Nutrition and metabolic profiles in Alzheimer's disease

de Leeuw, F.A.

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Chapter 5

Summary and general discussion

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In this thesis, we studied nutritional and metabolic determinants of Alzheimer's disease (AD) in brain, cerebrospinal fluid (CSF) and blood. We found that brain vitamin E levels were associated with lower activated microglia density and higher presynaptic protein levels in post-mortem brain tissue. In addition, we showed that plasma metabolites, including amino acids, lipoproteins and oxidative stress compounds, were associated with AD-type dementia diagnosis and imaging markers of AD. Finally, we found modest associations between nutritional biomarkers and clinical progression in a memory-clinic setting.

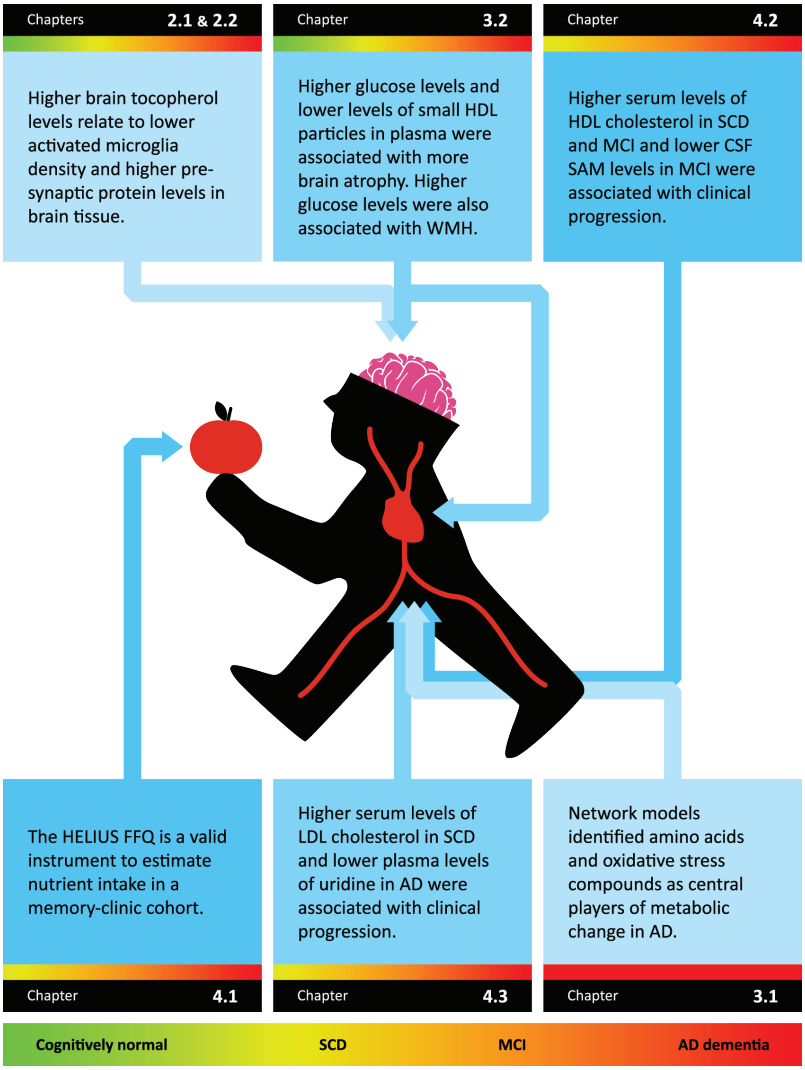

Figure 1 A summary of the key findings in this thesis (Illustration by Daan Janssen).

Summary

Nutrition and neuropathology

The aim of **chapter 2** was to gain more insight into the possible mechanisms underlying the relation between AD and vitamin E. In a group of 113 autopsied elderly we first investigated associations between brain α- and γ- tocopherol (vitamin E) levels and (activated) microglia density (**chapter 2.1**). We found that higher brain levels of α- and γ-tocopherol were associated with lower total and activated microglia density in cortical, but not in subcortical brain regions. The associations of cortical α-tocopherol and total microglia density remained significant after adjusting for amyloid load and neurofibrillary tangle severity. Secondly, we examined associations between brain α- and γ- tocopherol levels and presynaptic protein levels in post-mortem brain tissue (**chapter 2.2**). Eight distinct presynaptic proteins and the protein-protein interaction between synaptosomal-associated protein 25 (SNAP-25) and syntaxin were measured in two cortical brain regions. Composite scores were calculated for 1) three presynaptic proteins, all part of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex and 2) for synaptophysin and synaptotagmin. Higher brain γ-tocopherol levels were associated with higher levels of the SNARE protein composite, complexin-I, complexin-II, the synaptotagmin synaptophysin composite and septin-5 in the midfrontal cortex. These associations were independent of global Alzheimer's disease pathology, cerebral infarcts and Lewy body disease pathology. Our findings support the biological hypotheses that the anti-inflammatory and anti-oxidative properties of vitamin E might alleviate microglia activation and prevent presynaptic protein oxidation.

Metabolic profiles of Alzheimer's disease

Chapter 3 focused on metabolic determinants of clinical and imaging outcomes in AD. In **chapter 3.1**, we investigated plasma metabolite levels of 127 patients with AD dementia and 121 controls. We used mass spectrometry (MS) platforms to determine the plasma concentrations of 53 amine compounds, 22 organic acid compounds, 120 lipid compounds, and 40 oxidative stress compounds. Differential expression analysis showed lower levels of amino acids and triglycerides in AD in comparison to controls. Moreover, extracted network models of metabolites identified five hubs with a central role in metabolic dysregulation: tyrosine, glycylglycine, glutamine and the oxidative stress compounds lysophophatic acid C18:2 and platelet-activating factor C16:0. Stratified for apolipoprotein E (APOE) ε4 genotype, APOE ε4 negative AD patients had a more random and less cohesive network compared to APOE ε4 positive AD patients. Next, we aimed to investigate associations between plasma metabolites and imaging markers to gain more insight in biological pathways

between systemic metabolites and AD pathology (**chapter 3.2**). We studied associations of 143 plasma-based metabolites with imaging markers, using magnetic resonance imaging measures of brain and hippocampal atrophy and white matter hyperintensities (WMH) in almost 4000 participants from three independent cohort studies. Plasma metabolites were measured on a nuclear magnetic resonance (NMR) platform that allows simultaneous quantification of routine lipids, lipoprotein subclasses, fatty acids, amino acids, ketone bodies and gluconeogenesis-related metabolites [1]. In a meta-analysis, lower levels of three small high- density lipoprotein (HDL) particles and higher levels of glucose were associated with brain atrophy. In addition, higher glucose levels were also associated with more severe WMH. Hence, we discovered various metabolites that might play a role in AD, either as accelerating factor or as an early consequence of disease progression.

Nutritional determinants of Alzheimer's disease progression

In **chapter 4** we investigated the role of nutritional biomarkers in a memory-clinic setting. Firstly, we aimed to validate the HELIUS food frequency questionnaire (FFQ) in a memory-clinic cohort of patients with mild cognitive impairment (MCI), patients with AD dementia, and controls. (**chapter 4.1**). We found moderate associations between nutrient intake assessed by FFQ and nutrient status measured by nutritional biomarkers in blood. These associations were largely similar across diagnosis groups. Our findings suggest that the HELIUS FFQ provides valid estimates of nutrient intake in a memory-clinic cohort, similar to the general elderly population. Subsequently, we aimed to examine associations between nutritional biomarkers and clinical progression. In **chapter 4.2**, we investigated associations of 33 nutritional biomarkers in blood and/or CSF with clinical progression in a retrospective cohort of patients with subjective cognitive decline (SCD) and MCI. We found that in the total cohort, higher HDL cholesterol levels were associated with clinical progression and cognitive decline. In MCI, low CSF S-adenosylmethionine (SAM) and high theobromine were associated with clinical progression to dementia. In addition, we used a data-driven, integrative approach to study associations of nutritional biomarker profiles with clinical progression. We identified one profile for SCD and two profiles for MCI, which were both associated with clinical progression. In **chapter 4.3**, we expanded upon this work and measured 13 out of the 33 previously measured nutritional biomarkers in a prospective cohort of SCD, MCI and AD patients. Higher low-density lipoprotein (LDL) cholesterol levels in SCD and lower uridine levels in AD were associated with clinical progression. In addition, in a combined analysis of predementia stages in our prospective and retrospective cohort (**chapter 4.2** and **4.3**), higher HDL cholesterol levels and lower CSF SAM and S-adenosylhomocysteine levels in MCI were associated with clinical progression. Our work built on previous research in population-

based studies on the role of nutritional biomarkers, and showed that nutritional biomarkers are modestly associated with clinical progression in a memory-clinic setting.

Methodological considerations

Toward reproducible results in metabolomics

A large part of this thesis focused on the identification of metabolic determinants in AD. AD is biologically defined by accumulation of amyloid and tau in the brain, but the multiple biological processes leading to AD are complex [2]. Metabolomics studies can be used to investigate these biological processes, but have been challenged by reproducibility problems [3, 4]. A first step towards reproducible results will be the standardization of pre-analytical factors. This requires investigation of the effects of pre-analytical variation in storage procedures, processing steps and the blood product type (i.e. serum, plasma). Great efforts have been made towards the standardization of amyloid and tau measurements in CSF. Quality control programs that started in the CSF field may now be used to develop processing guidelines for blood biomarkers [5]. Secondly, metabolic change in AD is not likely to be a single metabolite defect. but involves many metabolic pathways. High throughput platforms (like those used in **chapter 3.1** and **3.2**) use NMR spectroscopy or MS for the rapid quantification of many metabolites, with limited sample preparation and at low costs. This is attractive but also results in the quantification of relatively unknown compounds. For example, in **chapter 3.2** we used an NMR platform that quantifies several lipid subclasses [1]. Current knowledge on the biological functions of lipid subclasses is, however, limited, which makes the interpretation of complex epidemiological findings difficult. In conclusion, we need standardized pre-analytical and analytical approaches and large-scale metabolomics studies to get repeatable and reliable metabolomics findings across populations. Initiatives such as the Alzheimer's Disease Metabolomics Consortium and the BioBanking for Medical Research Infrastructure of the Netherlands (BBMRI-NL) Metabolomics Consortium are working on a standardized measurement and data-analysis of metabolomics across cohorts and will aid in this challenge for the metabolomics field.

Integrative approaches

Nutrition is often studied as single nutrients, while in real life nutrients are consumed as complete meals and it is likely that the combined effects of nutrients are what influences brain health. This is also supported by several studies, which suggest that it is more an overall healthy diet rather than single nutrients that benefit brain health [6, 7]. In this thesis we performed two different data-driven approaches, to get a better view of the combined effect of nutritional

factors. In **chapter 3.1,** we used graphical modelling to construct metabolic networks. Graphical modelling can help us understand the complex relationships between determinants, and has already been frequently used to study brain grey-matter networks [8, 9]. This approach aids the identification of key players in metabolic change. In graph theory these central players within a network of other metabolites are referred to as hubs [8]. Alterations of a hub will most likely result in global metabolic change within the network. However, since many internal and external factors can cause metabolic change, (biological) interpretation of metabolic network changes can be complex. Future studies should aim to investigate if metabolic network changes relate to AD pathology, AD risk factors or are a consequence of disease progression. In **chapter 4.2** we used a different integrative approach, partial least square cox regression[10]. This statistical technique combines nutritional biomarkers that show a similar association with the outcome. While our approach in **chapter 3.1** is primarily useful to get more insight into potential underlying mechanisms of metabolic change, the approach in **chapter 4.2** is more directly focused on the clinical question of which combination of nutritional biomarkers can predict clinical progression. Disadvantages of these integrative approaches is that validation of the models is not straightforward. Since methods of integrative approaches are relatively new and quickly evolving, it is difficult to compare studies. Replication of these models can also be expensive, as it often requires the measurement of all nutritional biomarkers used to develop the model in an independent validation cohort. This limitation also hampered validation of the nutritional biomarker profiles developed in **chapter 4.2** in our prospective validation cohort (**chapter 4.3**), where we only measured a subset of the nutritional biomarkers used to develop the nutritional biomarker profiles. In summary, integrative methods are conceivably useful to profile global metabolic change, but strict replication of integrative models remains a challenge. In **chapter 4.3,** we therefore focused on the validation of single nutritional biomarker findings.

Nutritional biomarkers for clinical progression in AD

In **chapter 4.2** and **4.3** we aimed to identify promising nutritional biomarkers associated with clinical progression in a retrospective cohort and subsequently validate this panel in a prospective cohort. Our findings in the retrospective cohort were heterogeneous and not easy to fit in with biological hypotheses on the role of nutrition in AD. Therefore, selection of a panel of promising markers for further validation was complex. When we measured the selected panel in the prospective cohort, most findings in the retrospective cohort could not be replicated, although trends suggested that higher homocysteine levels and lower CSF SAM levels in MCI were associated with clinical progression in both cohorts. One explanation for this lack of reproducible findings might be that we validated only a subset of nutritional biomarkers in the prospective cohort

(**chapter 4.3**). Another explanation could be that the statistical power to detect subtle associations between nutritional biomarkers and clinical progression was too low in our studies. The cohorts of both studies were large, but rates of clinical progression were relatively low, especially in SCD. To increase statistical power, we oversampled patients with clinical progression to MCI or dementia in our retrospective cohort (**chapter 4.2**). **Chapter 4.3** had a prospective design and the incidence of clinical progression to MCI or dementia was relatively low, but we were able to use a broader definition of clinical progression, including nursing home admission and death. Furthermore, interindividual variability for the relation between nutritional biomarkers may be high and a larger sample may be required to properly investigate the factors that could explain this variability. For example, genetic factors or medication use might change the relation between nutritional biomarkers and clinical progression and should be further investigated.

Food in relation to AD

The relation between food intake and AD is influenced by both biological and behavioral factors. Food intake is affected by age, gender, location, physical activity, education, occupation, social economic status and many more external factors [11-13]. This can confound the studied associations between food and AD. Furthermore, there is large interindividual and intraindividual variability in the relation between food intake and uptake. For example, genetic factors and gut microbiome composition can influence the uptake and metabolism of nutrients, but intake of one nutrient can also affect the uptake of another nutrient [14-16]. Moreover, nutrient transport from the blood to the brain is modulated by the blood-brain barrier [17]. Thus, food intake does not perfectly correspond with blood levels and blood levels are not necessarily correlated with brain levels. This is also illustrated by our findings in **chapter 4.1**, where we found moderate associations between nutritional intake and nutritional biomarker levels in blood, similar to the general population (r≈0.2-0.7). In addition, in **chapter 2.1,** brain tocopherol levels were only marginally associated with supplement intake (r≈0.2) and not with food intake of vitamin E. These moderate associations indicate that it is important to investigate relations between food and AD on the level of intake (**chapter 4.1**), blood levels (**chapter 3, 4.2** and **4.3**), and brain levels (**chapter 2**). Further insight into the biological pathways between food and AD might be gained from animal models that facilitate the detailed quantification of nutrients across body (fluid) compartments.

Nutrition in a memory-clinic population

Most research on the role of nutrition in AD has been performed in community-dwelling elderly or institutionalized patients. In this thesis we aimed to close the gap between healthy and frail elderly by investigating nutritional factors in the

memory-clinic population. To this end, we studied patients with SCD, MCI and AD-type dementia from the NUDAD ('Nutrition, the unrecognized determinant in Alzheimer's disease') project [18]. The advantage of using a memory-clinic cohort as study population is that these patients are at increased risk for cognitive decline, which means rates of clinical progression are considerably higher than in population-based studies. Moreover, NUDAD patients underwent standardized cognitive screening and follow-up, which enabled the precise measurement of clinical progression [19]. Finally, these study participants would be the ideal target population for future dietary interventions or guidelines, as they visit the memory-clinic looking for guidance on maintaining or improving their brain health.

In recent years, the AD research field has been undergoing a shift from a syndromal staging system towards a biological definition of AD. In 2018, the National Institute on Aging and the Alzheimer's Association proposed a research framework in which the core biomarkers of AD are grouped in three categories of amyloid (A), tau (T) and neurodegeneration (N) [2]. This ATN scheme is independent of cognitive status and provides a useful framework to study biological trajectories in AD. We made the first steps to study nutritional biomarkers in the context of this ATN framework. For example, in **chapter 4.3** we performed a sensitivity analysis in amyloid positives only, which is referred to as the Alzheimer's continuum in the ATN framework. We found that our findings for nutritional biomarkers and clinical progression remained comparable in amyloid positives. Nutrition might, however, not always be amyloid specific as it relates to many other factors that influence cognitive health. For instance, a recent study in the British 1946 Birth Cohort showed that mid-life vascular health related to brain atrophy and WMH in, but not to, amyloid status [20]. Further evaluation of nutrition research within this framework will help to interpret epidemiological findings and to differentiate between factors that relate specifically to AD or neurodegeneration in general.

In **chapter 4.2** and **4.3** we found modest associations between nutritional biomarkers and clinical progression. The neurodegenerative disease process of AD starts years before clinical symptoms occur [21, 22]. It could be that the critical time frame to detect associations between nutritional exposure and AD lies before clinical symptoms occur, or even before pathology starts to accumulate. Vascular disease in mid-life is strongly related to diet, and an important risk factor for dementia [23, 24]. Recently, it was suggested that the predictive value of vascular health for brain atrophy and WMH in late-life was strongest at 36 years of age [20]. If the role of other nutritional factors is age range specific, is less well known. This should be further investigated in large longitudinal studies that include the biomarker categories from the ATN framework. Our findings

in **chapter 4.2** and **4.3** suggested, however, that nutritional biomarkers might have different roles at specific clinical stages of AD. For example, higher LDL cholesterol levels were associated with clinical progression in SCD, but not in MCI and AD-type dementia. Moreover, lower uridine levels were only associated with clinical progression in AD-type dementia and not in patients with SCD and MCI. These findings could suggest that the critical time window, during which micronutrients influence brain health, might differ per individual nutrient. It could, for instance, be that the associations of lower uridine levels with increased risk of clinical progression only develop in relation to homeostatic changes that occur during the late, symptomatic, phase. Moreover, cholesterol levels have been reported to decline before the onset of dementia, which might explain why higher LDL cholesterol levels are a risk factor in SCD, but this is less clear in MCI and AD [25].

Clinical implications

Health benefits of nutrition have gained a lot of attention in recent years. This is partly explained by the widely available media platforms that may announce preliminary results as if these findings have direct implications for individuals. Curcuma, green tea, ginger and coconut oil are just a few examples of food items for which protective effects for AD have been suggested by cell or animal studies, but have never been proven in humans [26-29]. Another explanation is that a disease modifying or curative treatment for AD is still unavailable and patients are eager to know what they can do to retain cognitive function. Specific lifestyle recommendations for memory-clinic patients are, however, unavailable. Large population-based studies have suggested primary preventive effects of nutrition, but the role of nutrition in clinical stages of AD is unclear. In this thesis we found that various nutritional biomarker concentrations were associated with prevalent and incident (predementia) AD. Our findings were subtle and heterogeneous however,, precluding a direct translation to clinical recommendations. Therefore, it is important to notify memory-clinic patients that current knowledge of the influence of nutrition on slowing down clinical progression in AD is limited. Current findings might be specific for clinical stages of disease (SCD/MCI/AD dementia) or certain subgroups (e.g. according to APOE ε4 genotype or vascular risk) and generalizability of findings needs to be confirmed. We should therefore strive to counter unproven food theories and create realistic expectations on the influence of nutrition.

Nonetheless, our findings give support to some of the existing general dietary recommendations. We found that higher glucose levels were associated with structural brain changes in preclinical and predementia stages of AD (**chapter 3.2**). Moreover, higher LDL cholesterol levels were associated with clinical

progression in SCD (**chapter 4.3**). These metabolites are also related to a higher risk of cardiovascular disease. A general healthy diet (Dutch food pyramid 'Schijf van Vijf' [30]) has been shown to ameliorate cardiovascular health and might also lower risk for dementia [31]. These guidelines overlap with the cornerstones of the Mediterranean diet, the most widely investigated dietary pattern in the context of cardiovascular health and AD [32-35]. Clinicians should inform patients that a general healthy or Mediterranean-style diet is the best recommendation currently available to lower risk for dementia.

Future perspectives

The results of this thesis provide several suggestions for future research. Firstly, identification of biological mechanisms underlying the relation between nutrients and AD is an exciting research field. Our findings suggest that vitamin E might influence inflammation, oxidative stress and synapse loss in AD brain tissue. Monitoring the effects of nutrients on these processes will improve our understanding of the pathological mechanisms by which nutrients relate to AD. Of note, the (blood) biomarker field is making quick progress in the validation of inflammatory, oxidative stress and synaptic markers, which can be used as objective outcome measures [36]. For example, one could consider GFAP, which reflects glial activation, or isoprostanes that relate to oxidative stress [37, 38]. A first next step would be to implement these markers in observational nutritional studies to evaluate their relation with intake of specific food groups and nutritional biomarkers. Eventually, this could lead to the identification of targets for dietary intervention trials.

Another approach to deepen our understanding on the relation between nutrition and brain health is to study the role of the gut microbiome in AD. Although this is a relatively new research field, first reports have shown remarkable differences in gut microbiome between patients with AD dementia and controls [39, 40]. Biological hypotheses on the gut-brain axis have largely been based on mice studies. For example, high salt intake led to gut-derived inflammatory responses that induced hyperphosphorylation of tau in mice [41, 42]. In other mice studies, oligomannate (a seaweed derived drug) has been suggested to suppress AD-related gut microbiome changes, reduce peripheral and central inflammation and improve cognitive function [43]. Recently, oligomannate has been tested in a Chinese phase III clinical trial and has been approved as symptomatic treatment for mild to moderate AD in China [43]. Clinical trials in Europe and North America will start in 2020 to further investigate the potential of oligomannate as treatment for AD.

A next step towards optimization of dietary intervention trials would be the identification of patients eligible for such trials. Previous studies have shown that selection of participants at increased risk for dementia based on cardiovascular risk factors, amyloid pathology or APOE ε4 genotype, might result in a higher success rate of these trials [44-46]. Additional selection criteria based on suboptimal nutritional biomarker levels might further improve participant selection. A recent study proposed a nutritional risk index, based on suboptimal levels of omega-3 fatty acids, vitamin D and homocysteine, that identified patients at increased risk for cognitive decline [47]. This nutritional risk score should be studied further to define its value for clinical trials.

Finally, the key towards successful strategies to prevent or slow AD might lay in multidomain interventions that combine nutritional interventions with physical and social activities. In the future, these interventions could also include pharmaceutical treatments. The Finnish FINGER trial is a promising example of a multidomain intervention, including dietary advice, exercise, cognitive training and vascular risk monitoring, that showed beneficial effects on cognitive outcomes [46]. A worldwide network of FINGER-like trials is now following this example, including FINGER-NL which is intended to start in the coming months [48, 49].

Conclusion

Over the past decades we learned that the AD process is multifactorial and is likely to involve different causal pathways. This thesis provides insights into the potential contributions of nutrition and metabolic change. We found that nutritional biomarkers are associated with prevalent and incident AD. Nonetheless, pinpointing nutritional risk factors for (clinical progression of) AD remains challenging. Further research that investigates associations of nutritional markers with gut- and brain derived inflammatory and oxidative stress responses and synaptic dysfunction might eventually lead to nutritional risk indexes for AD that could be of clinical value.

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