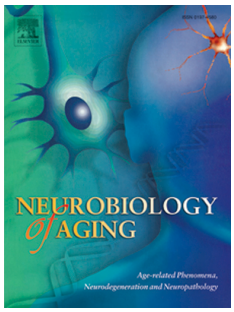
Accepted Manuscript



The effect of *APOE* genotype on Alzheimer's disease risk is influenced by sex and DHA status

Matthew Pontifex, David Vauzour, Anne-Marie Minihane

PII: S0197-4580(18)30178-7

DOI: 10.1016/j.neurobiolaging.2018.05.017

Reference: NBA 10253

To appear in: *Neurobiology of Aging*

Received Date: 21 February 2018

Revised Date: 23 April 2018

Accepted Date: 14 May 2018

Please cite this article as: Pontifex, M., Vauzour, D., Minihane, A.-M., The effect of *APOE* genotype on Alzheimer's disease risk is influenced by sex and DHA status, *Neurobiology of Aging* (2018), doi: 10.1016/j.neurobiolaging.2018.05.017.

The effect of *APOE* genotype on Alzheimer's disease risk is influenced by sex and DHA status

Matthew Pontifex, David Vauzour and Anne-Marie Minihane

Norwich Medical School, University of East Anglia, Norwich NR4 7UQ, United Kingdom

M.Pontifex@uea.ac.uk, D.Vauzour@uea.ac.uk , a.minihane@uea.ac.uk.

To whom correspondence should be addressed: Department of Nutrition and Preventive Medicine, Norwich Medical School, University of East Anglia, Norwich NR4 7UQ, UK. Email: m.pontifex@uea.ac.uk; Tel: +44 (0)1603 592389; Fax: +44 (0)1603 593752

Abbreviations: Alpha linoleic acid (ALA), Apolipoprotein E gene (APOE), Apolipoprotein E protein (apoE), β-site amyloid precursor protein cleavage enzyme (BACE1) Blood Brain Barrier (BBB), Brain derived neurotrophic factor (BDNF), Central nervous system (CNS) Cerebrospinal fluid (CSF), Cerebrovasculature (CV), Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA), Fatty acid binding protein (FABP), Fatty acid transporter protein (FATP), Free fatty acid (FFA), Late-onset Alzheimer's disease (LOAD), Lipoprotein receptor-related protein (LRP), Low density Lipoprotein (LDL), Lysophosphatidylcholine (LPC), Major facilitator superfamily domain containing 2A (MFSD2A), Mild cognitive impairment (MCI), Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phospholipid (PL), Positron emission tomography (PET), Randomised control trial (RCT), Targeted replacement (TR), Targeted replacement mouse (TR-Mouse), Triglyceride (TAG), Very low density lipoprotein (VLDL).

ABSTRACT

An *APOE*- ε*4* genotype is the strongest common genetic determinant of Alzheimer's disease (AD). The pleiotropic nature of apolipoprotein E, has made elucidation of the aetiological basis difficult to establish which is further complicated by the fact that the penetrance of the *APOE*- ε*4* allele is modulated by sex, age, and nutrition.

A greater metabolic consequence of the *APOE*- ε*4* allele is likely to contribute to the fact that two thirds of AD patients are female. A higher tissue status of the marine n-3 fatty acid docosahexaenoic acid (DHA), is associated with a lower AD risk. However, *APOE*- ε*4* carriers appear less sensitive to the neurocognitive benefits, which may be due to defective blood brain barrier transport of DHA exacerbated by ageing and possibly sex. This suggests higher DHA requirements in this large population subgroup. This narrative review will consider the influence of sex and DHA in modulating *APOE*- ε*4* mediated AD risk

Key words: Apolipoprotein E, Blood Brain Barrier, Docosahexaenoic acid, Lipid metabolism, Lipid transport, poly-unsaturated fatty acid's

1. Introduction

Dementias, of which Alzheimer's disease (AD) is the most common, are complex multifactorial disorders that manifest progressively over time, with deleterious behaviours and genetic predisposition contributing to compromised cognitive function. The apolipoprotein E ε4 allele (*APOE*-ε4) is the strongest prevalent genetic risk factor for sporadic late-onset Alzheimer's disease (LOAD) with possession of one or two *APOE-ε4* conferring respectively 3-4, and 8-12-fold increased risk, and reduced age of onset (Davidson et al., 2007; Heffernan et al., 2016). While a significant risk factor, possession of an *APOE*-ε4 does not categorically determine AD outcome (Corder et al., 1993; Liu et al., 2013) . Indeed, although *APOE*-ε4 prevalence within global AD populations varies considerably ranging from 41% - 61% (Crean et al., 2011; Farrer et al., 1997; R.M. Corbo, 1999), only half of *APOE*-ε4 homozygotes develop AD by age 90 years (Henderson et al., 1995). This indicates that the penetrance of the *E4* allele, it's influence on the rate of cognitive decline and the likelihood of transitioning to MCI and AD, is variable and therefore potentially modifiable (Fenesi et al., 2017; Moser and Pike, 2017; Singh et al., 2006; Ward et al., 2012).

Due to the pleiotropic nature of apolipoprotein E (apoE), possession of the deleterious *APOE- ε4* allele influences multiple biological processes, including inflammation, amyloid beta deposition, neurogenesis, synaptic function and lipid metabolism (including cholesterol and docosahexaenoic acid (DHA)) (Alata et al., 2015; Holtzman et al., 2012; Huang and Mahley, 2014; Theendakara et al., 2016). Originally described for its role in lipid transport, in contrast to the systemic circulation, apoE is the almost exclusive lipid transporter within the central nervous system (CNS)(Bu, 2012).

Regular consumption of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA, eicosapentaenoic acid (EPA) and DHA, found in higher concentration in oily fish) is associated with reduced AD risk (Barberger-Gateau et al., 2007; Zhang et al., 2016). Current evidence suggests that the cognitive responsiveness to DHA intake is lower in *APOE- ε4* individuals (Childs et al., 2014; Davis et al., 2017; Kofler et al., 2012; Metherel et al., 2009; Minihane, 2016; Slim et al., 2017; Walker et al., 2014).

Furthermore, the pathological impact of *APOE- ε4* carrier status appears to be modified by sex, with female carriers found to have increased MCI or AD risk between the ages of 55 and 70 years compared to their male counterparts (Farrer et al., 1997; Neu et al., 2017), suggesting a possible role of menopausal transition.

Therefore, those with an *APOE- ε4* genotype, particularly post-menopausal females, are a large 'at-risk' population group who should be targeted for preventive intervention, such as LC n-3 PUFA supplementation. Strategies capable of delaying disease onset by as little as two years would have profound implications on current disease burden (Brookmeyer et al., 1998). Recent predictive UK models suggest that achieving a two or five year delay would result in a respective 19% or 33% reduction in the predicted AD prevalence by 2050 (Lewis F, 2014), and alleviate the social and economic pressures associated with this debilitating disease.

This review will consolidate the current evidence of the interactive role of *APOE* genotype, DHA status and sex in the development of AD, highlighting research gaps and directions for future investigation.

2. Contribution of APOE genotype to AD risk

The human *APOE* gene, located on chromosome 19, has three common alleles, ε2, ε3 and ε4. Relative to the most common isoform *APOE- ε3* (allele frequency 78%) the rarer *APOE- ε2* (allele frequency 7%) is considered protective (Liu et al., 2013), whilst the *APOE- ε4* isoform (allele frequency 14%) predisposes to AD. Systematic reviews and meta-analyses consistently describe strong *APOE- ε4* -AD associations. For example using the *AlzGene* database, increased odds ratios (ORs) for both heterozygous (OR: 2.8, 95% CI: 2.3–3.5) and homozygotes (OR: 11.8, 95% CI: 7.0–19.8) *APOE- ε4* relative to 'neutral' *APOE- ε3* homozygotes were reported (Bertram et al., 2007). The *APOE- ε4* allele is concentrated within the AD population, with prevalence reaching in excess of 50%

relative to the global frequency estimated at 14% (in people <65 years of age) (D M Hallman, 1991; Eisenberg et al., 2010; Ward et al., 2012). Such associations are also apparent in mild cognitive impairment (MCI). *APOE- ε4* carriers are 3.0-3.7 times more likely to develop MCI compared to all other groups (Viticchi et al., 2017) and *APOE- ε4* MCI are more likely to convert to a more severe state of MCI (mMCI) or AD (Scarabino et al., 2016). *APOE- ε4* has been associated with hippocampal, amygdala and medial-temporal lobe atrophy (Lupton et al., 2016; Manning et al., 2014), which underlies the greater development and conversion rates in this genotype subgroup.

A consensus is developing that the impact of the *APOE- ε4* allele on AD risk diminishes upon reaching extreme ages (>90 years) (Corrada et al., 2013),(Valerio et al., 2014) which is reflected by the reduced allele frequency within the AD population (Corrada et al., 2013). Such a trend is unexpected given that the *APOE- ε4* variant is attributed to an increased risk and reduced age of onset. The phenomenon has been attributed to the survivor effect and the fact that these individuals have other phenotypic attributes which offer protection with many *APOE- ε4* carriers reaching extreme ages with normal cognition (Corder et al., 1994; Rebeck et al., 1994). The study of such indiviudals is likely to provide valuable insights into strategies to mitagte the effect of genotype at younger ages.

3. Impact of sex on AD risk

3.1 Sex disparity in MCI and AD incidence

Sex influences dementia risk and prevalence (Podcasy and Epperson, 2016). Above age 65 years there are approximately twice as many female AD cases (Seshadri et al., 1997). Although the higher prevalence has been attributed to longevity, the global five year longer lifespan in females (2017) can arguably only partially explain this phenomenon (Snyder et al., 2016). The reasons for this are still unclear, although it has been suggested that increased incidence in women may be related to

the loss, after menopause, of the neuroprotective effect of estrogens, important in maintaining synaptic plasticity, neurotransmission and blood brain barrier (BBB) integrity (Karp et al., 2017; Maggioli et al., 2016; McEwen and Milner, 2017). However, current clinical trials using hormone replacement therapy have failed to yield any promising results (Marjoribanks et al., 2017). Interestingly, analysis of murine hippocampal expression profiles reveals that key AD associated genes affecting energy and amyloid deposition are considerably altered prematurely in females, predisposing them to the development of the disease (Zhao et al., 2016).

A greater penetrance of an *APOE- ε4* genotype in females, first reported in the early 90s (Payami et al., 1994), could also explain these higher AD rates. A subsequent meta-analysis, found that carrying one *APOE- ε4* allele had a substantial effect on AD risk in females relative to non-carriers (OR: ≈4 at 65 years), whilst their male counterparts remained at similar risk (OR: ≈1 at 65 years) (Farrer et al., 1997) (Table 1 and Figure 1). This somewhat 'understudied' association, has been reiterated over the years (Bretsky et al., 1999; Gao et al., 1998; Holland et al., 2013; Xing et al., 2015), including work conducted by Altmann and colleagues who observed that the conversion of healthy controls to MCI/AD in *APOE- ε4* carriers was stronger in women (HR: Female = 1.81 Male = 1.27), with female *APOE- ε3/ ε4* more likely converting from MCI to AD (HR: Female = 2.17 and Male = 1.51 versus *APOE- ε 3/ ε3*) (Figure 1) (Altmann et al., 2014). A contemporary meta-analysis from the *Global Alzheimer's Association Interactive Network* (n=27 studies, 58,000 participants) has offered novel insight into this interaction. Despite no overall significant difference between men and women on *APOE- ε4* -AD in 55-85 year olds, the influence of sex emerged as being age-dependent. *APOE- ε4* females were at higher risk of MCI at ages 55-70 years and of AD at 65-75 years relative to *APOE- ε4* males, with the sexual dimorphism disappearing after 75 years (Neu et al., 2017). This indicates that a higher susceptibility to the *APOE- ε4* allele in females is most evident in the decade(s) following menopause. As with the overall reduction of penetrance of genotype on AD risk at older ages described above, a loss of effect of sex may be due to selective survival of those females less sensitive to genotype or the effect of genotype being lessened by an overall higher AD risk profile.

3.2 Female sex exacerbates the neurocognitive impact of an *APOE- ε4 genotype*

Limited human cognitive and biomarker data support the sexual dimorphism evident in epidemiological (incident disease) studies, indicating earlier onset and more extensive pathology in female *APOE- ε4* carriers. Differences in cerebrospinal fluid (CSF) tau and $A\beta_{42}$ load (Altmann et al., 2014; Li et al., 2017) along with aberrant Aβ/secretase profiles in autopsy samples (Nyarko et al., 2018) and brain hypometabolism and cortical thinning (Sampedro et al., 2015) have been observed between female and male *APOE- ε4* . Fleisher et al. observed reduced hippocampal volume and memory performance in female relative to male *APOE- ε4* carriers, in whom significant pathological changes only occurred when in possession of two *APOE- ε4* alleles (Fleisher et al., 2005). Analysis of >5000 brain samples of varying ages found that women carrying the *APOE- ε4* gene had more extensive neurofibrillary tangles and senile plaques, with onset of pathology beginning considerably earlier (Corder E. H., 2004). Neuroprotective immune cell (Aβ-specific CD4+ T cell) decline was found to occur 10-15 years earlier in female carriers compared to that of male carriers (Begum et al., 2014). Finally, levels of brain-derived neurotrophic factor (BDNF), an important modulator of neuron survival and growth in areas associated with memory, have been found to be significantly reduced in *APOE- ε4* females relative to age matched *APOE- ε4* males, and to correlate to poorer MMSE scores (Alvarez et al., 2014).

Rodent studies also highlight such a trend. It is widely accepted that female *APOE- ε4* mice present a more extreme phenotype (Raber et al., 1998; Rodriguez et al., 2013), with greater cognitive decline evident from their poorer performance on a battery of behavioural tests (Bour et al., 2008; Grootendorst et al., 2005; Heneka et al., 2015; Rodriguez et al., 2013), and a greater extent of neurodegeneration (Koutseff et al., 2014; Rijpma et al., 2013). For example Bour et al., demonstrated that 15-month female *APOE- ε4* targeted replacement (TR) mice had significantly greater deficits in spatial learning and memory compared with male *APOE- ε4* mice (Bour et al.,

2008). This is consistent with findings of Rijpma et al. who found middle aged *APOE- ε4* -TRfemale mice had decreased presynaptic density within the hippocampus, which was not found in *APOE- ε4* males (Rijpma et al., 2013). Wang et al, reported learning and memory impairment, occurring at a much younger age in APOE-ε4 /3xTg (sporadic and familial genes) females, which coincided with higher Aβ proteins and β-site amyloid precursor protein cleavage enzyme (BACE1) when compared to female nonTg, female 3xTg and male APOE-ε4 /3xTg mice(Wang et al., 2016).

To the best of our knowledge, the impact of sex on *APOE* genotype- DHA associations is currently completely unknown, but likely to be important, given the known role of sex on liver and systemic fatty acid synthesis and metabolism, and the differential impact of *APOE- ε4* on brain DHA uptake and status (addressed below).

3.2.1 Menopause and sex hormone dysregulation as a potential explanation for sex- *APOE- ε4* associations in AD

The process of menopause may offer an explanation for the greater AD susceptibility exhibited by females (Li et al., 2014). It may also help to explain why females appear to be predisposed to the effects of *APOE- ε4* carrier status (Mosconi et al., 2017; Moser and Pike, 2016; Yun et al., 2007; Zokaei et al., 2017). Earlier onset of menopause correlates with poorer cognition later in life (Ryan et al., 2014). The abrupt hormone dysregulation caused by the menopausal process, is likely to have significant implications on brain processes and cognition with the neuroprotective importance of oestrogens and progesterone well documented in the literature (reviewed extensively (Depypere et al., 2016; Vest and Pike, 2013)). A recent study using multimodal brain-imaging techniques highlighted the impact of menopause in AD development, with indicators of AD, such as hypo-metabolism, increased Aβ deposition and reduced grey and white matter brain volumes all evident to a greater extent as a result of menopause (menopause > perimenopause > no menopause) even after controlling for age and education (Mosconi et al., 2017). Interestingly, *APOE- ε4* carrier status

exacerbated menopausal Aβ deposition relative to other groups, which indicates an *APOE- ε4* - menopause interaction (Mosconi et al., 2017).

One would speculate that re-establishing the hormone profile altered in menopause would ameliorate these deleterious effects. However, inconsistent effects of hormone replacement therapy have been reported in both the general population and *APOE- ε4* carriers. These discrepancies are likely attributable to the heterogeneity in the initiation, treatment period, dose and combination of hormones used. Although currently inconclusive and sparce, the limited research available, addressing the impact of *APOE* genotype on the efficacy of hormone theapy appears to complicate this paradigm further with reports of both improvements (Jacobs et al., 2013; Kunzler et al., 2014; Yun et al., 2007), and no effect (Kang and Grodstein, 2012; Kunzler et al., 2014; Yaffe et al., 2000), in relation to cognition for carriers of *APOE- ε4*. Although oestrogen and activation of their receptors are known to alter *APOE* expression (Corbo et al., 2006; Wang et al., 2006), the molecular aetiology of possible *APOE* genotype-hormone-cognition interactions is not known.

Further research is needed to establish the benefits of hormone intervention during menopause and whether the effects are *APOE* genotype dependent, thereby warranting stratified approaches.

4. DHA: a dietary component with implications in AD

DHA, is a 22-carboxylic fatty acid consisting of 6 C=C double bonds (22:6n-3). An important constituent of the CNS, DHA can be synthesised from simpler precursors or obtained directly from the diet, predominantly from marine sources such as oily fish.

4.1 DHA in the brain

DHA is particularly concentrated in the brain (Arterburn et al., 2006), and accounts for 15% of the total fatty acids in the cerebral cortex. This dwarfs the levels found in the peripheral tissue such as the heart and liver, where DHA contributes about 2% (Arterburn et al., 2006). DHA is distinctly associated with the grey matter (Bradbury, 2011), and is highly concentrated within metabolically active neuronal regions (Bradbury, 2011; Crawford et al., 2013), including synaptic membranes, synaptic vesicles and mitochondria (Neuringer et al., 1988). DHA concentration varies across phospholipid species, and is abundant within phosphatidylserine and phosphatidylethanolamine, where DHA makes up around one third the total fatty acid composition (Lauritzen et al., 2001; Neuringer et al., 1988).

Synthesis of DHA in the brain occurs to a very limited extent (Igarashi et al., 2007), failing to increase even in times of n-3 PUFA deprivation (Igarashi et al., 2007). Brain DHA is supplied from the systemic circulation, with the DHA provided from the diet or synthesised mainly in the liver from its shorter chain precursor a-linoleic acid (ALA) (Domenichiello et al., 2015), through the actions of desaturase and elongase enzymes (Igarashi et al., 2006).

Upon entry into the brain, DHA is activated and esterified to the cell membrane phospholipids at the sn2 position, where it is believed to have a range of beneficial structural and functional roles. The unique highly polyunsaturated, kinked structure of DHA increases membrane fluidity, regulating the properties of the membrane and therefore affecting a range of properties including membrane protein function. DHA is also associated with promoting antioxidant processes, and altering gene expression profiles (Hashimoto et al., 2016). This occurs through the release of DHA from the membrane under the action of phospholipase A2. Although most is quickly re-esterified and conserved, release from the membrane allows DHA to partake in multiple signalling and regulatory processes, the most documented of which is DHA's role in neuroinflammation. The metabolism of DHA via enzymatic processes (e.g. cyclooxygenase, or lipoxygenase) or free radical oxidation,

transforms DHA into a plethora of bioactive lipid metabolites such as resolvins, protectins and maresins (Kuda, 2017), which are renowned for their anti-inflammatory properties.

4.2 Does DHA intake and status affect Dementia risk?

Prospective epidemiological studies, summarised by a number of meta-analyses, indicate that a higher consumption of fish and oily fish, the almost exclusive dietary source of LC n-3 PUFA is associated with reduced dementia and AD risk (Table 2) (Barberger-Gateau et al., 2002; Barberger-Gateau et al., 2007; Kalmijn et al., 1997; Morris et al., 2003; Zhang et al., 2016). To put this beneficial effect into perspective, the meta-analyses conducted by Wu and colleagues reported an 11% risk reduction in AD with each 100g increment of fish consumption per week (Wu et al., 2015), with Zhang et al. observing that a 0.1g increment in DHA per day was associated with 14% and 37% lower risks of dementia and AD respectively (Zhang et al., 2016).

Higher circulating DHA concentrations have been associated with improved cognition and reduced dementia and AD risk. Conquer et al. reported that AD patients have lower plasma DHA levels, in the total phospholipid, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions (Conquer et al., 2000). This was reiterated in the Framingham study where those in the top quartile of plasma PC-DHA had a 47% lower dementia risk relative to the bottom quartile (Schaefer et al., 2006), which is consistent with the *Rancho Bernado* study (Lopez et al., 2011). Interestingly those in the Framingham study in the lowest RBC DHA quartile also had lower total brain volume and performed poorer on a range of cognitive tests including visual memory, executive function and abstract thinking tasks (Tan et al., 2012). Further to this Yassine et al reported an association between serum DHA and cerebral amyloidosis and brain volumes (particularly those areas affected by AD) (Yassine et al., 2016a). Although not fully consistent, post mortem brain analysis also indicate that DHA levels are reduced in AD (Fraser et al., 2010; Söderberg et al., 1991). This beneficial assosiation appears to also continue into later life. For example Yassine et al. looked specifically at elderely individuals and

found participants within the lowest serum DHA quartile to have significantly more cerebral amyloidosis (Yassine et al., 2016a), However it appears there is little research examining the effects of DHA within the oldest old (85 + years), an area which warrents investigation.

A high oily fish and DHA consumption is associated with a higher socio-economic status, with such subgroups typically being more educated, engaging in higher physical activity, having lower prevalence of smoking and higher consumption of plant based foods, all of which have been shown to be neuroprotective. Although data analysis approaches correct for the impact of such co-variates, not all analyses are fully comprehensive and there may be other associated behavioural attributes which have not been considered. Therefore a proportion of the reported benefits of fish/DHA may in certain instances be attributable to the residual confounding effect of these associated factors.

Although collectively the observational data demonstrate that a high, habitual oily fish and DHA intake, and DHA status, are associated with a reduction in AD risk, these findings have not been consistently supported by randomised controlled trials (RCTs). RCTs have yielded conflicting results; some displaying promise (Lee et al., 2013; Stonehouse et al., 2013; Vedin et al., 2008; Yurko-Mauro et al., 2010), whilst others have failed to establish any beneficial effects (Joseph F. Quinn, 2010; Phillips et al., 2015; Quinn et al., 2010). Stonehouse et al. observed improved memory and reaction time in healthy young adults supplemented with 1.16g DHA for 6 months (Stonehouse et al., 2013). In the *Alzheimer's Disease Cooperative Study* (ADCS) which recruited those with mild to moderate AD, no cognitive benefits were evident following the consumption of 2g DHA daily for 18m (Quinn et al., 2010). RCTs to date have been predominately in individuals with existing MCI or AD who will have already experienced significant neuronal loss, thereby potentially missing the 'window' of preventive and therapeutic intervention. It is increasingly understood that the initial neuropathology which ultimately manifests in clinical AD, typically occurs some 20-30 years before any noticeable and measurable effects on cognition (Braak and Del Tredici, 2011). Therefore, interventions, such as

DHA, should target pre-clinical at-risk and prodromal groups, in order to maximise the individual and population benefits.

Apparent inconsistencies in RCT findings are also likely to be due to other heterogeneous elements of experimental design, such as DHA dose, habitual DHA status of trial participants (with likely greater benefit in those with a low baseline status, typical of western populations (Stonehouse et al., 2013)) and length of intervention. Brain DHA half-life is estimated to be up to 2.5 years, with mechanisms in place to conserve DHA in times of deprivation (Rao et al., 2007; Rapoport et al., 2007; Umhau et al., 2009). Many RCT are therefore too short to result in appreciable differences in brain DHA and associated neurocognitive benefits.

4.3 Pre-clinical and in vitro studies suggest DHA benefit

Interestingly, a number of cellular and pre-clinical studies demonstrate that DHA can ameliorate deleterious biological processes associated with AD, including some of those linked to an *APOE* genotype, as described above. Supplementing AD mouse models with a 0.6% DHA diet significantly reduced Aβ plaque formation (Lim et al., 2005; Teng et al., 2015) and affected amyloid precursor protein processing in Tg2576 mice (Lim et al., 2005). In addition, DHA was also observed to modulate Aβ aggregation by stabilising oligomers in APP/PS1 mice (Teng et al., 2015). This is consistent with *in vitro* findings (Hashimoto et al., 2009). Similarly, AD rodent models have revealed that DHA supplementation reduces aberrant phosphorylation of tau resulting in improved cognitive performance in 3xTg-AD mice (Arsenault et al., 2011). With DHA known to modulate cellular inflammation by multiple mechanisms (Calder, 2017), it is of no surprise that DHA supplementation reduces neuroinflammation. Combined EPA and DHA supplementation prevented cytokine expression and alterations in astrocyte morphology in aged wild type C57BL/6 mice (Labrousse VF, 2012). Hopperton and Thomas reported an effect of DHA supplementation on microglia activation (Hopperton et al., 2016) and inflammatory precursor expression (Thomas et al., 2013) in the same

model. It should be noted however that such rodent studies tend to employ large doses of DHA (human equivalent of ≈2-4g) (Arsenault et al., 2011; Chouinard-Watkins et al., 2017) for extended periods of time (in relation to a mouse lifespan ≈ 2 years) (4-8 months) (Arsenault et al., 2011; Lim et al., 2005; Teng et al., 2015). These strategies are utilised in the attempt to maximise the chance of observing cognitive benefit, therefore achieving such studies in humans may prove difficult or unfeasible. Despite their importance in the research of complex disease, current rodent models of AD have recognised limitations (McGowan et al., 2006), associated with dissimilar human-to-mouse lifespan and environmental conditions. As a result pathological protein profiles may differ chemically and morphologically, and human AD pathology is not fully recapitulated (Richardson and Burns, 2002). Such inconsistencies in regards to plaque pathology may produce 'effect sizes' in mouse models which cannot be reached in humans, which need to be appreciated when interpreting rodent data. In addition a wide range of rodent AD models exist, each with unique ageing profiles and pathological progressions, strengths and limitations These need to taken into account when collectively interpreting the results from rodent experimentation (Tai et al., 2011).

5. DHA availability and transport in the brain

5.1 Transport to the Brain

Effective circulatory transport and BBB uptake of DHA, is crucial for maintaining brain supply. DHA derived from the diet, synthesised in the liver or released from reserves in adipose tissue is packaged into various plasma pools for transportation (Lefkowitz et al., 2005), either in lipoproteins (esterified as triglycerides (TAG), diacylglycerol, phospholipids (PL) or cholesteryl esters or bound to albumin as non-esterified DHA or esterified to lysophosphatidylcholine (LPC) (Lagarde et al., 2001). The importance of each pool in supplying brain DHA is still widely debated; however, current evidence indicates that the albumin bound fractions are the predominant sources, with the non-esterified

DHA pool being quantitatively the major physiological pool despite LPC-DHA being preferentially incorporated after a single dose (Chen et al., 2015). The observation of adequate brain DHA status in LDL and VLDL receptor knockouts, indicates that direct lipoprotein uptake of DHA is not a significant source (Chen et al., 2008; Rahman et al., 2010). A more complete understanding of the ability of DHA pools and dietary sources to target the brain is of high therapeutic relevance, potentially leading to more effective use of DHA intervention to support healthy brain ageing.

5.1.1 DHA transport across the blood brain barrier

The movement of DHA across the BBB may be passive, transporter-mediated or a combination of both processes (Figure 2). Rodent studies and model membranes have demonstrated that un-esterified DHA can freely cross the BBB via simple diffusion (yellow arrow) (Ouellet et al., 2009), supporting the notion, that diffusion alone is adequate. Cluster of differentiation 36 (CD36) was previously believed to be a transporter of un-esterified DHA, however CD36 knockout mice do not display DHA deficits in the brain (3 – Red arrow) (Song et al., 2010). Similarly, the importance of lipoprotein mediated DHA uptake has been questioned; with LRP receptor knockout mice exhibiting normal DHA brain levels and supply (4 – Red arrow) (Chen et al., 2008; Rahman et al., 2010). Instead, it is now proposed that under the action of lipoprotein lipase at the BBB, lipoprotein DHA is released as free fatty acid enabling simple diffusion across the BBB (5) (Chen and Subbaiah, 2013; Sovic et al., 2005).

In contrast, major facilitator superfamily domain-containing protein 2 (Mfsd2a) provides evidence that transporter mediated routes are indeed necessary for sufficient DHA transport to the brain. Expressed in the BBB endothelium, Mfsd2a appears to be an integral part of DHA transport across the BBB and specifically transports LPC esterified DHA (6 – green arrow) (Nguyen et al., 2014). Mfsd2a's importance is evident from mice lacking the transporter. These knockout mice exhibit significantly reduced brain DHA levels, accompanied by hippocampal and cerebellum

neurodegeneration, which manifests as significant cognitive impairment (Nguyen et al., 2014; Wong et al., 2016).

In addition to Mfsd2a, various fatty acid binding proteins (FABP) have been implicated in DHA uptake and retention. Pan et al. discovered a role for the carrier protein FABP5, which is the most expressed FABP at the BBB (Lee et al., 2015). Confirming that FABP5 binds un-esterified DHA with high affinity, the group optimised an in *situ* trans-cardiac perfusion technique and deduced that transport of ^{14}C-DHA, was reduced by 36% in FABP5 deficient mice (Pan et al., 2015). They recently continued this line of research, describing how FABP5 knockout mice have a 14% reduction in cortical DHA levels. Loss of FABP5 and subsequent reduction in DHA transport translates to significant cognitive deficits, illustrating the importance of both FABP5 and DHA in cognitive health (Pan et al., 2016). Interestingly endothelial cell uptake of DHA was significantly impaired (48%) whilst cell transport was unaffected (Pan et al., 2016), providing evidence that FABP5 influences uptake, and not endothelial cell transport, as originally thought (7 – Green arrow and 8 – Red arrow). Finally, evidence has recently emerged that supports a role for fatty acid transport protein 1 (FATP1) in DHA supply to the brain (Ochiai et al., 2016). FATP1 appears to be a basal membrane transporter with initial cell studies suggesting it contributes to DHA transport across the BBB (9 – Green Arrow) (Ochiai et al., 2016). However, *in vivo* follow up experimentation, is required to confirm these initial findings.

In summary, although DHA has been observed to cross the BBB via simple diffusion, there appears to be protein mediated processers in place to ensure an adequate supply of this essential molecule, as evidenced by Mfsd2a and FABP5 knockout rodent model data. Further characterisation of these transporters in aged mice is required to confirm their importance in adult, non-developmental related DHA brain transport. Overcoming DHA transport deficits may enhance DHA supply to the brain, subsequently improving cognitive health. Potential methods to achieve this include: supplying DHA in a highly bioavailable pool, altering transporter BBB kinetics to favour DHA uptake, or

increasing overall blood DHA status. These methods could be utilised to compensate for the reduced delivery efficiency associated with BBB transport deficits e.g. associated with ageing (Graf et al., 2010) or *APOE* genotype.

5.2 The influence of *APOE* genotype on DHA metabolism and status

Human epidemiological evidence along with RCT data indicate that *APOE- ε4* carriers are less responsive to the neuroprotective benefits of higher DHA intakes (Huang et al., 2005; Quinn et al., 2010; Yassine et al., 2017a). Such findings are in agreement with recent results demonstrating that *APOE- ε4 TR* mice have significantly lower brain DHA concentrations (9% reduction in cortex compared to *APOE-* ε2) (Vandal et al., 2014), therefore indicating impaired DHA transport and/or metabolism associated with an *APO*E- *ε4* genotype. Uptake of DHA into the brain has been shown to be significantly reduced in *APOE- ε4 TR* mice and to progressively decline with age (Vandal et al., 2014), which may be suggestive of BBB transport impairment. This potential transport impairment in *APOE- ε4* rodents is consistent with findings from the *ADCS* trial in humans where lower DHA levels in the cerebrospinal fluid in *APOE- ε4* carriers following DHA supplementation was observed (Yassine et al., 2016b). The molecular aetiology of the proposed reduced BBB transport of DHA in *APOE- ε4,* and in particular any effect on the protein mediated transport mechanisms described above, is currently unknown.

A recently conducted PET study in humans, exploring the incorporation of ^{11}C-DHA surprisingly reported that young *APOE- ε4* carriers have increased DHA brain delivery and incorporation rates (Yassine et al., 2017b). Although these findings need to be confirmed in a larger study, this apparent increase, contradicts the suggested *APOE- ε4* transport deficits. This may represent a compensatory mechanism to overcome DHA loss via altered brain metabolism. The progression to further transport/metabolism deficits observed in aged *APOE- ε4* carriers may overwhelm this compensatory process leading to eventual reduced brain DHA concentrations and predisposing

carriers to AD. Further investigation is needed to fully establish the effect of *APOE- ε4 on* DHA transport and brain delivery and metabolism and how they are affected by age.

An altered systemic DHA metabolism, associated with lower overall DHA availability in *APOE- ε4 carriers* may also underlie a lower brain DHA status. Chouinard-Watkins et al, observed (using radiolabelled ^{13}C DHA) a reduced DHA plasma incorporation (31% less), and significantly increased DHA β-oxidation (80%) in elderly *APOE- ε4* carriers (Chouinard-Watkins et al., 2013) relative to *APOE- ε4* non-carriers. The greater hepatic uptake and β-oxidation of DHA potentially explains the lower plasma response observed in *APOE- ε4* carriers with high-BMI (Chouinard-Watkins et al., 2015). Altered DHA metabolism is apparent in the liver of *APOE- ε4 TR* mice, which express *APOE* under m-APOE promoter producing a physiologically relevant *APOE* model (Tai et al., 2011), in these mice PUFA homeostasis is significantly dysregulated, potentially as a result of altered fatty acid transporter expression, specifically FABP1 (Chouinard-Watkins et al., 2016). Interestingly supplementation with a relatively large dose (3g/day) of DHA restored these deficits observed in the liver (Chouinard-Watkins et al., 2016). In light of this, supplementation with larger doses of DHA (up to3 g/day) in *APOE- ε4* carriers may present a plausible route to overcome the transport and metabolism deficits associated with *APOE- ε4* (Chouinard-Watkins et al., 2017). Chouinard-Watkins and colleagues showed such supplementation prevented memory decline in APOE- ε4 rodent model with *APOE- ε4* TR mice performing similarly to both APOE- ε3 TR mice fed a normal diet and the diet highly enriched with DHA (Chouinard-Watkins et al., 2017; Yassine et al., 2017a). Given the apparent deficits in DHA transport and metabolism in those with an *APOE- ε4*, alternative intervention strategies should also be considered.

To our knowledge, the impact of sex on DHA BBB transport and its interaction with *APOE* genotype is currently completely unknown. It is proposed as a high priority research area to provide insight into the mechanistic basis for the greater effect of the *APOE- ε4 allele* in females.

6. Conclusion

The implications of the *APOE- ε4* genotype in cognition and AD risk are far-reaching and complex, detrimentally influencing a range of AD mechanisms from Aβ deposition to DHA metabolism. This makes the process of developing a mitigating intervention challenging. The extent of *APOE- ε4's* influence appears to be modulated to some extent by a triad of factors namely sex, age and DHA status. Targeted nutrition strategies may overcome the negative influence of these factors, attenuating genetic vulnerability and 'tipping the balance' in favour of AD prevention, particularly if such an intervention is implemented early in the disease process. From current evidence, advocating the consumption of oily fish and DHA appears a robust approach to reduce AD risk and in *APOE- ε4* carrier's higher doses may be warranted to achieve the benefit associated with non-carriers, due to impairment in transport and metabolism. This may also be true for the elderly who display an age dependant transport deficits, and females where the effects of the *APOE- ε4 genotype* are exacerbated.

Characterisation of the impact of sex and menopausal status on, brain DHA uptake and metabolism and responsiveness to DHA intervention, independent of, and according to *APOE* genotype status are identified as research priority area. Dose-response studies in sex by *APOE* genotype groups are needed to identify effective DHA intake doses and develop stratified nutrition intervention approaches

7. References

2017. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet (London, England) 390(10100), 1260-1344.

Alata, W., Ye, Y., St-Amour, I., Vandal, M., Calon, F., 2015. Human apolipoprotein E 4 expression impairs cerebral vascularization and blood-brain barrier function in mice. J Cereb Blood Flow Metab 35(1), 86-94.

Altmann, A., Tian, L., Henderson, V.W., Greicius, M.D., Alzheimer's Disease Neuroimaging Initiative, I., 2014. Sex modifies the APOE-related risk of developing Alzheimer disease. Ann Neurol 75(4), 563-573.

Alvarez, A., Aleixandre, M., Linares, C., Masliah, E., Moessler, H., 2014. Apathy and APOE4 are associated with reduced BDNF levels in Alzheimer's disease. J Alzheimers Dis 42(4), 1347-1355.

Arsenault, D., Julien, C., Tremblay, C., Calon, F., 2011. DHA improves cognition and prevents dysfunction of entorhinal cortex neurons in 3xTg-AD mice. PLoS One 6(2), e17397.

Arterburn, L.M., Hall, E.B., Oken, H., 2006. Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr 83(6 Suppl), 1467S-1476S.

Barberger-Gateau, P., Letenneur, L., Deschamps, V., Pérès, K., Dartigues, J.-F., Renaud, S., 2002. Fish, meat, and risk of dementia: cohort study. BMJ 325(7370), 932-933.

Barberger-Gateau, P., Raffaitin, C., Letenneur, L., Berr, C., Tzourio, C., Dartigues, J.F., Alperovitch, A., 2007. Dietary patterns and risk of dementia: the Three-City cohort study. Neurology 69(20), 1921-1930.

Bazinet, R.P., Laye, S., 2014. Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci 15(12), 771-785.

Begum, A.N., Cunha, C., Sidhu, H., Alkam, T., Scolnick, J., Rosario, E.R., Ethell, D.W., 2014. Women with the Alzheimer's risk marker ApoE4 lose Abeta-specific CD4(+) T cells 10-20 years before men. Transl Psychiatry 4, e414.

Bertram, L., McQueen, M.B., Mullin, K., Blacker, D., Tanzi, R.E., 2007. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat Genet 39(1), 17-23.

Bour, A., Grootendorst, J., Vogel, E., Kelche, C., Dodart, J.C., Bales, K., Moreau, P.H., Sullivan, P.M., Mathis, C., 2008. Middle-aged human apoE4 targeted-replacement mice show retention deficits on a wide range of spatial memory tasks. Behav Brain Res 193(2), 174-182.

Braak, H., Del Tredici, K., 2011. The pathological process underlying Alzheimer's disease in individuals under thirty. Acta Neuropathol 121(2), 171-181.

Bradbury, J., 2011. Docosahexaenoic acid (DHA): an ancient nutrient for the modern human brain. Nutrients 3(5), 529-554.

Breitner, J.C., Wyse, B.W., Anthony, J.C., Welsh-Bohmer, K.A., Steffens, D.C., Norton, M.C., Tschanz, J.T., Plassman, B.L., Meyer, M.R., Skoog, I., Khachaturian, A., 1999. APOE-epsilon4 count predicts age when prevalence of AD increases, then declines: the Cache County Study. Neurology 53(2), 321-331.

Bretsky, P.M., Buckwalter, J.G., Seeman, T.E., Miller, C.A., Poirier, J., Schellenberg, G.D., Finch, C.E., Henderson, V.W., 1999. Evidence for an interaction between apolipoprotein E genotype, gender, and Alzheimer disease. Alzheimer disease and associated disorders 13(4), 216-221.

Brookmeyer, R., Gray, S., Kawas, C., 1998. Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. Am J Public Health 88(9), 1337-1342.

Bu, G., 2012. ApoE and apoE receptors in brain lipid metabolism and AD. Molecular Neurodegeneration 7(Suppl 1), L10.

Calder, P.C., 2017. Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochemical Society transactions 45(5), 1105-1115.

Chen, C.T., Kitson, A.P., Hopperton, K.E., Domenichiello, A.F., Trepanier, M.O., Lin, L.E., Ermini, L., Post, M., Thies, F., Bazinet, R.P., 2015. Plasma non-esterified docosahexaenoic acid is the major pool supplying the brain. Sci Rep 5, 15791.

Chen, C.T., Ma, D.W., Kim, J.H., Mount, H.T., Bazinet, R.P., 2008. The low density lipoprotein receptor is not necessary for maintaining mouse brain polyunsaturated fatty acid concentrations. J Lipid Res 49(1), 147-152.

Chen, S., Subbaiah, P.V., 2013. Regioisomers of phosphatidylcholine containing DHA and their potential to deliver DHA to the brain: role of phospholipase specificities. Lipids 48(7), 675-686.

Childs, C.E., Kew, S., Finnegan, Y.E., Minihane, A.M., Leigh-Firbank, E.C., Williams, C.M., Calder, P.C., 2014. Increased dietary alpha-linolenic acid has sex-specific effects upon eicosapentaenoic acid status in humans: re-examination of data from a randomised, placebo-controlled, parallel study. Nutr J 13(1), 113.

Chouinard-Watkins, R., Conway, V., Minihane, A.M., Jackson, K.G., Lovegrove, J.A., Plourde, M., 2015. Interaction between BMI and APOE genotype is associated with changes in the plasma long-chain-PUFA response to a fish-oil supplement in healthy participants. Am J Clin Nutr 102(2), 505-513.

Chouinard-Watkins, R., Pincon, A., Coulombe, J.D., Spencer, R., Massenavette, L., Plourde, M., 2016. A Diet Rich in Docosahexaenoic Acid Restores Liver Arachidonic Acid and Docosahexaenoic Acid Concentrations in Mice Homozygous for the Human Apolipoprotein E epsilon4 Allele. J Nutr 146(7), 1315-1321.

Chouinard-Watkins, R., Rioux-Perreault, C., Fortier, M., Tremblay-Mercier, J., Zhang, Y., Lawrence, P., Vohl, M.C., Perron, P., Lorrain, D., Brenna, J.T., Cunnane, S.C., Plourde, M., 2013. Disturbance in uniformly 13C-labelled DHA metabolism in elderly human subjects carrying the apoE epsilon4 allele. Br J Nutr 110(10), 1751-1759.

Chouinard-Watkins, R., Vandal, M., Leveille, P., Pincon, A., Calon, F., Plourde, M., 2017. Docosahexaenoic acid prevents cognitive deficits in human apolipoprotein E epsilon 4-targeted replacement mice. Neurobiol Aging 57, 28-35.

Conquer, J.A., Tierney, M.C., Zecevic, J., Bettger, W.J., Fisher, R.H., 2000. Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. Lipids 35(12), 1305-1312.

Corbo, R.M., Gambina, G., Ruggeri, M., Scacchi, R., 2006. Association of estrogen receptor alpha (ESR1) PvuII and XbaI polymorphisms with sporadic Alzheimer's disease and their effect on apolipoprotein E concentrations. Dement Geriatr Cogn Disord 22(1), 67-72.

Corder E. H., G., E. Taylor, M. G. Thal, D. R. Ohm, T. G. and Braak H., 2004. The Biphasic Relationship between Regional Brain Senile Plaque and Neurofibrillary Tangle Distributions: Modification by Age, Sex, and APOE Polymorphism. Annals of the New York Academy of Sciences 1019, 24–28.

Corder, E.H., Saunders, A.M., Risch, N.J., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Jr., Rimmler, J.B., Locke, P.A., Conneally, P.M., Schmader, K.E., et al., 1994. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet 7(2), 180-184.

Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., Pericak-Vance, M.A., 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261(5123), 921-923.

Corrada, M.M., Paganini-Hill, A., Berlau, D.J., Kawas, C.H., 2013. Apolipoprotein E genotype, dementia, and mortality in the oldest old: the 90+ Study. Alzheimers Dement 9(1), 12-18.

Crawford, M.A., Broadhurst, C.L., Guest, M., Nagar, A., Wang, Y., Ghebremeskel, K., Schmidt, W.F., 2013. A quantum theory for the irreplaceable role of docosahexaenoic acid in neural cell signalling throughout evolution. Prostaglandins Leukot Essent Fatty Acids 88(1), 5-13.

Crean, S., Ward, A., Mercaldi, C.J., Collins, J.M., Cook, M.N., Baker, N.L., Arrighi, H.M., 2011. Apolipoprotein E epsilon4 prevalence in Alzheimer's disease patients varies across global populations: a systematic literature review and meta-analysis. Dement Geriatr Cogn Disord 31(1), 20-30.

D M Hallman, E.B., N Saha, C Sandholzer, H J Menzel, A Császár, G Utermann, 1991. The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet 49(2), 338–349.

Davidson, Y., Gibbons, L., Pritchard, A., Hardicre, J., Wren, J., Stopford, C., Julien, C., Thompson, J., Payton, A., Pickering-Brown, S.M., Pendleton, N., Horan, M.A., Burns, A., Purandare, N., Lendon, C.L., Neary, D., Snowden, J.S., Mann, D.M., 2007. Apolipoprotein E epsilon4 allele frequency and age at onset of Alzheimer's disease. Dement Geriatr Cogn Disord 23(1), 60-66.

Davis, D.J., Hecht, P.M., Jasarevic, E., Beversdorf, D.Q., Will, M.J., Fritsche, K., Gillespie, C.H., 2017. Sex-specific effects of docosahexaenoic acid (DHA) on the microbiome and behavior of socially-isolated mice. Brain Behav Immun 59, 38-48.

Depypere, H., Vierin, A., Weyers, S., Sieben, A., 2016. Alzheimer's disease, apolipoprotein E and hormone replacement therapy. Maturitas 94, 98-105.

Devore, E.E., Grodstein, F., van Rooij, F.J., Hofman, A., Rosner, B., Stampfer, M.J., Witteman, J.C., Breteler, M.M., 2009. Dietary intake of fish and omega-3 fatty acids in relation to long-term dementia risk. Am J Clin Nutr 90(1), 170-176.

Domenichiello, A.F., Kitson, A.P., Bazinet, R.P., 2015. Is docosahexaenoic acid synthesis from alpha-linolenic acid sufficient to supply the adult brain? Prog Lipid Res 59, 54-66.

Eisenberg, D.T., Kuzawa, C.W., Hayes, M.G., 2010. Worldwide allele frequencies of the human apolipoprotein E gene: climate, local adaptations, and evolutionary history. Am J Phys Anthropol 143(1), 100-111.

Farrer, L.A., Cupples, L., Haines, J.L., et al., 1997. Effects of age, sex, and ethnicity on the association between apolipoprotein e genotype and alzheimer disease: A meta-analysis. JAMA 278(16), 1349-1356.

Fenesi, B., Fang, H., Kovacevic, A., Oremus, M., Raina, P., Heisz, J.J., 2017. Physical Exercise Moderates the Relationship of Apolipoprotein E (APOE) Genotype and Dementia Risk: A Population-Based Study. J Alzheimers Dis 56(1), 297-303.

Fleisher, A., Grundman, M., Jack, C.R., Jr, et al., 2005. Sex, apolipoprotein e ε4 status, and hippocampal volume in mild cognitive impairment. Archives of Neurology 62(6), 953-957.

Fraser, T., Tayler, H., Love, S., 2010. Fatty acid composition of frontal, temporal and parietal neocortex in the normal human brain and in Alzheimer's disease. Neurochem Res 35(3), 503-513.

Gao, S., Hendrie, H.C., Hall, K.S., Hui, S., 1998. The relationships between age, sex, and the incidence of dementia and alzheimer disease: A meta-analysis. Archives of General Psychiatry 55(9), 809-815.

Graf, B.A., Duchateau, G.S., Patterson, A.B., Mitchell, E.S., van Bruggen, P., Koek, J.H., Melville, S., Verkade, H.J., 2010. Age dependent incorporation of 14C-DHA into rat brain and body tissues after dosing various 14C-DHA-esters. Prostaglandins Leukot Essent Fatty Acids 83(2), 89-96.

Grootendorst, J., Bour, A., Vogel, E., Kelche, C., Sullivan, P.M., Dodart, J.C., Bales, K., Mathis, C., 2005. Human apoE targeted replacement mouse lines: h-apoE4 and h-apoE3 mice differ on spatial memory performance and avoidance behavior. Behav Brain Res 159(1), 1-14.

Hashimoto, M., Hossain, S., Al Mamun, A., Matsuzaki, K., Arai, H., 2016. Docosahexaenoic acid: one molecule diverse functions. Crit Rev Biotechnol, 1-19.

Hashimoto, M., Shahdat, H.M., Katakura, M., Tanabe, Y., Gamoh, S., Miwa, K., Shimada, T., Shido, O., 2009. Effects of docosahexaenoic acid on in vitro amyloid beta peptide 25-35 fibrillation. Biochim Biophys Acta 1791(4), 289-296.

Heffernan, A.L., Chidgey, C., Peng, P., Masters, C.L., Roberts, B.R., 2016. The Neurobiology and Age-Related Prevalence of the epsilon4 Allele of Apolipoprotein E in Alzheimer's Disease Cohorts. J Mol Neurosci.

Henderson, A.S., Easteal, S., Jorm, A.F., Mackinnon, A.J., Korten, A.E., Christensen, H., Croft, L., Jacomb, P.A., 1995. Apolipoprotein E allele epsilon 4, dementia, and cognitive decline in a population sample. Lancet (London, England) 346(8987), 1387-1390.

Heneka, M.T., Carson, M.J., El Khoury, J., Landreth, G.E., Brosseron, F., Feinstein, D.L., Jacobs, A.H., Wyss-Coray, T., Vitorica, J., Ransohoff, R.M., Herrup, K., Frautschy, S.A., Finsen, B., Brown, G.C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., Petzold, G.C., Town, T., Morgan, D., Shinohara, M.L., Perry, V.H., Holmes, C., Bazan, N.G., Brooks, D.J., Hunot, S., Joseph, B., Deigendesch, N., Garaschuk, O., Boddeke, E., Dinarello, C.A., Breitner, J.C., Cole, G.M., Golenbock, D.T., Kummer, M.P., 2015. Neuroinflammation in Alzheimer's disease. Lancet Neurol 14(4), 388-405.

Holland, D., Desikan, R.S., Dale, A.M., McEvoy, L.K., Alzheimer's Disease Neuroimaging, I., 2013. Higher rates of decline for women and apolipoprotein E epsilon4 carriers. AJNR Am J Neuroradiol 34(12), 2287-2293.

Holtzman, D.M., Herz, J., Bu, G., 2012. Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. Cold Spring Harb Perspect Med 2(3), a006312.

Hopperton, K.E., Trepanier, M.O., Giuliano, V., Bazinet, R.P., 2016. Brain omega-3 polyunsaturated fatty acids modulate microglia cell number and morphology in response to intracerebroventricular amyloid-beta 1-40 in mice. J Neuroinflammation 13(1), 257.

Huang, T.L., Zandi, P.P., Tucker, K.L., Fitzpatrick, A.L., Kuller, L.H., Fried, L.P., Burke, G.L., Carlson, M.C., 2005. Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon4. Neurology 65(9), 1409-1414.

Huang, Y., Mahley, R.W., 2014. Apolipoprotein E: structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases. Neurobiol Dis 72 Pt A, 3-12.

Igarashi, M., DeMar, J.C., Jr., Ma, K., Chang, L., Bell, J.M., Rapoport, S.I., 2007. Docosahexaenoic acid synthesis from alpha-linolenic acid by rat brain is unaffected by dietary n-3 PUFA deprivation. J Lipid Res 48(5), 1150-1158.

Igarashi, M., Ma, K., Chang, L., Bell, J.M., Rapoport, S.I., DeMar, J.C., Jr., 2006. Low liver conversion rate of alpha-linolenic to docosahexaenoic acid in awake rats on a high-docosahexaenoate-containing diet. J Lipid Res 47(8), 1812-1822.

Jacobs, E.G., Kroenke, C., Lin, J., Epel, E.S., Kenna, H.A., Blackburn, E.H., Rasgon, N.L., 2013. Accelerated cell aging in female APOE-epsilon4 carriers: implications for hormone therapy use. PloS one 8(2), e54713.

Joseph F. Quinn, M.R.R., PhD; Ronald G. Thomas, PhD; Karin Yurko-Mauro, PhD; Edward B. Nelson, MD; Christopher Van Dyck, MD; James E. Galvin, MD; Jennifer Emond, MS; Clifford R. Jack, MD; Michael Weiner, MD; Lynne Shinto, ND; Paul S. Aisen, MD 2010. Docosahexaenoic Acid Supplementation and Cognitive Decline in Alzheimer Disease. JAMA 304(17), 1903-1911.

Kalmijn, S., Launer, L.J., Ott, A., Witteman, J.C., Hofman, A., Breteler, M.M., 1997. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol 42(5), 776-782.

Kang, J.H., Grodstein, F., 2012. Postmenopausal hormone therapy, timing of initiation, APOE and cognitive decline. Neurobiol Aging 33(7), 1129-1137.

Karp, N.A., Mason, J., Beaudet, A.L., Benjamini, Y., Bower, L., Braun, R.E., Brown, S.D.M., Chesler, E.J., Dickinson, M.E., Flenniken, A.M., Fuchs, H., Angelis, M.H., Gao, X., Guo, S., Greenaway, S., Heller, R., Herault, Y., Justice, M.J., Kurbatova, N., Lelliott, C.J., Lloyd, K.C.K., Mallon, A.M., Mank, J.E., Masuya, H., McKerlie, C., Meehan, T.F., Mott, R.F., Murray, S.A., Parkinson, H., Ramirez-Solis, R., Santos, L., Seavitt, J.R., Smedley, D., Sorg, T., Speak, A.O., Steel, K.P., Svenson, K.L., Wakana, S., West, D., Wells, S., Westerberg, H., Yaacoby, S., White, J.K., 2017. Prevalence of sexual dimorphism in mammalian phenotypic traits. Nat Commun 8, 15475.

Kofler, B.M., Miles, E.A., Curtis, P., Armah, C.K., Tricon, S., Grew, J., Napper, F.L., Farrell, L., Lietz, G., Packard, C.J., Caslake, M.J., Mathers, J.C., Williams, C.M., Calder, P.C., Minihane, A.M., 2012. Apolipoprotein E genotype and the cardiovascular disease risk phenotype: impact of sex and adiposity (the FINGEN study). Atherosclerosis 221(2), 467-470.

Koutseff, A., Mittelhaeuser, C., Essabri, K., Auwerx, J., Meziane, H., 2014. Impact of the apolipoprotein E polymorphism, age and sex on neurogenesis in mice: pathophysiological relevance for Alzheimer's disease? Brain Res 1542, 32-40.

Kuda, O., 2017. Bioactive metabolites of docosahexaenoic acid. Biochimie.

Kunzler, J., Youmans, K.L., Yu, C., Ladu, M.J., Tai, L.M., 2014. APOE modulates the effect of estrogen therapy on Abeta accumulation EFAD-Tg mice. Neurosci Lett 560, 131-136.

Labrousse VF, N.A., Joffre C, et al., 2012. Short-Term Long Chain Omega3 Diet Protects from Neuroinflammatory Processes and Memory Impairment in Aged Mice. PLoS One 7(5), e36861.

Lagarde, M., Bernoud, N., Thiès, F., Brossard, N., Lemaitre-Delaunay, D., Croset, M., Lecerf, J., 2001. Lysophosphatidylcholine as a carrier of docosahexaenoic acid to target tissues. World review of nutrition and dietetics 88, 173-177.

Lauritzen, L., Hansen, H.S., Jorgensen, M.H., Michaelsen, K.F., 2001. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 40(1-2), 1-94.

Lee, G.S., Kappler, K., Porter, C.J., Scanlon, M.J., Nicolazzo, J.A., 2015. Fatty Acid Binding Proteins Expressed at the Human Blood-Brain Barrier Bind Drugs in an Isoform-Specific Manner. Pharm Res 32(10), 3432-3446.

Lee, L.K., Shahar, S., Chin, A.V., Yusoff, N.A., 2013. Docosahexaenoic acid-concentrated fish oil supplementation in subjects with mild cognitive impairment (MCI): a 12-month randomised, double-blind, placebo-controlled trial. Psychopharmacology (Berl) 225(3), 605-612.

Lefkowitz, W., Lim, S.Y., Lin, Y., Salem, N., Jr., 2005. Where does the developing brain obtain its docosahexaenoic acid? Relative contributions of dietary alpha-linolenic acid, docosahexaenoic acid, and body stores in the developing rat. Pediatr Res 57(1), 157-165.

Lewis F, K.S.S., Sussex J, O'Neill P, Cockcroft L, 2014. The trajectory of dementia in the UK—Making a difference. OHE Consulting.

Li, G., Shofer, J.B., Petrie, E.C., Yu, C.E., Wilkinson, C.W., Figlewicz, D.P., Shutes-David, A., Zhang, J., Montine, T.J., Raskind, M.A., Quinn, J.F., Galasko, D.R., Peskind, E.R., 2017. Cerebrospinal fluid biomarkers for Alzheimer's and vascular disease vary by age, gender, and APOE genotype in cognitively normal adults. Alzheimers Res Ther 9(1), 48.

Li, R., Cui, J., Shen, Y., 2014. Brain sex matters: estrogen in cognition and Alzheimer's disease. Mol Cell Endocrinol 389(1-2), 13-21.

Lim, G.P., Calon, F., Morihara, T., Yang, F., Teter, B., Ubeda, O., Salem, N., Jr., Frautschy, S.A., Cole, G.M., 2005. A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. J Neurosci 25(12), 3032-3040.

Liu, C.C., Kanekiyo, T., Xu, H., Bu, G., 2013. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat Rev Neurol 9(2), 106-118.

Lopez, L.B., Kritz-Silverstein, D., Barrett Connor, E., 2011. High dietary and plasma levels of the omega-3 fatty acid docosahexaenoic acid are associated with decreased dementia risk: the Rancho Bernardo study. J Nutr Health Aging 15(1), 25-31.

Lupton, M.K., Strike, L., Hansell, N.K., Wen, W., Mather, K.A., Armstrong, N.J., Thalamuthu, A., McMahon, K.L., de Zubicaray, G.I., Assareh, A.A., Simmons, A., Proitsi, P., Powell, J.F., Montgomery, G.W., Hibar, D.P., Westman, E., Tsolaki, M., Kloszewska, I., Soininen, H., Mecocci, P., Velas, B., Lovestone, S., Brodaty, H., Ames, D., Trollor, J.N., Martin, N.G., Thompson, P.M., Sachdev, P.S., Wright, M.J., 2016. The effect of increased genetic risk for Alzheimer's disease on hippocampal and amygdala volume. Neurobiol Aging 40, 68-77.

Maggioli, E., McArthur, S., Mauro, C., Kieswich, J., Kusters, D.H., Reutelingsperger, C.P., Yaqoob, M., Solito, E., 2016. Estrogen protects the blood-brain barrier from inflammation-induced disruption and increased lymphocyte trafficking. Brain, behavior, and immunity 51, 212-222.

Manning, E.N., Barnes, J., Cash, D.M., Bartlett, J.W., Leung, K.K., Ourselin, S., Fox, N.C., 2014. APOE epsilon4 is associated with disproportionate progressive hippocampal atrophy in AD. PLoS One 9(5), e97608.

Marjoribanks, J., Farquhar, C., Roberts, H., Lethaby, A., Lee, J., 2017. Long-term hormone therapy for perimenopausal and postmenopausal women. Cochrane Database Syst Rev 1, CD004143.

McEwen, B.S., Milner, T.A., 2017. Understanding the broad influence of sex hormones and sex differences in the brain. Journal of neuroscience research 95(1-2), 24-39.

McGowan, E., Eriksen, J., Hutton, M., 2006. A decade of modeling Alzheimer's disease in transgenic mice. Trends in Genetics 22(5), 281-289.

Metherel, A.H., Armstrong, J.M., Patterson, A.C., Stark, K.D., 2009. Assessment of blood measures of n-3 polyunsaturated fatty acids with acute fish oil supplementation and washout in men and women. Prostaglandins Leukot Essent Fatty Acids 81(1), 23-29.

Minihane, A.M., 2016. Impact of Genotype on EPA and DHA Status and Responsiveness to Increased Intakes. Nutrients 8(3), 123.

Morris, M.C., Evans, D.A., Bienias, J.L., Tangney, C.C., Bennett, D.A., Wilson, R.S., Aggarwal, N., Schneider, J., 2003. Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 60(7), 940-946.

Mosconi, L., Berti, V., Quinn, C., McHugh, P., Petrongolo, G., Varsavsky, I., Osorio, R.S., Pupi, A., Vallabhajosula, S., Isaacson, R.S., de Leon, M.J., Brinton, R.D., 2017. Sex differences in Alzheimer risk: Brain imaging of endocrine vs chronologic aging. Neurology.

Moser, V.A., Pike, C.J., 2016. Obesity and sex interact in the regulation of Alzheimer's disease. Neurosci Biobehav Rev 67, 102-118.

Moser, V.A., Pike, C.J., 2017. Obesity Accelerates Alzheimer-Related Pathology in APOE4 but not APOE3 Mice. eNeuro 4(3).

Neu, S.C., Pa, J., Kukull, W., Beekly, D., Kuzma, A., Gangadharan, P., Wang, L.S., Romero, K., Arneric, S.P., Redolfi, A., Orlandi, D., Frisoni, G.B., Au, R., Devine, S., Auerbach, S., Espinosa, A., Boada, M., Ruiz, A., Johnson, S.C., Koscik, R., Wang, J.J., Hsu, W.C., Chen, Y.L., Toga, A.W., 2017. Apolipoprotein E Genotype and Sex Risk Factors for Alzheimer Disease: A Meta-analysis. JAMA Neurol 74(10), 1178-1189.

Neuringer, M., Anderson, G.J., Connor, W.E., 1988. The essentiality of n-3 fatty acids for the development and function of the retina and brain. Annu Rev Nutr 8, 517-541.

Nguyen, L.N., Ma, D., Shui, G., Wong, P., Cazenave-Gassiot, A., Zhang, X., Wenk, M.R., Goh, E.L., Silver, D.L., 2014. Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. Nature 509(7501), 503-506.

Nyarko, J.N.K., Quartey, M.O., Pennington, P.R., Heistad, R.M., Dea, D., Poirier, J., Baker, G.B., Mousseau, D.D., 2018. Profiles of beta-Amyloid Peptides and Key Secretases in Brain Autopsy Samples Differ with Sex and APOE epsilon4 Status: Impact for Risk and Progression of Alzheimer Disease. Neuroscience.

Ochiai, Y., Uchida, Y., Ohtsuki, S., Tachikawa, M., Aizawa, S., Terasaki, T., 2016. The blood-brain barrier fatty acid transport protein 1 (FATP1/SLC27A1) supplies docosahexaenoic acid to the brain, and insulin facilitates transport. J Neurochem.

Ouellet, M., Emond, V., Chen, C.T., Julien, C., Bourasset, F., Oddo, S., LaFerla, F., Bazinet, R.P., Calon, F., 2009. Diffusion of docosahexaenoic and eicosapentaenoic acids through the blood-brain barrier: An in situ cerebral perfusion study. Neurochem Int 55(7), 476-482.

Pan, Y., Scanlon, M.J., Owada, Y., Yamamoto, Y., Porter, C.J., Nicolazzo, J.A., 2015. Fatty Acid-Binding Protein 5 Facilitates the Blood-Brain Barrier Transport of Docosahexaenoic Acid. Mol Pharm 12(12), 4375-4385.

Pan, Y., Short, J.L., Choy, K.H., Zeng, A.X., Marriott, P.J., Owada, Y., Scanlon, M.J., Porter, C.J., Nicolazzo, J.A., 2016. Fatty Acid-Binding Protein 5 at the Blood-Brain Barrier Regulates Endogenous Brain Docosahexaenoic Acid Levels and Cognitive Function. J Neurosci 36(46), 11755-11767.

Payami, H., Montee, K.R., Kaye, J.A., et al., 1994. Alzheimers Disease, Apolipoprotein E4, and Gender. JAMA 271(17), 1316-1317.

Phillips, M.A., Childs, C.E., Calder, P.C., Rogers, P.J., 2015. No Effect of Omega-3 Fatty Acid Supplementation on Cognition and Mood in Individuals with Cognitive Impairment and Probable Alzheimer's Disease: A Randomised Controlled Trial. Int J Mol Sci 16(10), 24600-24613.

Podcasy, J.L., Epperson, C.N., 2016. Considering sex and gender in Alzheimer disease and other dementias. Dialogues Clin Neurosci 18(4), 437-446.

Quinn, J.F., Raman, R., Thomas, R.G., Yurko-Mauro, K., Nelson, E.B., Van Dyck, C., Galvin, J.E., Emond, J., Jack, C.R., Jr., Weiner, M., Shinto, L., Aisen, P.S., 2010. Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. JAMA 304(17), 1903-1911.

R.M. Corbo, R.S., 1999. Apolipoprotein E (APOE) allele distribution in the world. Is APOE*4 a 'thrifty' allele. Annals of Human Genetics 63(4), 277-381.

Raber, J., Wong, D., Buttini, M., Orth, M., Bellosta, S., Pitas, R.E., Mahley, R.W., Mucke, L., 1998. Isoform-specific effects of human apolipoprotein E on brain function revealed in ApoE knockout mice: increased susceptibility of females. Proc Natl Acad Sci U S A 95(18), 10914-10919.

Rahman, T., Taha, A.Y., Song, B.J., Orr, S.K., Liu, Z., Chen, C.T., Bazinet, R.P., 2010. The very low density lipoprotein receptor is not necessary for maintaining brain polyunsaturated fatty acid concentrations. Prostaglandins Leukot Essent Fatty Acids 82(2-3), 141-145.

Rao, J.S., Ertley, R.N., DeMar, J.C., Jr., Rapoport, S.I., Bazinet, R.P., Lee, H.J., 2007. Dietary n-3 PUFA deprivation alters expression of enzymes of the arachidonic and docosahexaenoic acid cascades in rat frontal cortex. Mol Psychiatry 12(2), 151-157.

Rapoport, S.I., Rao, J.S., Igarashi, M., 2007. Brain metabolism of nutritionally essential polyunsaturated fatty acids depends on both the diet and the liver. Prostaglandins Leukot Essent Fatty Acids 77(5-6), 251-261.

Rebeck, G.W., Perls, T.T., West, H.L., Sodhi, P., Lipsitz, L.A., Hyman, B.T., 1994. Reduced apolipoprotein epsilon 4 allele frequency in the oldest old Alzheimer's patients and cognitively normal individuals. Neurology 44(8), 1513-1516.

Richardson, J.A., Burns, D.K., 2002. Mouse Models of Alzheimer's Disease: A Quest for Plaques. ILAR Journal 43(2), 89-99.

Rijpma, A., Jansen, D., Arnoldussen, I.A., Fang, X.T., Wiesmann, M., Mutsaers, M.P., Dederen, P.J., Janssen, C.I., Kiliaan, A.J., 2013. Sex Differences in Presynaptic Density and Neurogenesis in Middle-Aged ApoE4 and ApoE Knockout Mice. J Neurodegener Dis 2013, 531326.

Rodriguez, G.A., Burns, M.P., Weeber, E.J., Rebeck, G.W., 2013. Young APOE4 targeted replacement mice exhibit poor spatial learning and memory, with reduced dendritic spine density in the medial entorhinal cortex. Learn Mem 20(5), 256-266.

Ryan, J., Scali, J., Carriere, I., Amieva, H., Rouaud, O., Berr, C., Ritchie, K., Ancelin, M.L., 2014. Impact of a premature menopause on cognitive function in later life. Bjog 121(13), 1729-1739.

Sampedro, F., Vilaplana, E., de Leon, M.J., Alcolea, D., Pegueroles, J., Montal, V., Carmona-Iragui, M., Sala, I., Sanchez-Saudinos, M.B., Anton-Aguirre, S., Morenas-Rodriguez, E., Camacho, V., Falcon, C., Pavia, J., Ros, D., Clarimon, J., Blesa, R., Lleo, A., Fortea, J., 2015. APOE-by-sex interactions on brain structure and metabolism in healthy elderly controls. Oncotarget 6(29), 26663-26674.

Scarabino, D., Broggio, E., Gambina, G., Maida, C., Gaudio, M.R., Corbo, R.M., 2016. Apolipoprotein E genotypes and plasma levels in mild cognitive impairment conversion to Alzheimer's disease: A follow-up study. Am J Med Genet B Neuropsychiatr Genet 171(8), 1131-1138.

Schaefer, E.J., Bongard, V., Beiser, A.S., Lamon-Fava, S., Robins, S.J., Au, R., Tucker, K.L., Kyle, D.J., Wilson, P.W., Wolf, P.A., 2006. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol 63(11), 1545-1550.

Seshadri, S., Wolf, P.A., Beiser, A., Au, R., McNulty, K., White, R., D'Agostino, R.B., 1997. Lifetime risk of dementia and Alzheimer's disease. The impact of mortality on risk estimates in the Framingham Study. Neurology 49(6), 1498-1504.

Singh, P.P., Singh, M., Mastana, S.S., 2006. APOE distribution in world populations with new data from India and the UK. Ann Hum Biol 33(3), 279-308.

Slim, K.E., Vauzour, D., Tejera, N., Voshol, P.J., Cassidy, A., Minihane, A.M., 2017. The effect of dietary fish oil on weight gain and insulin sensitivity is dependent on APOE genotype in humanized targeted replacement mice. Faseb j 31(3), 989-997.

Snyder, H.M., Asthana, S., Bain, L., Brinton, R., Craft, S., Dubal, D.B., Espeland, M.A., Gatz, M., Mielke, M.M., Raber, J., Rapp, P.R., Yaffe, K., Carrillo, M.C., 2016. Sex biology contributions to vulnerability to Alzheimer's disease: A think tank convened by the Women's Alzheimer's Research Initiative. Alzheimers Dement 12(11), 1186-1196.

Söderberg, M., Edlund, C., Kristensson, K., Dallner, G., 1991. Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. Lipids 26(6), 421.

Song, B.J., Elbert, A., Rahman, T., Orr, S.K., Chen, C.T., Febbraio, M., Bazinet, R.P., 2010. Genetic ablation of CD36 does not alter mouse brain polyunsaturated fatty acid concentrations. Lipids 45(4), 291-299.

Sovic, A., Panzenboeck, U., Wintersperger, A., Kratzer, I., Hammer, A., Levak-Frank, S., Frank, S., Rader, D.J., Malle, E., Sattler, W., 2005. Regulated expression of endothelial lipase by porcine brain capillary endothelial cells constituting the blood–brain barrier. Journal of Neurochemistry 94(1), 109-119.

Stonehouse, W., Conlon, C.A., Podd, J., Hill, S.R., Minihane, A.M., Haskell, C., Kennedy, D., 2013. DHA supplementation improved both memory and reaction time in healthy young adults: a randomized controlled trial. Am J Clin Nutr 97(5), 1134-1143.

Tai, L.M., Youmans, K.L., Jungbauer, L., Yu, C., LaDu, M.J., 2011. Introducing Human APOE into Aβ Transgenic Mouse Models. International Journal of Alzheimer’s Disease 2011, 9.

Tan, Z.S., Harris, W.S., Beiser, A.S., Au, R., Himali, J.J., Debette, S., Pikula, A., Decarli, C., Wolf, P.A., Vasan, R.S., Robins, S.J., Seshadri, S., 2012. Red blood cell omega-3 fatty acid levels and markers of accelerated brain aging. Neurology 78(9), 658-664.

Teng, E., Taylor, K., Bilousova, T., Weiland, D., Pham, T., Zuo, X., Yang, F., Chen, P.P., Glabe, C.G., Takacs, A., Hoffman, D.R., Frautschy, S.A., Cole, G.M., 2015. Dietary DHA supplementation in an APP/PS1 transgenic rat model of AD reduces behavioral and Abeta pathology and modulates Abeta oligomerization. Neurobiol Dis 82, 552-560.

Theendakara, V., Peters-Libeu, C.A., Spilman, P., Poksay, K.S., Bredesen, D.E., Rao, R.V., 2016. Direct Transcriptional Effects of Apolipoprotein E. J Neurosci 36(3), 685-700.

Thomas, J., Garg, M.L., Smith, D.W., 2013. Dietary supplementation with resveratrol and/or docosahexaenoic acid alters hippocampal gene expression in adult C57Bl/6 mice. J Nutr Biochem 24(10), 1735-1740.

Umhau, J.C., Zhou, W., Carson, R.E., Rapoport, S.I., Polozova, A., Demar, J., Hussein, N., Bhattacharjee, A.K., Ma, K., Esposito, G., Majchrzak, S., Herscovitch, P., Eckelman, W.C., Kurdziel, K.A., Salem, N., Jr., 2009. Imaging incorporation of circulating docosahexaenoic acid into the human brain using positron emission tomography. J Lipid Res 50(7), 1259-1268.

Valerio, D., Raventos, H., Schmeidler, J., Beeri, M.S., Villalobos, L.M., Bolanos-Palmieri, P., Carrion-Baralt, J.R., Fornaguera, J., Silverman, J.M., 2014. Association of apolipoprotein E-e4 and dementia declines with age. Am J Geriatr Psychiatry 22(10), 957-960.

Vandal, M., Alata, W., Tremblay, C., Rioux-Perreault, C., Salem, N., Jr., Calon, F., Plourde, M., 2014. Reduction in DHA transport to the brain of mice expressing human APOE4 compared to APOE2. J Neurochem 129(3), 516-526.

Vedin, I., Cederholm, T., Freund Levi, Y., Basun, H., Garlind, A., Faxen Irving, G., Jonhagen, M.E., Vessby, B., Wahlund, L.O., Palmblad, J., 2008. Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study. Am J Clin Nutr 87(6), 1616-1622.

Vest, R.S., Pike, C.J., 2013. Gender, sex steroid hormones, and Alzheimer's disease. Horm Behav 63(2), 301-307.

Viticchi, G., Falsetti, L., Buratti, L., Sajeva, G., Luzzi, S., Bartolini, M., Provinciali, L., Silvestrini, M., 2017. Framingham Risk Score and the Risk of Progression from Mild Cognitive Impairment to Dementia. J Alzheimers Dis.

Walker, C.G., Browning, L.M., Mander, A.P., Madden, J., West, A.L., Calder, P.C., Jebb, S.A., 2014. Age and sex differences in the incorporation of EPA and DHA into plasma fractions, cells and adipose tissue in humans. Br J Nutr 111(4), 679-689.

Wang, J.M., Hou, X., Zheng, B., Mosley, T.H., 2016. The high prevelance of Alzheimer's disease in female APOE4 allele carriers is contributed from a combination of APOE4 increased activity and female sex related high expression of BACE1 Alzheimer's & Dementia 12(7), P768-P769.

Wang, J.M., Irwin, R.W., Brinton, R.D., 2006. Activation of estrogen receptor alpha increases and estrogen receptor beta decreases apolipoprotein E expression in hippocampus in vitro and in vivo. Proc Natl Acad Sci U S A 103(45), 16983-16988.

Ward, A., Crean, S., Mercaldi, C.J., Collins, J.M., Boyd, D., Cook, M.N., Arrighi, H.M., 2012. Prevalence of apolipoprotein E4 genotype and homozygotes (APOE e4/4) among patients diagnosed with Alzheimer's disease: a systematic review and meta-analysis. Neuroepidemiology 38(1), 1-17.

Wong, B.H., Chan, J.P., Cazenave-Gassiot, A., Poh, R.W., Foo, J.C., Galam, D.L., Ghosh, S., Nguyen, L.N., Barathi, V.A., Yeo, S.W., Luu, C.D., Wenk, M.R., Silver, D.L., 2016. Mfsd2a Is a Transporter for the Essential omega-3 Fatty Acid Docosahexaenoic Acid (DHA) in Eye and Is Important for Photoreceptor Cell Development. J Biol Chem 291(20), 10501-10514.

Wu, S., Ding, Y., Wu, F., Li, R., Hou, J., Mao, P., 2015. Omega-3 fatty acids intake and risks of dementia and Alzheimer's disease: a meta-analysis. Neurosci Biobehav Rev 48, 1-9.

Xing, Y., Tang, Y., Jia, J., 2015. Sex Differences in Neuropsychiatric Symptoms of Alzheimer's Disease: The Modifying Effect of Apolipoprotein E epsilon4 Status. Behav Neurol 2015, 275256.

Yaffe, K., Haan, M., Byers, A., Tangen, C., Kuller, L., 2000. Estrogen use, APOE, and cognitive decline: evidence of gene-environment interaction. Neurology 54(10), 1949-1954.

Yassine, H.N., Braskie, M.N., Mack, W.J., Castor, K.J., Fonteh, A.N., Schneider, L.S., Harrington, M.G., Chui, H.C., 2017a. Association of Docosahexaenoic Acid Supplementation With Alzheimer Disease Stage in Apolipoprotein E epsilon4 Carriers: A Review. JAMA Neurol.

Yassine, H.N., Croteau, E., Rawat, V., Hibbeln, J.R., Rapoport, S.I., Cunnane, S.C., Umhau, J.C., 2017b. DHA brain uptake and APOE4 status: a PET study with [1-11C]-DHA. Alzheimers Res Ther 9(1), 23.

Yassine, H.N., Feng, Q., Azizkhanian, I., et al., 2016a. Association of serum docosahexaenoic acid with cerebral amyloidosis. JAMA Neurology 73(10), 1208-1216.

Yassine, H.N., Rawat, V., Mack, W.J., Quinn, J.F., Yurko-Mauro, K., Bailey-Hall, E., Aisen, P.S., Chui, H.C., Schneider, L.S., 2016b. The effect of APOE genotype on the delivery of DHA to cerebrospinal fluid in Alzheimer's disease. Alzheimers Res Ther 8, 25.

Yun, S.H., Park, K.A., Kwon, S., Woolley, C.S., Sullivan, P.M., Pasternak, J.F., Trommer, B.L., 2007. Estradiol enhances long term potentiation in hippocampal slices from aged apoE4-TR mice. Hippocampus 17(12), 1153-1157.

Yurko-Mauro, K., McCarthy, D., Rom, D., Nelson, E.B., Ryan, A.S., Blackwell, A., Salem, N., Jr., Stedman, M., Investigators, M., 2010. Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. Alzheimers Dement 6(6), 456-464.

Zhang, Y., Chen, J., Qiu, J., Li, Y., Wang, J., Jiao, J., 2016. Intakes of fish and polyunsaturated fatty acids and mild-to-severe cognitive impairment risks: a dose-response meta-analysis of 21 cohort studies. Am J Clin Nutr 103(2), 330-340.

Zhao, L., Mao, Z., Woody, S.K., Brinton, R.D., 2016. Sex differences in metabolic aging of the brain: insights into female susceptibility to Alzheimer's disease. Neurobiol Aging 42, 69-79.

Zokaei, N., Giehl, K., Sillence, A., Neville, M.J., Karpe, F., Nobre, A.C., Husain, M., 2017. Sex and APOE: A memory advantage in male APOE epsilon4 carriers in midlife. Cortex 88, 98-105.

8. Acknowledgements

Pontifex's PhD is funded by an Alzheimer's Society UK PhD studentship (AS-PhD-2015-023). Minihane's research in the area of n-3 fatty acids, APOE genotype and cognition is funded by the Biotechnology and Biological Sciences Research Council (BBSRC), UK, Alzheimer's Research UK (ARUK) and the Centre for

Nutrition Learning and Memory, University of Illinois, US. Vauzour's research in the area of n-3 fatty acids, APOE genotype and cognition is funded by the BBSRC, UK, and the Centre for Nutrition Learning and Memory, University of Illinois, US.

9. Figures

Figure 1: The impact of sex on Mild cognitive impairment (MCI) and Alzheimer's disease (AD) risk in *APOE-E4* carriers relative to non-carriers

From left to right in chronological order:

Farrer LA et al. 1997: Odds ratios (OR) for AD risk compared to *APOE-E3/E3*.

Altmann A. et al., 2014: Report the hazard ratios (HR) for the conversion from (a) healthy controls (HC) to MCI/AD in *APOE-E4 carriers* vs. non-carrier, or (b) MCI to AD conversion in *APOE-E3/E4* vs. *APOE-E3/E3*

Neu SC et al. 2017: OD for MCI or AD compared to *APOE-E3/E3.* Sex did not influence the sensitivity of *APOE-E3/E4*-AD association in the overall group (aged 55-85 years)(P=0.53). Females versus males *APOE-E3/A4* had a higher OR of MCI (P=0.05) at ages 55-70 years (P=0.05) and of AD at 65-75 years (P=0.002).

Study	Study type	Basic participant criteria	Follow up length	Outcome (OR/RR/HR)
Farrer **(Farrer et al., 1997)**	Meta-analysis N=14,537	40 research groups contributed patient data: • *APOE* genotype • Sex • Age at disease onset • Ethnic background	NA	• In comparison with *APOE- ε3/ ε3*, the sexual dimorphism in AD risk was 1.5 times greater in *APOE- ε3/ ε4* individuals (P=.01) • See Figure 1
Altmann **(Altmann et al., 2014)**	Prospective cohort N=8,084 Biomarkers N=980	• Subjects were healthy controls or MCI at initial assessment • *APOE* genotype available	Minimum one follow up at 12 months or later	• Female *APOE- ε4* carriers more likely to develop MCI/AD (HR=1.81 women; HR=1.27 men; P=0.011) • Compared to *APOE- ε3/ ε3, APOE- ε3/ ε4* females more likely to convert from MCI to AD (HR=2.17 women; HR=1.51 men, P=0.022) see figure 1 • In MCI, *APOE- ε4* -sex interaction, significant for tau load (total tau: P=0.009; tau:Aβ ratio P=0.020)
Sampedro **(Sampedro et al., 2015)**	Cross-sectional study N=274	• AD Neuroimaging Initiative (ADNI) database • aged 55 to 90 years • healthy elderly control individuals • available CSF and/or MRI and/or a FDG-PET analyses	NA	• Female *APOE- ε4* carriers showed brain hypometabolism in the temporal cortex P=0.001 • Female *APOE- ε4* carriers showed cortical thinning in AD vulnerable areas, P<0.001
Breitner **(Breitner et al., 1999)**	Cross-sectional N= 5,092	• Aged ≥65 years • Population of Cache County, Utah	NA	• Female sex a risk factor for AD only in those with *APOE- ε4* (OR=1.58, P=0.02)
Neu **(Neu et al., 2017)**	Meta-Analysis N= ≈58 000	• 27 independent research studies • White Participants only mainly non-Hispanic • Ages 55-85 years excluded all patients with a clinical history of, or comorbidity with any other known neurological disease	Maximum follow up 10 years	• No different between sex for *APOE- ε4 -AD* risk in whole group (OR=3.09 men and OR=3.31 women, P=0.53) • Female *E3/E4* had a higher risk of AD between the ages of 65 and 75 years (OR=3.14 men and OR=4.37, P=0.002) • Female *E3/E4* had higher a risk of MCI between the ages of 55 and 70 years (OR=1.07 men and

				OR=1.43, P=0.05) • See figure 1
Fleisher **(Fleisher et al., 2005)**	Cross-sectional study N= 193	• Aged 55 to 90 years • Took part in structural brain MRI • MCI at initial assessment • Good general health	NA	• Women with 1 or 2 *APOE- ε4* alleles were found to have significantly reduced hippocampal volume • Men only showed a significant reduction in hippocampal volume when carrying 2 *APOE- ε4* alleles. • Performance on delayed word recall task mirrored this trend
Alvarez **(Alvarez et al., 2014)**	Cross-sectional study N=362	• Recruited Healthy, MCI and AD patients • Excluded all patients with any other health conditions • not taking various neurological medications	NA	• Female *APOE- ε4* carriers showed lower BDNF levels ($p<0.01$) and MMSE scores ($p<0.01$) than non-*APOE- ε4* carriers • Males did not
Li **(Li et al., 2017)**	Cross-sectional study N=331	• Aged 21 to 100 • medically stable • no evidence or history of cognitive or functional decline	NA	• CSF total tau and p-tau181 had no gender differences • CSF Aβ42 had age× gender×*APOE* genotype interaction, p=0.047 • Male *APOE- ε4* , average CSF Aβ42 decreased gradually with age up to midlife and then levelled off. • Female *APOE- ε4* , average CSF Aβ42 remained relatively high through to age 50 and then had a rapid decline after

Table 1: Key studies supporting the notion of *an APOE-E4* -sex interaction

Abbreviations: MCI, Aβ, CSF, MRI, FDG-PET, MRI, BDNF, MMSE – Mild cognitive impairment, Amyloid beta, Cerebrospinal fluid, Magnetic resonance imaging, Fludeoxyglucose positron emission topography, Magnetic resonance imaging, Brain-derived neurotrophic factor, Mini Mental state examination

Study	Study type	Basic participant criteria	Follow up length	n-3 PUFA intake or status	Outcome (OR/RR/HR)
Barberger-Gateau (Barberger-Gateau et al., 2007)	Prospective cohort N=8085	• ≥65 years • Dementia free at baseline • Male and Female	4 years	1. Weekly consumption of fish 2. Regular n-3 rich oil intake	1. Reduced AD risk (HR 0.65, 95% CI 0.43 to 0.994) 2. Reduced all cause dementia risk (HR 0.60, 95% CI 0.40 to 0.90) in *APOE- ε4* non carriers only 3. Reduced dementia risk (HR 0.46, 95% CI 0.19 to 1.11)
Morris (Morris et al., 2003)	Prospective cohort N=815	• 65–94 years • Dementia free at baseline • Male and Female	Average 3.9 years	1. Weekly consumption of at least one portion of fish 2. Total intake of n-3 PUFA	1. Reduced AD risk (RR, 0.4; 95% CI 0.2 to 0.9) 2. Reduced AD risk with increasing n-3 PUFA intake (highest quintile RR, 0.3; 95% CI 0.1 to 0.9)
Schaefer (Schaefer et al., 2006)	Prospective cohort N=899	• 55-88 years • Dementia free at baseline • Male and Female	9.1 years	1. Highest quartile plasma PC-DHA levels – equating to 3 servings of fish per week	1. Reduced all cause Dementia risk (RR 0.53, 95% CI 0.29-0.97) and AD risk (RR 0.61, 95% CI 0.31 to1.18)
Huang (Huang et al., 2005)	Prospective cohort N=2233	• ≥65 years • Dementia free at baseline • Male and Female	0.1 to 8.4 years	1. Fatty fish consumption ≥ times per week	1. Nominal but not significant Reduction in dementia risk (HR 0.79; CI 0.53 to 1.20) in *APOE- ε4* non carriers only 2. Nominal but not significant Reduction in AD risk (HR 0.69; 0.91 to 1.22) In *APOE- ε4* non carriers only
Devore (Devore et al., 2009)	Prospective cohort N=5395	• ≥55 years • Dementia free at baseline • reported dietary information at baseline • Male and Female	Average 9.6 years	1. High fish intake 2. N-3 PUFA intake	1. Those who had a high fish intake (hazard ratio: 0.95; 95% CI: 0.76, 1.19) and those consuming fatty fish (HR: 0.98; 95% CI: 0.77 to 1.24) had a similar dementia risk when compared to those who typically ate no fish. 2. Participants in the lowest tertile of long-chain N-3 intake, had similar risk of dementia to those in the highest tertile (HR: 0.97; 95% CI: 0.77 to 1.21)
Wu (Wu et al.,	Meta-analysis of	• Prospective cohort	Minimum one year follow up	1. Higher intake of fish 2. Higher long-chain n-3	1. 36% reduced AD risk compared with lowest intake group (95% CI 8 to 56%) This increased

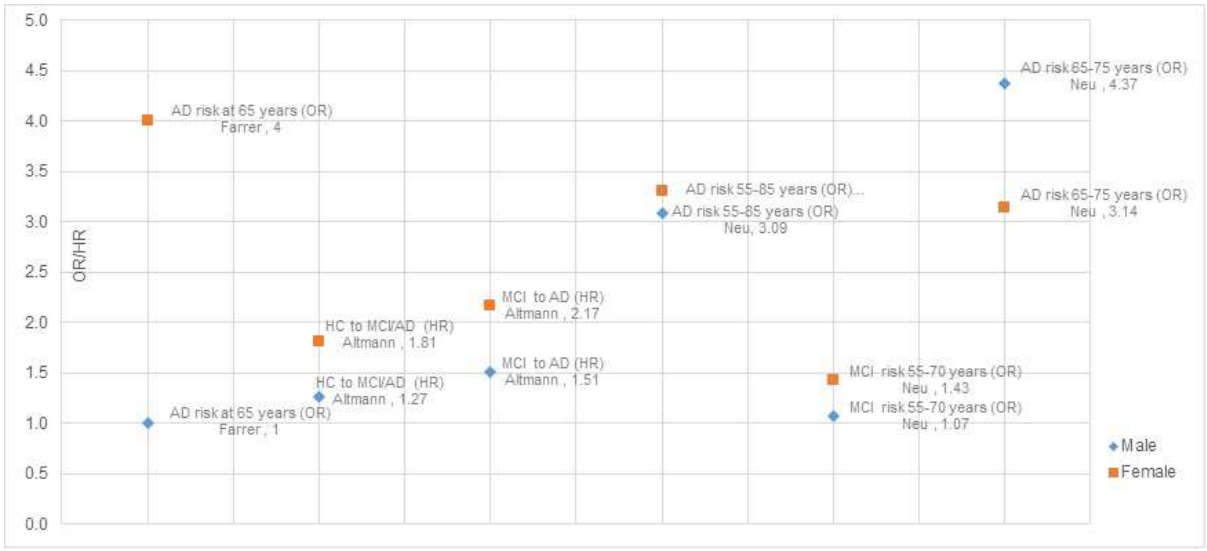
2015)	prospective cohort studies N=22402	studies		fatty acid intake 3. Increasing 100g per week increment of fish	further if using only studies with longer follow up. 2. No statistically significant association with long-chain omega-3 fatty acids intake and AD risk reduction (RR = 0.89, 95% CI 0.74–1.08). 3. 11% reduced AD risk (RR = 0.89, 95% CI 0.79 to 0.99).
Zhang (Zhang et al., 2016)	Meta-analysis of prospective cohort studies N= 181580	• Prospective cohort studies	2.1-21 ears	1. 1 serving of fish per week 2. 0.1-g per day increment of DHA	1. Lower dementia (RR: 0.95; 95% CI: 0.90 to 0.99), and AD risk (RR: 0.93; 95% CI: 0.90 to 0.95) 2. Lower dementia (RR: 0.86; 95% CI 0.76 to 0.96) and AD risk (RR: 0.63; 95% CI 0.51 to 0.76)

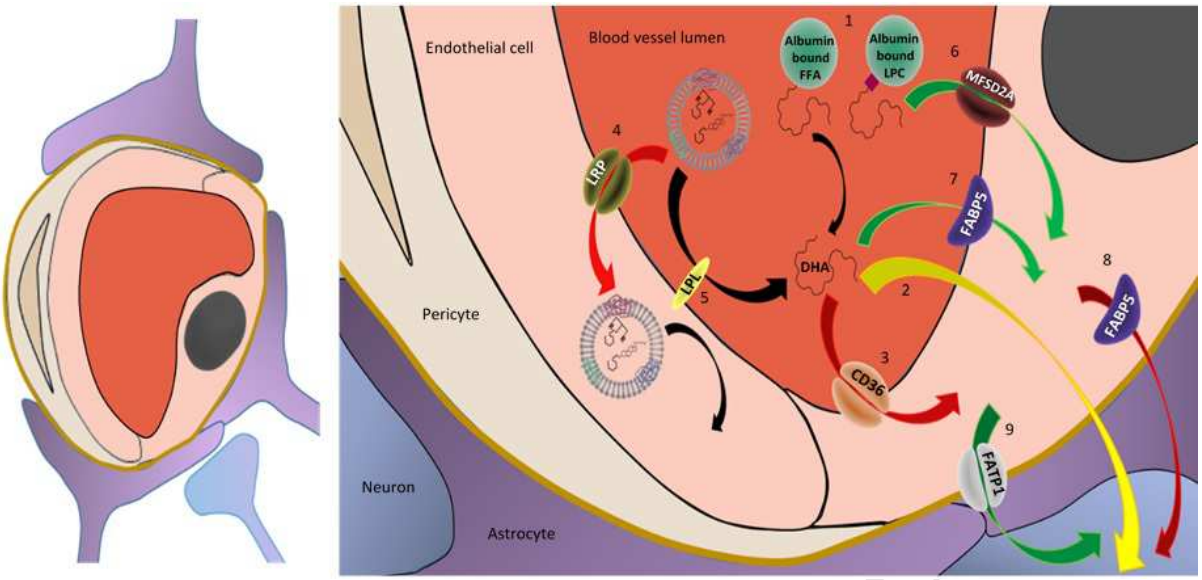
Table 2: Prospective cohort studies reporting on fish and DHA intakes and dementia risk

Abbreviations: PUFA, PC-DHA, HR, RR, CI – Polyunsaturated fatty acid, Phosphatidylcholine – docosahexaenoic acid, Hazard ratio, relative risk, confidence interval

Figure 2: DHA - BBB transport mechanisms – Plasma DHA is associated with lipoproteins or albumin depending on esterification status (1). Un-esterified DHA can cross the BBB via simple diffusion (2). The uptake of DHA via CD36 (3) and LRP (4) mediated processes are now considered insignificant. Lipoproteins may still contribute as a DHA pool under the action of lipoprotein lipase at the BBB (5). Mfsd2a specifically aids the uptake of LPC-DHA into the endothelium (6). FABP5 is implicated in the transport of DHA across the BBB; it is thought that this is achieved via endothelial uptake (7) and not cellular transport (8). FATP1 may enhance transport of DHA across the basal

membrane (9). Green arrow: Potential transport process Yellow arrow: Simple diffusion Red arrow: Disproved transport process. Adapted from Bazinet et al (Bazinet and Laye, 2014)





Highlights:

- The extent of APOE- ε4 's influence on Alzheimer's disease risk may be modulated by sex, age and DHA status
- The effects of the APOE- ε4 genotype are exacerbated in females in the decade(s) following menopause
- Consumption of oily fish, and the n-3 fatty acid DHA, reduces AD risk
- Higher doses of DHA may be warranted to achieve such benefit in APOE- ε4 carriers, the elderly and females