Dietary omega 3 polyunsaturated fatty acids and Alzheimer's disease: Interaction with apolipoprotein E genotype

Running title: Fatty acids and ApoE genotype

P. Barberger-Gateau (1,2), C. Samieri (1,2), C. Féart (1,2), M. Plourde (3)

(1) Inserm, U897, Bordeaux, F-33076 France

(2) Univ Victor Segalen Bordeaux 2, Bordeaux, F-33076 France

(3) Centre de recherche sur le vieillissement, Université de Sherbrooke, Québec, Canada

Corresponding author:

P. Barberger-Gateau

INSERM, U897, University Victor Segalen Bordeaux 2, case 11, 146 rue Léo-Saignat,

F-33076 Bordeaux cedex, France

Tel : + 33 5 57 57 15 96 Fax : + 33 5 57 57 14 86

Pascale.Barberger-Gateau@isped.u-bordeaux2.fr

Abstract (250 words)

Epidemiological studies suggest a protective role of omega-3 poly-unsaturated fatty acids (n-3 PUFA) against Alzheimer's disease (AD). However, most intervention studies of supplementation with n-3 PUFA have yielded disappointing results. One reason for such discordant results may result from inadequate targeting of individuals who might benefit from the supplementation, in particular because of their genetic susceptibility to AD. The ε4 allele of the apolipoprotein E gene (ApoE) is a genetic risk factor for late-onset AD. ApoE plays a key role in the transport of cholesterol and other lipids involved in brain composition and functioning. The action of n-3 PUFA on the aging brain might therefore differ according to ApoE polymorphism. The aim of this review is to examine the interaction between dietary fatty acids and ApoE genotype on the risk for AD.

Carriers of the ε4 allele tend to be the most responsive to changes in dietary fat and cholesterol. Conversely, several epidemiological studies suggest a protective effect of long-chain n-3 PUFA on cognitive decline only in those who do not carry the ε4 but with inconsistent results. An intervention study showed that only non-carriers had increased concentrations of long-chain n-3 PUFA in response to supplementation. The mechanisms underlying this gene-by-diet interaction on AD risk may involve impaired fatty acids and cholesterol transport, altered metabolism of n-3 PUFA, glucose or ketones, or modification of other risk factors of AD in ε4 carriers. Further research is needed to explain the differential effect of n-3 PUFA on AD according to ApoE genotype.

Key-words: Alzheimer's disease, apolipoprotein E, fatty acids, omega 3, nutrition,

1) Introduction

Prevention of age-related cognitive decline and dementia has become a public health challenge. Dementia results from a dynamic interaction between genetically determined, non-modifiable, pathological processes and environmental exposures that are potentially preventable or reversible [1]. Nutrition, a major lifelong environmental factor, offers promising perspectives for the prevention of late-life dementia. Long-chain poly-unsaturated fatty acids (PUFA) are major components of neuron membranes where they exert a functional and structural role [2-3]. Broadly speaking, PUFA of the omega-3 (n-3) and omega-6 (n-6) series have opposite effects on inflammation, atherogenesis, thrombosis, and gene expression, and their respective proportions influence the physical properties of biological membranes where they are incorporated [4]. A competition exists between the n-3 and n-6 families for the synthesis of long-chain PUFA from their respective precursors alpha-linolenic and linoleic acids [4]. Moreover, the biosynthesis of long-chain n-3 PUFA is very limited in humans [5]. These must therefore be obtained through diet. Fish and seafood are the main dietary source of long-chain n-3 PUFA under the form of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [6].

Several prospective epidemiological studies have suggested a protective role of fish consumption [7-11] or dietary n-3 PUFA [9] against risk of dementia or Alzheimer's disease (AD). However, conflicting results exist as well [12-15]. Moreover, most intervention studies based on supplementation with n-3 PUFA have failed to evidence any protective effect on AD [16-17] or cognitive decline [18-20] except in some patients with very mild cognitive impairment [16, 21-22]. We examined the reasons for such inconsistencies in a recent review [23]. A particular explanation for these discordant results may lie in ineffective targeting of individuals who might benefit from high doses of n-3 PUFA, in particular because of their genetic susceptibility to AD. The ε4 allele of the apolipoprotein E gene (ApoE) is the main genetic risk factor for late-onset AD [24]. The ApoE plays a key role in the transport of cholesterol and other lipids involved in brain composition and functioning [25]. The action of n-3 PUFA on the aging brain might therefore differ according to ApoE polymorphism, suggesting a gene-environment interaction. In this review we examine the interaction between dietary n-3 PUFA and ApoE genotype on the risk for AD at the light of biological data and human studies.

2) Role of ApoE genotype in AD

Despite the recent discovery of susceptibility genes for AD thanks to Genome Wide Assessment of large population-based samples, the main genetic risk factor for sporadic late-onset AD remains by far the ApoE ε4

allele (ApoE4) [26]. The ApoE gene has three different alleles: ε2, ε3 and ε4, that give rise to six genotypes: ε2/2, ε2/3, ε2/4, ε3/3, ε3/4, and ε4/4. ApoE3 is the most common isoform [27]. The frequency of the ApoE4 decreases following a North-to-South gradient in normal middle-aged populations as well as in patients with late-onset AD [28]. A meta-analysis showed that the relative increase in the frequency of ApoE4 in patients with AD compared with controls is substantially less in African Americans (32.2% vs. 19.0%) than Caucasians (36.7% vs. 13.6%) [29]. The ApoE4 increases the risk of AD by 15 times in Caucasian homozygotes [29]. Although ApoE4 is the major genetic risk factor for AD, its mode of action remains partly unknown [30] and several mechanisms have been proposed [27]. Indeed, ApoE4 is the only molecule that has been associated with all the biochemical disturbances characteristic of the disease: beta-amyloid (Aβ) peptide deposition, tangle formation, oxidative stress, inflammation, lipid homeostasis deregulation, synaptic plasticity loss and cholinergic dysfunction [27]. ApoE4 may increase Aβ in senile plaques and impair its clearance [30]. Recently, a study using voxel-based analysis of ^{11}C labelled Pittsburgh compound B (PIB)- positron emitting tomography (PET) and magnetic resonance imaging (MRI) showed higher levels of Aβ plaque deposition in AD patients with the ApoE4 allele compared to age-matched ApoE4-non carriers with similar levels of cognitive impairment and brain atrophy [31]. The neuropathological changes associated with AD occur as soon as 30 years of age, that is decades before the clinical onset of the disease, but in ApoE4 carriers the accumulation of Aβ peptide in senile plaques is more pronounced at every age except in the oldest old [32]. There is therefore a large window of action for environmental risk factors that will modulate the clinical expression of late-onset AD. In addition to their increased burden of Aβ, ApoE4 carriers may be particularly sensitive to some environmental factors, in particular dietary fat. We focus here on the antioxidant and immunomodulatory/anti-inflammatory properties of ApoE [33] that are particularly relevant to lipid metabolism given the high susceptibility to peroxidation and the anti-inflammatory properties of n-3 PUFA.

2.1 ApoE and oxidative stress

Increased oxidative stress is a key feature in AD [34]. Markers of oxidative damage precede pathological changes in experimental models and are observed very early in the clinical course of the disease [35-36]. Aβ is a potent generator of free radicals (reactive oxygen species, ROS, and reactive nitrogen species) [34]. The production of free radical species leads to peroxidation of membrane lipids. In synaptosomes isolated from mice that expressed only one of the ε2, ε3 or ε4 human ApoE alleles, treatment with Aβ induced a significantly higher increase in ROS and markers of lipid peroxidation in synaptosomes containing the ε4 allele [37].

Oxidative stress results from an imbalance between production of free radicals and anti-oxidant defence mechanisms. These defence mechanisms involve enzymatic systems such as glutathion peroxidase (GSH-Px) and catalase (CAT) and non-enzymatic antioxidant nutrients including vitamin E, carotenoids and selenium. In vitro, the ApoE isoforms are associated with different antioxidant capacities, the highest being observed with the ε2 and the lowest with the ε4 isoforms [38]. Plasma concentrations of vitamin E, a powerful fat-soluble antioxidant, vary according to ApoE genotype, the highest concentrations being found in ε2/2 individuals in one study [39]. In another study, no significant difference in vitamin E concentration was observed between ApoE4 carriers and non-carriers in older AD patients while in control subjects vitamin E level was significantly higher in ApoE4 carriers [40]. However, this difference disappeared after normalization according to triglycerides (TG) and cholesterol levels. For the authors, this increased plasma vitamin E level in ApoE4 controls could be associated with retention of vitamin E in plasma lipoproteins and a functional vitamin E deficiency in peripheral tissues [40]. The protective effect of some dietary antioxidants may also depend on ApoE genotype. Vitamin E from food, but not other antioxidants, was associated with a reduced risk of AD only among individuals without the ApoE4 allele [41]. Conversely, higher serum beta-carotene was associated with lower risk of cognitive decline only in ApoE4 carriers among high-functioning older persons [42]. In an elderly Chinese cohort, carriers of the ApoE4 allele had significantly lower Selenium levels measured in nails [43].

ApoE genotype may also influence activity of enzymes involved in antioxidant defence mechanisms such as GSH-Px and CAT. In AD patients as well as in age-matched controls, the ApoE4 carriers had significantly lower activities of GSH-Px and CAT, and lower serum total antioxidant status compared to non ApoE4 carriers [44]. The decreased antioxidant activity of ApoE4 could therefore contribute to its association with AD [33].

2.2 ApoE and inflammation

In AD, the accumulation of misfolded proteins leads to inflammatory damage [34]. In addition to low-grade inflammation associated with brain aging, Aβ deposition is accompanied by activation of microglia which will contribute to eliminate Aβ. In turn, chronically activated microglia will release chemokines and pro-inflammatory cytokines [34]. Acute systemic inflammation is also suspected to exacerbate chronic brain inflammation thereby increasing the rate of cognitive decline in AD patients [45]. The ApoE4 allele is also reported to directly promote inflammation which may contribute to its deleterious effect in AD [46]. The activation of the amyloid cascade in ApoE transgenic mice results in the activation of microgliosis and

astrogliosis in the hippocampus of ApoE4, but not in ApoE3 transgenic mice. This differential impact of ApoE genotype was limited to this brain area specifically affected in AD and was not observed in septal neurons [47]. Paradoxically, this exacerbation of brain inflammation linked to ApoE4 is not observed at peripheral level where inverse associations are reported. Indeed, in a comparative analysis of three large population-based studies, lower levels of plasma C-Reactive protein (CRP), a biomarker for inflammation, were observed in ApoE4 carriers [48]. Similarly, in a population of cognitively normal Italian elderly, ApoE4 carriers had a lower risk of high CRP than non-carriers [49]. The same association was observed in nonagerians [50]. However, there is an interaction between peripheral markers of inflammation and ApoE on cognitive performance. In a cross-sectional study conducted in older community dwellers, participants with at least one ε4 allele and CRP in the highest tertile had the greatest odds of impaired memory [51]. This finding suggests, as already hypothesized by Jofre-Monseny et al. [33], that the detrimental effect of ApoE might be partly counteracted by lower levels of CRP.

This large body of evidence suggests that ApoE4 is a key player in the pathogenesis of AD [27]. A large part of this effect might be explained by the major role of ApoE in lipid metabolism.

Figure 1

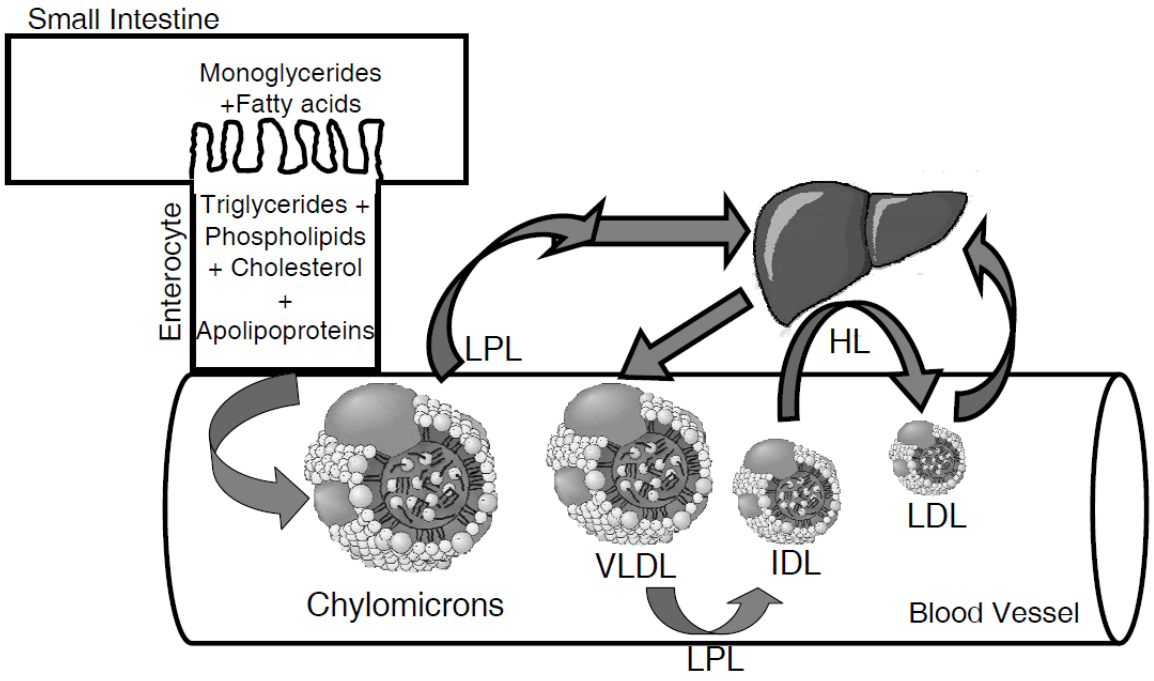

Figure 1. Metabolism of lipoproteins starting with absorption in the small intestine.

LPL = lipoprotein lipase, HL = hepatic lipase, VLDL = very low density lipoproteins, IDL = intermediate density lipoproteins, LDL = low density lipoproteins

2.3 ApoE genotype, lipid metabolism and AD

Most dietary fats are ingested in the form of TG. They are efficiently absorbed in the small intestine towards facilitated diffusion and uptaken by enterocytes in the forms of fatty acids and monoglycerides (Figure 1) [52]. Once fatty acids have entered the cell, they are re-esterified with glycerol back into TG and phospholipids (PL) for transport in the blood *via* chylomicrons to the liver and other tissues. All lipoproteins, with the exception of chylomicrons, are produced by the liver, and begin as molecules packed full with TG, with the exception of high density lipoprotein (HDL). Very low density lipoprotein (VLDL) is the most TG-rich lipoprotein, which through sequential lipolysis of TG by lipoprotein lipase leads to the formation of low density lipoprotein (LDL). Lipoproteins are composed of lipids and proteins named apolipoproteins which bind to specific receptors. ApoE is specific to LDL receptor which is at the root of cholesterol homeostasis but is also essential for normal catabolism of TG-rich lipoprotein constituent.

Throughout life and increasing with age, neurons must be remodelled and repair to maintain neurotransmission [30]. Through its lipid transport function, ApoE plays a key role in the transport and metabolism of cholesterol, TG and other lipids within many organs including the brain [25, 53]. ApoE is also the main ligand for LDL receptors in the periphery, while in the central nervous system it is the main ligand for the LDL receptor-related protein [53]. In the brain ApoE plays an important role in the maintenance and repair of neurons, by distributing lipids necessary for proliferation, synaptogenesis and myelinisation of axons [27]

Allelic variations in ApoE impair the protein structure, function and metabolism [54]. For instance, in ApoE4 carriers, substitution of an amino acid changes the protein structure because of the formation of a salt bridge between an arginine at position 61 and a glutamic acid at position 255, causing the isoform to bind preferentially to VLDL [54].

2.3.1 ApoE and cholesterol

Other mechanisms linking ApoE and risk of AD possibly involve cholesterol homeostasis in the brain since ApoE is its principal carrier [53]. In the brain, cholesterol has a crucial role in development of neuronal plasticity and function [55]. All the cholesterol used in the brain is synthesized within the central nervous system since the blood-brain barrier restricts direct transport of cholesterol from peripheral circulation, in the absence of vascular injury [55].

Cholesterol forms complexes with ApoE for delivery to neurons and is an essential component of neuronal membranes where it is concentrated in lipid rafts [34]. Rafts are membrane micro-environments for the assembly of β-secretases and γ-secretases and processing of amyloid precursor protein into Aβ [34]. ApoE isoforms influence the composition of membrane lipid rafts [56]. ApoE4 is the least effective of the three isoforms in promoting healthy lipid membrane turnover. ApoE4 also enhances Aβ production and decreases its clearance, leading to higher aggregation of Aβ in the brain especially when there is an overabundance of esterified cholesterol that decreases membrane lipid turnover [34].

Peripheral serum cholesterol levels are also influenced by ApoE genotype [57-59]. In a large sample of healthy European men, the ApoE genotypes ε2/2 and ε3/2, ε4/2, ε3/3, ε4/3, and ε4/4 were associated with respectively increasing levels of serum total and LDL cholesterol [60]. This relationship may however be disturbed in the presence of AD. In a case-control study, cholesterol levels were significantly higher in ApoE4 individuals within the control group, whereas no significant relationship was found between ApoE4 and cholesterol in the AD cases [40].

Hence, we could anticipate that higher plasma cholesterol levels would correlate with higher Aβ aggregation in the brain of ApoE4 AD patients. However, in post-mortem studies of AD patients, higher total cholesterol and LDL levels in the blood did correlate with higher Aβ42-amyloid levels in the brain but independently of ApoE genotype [61]. These correlations were not observed in healthy controls. In epidemiological studies, there is an association between higher serum cholesterol levels in midlife and increased risk of AD [62-63] but this association was independent of ApoE genotype [62]. Conversely, a decrease in serum total cholesterol from midlife to late-life was associated with early stages of dementia, independently of ApoE genotype [64-65]. Lower blood cholesterol in later life possibly indicates a poor nutritional status that could be a consequence rather than a cause of cognitive decline and dementia. Adding to the controversy, several studies show a modification of the association between serum cholesterol level and cognitive performance by ApoE genotype. In three cross-sectional studies conducted in various populations, higher plasma total and LDL cholesterol were associated with increased AD risk in the non carriers of the ApoE4 allele, whereas cholesterol was not associated with increased AD risk in the carriers [66-68]. Similarly, in a cross-sectional study conducted in very old (> 85 years) non-demented individuals, there was a significant positive correlation between higher total or LDL cholesterol and better memory performances in ApoE4 non-carriers [69]. No correlation was observed in ApoE4 carriers or with HDL cholesterol. The Longitudinal Aging Study Amsterdam yielded controversial results since in separate analyses by ApoE status, there was a significant positive association between total cholesterol level

and change in information processing speed only in the ApoE4 carriers, suggesting that a higher total cholesterol level at baseline was significantly associated with a slower rate of decline in these subjects [70]. A common limitation to these studies is the lack of longitudinal data on the interaction between ApoE genotype and change in serum cholesterol levels under treatment or dietary modification on the rate of cognitive decline or risk of dementia.

2.3.2 ApoE and fatty acids

Whereas cholesterol in the brain is produced and regulated endogenously [55], the fatty acid content of neuronal membranes can be modulated by dietary intake and ApoE polymorphism in animals [56]. ApoE4 is associated with the inhibition of lipid metabolism, inducing inefficient delivery of PUFA, in particular DHA, to neurons and altered lipid membrane homeostasis [56]. DHA is the most abundant component of membrane phospholipids in the brain. ApoE is a constituent of the TG rich lipoproteins VLDL but there are inconsistencies in the literature regarding the essentiality of ApoE in hepatic VLDL synthesis and secretion [71]. After the phospholipids, TG are the next richest source of DHA in plasma [72]. The most important role of ApoE in lipoprotein metabolism is to be a high-affinity ligand for receptor to the LDL-receptor family and to deliver cholesterol to neurons. Although the role of ApoE in brain cholesterol homeostasis is well documented, its potential role into fatty acid transport and delivery to the brain is less understood with the exception of mentioning that ApoE is important for synaptic plasticity [73]. Hence, at this point, no direct connection between ApoE genotypes and fatty acids delivered to the brain is yet possible to establish. This is supported by the data obtained by Fraser et al. [74] showing that the relative proportions of fatty acids in frontal, temporal and parietal cortex in AD and control brains was not significantly different by ApoE genotype. However, that study measured the overall fatty acid composition of the cerebral cortex without regard to subcellular distribution, especially in relation to membrane lipid rafts, which is a limitation, particularly for DHA [74].

3) Modification by ApoE genotype of the effect of dietary n-3 PUFA on cognition

3.1 Epidemiological studies

Few epidemiological studies have examined the interaction between n-3 PUFA status and ApoE on cognitive decline or risk for dementia or AD. They are summarized in table 1.

Table **1**. Association between n-3 PUFA and cognitive outcomes stratified by ApoE status in prospective epidemiological studies.

Reference	Exposure	Outcome	ApoE4 carriers		ApoE4 non-carriers	
			N	Measure of association (95% CI) or *P* value	N	Measure of association (95% CI) or *P* value
[75]	Fatty fish consumption: 2 to 4 servings per week	8-yr incidence of dementia	474	HR = 0.91 (0.48 to 1.71)	1570	HR = 0.60 (0.40 to 0.89)
[10]	Fish consumption: at least once a week	4-yr incidence of dementia	1479	HR = 1.28 (0.58 to 2.83)	5944	HR = 0.60 (0.41 to 0.89)
[76]	EPA + DHA in plasma cholesteryl esters (for 1 SD)	Cognitive decline on Word Fluency Test	650	OR=0.80 (0.45 to 1.63)	1601	OR = 0.61 (0.43 to 0.87)
[77].	Total n-3 PUFA in erythrocyte membranes (log)	Cognitive performance on a score derived by principal	38	$r = 0.02$ NS	75	$r = 0.35$ $P < 0.01$

		component analysis					
[78]	EPA and DHA in total plasma fatty acids (for 1 SD)	Cognitive decline on Benton Visual Retention Test	235	DHA: β=0.061, *P*=.01 EPA: β=0.077, *P*=.003 in subjects with low depressive symptoms; β=0.172, *P*<.001 in subjects with high depressive symptoms.	984	DHA: β=0.001, *P*=.93 EPA: β=0.001, *P*=.93 in subjects with low depressive symptoms; β=0.096, *P*=.04 in subjects with high depressive symptoms.

CI = confidence interval

HR = hazard ratio

OR = odds ratio

r = correlation coefficient

SD = standard deviation

NS = non significant

PUFA = poly-unsaturated fatty acid

EPA = eicosapentaenoic acid

DHA = docosahexaenoic acid

3.1.1 Dietary studies

The Cardiovascular Health Cognition Study was the first to evidence an interaction with borderline significance between consumption of fatty fish and presence of the ApoE4 allele on the risk for AD [75]. Fatty fish appeared to have little or no association with risk for AD in those with the ApoE4 but was associated with significantly lower risk in those without the ApoE4 allele. Similar results were observed in the French Three-City (3C) study which reported a significant protective effect of fish consumption against all-cause dementia only in ApoE4 non-carriers [10]. However, there was no statistical interaction between fish consumption and ApoE genotype on the risk for AD. Moreover, the same study showed a deleterious effect of n-6 rich oils (sunflower or grape seed oil) when their consumption was not counterbalanced by consumption of n-3 rich foods (oils or fish) among ApoE4 non-carriers. This detrimental effect was not observed in ApoE4 carriers.

Conversely, among ApoE4 carriers moderate intake of PUFA (2^{nd} quartile) at midlife was associated with decreased risk of dementia, while moderate intake of saturated fats was associated with an increased risk in the Cardiovascular Risk Factors, Aging, and Incidence of Dementia (CAIDE) study [79-80]. No association was found between intake of fat, whatever its type, and risk of dementia in ApoE4 non-carriers. However, that study did not discriminate between n-3 and n-6 PUFA and was therefore not included in table 1.

3.1.2 Biological studies

Most biological studies did not show any interaction between blood n-3 PUFA status and ApoE genotype on cognitive evolution in older persons. Indeed, among the five published or in press prospective epidemiological studies that examined whether ApoE4 may modify the relationship between long-chain n-3 PUFA blood status and dementia or cognitive decline, three [76], 14, 81] did not find any modification effect and only the first one [76] reported data stratified by ApoE status (table 1). In the Atherosclerosis Risk in Communities study [76], an inverse association was observed between higher EPA + DHA in plasma cholesterol esters and verbal fluency in ApoE4 non-carriers, but not in carriers despite a non-significant interaction term. The lack of significant protective association in ApoE4 carriers might therefore be attributed to a lack of power in this smaller subgroup. In the Canadian Study of Health and Aging [14], there was no significant modification of risk of dementia or AD associated with n-3 PUFA, DHA, or EPA concentrations, treated in quartiles or as continuous variables, by ApoE4 status in models adjusted for sex and education (data not shown by the authors and therefore not reproduced in Table 1). However, this study found a paradoxical protective association with higher blood mercury concentration, which can be considered as a proxy biomarker for fatty fish intake. The presence

of both high n-3 PUFA and high mercury concentration was associated with a significantly reduced risk by 43% for dementia and by 46% for AD, independently of ApoE genotype [14].

Two studies found a statistically significant interaction (table 1), with a protective effect in ApoE4 non-carriers only in one study [77] and conversely, a protective association only in ApoE4 carriers in the other study [78]. In the first study, children aged 11 years old with higher general intelligence had higher erythrocyte n-3 PUFA, but the correlation was significant only for those without the ApoE4 allele [77]. The same population was retested at 64 years old and their general intelligence was even more highly positively correlated with erythrocyte n-3 PUFA, but there was still no correlation in ApoE4carriers. The 3C study yielded inverse results since higher plasma EPA and DHA proportions were associated with slower decline in working memory assessed by Benton Visual Retention Test among ApoE4 carriers only [78]. Decline of performance in other cognitive domains (*i.e* global cognition, executive functioning and verbal fluency) was not related to plasma long-chain n-3 PUFA content, neither in the whole sample nor according to ApoE4 carrier status. Notably, in that study, baseline plasma EPA and DHA proportions did not differ according to ApoE genotype.

3.2 Intervention studies

One recent study evaluating the impact of fish oil on cognitive performance in the healthy elderly reported that after 26 weeks of supplementation with either 0.4 or 1.8 g/d of long-chain n-3 PUFA ApoE4 carriers had improved scores on tests of the cognitive domain of attention compared to the placebo contrarily to non-carriers [18]. Plasma EPA and DHA concentrations were given but no information was provided as to whether they changed differentially in relation to ApoE4 genotype.

Conversely, the promising results of a randomized controlled trial (RCT) comparing DHA (2 g/d) and placebo conducted by the National Institutes on Aging Alzheimer's Disease Cooperative Study Unit in mild to moderate AD which was presented at the International Conference on Alzheimer's disease 2009 [82] and reported by Joseph et al. [83] and Cole et al. [84], mentioned possible slowing of progression of cognitive decline in ApoE4 non-carriers, but no effect in ApoE4 carriers. In the Memory Improvement with DHA Study (MIDAS) RCT, improved learning and episodic memory functions were observed in older adults with mild memory complaints receiving 900 mg algal DHA per day but the publication does not mention whether ApoE genotype was taken into account [22].

Post-hoc analyses of RCT in small subgroups must be considered with caution when the randomisation was not stratified according to ApoE genotype. Analyses according to ApoE genotype should be systematically planned

in the protocol of trials of dietary supplementation with n-3 PUFA for the primary or secondary prevention of cognitive decline in AD.

4) Putative mechanisms underlying this interaction

4.1 Impaired fatty acids transport to the brain

The link between the way EPA and DHA are transported and cognition may involve delivery of fatty acids for membrane repair and signalling pathways within the brain. It is suggested that DHA-enriched LDL are taken up more efficiently by the brain than other lipoproteins [85-86], whereas others suggest that it is the unesterified form of DHA that most efficiently reaches the brain [87-88]. One major limitation to the delivery of fatty acids to the human brain is to cross the blood brain barrier. Two general models have been proposed: the first approach suggests a passive diffusion of unesterified plasma fatty acids [87-89] while the second suggests uptake of lipoproteins via a receptor-mediator process, likely the LDL-receptor [86, 88]. Since ApoE is the main ligand for LDL-receptor protein in the central nervous system [53] and that the ε4 allele seems to impact the efficiency of the binding of ApoE with the LDL-receptor, it is hence possible that the delivery of EPA and DHA to the brain is altered in ApoE4 carriers. However, this hypothesis yet has to be investigated in mice expressing human ApoE4.

4.2 Modification by ApoE of the effect of dietary EPA and DHA on blood lipids

Another mechanism explaining the interaction between dietary PUFA and ApoE genotype on rate of cognitive decline and risk for AD may lie in a modification by ApoE of the effect of EPA and DHA intake on blood lipid profile.

4.2.1. Modification by ApoE of the effect of dietary EPA and DHA on plasma fatty acid profile

A gene-by-diet interaction has recently been reported for the incorporation of EPA and DHA into plasma lipids of ApoE4 carriers and non-carriers [90]. The plasma fatty acid profiles of 8 carriers and 20 non-carriers of ApoE4 showed that ApoE4 carriers had significantly *higher* EPA and DHA in plasma TG at baseline [90]. However, after receiving a supplement of 1.9 g/d of EPA and 1.1g/d of DHA for 6 weeks, there were significant gene-by-diet interactions in the incorporation of both EPA in free fatty acids and DHA in plasma TG such that in carriers of ApoE4, increases in the two fatty acids was lower compared to the non-carriers [90]. The mechanism underlying the gene-by-diet interaction is possibly linked with the way EPA and DHA are transported by

lipoproteins. After dietary intake, most dietary lipids are transported by chylomicrons but small amounts circulate in the blood stream as free fatty acids bound to albumin. Chylomicrons are TG-rich lipoproteins produced in intestinal cells after the intake of a meal, while lipoproteins are produced by hepatic cells. All lipoproteins, with the exception of HDL, begin as molecules rich in TG. VLDL is the most TG-rich lipoprotein, which through sequential lipolysis of TG by lipoprotein lipase leads to the formation of LDL. Although many studies report the incorporation of EPA and DHA into blood total lipids, phospholipids, TG, cholesteryl esters or red blood cells, there are only a few studies that report how EPA and DHA are transported in the blood and how much is incorporated into each type of lipoprotein. In studies of supplementation with EPA + DHA, both fatty acids were incorporated into VLDL-, LDL- and HDL-TG within 4h after fish-oil intake [91-93]. Altogether, the limited number of available studies suggests that DHA is rapidly and preferentially incorporated into TG fraction of most lipoproteins after its intake, and that within a few hours its concentration tends to decrease. Hence, the metabolism of EPA and DHA is most of the time in transition and the lap of time of this transition may be altered by ApoE4 genotype. However, to our knowledge, no studies have evaluated the impact of ApoE4 genotype on the transport of EPA and DHA by lipoproteins.

4.2.2. Modification by ApoE of the effect of EPA and DHA on blood cholesterol

Some of the modifying effect of ApoE genotype on the relationship between dietary n-3 PUFA and risk of AD might be mediated through cholesterol. Indeed, blood cholesterol concentration is partly determined by dietary intake of n-3 and n-6 PUFA [94] but also by genetics, in particular ApoE polymorphism. A gene-nutrient interaction might therefore be expected.

Carriers of ApoE4 have been shown to have a greater lipid response to dietary changes [95]. In 2003, a systematic review of the literature reported the effect of genetic variation, including ApoE polymorphism, on the lipid response to dietary intervention [96]. The authors reviewed intervention studies to modify dietary cholesterol, dietary fat or other compounds of the diet. Of the 46 interventions that modified the fat composition of the diet, 15 found significantly different total, LDL- and HDL-cholesterol responses, with carriers of the ApoE4 allele tending to be the most responsive to changes in dietary fat. Recently, others have reported a gene-by-diet interaction such that total cholesterol in ApoE4 carriers was increased following DHA supplementation, which is possibly due to an increase in LDL-cholesterol [97]. In plasma $VLDL_2$ fraction of ApoE4 carriers, intake of DHA resulted in a significant 32% reduction in LDL uptake relative to control supporting alterations in the transport and uptake of lipoproteins [97]. However, this study did not investigate whether the incorporation

of DHA into the different types of lipoproteins was different based on ApoE4 genotype, so whether the possible changes are at the root of the LDL uptake differences still needs further investigations.

Altogether, these data suggest that ApoE genotype interacts with dietary PUFA in the transport and uptake of lipoproteins, thereby changing cholesterol levels.

4.2.3 Modification by ApoE of the effect of EPA and DHA on blood TG

A major effect of dietary n−3 PUFA on plasma lipids is a reduction of the concentration of plasma TG [98], an effect that may be modified by ApoE genotype. A study in healthy elderly Brazilian women showed higher TG serum levels in ε2 compared to ε4 carriers [99]. However, in the presence of high spontaneous intake of total fat or a low relative intake of PUFA, ApoE4 carriers lost protection against hypertriglyceridemia [99].

Administration of fish oil in a RCT showed a double treatment by sex by ApoE genotype interaction [100]. Indeed, the greatest decreases in TG concentration with either 0.7 g EPA+DHA/d or 1.8 g EPA+DHA/d were observed in ApoE4 males.

High TG are linked to cognitive decline and risk of dementia. An experimental study in obese mice showed that lowering TG can reverse their cognitive impairment [101]. In the 3C study we showed an association between hypertriglyceridemia and increased risk of all-cause and vascular dementia [102]. However, this effect was independent of ApoE4 genotype.

N-3 PUFA could therefore contribute to lower the risk of cognitive impairment and dementia associated with high TG in ApoE4 carriers. However, the potential modifying effect of ApoE genotype on this relationship still lacks scientific support.

4.2.4 Modification by ApoE of the susceptibility to lipid peroxidation

As discussed in section 2.1, ApoE4 carriers have a lower activity of defence mechanisms against oxidative stress. One hypothesis to explain why ApoE4 carriers are not protected against risk of cognitive decline by fish or DHA intake is possibly linked to their altered LDL metabolism leading to higher susceptibility to peroxidation [103]. Lipids are needed to repair neuronal membrane and ApoE4 genotype apparently fails to protect lipids from peroxidation. Therefore, the oxidised lipids needed to be removed from damaged neuronal membranes are possibly replaced by other oxidised lipids [103]. Moreover, formation of lipid peroxidation products may contribute to tau protein phosphorylation in AD [104]. Since EPA and DHA are susceptible to oxidation because they are highly unsaturated, their transport via LDL is thus possibly more vulnerable to oxidation in ApoE4

carriers. The residence time of LDL in ApoE4 carriers may also be longer since ApoE is the main ligand to the LDL-receptor in the periphery and that carrying the ε4 allele lowers the binding of ApoE to its LDL-receptor [71, 96]. The higher peroxidation of lipoproteins in ApoE4 carriers may hence contribute to their higher risk of AD.

4.3 Modification by ApoE of the effect of dietary EPA and DHA on other risk factors of dementia

Presence of the ApoE4 might accelerate the rate of cognitive decline through changes in the level of both AD pathology and vascular injury [1]. Inflammation and metabolic disorders belong to the large set of factors that contribute to the accumulation of neuro-pathological and vascular changes in late-life dementia [1]. N-3 PUFA may decrease the negative impact of these risk factors and enhance compensatory repair mechanisms [23]. In addition to its direct effects on lipid metabolism discussed above, ApoE genotype might also modulate the impact of dietary n-3 PUFA on inflammation, vascular risk factors and overall nutritional status. However, very few studies have analysed the interaction between EPA, DHA and ApoE genotype on these risk factors of dementia. The aim of this more speculative section is to raise new hypotheses that would deserve further research.

4.3.1 Inflammation

N-3 PUFA have potent anti-inflammatory properties [4]. Indeed, EPA is the precursor of anti-inflammatory eicosanoids and DHA is the precursor of neuroprotectin D1 (NPD1). NPD1 represses the activation of inflammatory signalling mediators such as prostaglandins synthesized from arachidonic acid (long-chain n-6 PUFA) by cyclooxygenase-2 [3].

As discussed in section 2.2, some studies suggest that inflammatory status might also be modulated by ApoE genotype. In the Cardiovascular Health Cognition Study, the reduction in risk for AD associated with use of non-steroidal anti-inflammatory drugs was greater among ApoE4 carriers [105]. Hence, ApoE4 carriers might also have an enhanced response to the anti-inflammatory effects of n-3 PUFA. This hypothesis was investigated in a small subsample of the OmegAD RCT which evaluated the impact of a supplementation with 2320 mg EPA+DHA /d on cognitive functioning in patients with mild to moderate AD [21, 106]. However, plasma biomarkers of inflammation (interleukin-6, tumor necrosis factor-α, CRP) did not differ between the intervention and placebo groups nor was there any difference for inflammation markers in cerebrospinal fluid. There was no interaction with ApoE genotype indicating a similar lack of impact of n-3 PUFA supplementation on biomarkers

of inflammation in ApoE4 carriers and non-carriers. However, this was a very small study (N=35) and the potential differential effect of n-3 PUFA on inflammation according to ApoE genotype still deserves further research.

4.3.2 Glucose metabolism

During healthy aging, brain glucose uptake may decrease significantly in specific cortical regions [107], an effect that may be more pronounced in the elderly with deteriorating cognitive function such as AD [108]. Reduction of cerebral metabolic rates for glucose was also found in asymptomatic ApoE4 carriers as young as 20 to 30 years old in the same brain regions as clinically affected AD patients [109]. These results are the earliest sign of brain hypometabolism abnormality yet found in living person at risk of AD and suggest that in ApoE4 carriers, hypometabolism precedes the onset of clinical AD and is not a consequence of the disease itself. Glucose is the main cerebral energy substrate and during fasting or low carbohydrate intake ketones are glucose's primary replacement fuel [110]. Hence, in the elderly and in ApoE4 carriers, increasing blood ketones and potentially their uptake by the brain may be a good strategy to counterbalance their lower brain glucose uptake. The capacity to produce ketones seems similar in the elderly (>70 y old) compared to the young (between 18-25 y old) and is potentially not depending upon ApoE genotype [111]. Higher blood ketones facilitated cognitive performance in ApoE4 non-carriers but not in the carriers [112]. Similar results in a larger scale study with 152 subjects diagnosed with mild to moderate AD confirmed that higher ketone levels in ApoE4 carriers had no specific changes in the scores of cognitive tests [113]. Whether increasing blood ketones improves cognition by raising brain uptake of ketone bodies needs investigation.

4.3.3 Hyperhomocysteinemia

Elevated serum homocysteine is a cardiovascular risk factor which is also associated with increased risk of cognitive decline [114-116], dementia [116-118] and AD [117-119]. Surprisingly, ApoE4 carriers tend to have a lower risk of hyperhomocysteinemia [49]. The presence of the ApoE4 allele also modified the relationship between hyperhomocysteinemia and cognitive performance in one study: the inverse association between total homocysteinemia and cognitive scores was consistently higher in ApoE4 carriers [120].

There is an inverse association between plasma homocysteine and platelet phospholipid DHA concentration [121]. Administration of fish oil significantly decreased plasma homocysteine in rats [122] and hyperlipemic men [123]. Conversely, DHA concentration may be affected by homocysteine concentration [124]. However, to

our knowledge, no published study examined the impact of ApoE genotype on the link between homocysteine and DHA in AD.

4.3.4 Obesity

There is increasing evidence that obesity at midlife is a risk factor for cognitive impairment [125-126] and dementia [127-130] later in life, although the latter association became non significant after further adjustment for vascular disorders and ApoE4 status in one study [130]. Obesity contributes to maintain a chronic inflammatory status. In vitro, DHA inhibits the differentiation of pre-adipocytes to adipocytes [131]. In obese adults, there was an inverse correlation between total n-3 PUFA, EPA and body mass index (BMI) which was not observed in normal weight individuals [132]. Moreover, obese individuals had lower n-3 PUFA concentrations which might contribute to their increased risk of cognitive decline.

However, the link between ApoE genotype, obesity, n-3 PUFA and dementia risk is not yet clearly established. Indeed, a recent review concluded that few studies have attempted to link human ApoE isoforms to obesity, and they have produced somewhat conflicting results [133]. Obesity results from an imbalance between energy intake and actual needs. One study evidenced an interaction between high energy intake and ApoE4 on the risk of dementia. Indeed, in the WHICAP higher calorie intake was associated with increased risk of AD only in those who had at least one ApoE4 allele [134]. High total fat intake was also associated with increased risk for AD in the same individuals. However, the modulating effect of n-3 PUFA on these relationships was not studied. Further research is therefore needed to understand how ApoE genotype could modulate the impact of n-3 PUFA on obesity and subsequent cognitive decline.

4.3.5 Global nutritional status

In older adults there is a U-shaped association between BMI and dementia, with increased dementia risk in obese and underweight individuals [135]. Poor nutritional status is associated with higher risk of dementia in older persons [135-137].

Although the underlying mechanisms are not clear, nutritional status may be associated with ApoE genotype. Surprisingly, in the Cache County Study on Memory and Aging participants with a diet that provided a greater variety of recommended foods were more likely to have at least one copy of the ε4 allele than were those with less varied diets [138]. This finding may be explained by selective survival of individuals whose healthy diet may have contributed to reverse the negative selection pressure associated with ApoE4. The quality of the diet

would be less essential for the survival of ApoE4 non-carriers. Significant weight loss and decrease in BMI were observed in older women with AD who were ApoE4 carriers but not in non-carriers [139]. In cognitively normal control women, no significant weight loss was observed whatever the ApoE4 status.

In AD patients included in the OmegAD RCT, weight gain and appetite improved in the EPA+DHA group independently of the ApoE genotype [140]. Not carrying the ApoE4 allele and higher DHA plasma levels during the treatment period were both positively associated with weight gain. Unfortunately, the same study did not report the differential effects of n-3 PUFA supplementation on cognition according to ApoE genotype [21]. Hence, DHA and/or EPA might also work through mechanisms that could contribute to improve overall nutritional status of AD patients. This effect might be modulated by ApoE4 genotype but the direction and magnitude of the interaction is not yet clearly established.

4.3.6 Mood

Older persons with depressive symptoms are at higher risk of cognitive decline and AD [141-143]. Moreover, several studies have reported an association between the ApoE4 allele and depression among older adults [144]. Studies examining the impact of long-chain n-3 PUFA on depressive symptoms have provided mixed results. While observational epidemiological studies suggest a protective effect of EPA and DHA [145-148] or fish consumption [147, 149-150] in various mood disorders, most RCT failed to evidence a significant impact of supplementation with EPA or DHA [19, 151-152] with some interesting recent exceptions [153]. This lack of apparent efficiency may be explained in part by a differential effect of n-3 PUFA according to ApoE genotype which could mask a protective association in some individuals. In the 3C study, higher plasma EPA was associated with slower decline on Benton Visual Retention Test in ApoE4 carriers, or in subjects with high depressive symptoms at baseline [78]. Both interactions were independent, i.e. the highest protective effect was observed in depressive ApoE4 carriers. This finding suggests that the impact of n-3 PUFA on cognition in ApoE4 carriers is mediated by more than a decrease in depressive symptoms.

In the OmegAD RCT, no significant overall treatment effects on neuropsychiatric symptoms of AD patients were found [154]. However, significant positive treatment effects on the scores in the Neuro-Psychiatric Inventory agitation domain in ApoE4 carriers and on the Montgomery-Asberg Depression Rating Scale in ApoE4 non-carriers were found. Such inconsistent results emphasize the need for further research on the mechanisms underlying the effect of n-3 PUFA on mood disorders in AD patients according to ApoE genotype and their potential impact on subsequent cognitive decline.

5) General discussion and conclusion

As suggested in a recent review, the presence of the ApoE4 allele might alter the course of late-life cognitive decline through changes in levels of both AD pathology and vascular injury, as well as through modifications in compensatory repair mechanisms [1]. AD likely occurs when many contributing risk factors, including genetics, converge and are counterbalanced by few protective factors [155]. Dietary n-3 PUFA are now understood to modify some of these risk factors. The ApoE4 interaction with PUFA metabolism is a nutrient-gene interaction which must be taken into account when assessing the risk for AD and evaluating the impact of dietary intake or supplementation with n-3 PUFA. Statistical analyses should systematically search for interactions with, and not only adjust for, ApoE genotype. In case of a significant interaction, stratified analyses should be provided.

The reasons why some effects of n-3 PUFA are observed in the ApoE4 carriers and others in non-carriers, as summarized in table 2, are not yet fully understood. It has been suggested that ApoE4 carriers may be more vulnerable to environmental factors [80] because of compromised brain reserve [156] or poor brain protection and repair mechanisms. Indeed, ApoE4 carriers have higher serum total and LDL cholesterol, impaired brain metabolism, and more often depressive symptoms. Nevertheless, ApoE4 carriers often have a lower level of some risk factors related to cognitive decline or dementia such as inflammation, hyperhomocysteinemia, and overweight, with inconsistent results. When considering the impact of dietary supplementation with n-3 PUFA on these biomarkers, ApoE4 carriers tend to be more responsive but few data are available from epidemiological or intervention studies. Paradoxically, ApoE4 carriers are less sensitive to the protective effects of n-3 PUFA on cognition in most studies. Further research is needed to understand the patho-physiological mechanisms underlying the differential effect of dietary PUFA on AD risk or cognitive decline according to ApoE genotype in order to better target intervention studies and nutritional recommendations on potential beneficiaries.

Table **2**. Impact of n-3 PUFA on risk factors of dementia and AD according to ApoE genotype: summary of findings.

Effect of dietary n-3 PUFA on:	Stronger effect of n-3 PUFA in:	
	ApoE4 carriers	ApoE4 non-carriers
Incorporation of EPA and DHA in blood TG		X
Blood total, LDL or HDL cholesterol	X	
Lowering blood TG concentration	X	
Increasing susceptibility to lipid peroxidation	X	
Lowering inflammation	=	=
Lowering homocysteine concentration	?	?
Increasing appetite and weight gain in AD patients	=	=
Improving neuro-psychiatric symptoms	X	
Improving depressive symptoms		X

Legend:

X stronger effect

= similar effect in both groups

? unknown or discordant results

List of abbreviations

AD	Alzheimer's disease
ApoE	Apolipoprotein E
ApoE4	ε4 allele of the Apoliprotein E gene
BMI	Body mass index
CAT	Catalase
CRP	C-reactive protein
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
GSH-Px	Glutathion peroxidase
HL	Hepatic lipase
IDL	Intermediate density lipoproteins
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
MRI	Magnetic resonance imaging
n-3	Omega-3
n-6	Omega-6
PET	Positron emitting tomography
PIB	Pittsburgh compound B
PUFA	Poly-unsaturated fatty acids
RCT	Randomized controlled trial
ROS	Reactive oxygen species
TG	Triglycerides
VLDL	Very low density lipoprotein
WHICAP	Washington Heights-Inwood Columbia Aging Project

Conflict of interest (if any)

The authors report no conflict of interest

Acknowledgments: We thank S. Cunnane, Research Center on Aging, University of Sherbrooke, Sherbrooke, Canada, for his careful revision of the manuscript and relevant comments.

CF benefited from financial support of the « ANR- *Agence Nationale de la Recherche* - The French National Research Agency » under the « *Programme National de Recherche en Alimentation et nutrition humaine* », project « COGINUT ANR-06-PNRA-005».

CS was funded by the Carnot Institute LISA (Lipids for Industry, Safety and Health).

MP was supported by a fellowship given by the Fonds de la recherche en santé du Québec and her research is currently supported by Advanced Foods and Materials Network.

References

[1] Fotuhi M, Hachinski V, Whitehouse PJ. Changing perspectives regarding late-life dementia. Nat Rev Neurol 5; 649-58 (2009)

[2] Rapoport SI. Arachidonic acid and the brain. J Nutr 138; 2515-2520 (2008)

[3] Lukiw WJ, Bazan NG. Docosahexaenoic acid and the aging brain. J Nutr 138; 2510-2514 (2008)

[4] Schmitz G, Ecker J. The opposing effects of n-3 and n-6 fatty acids. Prog Lipid Res 47; 147 (2008)

[5] Plourde M, Cunnane S. Extremely limited conversion of long chain omega 3 polyunsaturates in adults: implications for their dietary essentiality and use as supplements. Appl Physiol Nutr Metab 32; 619-634 (2007)

[6] Bourre JM, Paquotte P. Contributions (in 2005) of marine and fresh water products (finfish and shellfish, seafood, wild and farmed) to the French dietary intakes of vitamins D and B12, selenium, iodine and docosahexaenoic acid: impact on public health. Int J Food Sci Nutr 59; 491-501 (2008)

[7] Kalmijn S, Launer LJ, Ott A, Witteman JCM, Hofman A, Breteler MMB. Dietary fat intake and the risk of incident dementia in the Rotterdam study. Ann Neurol 42; 776-782 (1997)

[8] Barberger-Gateau P, Letenneur L, Deschamps V, Peres K, Dartigues J-F, Renaud S. Fish, meat, and risk of dementia: cohort study. BMJ 325; 932-933 (2002)

[9] Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Wilson RS, *et al.* Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 60; 940-946 (2003)

[10] Barberger-Gateau P, Raffaitin C, Letenneur L, Berr C, Tzourio C, Dartigues JF, *et al.* Dietary patterns and risk of dementia: the Three-City cohort study. Neurology 69; 1921-1930 (2007)

[11] Albanese E, Dangour AD, Uauy R, Acosta D, Guerra M, Guerra SSG, *et al.* Dietary fish and meat intake and dementia in Latin America, China, and India: a 10/66 Dementia Research Group population-based study. Am J Clin Nutr 90; 392-400 (2009)

[12] Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JCM, *et al.* Diet and risk of dementia: Does fat matter? The Rotterdam Study. Neurology 59; 1915-1921 (2002)

[13] Devore EE, Grodstein F, van Rooij FJA, Hofman A, Rosner B, Stampfer MJ, *et al.* Dietary intake of fish and omega-3 fatty acids in relation to long-term dementia risk. Am J Clin Nutr 90; 170-176 (2009)

[14] Kroger E, Verreault R, Carmichael P-H, Lindsay J, Julien P, Dewailly E, *et al.* Omega-3 fatty acids and risk of dementia: the Canadian Study of Health and Aging. Am J Clin Nutr 90; 184-192 (2009)

[15] van de Rest O, Spiro A, III, Krall-Kaye E, Geleijnse JM, de Groot LCPGM, Tucker KL. Intakes of (n-3) Fatty Acids and Fatty Fish Are Not Associated with Cognitive Performance and 6-Year Cognitive Change in Men Participating in the Veterans Affairs Normative Aging Study. J Nutr 139; 2329-2336 (2009)

[16] Kotani S, Sakaguchi E, Warashina S, Matsukawa N, Ishikura Y, Kiso Y, *et al.* Dietary supplementation of arachidonic and docosahexaenoic acids improves cognitive dysfunction. Neurosci Res 56; 159-64 (2006)

[17] Chiu C-C, Su K-P, Cheng T-C, Liu H-C, Chang C-J, Dewey ME, *et al.* The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: A preliminary randomized double-blind placebo-controlled study. Prog Neuropsychopharmacol Biol Psychiatry 32; 1538 (2008)

[18] van de Rest OM, Geleijnse JMP, Kok FJP, van Staveren WAP, Dullemeijer CM, OldeRikkert MGMMDP, *et al.* Effect of fish oil on cognitive performance in older subjects: A randomized, controlled trial. Neurology 71; 430-438 (2008)

[19] Rogers PJ, Appleton KM, Kessler D, Peters TJ, Gunnell D, Hayward RC, *et al.* No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial. Br J Nutr 99; 421-31 (2008)

[20] Dangour AD, Allen E, Elbourne D, Fasey N, Fletcher AE, Hardy P, *et al.* Effect of 2-y n-3 long-chain polyunsaturated fatty acid supplementation on cognitive function in older people: a randomized, double-blind, controlled trial. Am J Clin Nutr 91; 1725-1732 (2010)

[21] Freund-Levi Y, Eriksdotter-Jonhagen M, Cederholm T, Basun H, Faxen-Irving G, Garlind A, *et al.* Omega-3 Fatty Acid Treatment in 174 Patients With Mild to Moderate Alzheimer Disease: OmegAD Study: A Randomized Double-blind Trial. Arch Neurol 63; 1402-1408 (2006)

[22] Yurko-Mauro K, McCarthy D, Rom D, Nelson EB, Ryan AS, Blackwell A, *et al.* Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. Alzheimers Dement 6 : 456-464

[23] Cunnane SC, Plourde M, Pifferi F, Bégin M, Féart C, Barberger-Gateau P. Fish, docosahexaenoic acid and Alzheimer's disease. Prog Lipid Res 48; 239-256 (2009)

[24] Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. Lancet 368; 387-403 (2006)

[25] Solfrizzi V, D'Introno A, Colacicco AM, Capurso C, Todarello O, Pellicani V, *et al.* Circulating biomarkers of cognitive decline and dementia. Clin Chim Acta 364; 91 (2006)

[26] Lambert J-C, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, *et al.* Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 41; 1094-U68 (2009)

[27] Cedazo-Mínguez A. Apolipoprotein E and Alzheimer's disease: molecular mechanisms and therapeutic opportunities. J Cell Mol Med 11; 1227-1238 (2007)

[28] Panza F, Solfrizzi V, Torres F, Mastroianni F, DelParigi A, Colacicco AM, *et al.* Decreased frequency of apolipoprotein E epsilon 4 allele from Northern to Southern Europe in Alzheimer's disease patients and centenarians. Neurosci Lett 277; 53-56 (1999)

[29] Farrer LAP, Cupples LAP, Haines JLP, Hyman BMDP, Kukull WAP, Mayeux RMD, *et al.* Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: a meta-analysis. Jama 278; 1349-1356 (1997)

[30] Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. Proc Natl Acad Sci U S A 103; 5644-51 (2006)

[31] Drzezga A, Grimmer T, Henriksen G, Muhlau M, Perneczky R, Miederer I, *et al.* Effect of APOE genotype on amyloid plaque load and gray matter volume in Alzheimer disease. Neurology 72; 1487-1494 (2009)

[32] Kok E, Haikonen S, Luoto T, Huhtala H, Goebeler S, Haapasalo H, *et al.* Apolipoprotein E-dependent accumulation of Alzheimer disease-related lesions begins in middle age. Ann Neurol 65; 650-657 (2009)

[33] Jofre-Monseny L, Minihane A-M, Rimbach G. Impact of apoE genotype on oxidative stress, inflammation and disease risk. Mol Nutr Food Res 52; 131-145 (2008)

[34] Querfurth HW, LaFerla FM. Alzheimer's Disease. N Engl J Med 362; 329-344 (2010)

[35] Montine TJ, Neely MD, Quinn JF, Beal MF, Markesbery WR, Roberts LJ, *et al.* Lipid peroxidation in aging brain and Alzheimer's disease. Free Radic Biol Med 33; 620-6 (2002)

[36] Markesbery WR, Kryscio RJ, Lovell MA, Morrow JD. Lipid peroxidation is an early event in the brain in amnestic mild cognitive impairment. Ann Neurol 58; 730-5 (2005)

[37] Lauderback CM, Kanski J, Hackett JM, Maeda N, Kindy MS, Butterfield DA. Apolipoprotein E modulates Alzheimer's A[beta](1-42)-induced oxidative damage to synaptosomes in an allele-specific manner. Brain Research 924; 90-97 (2002)

[38] Miyata M, Smith JD. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. Nat Genet 14; 55-61 (1996)

[39] Ortega H, Castilla P, Gomez-Coronado D, Garces C, Benavente M, Rodriguez-Artalejo F, *et al.* Influence of apolipoprotein E genotype on fat-soluble plasma antioxidants in Spanish children. Am J Clin Nutr 81; 624-632 (2005)

[40] Mas E, Dupuy AM, Artero S, Portet F, Cristol JP, Ritchie K, *et al.* Functional vitamin E deficiency in ApoE4 patients with Alzheimer's disease. Dement Geriatr Cogn Disord 21 (3); 198-204 (2006)

[41] Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal N, *et al.* Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease on a biracial community study. Jama 287; 3230-3237 (2002)

[42] Hu P, Bretsky P, Crimmins EM, Guralnik JM, Reuben DB, Seeman TE. Association between serum beta-carotene levels and decline of cognitive function in high-functioning older persons with or without apolipoprotein E 4 alleles: MacArthur studies of successful aging. J Gerontol A Biol Sci Med Sci 61; 616-20 (2006)

[43] Gao S, Jin Y, Hall KS, Liang C, Unverzagt FW, Ma F, *et al.* Selenium level is associated with apoE epsilon4 in rural elderly Chinese. Public Health Nutr 12; 2371-6 (2009)

[44] Kharrazi H, Vaisi-Raygani A, Rahimi Z, Tavilani H, Aminian M, Pourmotabbed T. Association between enzymatic and non-enzymatic antioxidant defense mechanism with apolipoprotein E genotypes in Alzheimer disease. Clin Biochem 41; 932-936 (2008)

[45] Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, Kerr S, *et al.* Systemic inflammation and disease progression in Alzheimer disease. Neurology 73; 768-774 (2009)

[46] Finch CE, Morgan TE. Systemic inflammation, infection, ApoE alleles, and Alzheimer disease: a position paper. Curr Alzheimer Res 4; 185-9 (2007)

[47] Belinson H, Michaelson DM. ApoE4-dependent Abeta-mediated neurodegeneration is associated with inflammatory activation in the hippocampus but not the septum. J Neural Transm 116; 1427-34 (2009)

[48] Chasman DI, Kozlowski P, Zee RY, Kwiatkowski DJ, Ridker PM. Qualitative and quantitative effects of APOE genetic variation on plasma C-reactive protein, LDL-cholesterol, and apoE protein. Genes Immun 7; 211-219 (2006)

[49] Ravaglia G, Forti P, Maioli F, Chiappelli M, Montesi F, Bianchin M, *et al.* Apolipoprotein E e4 allele affects risk of hyperhomocysteinemia in the elderly. Am J Clin Nutr 84; 1473-80 (2006)

[50] Rontu R, Ojala P, Hervonen A, Goebeler S, Karhunen PJ, Nikkilä M, *et al.* Apolipoprotein E genotype is related to plasma levels of C-reactive protein and lipids and to longevity in nonagenarians. Clin Endocrinol (Oxf) 64; 265-270 (2006)

[51] Noble JM, Manly JJ, Schupf N, Tang MX, Mayeux R, Luchsinger JA. Association of C-reactive protein with cognitive impairment. Arch Neurol 67; 87-92 (2010)

[52] Mu H, Hoy CE. The digestion of dietary triacylglycerols. Prog Lipid Res 43; 105-33 (2004)

[53] Hooijmans CR, Kiliaan AJ. Fatty acids, lipid metabolism and Alzheimer pathology. Eur J Pharmacol 585; 176-196 (2008)

[54] Mahley RW, Rall SC. Apoliprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 1; 507-537 (2000)

[55] Shobab LA, Hsiung G-YR, Feldman HH. Cholesterol in Alzheimer's disease. Lancet Neurol 4; 841-852 (2005)

[56] Lane RM, Farlow MR. Lipid homeostasis and apolipoprotein E in the development and progression of Alzheimer's disease. J Lipid Res 46; 949-68 (2005)

[57] Garry PJ, Baumgartner RN, Brodie SG, Montoya GD, Liang HC, Lindeman RD, *et al.* Estrogen replacement therapy, serum lipids, and polymorphism of the apolipoprotein E gene. Clin Chem 45; 1214-1223 (1999)

[58] Staehelin HB, PerrigChiello P, Mitrache C, Miserez AR, Perrig WJ. Apolipoprotein E genotypes and cognitive functions in healthy elderly persons. Acta Neurol Scand 100; 53-60 (1999)

[59] Wehr H, Parnowski T, Puzynski S, BednarskaMakaruk M, Bisko M, KotapkaMinc S, *et al.* Apolipoprotein E genotype and lipid and lipoprotein levels in dementia. Dement Geriatr Cogn Disord 11; 70-73 (2000)

[60] Giltay EJ, van Reedt Dortland AKB, Nissinen A, Giampaoli S, van Veen T, Zitman FG, *et al.* Serum cholesterol, apolipoprotein E genotype and depressive symptoms in elderly European men: The FINE study. J Affect Disord 115; 471-477 (2009)

[61] Kuo YM, Emmerling MR, Bisgaier CL, Essenburg AD, Lampert HC, Drumm D, *et al.* Elevated low-density lipoprotein in Alzheimer's disease correlates with brain A beta 1-42 levels. Biochem Biophys Res Commun 252; 711-715 (1998)

[62] Kivipelto M, Helkala E-L, Laakso MP, Hanninen T, Hallikainen M, Alhainen K, *et al.* Apolipoprotein E epsilon 4 allele, elevated midlife total cholesterol level, and high midlife systolic blood pressure are independent risk factors for late-life Alzheimer disease. Ann Intern Med 137; 149-155 (2002)

[63] Solomon A, Kivipelto M, Wolozin B, Zhou J, Whitmer RA. Midlife serum cholesterol and increased risk of Alzheimer's and vascular dementia three decades later. Dement Geriatr Cogn Disord 28; 75-80 (2009)

[64] Solomon A, Kareholt I, Ngandu T, Winblad B, Nissinen A, Tuomilehto J, *et al.* Serum cholesterol changes after midlife and late-life cognition - Twenty-one-year follow-up study. Neurology 68; 751-756 (2007)

[65] Stewart R, White LR, Xue QL, Launer LJ. Twenty-six-year change in total cholesterol levels and incident dementia - The Honolulu-Asia Aging Study. Arch Neurol 64; 103-107 (2007)

[66] Evans RM, Emsley CL, Gao S, Sahota A, Hall KS, Farlow MR, *et al.* Serum cholesterol, APOE genotype, and the risk of Alzheimer's disease: A population-based study of African Americans. Neurology 54; 240-242 (2000)

[67] Hall K, Murrell J, Ogunniyi A, Deeg M, Baiyewu O, Gao S, *et al.* Cholesterol, APOE genotype, and Alzheimer disease: An epidemiologic study of Nigerian Yoruba. Neurology 66; 223-227 (2006)

[68] Dufouil C, Richard F, Fievet N, Dartigues JF, Ritchie K, Tzourio C, *et al.* APOE genotype, cholesterol level, lipid-lowering treatment, and dementia: the Three-City Study. Neurology 64; 1531-8 (2005)

[69] West R, Beeri MS, Schmeidler J, Hannigan CM, Angelo G, Grossman HT, *et al.* Better memory functioning associated with higher total and low-density lipoprotein cholesterol levels in very elderly subjects without the apolipoprotein e4 allele. Am J Geriatr Psychiatry 16; 781-785 (2008)

[70] van den Kommer TN, Dik MG, Comijs HC, Fassbender K, Lutjohann D, Jonker C. Total cholesterol and oxysterols: Early markers for cognitive decline in elderly? Neurobiol Aging 30; 534-545 (2009)

[71] Minihane AM, Jofre-Monseny L, Olano-Martin E, Rimbach G. ApoE genotype, cardiovascular risk and responsiveness to dietary fat manipulation. Proc Nutr Soc 66; 183-97 (2007)

[72] Plourde M, Fortier M, Vandal M, Tremblay-Mercier J, Freemantle E, Bégin M, *et al.* Unresolved issues in the link between docosahexaenoic acid and Alzheimer's disease. Prostaglandins Leukot Essent Fatty Acids 77; 301 (2007)

[73] Kim J, Basak JM, Holtzman DM. The role of apolipoprotein E in Alzheimer's disease. Neuron 63; 287-303 (2009)

[74] Fraser T, Tayler H, Love S. Fatty acid composition of frontal, temporal and parietal neocortex in the normal human brain and in Alzheimer's disease. Neurochem Res 35; 503-13 (2010)

[75] Huang TL, Zandi PP, Tucker KL, Fitzpatrick AL, Kuller LH, Fried LP, *et al.* Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon4. Neurology 65; 1409-1414 (2005)

[76] Beydoun MA, Kaufman JS, Satia JA, Rosamond W, Folsom AR. Plasma n-3 fatty acids and the risk of cognitive decline in older adults: the Atherosclerosis Risk in Communities Study. Am J Clin Nutr 85; 1103-1111 (2007)

[77] Whalley LJ, Deary IJ, Starr JM, Wahle KW, Rance KA, Bourne VJ, *et al.* n-3 Fatty acid erythrocyte membrane content, APOE epsilon4, and cognitive variation: an observational follow-up study in late adulthood. Am J Clin Nutr 87; 449-454 (2008)

[78] Samieri C, Feart C, Proust-Lima C, Peuchant E, Dartigues JF, Amieva H, *et al.* Omega-3 fatty acids and cognitive decline: modulation by ApoE epsilon4 allele and depression. Neurobiol Aging; (in press)

[79] Laitinen MH, Ngandu T, Rovio S, Helkala EL, Uusitalo U, Viitanen M, *et al.* Fat intake at midlife and risk of dementia and Alzheimer's disease: a population-based study. Dement Geriatr Cogn Disord 22; 99-107 (2006)

[80] Kivipelto M, Rovio S, Ngandu T, Kåreholt I, Eskelinen M, Winblad B, *et al.* Apolipoprotein epsilon4 magnifies lifestyle risks for dementia: a population-based study. J Cell Mol Med 12; 2762-2771 (2008)

[81] Samieri C, Feart C, Letenneur L, Dartigues J-F, Peres K, Auriacombe S, *et al.* Low plasma eicosapentaenoic acid and depressive symptomatology are independent predictors of dementia risk. Am J Clin Nutr 88; 714-721 (2008)

[82] Quinn JF, Raman R, Thomas RG, Yurko-Mauro K, Nelson EB, Van Dyck C, *et al.* Docosahexaenoic acid supplementation and cognitive decline in Alzheimer Disease: A randomized trial. *JAMA* 304: 1903-1911 (2010)

[83] Joseph J, Cole G, Head E, Ingram D. Nutrition, Brain Aging, and Neurodegeneration. J Neurosci 29; 12795-12801 (2009)

[84] Cole GM, Frautschy SA. DHA May Prevent Age-Related Dementia. J Nutr 140; 869-874 (2010)

[85] Polozova A, Salem N, Jr. Role of liver and plasma lipoproteins in selective transport of n-3 fatty acids to tissues: a comparative study of 14C-DHA and 3H-oleic acid tracers. J Mol Neurosci 33; 56-66 (2007)

[86] Edmond J. Essential polyunsaturated fatty acids and the barrier to the brain: the components of a model for transport. J Mol Neurosci 16; 181-93; discussion 215-21 (2001)

[87] Hamilton JA, Brunaldi K. A model for fatty acid transport into the brain. J Mol Neurosci 33; 12-7 (2007)

[88] Spector AA. Plasma free fatty acid and lipoproteins as sources of polyunsaturated fatty acid for the brain. J Mol Neurosci 16; 159-65; discussion 215-21 (2001)

[89] Hamilton JA, Hillard CJ, Spector AA, Watkins PA. Brain uptake and utilization of fatty acids, lipids and lipoproteins: application to neurological disorders. J Mol Neurosci 33; 2-11 (2007)

[90] Plourde M, Vohl M-C, Vandal M, Couture P, Lemieux S, Cunnane SC. Plasma n-3 fatty acid response to an n-3 fatty acid supplement is modulated by apoE 4 but not by the common PPAR-alpha L162V polymorphism in men. Br J Nutr 102; 1121-1124 (2009)

[91] Sadou H, Leger CL, Descomps B, Barjon JN, Monnier L, Crastes de Paulet A. Differential incorporation of fish-oil eicosapentaenoate and docosahexaenoate into lipids of lipoprotein fractions as related to their glyceryl esterification: a short-term (postprandial) and long-term study in healthy humans. Am J Clin Nutr 62; 1193-200 (1995)

[92] Gibney MJ, Daly E. The incorporation of n-3 polyunsaturated fatty acids into plasma lipid and lipoprotein fractions in the postprandial phase in healthy volunteers. Eur J Clin Nutr 48; 866-72 (1994)

[93] Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN. Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. J Lipid Res 44; 2065-72 (2003)

[94] Motoyama KR, Curb JD, Kadowaki T, El-Saed A, Abbott RD, Okamura T, *et al.* Association of serum n-6 and n-3 polyunsaturated fatty acids with lipids in 3 populations of middle-aged men. Am J Clin Nutr 90; 49-55 (2009)

[95] Anil E. The impact of EPA and DHA on blood lipids and lipoprotein metabolism: influence of apoE genotype. Proc Nutr Soc 66; 60-8 (2007)

[96] Masson LF, McNeill G, Avenell A. Genetic variation and the lipid response to dietary intervention: a systematic review. Am J Clin Nutr 77; 1098-1111 (2003)

[97] Olano-Martin E, Anil E, Caslake MJ, Packard CJ, Bedford D, Stewart G, *et al.* Contribution of apolipoprotein E genotype and docosahexaenoic acid to the LDL-cholesterol response to fish oil. Atherosclerosis 209; 104-10 (2010)

[98] Nagao K, Yanagita T. Bioactive lipids in metabolic syndrome. Prog Lipid Res 47; 127 (2008)

[99] Paula R, Souza V, Benedet A, Souza E, Toledo J, Moraes C, *et al.* Dietary fat and apolipoprotein genotypes modulate plasma lipoprotein levels in Brazilian elderly women. Mol Cell Biochem 337; 307-315 (2010)

[100] Caslake MJ, Miles EA, Kofler BM, Lietz G, Curtis P, Armah CK, *et al.* Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. Am J Clin Nutr 88; 618-629 (2008)

[101] Farr SA, Yamada KA, Butterfield DA, Abdul HM, Xu L, Miller NE, *et al.* Obesity and hypertriglyceridemia produce cognitive impairment. Endocrinology 149; 2628-36 (2008)

[102] Raffaitin C, Gin H, Empana JP, Helmer C, Berr C, Tzourio C, *et al.* Metabolic syndrome and risk for incident Alzheimer's disease or vascular dementia: the Three - City study. Diabetes Care 32; 169-174 (2009)

[103] Cooper JL. Dietary lipids in the aetiology of Alzheimer's disease: implications for therapy. Drugs Aging 20; 399-418 (2003)

[104] GomezRamos A, DiazNido J, Smith MA, Perry G, Avila J. Effect of the lipid peroxidation product acrolein on tau phosphorylation in neural cells. J Neurosci Res 71; 863-870 (2003)

[105] Szekely CA, Breitner JCS, Fitzpatrick AL, Rea TD, Psaty BM, Kuller LH, *et al.* NSAID use and dementia risk in the cardiovascular health study - Role of APOE and NSAID type. Neurology 70; 17-24 (2008)

[106] Freund-Levi Y, Hjorth E, Lindberg C, Cederholm T, Faxen-Irving G, Vedin I, *et al.* Effects of Omega-3 Fatty Acids on Inflammatory Markers in Cerebrospinal Fluid and Plasma in Alzheimer's Disease: The OmegAD Study. Dement Geriatr Cogn Disord 27; 481-490 (2009)

[107] Kalpouzos G, Chetelat G, Baron JC, Landeau B, Mevel K, Godeau C, *et al.* Voxel-based mapping of brain gray matter volume and glucose metabolism profiles in normal aging. Neurobiol Aging 30; 112-24 (2009)

[108] Kalpouzos G, Eustache F, de la Sayette V, Viader F, Chetelat G, Desgranges B. Working memory and FDG-PET dissociate early and late onset Alzheimer disease patients. J Neurol 252; 548-58 (2005)

[109] Reiman EM, Chen K, Alexander GE, Caselli RJ, Bandy D, Osborne D, *et al.* Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. Proc Natl Acad Sci U S A 101; 284-9 (2004)

[110] Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG, Cahill GF, Jr. Brain metabolism during fasting. J Clin Invest 46; 1589-95 (1967)

[111] Freemantle E, Vandal M, Tremblay-Mercier J, Plourde M, Poirier J, Cunnane S. Metabolic response to a ketogenic breakfast in the healthy elderly. J Nutr Health Aging 13; 293 (2009)

[112] Reger MA, Henderson ST, Hale C, Cholerton B, Baker LD, Watson GS, *et al.* Effects of [beta]-hydroxybutyrate on cognition in memory-impaired adults. Neurobiol Aging 25; 311-314 (2004)

[113] Henderson ST, Vogel JL, Barr LJ, Garvin F, Jones JJ, Costantini LC. Study of the ketogenic agent AC-1202 in mild to moderate Alzheimer's disease: a randomized, double-blind, placebo-controlled, multicenter trial. Nutr Metab (Lond) 6; 31 (2009)

[114] Tucker KL, Qiao N, Scott T, Rosenberg I, Spiro A, 3rd. High homocysteine and low B vitamins predict cognitive decline in aging men: the Veterans Affairs Normative Aging Study. Am J Clin Nutr 82; 627-35 (2005)

[115] Clarke R, Birks J, Nexo E, Ueland PM, Schneede J, Scott J, *et al.* Low vitamin B-12 status and risk of cognitive decline in older adults. Am J Clin Nutr 86; 1384-1391 (2007)

[116] Haan MN, Miller JW, Aiello AE, Whitmer RA, Jagust WJ, Mungas DM, *et al.* Homocysteine, B vitamins, and the incidence of dementia and cognitive impairment: results from the Sacramento Area Latino Study on Aging. Am J Clin Nutr 85; 511-7 (2007)

[117] Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, *et al.* Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 346; 476-83 (2002)

[118] Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, *et al.* Homocysteine and folate as risk factors for dementia and Alzheimer disease. Am J Clin Nutr 82; 636-43 (2005)

[119] Ravaglia G, Forti P, Maioli F, Chiappelli M, Montesi F, Tumini E, *et al.* Blood inflammatory markers and risk of dementia: The conselice study of brain aging. Neurobiol Aging 28; 1810-1820 (2007)

[120] Elias MF, Robbins MA, Budge MM, Elias PK, Dore GA, Brennan SL, *et al.* Homocysteine and cognitive performance: Modification by the ApoE genotype. Neurosci Lett 430; 64-69 (2008)

[121] Li D, Yu XM, Xie HB, Zhang YH, Wang Q, Zhou XQ, *et al.* Platelet phospholipid n-3 PUFA negatively associated with plasma homocysteine in middle-aged and geriatric hyperlipaemia patients. Prostaglandins Leukot Essent Fatty Acids 76; 293-297 (2007)

[122] Huang T, Wahlqvist ML, Li D. Docosahexaenoic acid decreases plasma homocysteine via regulating enzyme activity and mRNA expression involved in methionine metabolism. Nutrition 26; 112-9 (2010)

[123] Olszewski AJ, McCully KS. Fish oil decreases serum homocysteine in hyperlipemic men. Coron Artery Dis 4; 53-60 (1993)

[124] Selley ML. A metabolic link between S-adenosylhomocysteine and polyunsaturated fatty acid metabolism in Alzheimer's disease. Neurobiol Aging 28; 1834-9 (2007)

[125] Kanaya AM, Lindquist K, Harris TB, Launer L, Rosano C, Satterfield S, *et al.* Total and regional adiposity and cognitive change in older adults: The Health, Aging and Body Composition (ABC) study. Arch Neurol 66; 329-35 (2009)

[126] Sabia S, Kivimaki M, Shipley MJ, Marmot MG, Singh-Manoux A. Body mass index over the adult life course and cognition in late midlife: the Whitehall II Cohort Study. Am J Clin Nutr 89; 601-607 (2009)

[127] Beydoun MA, Lhotsky A, Wang Y, Forno GD, An Y, Metter EJ, *et al.* Association of Adiposity Status and Changes in Early to Mid-Adulthood With Incidence of Alzheimer's Disease. Am J Epidemiol 168; 1179-1189 (2008)

[128] Whitmer RA, Gustafson DR, Barrett-Connor E, Haan MN, Gunderson EP, Yaffe K. Central obesity and increased risk of dementia more than three decades later. Neurology 71; 1057-1064 (2008)

[129] Fitzpatrick AL, Kuller LH, Lopez OL, Diehr P, O'Meara ES, Longstreth WT, Jr., *et al.* Midlife and late-life obesity and the risk of dementia: cardiovascular health study. Arch Neurol 66; 336-42 (2009)

[130] Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kareholt I, Winblad B, *et al.* Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Arch Neurol 62; 1556-60 (2005)

[131] Kim H-K, Della-Fera M, Lin J, Baile CA. Docosahexaenoic Acid Inhibits Adipocyte Differentiation and Induces Apoptosis in 3T3-L1 Preadipocytes. J Nutr 136; 2965-2969 (2006)

[132] Micallef M, Munro I, Phang M, Garg M. Plasma n-3 Polyunsaturated Fatty Acids are negatively associated with obesity. Br J Nutr 102; 1370-4 (2009)

[133] Kypreos KE, Karagiannides I, Fotiadou EH, Karavia EA, Brinkmeier MS, Giakoumi SM, *et al.* Mechanisms of obesity and related pathologies: Role of apolipoprotein E in the development of obesity. FEBS J 276; 5720-5728 (2009)

[134] Luchsinger JA, Tang MX, Shea S, Mayeux R. Caloric intake and the risk of Alzheimer disease. Arch Neurol 59; 1258-1263 (2002)

[135] Beydoun MA, Beydoun HA, Wang Y. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. Obes Rev 9; 204-18 (2008)

[136] Nourhashemi F, Deschamps V, Larrieu S, Letenneur L, Dartigues JF, Barberger-Gateau P. Body mass index and incidence of dementia: the PAQUID study. Neurology 60; 117-119 (2003)

[137] Atti AR, Palmer K, Volpato S, Winblad B, De Ronchi D, Fratiglioni L. Late-life body mass index and dementia incidence: Nine-year follow-up data from the kungsholmen project. J Am Geriatr Soc 56; 111-116 (2008)

[138] Wengreen HJ, Neilson C, Munger R, Corcoran C. Diet Quality Is Associated with Better Cognitive Test Performance among Aging Men and Women. J Nutr 139; 1944-1949 (2009)

[139] Vanhanen M, Kivipelto M, Koivisto K, Kuusisto J, Mykkänen L, Helkala EL, *et al.* APOE-ε4 is associated with weight loss in women with AD. A population-based study. Neurology 56; 655-659 (2001)

[140] Faxen Irving G, Freund-Levi Y, Eriksdotter-Jonhagen M, Basun H, Brismar K, Hjorth E, *et al.* Omega-3 Fatty Acid Supplementation Effects on Weight and Appetite in Patients with Alzheimer's Disease: The Omega-3 Alzheimer's Disease Study. J Am Geriatr Soc; (2008)

[141] Jorm AF. Is depression a risk factor for dementia or cognitive decline? A review. Gerontology 46; 219-227 (2000)

[142] Luchsinger JA, Honig LS, Tang MX, Devanand DP. Depressive symptoms, vascular risk factors, and Alzheimer's disease. Int J Geriatr Psychiatry 23; 922-928 (2008)

[143] Modrego PJ, Ferrandez J. Depression in patients with mild cognitive impairment increases the risk of developing dementia of Alzheimer type - A prospective cohort study. Arch Neurol 61; 1290-1293 (2004)

[144] Slifer MA, Martin ER, Gilbert JR, Haines JL, Pericak-Vance MA. Resolving the relationship between Apolipoprotein E and depression. Neurosci Lett 455; 116-119 (2009)

[145] Tiemeier H, van Tuijl HR, Hofman A, Kiliaan AJ, Breteler MM. Plasma fatty acid composition and depression are associated in the elderly: the Rotterdam Study. Am J Clin Nutr 78; 40-46 (2003)

[146] Bourre JM. Dietary Omega-3 fatty acids and psychiatry: mood, behaviour, stress, depression, dementia and aging. J Nutr Health Aging 9; 31-38 (2005)

[147] Astorg P, Couthouis A, Bertrais S, Arnault N, Meneton P, Guesnet P, *et al.* Association of fish and long-chain n-3 polyunsaturated fatty acid intakes with the occurrence of depressive episodes in middle-aged French men and women. Prostaglandins Leukot Essent Fatty Acids 78; 171 (2008)

[148] Feart C, Peuchant E, Letenneur L, Samieri C, Montagnier D, Fourrier-Reglat A, *et al.* Plasma eicosapentaenoic acid is inversely associated with severity of depressive symptomatology in the elderly: data from the Bordeaux sample of the Three-City Study. Am J Clin Nutr 87; 1156-1162 (2008)

[149] Hibbeln JR. Fish consumption and major depression. Lancet 351; 1213 (1998)

[150] Tanskanen A, Hibbeln JR, Tuomilehto J, Uutela A, Haukkala A, Viinamaki H, *et al.* Fish consumption and depressive symptoms in the general population in Finland. Psychiatr Serv 52; 529-531 (2001)

[151] Lin PY, Su KP. A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids. J Clin Psychiatry 68; 1056-61 (2007)

[152] van de Rest O, Geleijnse JM, Kok FJ, van Staveren WA, Hoefnagels WH, Beekman ATF, *et al.* Effect of fish-oil supplementation on mental well-being in older subjects: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 88; 706-713 (2008)

[153] Lucas M, Asselin G, Merette C, Poulin M-J, Dodin S. Ethyl-eicosapentaenoic acid for the treatment of psychological distress and depressive symptoms in middle-aged women: a double-blind, placebo-controlled, randomized clinical trial. Am J Clin Nutr 89; 641-651 (2009)

[154] Freund-Levi Y, Basun H, Cederholm T, Faxén-Irving G, Mikaela AG, Vedin GI, *et al.* Omega-3 supplementation in mild to moderate Alzheimer's disease: effects on neuropsychiatric symptoms. Int J Geriatr Psychiatry 23; 161-169 (2008)

[155] Corder EH, Caskey J. Early intervention in Alzheimer disease: The importance of APOE4 plus family history. Neurology 73; 2054-2055 (2009)

[156] Stern Y. Cognitive reserve and Alzheimer disease. Alz Dis Assoc Disorder 20; 112-117 (2006)