Nourishing neurons

van Wijk, N.

2018

document version
Publisher's PDF, also known as Version of record

Summary and general discussion

Chapter 11

General objective

Patients with Alzheimer's disease (AD) likely have an increased nutritional need to counter synapse loss and to reduce membrane-related pathology. Previous preclinical *in vivo* and *in vitro* studies already revealed that specific nutrients can act synergistically to increase synapse formation and modulate membrane-related processes. Concurrently, epidemiological studies indicated that AD patients have lower plasma levels of several of these nutrients compared to healthy controls. The research presented in this thesis built further on these previous observations and aimed to gain more insight into the possible AD-specific nutritional need to counter synaptic pathology. Therefore, the mechanism of action and effectiveness of combinations of nutrients to increase markers of synapse formation and functioning was further explored and reviewed. In addition, the nutritional status of AD and mild cognitive impairment (MCI) patients was investigated, the effectiveness of a nutritional intervention on plasma phospholipid levels in AD patients was tested, and the potential of nutritional approaches in AD was reviewed.

Summary of the findings

Synapse loss and related membrane breakdown are part of AD pathogenesis and represent potential targets for intervention. Synapses consist principally of pre- and postsynaptic membranes and the formation of new synapses therefore requires the synthesis of new synaptic membranes and of constituent phospholipids. Interventions that support synaptic membrane formation and function, theoretically counter synaptic dysfunction in AD. **Chapter 2** reviews the collective experimental support for the mechanism of action of nutritional combinations on enhancing synaptic membrane formation and function. *In vitro* and *in vivo* studies have demonstrated that combined supplementation of the membrane phospholipid precursors docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), uridine monophosphate (UMP), and choline increases brain phospholipid levels, by up to twice the levels without supplementation. These precursors act by enhancing the substrate-saturation of the enzymes that catalyze the rate-limiting steps in phospholipid syntheses. The increased phospholipid levels were accompanied by increased neurite outgrowth, levels of specific pre- and postsynaptic proteins, and number of dendritic spines, all indicative of new synapse formation. Other nutrients, i.e. folate, vitamins B6, B12, C, and E, selenium, and lecithin can further increase levels of membrane phospholipids by increasing the availability of phospholipid precursors or by directly affecting the neuronal membrane or membrane synthesis. Potential mechanisms of action

of these nutrients are discussed in more detail in chapters 3 to 6. Combinations of phospholipid precursors and other nutrients were also shown to improve membrane-related processes, such as neurotransmission, amyloid-β (Aβ)-related pathology, and ultimately cognitive performance. It was repeatedly shown that combined supplementation of nutrients needed for phospholipid synthesis induces a more than additive effect compared to single nutrient supplementation. Based on these insights, the specific nutrient combination Fortasyn® Connect (UMP, DHA, EPA, choline, lecithin, folate, vitamins B6, B12, C and E, and selenium) was designed to ameliorate synapse loss and synaptic dysfunction in AD. The complete nutrient combination of Fortasyn Connect (FC) has been tested in many preclinical *in vitro* and *in vivo* studies and the results confirm the hypothesis that nutrients in FC act in concert to enhance synapse formation and functioning, and to ameliorate cognitive dysfunction. One of these studies is described in chapter 7, in which the effects of FC on hippocampal cholinergic functioning in aged rats is investigated. The review in chapter 2 ends with what is discussed in more detail in chapter 8: a perspective on nutritional requirements in AD, the clinical utility of FC, and a short overview of the clinical studies with the medical food Souvenaid®, containing FC.

The thesis continues by providing experimental evidence that dietary folate, vitamin B6, and vitamin B12 influences the systemic levels of nutritional phospholipid precursors, i.e. choline (**chapter 3**) and DHA (**chapter 4**). Folate, vitamin B12, and vitamin B6 are essential nutritional components in one-carbon metabolism and their availability affects methylation capacity. These vitamins may therefore affect the methylation rate of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) by phosphatidylethanolamine-*N*-methyltransferase (PEMT) in the liver. Increasing PC synthesis by PEMT could not only increase *de novo* choline synthesis (Zeisel and Blusztajn 1994), but also increases the transport of polyunsaturated fatty acids (PUFAs) from the liver to the plasma and other tissues (Selley 2007, Watkins et al. 2003, Yan et al. 2011). Hence, by increasing PC synthesis by PEMT, B-vitamin supplementation theoretically increases choline and DHA availability. To test this hypothesis, the effect of varying dietary levels of the three B-vitamins on plasma choline, DHA, and homocysteine concentration in rats was investigated. The experiments demonstrated that rats supplemented with dietary B-vitamins had lower plasma homocysteine concentrations and higher plasma concentrations of choline and DHA, where plasma DHA was dose-dependently increased. In conclusion, systemic availability of phospholipid precursors DHA and choline is not only affected by their nutritional intake, but also by the intake of B-vitamins.

Evidence on the added value of dietary lecithin for increasing phospholipid precursor availability is presented in **chapter 5**. Theoretically, dietary lecithin (i.e. phospholipids) could increase systemic availability of dietary omega-3 PUFA, since (biliary) phospholipids are required for the absorption of fat from the gut lumen into the enterocytes and the lymph (O'Doherty et al. 1973, Werner et al. 2006). To test the hypothesized additive effects of dietary lecithin on top of dietary DHA, plasma and red blood cell (RBC) DHA levels were determined after dietary supplementation of DHA-containing oils with and without concomitant dietary supplementation of lecithin to rats. The results demonstrated that supplementation of lecithin, which itself does not contain any DHA, increased levels of DHA in plasma and RBC of rats fed DHA oil-containing diets. Thus, while dietary lecithin supplementation alone did not affect DHA levels in plasma and RBC, lecithin slightly further increased the rise in DHA induced by dietary DHA supplementation. By increasing the systemic availability of dietary DHA, dietary lecithin may increase the efficacy of DHA supplementation when their intake is combined.

Taken together, chapters 3 to 5 indicate that the availability of the phospholipid precursors DHA and choline is not only affected by their nutritional intake but also by the intake of B-vitamins and lecithin which influence precursor uptake and metabolism. Because levels of DHA (Rapoport et al. 2001) and choline (Klein et al. 1990) in the brain are affected by their plasma concentrations, supplemental dietary B-vitamins and lecithin could ultimately sustain brain levels of DHA and choline. Small increases in the local availability of phospholipid precursors are already relevant since most enzymes involved in phospholipids synthesis have low affinities for their substrates. Consequently, addition of B-vitamins and lecithin potentially facilitates the synthesis of membrane phospholipids by enhancing the availability of the rate-limiting precursors.

Chapter 6 provides experimental evidence on the added value of dietary vitamin C, vitamin E, and selenium on increasing synaptic membrane formation induced by phospholipid precursors. In theory these antioxidants affect the breakdown or synthesis of neuronal membranes through several mechanisms, including the prevention of lipid peroxidation, increasing the activity of Kennedy pathway enzymes, and by affecting the neuronal membranes directly. To obtain a proof-of-principle, the effects of dietary supplementation with the phospholipid precursors DHA, EPA, and uridine on synaptic membrane levels in rats were assessed using diets that were either nearly devoid of vitamin C, vitamin E, and selenium or supplemented with high levels of these antioxidants.

The data showed that levels of brain phospholipids and synaptic proteins were substantially enhanced in rats supplemented with DHA, EPA, and uridine only if the diet also contained sufficient quantities of the antioxidants vitamin C, vitamin E, and selenium. The formation of new synapses might therefore be influenced by the concerted availability of phospholipid precursors and antioxidants.

The studies in the above mentioned chapters illustrate physiological and biochemical interactions between nutrients and indicate that a higher efficacy can be reached with combined supplementation. Stimulating the formation and function of synaptic membranes might require a combined increased supply of phospholipid precursors and facilitating nutrients. The study described in **chapter 7** investigated the effects of dietary supplementation of such a combination (FC) on hippocampal cholinergic functioning in aged rats. Results revealed that supplementation with the FC diet for 4 or 6 weeks, induced a substantial and time-dependent increase in basal and stimulated hippocampal acetylcholine (ACh) release, ACh tissue levels, and choline acetyltransferase (ChAT) tissue levels, along with equally increased levels of phospholipids and synaptic proteins. These data show that the FC diet enhances hippocampal cholinergic neurotransmission in aged rats, which probably can be ascribed to the concurrently observed enhanced synaptic membrane formation.

Experimental studies, including the ones described in chapters 2 to 7, demonstrated that nutrients and combinations thereof affect markers of synaptic membrane formation and function and which leads to improved membrane-dependent processes. This implies a potential role for nutritional intervention in patients with AD. The clinical utility of FC and other single- and multi-nutrient approaches for risk reduction and management of AD patients is discussed in **chapter 8**. Hypothetically, the need for and utilization of specific nutrients for synaptic membrane and synapse formation is higher to compensate for the increased loss of synapses in AD. This increased need might in turn be compromised by a lower nutritional status in AD patients. The chapter also discusses the paradigm shift to intervene in earlier stages of AD, facilitated by the advancement of diagnostic techniques and before pathological changes have accumulated to an irreversible degree. This especially provides opportunities for nutritional interventions due to the low risk for adverse effects and necessarily longer exposure time in early intervention studies.

Thus, AD patients may have disease-specific nutritional requirements that are associated with pathological processes, such as synapse loss. Studies on nutritional status in AD have generally shown lower blood levels of most nutrients that are required for synaptic membrane synthesis (Blass et al. 1985, de Wilde et al. 2017, Lopes da Silva et al. 2014, Olde Rikkert et al. 2014). The cross-sectional study described in **chapter 9** adds to these observations by showing lower levels of nutrients involved in phospholipid synthesis in both blood and cerebrospinal fluid (CSF), and in both AD and MCI patients. More specifically, this study showed that compared with control subjects, subjects with AD had lower CSF uridine and plasma choline and higher CSF homocysteine concentrations, while subjects with MCI had lower plasma and CSF uridine and serum and CSF folate and higher CSF homocysteine concentrations. Concentrations of these metabolites did not differ between MCI and AD. Hence, disease-specific nutritional deficits might already be present in MCI, which lends support to the application of nutritional strategies in the management of early stages of AD.

Souvenaid, containing FC, has been tested in several randomized controlled clinical trials. In the Souvenir II trial, drug-naive patients with very mild to mild AD received Souvenaid for 24 weeks. **Chapter 10** reports levels of a set of phospholipids in baseline and 24-week plasma samples from Souvenir II. This biomarker set of phospholipids, specifically phosphatidylcholines, was previously reported to accurately detect preclinical AD (Mapstone et al. 2014), although other investigators were unsuccessful in replicating the high accuracy level (Casanova et al. 2016, D. Li et al. 2017). The reduced levels of these plasma biomarker phospholipids in these subjects with preclinical AD probably reflects altered phospholipid metabolism in the brain and periphery. Intervention with Souvenaid for 24 weeks increased 5 of the 7 measured biomarker PC species by 1.3 to 2.3-fold of baseline levels. These results indicate that a plasma biomarker profile reflecting disturbed phospholipid metabolism can be improved by providing nutrients needed for phospholipid synthesis. The increased phospholipids levels may be indicative of the changes induced concomitantly in the brain, e.g. increased synaptic membrane synthesis.

Discussion of the findings

Phospholipid biosynthesis

Central to this thesis is the effect of nutritional interventions on phospholipid synthesis. Phospholipids can be subdivided into individual classes, like PC, PE, phosphatidylserine

(PS), phosphatidylinositol (PI), and sphingomyelin (SM). Each individual class represents a large subset of compounds with varying fatty acid chains that are differentially linked to the glycerol backbone.

There are several biochemical pathways for the synthesis of the different phospholipid classes. PC and PE, the most abundant phospholipids in the mammalian brain, are primarily generated *de novo via* the cytidine diphosphate (CDP)-choline and CDP-ethanolamine pathways (the Kennedy pathway) (Kennedy and Weiss 1956). The synthesis of PC *via* the CDP-choline pathway is initiated with the phosphorylation of choline. This phosphocholine then combines with cytidine triphosphate (CTP) to form CDP-choline. CTP can be formed from several pyrimidines (e.g. cytidine or uridine). The phosphocholine unit of CDP-choline is then transferred to diacylglycerol (DAG) to yield PC. PC can also be synthesized by methylation of ethanolamine in PE by PEMT. In animals PS is made *via* a base-exchange reaction, in which serine is exchanged by the choline or ethanolamine moiety in PC or PE, respectively. The *de novo* pathway of PI starts with the formation of CDP-diacylglycerol from diacylglycerol 3-phosphate and CTP. The activated DAG unit then reacts with inositol to form PI. SM is usually formed from the combination of ceramide and the phosphocholine unit of PC (Gurr et al. 2002). These are simplified descriptions of the main pathways but it should be noted that in reality, phospholipid synthesis and breakdown form a complex network of reactions with various enzymes and intermediates that are also involved in several other pathways.

The synthesis of all major membrane phospholipids is dependent on circulating nutritional precursors. For example, the synthesis of PC may utilize choline, a pyrimidine, and DAG. These precursors act by enhancing the substrate-saturation of the enzymes that catalyze the steps in phospholipid synthesis (Wurtman et al. 2009). Most of the enzymes involved in the Kennedy cycle have low affinities for their substrates (as reviewed in Wurtman et al. 2009), meaning that the enzymes are unsaturated at normal levels of the precursors and that the available levels of these precursors are rate-limiting for the amount of end products.

In theory, supplementation of nutritional precursors could increase phospholipid synthesis in the brain *via* increasing substrate-saturation of the enzymes involved; however several elements in this hypothesis need further elucidation. Firstly, one of the Kennedy pathway enzymes, choline kinase, may already be saturated at normal brain choline levels as

indicated by the only available human data set from autopsy material from 3-4 subjects with different causes of death (Ross et al. 1997). This is in contrast to what is reported in rodents (Wurtman et al. 2009). Secondly, the activity of another Kennedy pathway enzyme, CTP:phosphocholine cytidylyltransferase, is also determined by the regulation of its location: the cytosolic form is inactive, whereas the membrane-bound form is active. Thirdly, some of the biochemical reactions in phospholipid synthesis are reversible and end-product inhibition affects the affinity of the enzyme for their substrates. Finally, as indicated before, the nutritional precursors and intermediates of the pathways for phospholipid synthesis are also involved in other biochemical pathways and may therefore be utilized in other ways.

In practice, supplementation of nutritional phospholipid precursors has repeatedly been shown to synergistically increase phospholipid levels in the brains of rodents (see also chapters 2, 6, and 7) (Cansev et al. 2009, Cansev et al. 2008, Cansev and Wurtman 2007, Holguin et al. 2008a, Holguin et al. 2008b, Sakamoto et al. 2007, Shahdat et al. 2004, Wurtman et al. 2006). Although this provides ample evidence for the opportunity of phospholipid precursors to increase brain phospholipid levels, these studies were mostly performed in healthy adult or aged gerbils or rats. Hence, current evidence could be strengthened by replication in other animal models. Clinical studies showing that phospholipid precursors affect brain phospholipid metabolism to date (Agarwal et al. 2010, Rijpma et al. 2017, Silveri et al. 2008), confirm the effects in humans. Nevertheless, more clinical evidence supporting both the mechanism of action and effectiveness on phospholipid synthesis would be valuable.

Altered phospholipid metabolism in AD

There is a growing amount of evidence showing that AD is associated with decreased phospholipid levels in the brain (Farooqui et al. 1997, Ginsberg et al. 1995, Gottfries et al. 1996, Grimm et al. 2011a, Grimm et al. 2011b, Guan et al. 1999a, Han et al. 2001, Igarashi et al. 2011, Kou et al. 2011, Miatto et al. 1986, Nitsch et al. 1992, Pettegrew et al. 2001, Prasad et al. 1998, Wells et al. 1995, Wood et al. 2015, Yuki et al. 2014) and CSF (Fonteh et al. 2013, C. Mulder et al. 2003, M. Mulder et al. 1998). Altered phospholipid levels are also observed in plasma of AD patients (Casanova et al. 2016, Fiandaca et al. 2015, Gonzalez-Dominguez et al. 2014a, Gonzalez-Dominguez et al. 2014b, Gonzalez-Dominguez et al. 2016, Goodenowe et al. 2007, Kim et al. 2017, Klavins et al. 2015, D. Li et al. 2016, D. Li et al. 2017, Mapstone et al. 2014, Mapstone et al. 2017, Olazaran et al. 2015, Oresic et al.

2011, Proitsi et al. 2017, Toledo et al. 2017, Whiley et al. 2014, Wood et al. 2010). Plasma phospholipid changes are now explored for their potential in detecting preclinical, prodromal, or clinical AD, i.e. as less invasive and less costly diagnostic or prognostic biomarkers for AD, with mixed results (e.g. Casanova et al. 2016, Fiandaca et al. 2015, Mapstone et al. 2014). Whether changes in plasma phospholipid levels directly originate from disturbed phospholipid metabolism in the brain, or are caused by disturbed peripheral phospholipid metabolism (e.g. liver) remains to be elucidated.

The reported decreases in brain and periphery range from total phospholipid levels to levels of individual classes, plasmalogens, ether phospholipids, and specific phospholipid species, which probably depends on the focus of the study, methodology used and, not in the least, the study population. A systematic review of all these publications to get a view on the consistency of changes is still lacking. A bird's eye view, however, reveals recurrent reports of lower levels of PC species containing DHA. Taken together, these publications clearly show that phospholipid metabolism in AD is disturbed and most authors suggest that it reflects the breakdown of neuronal membranes. Phospholipid changes in the brain and periphery are also reportedly related to disease severity (Goodenowe et al. 2007, Han et al. 2001, Wood et al. 2010).

Numerous metabolic changes have been suggested to contribute to the phospholipid changes in the AD brain, ranging from lower levels of substrates for phospholipid synthesis (Ellison et al. 1987, Nitsch et al. 1992), to peroxisomal dysfunction (Goodenowe et al. 2007, Grimm et al. 2011b, Kou et al. 2011), lower PEMT activity (Guan et al. 1999b), breakdown of PC for utilization of choline for ACh synthesis (Blusztajn et al. 1986), and increased phospholipase activity (Farooqui et al. 1997). Several publications also indicate possible compensatory phospholipid metabolism changes to counter the loss of phospholipids, such as increased activity and/or expression of phospholipid-synthesizing enzymes and lower activity of catabolic enzymes (Andreev et al. 2012, Ross et al. 1998, Twine et al. 2011). However, this can only be partly effective due to the rate-limiting levels of phospholipid precursors. Further research is needed to reconcile all these observed changes.

Synapse loss in AD may involve the degeneration of neuronal membranes and depletion of membrane phospholipids or changes in membrane phospholipid composition (Bennett et al. 2013, Kosicek and Hecimovic 2013, Naudi et al. 2015). In fact, it has been suggested

that changes in membrane phospholipid metabolism might mechanistically contribute to synaptic dysfunction in AD (Bennett et al. 2013). For example, the depletion of PC(18:0/22:6) in the gray matter in the AD brain was correlated with the loss of postsynaptic density-95 (PSD-95) and with disease duration (Yuki et al. 2014). Underlying relationships and timing of the loss of synapses and phospholipids are unclear and need to be determined.

Protective dietary patterns

Identifying and reducing modifiable risk factors that could prevent or delay the onset and progression of AD could be a preventative strategy to combat the rising prevalence of the disease. Risk factors of nutritional origin are extensively studied for their possible role in AD onset and progression. Although epidemiological studies show that higher intake of certain nutrients, such as vitamin C, vitamin E, B-vitamins, and unsaturated fatty acids, are associated with a lower risk for AD (N. Hu et al. 2013, Morris 2009), most single nutrient clinical intervention trials in AD showed limited benefits (e.g. Burckhardt et al. 2016, Farina et al. 2012, M.M. Li et al. 2014). Presumably efficacy requires the combined availability of various nutritional components (Gustafson et al. 2015). Diet is per definition multi-nutrient. Thus, investigating the impact of dietary patterns on AD, rather than single nutrients, might be a better approach that takes into account possible correlation, interaction, or synergy between nutrients (F.B. Hu 2002).

The most extensively studied protective dietary pattern is the Mediterranean diet. This dietary pattern is characterized by high intake of fruits, vegetables, cereals, and legumes, moderate fish intake, high intake of olive oil, low intake of saturated fats and, low to moderate intake of dairy products, low intake of red meat, and moderate intake of wine (van de Rest et al. 2015). Systematic reviews have concluded that a greater adherence to a Mediterranean diet is associated with less cognitive decline and lower risk of AD (Cao et al. 2016, Lourida et al. 2013, van de Rest et al. 2015). Results of a randomized controlled trial with a Mediterranean diet in cognitively healthy elderly suggested that this diet (supplemented with olive oil or nuts) may counteract age-related cognitive decline (Valls-Pedret et al. 2015). Many other *a priori* and data-driven *a posteriori* dietary patterns have also been shown to reduce risk for developing AD (van de Rest et al. 2015). Notably, in a head-to-head comparison study of the DASH diet (Dietary Approaches to Stop Hypertension), the MIND diet (Mediterranean-DASH Intervention for Neurodegenerative Delay), and a Mediterranean diet showed that high adherence to either diet reduces AD

risk, with superior protective effects of the MIND diet (Morris et al. 2015). This study indicated that even high quality diets can further be modified to provide even better protection against AD. More research is needed to find more effective nutritional patterns and respective intake levels.

Hence, nutritional advice or nutritional intervention could be a viable approach in AD risk reduction. Combining nutritional intervention with other interventions targeting additional modifiable risk factors may provide an even more powerful approach. First studies with such multidomain lifestyle interventions have now been finalized. The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) showed that in elderly at risk for cognitive decline a multidomain intervention on several risk factors (diet, exercise, cognitive training, and vascular risk monitoring) can improve or maintain cognitive functioning and reduce the risk of cognitive decline (Ngandu et al. 2015). In another study, several lifestyle factors (maintenance of normal body weight, regular physical activity, and healthy diet) were linked to lower levels of AD biomarkers (plaques and tangles) in the brains of people with MCI or with memory complaints, suggesting protective effects of these factors (Merrill et al. 2016). However, recently the Multidomain Alzheimer Preventive Trial (MAPT) showed no effects on cognitive decline in elderly people with memory complaints after multidomain intervention (cognitive training, physical activity, nutritional advice, and three preventive consultations) and fish oil supplementation, either alone or in combination (Andrieu et al. 2017). The MIND-AD (JPND #733051041) consortium is initiating up a secondary prevention trial to test the feasibility of compliance to a multidomain range of lifestyle changes (diet, exercise, cognitive training) in order to postpone further deterioration in subjects with MCI due to AD. Besides nutritional advice, this trial will investigate the effects of Souvenaid as part of a multidomain intervention. Besides the latter initiative, more research is needed to determine which population is responsive to which (multidomain) intervention for the prevention or delayed onset of AD. A more personalized approach may be necessary, e.g. by supplementing those subjects that have lower blood levels of specific nutrients.

Combined supplementation is required for (higher) efficacy

As discussed above, targeting pathological processes with multiple nutrients that interact or synergize are more likely to be effective than a single nutrient intervention. On the one hand, each individual nutritional component can have individual effects, and together the nutrients' effects culminate to higher efficacy on a relevant parameter. On the other hand,

physiological and biochemical interactions between nutrients can enhance the rate of a cellular process which normally is rate-limiting.

In this thesis (chapters 2 to 7) it was discussed that combined supplementation of nutritional phospholipid precursors and other nutrients synergistically increases synaptic membrane formation and function. The studies indicate that more pronounced effects can be reached with combined supplementation by (among other processes) simultaneously increasing the availability of phospholipid precursors and enhancing the substrate-saturation in phospholipid synthesis. Although exhaustive comparisons of possible combinations of nutrients, doses, and time-courses, to search for a maximal efficacy are not available, supplementation with the FC combination was repeatedly shown to be superior to single-nutrient (e.g. DHA alone) or partial combinations (e.g. DHA+uridine) (Broersen et al. 2013, Janickova et al. 2015, Jansen et al. 2013, Koivisto et al. 2014, Perez-Pardo et al. 2017, Savelkoul et al. 2012, Zerbi et al. 2014).

In contrast, a deficit of only one nutritional compound can counteract the beneficial effects of other nutritional compounds or combinations thereof. For example, the efficacy of DHA and choline supplementation in AD might be hampered by concurrent B-vitamin deficiencies, as commonly observed in AD (van Dam and van Gool 2009) since B-vitamin deficiencies impair the transport of DHA from the liver, decrease the synthesis of choline, and increase the utilization of choline. Indispensability between DHA and B-vitamins was also underscored by the results of a clinical trial showing that supplementation with folic acid, vitamin B6, and vitamin B12 was not effective in reducing brain atrophy (Jerneren et al. 2015) and cognitive decline (Oulhaj et al. 2016) in MCI subjects with low plasma omega-3 fatty acids levels, as opposed to subjects with higher plasma levels.

Update on preclinical studies with Fortasyn Connect

Chapter 2 presented a comprehensive overview of studies that led to the development of FC and of several studies showing efficacy of this combination in several preclinical *in vivo* and *in vitro* models. Since then, several other studies in mice models of AD have been published with additional preclinical evidence supporting the mode of action and effectiveness of FC.

Dietary supplementation with FC shows a consistent effect on brain fatty acid composition: in transgenic APP/PS1 and ApoE-4, ApoE-KO, and wild type mice, FC

increases brain levels of DHA and total omega-3 and reduces brain levels of arachidonic acid and total omega-6 PUFA levels (Jansen et al. 2013, Jansen et al. 2014, Wiesmann et al. 2016, Zerbi et al. 2014). Adding to the evidence on improving hippocampal cholinergic neurotransmission in aged rats (chapter 7), FC also enhanced hippocampal density of muscarinic receptors and activity of ChAT and acetylcholinesterase (AChE) in transgenic APP/PS1 mice (Janickova et al. 2015). In another study in transgenic APP/PS1 mice, FC preserved diffusion tensor magnetic resonance imaging (MRI) measures of brain white and gray matter integrity that are known to correlate with loss of myelin, decreased axonal density and connectivity in white matter, and neuronal loss in gray matter (Zerbi et al. 2014). In aged wild-type and transgenic ApoE-4 mice, FC increased functional connectivity in hippocampal and cortex areas as measured by resting state functional MRI (Wiesmann et al. 2016). In addition, FC increased hippocampal and cortical PSD-95 levels in these mice (wild-type and transgenic) (Wiesmann et al. 2016). FC also had profound and consistent effects on cerebral blood flow (Wiesmann et al. 2016, Zerbi et al. 2014) and cerebral blood volume (Jansen et al. 2014) in wild-type, transgenic APP/PS1 and ApoE-4, and ApoE-KO mice. Furthermore, FC restored neurogenesis in APP/PS1 mice (Jansen et al. 2013). Finally, several studies showed behavioral effects of FC supplementation. FC improved exploratory behavior in wild-type, transgenic APP/PS1, and ApoE-KO mice (Jansen et al. 2013, Jansen et al. 2014) and improved learning and memory in wild-type and transgenic APP/PS1 mice (Koivisto et al. 2014, Wiesmann et al. 2013).

Although the therapeutic potential of FC has focused predominantly on AD, it is well recognized that synaptic dysfunctions are similarly pivotal in other indications. First preclinical *in vivo* results point towards a broader applicability of this nutritional combination In rats with experimentally-induced spinal cord injury, therapeutic intervention (thus after injury onset) with FC induced a dose-dependent enhancement of locomotor and bladder function recovery, and protection of spinal cord tissue (Pallier et al. 2015). Supplementation with FC after experimentally-induced ischemic stroke in mice improved recovery by increasing cerebral blood flow, protecting white and gray matter integrity, restoring functional connectivity, increasing neurogenesis, decreasing the neuroinflammatory response, and improving motor skills and muscle strength (Wiesmann et al. 2017). In the intrastriatal rotonone model of Parkinson's disease in mice, therapeutic intervention with an FC combination normalized the model-induced decline in motor function, spatial memory, and intestinal function and improved histological parameters, such as striatal dopamine transporter density (Perez-Pardo et al. 2017).

Interspecies translation of Fortasyn Connect nutrient levels

In translating supplemental nutrient levels from animals to humans and back, many factors should be considered, including species differences between estimations of metabolic rate, minimal requirements per nutrient, upper limit levels, basal nutrient intake, and prior dose-response evidence.

Chapters 3 to 6 mostly describe proof-of-concept studies in which varying levels of nutrients are investigated ranging from deficient to adequate and high, based upon the nutrient requirements of the animals. In chapter 2 and 7, however, rodent studies with FC are discussed. The FC nutrients are normally supplemented to a standardized AIN93-based (Reeves et al. 1993), resulting in a 2 to 30-fold increase of the respective nutrient levels. Obviously, the supplemented levels of these nutrients are not equal to the levels of FC in Souvenaid for human application, but can be translated back and forth with varying considerations per nutrient. For instance, Souvenaid provides 1.5 g of DHA plus EPA over and above the normal diet with a recommended daily intake of 0.25 to 0.50 g (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA) 2012). In rats, the FC diet provides approximately 10 g of DHA plus EPA per kg diet, while 2 g of total omega-3 PUFAs per kg diet are thought to be essential (National Research Council 1995), although there are no specific recommendations for DHA and EPA. Thus, supplementation in both humans and rats leads to a total intake of approximately 5x the recommended levels.

Nutritional requirements in AD

In this thesis (chapters 2, 8, and 9) it is postulated that AD patients may have a disease-specific nutritional requirement. Factors contributing to higher need for specific nutrients in AD may include a disease-specific lower nutritional status and the increased utilization of nutrients to counter synapse loss (Gustafson et al. 2015, Vandewoude et al. 2016). Hence, even if the nutritional status of a patient with AD is comparable to a healthy person of the same age and gender, it might still be that this patient has an insufficient availability of certain nutrients. Disease-specific nutritional requirements also go beyond the nutritional recommendations set for the healthy population. The Population Reference Intakes (PRI) used in the EU (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA) 2010) or the Recommended Dietary Allowance (RDA) used in the US (Institute of Medicine Committee on Use of Dietary Reference Intakes in Nutrition Labeling 2003) is defined as the level of intake of a specific nutrient that is adequate to satisfy the physiological requirements (e.g. to prevent clinical deficiencies) of 97.5% of the

healthy individuals in the population. It is acknowledged that specific groups have different requirements as there are specific recommendations per age group, gender, and pregnant and lactating women. In addition, in some countries specific recommendations exist for taking supplemental vitamins for specific groups to reach the recommend levels, for example for infants, pregnant women, vegans, and dark-skinned persons living at higher latitudes. Most likely, requirements are also different in a disease state in which metabolic changes and compensation mechanisms result in a different utilization or endogenous synthesis of nutrients. However, current guidelines on dietary recommendation for patients with AD advise to eat a healthy balanced diet (e.g. Volkert et al. 2015) and do not differ from the recommended daily intake for a healthy population. Meeting the recommended daily intake is already very challenging for large proportions of the healthy population, let alone for AD patients that are the least able to make cognitive and physical changes and experience worsening of appetite, taste, and smell (Shatenstein et al. 2007). Hence, meeting the proposed disease-specific nutritional requirement in AD is practically impossible to reach with adaptation of the diet. Nutritional intervention in AD by means of dietary supplements or medical foods provide a practical approach to increase the availability of nutrients required to support synapse formation and function.

Future perspectives

Key to the near future perspective of medical treatment of AD are the results of upcoming large drug trials with monoclonal antibodies and beta-secretase inhibitors. However, regardless of their outcomes, on the longer term preventative strategies will be the way forward. Prevention strategies require an early identification of people at high risk or with preclinical or prodromal disease and an effective approach to prevent or postpone the onset of AD. Primary prevention strategies could include practical changes in modifiable risk factors with low adverse effects, such as a nutritional intervention, in people at high risk of developing AD. These individuals could be identified by an easy to apply tool, such as a blood test for having increased risk (by analogy to the cholesterol test for cardiovascular disease risk). For secondary prevention in preclinical or prodromal AD, i.e. in patients that will in all likelihood develop AD, therapeutic intervention might include radical (drug-induced) changes in modifiable risk factors, (a combination of) drugs that combat disease pathology, interventions that stimulate neuronal regeneration, or a combination of all. A prerequisite for application of such therapies is a highly accurate diagnosis of (sub)populations with preclinical AD. In the distant future, a near 100%-

accurate peripheral biomarker (e.g. blood-based) for preclinical AD that is less invasive and less costly than the brain imaging and CSF sampling would pave the way for large scale population screening programs.

The clinical studies to date indicate that Souvenaid offers greater potential when applied to earlier stages of AD when pathological damage is less advanced. To explore the application of Souvenaid in AD risk reduction, a long-term intervention trial in asymptomatic subjects at high risk for developing AD is warranted. Intervention in such subjects is feasible providing the positive safety profile of Souvenaid. A most responsive population should be selected, perhaps also based on blood levels of certain nutrients.

Recent lipidomics and metabolomics studies in AD have proven that AD is accompanied by many metabolic disturbances. Phospholipid disturbances might mediate synaptic dysfunction in AD. This field has only started to unravel the complexity of the interrelation of the AD-specific metabolism and classical pathology. Hence, much more research, perhaps requiring machine learning, is needed to extract a therapeutic option from the vast amount of data that is being collected.

Additional research is currently generated in the Nutrition the Unrecognized Determinant for Alzheimer's Disease (NUDAD) project on factors that contribute an AD-specific nutritional need, such as compromised nutrient intake, uptake, metabolism, and utilization. Such research might eventually result in disease-specific nutritional recommendations in parallel to the already existing recommendations for the general population.

More specifically for the FC evidence base and building further on the studies described in this thesis, it would be of interest to have more fundamental understanding of the phospholipid synthesizing and breakdown pathways and AD-specific changes, better comprehension of the link between synapse loss and phospholipid loss across the whole AD spectrum, clarification on phospholipid disturbances in the periphery and the brain in AD, further replication and extension of preclinical and clinical effects of nutritional combinations on phospholipid metabolism and synaptic membrane formation, and more clinical evidence on the effect of nutritional intervention on markers of synaptic functioning in AD.

List of abbreviations

ACh, acetylcholine
AChE, acetylcholinesterase
AD, Alzheimer's disease
Aβ, amyloid-β
CDP, cytidine diphosphate
ChAT, choline acetyltransferase
CSF, cerebrospinal fluid
CTP, cytidine triphosphate
DAG, diacylglycerol
DHA, docosahexaenoic acid
EPA, eicosapentaenoic acid
FC, Fortasyn Connect
MCI, mild cognitive impairment
MRI, magnetic resonance imaging
PC, phosphatidylcholine
PE, phosphatidylethanolamine
PEMT, phosphatidylethanolamine-*N*-methyltransferase
PI, phosphatidylinositol
PRI, population reference intakes
PS, phosphatidylserine
PSD-95, postsynaptic density-95
PUFA(s), polyunsaturated fatty acid(s)
RBC, red blood cell(s)
RDA, recommended dietary allowance
SM, sphingomyelin
UMP, uridine monophosphate

References

Agarwal N, Sung YH, Jensen JE, daCunha G, Harper D, Olson D, and Renshaw PF. Short-term administration of uridine increases brain membrane phospholipid precursors in healthy adults: a 31-phosphorus magnetic resonance spectroscopy study at 4T. *Bipolar Disord*. 2010;12:825-33.

Andreev VP, Petyuk VA, Brewer HM, Karpievitch YV, Xie F, Clarke J, Camp D, Smith RD, Lieberman AP, Albin RL, Nawaz Z, El Hokayem J, and Myers AJ. Label-free quantitative LC-MS proteomics of Alzheimer's disease and normally aged human brains. *J Proteome Res*. 2012;11:3053-67.

Andrieu S, Guyonnet S, Coley N, Cantet C, Bonnefoy M, Bordes S, Bories L, Cufi MN, Dantoine T, Dartigues JF, Desclaux F, Gabelle A, Gasnier Y, Pesce A, Sudres K, Touchon J, Robert P, Rouaud O, Legrand P, Payoux P, Caubere JP, Weiner M, Carrie I, Ousset PJ, Vellas B, and Group MS. Effect of long-term omega 3 polyunsaturated fatty acid supplementation with or without multidomain intervention on cognitive function in elderly adults with memory complaints (MAPT): a randomised, placebo-controlled trial. *Lancet Neurol*. 2017;16:377-89.

Bennett SA, Valenzuela N, Xu H, Franko B, Fai S, and Figeys D. Using neurolipidomics to identify phospholipid mediators of synaptic (dys)function in Alzheimer's Disease. *Front Physiol*. 2013;4:168.

Blass JP, Hanin I, Barclay L, Kopp U, and Reding MJ. Red blood cell abnormalities in Alzheimer disease. *J Am Geriatr Soc*. 1985;33:401-5.

Blusztajn JK, Holbrook PG, Lakher M, Liscovitch M, Maire JC, Mauron C, Richardson UI, Tacconi M, and Wurtman RJ. "Autocannibalism" of membrane choline-phospholipids: physiology and pathology. *Psychopharmacol Bull*. 1986;22:781-6.

Broersen LM, Kuipers AA, Balvers M, van Wijk N, Savelkoul PJ, de Wilde MC, van der Beek EM, Sijben JW, Hageman RJ, Kamphuis PJ, and Kiliaan AJ. A specific multi-nutrient diet reduces Alzheimer-like pathology in young adult AbetaPPswe/PS1dE9 mice. *J Alzheimers Dis*. 2013;33:177-90.

Burckhardt M, Herke M, Wustmann T, Watzke S, Langer G, and Fink A. Omega-3 fatty acids for the treatment of dementia. *Cochrane Database Syst Rev*. 2016;4:Cd009002.

Cansev M and Wurtman RJ. Chronic administration of docosahexaenoic acid or eicosapentaenoic acid, but not arachidonic acid, alone or in combination with uridine, increases brain phosphatide and synaptic protein levels in gerbils. *Neuroscience*. 2007;148:421-31.

Cansev M, Ulus IH, Wang L, Maher TJ, and Wurtman RJ. Restorative effects of uridine plus docosahexaenoic acid in a rat model of Parkinson's disease. *Neurosci Res*. 2008;62:206-9.

Cansev M, Marzloff G, Sakamoto T, Ulus IH, and Wurtman RJ. Giving uridine and/or docosahexaenoic acid orally to rat dams during gestation and nursing increases synaptic elements in brains of weanling pups. *Dev Neurosci*. 2009;31:181-92.

Cao L, Tan L, Wang HF, Jiang T, Zhu XC, Lu H, Tan MS, and Yu JT. Dietary patterns and risk of dementia: a systematic review and meta-analysis of cohort studies. *Mol Neurobiol*. 2016;53:6144-54.

Casanova R, Varma S, Simpson B, Kim M, An Y, Saldana S, Riveros C, Moscato P, Griswold M, Sonntag D, Wahrheit J, Klavins K, Jonsson PV, Eiriksdottir G, Aspelund T, Launer LJ, Gudnason V, Legido Quigley C, and Thambisetty M. Blood metabolite markers of preclinical Alzheimer's disease in two longitudinally followed cohorts of older individuals. *Alzheimers Dement*. 2016;12:815-22.

de Wilde MC, Vellas B, Girault E, Cetinyurek-Yavuz A, and Sijben JW. Lower brain and blood nutrient status in Alzheimer's disease: Results from meta-analyses. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*. 2017;3:416-31.

EFSA Panel on Dietetic Products Nutrition and Allergies (NDA). Scientific Opinion on principles for deriving and applying Dietary Reference Values. *EFSA Journal*. 2010;8:1458.

EFSA Panel on Dietetic Products Nutrition and Allergies (NDA). Scientific opinion on the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). *EFSA Journal*. 2012;10:2815.

Ellison DW, Beal MF, and Martin JB. Phosphoethanolamine and ethanolamine are decreased in Alzheimer's disease and Huntington's disease. *Brain Res*. 1987;417:389-92.

Farina N, Isaac MG, Clark AR, Rusted J, and Tabet N. Vitamin E for Alzheimer's dementia and mild cognitive impairment. *Cochrane Database Syst Rev*. 2012;11:Cd002854.

Farooqui AA, Rapoport SI, and Horrocks LA. Membrane phospholipid alterations in Alzheimer's disease: deficiency of ethanolamine plasmalogens. *Neurochem Res*. 1997;22:523-7.

Fiandaca MS, Zhong X, Cheema AK, Orquiza MH, Chidambaram S, Tan MT, Gresenz CR, FitzGerald KT, Nalls MA, Singleton AB, Mapstone M, and Federoff HJ. Plasma 24-metabolite panel predicts preclinical transition to clinical stages of Alzheimer's disease. *Front Neurol*. 2015;6:237.

Fonteh AN, Chiang J, Cipolla M, Hale J, Diallo F, Chirino A, Arakaki X, and Harrington MG. Alterations in cerebrospinal fluid glycerophospholipids and phospholipase A2 activity in Alzheimer's disease. *J Lipid Res*. 2013;54:2884-97.

Ginsberg L, Rafique S, Xuereb JH, Rapoport SI, and Gershfeld NL. Disease and anatomic specificity of ethanolamine plasmalogen deficiency in Alzheimer's disease brain. *Brain Res*. 1995;698:223-6.

Gonzalez-Dominguez R, Garcia-Barrera T, and Gomez-Ariza JL. Combination of metabolomic and phospholipid-profiling approaches for the study of Alzheimer's disease. *J Proteomics*. 2014a;104:37-47.

Gonzalez-Dominguez R, Garcia-Barrera T, and Gomez-Ariza JL. Metabolomic study of lipids in serum for biomarker discovery in Alzheimer's disease using direct infusion mass spectrometry. *J Pharm Biomed Anal*. 2014b;98:321-6.

Gonzalez-Dominguez R, Ruperez FJ, Garcia-Barrera T, Barbas C, and Gomez-Ariza JL. Metabolomic-driven elucidation of serum disturbances associated with Alzheimer's disease and mild cognitive impairment. *Curr Alzheimer Res*. 2016;13:641-53.

Goodenowe DB, Cook LL, Liu J, Lu Y, Jayasinghe DA, Ahiahonu PW, Heath D, Yamazaki Y, Flax J, Krenitsky KF, Sparks DL, Lerner A, Friedland RP, Kudo T, Kamino K, Morihara T, Takeda M, and Wood PL. Peripheral ethanolamine plasmalogen deficiency: a logical causative factor in Alzheimer's disease and dementia. *J Lipid Res*. 2007;48:2485-98.

Gottfries CG, Karlsson I, and Svennerholm L. Membrane components separate early-onset Alzheimer's disease from senile dementia of the Alzheimer type. *Int Psychogeriatr*. 1996;8:365-72.

Grimm MO, Grosgen S, Riemenschneider M, Tanila H, Grimm HS, and Hartmann T. From brain to food: analysis of phosphatidylcholins, lyso-phosphatidylcholins and phosphatidylcholin-plasmalogens derivates in Alzheimer's disease human post mortem brains and mice model via mass spectrometry. *J Chromatogr A*. 2011a;1218:7713-22.

Grimm MO, Kuchenbecker J, Rothhaar TL, Grosgen S, Hundsdorfer B, Burg VK, Friess P, Muller U, Grimm HS, Riemenschneider M, and Hartmann T. Plasmalogen synthesis is regulated via alkyl-dihydroxyacetonephosphate-synthase by amyloid precursor protein processing and is affected in Alzheimer's disease. *J Neurochem*. 2011b;116:916-25.

Guan Z, Wang Y, Cairns NJ, Lantos PL, Dallner G, and Sindelar PJ. Decrease and structural modifications of phosphatidylethanolamine plasmalogen in the brain with Alzheimer disease. *J Neuropathol Exp Neurol*. 1999a;58:740-7.

Guan Z, Wang Y, Xiao K, Hu P, and Liu J. Activity of phosphatidylethanolamine-N-methyltransferase in brain affected by Alzheimer's disease. *Neurochem Int*. 1999b;34:41-7.

Gurr MI, Harwood JL, and Frayn KN. *Lipid biochemistry, An introduction*. 5th ed. Oxford: Blackwell Publishing; 2002.

Gustafson DR, Clare Morris M, Scarmeas N, Shah RC, Sijben J, Yaffe K, and Zhu X. New perspectives on Alzheimer's disease and nutrition. *J Alzheimers Dis*. 2015;46:1111-27.

Han X, Holtzman DM, and McKeel DW, Jr. Plasmalogen deficiency in early Alzheimer's disease subjects and in animal models: molecular characterization using electrospray ionization mass spectrometry. *J Neurochem*. 2001;77:1168-80.

Holguin S, Huang Y, Liu J, and Wurtman R. Chronic administration of DHA and UMP improves the impaired memory of environmentally impoverished rats. *Behav Brain Res*. 2008a;191:11-6.

Holguin S, Martinez J, Chow C, and Wurtman R. Dietary uridine enhances the improvement in learning and memory produced by administering DHA to gerbils. *FASEB J*. 2008b;22:3938-46.

Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol*. 2002;13:3-9.

Hu N, Yu JT, Tan L, Wang YL, Sun L, and Tan L. Nutrition and the risk of Alzheimer's disease. *Biomed Res Int*. 2013;2013:524820.

Igarashi M, Ma K, Gao F, Kim HW, Rapoport SI, and Rao JS. Disturbed choline plasmalogen and phospholipid fatty acid concentrations in Alzheimer's disease prefrontal cortex. *J Alzheimers Dis*. 2011;24:507-17.

Institute of Medicine Committee on Use of Dietary Reference Intakes in Nutrition Labeling. Dietary Reference Intakes: Guiding principles for nutrition labeling and fortification. Washington (DC): National Academies Press (US); 2003.

Janickova H, Rudajev V, Dolejsi E, Koivisto H, Jakubik J, Tanila H, El-Fakahany EE, and Dolezal V. Lipid-based diets improve muscarinic neurotransmission in the hippocampus of transgenic APPswe/PS1dE9 mice. *Curr Alzheimer Res*. 2015;12:923-31.

Jansen D, Zerbi V, Arnoldussen IA, Wiesmann M, Rijpma A, Fang XT, Dederen PJ, Mutsaers MP, Broersen LM, Lutjohann D, Miller M, Joosten LA, Heerschap A, and Kiliaan AJ. Effects of specific multi-nutrient enriched diets on cerebral metabolism, cognition and neuropathology in AbetaPPswe-PS1dE9 mice. *PLoS One*. 2013;8:e75393.

Jansen D, Zerbi V, Janssen CI, van Rooij D, Zinnhardt B, Dederen PJ, Wright AJ, Broersen LM, Lutjohann D, Heerschap A, and Kiliaan AJ. Impact of a multi-nutrient diet on cognition, brain metabolism, hemodynamics, and plasticity in apoE4 carrier and apoE knockout mice. *Brain Struct Funct*. 2014;219:1841-68.

Jerneren F, Elshorbagy AK, Oulhaj A, Smith SM, Refsum H, and Smith AD. Brain atrophy in cognitively impaired elderly: the importance of long-chain omega-3 fatty acids and B vitamin status in a randomized controlled trial. *Am J Clin Nutr*. 2015;102:215-21.

Kennedy EP and Weiss SB. The function of cytidine coenzymes in the biosynthesis of phospholipides. *J Biol Chem*. 1956;222:193-214.

Kim M, Nevado-Holgado A, Whiley L, Snowden SG, Soininen H, Kloszewska I, Mecocci P, Tsolaki M, Vellas B, Thambisetty M, Dobson RJ, Powell JF, Lupton MK, Simmons A, Velayudhan L, Lovestone S, Proitsi P, and Legido-Quigley C. Association between plasma ceramides and phosphatidylcholines and hippocampal brain volume in late onset Alzheimer's disease. *J Alzheimers Dis*. 2017;60:807-17.

Klavins K, Koal T, Dallmann G, Marksteiner J, Kemmler G, and Humpel C. The ratio of phosphatidylcholines to lysophosphatidylcholines in plasma differentiates healthy controls from patients with Alzheimer's disease and mild cognitive impairment. *Alzheimers Dement*. 2015;1:295-302.

Klein J, Koppen A, and Loffelholz K. Small rises in plasma choline reverse the negative arteriovenous difference of brain choline. *J Neurochem*. 1990;55:1231-6.

Koivisto H, Grimm MO, Rothhaar TL, Berkecz R, Lutjohann DD, Giniatullina R, Takalo M, Miettinen PO, Lahtinen HM, Giniatullin R, Penke B, Janaky T, Broersen LM, Hartmann T, and Tanila H. Special lipid-based diets alleviate cognitive deficits in the APPswe/PS1dE9 transgenic mouse model of Alzheimer's disease independent of brain amyloid deposition. *J Nutr Biochem*. 2014;25:157-69.

Kosicek M and Hecimovic S. Phospholipids and Alzheimer's disease: alterations, mechanisms and potential biomarkers. *Int J Mol Sci*. 2013;14:1310-22.

Kou J, Kovacs GG, Hoftberger R, Kulik W, Brodde A, Forss-Petter S, Honigschnabl S, Gleiss A, Brugger B, Wanders R, Just W, Budka H, Jungwirth S, Fischer P, and Berger J. Peroxisomal alterations in Alzheimer's disease. *Acta Neuropathol*. 2011;122:271-83.

Li D, Misialek JR, Boerwinkle E, Gottesman RF, Sharrett AR, Mosley TH, Coresh J, Wruck LM, Knopman DS, and Alonso A. Plasma phospholipids and prevalence of mild cognitive impairment and/or dementia in the ARIC Neurocognitive Study (ARIC-NCS). *Alzheimers Dement*. 2016;3:73-82.

Li D, Misialek JR, Boerwinkle E, Gottesman RF, Sharrett AR, Mosley TH, Coresh J, Wruck LM, Knopman DS, and Alonso A. Prospective associations of plasma phospholipids and mild cognitive impairment/dementia among African Americans in the ARIC Neurocognitive Study. *Alzheimers Dement*. 2017;6:1-10.

Li MM, Yu JT, Wang HF, Jiang T, Wang J, Meng XF, Tan CC, Wang C, and Tan L. Efficacy of vitamins B supplementation on mild cognitive impairment and Alzheimer's disease: a systematic review and meta-analysis. *Curr Alzheimer Res*. 2014;11:844-52.

Lopes da Silva S, Vellas B, Elemans S, Luchsinger J, Kamphuis P, Yaffe K, Sijben J, Groenendijk M, and Stijnen T. Plasma nutrient status of patients with Alzheimer's disease: Systematic review and meta-analysis. *Alzheimers Dement*. 2014;10:485-502.

Lourida I, Soni M, Thompson-Coon J, Purandare N, Lang IA, Ukoumunne OC, and Llewellyn DJ. Mediterranean diet, cognitive function, and dementia: a systematic review. *Epidemiology*. 2013;24:479-89.

Mapstone M, Cheema AK, Fiandaca MS, Zhong X, Mhyre TR, Macarthur LH, Hall WJ, Fisher SG, Peterson DR, Haley JM, Nazar MD, Rich SA, Berlau DJ, Peltz CB, Tan MT, Kawas CH, and Federoff HJ. Plasma phospholipids identify antecedent memory impairment in older adults. *Nat Med*. 2014;20:415-8.

Mapstone M, Lin F, Nalls MA, Cheema AK, Singleton AB, Fiandaca MS, and Federoff HJ. What success can teach us about failure: the plasma metabolome of older adults with superior memory and lessons for Alzheimer's disease. *Neurobiol Aging*. 2017;51:148-55.

Merrill DA, Siddarth P, Raji CA, Emerson ND, Rueda F, Ercoli LM, Miller KJ, Lavretsky H, Harris LM, Burggren AC, Bookheimer SY, Barrio JR, and Small GW. Modifiable risk factors and brain positron emission tomography measures of amyloid and tau in nondemented adults with memory complaints. *Am J Geriatr Psychiatry*. 2016;24:729-37.

Miatto O, Gonzalez RG, Buonanno F, and Growdon JH. In vitro 31P NMR spectroscopy detects altered phospholipid metabolism in Alzheimer's disease. *Can J Neurol Sci*. 1986;13:535-9.

Morris MC. The role of nutrition in Alzheimer's disease: epidemiological evidence. *Eur J Neurol*. 2009;16 Suppl 1:1-7.

Morris MC, Tangney CC, Wang Y, Sacks FM, Bennett DA, and Aggarwal NT. MIND diet associated with reduced incidence of Alzheimer's disease. *Alzheimers Dement*. 2015;11:1007-14.

Mulder C, Wahlund LO, Teerlink T, Blomberg M, Veerhuis R, van Kamp GJ, Scheltens P, and Scheffer PG. Decreased lysophosphatidylcholine/phosphatidylcholine ratio in cerebrospinal fluid in Alzheimer's disease. *J Neural Transm*. 2003;110:949-55.

Mulder M, Ravid R, Swaab DF, de Kloet ER, Haasdijk ED, Julk J, van der Boom JJ, and Havekes LM. Reduced levels of cholesterol, phospholipids, and fatty acids in cerebrospinal fluid of Alzheimer disease patients are not related to apolipoprotein E4. *Alzheimer Dis Assoc Disord*. 1998;12:198-203.

National Research Council. *Nutrient requirements of laboratory animals*. 4th revised ed. Washington: National Academic Press; 1995.

Naudi A, Cabre R, Jove M, Ayala V, Gonzalo H, Portero-Otin M, Ferrer I, and Pamplona R. Lipidomics of human brain aging and Alzheimer's disease pathology. *Int Rev Neurobiol*. 2015;122:133-89.

Ngandu T, Lehtisalo J, Solomon A, Levalahti E, Ahtiluoto S, Antikainen R, Backman L, Hanninen T, Jula A, Laatikainen T, Lindstrom J, Mangialasche F, Paajanen T, Pajala S, Peltonen M, Rauramaa R, Stigsdotter-Neely A, Strandberg T, Tuomilehto J, Soininen H, and Kivipelto M. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. *Lancet*. 2015;385:2255-63.

Nitsch RM, Blusztajn JK, Pittas AG, Slack BE, Growdon JH, and Wurtman RJ. Evidence for a membrane defect in Alzheimer disease brain. *Proc Natl Acad Sci U S A*. 1992;89:1671-5.

O'Doherty PJ, Kakis G, and Kuksis A. Role of luminal lecithin in intestinal fat absorption. *Lipids*. 1973;8:249-55.

Olazaran J, Gil-de-Gomez L, Rodriguez-Martin A, Valenti-Soler M, Frades-Payo B, Marin-Munoz J, Antunez C, Frank-Garcia A, Acedo-Jimenez C, Morlan-Gracia L, Petidier-Torregrossa R, Guisasola MC, Bermejo-Pareja F, Sanchez-Ferro A, Perez-Martinez DA, Manzano-Palomo S, Farquhar R, Rabano A, and Calero M. A blood-based, 7-metabolite signature for the early diagnosis of Alzheimer's disease. *J Alzheimers Dis*. 2015;45:1157-73.

Olde Rikkert MG, Verhey FR, Sijben JW, Bouwman FH, Dautzenberg PL, Lansink M, Sipers WM, van Asselt DZ, van Hees AM, Stevens M, Vellas B, and Scheltens P. Differences in nutritional status between very mild Alzheimer's disease patients and healthy controls. *J Alzheimers Dis*. 2014;41:261-71.

Oresic M, Hyotylainen T, Herukka SK, Sysi-Aho M, Mattila I, Seppanan-Laakso T, Julkunen V, Gopalacharyulu PV, Hallikainen M, Koikkalainen J, Kivipelto M, Helisalmi S, Lotjonen J, and Soininen H. Metabolome in progression to Alzheimer's disease. *Transl Psychiatry*. 2011;1:e57.

Oulhaj A, Jerneren F, Refsum H, Smith AD, and de Jager CA. Omega-3 fatty acid status enhances the prevention of cognitive decline by B vitamins in mild cognitive impairment. *J Alzheimers Dis*. 2016;50:547-57.

Pallier PN, Poddighe L, Zbarsky V, Kostusiak M, Choudhury R, Hart T, Burguillos MA, Musbahi O, Groenendijk M, Sijben JW, deWilde MC, Quartu M, Priestley JV, and Michael-Titus AT. A nutrient combination designed to enhance synapse formation and function improves outcome in experimental spinal cord injury. *Neurobiol Dis*. 2015;82:504-15.

Perez-Pardo P, de Jong EM, Broersen LM, van Wijk N, Attali A, Garssen J, and Kraneveld AD. Promising effects of neurorestorative diets on motor, cognitive, and gastrointestinal dysfunction after symptom development in a mouse model of Parkinson's disease. *Front Aging Neurosci*. 2017;9:57.

Pettegrew JW, Panchalingam K, Hamilton RL, and McClure RJ. Brain membrane phospholipid alterations in Alzheimer's disease. *Neurochem Res*. 2001;26:771-82.

Prasad MR, Lovell MA, Yatin M, Dhillon H, and Markesbery WR. Regional membrane phospholipid alterations in Alzheimer's disease. *Neurochem Res*. 1998;23:81-8.

Proitsi P, Kim M, Whiley L, Simmons A, Sattlecker M, Velayudhan L, Lupton MK, Soininen H, Kloszewska I, Mecocci P, Tsolaki M, Vellas B, Lovestone S, Powell JF, Dobson RJ, and Legido-Quigley C. Association of blood lipids with Alzheimer's disease: A comprehensive lipidomics analysis. *Alzheimers Dement*. 2017;13:140-51.

Rapoport SI, Chang MC, and Spector AA. Delivery and turnover of plasma-derived essential PUFAs in mammalian brain. *J Lipid Res*. 2001;42:678-85.

Reeves PG, Nielsen FH, and Fahey GC, Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr*. 1993;123:1939-51.

Rijpma A, van der Graaf M, Lansbergen MM, Meulenbroek O, Cetinyurek-Yavuz A, Sijben JW, Heerschap A, and Olde Rikkert MGM. The medical food Souvenaid affects brain phospholipid metabolism in mild Alzheimer's disease: results from a randomized controlled trial. *Alzheimers Res Ther*. 2017;9:51.

Ross BM, Moszczynska A, Blusztajn JK, Sherwin A, Lozano A, and Kish SJ. Phospholipid biosynthetic enzymes in human brain. *Lipids*. 1997;32:351-8.

Ross BM, Moszczynska A, Erlich J, and Kish SJ. Phospholipid-metabolizing enzymes in Alzheimer's disease: increased lysophospholipid acyltransferase activity and decreased phospholipase A2 activity. *J Neurochem*. 1998;70:786-93.

Sakamoto T, Cansev M, and Wurtman RJ. Oral supplementation with docosahexaenoic acid and uridine-5'-monophosphate increases dendritic spine density in adult gerbil hippocampus. *Brain Res*. 2007;1182:50-9.

Savelkoul PJ, Janickova H, Kuipers AA, Hageman RJ, Kamphuis PJ, Dolezal V, and Broersen LM. A specific multi-nutrient formulation enhances M1 muscarinic acetylcholine receptor responses in vitro. *J Neurochem*. 2012;120:631-40.

Selley ML. A metabolic link between S-adenosylhomocysteine and polyunsaturated fatty acid metabolism in Alzheimer's disease. *Neurobiol Aging*. 2007;28:1834-9.

Shahdat H, Hashimoto M, Shimada T, and Shido O. Synaptic plasma membrane-bound acetylcholinesterase activity is not affected by docosahexaenoic acid-induced decrease in membrane order. *Life Sci*. 2004;74:3009-24.

Shatenstein B, Kergoat MJ, and Reid I. Poor nutrient intakes during 1-year follow-up with community-dwelling older adults with early-stage Alzheimer dementia compared to cognitively intact matched controls. *J Am Diet Assoc*. 2007;107:2091-9.

Silveri MM, Dikan J, Ross AJ, Jensen JE, Kamiya T, Kawada Y, Renshaw PF, and Yurgelun-Todd DA. Citicoline enhances frontal lobe bioenergetics as measured by phosphorus magnetic resonance spectroscopy. *NMR Biomed*. 2008;21:1066-75.

Toledo JB, Arnold M, Kastenmuller G, Chang R, Baillie RA, Han X, Thambisetty M, Tenenbaum JD, Suhre K, Thompson JW, John-Williams LS, MahmoudianDehkordi S, Rotroff DM, Jack JR, Motsinger-Reif A, Risacher SL, Blach C, Lucas JE, Massaro T, Louie G, Zhu H, Dallmann G, Klavins K, Koal T, Kim S, Nho K, Shen L, Casanova R, Varma S, Legido-Quigley C, Moseley MA, Zhu K, Henrion MYR, van der Lee SJ, Harms AC, Demirkan A, Hankemeier T, van Duijn CM, Trojanowski JQ, Shaw LM, Saykin AJ, Weiner MW, Doraiswamy PM, Kaddurah-Daouk R, Alzheimer's Disease Neuroimaging I, and the Alzheimer Disease Metabolomics C. Metabolic network failures in Alzheimer's disease: A biochemical road map. *Alzheimers Dement*. 2017;13:965-84.

Twine NA, Janitz K, Wilkins MR, and Janitz M. Whole transcriptome sequencing reveals gene expression and splicing differences in brain regions affected by Alzheimer's disease. *PLoS One*. 2011;6:e16266.

Valls-Pedret C, Sala-Vila A, Serra-Mir M, Corella D, de la Torre R, Martinez-Gonzalez MA, Martinez-Lapiscina EH, Fito M, Perez-Heras A, Salas-Salvado J, Estruch R, and Ros E. Mediterranean diet and age-Related cognitive decline: A randomized clinical trial. *JAMA Intern Med*. 2015;175:1094-103.

van Dam F and van Gool WA. Hyperhomocysteinemia and Alzheimer's disease: A systematic review. *Arch Gerontol Geriatr*. 2009;48:425-30.

van de Rest O, Berendsen AA, Haveman-Nies A, and de Groot LC. Dietary patterns, cognitive decline, and dementia: a systematic review. *Adv Nutr*. 2015;6:154-68.

Vandewoude M, Barberger-Gateau P, Cederholm T, Mecocci P, Salvà A, Sergi G, Topinkova E, and Van Asselt D. Healthy brain ageing and cognition: Nutritional factors. *Eur Geriatr Med*. 2016;7:77-85.

Volkert D, Chourdakis M, Faxen-Irving G, Fruhwald T, Landi F, Suominen MH, Vandewoude M, Wirth R, and Schneider SM. ESPEN guidelines on nutrition in dementia. *Clin Nutr*. 2015;34:1052-73.

Watkins SM, Zhu X, and Zeisel SH. Phosphatidylethanolamine-N-methyltransferase activity and dietary choline regulate liver-plasma lipid flux and essential fatty acid metabolism in mice. *J Nutr*. 2003;133:3386-91.

Wells K, Farooqui AA, Liss L, and Horrocks LA. Neural membrane phospholipids in Alzheimer disease. *Neurochem Res*. 1995;20:1329-33.

Werner A, Havinga R, Perton F, Kuipers F, and Verkade HJ. Lymphatic chylomicron size is inversely related to biliary phospholipid secretion in mice. *Am J Physiol Gastrointest Liver Physiol*. 2006;290:G1177-85.

Whiley L, Sen A, Heaton J, Proitsi P, Garcia-Gomez D, Leung R, Smith N, Thambisetty M, Kloszewska I, Mecocci P, Soininen H, Tsolaki M, Vellas B, Lovestone S, Legido-Quigley C, and AddNeuroMed C. Evidence of altered phosphatidylcholine metabolism in Alzheimer's disease. *Neurobiol Aging*. 2014;35:271-8.

Wiesmann M, Jansen D, Zerbi V, Broersen LM, Garthe A, and Kiliaan AJ. Improved spatial learning strategy and memory in aged Alzheimer AbetaPPswe/PS1dE9 mice on a multi-nutrient diet. *J Alzheimers Dis*. 2013;37:233-45.

Wiesmann M, Zerbi V, Jansen D, Haast R, Lutjohann D, Broersen LM, Heerschap A, and Kiliaan AJ. A dietary treatment improves cerebral blood flow and brain connectivity in aging apoE4 mice. *Neural Plast*. 2016;2016:6846721.

Wiesmann M, Zinnhardt B, Reinhardt D, Eligehausen S, Wachsmuth L, Hermann S, Dederen PJ, Hellwich M, Kuhlmann MT, Broersen LM, Heerschap A, Jacobs AH, and Kiliaan AJ. A specific dietary intervention to restore brain structure and function after ischemic stroke. *Theranostics*. 2017;7:493-512.

Wood PL, Mankidy R, Ritchie S, Heath D, Wood JA, Flax J, and Goodenowe DB. Circulating plasmalogen levels and Alzheimer Disease Assessment Scale-Cognitive scores in Alzheimer patients. *J Psychiatry Neurosci*. 2010;35:59-62.

Wood PL, Barnette BL, Kaye JA, Quinn JF, and Woltjer RL. Non-targeted lipidomics of CSF and frontal cortex grey and white matter in control, mild cognitive impairment, and Alzheimer's disease subjects. *Acta Neuropsychiatr*. 2015;27:270-8.

Wurtman RJ, Ulus IH, Cansev M, Watkins CJ, Wang L, and Marzloff G. Synaptic proteins and phospholipids are increased in gerbil brain by administering uridine plus docosahexaenoic acid orally. *Brain Res*. 2006;1088:83-92.

Wurtman RJ, Cansev M, Sakamoto T, and Ulus IH. Use of phosphatide precursors to promote synaptogenesis. *Annu Rev Nutr*. 2009;29:59-87.

Yan J, Wang W, Gregory JF, 3rd, Malysheva O, Brenna JT, Stabler SP, Allen RH, and Caudill MA. MTHFR C677T genotype influences the isotopic enrichment of one-carbon metabolites in folate-compromised men consuming d9-choline. *Am J Clin Nutr*. 2011;93:348-55.

Yuki D, Sugiura Y, Zaima N, Akatsu H, Takei S, Yao I, Maesako M, Kinoshita A, Yamamoto T, Kon R, Sugiyama K, and Setou M. DHA-PC and PSD-95 decrease after loss of synaptophysin and before neuronal loss in patients with Alzheimer's disease. *Sci Rep*. 2014;4:7130.

Zeisel SH and Blusztajn JK. Choline and human nutrition. *Annu Rev Nutr*. 1994;14:269-96.

Zerbi V, Jansen D, Wiesmann M, Fang X, Broersen LM, Veltien A, Heerschap A, and Kiliaan AJ. Multinutrient diets improve cerebral perfusion and neuroprotection in a murine model of Alzheimer's disease. *Neurobiol Aging*. 2014;35:600-13.