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 e3 frequency in the Mediterranean area, whereas the ApoE e4 genotype is enriched in Northern Europe. The ApoE e4 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 e3 but not the ApoE e4 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 e4 than e3 subjects indicating a certain advantage of e4 over e3. 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-receptor¹². ApoE also binds to cell surface located glycosaminoglycans such as heparin sulphate proteoglycans to facilitate lipoprotein uptake³. In addition to regulation of extrahepatic cholesterol metabolism apoE is centrally involved in chylomicron clearance through uptake of remnants by the liver⁴. 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 walls⁵.

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 protein⁶. There are three major protein isoforms (E2, E3 and E4) arising from the three possible genetic variants ε2, ε3 and ε4. Although other mammals express apoE, allelic variation was only found in human subjects. Sequence analysis revealed that
primate apoE is identical to human apoE ε4 at the sites coding for Arg at positions 112 and 158\(^\text{7}\). 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\(^\text{8}\). As a result of combination of the allelic variants three homozygous (ε2ε2, ε3ε3 and ε4ε4) and three heterozygous (ε2ε3, ε2ε4, ε3ε4) 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\(^\text{9}\).

**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\(^\text{10}\). Highest apoE ε4 frequencies are found in Central Africa (including Pygmies (0.407)\(^\text{9}\) and Tutsi (0.385)\(^\text{10}\)); Oceania (including Papuans (0.368) and Australian Aborigines (0.260)) and in Saami people (0.310)\(^\text{9}\). 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-Saharan, Malaysian and Papuan\(^\text{10}\). 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\(^\text{10}\). Although in China existence of a south-to-north gradient in ε4 frequency was reported\(^\text{11}\). 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

<table>
<thead>
<tr>
<th>Transcript variation</th>
<th>Protein variation</th>
<th>Allele frequency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε4</td>
<td>T</td>
<td>Arg</td>
</tr>
<tr>
<td>ε3</td>
<td>C</td>
<td>Cys</td>
</tr>
<tr>
<td>ε2</td>
<td>C</td>
<td>Cys</td>
</tr>
</tbody>
</table>

*Data from Corbo and Scacchi\(^\text{9}\).
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.

In the apoE4 isoform, the positive charge of Arg112 facilitates a domain interaction 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.

ApoE4 prefers binding VLDL and intermediate density lipoproteins, while apoE3 and apoE2 display a preference for cholesterol-rich HDL particles.

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.
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 e4 carriers with 40% increased incidence as compared with the e3 genotype. This has been attributed to modestly elevated LDL-cholesterol in the e4 genotype, although mechanisms underlying apoE e4-CVD-risk associations may be more complex. Several lines of evidence suggest that apoE e4 potentiates adverse effects of CVD-related risk factors such as smoking and physical inactivity(21,22). In Alzheimer’s disease (AD) association of apoE e4 and disease prevalence is even more striking. Presence and number of apoE e4 alleles increased AD risk (OR of 3.2 (e4/e3) and 14.9 (e4/e4) relative to e3/e3) with each additional e4 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 e4 and AD development(25-27). In contrast apoE e2 appears to be protective compared with e3 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 e4 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 e4 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 e4 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 e2/e2, e2/e3, e2/e4, e3/e3, e3/e4 and e4/e4) with LDL-cholesterol concentrations and with CHD risk(39). The LDL-cholesterol concentrations were approximately 30% lower in people with e2/e2 than with e4/e4 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 e2 allele is associated with lower blood concentrations of apoB and increased concentrations of TAG and apoE when compared with the e3 allele51. Similarly, apoE e2/e2 and e2/e3 are associated with lower concentrations of LDL-cholesterol when compared with e3/e352. The increased concentrations of TAG and apoE are consistent with an impaired clearance of remnant particles53. The metabolic explanation for the reduced LDL-cholesterol concentrations is less clear. Individuals with the e2/e2 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 e2/e2 genotype is present in about 1% of the general population, less than 5% of individuals with e2/e2 develop a type III hyperlipoproteinemia. Several secondary factors may thus promote type III hyperlipoproteinaemia in individuals with e2/e2 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 e4/e3 and e4/e4 genotypes compared with e3/e3 individuals(54). The low TAG is consistent with the fact that carriers of the e4 allele clear circulating chylomicron remnants into the liver more rapidly than e3/e3 individuals and twice as fast as e3/e2 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 e4/e3 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 e4 allele may more efficiently and rapidly deliver dietary fat to the liver. Faster hepatic clearance of dietary fat in apoE e4/e3 subjects could cause the down-regulation of LDL-receptors and an increase in plasma LDL-cholesterol55.
Table 2. The impact of apoE isoform on serum/plasma concentrations of lipids and lipoproteins in human subjects

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>E2 (mmol/L)</th>
<th>E3 (mmol/L)</th>
<th>E4 (mmol/L)</th>
<th>Significant inter-group differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almeida et al.(40)</td>
<td>285 postmenopausal women; HRT +: mean age 56 (so 6:7) years; HRT -: mean age 58 (so 9:8) years</td>
<td>TAG 1:5 (so 0:02)</td>
<td>TAG 1:22 (so 0:01)</td>
<td>TAG 1:45 (so 0:02)</td>
<td>HRT +: HRT -:</td>
</tr>
<tr>
<td>Carvalho-Wells et al.(41)</td>
<td>251 healthy adults; mean age 53 (so 1) years</td>
<td>TAG 1:74 (so 0:12)</td>
<td>TAG 1:49 (so 0:06)</td>
<td>TAG 1:88 (so 0:12)</td>
<td>e4 carriers had higher plasma TAG concentrations compared to the e3/e3 group; e4 carriers had higher plasma LDL-C concentrations compared to the E2 group.</td>
</tr>
<tr>
<td>Corella et al.(42)</td>
<td>Framingham Offspring Study</td>
<td>Men: Men:</td>
<td>Men:</td>
<td>Tag 1:99 (so 1:43)</td>
<td>Tag 1:71 (so 1:22)</td>
</tr>
<tr>
<td>Corella et al.(43)</td>
<td>272 CHD cases and 496 controls; mean age 54 years</td>
<td>CHD cases: Controls:</td>
<td>CHD cases: Controls:</td>
<td>Tag 1:89 (so 0:65)</td>
<td>Tag 1:75 (so 1:37)</td>
</tr>
<tr>
<td>Dietrich et al.(44)</td>
<td>274 healthy adults; mean age 46-9 (so 13:0) years</td>
<td>–</td>
<td>–</td>
<td>Tag 1:28 (so 0:79)</td>
<td>Tag 1:37 (so 0:80)</td>
</tr>
<tr>
<td>Eger et al.(45)</td>
<td>Ninety-three patients with metabolic syndrome traits; mean age 45 (so 10:5) years</td>
<td>TAG 1:85 (so 0:60)</td>
<td>TAG 2:01 (so 1:05)</td>
<td>Tag 2:89 (so 1:36)</td>
<td>Higher serum TAG concentrations in the APOE4 group compared with the E3 group.</td>
</tr>
<tr>
<td>Huebbe et al.(46)</td>
<td>699; general German population sample; mean age 63 (so 7) years</td>
<td>–</td>
<td>–</td>
<td>Tag 1:54 (so 1:3)</td>
<td>Tag 1:36 (so 1:7)</td>
</tr>
<tr>
<td>Minihane et al.(47)</td>
<td>50 males with an atherogenic lipoprotein phenotype; mean age 56 (so 1) years</td>
<td>TAG 2:41 (so 0:16)</td>
<td>TAG 2:56 (so 0:21)</td>
<td>Tag 2:42 (so 0:19)</td>
<td>Lower plasma HDL-C concentrations in the APOE4 group compared with the APOE2 and APOE3 group.</td>
</tr>
<tr>
<td>Miltiadou et al.(48)</td>
<td>200 normolipaemic individuals; mean age 36 years</td>
<td>–</td>
<td>–</td>
<td>Tag 1:54 (so 1:3)</td>
<td>Tag 1:36 (so 1:7)</td>
</tr>
<tr>
<td>Scuteri et al.(49)</td>
<td>306 healthy men; mean age 58 years</td>
<td>–</td>
<td>–</td>
<td>Tag 1:30 (so 0:84)</td>
<td>Tag 1:35 (so 0:81)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Compared apoE isoforms</th>
<th>Biomarker</th>
<th>Significant inter-group differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golledge et al.</td>
<td>1278 men</td>
<td>Non-E4 v. E4</td>
<td>Serum CRP</td>
<td>Non-E4 &gt; E4</td>
</tr>
<tr>
<td>Hubacek et al.</td>
<td>6108 randomly selected adults</td>
<td>E3 v. E4</td>
<td>Plasma CRP</td>
<td>E3 &gt; E4</td>
</tr>
<tr>
<td>Miles et al.</td>
<td>312 healthy adults</td>
<td>E2 v. E3 v. E4</td>
<td>Plasma CRP</td>
<td>E2 &gt; E3 &gt; E4</td>
</tr>
<tr>
<td>Kravitz et al.</td>
<td>227 people aged 90 years and older</td>
<td>Non-E4 v. E4</td>
<td>Serum CRP</td>
<td>Non-E4 = E4</td>
</tr>
<tr>
<td>Golledge et al.</td>
<td>1278 men</td>
<td>Non-E4 v. E4</td>
<td>Serum CRP</td>
<td>Non-E4 &gt; E4</td>
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<tr>
<td>Hubacek et al.</td>
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</tr>
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<td>Golledge et al.</td>
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</tr>
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<td>Hubacek et al.</td>
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</tr>
<tr>
<td>Miles et al.</td>
<td>312 healthy adults</td>
<td>E2 v. E3 v. E4</td>
<td>Plasma CRP</td>
<td>E2 &gt; E3 &gt; E4</td>
</tr>
<tr>
<td>Haan et al.</td>
<td>1398 Latinos aged between 60–101 years</td>
<td>non-E4 v. E4</td>
<td>Blood CRP</td>
<td>Non-E4 &gt; E4</td>
</tr>
<tr>
<td>Park et al.</td>
<td>394 adults (275 stroke cases 119 controls)</td>
<td>non-E2 v. E2</td>
<td>Serum CRP</td>
<td>Stroke: NS</td>
</tr>
<tr>
<td>Kahlri et al.</td>
<td>368 adults (211 low-HDL-C subjects; 157 normolipidemic subjects)</td>
<td>E3 v. E4</td>
<td>Serum CRP</td>
<td>E3 &gt; E4</td>
</tr>
<tr>
<td>Ravaglia et al.</td>
<td>671 adults aged 65 years and older</td>
<td>Non-E4 v. E4</td>
<td>Serum CRP</td>
<td>NS</td>
</tr>
<tr>
<td>Paschos et al.</td>
<td>50 dyslipidemiac men</td>
<td>E2/E3 v. E3/E4 v. E3/E4</td>
<td>Serum CRP, IL-6, MCF6, SAA</td>
<td>NS</td>
</tr>
<tr>
<td>Austin et al.</td>
<td>552 Japanese Americans</td>
<td>E2 v. E3 v. E4</td>
<td>Plasma CRP</td>
<td>E2 &gt; E4</td>
</tr>
<tr>
<td>Marz et al.</td>
<td>1309 adults (571 controls 738 cases of coronary artery disease)</td>
<td>E2 v. E3 v. E4</td>
<td>Serum CRP</td>
<td>E2 &gt; E4</td>
</tr>
<tr>
<td>Pertovaara et al.</td>
<td>63 pSS patients</td>
<td>Non-E4 v. E4</td>
<td>Plasma CRP, IL-6, TNFα</td>
<td>NS</td>
</tr>
</tbody>
</table>
susceptibility of plasma lipoproteins to stress was found in apoE-deficient mice with increased presence of apoE4 than apoE3 (62–64). Biomarkers of oxidative stress are elevated in apoE that the 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

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 Plasma TNFα Plasma MCP-1 NS (non-E4 < E4)*

Drabe et al. (98) 22 patients undergoing cardiopulmonary bypass Non-E4 v. E4 CSF IL-6 and MCP-1 NS


Egert et al. (46) 93 patients with metabolic syndrome traits E3 v. E4 Serum CRP E3 > E4

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 antioxidative effects. The presence of Arg112 rather than the absence of any cysteinyln 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 target genes (70). Although data are sometimes conflicting nuclear 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 (0.05 < P < 0.1).
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 apoE4 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 apoE4 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 apoE4 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 e4 as compared with non-e4 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 e4 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 e3 are highly responsive towards the blood pressure lowering effects of dietary quercetin supplementation, whereas apoE e4 carriers, by large, do not benefit\(^{45}\) (Table 5). We hypothesised that quercetin supplementation may have resulted in higher endothelial NO levels in apoE e3 v. apoE e4 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 e4 allele carriers but not in homozygous e3/A3. Moreover, our recent findings in apoE3- and apoE4-targeted gene replacement mice indicated that apoE3 animals were more responsive to the TNF-α-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 4. Effects of the apoE isomorph on parameters of vitamin D and Ca status evident in apoE4 compared with apoE3 targeted gene replacement mice\(^{46}\)**

<table>
<thead>
<tr>
<th>Parameter of vitamin D and Ca status</th>
<th>APOE4 &gt; APOE3</th>
<th>APOE4 &lt; APOE3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 25-(hydroxy)-vitamin D level mRNA level of genes encoding proteins involved in Bile acid production (Cyp7a1) Vitamin D binding in kidney (Lrp2) and serum (Gc) Renal absorption of Ca from primary urine (Trpv6, S100g) Bone Ca concentration Intestinal Ca absorption</td>
<td>≥</td>
<td>&lt;</td>
</tr>
<tr>
<td>Cyp7a1, cholesterol-7-α-hydroxylase; Lrp2, LDL-receptor-related protein 2; Gc, vitamin D binding protein; Trpv6, Ca transport protein 1; S100g, calbindin D9K.</td>
<td>≥</td>
<td>&lt;</td>
</tr>
</tbody>
</table>
### Table 5. ApoE isoform and responsiveness to flavonoid manipulation in human subjects – evidence from randomised controlled intervention studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects analysed</th>
<th>Study design</th>
<th>Intervention and duration</th>
<th>CVD biomarkers</th>
<th>Significant effects of intervention (E2 vs. E3 vs. E4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atkinson et al. (121)</td>
<td>177 menopausal women; mean age 55-1 years; retrospectively genotyped</td>
<td>Randomised, double-blind, placebo-controlled, parallel, two groups</td>
<td>43.5 mg/d red clover-derived isoflavones or placebo for 12 months</td>
<td>Fasting serum Chol, LDL-C, HDL-C, TAG, fibrinogen, PAI-1; SBP, DBP</td>
<td>Interactions between apoE and treatment for changes in Chol and LDL-C tended to be significant (P = 0.06 and P = 0.05, respectively). Women with the e2/e3 genotype appeared to respond more favourably to the intervention than women with the e3/e3 or e3/e4 genotypes.</td>
</tr>
<tr>
<td>Egert et al. (45)</td>
<td>93 patients with metabolic syndrome traits; mean age 45 (± 10-5) years; retrospectively genotyped</td>
<td>Randomised, double-blind, placebo-controlled, crossover, two treatments</td>
<td>150 mg/d quercetin or placebo for 6 weeks separated by a 5-week wash-out period</td>
<td>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</td>
<td>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. Quercetin reduced BMI, body weight and waist circumference in apoE3 but not in apoE4 subjects.</td>
</tr>
<tr>
<td>Pfeuffer et al. (122)</td>
<td>49 healthy male subjects; mean age 59-4 (± 0-9) years; prospectively recruited according to apoE genotype</td>
<td>Randomised, double-blind, placebo-controlled, crossover, two treatments</td>
<td>150 mg/d quercetin or placebo for 8 weeks separated by a 3-week wash-out period</td>
<td>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</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects analysed</th>
<th>Study design</th>
<th>Intervention and duration</th>
<th>CVD biomarkers</th>
<th>Significant effects of intervention (apoE2 v. E3 v. E4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paschos et al.(^{(93)})</td>
<td>50 dyslipidaemic patients; mean age 50-4 (SD 7-3) years; retrospectively genotyped</td>
<td>Dietary intervention study, one treatment group</td>
<td>Supplementation of the diet with 15 ml/d flaxseed oil (8.1 g/d ALA) for 12 weeks</td>
<td>Fasting serum Chol, LDL-C, HDL-C, TAG, apoA1, apoB, SAA, CRP, MCSF, IL-6; LDL density</td>
<td>ALA decreased HDL-C and apoA1 in the ε3ε3 heterozygotes; ALA decreased SAA and MCSF in the subgroups ε3ε3 and ε3ε4; in addition, ALA decreased CRP and IL-6 in ε3ε3 individuals.</td>
</tr>
<tr>
<td>Caslake et al.(^{(130)})</td>
<td>312 healthy adults; mean age 45-0 (SD 0-7) years; prospectively recruited according to age, sex and apoE genotype</td>
<td>Randomised, double-blind, placebo-controlled, crossover, three treatments</td>
<td>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</td>
<td>Fasting plasma Chol, VLDL-C, LDL-C, HDL-C, TAG, LDL and HDL subclasses, NEFA, glucose, apoE, apoB, apoA1, insulin, α-tocopherol, ox-LDL</td>
<td>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.</td>
</tr>
<tr>
<td>Minihane et al.(^{(47)})</td>
<td>50 males with an atherogenic lipoprotein phenotype; mean age 56 (SD 1) years; retrospectively genotyped</td>
<td>Randomised, double-blind, placebo-controlled, crossover, two treatments</td>
<td>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</td>
<td>Fasting and postprandial plasma concentrations of Chol, LDL-C, HDL-C, TAG, NEFA; LPL activity</td>
<td>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.</td>
</tr>
<tr>
<td>Olano-Martin et al.(^{(131)})</td>
<td>38 healthy males; mean age 42-7 (SD 2-2) years; prospectively recruited on the basis of apoE genotype</td>
<td>Randomised, double-blind, placebo-controlled, crossover, three treatments</td>
<td>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</td>
<td>Fasting plasma Chol, LDL-C, HDL-C, TAG, non-HDL-C, Lp(a), %LDL3, LDL mass, %HDL3, HDL mass, apoB, apoE, plasma lipoprotein compositions</td>
<td>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 = 0.089)) did not reach significance, within DHA treatment analysis indicated a 10% increase in LDL-C ((P = 0.029)) in E4 carriers with a non-significant 4% reduction in ε3ε3 individuals. A genotype-independent increase in LDL mass was observed following DHA intervention.</td>
</tr>
</tbody>
</table>

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.
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 ε3/ε3 and apoE ε3/ε4 genotypes, but not in carriers of the apoE ε2 allele\(^{93}\). Owing to the limited number of ε2 (7%) and ε4 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 ε3 have been put down to decreased susceptibility to AD and CVD in later life compared with apoE ε4; 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 ε3 allele, but not in ε4 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 ε4 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 ε4 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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References


ApoE genotype and dietary responsiveness


