Genetic and environmental determinants of cardiometabolic health

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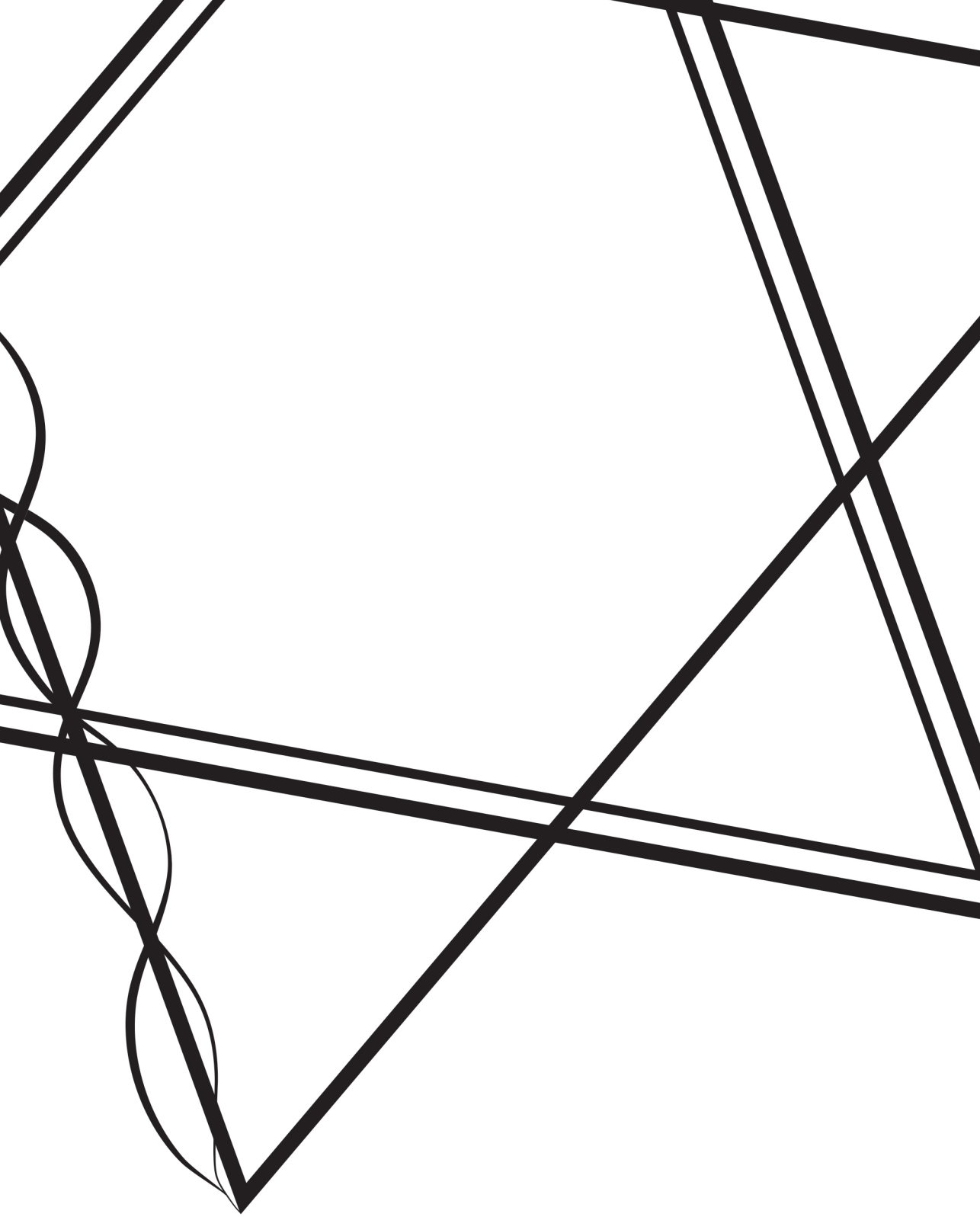
PART III

APOE



CHAPTER 4.1

The ApoE ε4 Isoform: Can the Risk of Diseases be Reduced by Environmental Factors?

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ABSTRACT

Candidate gene studies and genome-wide association studies found that genetic variation in *APOE* is robustly associated with multiple age-related diseases and longevity. Apolipoprotein E (ApoE) is an apolipoprotein that plays an important role in triglyceride and cholesterol metabolism. In literature, especially the ApoE ε4 isoform has been associated with an increased risk of mortality and age-related diseases such as Alzheimer's disease (AD), cardiovascular diseases (CVD) , as compared to the 'neutral' ApoE ε3 isoform. There are, however, large differences in the deleterious effects of the ApoE ε4 isoform between ancestries and populations, which might be explained by differences in environmental and lifestyle exposures. In this respect, poor nutrition and physical inactivity are two important lifestyle factors that have been associated with increased risks for AD and CVD. Therefore, in this narrative review we discuss how omega-3 fatty acid intake and physical activity, may modify the impact of ApoE ε4 on AD and CVD risk.

INTRODUCTION

Genetic variation in *APOE* is robustly associated with human longevity[1, 2]. The *APOE* gene, located on chromosome 19, consists of three different isoforms, notably ApoE ε2 (Cys^{112}, Cys^{158}), ApoE ε3 (Cys^{112}, Arg^{158}) and ApoE ε4 (Arg^{112}, Arg^{158}), of which the ApoE ε3 isoform is generally considered the 'neutral' isoform[3]. With respect to longevity, the ApoE ε2 isoform has been associated with an increased survival and with a more beneficial lipid profile[4, 5]. In contrast, compared with ApoE ε3 carriers, carriers of the ApoE ε4 isoform have higher mean total serum cholesterol levels[6]. Moreover, previous research indicated that the ApoE ε4 isoform decreases the efficacy of cholesterol lowering statin therapy[7, 8]. ApoE ε4 is an established risk factor for ageing and various age-related diseases, such as multiple types of dementia (including Alzheimer's disease (AD)) and cardiovascular disease (CVD)[6, 9]. In a study comprising individuals of European ancestry (5,107 AD patients and 6,262 controls), ApoE ε3/ε4 carriers had a 3.2-fold increased risk and ApoE ε4/ε4 carriers had a 14.9-fold increased risk to develop AD compared to ApoE ε3/ε3 carriers[6]. Furthermore, in a meta-analysis of studies from different ancestries (15,492 cases and 32,965 controls), it was shown that both ApoE ε3/ε4 carriers and ApoE ε4/ε4 carriers had a 1.4-fold higher risk to develop coronary artery disease[10]. Most interestingly, the increased risk of disease associated with ApoE ε4 seems to be variable between individuals of different ancestries with Kenyan or Nigerian ancestry individuals having no harmful effects of ApoE ε4[11, 12]. Strikingly, Nigerian ancestry individuals have the highest frequency of the ApoE ε4, but a relatively low incidence of AD[13].

Possibly, the differences observed in risk conferred by ApoE genotype between individuals of different ancestries could be attributable to environmental- and lifestyle-factors. Therefore, for our biological understanding and to be eventually of added value to public health, it is of interest to disentangle the mechanisms resulting in the lower disease risk conferred by ApoE ε4 in certain populations. Lifestyle factors vary between populations, and are associated with increased risks of disease. Because of the broad definition of lifestyle, we will only elaborate on nutritional intake and physical activity in the context of ApoE and age-related disease. Therefore, the primary aim of this narrative review is to discuss potential pathways that might attenuate the effects of the genetic susceptibility for AD and CVD in ApoE ε4 carriers. To the best of our knowledge, this is the first narrative review to discuss the current (biological) evidence of APOE-lifestyle interactions in the pathophysiology of age-related diseases. First, we will provide a short overview of the ApoE protein, the different isoforms and their function. Next, we will focus on how nutrition and physical activity could modify the effect of genetic predisposition of individuals carrying the ApoE ε4 risk allele.

The ApoE protein and isoform prevalence

ApoE is a 299-residue protein, which is predominantly produced by hepatocytes, macrophages and astrocytes[14, 15]. Human ApoE comprises multiple amphipathic α-helices and is known to contain a low-density lipoprotein receptor (LDLR) binding site on the fourth helix[16]. ApoE is a major apolipoprotein found in plasma and is presented on chylomicron remnants, very low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDL), and high-density lipoproteins (HDL) to mediate their receptor-mediated uptake from the circulation. ApoE is also involved in VLDL assembly and secretion by hepatocytes [17]. Moreover, ApoE is present in the central nervous system[18, 19] where it plays an important role in the transport of cholesterol and in cellular reparative processes (e.g. neuronal repair)[18].

Figure 1 provides a schematic overview of the role of ApoE in lipoprotein metabolism. In the exogenous pathway, dietary triglycerides and cholesterol are absorbed by enterocytes and are used for lipidation of ApoB48 to generate chylomicrons, which enter the blood circulation via the lymphatic system. In the circulation, lipoprotein lipase (LPL) on metabolically active tissues, hydrolyses triglycerides (TG) within these particles to release free fatty acids that are taken up by these tissues (including the heart, skeletal muscles, white adipose tissue and brown adipose tissue). As a consequence of LPL-mediated lipolysis, smaller chylomicron remnant particles are formed, which become enriched with ApoE that is acquired from HDL. Enrichment with ApoE abrogates the lipolysis by LPL and mediates the subsequent uptake of the chylomicron remnants by hepatocytes through receptor-mediated endocytosis via the LDLR, and the LDLR-related protein (LRP)[20]. In addition to these high-affinity receptors, heparan sulfate proteoglycans (HSPG) also play a role in the low affinity/high capacity binding and internalization of chylomicron remnants[21].

In the endogenous pathway, VLDL particles are synthesized in the liver by lipidation of ApoB100 with cholesterol and triglycerides, and serve to deliver endogenous fatty acids as well as cholesterol towards peripheral tissues. After secretion from the liver into the plasma, LPL hydrolyses VLDL similarly to chylomicrons, which results in the formation of VLDL remnants. ApoE mediates the uptake of VLDL remnants (also termed 'intermediate-density lipoproteins; IDL) via hepatic receptors and binding sites in a similar fashion as to chylomicron remnants. VLDL remnants that escape uptake by the liver are completely lipolysed by LPL to generate low-density lipoprotein (LDL) particles that mainly carry cholesteryl esters. The main apolipoprotein in LDL is ApoB100, as all other apolipoproteins including ApoE are lost during lipolysis. LDL particles are taken up via recognition of ApoB100 through the LDLR on the liver and peripheral tissues (e.g. adrenals, testes and ovaria), which need cholesterol for e.g. steroid hormone synthesis.

Both the liver and intestines produce lipid-poor HDL particles containing ApoAI and ApoAII in addition to ApoE. Through these apolipoproteins, HDL can induce the efflux of cholesterol from peripheral tissues via ATP-Binding Cassette Transporter A1 (ABCA1) and ATP-binding Cassette Transporter G1 (ABCG1) and transport the cholesterol to the liver via Scavenger Receptor Class B Member 1 (SR-BI), after which cholesterol can be converted into bile acids. Collectively, this pathway is called reverse cholesterol transport (RCT)[16]. Alternatively, cholesterol from peripheral tissues can reach the liver after transfer of cholesteryl esters from HDL to VLDL via the cholesteryl ester transfer protein (CETP), with subsequent receptor-mediated uptake of remnants by the liver.

Table 1. *ApoE isoforms and their properties*

Isoform	2	3	4
Residue 112	Cysteine	Cysteine	Arginine
Residue 158	Cysteine	Arginine	Arginine
Overall frequency (mean %)	7	79	14
Plasma triglycerides (mmol/L)	Higher	Normal	Higher
Plasma cholesterol (mmol/L)	Lower	Normal	Higher
ApoE stability	Higher	Normal	Lower
Associated disorders	Type III hyper-lipoproteinaemia, PVC, ASCVD	Normal	Hypercholesterolemia, CVD, AD
LDLR binding affinity (%)	1	100	100
Lipid binding ability	Normal	Normal	Higher
Binding preference	HDL	HDL	VLDL

Abbreviations: AD, Alzheimer's disease; ASCVD, atherosclerotic cardiovascular disease; CVD, cardiovascular disease, HDL, high-density lipoprotein, PVC, peripheral vascular disease; VLDL, very-low-density lipoprotein.

APOE isoforms and prevalence

Table 1 provides an overview of the different isoforms and their characteristics. The frequencies of the different ApoE isoforms vary greatly between populations, but ApoE ε3 is most common in all (mean global frequency ≈79%) followed by the ε4 isoform (≈14%) and the ε2 isoform (≈7%)[24, 25]. For example, ApoE ε3 frequency ranges from 54% in African Pygmies to 91% in Mayans[26, 27], while ApoE ε4 frequency ranges from 5% in Sardinians to 41% in African Pygmies[26]. Importantly, among European populations, ApoE ε4 frequency is higher in northern European countries than in Southern countries[28, 29]. Compared to ApoE ε3 (normal plasma cholesterol levels[3]), ApoE ε4 is associated with altered plasma lipid levels and lipoprotein particle

distributions as the resulting ApoE protein has altered binding affinity for either lipoprotein particles or the low-density lipoprotein receptor (LDLR)[30]. The ApoE ε4 isoform is associated with higher total cholesterol levels, higher LDL-cholesterol levels and lower HDL-cholesterol levels[24]. ApoE ε4 has a higher binding affinity for larger TG-rich lipoproteins (such as VLDL and chylomicron remnants)[22, 23]. The ApoE ε2 isoform displays about 1% of the binding affinity to the LDLR compared to ApoE ε3 and ε4[31]. Moreover, ApoE ε2 carriers have a lower hepatic VLDL assembly and VLDL uptake, resulting in diminished clearance from the blood and subsequent type III hyperlipoproteinemia[32, 33]. *In vitro* studies have shown that ApoE ε2 can protect cells from oxidative stress induced cell death. Moreover, ApoE is important in neural injury repair by initiating membrane repair by redistribution of lipids [34]. The molecular stability of ApoE is lower for ApoE ε4 as compared to the ε2 and ε3 isoform[35]. The structural differences of the ApoE isoforms result in different susceptibility to proteolytic cleavage, by which neurotoxic fragments are formed. Proteolytic cleavage is lowest for ApoE ε2[34,36-38] and highest for ApoE ε4, providing a potential mechanism explaining the higher risk of AD in ApoE ε4 carriers[34]. Moreover, it was shown that HDL-induced recycling of ApoE ε4-containing TG-rich lipoproteins is strongly reduced in hepatocytes, resulting in increased intracellular cholesterol levels[39]. As ApoE ε4 has a preference to bind VLDL and chylomicrons, this results in an enhanced uptake of these particles by the hepatocytes thereby competing with the uptake of LDL particles, resulting in increased LDL concentration[19]. Since this review focuses on strategies to alleviate the health risk associated with ApoE ε4, a detailed discussion of the ApoE ε2 isoform is beyond the scope of this review.

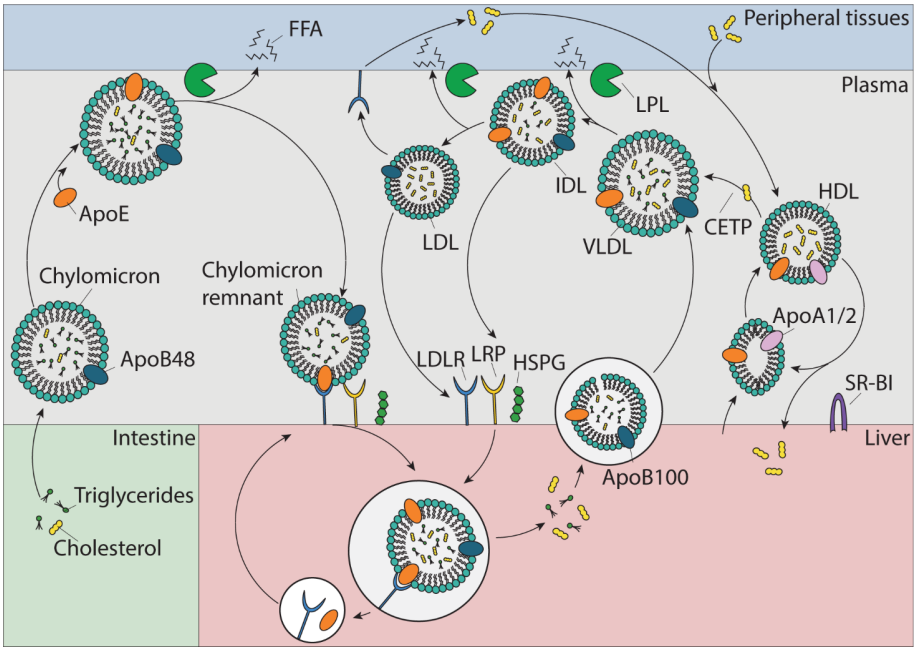

Figure 1. *Schematic overview of lipoprotein metabolism*. In the exogenous pathway, dietary cholesterol and triglycerides (TG) are absorbed by the small intestine and incorporated into chylomicrons within enterocytes. Via the lymphatic system, chylomicrons reach the circulation, where their triglycerides are hydrolysed by lipoprotein lipase (LPL) present on metabolically active tissues, to deliver exogenous fatty acids to these tissues[20]. This results in formation of smaller remnant particles that are enriched with ApoE to mediate subsequent internalisation by hepatocytes via the LDL receptor (LDLR), the LDLR-related protein (LRP), and heparan sulfate proteoglycans (HSPG)[21]. In the endogenous pathway, very-low-density lipoproteins (VLDL) are assembled and secreted by the liver to deliver endogenous fatty acids towards metabolically active tissues. Similar to chylomicrons, VLDL are lipolysed by LPL and their remnants can be taken up by hepatocytes via ApoE. Alternatively, the particles can be further processed by LPL to yield low-density lipoproteins (LDL) as lipolytic end product. LDL lacks ApoE and therefore ApoB100 serves as a ligand to bind exclusively the LDLR. The free fatty acids (FFA) derived from lipolysis are used in the peripheral tissues. High density lipoproteins (HDL) are synthesized as discoidal precursors by the liver and small intestine. Also, surface remnants that are produced during lipolysis of chylomicrons and VLDL can contribute to the HDL pool. HDL can acquire cholesterol from peripheral tissues and transport the cholesterol back to the liver through the direct pathway of reverse cholesterol transport (RCT) via scavenger receptor class B member 1 protein (SR-BI), which selectively takes up cholesteryl esters from HDL, after which liberated cholesterol can be converted into bile acids and secreted into the feces[16]. Reverse cholesterol transport can also occur through the indirect pathway, whereby cholesterol is transported via CETP from HDL to VLDL and LDL. Compared to the ApoE ε3 isoform, the ApoE ε4 isoform binds preferentially to VLDL particles and slows down lipolysis. This results in higher VLDL concentrations and lower HDL concentrations[22, 23].

Effect modifiers of ApoE isoforms

Nutritional intake and physical activity have been hypothesized to modify the metabolic effects of ApoE ε4. Therefore, it is of interest to elaborate more on these effect modifiers in relation to ApoE genotype.

Fatty acids and disease risk

A meta-analysis comprising 11 prospective cohort studies (371,965 participants from general populations and 31,185 death events) showed that higher dietary intake and higher circulating levels of n-3 long-chain polyunsaturated fatty acids were associated with a lower risk for all-cause mortality[40]. Notably, it was found that a 0.3 g daily increase in dietary intake of n-3 long-chain polyunsaturated fatty acids (also called omega-3 fatty acids) was associated with 6% lower risk of all-cause mortality in the general population. Furthermore, a 1% increase in circulating levels of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were associated with a 20% and 21% risk reduction of all cause-mortality, respectively[40]. In addition, emerging evidence suggests that dietary factors and cognitive function are related[41]; several epidemiological studies showed that higher fish intake is associated with a lower risk for cognitive decline and dementia during follow-up[42, 43]. In more detail, a study comprising 2,031 Norwegian individuals aged 70-74 years, recruited from the general population, showed that fish intake was associated with a better cognitive function in a dose-dependent manner, with 75 g fish a day as optimum[44]. Omega-3 fatty acids not only exert a beneficial effect on cognition, but also influence cardiovascular risk in the general population. Overall, omega-3 fatty acids are able to reduce CVD mortality by 37%[45, 46]. However, the effects of omega-3 fatty acid intake on disease risk are conflicting with studies indicating positive, null or negative effects[47-50]. For example, no significant difference in triglyceride concentration has been observed upon omega-3 fatty acids supplementation in elderly[48]. However, a linear correlation between higher doses of omega-3 fatty acid intake and a triglyceride lowering effect has also been observed[47]. Therefore, given this heterogeneity in research findings, there are yet no definite conclusions on the role of omega-3 fatty acids in CVD and neurodegenerative disease. Importantly, these studies did not take any specific ApoE isoform into account.

Fatty acids, disease risk and APOE genotype

In the following paragraph, we discuss potential effect modification of the association between fatty acid intake and disease risk by *APOE*. Kariv-Inbal *et al.*[43] described that the detrimental effects of the ApoE ε4 isoform on AD risk could be reduced by a diet enriched with fish oil (DHA) in ApoE ε4-targeted replacement mice. Another study, conducted in humans, determined the association between seafood and n-3 fatty acid intake and cognitive decline in relation to the ApoE ε4 isoform[51]. This longitudinal, community-

based epidemiologic study in 915 elderly participants of Caucasian ancestry (recruited from retirement communities in Illinois, USA), demonstrated that ApoE ε4 carriers had a slower decline in multiple cognitive domains with weekly seafood consumption and moderate to high intake of n-3 fatty acids than ε3 and ε2 carriers consuming the same amount of seafood after an average follow up of 4.9 ± 2.5 years[51]. Intake of vegetable α-linolenic acid, which is used by the body to form long chain n-3 fatty acids, was also associated with slower cognitive decline only in carriers of the ApoE ε4 isoform[51]. These studies indicate that omega-3 fatty acids are beneficial in preventing cognitive decline and suggest that individuals with the ApoE ε4 isoform may especially benefit from higher n-3 fatty acid consumption for the prevention of cognitive decline and AD.

The possible mechanisms of action of unsaturated fatty acids

It is of interest to elaborate more on how polyunsaturated 3-n fatty acids may be beneficial in slowing AD and CVD development related to ApoE ε4, and healthy aging in general, which may be through multiple biological pathways. For example, in the brain, omega-3 fatty acids are incorporated in phospholipids where they replace omega-6 fatty acids, which increases fluidity of membranes of neuronal cells[52, 53]. This increased fluidity allows for better signal transduction between the neuronal cells. Omega-3 fatty acids also improve neurotransmission by increasing receptor binding affinity and increasing the number of receptors of ion channels[54], which therefore counteracts the synaptic deficits associated with ApoE ε4[55].

Another biological mechanism might work via the ability of omega-3 fatty acids to lower the synthesis of new VLDL particles and triglycerides from the liver[56], as illustrated **in Figure 2A** and **2B**. ApoE ε4 isoform carriers have a faster clearance of VLDL particles compared to ApoE ε3 carriers[57], which by competition for the hepatic clearance of LDL raises LDL-cholesterol. We therefore hypothesize that omega-3 fatty acids could possibly lower the synthesis of VLDL particles, whereby competition for hepatic uptake between VLDL remnants and LDL is reduced, and the uptake of LDL particles by the liver is increased. Subsequently, this might lead to lower serum LDL-cholesterol concentrations to be of specific importance to ApoE ε4 carriers.

The positive effects of polyunsaturated fatty acids may also be explained through inflammatory pathways. In relation to ApoE ε4, increased inflammation and oxidative stress has been observed in cell lines, rodents and human volunteers[58]. It was previously reported in animal studies that fish oil has beneficial effects on triglyceride levels and inflammatory factors by downregulation of inflammatory genes and upregulation of peroxisome proliferator-activated receptor-gamma (PPAR-γ)[59]. The PPARs belong to a nuclear receptor group that act as lipid-activated transcription

factors. Increasing evidence suggests a protective role of PPAR-γ signaling in atherosclerosis by decreasing inflammatory cytokine production and mediating lipid metabolism[60, 61]. Moreover, a placebo-controlled study in hyperlipidemic individuals demonstrated that n-3 polyunsaturated fatty acids in combination with plant sterols were able to reduce several inflammatory markers, such as C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and leukotriene B(4) (LTB(4))[62]. In the same study, the overall CVD risk was reduced, suggesting that higher n-3 polyunsaturated fatty acid intake works in a cardio-protective manner possibly through reduced inflammation[62].

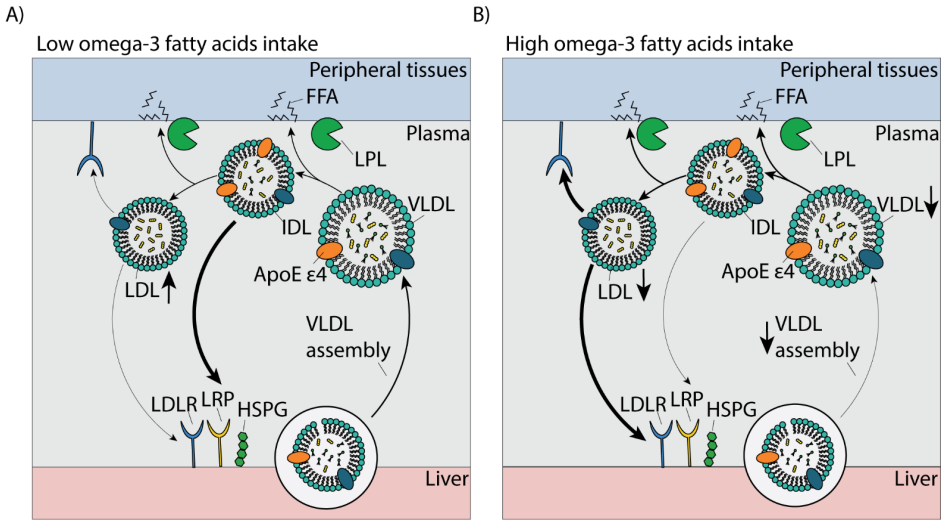

Figure 2. *The effects of omega-3 fatty acid intake on the ApoE ε4 isoform* A) ApoE ε4 has a higher affinity for the LDLR receptor than LDL itself, and therefore binds preferentially to this receptor, resulting in receptor-mediated endocytosis of VLDL instead of LDL. This preferential uptake of VLDL results in higher levels of LDL in the plasma[19]. B) Unsaturated fatty acid intake decreases VLDL assembly, resulting in a lower concentration of VLDL and thereby increases the uptake of LDL and IDL by the liver, leading to lower LDL levels[56].

Physical activity and disease risk

It has been argued that a high level of aerobic exercise can attenuate the process of aging by reducing amyloid plaque formation and increasing overall vascular health[63]. Previous studies in healthy older adults demonstrated that high physical activity is associated with a preservation of both cognitive function and hippocampal volume[64-66]. Moreover, cognitive function and amyloid plaque formation in elderly AD patients benefits from daily exercise[67].

Physical activity, disease risk and APOE genotype

The following paragraph will focus on physical activity in relation to cognitive and cardiovascular health with respect to ApoE ε4. In cognitively healthy adults, a more sedentary lifestyle was associated with higher amyloid deposition[68]. Interestingly, this finding was only observed in ApoE ε4 carriers and not in carriers of the other ApoE genotypes[68]. Moreover, in a study of 78 cognitive healthy older adults with 18 months follow-up, high physical activity was associated with a slower decline in hippocampal volume. Again, this effect was specifically observed only in ApoE ε4 carriers[69]. In addition, aerobic exercise was associated with a slower cognitive decline, and decreased risks of various types of dementia, including AD, but specifically in ApoE *ε4 carriers*[70-72]. In relation to cardiovascular health, it has been shown that age-related changes in cholesterol and LDL-cholesterol were counteracted by life-long endurance exercise in 15 old trained healthy men as compared to 12 old untrained, 10 young trained and 12 young untrained men[73]. Compared to a group of mild-to-moderate physically active men who maintained their physical exercise level, men that increased their exercise during a 1-year follow-up had a more favorable lipid profile[74]. Furthermore, it was shown that HDL-cholesterol levels increased directly after exercise training in 17 overweight men, possibly through reduction in HDL protein catabolism[75]. However, these intervention studies have been generally conducted in small samples and with short follow-up. In a population-based cross-sectional study (N=1,708, aged 35-74 years), higher physical activity was associated with higher HDL cholesterol and lower triglyceride levels in specifically ApoE ε4 carriers[76]. However, these results have not been confirmed in a subsequent study[77]. These discrepancies require further research and might yield insights in other mechanisms associated with physical activity.

The possible mechanisms of action of physical activity

One possible mechanistic explanation underlying the association between physical activity and AD specifically in ApoE ε4 carriers, is based on the finding that neuronal ApoE ε4 has an increased susceptibility for proteolytic cleavage compared to ApoE ε3[36-38]. In brain tissue samples of AD patients, fragments of the ApoE protein are present in much higher concentrations as compared to those of controls[36, 38]. Physical exercise in ApoE ε4 carriers is able to reduce the neuronal level of ApoE ε4 and thereby lower the total amount of ApoE ε4 fragments in the brains of these individuals. Subsequently, the risk of developing AD in these individuals might be reduced. However, this is merely a hypothesis based on a small number of studies[36, 38]. Additional research is warranted to disentangle the protective mechanism of physical exercise on ApoE ε4 concentrations in the brain.

Based on previous studies, we are able to hypothesize the biological mechanism through which physical activity might modify the detrimental effects of ApoE ε4 carriership (visualized in **Figure 3A** and **3B**). Exercise enhances the LPL-dependent flux of triglyceride-derived fatty acids from chylomicrons and VLDL to myocytes, which decreases the level of serum triglycerides[16]. As a consequence, excess surface lipids are released from chylomicrons and VLDL as surface remnants that mainly contain phospholipids and unesterified cholesterol. These surface remnants are precursors of HDL that subsequently accept additional cholesterol from peripheral tissues, thereby increasing total HDL cholesterol levels[78, 79]. In this way, increased LPL activity may decrease serum triglyceride levels in more physically active ApoE ε4 carriers. Indeed, it was shown that physical exercise decreases VLDL particle size, which is consistent with hydrolysis of these particles by LPL[80].

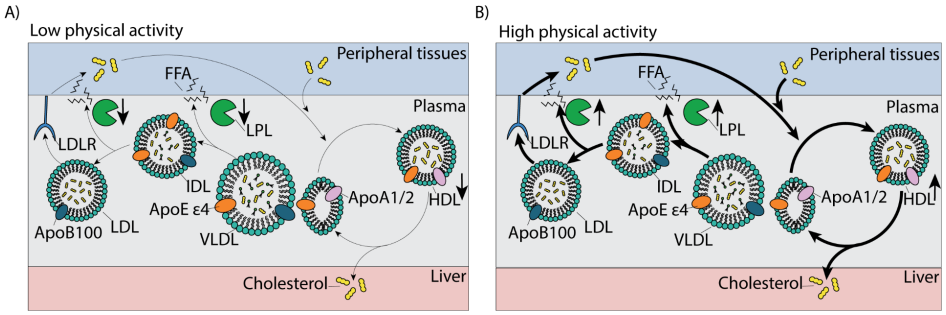

Figure 3. *The effects of physical activity on the ApoE ε4 isoform.* A) The ApoE ε4 isoform binds preferably to large lipoprotein particles, such as VLDL, due to higher lipid binding ability. The increased binding to VLDL slows down lipolysis of these particles[22, 23]. B) Physical activity enhances LPL activity. This increased activity enhances lipolysis of VLDL particles and thereby decreases the VLDL concentration. Generation of more surface remnants increases the level of HDL that can accept cholesterol from peripheral tissues[16, 78, 79].

FUTURE PERSPECTIVES

Due to advances in technology and availability of large datasets, multiple novel genetic determinants of diseases are being identified. ApoE ε4 carriership is the strongest genetic risk factor for multiple age-related diseases, including diseases for which no drug treatments are (currently) available. In the present narrative review, we described several biological mechanisms on how unsaturated fatty acids and physical activity may prevent or delay cognitive decline and CVD, and discuss how these effects extend to and are possibly even stronger in carriers of the ApoE ε4 risk allele.

On the one hand, the general public is becoming increasingly aware of the impact of nutrition and physical activity on their health. However, on the other hand, current consumption of omega-3 fatty acids is low due to modern agriculture and a Western diet[81] and a large part of modern society is now adapted to a sedentary lifestyle whereby the largest proportion of adults does not even meet the proposed physical activity guidelines[82]. In line, a higher incidence of cognitive decline, AD, and other age-related diseases in relation to the Western diet is observed[83-85]. This is especially of importance when populations that still have a high prevalence of the ApoE ε4 isoform (e.g., Nigerian ancestry or Northern European countries) adapt to a more sedentary lifestyle, because an even higher increase in CVD and AD may occur in these at-risk individuals[63]. In agreement with this hypothesis, African populations that move to cities and reduce their physical activity are much more susceptible to acquire CVD and AD than Western populations [86]. Therefore, it seems that individuals carrying the ApoE ε4 isoform could specifically benefit from increasing their physical activity and/or increasing their omega-3 fatty acid intake.

In order to assess if an individual is a carrier of the ApoE ε4 isoform, screening for this genotype has to be implemented. However, screening of ApoE ε4 carriers runs into a vast amount of ethical, methodological, and economic aspects that need to be addressed first in order to make the implementation of these models feasible as well as cost effective. For example, important questions, such as the clinical meaning and implications of such screening and which professional figures should manage the implementation, are only some of many questions that have to be answered first. However, there is an increasing body of evidence suggesting that lifestyle may influence genetic susceptibility to several chronic diseases that may not be left unnoticed. Therefore, in line with the evidence as discussed in this narrative review, next to focusing on the general population to increase their omega-3 fatty acid intake and enhance their physical activity, it may be valuable to specifically focus on at-risk individuals and/or families that have a higher susceptibility to carry ApoE ε4. An example of a at-risk group

may be certain families with a high incidence of AD, in which a higher prevalence of hypertension, pro-inflammatory markers and ApoE ε4 genotype has been observed. These factors may be early risk factors for AD in old age, as those have been observed already at middle-age before the onset of AD[87]. Specifically, increasing awareness of physicians and general practitioners may lead them to stress the importance of adhering to a healthier lifestyle in at-risk individuals. For example, in previous research, it was demonstrated that lifestyle interventions to improve physical activity and/or nutritional habits, even in older adults, seem promising[88, 89]. A 13-weeks lifestyle program already induced metabolic health benefits, which might increase the positive adaption of lifestyle changes in the general population as effects occurred relatively fast[88]. These studies suggest that diminishing the occurrence of non-communicable diseases associated with ApoE ε4 via improving physical activity seems possible. Next to increasing physical activity, we hypothesize that a diet rich in polyunsaturated fatty acids will benefit ApoE ε4 isoform carriers. For example, the Mediterranean diet is a plant-based diet rich of unsaturated fatty acids and antioxidants. The Mediterranean diet is characterized by a high content of olive oil, high intake of fruits and vegetables, moderate-to-high fish and seafood consumption, low intake of dairy products, low meat consumption and a regular intake of red wine[90]. The Mediterranean diet is associated with a lower risk of AD and cognitive decline[91, 92] and has beneficial effects on overall health[93]. In a randomized clinical trial in healthy elderly, Valls-Pedret *et al.*[94] showed that a Mediterranean diet supplemented with olive oil and mixed nuts was able to improve cognitive function. The Mediterranean diet is rich in bioactive phytochemicals that are known to have antioxidant and anti-inflammatory properties. For example, olive oil is rich in phenolic compounds that may counteract oxidative stress processes in the brain and thereby decrease neurodegeneration[94].

There are still many questions remaining to be addressed in future research. For example, it needs to be investigated whether short term or only prolonged physical activity is beneficial in ApoE ε4 carriers and at what age it can still restore the metabolic consequences of ApoE ε4. As only life-long high aerobic exercise exerts a protective effect, the overall health benefits of increasing physical activity at high age might be lower than those in younger individuals[73]. In this review, we described the effects of unsaturated fatty acids intake and physical activity separately. However, further research should also warrant attention to the combined effect of these lifestyle factors to disentangle those mechanisms. For example, a synergistic effect of these two lifestyle-factors on ApoE ε4-related disorders might exist, or one of the two might have a higher impact on these outcomes. Not only omega-3 fatty acids, but also other macro- and micronutrients might be of interest in relation to healthy aging in ApoE ε4 carriers and the general population, here further research is also warranted[19, 95]. Moreover, this review only focused on two

lifestyle-related factors. However, other lifestyle-factors (e.g. sleep), but also cultural- and environmental factors and medication use may modify ApoE ε4 related effects. A recent trial in high-risk individuals investigating the effect of a multidomain lifestyle intervention program on cognition in different *APOE* genotype subgroups did not show specific beneficial effects on cognition in ApoE ε4 carriers[96]. However, sample size and follow-up duration might have been limited. We acknowledge, however, that in general it is very difficult for individuals to alter their lifestyle, and adherence to the intervention might be an issue to longer follow-ups. Alternatively, medication has been suggested to specifically target the mechanisms described in this review. For example, it has been described that drugs like CETP inhibitors and APOC3 antisense may work through similar pathways as described in this narrative review. The efficacy and safety of APOC3 antisense for the treatment of hypertriglyceridemia is currently being tested in phase 3 trials[97]. CETP inhibitors, however, have not been able to demonstrate clinical benefit and were found to have effects that are modest at best in phase 3 clinical trials[97, 98]. An important area of future research comprises assessment of interactions between genes, lifestyle and medication use. Especially in a medical world trying to de-prescribe, these future studies focusing on lifestyle and its interactions may have considerable value.

CONCLUDING REMARKS

In this review, we discussed lifestyle-related factors and their contribution to the effects of genetic variation in the *APOE* gene on age-related diseases. This review provides an overview of the current literature, however, some limitations should be mentioned. First, because of the consistently growing body of evidence regarding this topic we may have missed important results that could influence our conclusions. Moreover, the authors are aware that the discussed epidemiological studies differ in their design and their study population, which may therefore cause the results to not be directly comparable. For example, there may be differences in the methods of administering omega-3 fatty acids (EPA, DHA, fish oil etc.) and in the amount of time exposed to physical activity (long-term, high-intensity, low-intensity etc.). In addition, most studies included in this narrative review did not take into account the effect of *APOE* genotype heterozygosity, which have been found previously to be of importance[6, 10]. However, studies addressing the relation of *APOE* genotype heterozygosity with omega-3 fatty acid intake and physical activity are scarce. Further research should consider this heterozygosity in relation to lifestyle factors and disease risk.

Taken together, an increasing body of evidence suggests a protective role for omega-3 fatty acids and physical activity in carriers of the ε4 allele. The risks associated with the ApoE ε4 isoform consist of several components that jointly contribute to disease onset. By modifying the risk of the ApoE ε4 isoform, disease burden associated with this risk allele might be decreased in the general population. This information is of interest as it now seems that the risks associated with the ApoE ε4 isoform are modifiable which may stimulate risk-reducing behaviors.

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