General introduction and thesis outline
Chapter 1

Introduction

Alzheimer’s disease (AD) is as yet incurable, the precise etiology of the disease is still unclear, and the drug development pipeline has a dramatically high rate of failure. Without intervention, AD prevalence will triple the coming 30 years (Alzheimer’s Disease International 2015). Hence, new therapies or preventative strategies are needed to address this vast public health challenge. Fortunately, many research efforts are still made to find effective treatments or ways to prevent AD. Mounting evidence suggests a role for nutritional intervention in both prevention and management of AD. The present thesis focuses on how specific nutrients and their combinations can affect aspects of AD pathophysiology, such as synapse loss.

AD is a multifactorial neurodegenerative disorder involving genetic and environmental factors. Several interacting processes contribute to the neurodegenerative process, including abnormal protein processing and neuronal membrane degeneration (Ballard et al. 2011, Pereira et al. 2005). Pathophysiological processes of AD may start decades before the clinical diagnosis of dementia (Sperling et al. 2011). Hallmarks of AD include protein accumulations of amyloid-β (Aβ) in extracellular plaques and hyperphosphorylated tau in intracellular neurofibrillary tangles, probably resulting in a continuous loss of neurons (Ballard et al. 2011, Pereira et al. 2005). Aβ aggregation, into oligomers or plaques, is considered an upstream event in the cascade that leads to synaptic loss and synaptic dysfunction (Haass and Selkoe 2007, Koffie et al. 2011, Selkoe 2002, Sperling et al. 2011). AD-related synapse loss progresses during the course of the disease, with estimations of 15 to 40% loss of synapses (de Wilde et al. 2016). This progressive loss leads to an advancing impairment of brain performance and worsening of clinical symptoms (Arendt 2009, Selkoe 2002, Terry 2006). Degenerating neurons and synapses are primarily located within the regions with projections to or from areas that display high densities of plaques and tangles. Various neurotransmitter systems are affected, including the cholinergic system. The loss of cholinergic neurotransmission in several brain areas is considered to contribute to cognitive impairment and behavioral symptoms (Blennow et al. 2006, Francis et al. 1999, Kar et al. 2004).

Currently there is no cure for AD. The pharmacological treatment options for AD that are available do not affect the underlying disease process, but offer modest symptomatic relief. Introduced in the late ‘90s and early 2000s, acetylcholinesterase inhibitors (AChEI) and one N-methyl-D-aspartate (NMDA) receptor antagonist are currently the mainstays of
AD pharmacotherapy (Lleo et al. 2006, Schneider 2013). The AD drug development pipeline is relatively narrow in terms of number of agents and potential targets that are under investigation and the overall success rate for past AD trials is less than 1% (Cummings et al. 2016). The next 3 to 5 years will be decisive for whether or not targeting amyloid is a viable disease modifying approach, since by then definitive phase III trial results will be available for still promising agents, such as the monoclonal antibodies aducanumab (Sevigny et al. 2016) and gantenerumab (Lasser et al. 2015), and the beta-secretase inhibitor verubecestat (Kennedy et al. 2016).

The primary risk factors for late-onset AD are age, a family history of AD, female gender, and genetic susceptibility, such as with the ApoE4 genotype. Other risk factors include, occurrence of traumatic brain injury, obesity, hypertension, smoking, and diabetes, whereas a healthy diet, physical activity, and intellectual and social activity are known protective factors (Anstey et al. 2015, Baumgart et al. 2015, Reitz and Mayeux 2014). To a certain extent these factors can be modified by medical interventions or by behavioral adaptations. It was estimated that up to half of AD cases could be attributable to modifiable risk factors (Barnes and Yaffe 2011). This provides leads for primary prevention strategies and also for secondary prevention strategies, i.e. interventions to prevent disease onset or delay disease progression (Solomon et al. 2014).

Diet is increasingly recognized as an important factor in the etiology and progression of AD (Gustafson et al. 2015, Vandewoude et al. 2016). Epidemiological studies have suggested that specific macro- and micronutrients affect the decline of cognitive function and risk of developing AD (Reitz and Mayeux 2014, Scarmeas et al. 2009). Furthermore, studies indicated that the influence of nutrition is associated with dietary patterns as a whole rather than the intake of specific individual nutrients (Cao et al. 2016, Lourida et al. 2013, Morris et al. 2015, Shakersain et al. 2016, van de Rest et al. 2015). In fact, most trials of single-nutrient interventions in AD have not been successful (e.g. Burckhardt et al. 2016, Farina et al. 2012, Li et al. 2014), presumably because efficacy requires the combined availability of various nutritional components (Gustafson et al. 2015). For example, in elderly with mild cognitive impairment (MCI), a 40% reduction in brain atrophy after 2 years of oral supplementation with folic acid, vitamin B6 and B12, was only observed in subjects with high plasma omega-3 fatty acids levels (Jerneren et al. 2015). This suggests that there are indispensable interactions between nutritional components. The necessity
of multiple nutritional compounds to be present in sufficient quantities might have masked potential protective effects in previous single-nutrient intervention studies.

Dietary composition can affect normal function and structure of the brain via various mechanisms (Bourre 2006a, Bourre 2006b, Gomez-Pinilla and Tyagi 2013). Nutrients can influence neuronal functions by regulating neurotransmitter synthesis, synaptic transmission, membrane fluidity, and signal-transduction pathways. For example, docosahexaenoic acid (DHA), which is present in high concentrations in retinal and neuronal membranes, influences membrane fluidity and, therefore, membrane-related processes such as neurotransmission. Choline is a precursor for acetylcholine and phosphatidylcholine, while the amino acids tryptophan and tyrosine are precursors for the neurotransmitters serotonin and catecholamines, respectively. In particular, neurons require specific nutrients for the formation and maintenance of their membranes. Phospholipids are the main constituents of membranes and also of neuronal membrane structures, like the pre- and post-synaptic membranes of synapses. As will be described in this thesis, synaptic membrane synthesis depends on the availability of several nutrients, for example, DHA, choline, and uridine, which fuel the metabolic pathways for the formation of constituent phospholipids.

In AD, degeneration of neuronal membranes and depletion of membrane phospholipids or changes in membrane phospholipid composition have been linked to synapse loss (Bennett et al. 2013, Kosicek and Hecimovic 2013, Naudi et al. 2015). Hence, membrane-related pathology and synapse loss play a central role in the pathogenesis of AD, and consequently provide viable interventional targets. On the one hand, insufficient availability of certain nutrients hypothetically limits, among other processes, membrane formation and could contribute to synaptic dysfunction. On the other hand, the need for and utilization of specific nutrients for synaptic membrane and synapse formation might be higher to compensate for the increased loss of synapses in AD. Hence, AD patients may have disease-specific nutritional requirements that are associated with pathological processes. This nutritional need may be addressed by specific dietary management which may be most effective when applied to earlier stages of AD, before synaptic pathological changes have accumulated to an irreversible degree.
Aims of this thesis
The overall aim of this thesis was to gain more insight into the possible AD-specific nutritional need to counter synapse loss and to reduce membrane-related pathology. To this end, the following was investigated:

- the mechanism of action and effectiveness of combinations of nutrients to increase markers of synaptic membrane formation and synaptic functioning in preclinical in vivo models
- the nutritional status of AD and MCI patients in a cross-sectional study
- plasma phospholipid levels of AD patients after nutritional intervention in a randomized controlled trial
- nutritional approaches in the risk reduction and management of AD

Thesis outline
Chapter 2 of this thesis is a review on how nutrients and their specific combinations affect the synthesis and composition of synaptic membranes, leading to improved membrane-dependent processes of potential relevance for AD patients. It provides a comprehensive overview of basic scientific studies that led to the creation of a specific nutrient combination Fortasyn Connect (Nutricia N.V., Zoetermeer, the Netherlands).

In chapters 3, 4, and 5 the results of studies in rats are presented demonstrating that dietary supplementation of B-vitamins and lecithin increases the systemic availability of nutritional phospholipid precursors, i.e. DHA and choline. These studies indicate that combined intake of B-vitamins, lecithin, DHA, and choline would be more effective in increasing DHA and choline availability than intake of DHA and choline alone.

Chapter 6 describes the results of a study in rats showing that the increase in synaptic membrane formation induced by phospholipid precursors depends on adequate intake of vitamin C, vitamin E, and selenium. This study illustrates dependence and/or synergy between nutrients and that combined supplementation is needed for their effectiveness.

Chapter 7 describes a study in aged rats in which dietary supplementation of the nutrient combination Fortasyn Connect enhanced hippocampal cholinergic neurotransmission which was paralleled by increased synthesis of synaptic membranes. This study provides
further insight into cellular and molecular mechanisms by which combined supplementation of nutrients can affect synapse formation.

Chapter 8 is a review in which nutritional approaches for the risk reduction and management of AD patients are discussed. Several factors are proposed to contribute to a disease-specific nutritional requirement in AD, including compromised nutrient intake, uptake, metabolism, and utilization.

Chapter 9 describes the results of a cross-sectional study in MCI, AD, and control subjects and shows that compared with controls, patients with MCI and AD have lower blood and CSF concentrations of nutrients involved in phospholipid synthesis, i.e. uridine, folate, and choline. This study indicates putative nutritional deficits in both AD and MCI patients.

Chapter 10 presents results of a randomized clinical trial (Souvenir II trial) in which a 24-week nutritional intervention in AD patients significantly increased levels of plasma phosphatidylcholine species that were previously reported to be predictive for phenoconversion from cognitive normal aged adults to MCI or AD. These results indicate that a biomarker profile reflecting disturbed phospholipid metabolism in AD can be improved by providing nutrients needed for phospholipid synthesis.

In chapter 11, the main findings of this thesis are summarized and discussed, and a future perspective is provided.
**List of abbreviations**

AChEI, acetylcholinesterase inhibitor
AD, Alzheimer’s disease
Aβ, amyloid-β
DHA, docosahexaenoic acid
MCI, mild cognitive impairment
NMDA, N-methyl-D-aspartate
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