REVIEW

Implications of apolipoprotein E genotype on inflammation and vitamin E status

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In Western societies the apolipoprotein E4 (apoE4) genotype is associated with increased morbidity and mortality and represents a significant risk factor for cardiovascular and Alzheimer's disease. In a recent study we observed significantly lower tissue α-tocopherol (α-TOH) concentrations in apoE4 compared with apoE3 mice. Furthermore, genes encoding for proteins involved in peripheral α-TOH transport and degradation were affected by the apoE genotype. Thus, the apoE4 genotype may be associated with lower vitamin E retention in peripheral tissues. This is possibly related to an altered lipoprotein metabolism including increased α-TOH retention in LDL, a decreased expression of lipoprotein receptors and impaired cellular vitamin E delivery system, and a greater intracellular degradation of tocopherols in the apoE4 genotype. An increasing number of studies in cultured cells, transgenic mice and human volunteers indicate a more pro-inflammatory state associated with the apoE4 allele. In apoE4 macrophages there is an enhanced transactivation of the key redox sensitive transcription factor NF-κB accompanied by a higher production of pro-inflammatory molecules (tumor necrosis factor α, interleukin 1β, macrophage inflammatory protein 1-α) and a lower production of anti-inflammatory interleukin 10, as compared with apoE3 macrophages. Both tissue vitamin E retention and biomarkers of chronic inflammation may be affected by the apoE genotype.

Keywords:
Apolipoprotein E genotype / Cytokine / Inflammation / Tocopherol / Vitamin E

1 Apolipoprotein E

Apolipoprotein E (apoE) has raised particular interest since apoE polymorphisms are related to several chronic disorders such as Alzheimer's disease (AD) [1] and cardiovascular disease (CVD) [2], and in general an apoE4 genotype is negatively associated with longevity [3]. A meta-analysis of 48 studies published between 1996 and 2004 reported a 42% increased incidence of CVD in apoE4 carriers relative to the E3/E3 genotype, with a RR > 5 in E4/E4 individuals (~2% population) [2]. The impact on AD risk is even more dramatic with RR of 3.2 and 14.9 reported for E3/E4 and E4/E4 individuals, respectively [4]. ApoE is a major constituent of lipoproteins and a key regulator of lipid and cholesterol metabolism. ApoE is involved in the assembly of lipoproteins and serves as a high-affinity ligand for cellular lipoprotein uptake *via* members of several receptor classes including the apoE and LDL receptors. Apart from the liver, where apoE is mainly synthesised, several extra-hepatic tissues are able to produce significant amounts of apoE including the brain and monocyte-derived macrophages [5, 6].

The apoE gene is subjected to numerous polymorphisms of which the most common gives rise to three isoforms apoE2, E3 and E4. ApoE allelic distribution varies worldwide, but the apoE3 allele is the most abundant with a

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Abbreviations: α-TOH, α-tocopherol; **AD,** Alzheimer's disease; **apoE,** apolipoprotein E; **CM,** chylomicrons; **CVD,** cardiovascular disease; **CYP3A,** cytochrome P450 3A; **LPL,** lipoprotein lipase; **SR-B1,** scavenger receptor class B type 1

frequency of 60–90% and also referred to as the wild type. In Caucasian populations the apoE4 allelic frequency is reported to be 14%, while the apoE2 allele reaches a frequency of 8% [7]. However, in Europe there exists a geographic shift towards a more than twofold higher prevalence of the apoE4 allele in northern countries (*e.g.* Norway 19.8%) compared with southern countries (*e.g.* Italy 6.3%). Interestingly, there is also a north-south gradient in Europe in the incidence of CVD that is positively correlated with the prevalence of the apoE4 genotype. The distribution of apoE alleles in Germany is summarised in Fig. 1.

The apoE isoforms differ in the amino acid residue at positions 112 and 158 with apoE3 having cysteine and arginine at these sites. Substitution from cysteine to arginine at position 112 in the apoE4 isoform affects a side chain interaction ($61 \rightarrow 255$) within the protein and is supposed to impact on the protein stability forming a so-called ''molten globule'' configuration [8, 9]. In addition to reduced stability and higher susceptibility to degradation, the lipoprotein-binding preference of apoE4 differs compared with apoE3 and apoE2 due to the altered protein folding. Thus, apoE4 prefers binding to larger triglyceride-rich lipoproteins (VLDL and chylomicron (CM) remnants), whereas apoE3 and apoE2 preferentially bind to smaller cholesterol-rich particles such as HDL [10]. ApoE4 has been reported to have a 25-fold higher affinity to the LDL receptor than LDL itself [11], implicating that in apoE4 carriers, where the apoE4 protein is mainly associated to triglyceride-rich particles, uptake of LDL-cholesterol by the LDL receptor would be delayed in favour of VLDL and CM clearance. Moreover, LDL receptor expression is supposed to be down-regulated in the apoE4 *versus* apoE3 genotype [12] aggravating the competition for LDL receptor-mediated clearance. Finally, hepatic lipase mediated conversion of VLDL to LDL is significantly increased in the apoE4 compared with the apoE3 genotype [13].

All these findings may explain the trend towards higher LDL cholesterol levels in apoE4 carriers that has been reported in numerous studies, although not consistently significant.

However, the relatively moderate increase in LDL cholesterol (8%) cannot solely explain the disease differential between apoE4 and non-apoE4 carriers [14]. Thus, other mechanisms have been suggested to contribute to the increased disease risk associated with the apoE4 genotype [15] including increased chronic inflammation and activation of redox regulated transcription factors, decreased neuronal repair and decreased responsiveness towards dietary regimens such as flavonoids and fish oil (Table 1).

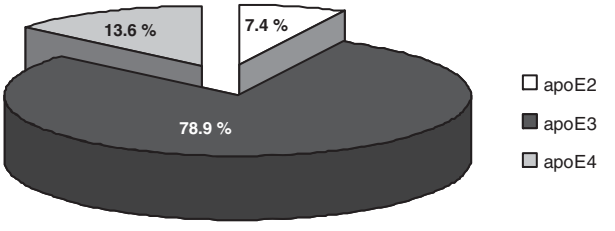

Figure 1. ApoE allele distribution in the German population. Data from Assman *et al.* [66], Boerwinkle and Utermann [67], and Orth *et al.* [68] were pooled.

The apoE protein shows antioxidant activity *in vitro*, although its *in vivo* relevance remains to be established. Interestingly, the antioxidant activity appears to be allele specific. Miyata and Smith found that at physiological concentrations all three apoE proteins significantly reduced H_2O_2-induced cell death, but in the following order of effectiveness $E2 > E3 > E4$, with the E2 protein being approximately twofold more effective compared with E4 [16]. However, evidence regarding the contribution of apoE genotype to oxidative stress-dependent processes is limited. In the Northwick Park Heart Study II the authors speculated that the 2.79-fold increased risk of CVD events in apoE4 smokers relative to the non-smoking group may be due to the different antioxidant capacity of the apoE protein isoforms [17], although no direct evidence was provided. Importantly, we have observed significantly higher (29%) circulating levels of F_2-isoprostanes, a surrogate biomarker of lipid peroxidation, in mildly hypercholesterolemic (> 5.6 mmol/L) apoE4 as compared with non-apoE4 carriers [18]. This finding is in line with other studies reporting higher plasma concentrations of oxidised LDL and malondialdehyde-modified LDL in apoE4 *versus* non-apoE4 allele carriers [19, 20]. Cigarette smoking has been associated with a higher consumption of plasma antioxidants related to increased oxidative stress [21]. Thus, it is of particular interest that smoking cigarettes is apparently more detrimental for apoE4 than apoE3 allele carriers as far as cardiovascular events are concerned [17]. This has been attributed to a dysregulation of the cellular oxidant/antioxidant status in apoE4 carriers.

In cell culture studies, apoE4 macrophages exhibited increased membrane oxidation and produced more reactive oxygen and nitrogen species upon stimulation with the phorbol ester PMA and bacterial LPS, respectively [22]. A higher transactivation of the key redox sensitive transcription factor NF-κB was evident in apoE4 as compared with apoE3 macrophages and was accompanied by a higher production of pro-inflammatory molecules (tumor necrosis factor α, interleukin 1β, macrophage inflammatory protein 1 α). Amounts of the anti-inflammatory cytokine interleukin 10 produced in apoE4 macrophages were lower than in apoE3 cells [23]. These data may indicate that apoE4 macrophages have an altered inflammatory response, which may contribute to the higher CVD risk observed in apoE4 carriers.

AD is characterised by massive neuronal loss in brain regions associated with memory and learning. Beyond others oxidative damage to neurons and sustained activation of microglia leading to neuroinflammation are believed to contribute to neuronal death in AD brains [24, 25]. In AD patients, the apoE4 genotype aggravates oxidative damage and decreases activity of antioxidative enzymes in the brain

Table 1. Effects of the apoE4 genotype possibly contributing to the increased disease risk

Model	Outcome	Author and year
ApoE3- and apoE4-transfected macrophages	Increased activity of the redox-sensitive transcription factor NF-κB	Jofre-Monseny *et al.* 2007 [23]
ApoE3- and apoE4-transfected macrophages	Increased production of pro-inflammatory proteins (TNF-α, IL1β, IL6, MIP1α), decreased production of anti-inflammatory IL10	Jofre-Monseny *et al.* 2007 [23]
ApoE3- and apoE4-transfected macrophages	Higher production of superoxide anions, increased membrane oxidation	Jofre-Monseny *et al.* 2007 [22]
Neuronal cells incubated with apoE3- and apoE4-conditioned medium	Increased susceptibility towards oxidative damage (H_2O_2 induced toxicity)	Huebbe *et al.* 2007 [28]
ApoE3- and apoE4-transfected neuroblastoma cells	Impaired neuronal remodelling (inhibition of neurite outgrowth)	Bellosta *et al.* 1995 [63]
Primary cell cultures from apoE3 and apoE4 transgenic mice	Increased vulnerability of neurons towards inflammation-related stress (neurotoxicity by activated microglia)	Maezawa *et al.* 2006 [30]
ApoE3 and apoE4 transgenic mice	Poorer dendritic recovery of hippocampal neurons following activation of innate immune response	Maezawa *et al.* 2006 [30]
ApoE3 and apoE4 transgenic mice	Decreased cognitive performance (poorer outcome in spatial learning and memory tests)	Raber *et al.* 2000 [31]
Human subjects, apoE4 *versus* non-apoE4 carriers	Increased level of circulating F_2-isoprostanes	Dietrich *et al.* 2005 [18]
Smokers with apoE4 *versus* apoE3 and apoE2 genotype	Increased level of oxidised LDL and decreased antioxidant status	Talmud *et al.* 2005 [19]
ApoE3 and apoE4 transgenic mice	Reduced responsiveness towards the TNF-α lowering effect of dietary quercetin	Boesch-Saadatmandi *et al.* 2008 [64]
Human subjects, apoE4 *versus* apoE3 and apoE2 genotype	No effects of dietary fish oil supplementation regarding an improvement of the LDL/HDL-cholesterol ratio	Minihane *et al.* 2000 [65]

when compared with non-apoE4 genotypes [26, 27]. Also in cultured neuronal cells, apoE4 increases vulnerability towards oxidative agonists. Hydrogen peroxide and glutamate induced cytotoxicity was higher in neuronal cells cultured with apoE4 than with apoE3 conditioned media [28]. Furthermore, vulnerability towards glia-induced inflammation was more pronounced in primary neurons from the brain of apoE4 transgenic mice [29] and dendritic recovery was impaired in the hippocampus of apoE4 *versus* apoE3 mice [30]. Higher neuronal susceptibility towards oxidative insults and poorer neuronal repair are suggested to at least partly underlie the increased AD risk associated with the apoE4 genotype. In consistence apoE4 transgenic mice exhibited a decreased cognitive performance including poorer outcome in spatial memory and learning tests similar as in AD pathology compared with apoE3 mice [31].

2 ApoE genotype and vitamin E status

The metabolism of vitamin E is linked to the metabolism of lipids and cholesterol in the body. In the small intestine dietary tocopherol esters are hydrolysed and internalised into CMs along with dietary lipids such as triglycerides and cholesterol. Recent studies show that vitamin E uptake in enterocytes is most likely mediated by two different receptors namely scavenger receptor class B type 1 (SR-B1) [32] and Niemann-Pick C1-like 1 (NPC1L1) [33]. Vitamin E efflux from enterocytes mainly occurs *via* secretion of CMs into lymph and to a lesser extent *via* the HDL pathway involving ABCA1 and apoA-I into the circulation [34]. Circulating CM are hydrolyzed by endothelial lipoprotein lipase (LPL) releasing fatty acids and vitamin E that can be absorbed by peripheral tissue [35]. CM also interact with discoid HDL particles transferring cholesterol and vitamin E to HDL. In the liver, hepatocytes internalise CM and HDL *via* apoE receptors and SR-B1, respectively, whereupon vitamin E reaches the endosomal/lysosomal system. Hepatic α-tocopherol (α-TOH) transport protein, which is co-localised with the endosomal compartment [36], preferentially binds *RRR*-α-TOH and facilitates hepatic secretion of α-TOH by two conceivable pathways including incorporation into VLDL and efflux *via* ABCA1 into extracellular HDL [37]. Circulating VLDL are catabolised by LPL releasing α-TOH that can be then taken up by peripheral tissues. VLDL remnants are internalised again by the liver *via* an apoE-receptor mediated pathway and are converted to LDL particles. LDL is considered as the major carrier of α-TOH to the peripheral tissue. However, there is a constant flux of α-TOH between the different lipoproteins with varying efficiency of α-TOH retention and release [38].

As SR-B1 has been repeatedly shown to mediate selective lipid uptake from HDL and LDL [39, 40] this receptor is considered to be crucial in α-TOH tissue delivery. SR-B1 is predominantly expressed in those tissues with high lipoprotein α-TOH uptake and α-TOH content, and the expression of SR-B1 has been shown to be regulated by α-TOH exposure [39, 41, 42]. Furthermore, SR-B1-deficient mice have 1.4-fold higher circulating α-TOH concentrations relative to wild-type controls (attributed to the elevated α-TOH content in HDL), but a 65–80% decrease in the α-TOH content of several tissues [43]. Therefore, it is evident that the complex mechanisms that mediate lipoprotein metabolism and delivery are likely to have a significant impact on α-TOH transport and tissue uptake.

In summary, there are different mechanisms by which peripheral tissues obtain vitamin E including (i) LPL-mediated release of vitamin E from particularly CM and VLDL, (ii) LDL receptor and related receptor classes mediated uptake from particularly VLDL and (iii) transfer *via* SR-B1 from HDL [44].

Based on the fact that vitamin E is closely related to lipoprotein transport and that the apoE genotype affects lipoprotein metabolism it is likely that the apoE genotype also impacts on vitamin E status. There is some experimental evidence demonstrating an interaction between apoE and vitamin E. Vatassery *et al.* found lower brain α-TOH levels in apoE deficient compared with control mice [45] and reported that the uptake and retention of radioactive tocopherol in different brain areas is altered in apoE deficiency [46] strongly suggesting a direct effect of apoE on α-TOH dynamics in the brain. A number of studies have assessed the influence of apoE genotype on vitamin E levels in humans with contrasting results. Gómez-Coronado *et al.* found no significant association between apoE genotype and lipid adjusted vitamin E levels in adult males and females, although male apoE4 individuals displayed slightly higher values than the other genotypes (mean plasma vitamin E of 29.4, 30.1 and 32.8 μmol/L in men with apoE2, E3 and E4 genotype, respectively) [47]. More recently, however, Borel *et al.* described significant associations between apoE genotype and plasma vitamin E levels. Following adjustment, mean vitamin E concentrations for each genotype were: E2/2 18.7 μmol/L, E3/2 23.0 μmol/L, E3/3 27.1 μmol/L, E4/3 27.4 μmol/L, E4/2 32.8 μmol/L and E4/4 20.6 μmol/L, the E2/2 and E4/2 individuals having the significantly lowest and highest vitamin E concentrations respectively compared with the other genotypes [48]; however, the number of subjects with these genotypes was low (3 and 2 respectively, based on frequency). As apoE4 carriers have a significantly higher risk of AD [1] a number of studies have compared blood vitamin E levels between apoE genotypes in AD patients. Fernandes *et al.* found no significant differences between apoE genotype and vitamin E levels in plasma, erythrocytes and platelets in AD patients [49]. Similarly, Battino *et al.* also found no association between plasma vitamin E levels and apoE genotype in AD patients [50]. However, there are no data available on vitamin E tissue concentrations, particularly of the brain, in presence of different apoE genotypes.

However, as plasma levels are not ideal measures of vitamin E status, we have investigated the influence of apoE

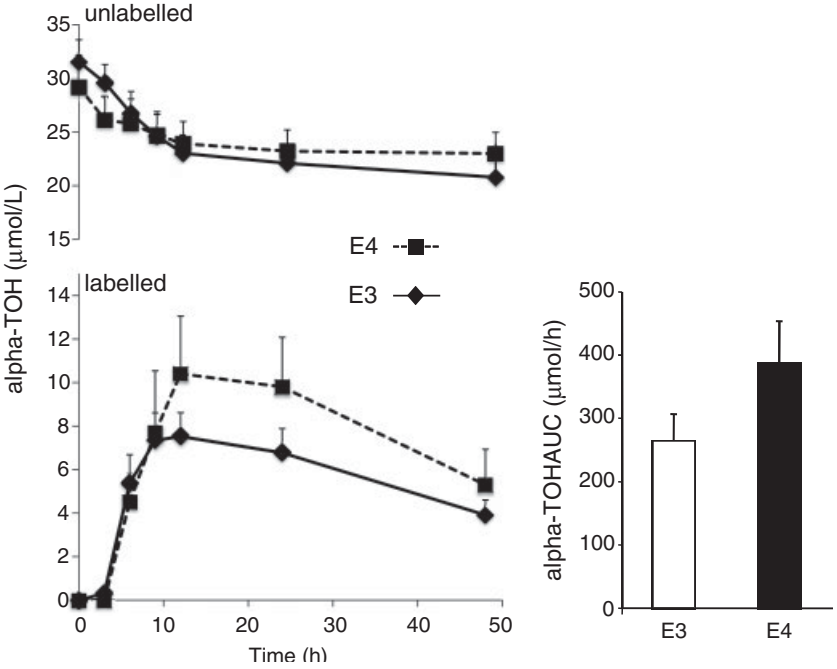

Figure 2. Influence of apoE genotype on vitamin E biokinetics. Plasma unlabelled and labelled α-TOH concentrations, standardised to cholesterol (TC), following ingestion of 150 mg deuterated *RRR*-α-tocopheryl acetate in apoE3 and apoE4 carriers. The area under the curve from the labelled α-TOH concentration/time profile is also shown. Values are means+SEM.

Table 2. Plasma level and tissue retention of α-tocopherol and mRNA levels of genes involved in the uptake and degradation of α-tocopherol in the lung are different between the apoE3 and apoE4 genotype in targeted gene replacement mice

Biomarker	Outcome
Plasma level of α-tocopherol	apoE3<apoE4
Peripheral tissue retention of α-tocopherol	apoE3>apoE4
LDL receptor	apoE3>apoE4
LDL receptor related protein 1	apoE3>apoE4
Scavenger receptor class B1	apoE3>apoE4
Cytochrome P450 family 3A	apoE3<apoE4

genotype using a stable-isotope biokinetic approach [44]. Our experimental data suggest a different processing of vitamin E in response to the apoE genotype. Figure 2 shows the unlabelled (endogenous) and deuterium labelled α-TOH concentration over 48 h following ingestion of deuterium labelled α-TOH. Although baseline unlabelled α-TOH concentrations were similar between apoE3 and E4 carriers, as previously observed, plasma uptake of newly absorbed (deuterium labelled) α-TOH was significantly higher in the apoE4 carriers compared with the apoE3 carriers, as demonstrated by the higher concentration of labelled α-TOH at each time point, and the higher area under the curve (Fig. 2) [44].

It was hypothesised that the higher concentration of newly absorbed α-TOH in the apoE4 genotype could be indicative for an increased α-TOH retention in LDL and thus, an impaired vitamin E delivery to peripheral tissues. Furthermore, higher levels of newly absorbed α-TOH could also be due to higher plasma levels of LDL and reduced LDL catabolism as already been described for the apoE4 genotype. This is in line with recent data showing increased total cholesterol accompanied by increased vitamin E levels in the plasma of healthy apoE4 *versus* non-apoE4 carriers clearly indicating a relationship between lipid and vitamin E metabolism [51]. Alternatively, a defective SR-B1 delivery system could also contribute to the difference between genotypes. ApoE acts as a high affinity ligand for SR-B1 [52, 53]. In apoE4 individuals the preferential association of apoE with VLDL rather than HDL may have a detrimental impact on the binding of HDL to SR-B1 and subsequent cellular α-TOH uptake. This would result in lower tissue α-TOH, but potentially higher levels in the circulation. This is in line with our recent observations in apoE transgenic mice where significantly lower α-TOH tissue levels were found in the lung of apoE4 compared with apoE3 mice although plasma α-TOH concentrations were similar between the two genotypes [54]. These data suggest that plasma vitamin E levels may not necessarily display peripheral vitamin E tissue status and may therefore be of limited validity.

Investigating vitamin E tissue uptake we determined the mRNA expression of several lipoprotein receptors and found significantly lower levels of SR-B1 and LDL receptor related protein in apoE4 compared with apoE3 mice [55]. Thus, decreased expression of receptors mediating vitamin E uptake in the extra-hepatic tissue of apoE4 mice is likely to contribute to decreased vitamin E tissue concentrations.

Proteins of the cytochrome P450 3A (CYP3A) family initiate microsomal degradation of vitamin E [56] and are up-regulated upon α-TOH supplementation [57, 58]. However, CYP3A mRNA levels were higher in the lung of apoE4 mice in spite of decreased lung α-TOH concentration

compared with apoE3 mice [55]. Increased tissue α-TOH degradation due to higher CYP3A levels may also contribute to lower peripheral vitamin E concentration in apoE4 animals (Table 2).

3 Concluding remarks

We have previously suggested that genetic heterogeneity is an important determinant of vitamin E status and greatly contributes to inter-individual variation in response to vitamin E supplementation [59]. Based on literature data and our own studies in targeted gene replacement mice and humans we hypothesise that the apoE4 genotype is associated with lower retention of vitamin E in peripheral tissues. Lower α-TOH tissue levels observed in the apoE4 genotype may be due to (i) altered lipoprotein metabolism including decreased catabolism of LDL, and thus increased α-TOH retention in LDL, (ii) decreased expression of lipoprotein receptors, which will in consequence hinder the cellular uptake of vitamin E in apoE4 carriers, (iii) greater intracellular degradation of tocopherols in the apoE4 genotype contributing to lower vitamin E concentrations in peripheral tissues and (iv) an impaired cellular vitamin E delivery system through the SR-B1 receptor and lower levels of SR-B1. A lower extra-hepatic α-TOH status accompanied by chronic inflammation may contribute to an increased disease risk as observed in apoE4 carriers.

α-Tocopherol has been shown to regulate gene expression [60–62] including those genes shown to be affected by the apoE genotype such as SR-B1 and CYP3A. Therefore, future studies should focus on α-TOH mediated gene expression of known α-TOH-sensitive genes (*e.g.* CD36, PXR, γ-GCS) in response to the apoE genotype.

The authors have declared no conflict of interest.

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