans to facilitate lipoprotein uptake[3]. In addition to regulation of extrahepatic cholesterol metabolism apoE is centrally involved in chylomicron clearance through uptake of remnants by the liver[4]. The apoE protein is produced in various tissues with particular high concentrations in liver, brain, kidney, lymphocytes and adipose tissue. Beyond its known function in lipid and cholesterol metabolism apoE is believed to modulate many aspects of ageing in brain and artery walls[5].

ApoE *allelic variation*

The human *apoE* gene is polymorphic with two major SNP (rs429358C>T, rs7412C>T) in the coding region of exon 4. The two nucleotide exchanges are revealed at the protein level as amino acid substitution (Arg→Cys) at positions 112 and 158 of the mature apoE protein[6]. There are three major protein isoforms (E2, E3 and E4) arising from the three possible genetic variants ε*2,* ε*3* and ε*4.* Although other mammals express apoE, allelic variation was only found in human subjects. Sequence analysis revealed that

Abbreviations: AD, Alzheimer's disease; ALA, α-linolenic acid.

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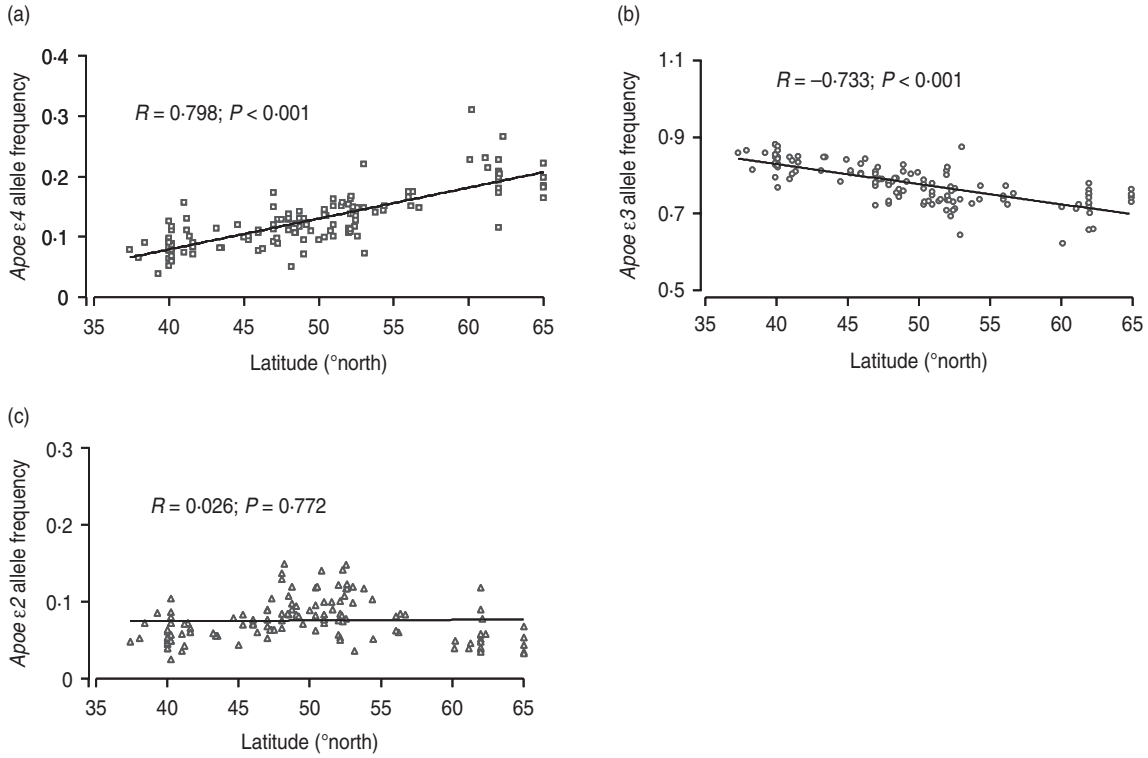

Fig. 1. Correlation of (a) *apoE ε4*, (b) *apoE ε3* and (c) *apoE ε2* allele frequencies in Europe with respective latitudes. Data on allele frequency and latitude were adapted from Singh *et al.*[10] and Rodrigues *et al.*[13]. Linear regression of *apoE* allele frequency and latitude was calculated applying Pearson's correlation analysis and is given as regression coefficient *R* with corresponding *P*-values.

primate *apoE* is identical to human *apoE ε4* at the sites coding for Arg at positions 112 and 158[7]. Therefore, *apoE ε4* is considered as the ancestral human allele that, after the human and primate lineages split, was modified by single successive mutations breeding the *ε3* and *ε2* alleles[8]. As a result of combination of the allelic variants three homozygous (*ε2/ε2*, *ε3/ε3* and *ε4/ε4*) and three heterozygous (*ε2/ε3*, *ε2/ε4*, *ε3/ε4*) genotypes emerge with varying frequency throughout human populations. *ApoE ε4* is always the minor allele when compared with *apoE ε3*, whereas *apoE ε2* is least common and even absent in particular aborigine populations[9].

Geographic distribution of apoE *genotypes*

Distribution of the three major *apoE* alleles varies worldwide (Table 1); however, the *ε3* variant is most abundant in all human populations and ranges between 0·968 in Indians and 0·356 in Papuans[10]. Highest *apoE ε4* frequencies are found in Central Africa (including Pygmies (0·407)[9] and Tutsi (0·385)[10]); Oceania (including Papuans (0·368) and Australian Aborigines (0·260)) and in Saami people (0·310)[9]. Particularly low *apoE ε4* frequencies are found in Mediterranean and several Asian populations (<0·10). The *ε2* allele is rare or absent in Inuits, South Americans, Siberians and Mongolians, but relatively frequent in sub-Saharans, Malaysian and Papuans[10]. At the continental level, allele frequencies of *apoE ε3* and *ε4* are inversely correlated in Europe, Africa and North America. In Asian and Oceanian populations, both *ε2* and *ε4* frequencies rise, when *ε3* is less abundant. Of particular importance is the non-random north-to-south gradient of *ε4* and *ε3* alleles in Europe as shown in Fig. 1. The frequency of *apoE ε4* increases with increasing latitude, whereas the *ε3* allele frequency is negatively correlated with latitude. The occurrence of *apoE ε2* is independent of the European latitude. The significant pattern in latitudinal *apoE ε4* and *ε3* allele distribution is also found in North but not in South America and Asia[10]. Although in China existence of a south-to-north gradient in *ε4* frequency was reported[11]. A more recent study additionally modelled a curvilinear relationship where worldwide *ε4* allele frequencies first decrease with distance from the equator and then increase again at absolute latitudes higher than 35°. Importantly the population variation in *apoE ε4* frequency was suggested to be shaped by natural

Table 1. Allelic variation of apoE at the transcript and protein level and ranges of worldwide allele frequencies

	Transcript variation		Protein variation		Allele frequency*
	388	487	112	158	
apoE ε4	T	T	Arg	Arg	0·052–0·407
apoE ε3	C	T	Cys	Arg	0·553–0·911
apoE ε2	C	C	Cys	Cys	0–0·145

*Data from Corbo and Scacchi[9].

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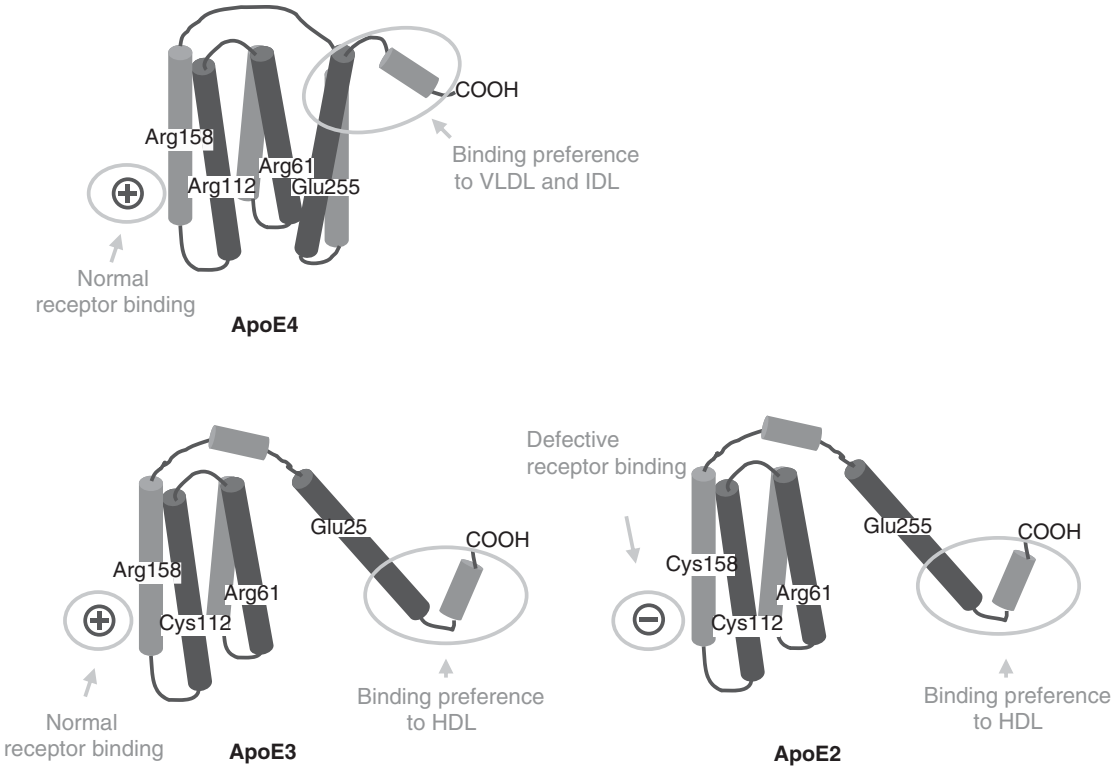

Fig. 2. Schematic protein structures of apoE4, apoE3 and apoE2 (adapted from Ye *et al.*[19]) showing amino acid residues that distinguish between the isoforms. Arg112 facilitates bridge formation (Arg61–Glu255) leading to domain interaction in the apoE4 isoform. Mutation at position 158 in apoE2 (Arg→Cys) changes domain charge from positive to negative (oval marking) and interferes with receptor binding. Additional oval marking of the C-terminal region that is responsible for apoE protein structure, self-association and ability to bind lipids and lipoprotein particles differentially organised in apoE4 and apoE3.

selection and not due to underlying population structure[12].

Generally *apoE ε4* is more present in people either with dark skin pigmentation or living in regions with low insolation, while lower presence is found in people with moderate melanin pigmentation but exposed to relatively high solar irradiation. Therefore, the capability of better enduring low UV concentrations may be an advantage of the ancestral *apoE ε4* compared with the new *ε3* genotype. The evolution of *apoE ε3* about 200 000 years ago[8] was accompanied by the establishment of more agricultural communities, as distinguished from simple hunters and gatherers, and subsequent emigration of the modern *Homo sapiens* from Africa[14]. However, it is uncertain precisely when and why *apoE ε3* began to expand in frequency and supersede the ancestral *ε4*. Furthermore, emergence of the recent *ε2* allele cannot be dated exactly yet. Due to the fact that *apoE ε2* is absent in people coming from north Asia settled in Arctic regions and America 40 000–10 000 years ago, *ε2* may likely first have emerged subsequent to this event[8].

ApoE protein isoforms

The mature apoE protein (34 kDa, 299 amino acids) comprises two structural helical domains, a bigger amino (N)-terminal (1–191) and a carboxyl (C)-terminal (216–299) region that are connected by a non-helical hinge region. The region responsible for receptor binding is determined in the N-terminal domain known to be rich in basic amino acids, whereas the region spanning residues 261–272 of the C-terminus determines lipoprotein and lipid-binding properties of apoE[15]. In the apoE4 isoform, the positive charge of Arg112 facilitates a domain interaction within the protein determined by a salt bridge formation between residues Arg61 and Glu255. The Arg61–Glu255 salt bridge is not present in apoE3 and apoE2 as Arg is substituted by Cys at position 112 (Fig. 2). Due to the domain interaction (Arg61–Glu255), the C-terminal domain is organised differentially in apoE4 compared with apoE3 and apoE2 and therefore, lipoprotein-binding affinity is also altered[16]. ApoE4 prefers binding VLDL and intermediate density lipoproteins, while apoE3 and apoE2 display a preference for cholesterol-rich HDL particles[17]. In the apoE2 isoform, the mutation at position 158 (Arg→Cys) causes a salt bridge formation revealing conformational changes that affect its LDL-receptor-binding domain[18]. Interestingly, although primate apoE holds an Arg residue at position 112 (similar to apoE4), there is a Thr at position 61 (instead of Arg in human apoE) preventing the interaction with the C-terminal domain. Therefore, primate apoE is in terms of function more related to human apoE3 than apoE4.

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ApoE *genotype and disease risk*

Beyond genotype-dependent effects on blood lipids, which will be reviewed in the following section, the apoE polymorphism is associated with age-related chronic as well as infectious diseases. Risk of CVD is dramatically increased in *apoE* ε*4* carriers with 40% increased incidence as compared with the ε*3* genotype[20]. This has been attributed to modestly elevated LDL-cholesterol in the ε*4* genotype, although mechanisms underlying *apoE* ε*4*-CVD-risk associations may be more complex. Several lines of evidence suggest that *apoE* ε*4* potentiates adverse effects of CVD-related risk factors such as smoking and physical inactivity[21,22]. In Alzheimer's disease (AD) association of *apoE* ε*4* and disease prevalence is even more striking. Presence and number of *apoE* ε*4* alleles increased AD risk (OR of 3·2 (ε*4*/ε*3*) and 14·9 (ε*4*/ε*4*) relative to ε*3*/ε*3*)[23] with each additional ε*4* allele shifting disease onset to younger age[24]. Poor neuronal repair, increased amyloid plaque burden and higher susceptibility towards oxidative insults have been suggested to underlie the positive association of *apoE* ε*4* and AD development[25–27]. In contrast *apoE* ε*2* appears to be protective compared with ε*3* both in CVD and AD[21,23].

There is increasing body of evidence that apoE may modulate susceptibility to viral infections in an isoform-dependent manner (extensively reviewed in Kuhlmann *et al.*[28]). ApoE4 increases fusion rate and cell entry of the HIV resulting in faster disease progression relative to apoE3, though the risk of acquiring HIV infection is independent of the apoE isoform[29]. Risk of herpes labialis and development of herpes simplex-associated AD is potentiated in *apoE* ε*4* carriers[30,31]. In contrast apoE4 protects against hepatitis C-induced liver damage and increases virus clearance attenuating chronic infection risk compared with apoE3[32,33]. Although data are scarce, it was suggested that apoE4 may also reduce heavy burden of early childhood diarrhoea and improve disease outcome in children in the first 2 years[34].

Overall the *apoE* ε*4* genotype is associated with increased morbidity and mortality in the elderly and the allele frequency is significantly declining from 85 years of age[35,36]. The influence of the ε*4* allele on mortality is even increasing in advanced age (92–103 years)[37]. Adverse effects of apoE4 may be attributed to altered lipid metabolism, but may also be mediated by differences in biomarkers of oxidative stress, inflammation and nuclear factor (erythroid-derived 2)-like 2-signalling.

Metabolic and molecular mechanisms of apoE isoforms

Lipid metabolism

Prospective cohort studies and human intervention studies have shown that the apoE polymorphism has a substantial effect on plasma lipids and lipoproteins (Table 2). Specifically, the apoE phenotypes have been associated with the variability of plasma total cholesterol concentrations and contribute to 4–12% of the variability of LDL-cholesterol concentrations in several populations[38]. In addition, a recent comprehensive meta-analysis demonstrated approximately linear relationships of *apoE* genotypes (when ordered ε*2*/ε*2*, ε*2*/ε*3*, ε*2*/ε*4*, ε*3*/ε*3*, ε*3*/ε*4* and ε*4*/ε*4*) with LDL-cholesterol concentrations and with CHD risk[39]. The LDL-cholesterol concentrations were approximately 30% lower in people with ε*2*/ε*2* than with ε*4*/ε*4* genotypes, a difference comparable with that produced by 'statin' therapy.

The impact of the different apoE isoforms on blood concentrations of lipids and lipoproteins has been explained by several mechanisms including (i) receptor-binding affinities of the different apoE-containing lipoproteins, (ii) dietary fat clearance, (iii) differences in the clearance of LDL apoB, and (iv) differences in the efficiency of intestinal cholesterol absorption (for review, see[38,50]).

In addition to the lower blood concentration of LDL-cholesterol (discussed earlier), the ε*2* allele is associated with lower blood concentrations of apoB and increased concentrations of TAG and apoE when compared with the ε*3* allele[51]. Similarly, *apoE* ε*2*/ε*2* and ε*2*/ε*3* are associated with lower concentrations of LDL-cholesterol when compared with ε*3*/ε*3*[52]. The increased concentrations of TAG and apoE are consistent with an impaired clearance of remnant particles[53]. The metabolic explanation for the reduced LDL-cholesterol concentrations is less clear. Individuals with the ε*2*/ε*2* genotype can develop a type III hyperlipoproteinemia. This is characterised by an accumulation of remnants of TAG-rich lipoprotein particles in plasma. It has been associated with several genetic abnormalities affecting lipoprotein metabolism including hepatic lipase deficiency and defects in the lipoprotein remnant receptor[52,54]. However, it should be noted that although the *apoE* ε*2*/ε*2* genotype is present in about 1% of the general population, less than 5% of individuals with ε*2*/ε*2* develop a type III hyperlipoproteinemia. Several secondary factors may thus promote type III hyperlipoproteinemia in individuals with ε*2*/ε*2* genotype such as a hormonal disturbance (e.g. hypothyroidism, oestrogen withdrawal and pregnancy), environmental factors (e.g. positive energy balance leading to obesity) or changes associated with increasing age[52,54].

Higher LDL-cholesterol, low TAG and apoE concentrations typically occur in individuals with ε*4*/ε*3* and ε*4*/ε*4* genotypes compared with ε*3*/ε*3* individuals[54]. The low TAG is consistent with the fact that carriers of the ε*4* allele clear circulating chylomicron remnants into the liver more rapidly than ε*3*/ε*3* individuals and twice as fast as ε*3*/ε*2* individuals[55]. ApoE is not a constituent of LDL particles, but it seems to have an indirect influence on LDL-cholesterol concentrations. In the fasting state, most plasma apoE resides in HDL particles. After intake of dietary fat, apoE shifts from HDL to postprandial particles. In ε*4*/ε*3* individuals, VLDL and HDL are enriched in apoE protein[56]. ApoE4 preferentially associated with VLDL is removed from the circulation more rapidly than apoE3[57]. Accordingly, it is supposed that individuals with the ε*4* allele may more efficiently and rapidly deliver dietary fat to the liver. Faster hepatic clearance of dietary fat in *apoE* ε*4*/ε*3* subjects could cause the down-regulation of LDL-receptors and an increase in plasma LDL-cholesterol[55].

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Table 2. The impact of apoE isoform on serum/plasma concentrations of lipids and lipoproteins in human subjects

Reference	Subjects	E2 (mmol/L)	E3 (mmol/L)	E4 (mmol/L)	Significant inter-group differences
Almeida *et al.*[40]	285 postmenopausal women; HRT+: mean age 56 (SD 6·7) years; HRT−: mean age 58 (SD 9·8) years	HRT+: TAG 1·5 (SD 0·02) LDL-C 3·04 (SD 1·07) HDL-C 1·62 (SD 0·33) HRT−: TAG 1·33 (SD 0·02) LDL-C 3·66 (SD 0·96) HDL-C 1·29 (SD 0·44)	HRT+: TAG 1·22 (SD 0·01) LDL-C 3·35 (SD 0·74) HDL-C 1·60 (SD 0·36) HRT−: TAG 1·41 (SD 0·02) LDL-C 3·87 (SD 0·94) HDL-C 1·24 (SD 0·29)	HRT+: TAG 1·45 (SD 0·02) LDL-C 3·56 (SD 0·72) HDL-C 1·57 (SD 0·36) HRT−: TAG 1·53 (SD 0·02) LDL-C 4·49 (SD 1·05) HDL-C 1·21 (SD 0·29)	HRT+: NS HRT−: LDL-C serum concentrations were higher in *ε4* carriers than in E2 and E3 groups.
Carvalho-Wells *et al.*[41]	251 healthy adults; mean age 53 (SD 1) years	TAG 1·74 (SD 0·12) LDL-C 3·51 (SD 0·16) HDL-C 1·33 (SD 0·06)	TAG 1·49 (SD 0·06) LDL-C 3·70 (SD 0·08) HDL-C 1·32 (SD 0·03)	TAG 1·88 (SD 0·12) LDL-C 3·82 (SD 0·12) HDL-C 1·31 (SD 0·06)	*ε4* carriers had higher plasma TAG concentrations compared to the *ε3/ε3* group; *ε4* carriers had higher plasma LDL-C concentrations compared to the E2 group.
Corella *et al.*[42] Framingham Offspring Study	1014 healthy men and 1133 healthy women; mean age 54 years	Men: TAG 1·99 (SD 1·43) LDL-C 2·92 (SD 0·86) HDL-C 1·14 (SD 0·32) *Women:* TAG 1·51 (SD 0·74) LDL-C 2·88 (SD 0·90) HDL-C 1·51 (SD 0·43)	Men: TAG 1·71 (SD 1·22) LDL-C 3·37 (SD 0·77) HDL-C 1·14 (SD 0·29) *Women:* TAG 1·46 (SD 0·87) LDL-C 3·25 (SD 0·85) HDL-C 1·47 (SD 0·39)	Men: TAG 1·87 (SD 1·31) LDL-C 3·43 (SD 0·83) HDL-C 1·08 (SD 0·28) *Women:* TAG 1·61 (SD 1·17) LDL-C 3·35 (SD 0·82) HDL-C 1·43 (SD 0·40)	Both male and female subjects with the *ε2* allele had lower plasma LDL-C concentrations than subjects with the *ε3* or *ε4* allele. No differences in means were observed between subjects with the *ε3* and *ε4* alleles.
Corella *et al.*[43] nested case-control study in the Spanish EPIC cohort	272 CHD cases and 496 controls; mean age 54 years	*CHD cases:* TAG 1·89 (SD 0·65) LDL-C 3·58 (SD 1·01) HDL-C 1·20 (SD 0·25) *Controls:* TAG 1·21 (SD 1·0) LDL-C 3·18 (SD 0·88) HDL-C 1·47 (SD 0·40)	*CHD cases:* TAG 1·75 (SD 1·37) LDL-C 4·05 (SD 0·86) HDL-C 1·28 (SD 0·39) *Controls:* TAG 1·36 (SD 0·78) LDL-C 3·71 (SD 0·82) HDL-C 1·39 (SD 0·35)	*CHD cases:* TAG 1·93 (SD 1·41) LDL-C 4·43 (SD 0·92) HDL-C 1·24 (SD 0·40) *Controls:* TAG 1·51 (SD 1·0) LDL-C 3·84 (SD 0·77) HDL-C 1·30 (SD 0·33)	In both incident CHD cases and controls, plasma LDL-C in *ε4* carriers > *ε3* carriers > *ε2* carriers.
Dietrich *et al.*[44]	274 healthy adults; mean age 46·9 (SD 13·0) years	– – –	TAG 1·28 (SD 0·79) LDL-C 3·36 (SD 0·95) HDL-C 1·08 (SD 0·29)	TAG 1·37 (SD 0·80) LDL-C 3·59 (SD 1·12) HDL-C 1·07 (SD 0·37)	NS
Egert *et al.*[45]	Ninety-three patients with metabolic syndrome traits; mean age 45 (SD 10·5) years	TAG 1·85 (SD 0·60) LDL-C 3·85 (SD 0·92) HDL-C 1·27 (SD 0·16)	TAG 2·01 (SD 1·05) LDL-C 3·35 (SD 0·94) HDL-C 1·47 (SD 0·47)	TAG 2·89 (SD 1·36) LDL-C 3·40 (SD 0·86) HDL-C 1·36 (SD 0·47)	Higher serum TAG concentrations in the APOE4 group compared with the E3 group.
Huebbe *et al.*[46]	699; general German population sample; mean age 63 (SD 7) years	– –	no APOE4 LDL-C 3·64 (SD 0·94) HDL-C 1·79 (SD 0·47)	APOE4 LDL-C 3·88 (SD 0·97) HDL-C 1·76 (SD 0·51)	NS
Minihane *et al.*[47]	50 males with an atherogenic lipoprotein phenotype; mean age 56 (SD 1) years	TAG 2·41 (SD 0·16) LDL-C 4·23 (SD 0·33) HDL-C 1·02 (SD 0·08)	TAG 2·56 (SD 0·21) LDL-C 4·35 (SD 0·17) HDL-C 1·00 (SD 0·03)	TAG 2·42 (SD 0·19) LDL-C 4·72 (SD 0·22) HDL-C 0·91 (SD 0·03)	Lower plasma HDL-C concentrations in the APOE4 group compared with the APOE2 and APOE3 group.
Miltiadous *et al.*[48]	200 normolipidaemic individuals; mean age 36 years	– – –	Non-E4 TAG 1·54 (SD 1·3) LDL-C 3·52 (SD 1·3) HDL-C 1·23 (SD 0·5)	E4 TAG 1·36 (SD 1·7) LDL-C 3·48 (SD 1·0) HDL-C 1·15 (SD 0·4)	NS
Scuteri *et al.*[49]	306 healthy men; mean age 58 years	– – –	Non-E4 TAG 1·30 (SD 0·84) LDL-C 3·09 (SD 0·79) HDL-C 1·11 (SD 0·27)	E4 TAG 1·35 (SD 0·81) LDL-C 3·27 (SD 0·90) HDL-C 1·08 (SD 0·27)	NS

Abbreviations: EPIC, European Prospective Investigation into Cancer and Nutrition; HDL-C, HDL-cholesterol; HRT, hormonal replacement therapy; HRT+, postmenopausal women HRT users; HRT−, postmenopausal women HRT non-users; LDL-C, LDL-cholesterol.

Table 3. The impact of apoE isoform on biomarkers of inflammation in human subjects

Reference	Subjects	Compared apoE isoforms	Biomarker	Significant inter-group differences
Golledge *et al.*[79]	1278 men	Non-E4 *v.* E4	Serum CRP	Non-E4>E4
Hubacek *et al.*[80]	6108 randomly selected adults	E3 *v.* E4	Plasma CRP	E3>E4
Miles *et al.*[81]	312 healthy adults	E2 *v.* E3 *v.* E4	Plasma CRP	E2>E3>E4
			Plasma IL-6	NS
			Plasma IL-10	NS
			Plasma TNFα	NS
Kravitz *et al.*[82]	227 people aged 90 years and older	Non-E4 *v.* E4	Serum CRP	Non-E4 = E4
Ojala *et al.*[83]	39 (26 AD, 4 vascular dementia, 9 control)	E3/E3 *v.* E4/E(2/3) *v.* E4/E4	Brain IL-18	NS
Angelopoulos *et al.*[84]	117 healthy adults	E2/E3 *v.* E3/E3 *v.* E3/E4	Serum CRP	E2/E3>E3/E4
Gronroos *et al.*[85]	1221 randomly selected Finns	E3/E2 *v.* E4/E2 *v.* E3/E3 *v.* E4/E3 *v.* E4/E4	Serum CRP	*Childhood:* E3/E2>E4/E2 = E3/E3> E4/E3>E4/E4 *Adulthood:* E3/E2>E4/E2 = E4/E3> E4/E4
Haan *et al.*[86]	1398 Latinos aged between 60–101 years	non-E4 *v.* E4	Blood CRP	Non-E4>E4
Park *et al.*[87]	394 adults (275 stroke cases 119 controls)	non-E2 *v.* E2	Serum CRP	Stroke: NS Control: NS
			Serum MMP-9	Stroke: non-E2<E2 Control: NS
			Serum TIMP-1	Stroke: (non-E2<E2)* Control: NS
		Non-E4 *v.* E4	Serum CRP, MMP-9 and TIMP-1	Stroke and control: NS
		Eiriksdottir *et al.*[88]	2251 adults	Non-E4 *v.* E3/E4 *v.* E4/E4
Blood CRP	Non-E4>E3/E4 Non-E4>E4/E4			
Kahri *et al.*[89]	368 adults (211 low-HDL-C subjects; 157 normolipidemic subjects)	E3 *v.* E4	Serum CRP	E3>E4
			Serum VCAM-1, I CAM-1 and E-selectin	NS
Mooijaart *et al.*[90]	546 adults aged 85 years	E2/E2 *v.* E2/E3 *v.* E2/E4 *v.* E3/E3 *v.* E3/E4 *v.* E4/E4	Plasma CRP	NS
Ravaglia *et al.*[91]	671 adults aged 65 years and older	Non-E4 *v.* E4	Serum CRP	NS
Tziakas *et al.*[92]	70 chronic stable angina patients	E2/E3 *v.* E3/E3 *v.* E3/E4	Serum CRP	E3/E3>E3/E4
			Serum IL-10	(E2/E3>E3/E4)* E2/E3 = E3/E3 E2/E3>E3/E4 E2/E3>E3/E3 (E3/E3>E3/E4)*
Tziakas *et al.*[92]	166 patients with acute coronary syndrome	E2/E3 *v.* E3/E3 *v.* E3/E4	Serum CRP	E2/E3 = E3/E3>E3/E4
			Serum IL-10	E3/E3>E3/E4 (E2/E3>E3/E4)* E2/E3 = E3/E3
Paschos *et al.*[93]	50 dyslipidemic men	E2/E3 *v.* E3/E3 *v.* E3/E4	Serum CRP, IL-6, MCFS, SAA	NS
Austin *et al.*[94]	552 Japanese Americans	E2 *v.* E3 *v.* E4	Plasma CRP	E2>E4
Marz *et al.*[95]	1309 adults (571 controls 738 cases of coronary artery disease)	E2 *v.* E3 *v.* E4	Serum CRP	E2>E4 E3>E4 E2 = E3
Pertovaara *et al.*[96]	63 pSS patients	Non-E4 *v.* E4	Plasma CRP, IL-6, TNFα	NS

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Table 3 (Continued)

Reference	Subjects	Compared apoE isoforms	Biomarker	Significant inter-group differences
Sun *et al.*[97]	141 'probable AD'	Non-E4 *v.* E4	Plasma IL-6	NS
			Plasma TNFα	(non-E4<E4)*
			Plasma MCP-1	NS
			CSF IL-6 and MCP-1	NS
Drabe *et al.*[98]	22 patients undergoing cardiopulmonary bypass	Non-E4 *v.* E4	Plasma IL-8	Non-E4<E4
			Plasma TNFα	Non-E4<E4
Manttari *et al.*[99]	272 adults (136 myocardial infarction or coronary death; 132 controls)	E3/E2 *v.* E3/E3 *v.* E4/E2 *v.* E4/E3 *v.* E4/E4	Serum CRP	E3/3>E3/2>E4/3>E4/2>E4/4
Egert *et al.*[45]	93 patients with metabolic syndrome traits	E3 *v.* E4	Serum CRP	E3>E4

AD, Alzheimer's disease; CRP, C-reactive protein; CSF, cerebrospinal fluid; ICAM-1, intercellular adhesion molecule 1; MCFS, macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein; MMP: matrix metalloproteinase; pSS, primary Sjögren's syndrome; SAA, serum amyloid A; TIMP-1, tissue inhibitor of metalloproteinase-1; VCAM-1, vascular cell adhesion molecule-1.
*Trend ($0{\cdot}05<P<0{\cdot}1$).

Oxidative stress, antioxidant defence and chronic inflammation

The first evidence that apoE may protect against oxidative stress was found in apoE-deficient mice with increased susceptibility of plasma lipoproteins to *in vitro* oxidation compared with wild-type mice[58]. Miyata and Smith[59] then postulated that antioxidative activity of apoE would be isoform dependent and that E4 was least and E2 most effective. The authors suggested different metal-binding capacities of the individual apoE isoforms that were possibly involved in the observed antioxidant effects. The presence of Arg112 rather than the absence of any cysteinyl groups in the protein appears to contribute to the increased oxidative susceptibility (due to altered protein stability) of apoE4 compared with apoE3 and its associated lipoproteins[60,61]. Neuronal cells cultured in apoE4 conditioned medium were more susceptible towards oxidative stress-induced cytotoxicity than in apoE3 conditioned medium[27]. Furthermore, innate immune cells produce higher levels of reactive oxygen or nitrogen species in the presence of apoE4 than apoE3[62–64]. Biomarkers of oxidative stress are elevated in *apoE ε4* carriers notably in subjects suffering from AD or CVD[44,65–68]. Expression of anti-atherogenic paraoxonase 1, which inhibits and reverses LDL-oxidation, is also lower in apoE4 than apoE3-targeted replacement mice[69]. Recent evidence suggests that the *apoE ε4* genotype is associated with lower expression of the antioxidant enzyme heme oxygenase 1 and other nuclear factor (erythroid-derived 2)-like 2 target genes[70]. Although data are sometimes conflicting nuclear factor (erythroid-derived 2)-like 2 may play a role in preventing atherosclerosis[71]. In summary, modulation of oxidative stress and antioxidant defence mechanisms may be a relevant physiological function of apoE, which is implemented in an isoform-dependent manner.

A number of studies have been conducted investigating the role of apoE in inflammatory processes mostly in models of neurodegeneration. Indeed chronic inflammation is associated with neurodegenerative disorders such as AD[72]. ApoE has been shown to modulate inflammatory response in either direction, pro- and anti-inflammatory[73]. However, expression of pro-inflammatory markers such as cytokines and NO in stimulated microglia and macrophages was higher in the presence of apoE4 than apoE3[74–76]. Higher pro-inflammatory response in apoE4 may be mediated by increased and prolonged activation of the redox-sensitive transcription factor NF-κB[74,75]. Chronic inflammation in the brain coincides with amyloid plaque pathology and both are more pronounced in apoE4 than apoE3 transgenic mice[77]. *ApoE ε4* is significantly associated with higher serum amyloid P (acute phase protein) in mice[78] suggesting an elevated level of chronic low grade inflammation which may contribute to the increased chronic disease risk of *ε4* as compared with non-*ε4* carriers (Table 3).

Responsiveness of the *apoE* genotype to dietary factors

Vitamin E

Vitamin E comprises eight different tocopherols and tocotrienols, α-tocopherol being biologically the most important vitamer (herein after referred to as vitamin E). Dietary vitamin E is postprandially delivered to the plasma via chylomicrons released from enterocytes or via VLDL following hepatic secretion. Under basal conditions, vitamin E is mainly associated with LDL particles with a constant flux existing between the different lipoprotein classes. Since apoE polymorphism affects concentration and clearance of plasma lipoproteins, it is conceivable that vitamin E metabolism is also impacted by the *apoE* genotype. Although a few studies found no difference in plasma vitamin E levels between *apoE* genotypes under baseline conditions[100,101], a biokinetic approach using stable isotopes observed higher newly absorbed vitamin E levels among *ε4* as compared with *ε3* carriers[102]. Extra-hepatic

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Table 4. Effects of the apoE isoform on parameters of vitamin D and Ca status evident in apoE4 compared with apoE3 targeted gene replacement mice[46]

Parameter of vitamin D and Ca status	
Serum 25-(hydroxy)-vitamin D level	APOE4>APOE3
mRNA level of genes encoding proteins involved in	
Bile acid production (*Cyp7a1*)	APOE4>APOE3
Vitamin D binding in kidney (*Lrp2*) and serum (*Gc*)	APOE4>APOE3
Renal absorption of Ca from primary urine (*Trpv6*, *S100g*)	APOE4<APOE3
Bone Ca concentration	APOE4>APOE3
Intestinal Ca absorption	APOE4>APOE3

Cyp7a1, cholesterol-7-α-hydroxylase; *Lrp2*, LDL-receptor-related protein 2; *Gc*, vitamin D binding protein; *Trpv6*, Ca transport protein 1; *S100g*, calbindin D9K.

vitamin E concentration is lower in apoE4- than apoE3-targeted replacement mice, which is most likely due to the lower expression of LDL-receptor and related receptor classes mediating vitamin E uptake[103]. Furthermore, degradation of vitamin E may be increased in the *apoE ε4* genotype contributing to lower tissue retention and therefore possibly lower vitamin E status in peripheral tissues[104].

Vitamin D

Unlike vitamin E the impact of the apoE polymorphism on vitamin D status is more pronounced. We recently provided first experimental and epidemiological evidence suggesting the *apoE ε4* genotype is associated with higher circulating vitamin D levels[46]. In targeted gene replacement, mice expressing human apoE4 serum 25-hydroxy-vitamin D concentration was significantly higher compared with apoE3- and apoE2-expressing mice. The observed higher serum concentration may be a result of increased intestinal absorption of dietary vitamin D as the mRNA level encoding for the key enzyme in bile acid production was higher in apoE4 than E3 and E2 mice. Elevated renal reabsorption of vitamin D from primary urine may also contribute to better vitamin D status as loss of vitamin D due to renal excretion would be reduced in the *apoE ε4* genotype. Furthermore, a higher femoral Ca concentration was evident in apoE4 *v.* apoE3 mice accompanied by relatively higher dietary Ca absorption. Supportive of a better vitamin D and Ca status, apoE4 mice showed increased renal Ca excretion and lower mRNA levels of renal Ca absorption genes that would have been induced upon hypocalcaemia (Table 4). These data illustrate that apoE4 compared with apoE3 mice have a better vitamin D and Ca status while dietary supply of both nutrients was similar in the apoE genotype groups[46]. In addition, circulating vitamin D was assessed in two independent human samples from northern Germany. Serum concentration of 25-hydroxy-vitamin D was significantly higher in subjects carrying *ε4* as compared with non-ε4 carriers. Mean 25-hydroxy-vitamin D concentration of both samples was <50 nmol/l suggesting a mild vitamin D deficiency throughout study participants. Mild vitamin D deficiency is relatively common in people inhabiting the same geographic latitude and has been reported before[105,106]. Our data suggest *apoE ε4* as a modulator of vitamin D and Ca status in apoE transgenic mice and in a population with insufficient vitamin D supply, which in the light of evolutionary aspects may explain non-random geographic distribution of *apoE* alleles (see subsection 'Geographic distribution of *apoE* genotypes').

Flavonoids

Flavonoids are a large group of secondary plant metabolites with >6000 distinct flavonoids identified to date[107]. Epidemiological studies, together with data from animal models and some clinical trials, suggest a role of dietary flavonoids in the prevention of CVD and other age-related chronic diseases[108–110]. The flavonol quercetin exhibits a wide range of physiological effects such as inhibition of LDL-oxidation, lowering of arterial blood pressure and platelet aggregation, and improvement of endothelial function as shown in animal models and in human subjects[111–117]. Furthermore, cell culture and animal studies indicate a potent anti-inflammatory activity of quercetin[116,118–120].

Current scientific evidence from human and animal studies indicates that the *apoE* genotype may be an important determinant of the responsiveness to dietary quercetin. We have recently found that overweight and obese patients with metabolic syndrome traits carrying the *apoE ε3* are highly responsive towards the blood pressure lowering effects of dietary quercetin supplementation, whereas *apoE ε4* carriers, by large, do not benefit[45] (Table 5). We hypothesised that quercetin supplementation may have resulted in higher endothelial NO levels in *apoE ε3 v. apoE ε4* carriers due to potential differences in the cellular redox and inflammatory states between the two genotypes. In addition, we found *apoE* genotype-specific effects of quercetin on fasting serum concentrations of HDL-cholesterol and apoA1 and on the ratio of LDL:HDL-cholesterol. Quercetin significantly decreased serum HDL-cholesterol and ApoA1 in *apoE* ε4 allele carriers but not in homozygous *ε3/ε3*. Moreover, our recent findings in apoE3- and apoE4-targeted gene replacement mice indicated that apoE3 animals were more responsive to the TNFα-lowering properties of dietary quercetin supplementation compared with apoE4 animals[119]. Therefore, the *apoE* genotype may in part explain the large heterogeneity of studies regarding potential health effects of flavonoids in human subjects where cohorts are not genotyped for apoE polymorphisms.

Plant and marine n-3 *fatty acids*

A large body of epidemiological data and evidence from randomised controlled human trials has demonstrated the cardioprotective effects of the marine *n*-3 fatty acids EPA and DHA[123–125]. For example, EPA and DHA have been shown to improve dyslipidaemia, to lower blood pressure and heart rate, to reduce inflammation, and to improve vascular function[125–127]. An alternative (*n*-3) PUFA is

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Table 5. ApoE isoform and responsiveness to flavonoid manipulation in human subjects – evidence from randomised controlled intervention studies

Reference	Subjects analysed	Study design	Intervention and duration	CVD biomarkers	Significant effects of intervention (E2 *v.* E3 *v.* E4)
Atkinson *et al.*[121]	177 menopausal women; mean age 55·1 years; retrospectively genotyped	Randomised, double-blind, placebo-controlled, parallel, two groups	43·5 mg/d red clover-derived isoflavones or placebo for 12 months	Fasting serum Chol, LDL-C, HDL-C, TAG, fibrinogen, PAI-1; SBP, DBP	Interactions between apoE and treatment for changes in Chol and LDL-C tended to be significant ($P = 0{\cdot}06$ and $P = 0{\cdot}05$, respectively). Women with the *ε2/ε3* genotype appeared to respond more favourably to the intervention than women with the *ε3/ε3* or *ε3/ε4* genotypes.
Egert *et al.*[45]	93 patients with metabolic syndrome traits; mean age 45 (SD 10·5) years; retrospectively genotyped	Randomised, double-blind, placebo-controlled, crossover, two treatments	150 mg/d quercetin or placebo for 6 weeks separated by a 5-week wash-out period	Fasting serum Chol, LDL-C, HDL-C, TAG, apoB, apoA1, glucose, uric acid, CRP, TNFα, plasma ox-LDL, waist circumference, body composition, resting SBP and DBP	In contrast to placebo, quercetin decreased SBP in the apoE3 group, whereas no effect was observed in the apoE4 group. In the apoE4 group, quercetin decreased HDL-C and apoA1, whereas both variables remained unchanged in the apoE3 group.
Pfeuffer *et al.*[122]	49 healthy male subjects; mean age 59·4 (SD 0·9) years; prospectively recruited according to apoE genotype	Randomised, double-blind, placebo-controlled, crossover, two treatments	150 mg/d quercetin or placebo for 8 weeks separated by a 3-week wash-out period	Fasting and postprandial vascular endothelial function, serum Chol, LDL-C, HDL-C, TAG, glucose, insulin, sVCAM, sICAM, sE-selectin, CRP, TNFα, plasma ox-LDL; urinary 8-iso-PGF2α, erythrocyte glutathione, waist circumference, resting SBP and DBP	Quercetin reduced BMI, body weight and waist circumference in apoE3 but not in apoE4 subjects.

Chol, cholesterol; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; ox-LDL, oxidized LDL; PAI-1, plasminogen activator inhibitor type 1; SBP, systolic blood pressure; sE-selectin, soluble endothelial-selectin; sICAM, soluble intracellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule; urinary 8-iso-PGF2α, 8-epimer of PG F2α.

Table 6. ApoE isoform and responsiveness to *n*-3 fatty acid manipulation in human subjects – evidence from randomised controlled intervention studies

Reference	Subjects analysed	Study design	Intervention and duration	CVD biomarkers	Significant effects of intervention (apoE2 *v.* E3 *v.* E4)
ALA					
Paschos *et al.*[93]	50 dyslipidaemic patients; mean age 50·4 (SD 7·3) years; retrospectively genotyped	Dietary intervention study, one treatment group	Supplementation of the diet with 15 ml/d flaxseed oil (8·1 g/d ALA) for 12 weeks	Fasting serum Chol, LDL-C, HDL-C, TAG, apoA1, apoB, SAA, CRP, MCSF, IL-6; LDL density	ALA decreased HDL-C and apoA1 in the *ε3/ε3* homozygotes; ALA decreased SAA and MCSF in the subgroups *ε3/ε3* and *ε3/ε4*; in addition, ALA decreased CRP and IL-6 in *ε3/ε3* individuals.
EPA/DHA					
Caslake *et al.*[130]	312 healthy adults; mean age 45·0 (SD 0·7) years; prospectively recruited according to age, sex and apoE genotype	Randomised, double-blind, placebo-controlled, crossover, three treatments	0·7 g/d EPA/DHA or 1·8 g/d EPA/DHA or control oil (placebo) for 8 weeks separated by 12-week wash-out periods	Fasting plasma Chol, VLDL-C, LDL-C, HDL-C, TAG, LDL and HDL subclasses, NEFA, glucose, apoE, apoB, apoA1, insulin, α-tocopherol, ox-LDL	In the group as a whole, 8 and 11% lower plasma TAG concentrations were evident after 0·7 EPA/DHA and 1·8 EPA/DHA, respectively: significant sex × treatment and sex × genotype × treatment interactions were observed, and the greatest TAG-lowering responses were evident in apoE4 men.
Minihane *et al.*[47]	50 males with an atherogenic lipoprotein phenotype; mean age 56 (SD 1) years; retrospectively genotyped	Randomised, double-blind, placebo-controlled, crossover, two treatments	6 g/d fish oil (3 g/d EPA/DHA) or 6 g/d olive oil (placebo) for 6 weeks separated by a 12-week wash-out period	Fasting and postprandial plasma concentrations of Chol, LDL-C, HDL-C, TAG, NEFA; LPL activity	Individuals with an apoE2 allele displayed a marked reduction in postprandial incremental TAG response and a trend towards an increase in LPL activity relative to non-E2 carriers. In apoE4 individuals, a significant increase in Chol and a trend towards a reduction in HDL-C relative to the homozygous E3/E3 profile was evident.
Olano-Martin *et al.*[131]	38 healthy males; mean age 42·7 (SD 2·2) years; prospectively recruited on the basis of apoE genotype	Randomised, double-blind, placebo-controlled, crossover, three treatments	3·3 g/d EPA or 3·7 g/d DHA or control oil (placebo) for 4 weeks separated by 10-week wash-out periods	Fasting plasma Chol, LDL-C, HDL-C, TAG, non-HDL-C, Lp(a), %LDL3, LDL mass, %HDL3, HDL mass, apoB, apoE, plasma lipoprotein compositions	For Chol, no treatment effects were evident; however, a genotype by treatment interaction emerged, with a differential response to EPA and DHA in *ε4* carriers. Although the genotype × treatment interaction for LDL-C ($P = 0{\cdot}089$) did not reach significance, within DHA treatment analysis indicated a 10% increase in LDL-C ($P = 0{\cdot}029$) in E4 carriers with a non-significant 4% reduction in *ε3/ε3* individuals. A genotype-independent increase in LDL mass was observed following DHA intervention.

ALA, α-linolenic acid; Chol, total cholesterol; CRP, C-reactive protein; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; LPL, lipoprotein lipase; MCSF, macrophage colony stimulating factor; ox-LDL, oxidized LDL; SAA, serum amyloid A; VLDL-C, VLDL-cholesterol.

plant-derived α-linolenic acid (ALA), which in stable-isotope studies in human subjects was shown to be desaturated and elongated to long-chain (*n*-3) PUFA[128]. ALA may also protect against CHD. However, the data concerning the protective role of ALA are less definitive than that for the long-chain *n*-3 PUFA, EPA and DHA[129].

Recent evidence suggests that the *apoE* genotype may predict the lipid and lipoprotein response to *n*-3 fatty acid interventions (Table 6). Minihane *et al.*[47] examined the effect of apoE polymorphism and fish oil supplementation on volunteers with an atherogenic lipoprotein phenotype, which is characterised by moderate hypertriacylglycerolaemia, low concentrations of HDL-cholesterol and a predominance of small dense LDL3 particles. Fish oil (3 g/d EPA and DHA) was found to lower fasting and postprandial TAG responses, with a tendency towards greater responsiveness in individuals with the *apoE* ε*2* 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 *apoE* ε*4* 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. This study demonstrated for the first time 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 *apoE* ε*4* carriers.

In a subsequent trial using a prospectively genotyped cohort of metabolically healthy participants, Caslake *et al.*[130] systematically investigated the effect of apoE polymorphism, sex and age on lipid responses to modest fish oil supplementation (0·7 or 1·8 g/d EPA and DHA). In contrast with the previously described data[47], there was no significant effect of *apoE* genotype on LDL-cholesterol. It was speculated that the effect of *apoE* genotype on LDL-cholesterol response may be dose dependent[130]. In addition, there was no significant effect of *apoE* genotype on the responsiveness to TAG lowering by EPA and DHA. However, there was a trend towards greater responsiveness in carriers of the ε*4* allele: a significant sex × genotype × treatment interaction was seen, and 15 and 23 % reductions in TAG were evident in male ε*4* carriers, respectively. It was speculated that the selective affinity of the E4 protein isoform for VLDL, in contrast with the E2 and E3 isoforms, which have a preference for the more lipid-poor large HDL protein, may explain the apparently greater TAG lowering in apoE4 subjects[130]. A recent study of the same research group systematically examined the individual impact of EPA- *v.* DHA-rich oils fed separately on plasma lipids in ε*3*/ε*3* *v.* ε*3*/ε*4* normolipidaemic males[131]. In the ε*3*/ε*4* group, within-treatment group analysis showed that DHA treatment, but not EPA, resulted in a significant increase in LDL-cholesterol, with a non-significant decrease in the ε*3*/ε*3* group. As this proatherogenic shift may negate the cardioprotective actions of DHA, it was suggested that EPA-rich oils may be a more suitable therapy for apoE4 subjects[131].

The gene–nutrient interaction between apoE polymorphism and ALA and their subsequent effect on lipid metabolism and further CVD biomarkers has not been extensively studied until now. There is only one, uncontrolled study (no control group) in dyslipidaemic patients indicating that ALA may have beneficial effects on biomarkers of inflammation in carriers of the *apoE* ε*3*/ε*3* and *apoE* ε*3*/ε*4* genotypes, but not in carriers of the *apoE* ε2 allele[93]. Owing to the limited number of ε*2* (7 %) and ε*4* allele carriers (10 %) these results need confirmation in larger, well-controlled and well-powered studies in prospectively genotyped participants.

Conclusions

In the present review, we have considered current data on apoE polymorphism. We have summarized metabolic impacts of the apoE isoforms and their responsiveness to dietary factors possibly underlying the geographic distribution and varying disease risk of the major apoE isoforms. The emergence and successful distribution of *apoE* ε*3* have been put down to decreased susceptibility to AD and CVD in later life compared with *apoE* ε*4*; however, it could also be a result of varying responsiveness to dietary factors already present in younger life. The beneficial effects of quercetin and *n*-3 fatty acids were observed in individuals carrying the ε*3* allele, but not in ε*4* carriers indicating apoE3 a more flexible and responsive phenotype than apoE4. On the other hand, due to better clearance of dietary fat and reduced LDL uptake, the *apoE* ε*4* genotype is associated with better vitamin D status and may provide protection against several infectious diseases. This could help to understand why the frequency of the ε*4* allele follows a distinct pattern of geographic distribution and is enriched in particular regions (e.g. Northern Europe with insufficient UV-exposure in autumn and winter). Taken together, the *apoE* genotype appears to be an important determinant of individual responsiveness to dietary factors; however, large prospectively genotyped cohorts are required to confirm present data and to assess the clinical relevance of apoE isoform-dependent effects.

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