Lifestyle and Environmental Factors in the Risk of Dementia

A population-based approach

Tosca O.E. de Crom

Cover design: Tosca O.E. de Crom & Erwin Timmerman
Lay-out: Tosca O.E. de Crom
Printing: Optima Grafische Communicatie, Rotterdam.

ISBN: 978-94-6361-984-4

Lifestyle and Environmental Factors in the Risk of Dementia
A population-based approach

Levensstijl en Omgevingsfactoren bij het Risico op Dementie
Een populatie-gebaseerde benadering

Proefschrift
ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof. dr. A.L. Bredenoord

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

dinsdag 4 juni 2024 om 15:30 uur

door

Tosca Oda Elisabeth de Crom
geboren te Bergen op Zoom.

Erasmus University Rotterdam

PROMOTIECOMMISSIE

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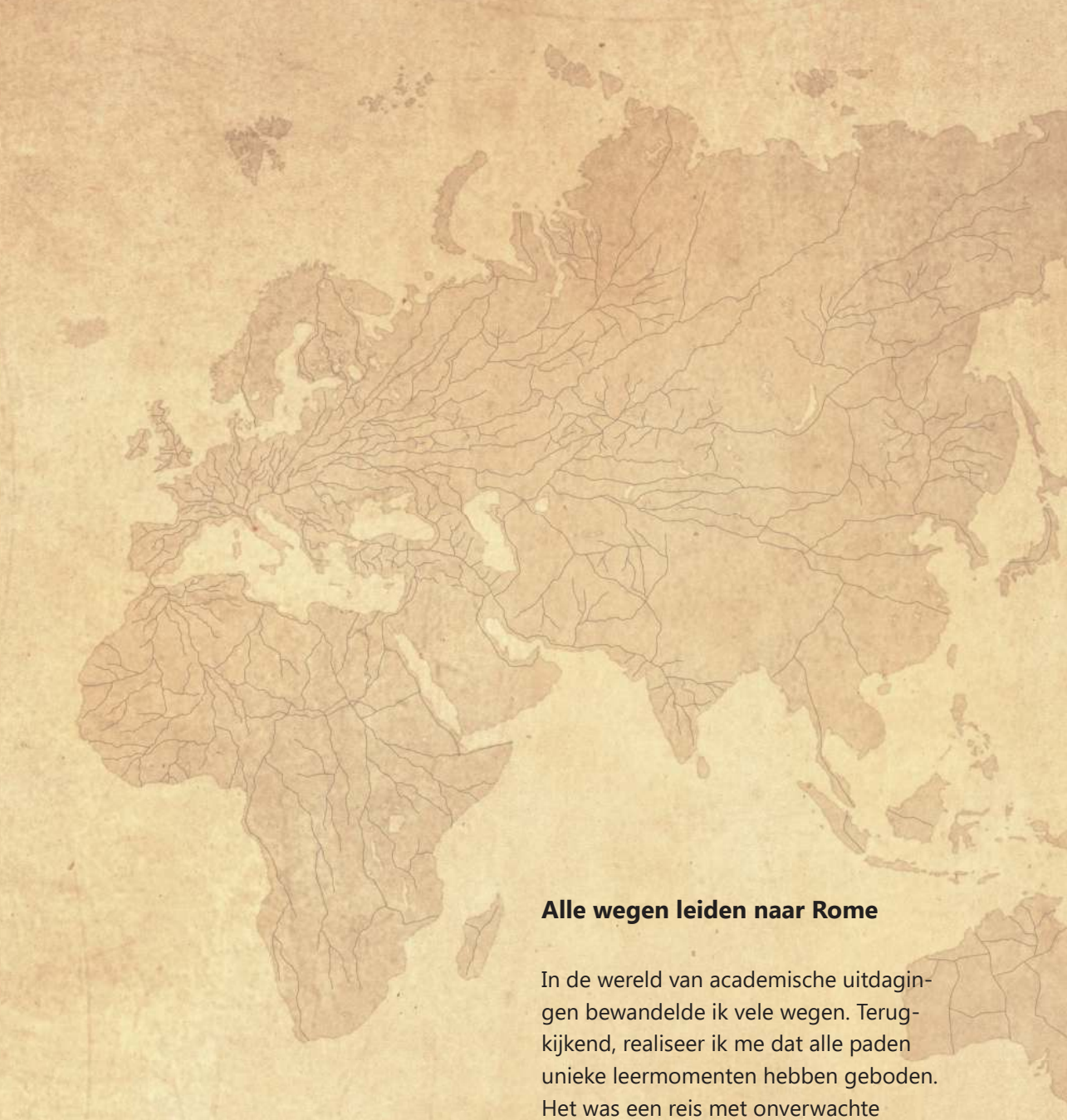
ACKNOWLEDGEMENTS

The work described in this PhD-thesis was conducted at the Department of Epidemiology of the Erasmus MC – University Medical Center Rotterdam, Rotterdam, the Netherlands. Studies described in this thesis use data from the Rotterdam Study, which is supported by: Erasmus Medical Centre and Erasmus University, Rotterdam; the Netherlands Organization for the Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE); the Ministry of Education, Culture and Science; the Ministry for Health, Welfare and Sports; the European Commission (DG XII); and the Municipality of Rotterdam. This thesis was performed as part of the ADAIR project (grant #JPND2019-466-037), an EU Joint Programme - Neurodegenerative Disease Research (JPND) project. The project is supported through the following funding organisations under the aegis of JPND - www.jpnd.eu (Ministry of Education, Youth and Sports, Czech Republic; Academy of Finland, Finland; National Research, Development and Innovation Office, Hungary; Ministry of Education, Universities and Research, Italy; ZonMW – The Netherlands Organisation for Health Research and Development, The Netherlands; Swedish Research Council, Sweden). None of the funding organizations were involved in the study design, data collection and analysis, writing of this thesis or the decision to submit chapters for publication.

Financial support by the Erasmus Medical Center Rotterdam, Erasmus University Rotterdam, Stichting Alzheimer Nederland, and Erasmus Trustfonds for publication of this thesis is gratefully acknowledged.



Alle wegen leiden naar Rome

In de wereld van academische uitdagingen bewandelde ik vele wegen. Terugkijkend, realiseer ik me dat alle paden unieke leermomenten hebben geboden. Het was een reis met onverwachte wendingen en uitdagingen die niet altijd even voorspelbaar waren. Door hard te werken heb ik veel obstakels overwonnen en kijk ik met trots terug op mijn afgeronde PhD. Het was geen eenvoudige reis, maar elke hobbel en omweg heeft bijgedragen aan mijn persoonlijke en professionele ontwikkeling.

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de Crom TOE, Mooldijk SS, Ikram MK, Ikram MA, Voortman T. MIND diet and the risk of dementia: a population-based study. *Alzheimer's Research & Therapy*. 2022;14(1):1-10.

de Crom TOE, Steur M, Ikram MK, Ikram MA, Voortman T. Plant-based dietary patterns and the risk of dementia: A population-based study. *Age and Ageing*. 2023;52(9):178.

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de Crom TOE*, Mooldijk SS*, Ikram MK, Ikram MA, Voortman T. Adiposity in the older population and the risk of dementia: The Rotterdam Study. *Alzheimer's & Dementia*. 2023;19(5):2047-2055.

de Crom TOE, Ghanbari M, Voortman T, Ikram MA. Body composition and plasma total-tau, neurofilament light chain, and amyloid-β: A population-based study. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*. 2023;16(1), e12519.

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[#]These second authors contributed equally to the respective manuscript


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In het complexe doolhof van het leven dient het kompas als een betrouwbare gids, altijd klaar om ons de juiste weg te wijzen. Het kompas symboliseert richting, oriëntatie en het vermogen om onze koers te bepalen. Maar wat als dit essentiële instrument plotseling begint te haperen?

Dementie, een genadeloze aandoening die herinneringen en identiteit langzaam wegneemt, wordt vaak vergeleken met het breken van dit innerlijke kompas. Het kompas dat ooit zo zeker leek, verliest zijn betrouwbaarheid en leidt niet langer naar bekende plekken...

Chapter 1

General introduction

GENERAL INTRODUCTION

The term dementia is derived from the Latin word "demens", which means "out of your mind" or "senseless". Although dementia was not recognized as a clinical disorder until the 18th century,[1] references to memory loss and cognitive decline accompanying the aging process were already found in ancient Egyptian, Greek and Roman texts.[2] In the Middle Ages, it was thought that dementia was caused by the possession of evil spirits and treatment options involved exorcism and other religious rituals.[3] In 1906, the German psychiatrist and neuropathologist Alois Alzheimer provided his first case report of a patient experiencing profound memory loss in the presence of senile plaques and neurofibrillary tangles in the brain.[4] This seminal work led to the recognition of what is now known as Alzheimer's disease, the most common cause of dementia.

Currently, over 55 million people are living with dementia worldwide.[5] Given the projected increase in population ageing and growth, the prevalence of dementia is predicted to increase rapidly in the coming years.[6] Accordingly, the already enormous physical, emotional and economic burden of dementia on patients, their family and society will increase further. This emphasizes the urgent need to prioritize exploring strategies to curb the dementia pandemic.

Over the past century, significant advancements have been made in understanding the complex etiology of dementia, but for many years, treatment options have been limited to symptom management.[7] Recent breakthroughs, however, have led to the discovery of putative disease-modifying therapies that potentially slows down the progression of dementia.[8, 9] As our understanding in mechanisms underlying dementia keeps expanding, researchers continuously strive to develop new therapeutic targets that can slow down or even reverse the progression of the disease. Yet, as Desiderius Erasmus wrote: "prevention is better than cure".

It is well known that dementia is largely determined by a combination of genetic, lifestyle, and environmental factors (**Figure 1**).[10, 11] While genetic factors contribute to a person's susceptibility to dementia, lifestyle and environmental factors play an important role in modulating its development and progression. The aim of this thesis is to study the role of lifestyle and environmental factors in the risk of dementia, with the ultimate goal to enhance our understanding in preventive opportunities against dementia. More specifically, I shall focus on the role of nutrition, which is possibly the most important lifestyle factor in the prevention of any chronic disease,[12] and air pollution, an increasing

public health problem. Furthermore, the focus shall be on the role of body composition, which is largely determined by lifestyle and potentially in part by environmental factors.[13, 14]

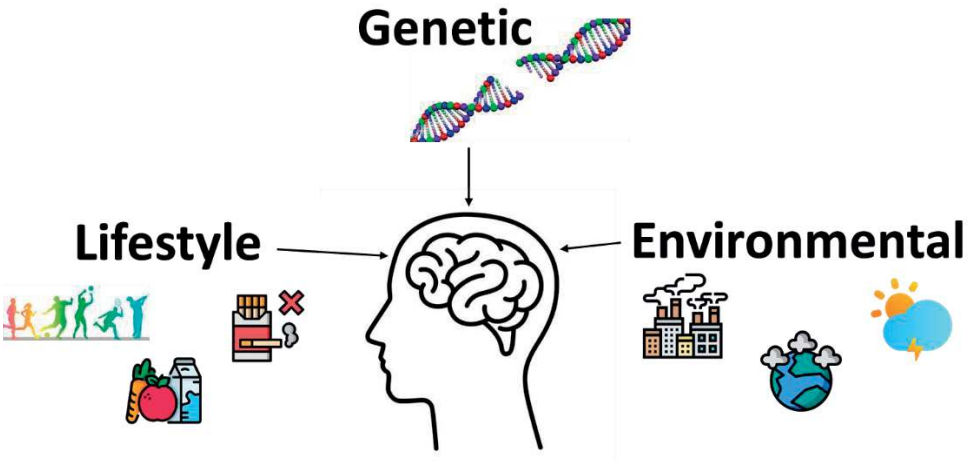

Figure 1. Overview of risk factors for dementia

Studies that form the basis of this thesis are all embedded within the framework of the Rotterdam Study. The Rotterdam Study, in Dutch known as the Erasmus Rotterdam Gezondheid Onderzoek (ERGO), is an ongoing prospective population-based cohort study designed to investigate the determinants and consequences of common diseases, including dementia.[15] The study was initiated in the year 1990 and currently comprises approximately 18,000 individuals aged 40 years and older, residing in the well-defined Ommoord district of Rotterdam. At study entry, participants underwent home interviews, followed by extensive clinical examinations at the research center. Every 3 to 6 years, participants are invited to undergo follow-up examinations and clinical outcomes are continuously monitored through a link with general practitioner records. This comprehensive cohort study embraces valuable data to determine the temporal relationships between potential risk factors and the incidence of dementia.

Chapter 2 – Nutrition

"Let food be thy medicine and medicine be thy food"

This famous quote is often attributed to Hippocrates, also known as the father of medicine. By this quote, he probably has been the first to acknowledge the importance of nutrition in health and disease. To date, many researchers and health professionals recognize a healthy diet as a cornerstone of overall health and a key factor in the prevention of several chronic diseases, including brain diseases.[16] Nevertheless, in 2020, the Lancet Commission

on Dementia Prevention, Intervention, and Care did not include dietary habits in its list of modifiable risk factors for dementia.[11] This omission is attributed to the largely inconclusive connection between nutrition and brain health, which is primarily due to the complex and multifactorial nature of nutrition.[17, 18] Further research is warranted to attain a more comprehensive understanding of the modulating effect of nutrition in the development of dementia.

In **Chapter 2.1**, I therefore first investigated dietary nitrate as potential determinant of dementia. Dietary nitrate can be metabolized into nitric oxide, a free radical that plays a key role in regulating vascular health.[19, 20] With cardiovascular disease being established as an important determinant in the multifactorial etiology of dementia,[21] the underlying hypothesis is that a higher dietary nitrate intake decreases the risk of dementia by improving vascular brain health.

Given that nutrients are usually consumed in combination with other nutrients and interactions among nutrients may enhance or diminish potential beneficial effects, a growing body of research focusses on the potential role of dietary patterns in the development of dementia.[22, 23] Nonetheless, commonly defined dietary patterns considered as healthy, like the Mediterranean diet, may not be optimal for the brain. Therefore, the Mediterranean-Dietary Approaches to Stop Hypertension (DASH) Intervention for Neurodegenerative Delay (MIND) diet has been proposed.[24] This diet is derived from the Mediterranean and DASH diet and uniquely emphasizes foods considered to be brain-healthy, including green leafy vegetables and berries.[16] In **Chapter 2.2** I discuss a study that explores whether individuals adhering to the MIND diet are indeed at a lower risk of developing dementia.

Apart from the MIND diet, dietary patterns with relatively more plant- and fewer animal-based foods are gaining popularity in Western countries. This shift in dietary choices is driven by their environmental sustainability benefits, and associated health advantages, particularly in terms of cancer and cardiometabolic heath.[25-27] In **Chapter 2.3** I describe a study investigating whether such beneficial effects of a more plant-based dietary pattern extend to the risk of dementia.

It is generally known that the production of animal-based foods contributes substantially to greenhouse gas emission, water pollution and biodiversity loss,[28, 29] and that excessive consumption of animal-based foods has implications for human health and animal wellbeing.[26, 27, 30] Nonetheless, the majority of the Western population still consumes substantial amounts of animal-based foods on a daily basis.[31, 32] **Chapter 2.4** is therefore

devoted to the identification of barriers and facilitators to adopt a more plant-based dietary pattern.

Chapter 3 – Air pollution

Air pollution is another pressing environmental concern with far-reaching implications for our planet, but also for human health. With more than 90% of the global population residing in regions where levels of air pollution exceed the World Health Organization's guidelines,[33] an estimated 4.2 million deaths could be attributed to excessive exposure to air pollution in 2015.[34] The detrimental effects of air pollution predominantly manifest in the form of respiratory diseases,[35] cancer[36] and cardiovascular disorders.[37, 38] In addition, a growing body of evidence supports a potential adverse effect of air pollution on cognitive health and the incidence of dementia.[39, 40] This is primarily thought to be attributable to fine particulate matter, but other air pollutants, including nitrogen dioxide and ozone, may also affect the brain.[39] To properly inform policy makers on which efforts should be made in terms of reducing air pollutant levels to minimize cognitive decline, it is of critical importance to fully understand the role of air pollution in the development of dementia.

In **Chapter 3.1**, I therefore first describe a study assessing the association of exposure to air pollution with the risk of dementia and cognitive decline in a study area with relatively low variation in air pollutant levels. In **Chapter 3.2**, I further explore mechanisms that may underlie the toxic effects of air pollution on human health by studying the link between exposure to several air pollutants and concentrations of circulating metabolites.

Chapter 4 – Body composition

The proportion of body tissues, including adipose and muscle tissue, do not only reflect an individual's physical appearance but also has profound implications for human health.[41] Given that body composition is largely determined by lifestyle and environmental factors, the proportions and distributions of adipose and muscle tissue may serve as a mediator in the link of lifestyle choices and environmental exposures with the risk of dementia.[42, 43]

With adiposity being one of the most firmly established risk factor for cardiovascular disease,[44] and cardiovascular health being a key determinant in the development of dementia, it is generally thought that adiposity increases the risk of dementia.[45] Although a large body of literature indeed suggest that excessive adipose tissue during mid-life increases the risk of dementia,[46] observational studies have consistently linked a higher body mass at older age to a lower risk of dementia.[47] This seemingly protective effect of a higher body mass may be explained by the differential effects of lean and fat mass on the brain.[48] The aging process is generally accompanied by changes in body composition,

characterized by increased adipose tissue and decreased muscle mass. A higher body mass at older age may indicate higher lean mass, which can be considered as a general marker for overall health during the aging process.[49] Alternative explanations for this paradoxical phenomenon include reverse causality, meaning that participants lose weight as a result of clinical dementia symptoms; or competing risk by mortality, meaning that mortality precludes a dementia diagnosis, concealing potential harmful effects.[50] Nonetheless, a true protective effect of adiposity on brain health has also been suggested trough the excretion of certain hormones.[51]

To further elucidate the complex relation between body composition measures and the risk of dementia, while taking into account methodological challenges, **Chapter 4.1** focusses on a study investigating the association of total body and fat mass, as well as regional fat mass with the risk of dementia. Next, an approach was used in which trajectories of adiposity measures were estimated before the clinical diagnosis of dementia and these were compared to trajectories of dementia-free controls. In **Chapter 4.2** I further elucidated the link between body composition and brain health by linking total body, fat and lean mass to preclinical markers of dementia.

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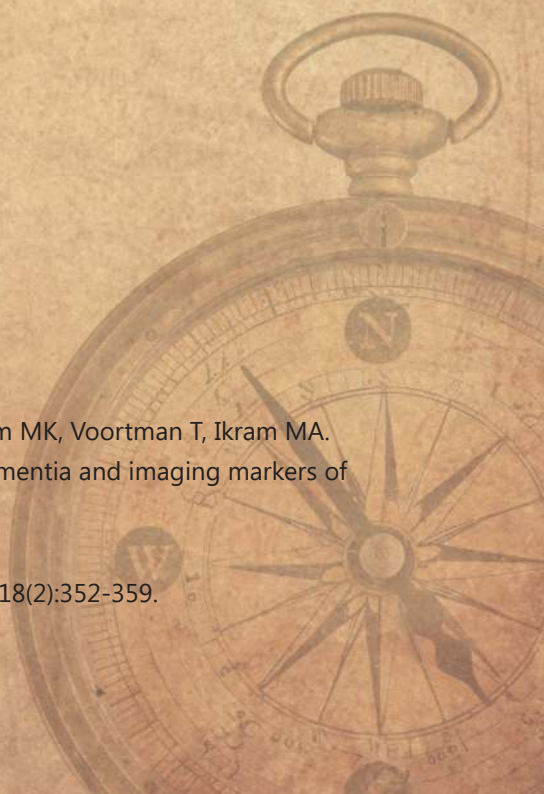
Chapter 2

Nutrition

Chapter **2.1**

Dietary nitrate and dementia



de Crom TOE, Blekkenhorst L, Vernooij MW, Ikram MK, Voortman T, Ikram MA. Dietary nitrate intake in relation to the risk of dementia and imaging markers of vascular brain health: A population-based study.

The American Journal of Clinical Nutrition. 2023;118(2):352-359.

ABSTRACT

Introduction: Nitric oxide is a free radical that can be produced from dietary nitrate and positively affects cardiovascular health. With cardiovascular health playing an important role in the etiology of dementia, we hypothesized a link between dietary nitrate intake and the risk of dementia.

Objective: To study the association of total, vegetable and non-vegetable dietary nitrate intake with the risk of dementia and imaging markers of vascular brain health, including total brain volume, global cerebral perfusion, white matter hyperintensity volume, microbleeds and lacunar infarcts.

Methods: Between 1990 and 2009, dietary intake was assessed using food frequency questionnaires in 9,543 dementia-free participants (mean age 64 years, 58% female) from the prospective population-based Rotterdam Study. Participants were followed-up for incidence dementia until January 2020. We used Cox models to determine the association between dietary nitrate intake and incident dementia. Using linear mixed models and logistic regression models, we assessed the association of dietary nitrate intake with changes in imaging markers across three consecutive examination rounds (average interval between images 4.6 years).

Results: Participants median dietary nitrate consumption was 85 milligrams per day (interquartile range: 55), derived on average for 81% from vegetable sources. During a mean follow-up of 14.5 years, 1,472 participants developed dementia. A higher intake of total and vegetable dietary nitrate was associated with a lower risk of dementia (hazard ratio (HR) [95% confidence interval (CI)] per 50 mg/day increase: 0.92 [0.87-0.98] and 0.92 [0.86-0.97], respectively), but not with changes in neuroimaging markers. No association between non-vegetable dietary nitrate intake and the risk of dementia (HR [95% CI]: 1.15 [0.64-2.07]) or changes in neuroimaging markers were observed.

Conclusion: A higher dietary nitrate intake from vegetable sources was associated with a lower risk of dementia. We found no evidence that this association was driven by vascular brain health.

INTRODUCTION

Nitric oxide is a free radical that regulates vasodilation, has an inhibitory effect on platelet aggregation, and thereby improves blood circulation.[1, 2] Nitric oxide can be produced endogenously by nitric oxide synthases via the oxidation of l-arginine.[3, 4] Alternatively, dietary nitrate can be metabolized into nitric oxide via the nitrate-nitrite-nitric oxide pathway.[5] In particular, dietary nitrate from vegetable sources may convert easily to nitric oxide owing to the enhancing effects of accompanying bioactive compounds like vitamin C and polyphenols.[6, 7]

Given these effects of nitric oxide on the vasculature, a higher dietary nitrate intake has been linked to a lower risk of various cardiovascular adverse health outcomes, such as hypertension and coronary heart disease.[8-10] With cardiovascular disease now established in the multifactorial etiology of dementia, a link between dietary nitrate intake and risk of dementia has been hypothesized, but direct evidence remains scarce.[11, 12]

Previous randomized controlled trials have investigated the effect of nitrate or nitrite supplementation on cerebral blood perfusion and cognitive performance, but yielded inconsistent results.[13-20] A recent meta-analysis concluded that this may be explained by the small sample sizes and short intervention timeframes of these trials.[12] Large and long-term experiments have not yet been conducted owing to challenges such as high attrition rates and high costs. This highlights the need to verify the link between nitrate intake and the risk of dementia using large observational cohort studies with long follow-up periods.

Therefore, we studied the association of total, vegetable and non-vegetable dietary nitrate intake with the risk of dementia within a large prospective population-based cohort study. We further explored the association between dietary nitrate intake and imaging markers of vascular brain health, including total brain volume, cerebral perfusion, white matter hyperintensity volume, microbleeds and lacunar infarcts.

METHODS

Study population

This study was embedded within the prospective population-based Rotterdam Study, of which details have been described elsewhere.[21] Briefly, the initial study was established in 1990 with 7,983 participants aged ≥55 years, who were living in the district of Ommoord

in Rotterdam, the Netherlands. In 2000, the study was enlarged with a second cohort including 3,011 participants who turned 55 years or moved into the study area, and again in the year 2005 with a third cohort comprising 3,932 persons aged ≥45 years. Every 3 to 6 years, all participants are invited to undergo extensive follow-up examinations.

The Rotterdam Study has been approved by the Medical Ethics Committee of Erasmus Medical Center and by the board of the Netherlands Ministry of Health, Welfare, and Sports. Written informed consent was obtained from all participants.

At study entry of the three cohorts, dietary data was collected for 9,737 participants. From this sample, we excluded participants who reported an unreliable energy intake of <500 or >5000 kcal per day (n=38), had prevalent dementia (n=38), were insufficiently screened for dementia (n=58) or did not sign informed consent to link the study database to medical records (n=60). This resulted in a sample comprising 9,543 participants who were eligible to be followed-up for incident dementia (dementia sample, **Figure 1A**). From 2005 onwards, brain magnetic resonance imaging (MRI) was implemented in the core protocol of the Rotterdam Study. Between 2006 and 2012, corresponding to the fifth examination round of the first cohort, the third examination round of the second cohort and study entry of the third cohort, dietary data was collected for 5,425 dementia-free participants, after which 4,228 participants underwent at least one MRI scan. We excluded participants with insufficient segmentations (n=162) and prevalent cortical infarcts (n=117), resulting in an additional sample of 3,949 participants (brain imaging sample, **Figure 1B**).

Dietary assessment

Dietary intake was assessed for all three sub-cohorts at study entry, which constituted the baseline for the dementia sample. For the first and second sub-cohort dietary intake was again assessed between 2009 and 2012, which constituted together with the study entry of the third sub-cohort as baseline for the brain imaging sample. The approach used to quantify dietary intake at study entry for the first and second cohort was slightly different compared to the method used at the other dietary assessment visits (**Figure 1**). At study entry for the first and second cohort, participants first completed a self-administered food frequency questionnaire (FFQ) including 170-food items followed by a structured interview with a trained dietician to specify food items, preparation methods and identify in which frequencies and amounts the food items were consumed. At the other dietary assessment visits, participants completed a self-administered semi-quantitative 389-item FFQ. The FFQ includes questions on main food items, its subtypes, preparation methods, cooking fats and condiments. Overall, the questionnaire is well-structured according to meals, with questions on frequency and portion sizes in household units as part of the food item

instead of as a separate list. These FFQs have been validated against other dietary assessment methods which showed that both FFQs can adequately rank individuals according to their intake of several nutrients.[22-24] Unfortunately, nitrate intake was not evaluated in these validation studies.

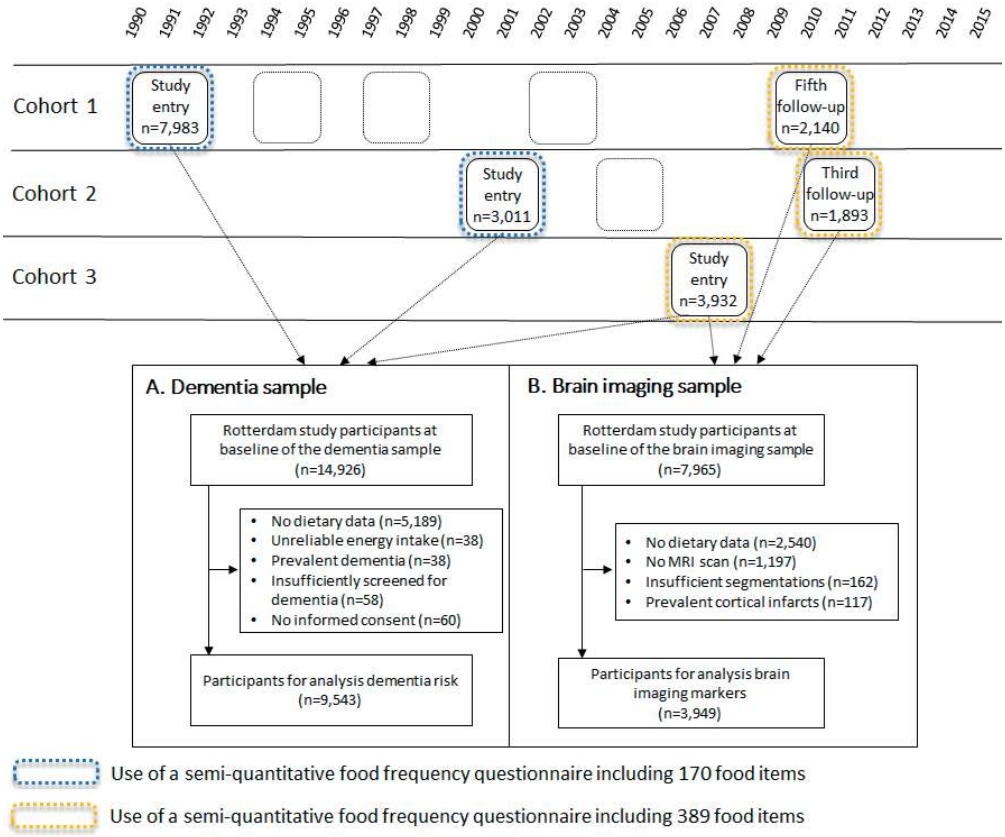


Figure 1. Flowchart of participants included in the dementia sample and brain imaging sample.

Dietary nitrate intake

In the present study, all foods derived from plants, including potatoes, but excluding fruits, nuts and cereals, were defined as vegetables. Dietary nitrate content of vegetables obtained from the FFQs was calculated using a comprehensive vegetable nitrate database, which was developed by means of a systematic review including studies measuring nitrate concentrations in vegetables between 1980 and 2016.[25] Measurements were mostly chemical analyzes performed in accredited laboratories. The final database summarizes data from 255 studies, with information on 178 vegetables. From these studies, 12 were

conducted in the Netherlands, covering 557 entries from 26 different vegetables. Overall, dietary nitrate concentrations in vegetables vary substantially across as well as within countries, for instance as a result of differences in agricultural practices, soil composition, farming methods and fertilizer use.[26] Given that one entry per vegetable in the Netherlands presumably does not provide a reasonable proxy for the dietary nitrate content, we considered the median nitrate concentration of each vegetable that was measured in the Netherlands and surrounding countries. The median was taken as the distribution of the obtained dietary nitrate concentrations for each vegetable was quite skewed. For 30% of the vegetables from which 16% of the total calculated dietary nitrate was derived, less than three entries were available for the Netherlands and surrounding counties. For these vegetables, the median nitrate concentration of all European countries was used. For 12% of the vegetables from which 0.5% of the total calculated dietary nitrate was derived, less than three entries were available for all European countries. For these vegetables, the median nitrate concentration of all countries was used. The database included season specific estimates, as nitrate in vegetables varies between seasons.[26] However, given that we are interested in habitual nitrate intake and we have previously shown that vegetable consumption across different seasons remains constant,[27] average nitrate concentrations throughout the year were used. Moreover, during the preparation processes boiling or cooking, approximately 50% of the nitrate content in vegetables will be lost.[28] We therefore divided the obtained nitrate concentration of the corresponding vegetable by two if such preparation method was used. This approach has been validated against urinary nitrate excretion samples in individuals from Australia, which showed that FFQs can adequately rank individuals according to their dietary nitrate intake.[25]

The dietary nitrate content of non-vegetable food items obtained from the FFQs was determined based on a comprehensive database, which was developed by means of a systematic review including 26 studies that have used chemical analyses to measure nitrate concentrations of foods between 1967 and 2008.[29] Data from Western countries only was used as dietary nitrate concentrations in non-vegetable foods differ substantially between high and middle income counties, but less variation is seen across high-income countries.[30]

Follow-up for dementia

Participants were screened for dementia at baseline and every 3 to 6 years during follow-up examinations using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Those with an MMSE score of <26 or a GMS organic level score of >0 were further examined using the Cambridge Examination for Mental Disorders in the Elderly diagnostic interview. Participants were also monitored for dementia on a continuous basis through an electronic link between the study database

and medical records from general practitioners and the Regional Institute of Outpatients Mental health Care. The final diagnosis was established by a consensus panel led by a neurologist, according to standard criteria for dementia (DSM-III-R), and for sub-diagnosis of Alzheimer's disease (NINCDS-ADRDA). Follow-up was virtually completed until January 1st 2020 for 95.9% of the potential person-years.

Acquisition and post-processing of brain MRI

Brain MRI scanning was performed on a 1.5-Tesla MRI scanner (GE Signa Excite; General Electric Healthcare, Milwaukee, USA) according to standardized protocols to determine total brain volume, global cerebral perfusion, white matter hyperintensity volume, microbleeds and lacunar infarcts.[31] There were no hardware or software updates over the study period. The scan protocol, sequence details and processing of the data have been described in detail previously.[31]

Covariables

Energy and alcohol intake were calculated from the obtained dietary data based on the Dutch Nutrient Database tables. Using structured interviews, data on educational attainment and smoking status were obtained. Education attainment was classified into four levels: primary, lower, intermediate and higher. Smoking status was categorized as never, current or former. Physical activity was measured using a validated adapted version of the Zutphen Physical Activity Questionnaire at study entry for the first and second cohort and the LASA Physical Activity Questionnaire at the other visits. A diet quality score reflecting adherence to the Dutch Dietary Guidelines was calculated by adding adherence scores for 14 food recommendations, as described in detail elsewhere.[32] A plant-based dietary index was derived from the dietary intake data as described in detail elsewhere.[33] A higher index reflect relatively higher consumption of plant-based and lower consumption of animal-based foods. Height and weight were measured at the research center and body mass index was calculated. *APOE* genotype was obtained using polymerase chain reaction of coded DNA samples for the first cohort and with bi-allelic TaqMan assay for the second and third cohort.

Statistical analysis

Descriptive statistics are presented as mean (standard deviation) for normally distributed continuous variables, median (interquartile range) for non-normally distrusted continuous variables and number (percentage) for categorical variables. The interquartile range is provided as a measure of spread, i.e. the difference between the upper quartile and lower quartile.[34]

As the distribution of dietary nitrate intake was right-skewed, we performed a natural-log transformation to obtain a roughly normal distribution. Moreover, to account for energy intake, we applied the nutrient residual method to calculate energy-adjusted nitrate intake. However, given that both the natural-log transformation and nutrient residual method did not affect our results, effect estimates based on crude dietary nitrate intake variables are provided in the manuscript for interpretation purposes.

We determined the association between dietary nitrate intake at baseline and the risk of dementia and Alzheimer's disease using Cox proportional hazard models. We verified that the proportional hazard assumption was met based on Schoenfeld residuals. Dietary nitrate intake was included in the model per 50 mg/day increase, equivalent to circa 30 grams of nitrate rich vegetables and circa 250 grams of nitrate poor vegetables,[25] as well as per quartile. To further explore potential non-linear associations, we included dietary nitrate intake as a quadratic term in the models and tested whether this improved the fit of the models using ANOVA. All analyses were adjusted for age, sex and energy intake (model I) and additionally for educational attainment, diet quality, alcohol intake, physical activity, smoking status, body mass index, and *APOE* ε4 status (model II).

To assess potential effect modification, we added an interaction term between dietary nitrate intake and sex, *APOE* ε4 status, dietary intake of vitamin C and body mass index (as adiposity is associated with impaired nitric oxide availability [35]). Moreover, to evaluate the robustness of our findings, we performed several sensitivity analyses. First, to evaluate whether reverse causality accounted for potential associations, we repeated the analysis after excluding the first 5 years of follow-up. Second, given that for the first and second sub-cohort a different FFQ has been used to asses dietary nitrate intake than for the third sub-cohort, we repeated the analyses in the first and second sub-cohort only. Third, to enhance comparability between the dementia risk results and brain imaging results, we also repeated the analyses within the brain imaging sample. Fourth, we repeated the analyses while additionally correcting for vegetable intake (grams/day) and after replacing the overall diet quality score in the models by a plant-based dietary index. Last, we repeated the analyses for dietary nitrate intake derived from diverse vegetable groups separately.

We determined the association of dietary nitrate intake at baseline with total brain volume, cerebral perfusion and white matter hyperintensity over time, using linear mixed models with a random intercept and slope. The natural logarithm of white matter hyperintensity volume was taken to reach an approximately normal distribution. Time in years between the consecutive MRI scans was used as underlying time scale. We included dietary nitrate

intake in the model as fixed effect (main effect), representing overall differences in trajectories of imaging markers during follow-up. In addition, we included an interaction term between dietary nitrate intake and time (slope effect), allowing imaging markers to change differentially over time for different dietary nitrate intake levels. To correct for confounders, all covariables mentioned above were included in the models and intracranial volume, as proxy of head size, was additionally added. We further included an interaction term between age and time, as changes in MRI markers are exponentially related to age.[36] To study the association between dietary nitrate intake with the prevalence and incidence of any microbleeds and lacunar infarcts, we used logistic regression models. All covariables mentioned above were included in the models and in the models assessing incidence, time between dietary nitrate intake assessment and the MRI scan on which the incidence was detected was added.

Missing data on covariables were imputed using five-fold multiple imputation. In the dementia sample, 19% of the physical activity data, 5% of the *APOE* ε4 status data, and less than 1% of all other covariables was missing. In the brain imaging sample, 6.5% of the *APOE* ε4 status data, 4.8% of the physical activity data and less than 1% of all other covariables was missing. The distribution of covariables in the imputed dataset was similar as in the non-imputed dataset. All statistical analyses were conducted using R Statistical Software version 4.0.3.

RESULTS

Baseline characteristics of the total study population are provided in **Table 1** and per quartile of dietary nitrate intake in **Supplementary Table 1**. The mean age of the participants in the dementia sample was 64.1 years (standard deviation: 8.6 years) and 58% were female. Participants median dietary nitrate consumption was 85 milligrams per day (interquartile range: 55), derived on average for 81% from vegetable sources. Total dietary nitrate and dietary nitrate from vegetable sources were positively correlated with total vegetable intake (r: 0.63 and 0.64, respectively). Overall, dietary nitrate was mainly derived from the category other vegetables, followed by dark green vegetables, starchy vegetables, red and orange vegetables, and beans and peas (**Supplementary Table 2**).

Table 1. Baseline characteristics of the study population.

Characteristics	Dementia sample (N=9,543)	Brain imaging sample (N=3,949)
Age, years	64.1 (8.6)	65.2 (10.8)
Female sex	5,530 (58)	2,264 (57)
Energy intake, kcal/day	2,099 (595)	2,144 (656)
Education attainment		
Primary	1,463 (15)	328 (8)
Lower	3,900 (41)	1,473 (38)
Intermediate	2,662 (28)	1,178 (30)
Higher	1,463 (15)	927 (24)
Diet quality score	6.7 (1.9)	6.9 (1.9)
Alcohol intake, grams/day	5 [17]	7 [17]
Physical activity, MET hours/week	69 [63]	42 [65]
Smoking status		
Never	2,231 (23)	701 (18)
Former	4,152 (44)	1,974 (50)
Current	3,114 (33)	1,268 (32)
Body mass index, kg/m^2	26.8 (4.0)	27.3 (4.1)
APOE ε4 status		
No allele	6,482 (72)	2,656 (72)
1 allele	2,353 (26)	957 (26)
2 allele	205 (2)	81 (2)
Total dietary nitrate intake, mg/day	85 [55]	95 [75]
Vegetable dietary nitrate intake, mg/day	70 [53]	78 [71]
Non-vegetable dietary nitrate intake, mg/day	6 [7]	16 [10]
Total brain volume, mL	-	934.5 (99.8)
Cerebral perfusion, mL	-	524,7 (102.4)
White matter hyperintensity volume, mL	-	3.2 [2.01]
Any microbleeds	-	808 (20)
Any lacunar infarcts	-	293 (7)

Data are shown for non-imputed data and are presented as mean (standard deviation) for normally distributed continuous variables, medium [interquartile range] for non-normally distributed continuous variables, and number (percentages) for categorical variables. Abbreviations: MET, metabolic equivalent of task; MRI, magnetic resonance imaging; N, number of participants.

During a mean follow-up of 14.5 years, 1,472 participants developed dementia (incidence rate 10.6 per 1000 person-years) among whom 1,078 had Alzheimer's disease. A higher intake of total and vegetable dietary nitrate intake was associated with a lower risk of dementia (hazard ratio (HR) [95% confidence interval (CI)] per 50 mg/day increase: 0.92 [0.87-0.98] and 0.92 [0.86-0.97], respectively, **Table 2**). No association between non-vegetable dietary nitrate intake and the risk of dementia was observed (HR [95% CI]: 1.15

[0.64-2.07]). Analyzing dietary nitrate intake into quartiles showed no indication for non-linearity, neither did adding a quadratic term in the models (p-values >0.4).

Table 2. Dietary nitrate intake and the risk of dementia.

		Hazard ratio (95% confidence interval)	
	n/N	Model I	Model II
Total dietary nitrate intake			
Per 50 mg/day increase	1,472/9,543	0.91 (0.86-0.96)	0.92 (0.87-0.98)
Per quartile:			
Quartile 1 (1.9-64.3 mg/day)	434/2,386	Reference	Reference
Quartile 2 (64.3-85.4 mg/day)	449/2,386	0.98 (0.86-1.12)	0.99 (0.87-1.13)
Quartile 3 (85.4-119.0 mg/day)	383/2,386	0.93 (0.80-1.07)	0.93 (0.81-1.08)
Quartile 4 (119.1-1063.5 mg/day)	206/2,385	0.84 (0,71-1.00)	0.88 (0.74-1.05)
Vegetable dietary nitrate intake			
Per 50 mg/day increase	1,472/9,543	0.90 (0.85-0.96)	0.92 (0.86-0.97)
Per quartile:			
Quartile 1 (0.0-50.1 mg/day)	443/2,386	Reference	Reference
Quartile 2 (50.1-70.4 mg/day)	436/2,386	0.95 (0.83-1.09)	0.96 (0.84-1.11)
Quartile 3 (70.4-103.0 mg/day)	381/2,386	0.89 (0.77-1.02)	0.91 (0.79-1.05)
Quartile 4 (103.1- 1052.0 mg/day)	212/2,385	0.83 (0.70-0.98)	0.87 (0.73-1.03)
Non-vegetable dietary nitrate intake			
Per 50 mg/day increase	1,472/9,543	1.17 (0.66-2.07)	1.15 (0.64-2.07)
Per quartile:			
Quartile 1 (1.24-11.4 mg/day)	384/2,386	Reference	Reference
Quartile 2 (11.4-14.3 mg/day)	419/2,386	1.03 (0.90-1.19)	1.05 (0.91-1.21)
Quartile 3 (14.3-18.1 mg/day)	393/2,386	1.15 (0.99-1.33)	1.13 (0.97-1.32)
Quartile 4 (18.1-82.0 mg/day)	276/2,385	1.06 (0.89-1.25)	1.07 (0.90-1.27)

Cox proportional hazards models were used to obtain hazard ratios and 95% confidence intervals. Model I is adjusted for age, sex, and energy intake. Model II is further adjusted for educational attainment, diet quality, alcohol intake, physical activity, smoking status, body mass index, and *APOE* ε4 status. Abbreviations: n, number of incident cases; N, number of participants at risk.

No evidence for effect modification by sex, *APOE* ε4 status, dietary intake of vitamin C or body mass index was found (p-values for interaction >0.45). Furthermore, effect estimates were similar for Alzheimer's disease, after excluding the first 5 years of follow-up, when restricting the analyses to the first and second sub-cohort, and when repeating the analyses in the brain imaging sample (**Supplementary Figure 1**). Additional adjustment for vegetable consumption or replacing the diet quality score by a plant-based dietary index did also not affect our results. In addition, effect estimates were somewhat stronger for dietary nitrate intake derived from dark green vegetables and starchy vegetables than for other vegetables (**Supplementary Figure 2**).

Participants in the brain imaging sample were on average 65.2 years old (standard deviation: 10.8) and 57% were female (**Table 1**). Baseline characteristics per quartile of dietary nitrate intake are provided in **Supplementary Table 3**. Among the 3,949 participants, 2,433 participants underwent a second and 1,107 participants a third MRI scan. The average interval between scans was 4.6 years. Participants with a second and third MRI scan were on average younger compared to those without an additional MRI scan, whereas other baseline characteristics were similar (**Supplementary Table 4**). Dietary nitrate intake was not associated with cerebral brain perfusion or white matter hyperintensity volume over time (**Table 3**). A higher non-vegetable, but not total or vegetable, dietary nitrate intake was associated with a higher total brain volume (mean difference in z-score of main effect [95% CI] per 50 mg/day increase: 0.11 [0.03-0.20]), but not with changes in total brain volume over time (mean difference in z-score of slope [95% CI]: 0.01 [0.00-0.01]). No association between dietary nitrate intake and either prevalent or incident microbleeds and lacunar infarcts was observed (**Table 4**).

Table 3. Dietary nitrate intake and total brain volume, cerebral perfusion and white matter hyperintensity volume (N=3,949).

	Total brain volume	**Cerebral perfusion**	**White matter hyperintensity volume**
	Main effect		
Per 50 mg/day increase in:	Mean difference in z-score (95% confidence interval)		
Total dietary nitrate intake	0.00 (-0.01-0.01)	0.01 (-0.02-0.04)	-0.01 (-0.03-0.02)
Vegetable dietary nitrate intake	0.00 (-0.01-0.01)	0.01 (-0.02-0.04)	0.00 (-0.03-0.02)
Non-vegetable dietary nitrate intake	0.11 (0.03-0.20)	-0.14 (-0.38-0.10	-0.02 (-0.24-0.20)
	Slope		
Per 50 mg/day increase in:	Mean difference in z-score (95% confidence interval)		
Total dietary nitrate intake	0.00 (0.00-0.00)	0.00 (-0.01-0.00)	0.00 (0.00-0.00)
Vegetable dietary nitrate intake	0.00 (0.00-0.00)	0.00 (-0.01-0.00)	0.00 (0.00-0.00)
Non-vegetable dietary nitrate intake	0.01 (0.00-0.01)	-0.03 (-0.08-0.02)	0.00 (-0.01-0.02)

Linear mixed models with a random intercept and slope were used to obtain mean differences of the main effect (i.e. dietary nitrate included in the models as fixed effect) and slope effect (i.e. interaction between dietary nitrate intake and time). Models are adjusted for age, sex, energy intake, educational attainment, diet quality, alcohol intake, physical activity, smoking status, body mass index, *APOE* ε4 status, and intracranial volume.

Table 4. Dietary nitrate intake and microbleeds and lacunar infarcts (N=3,949).

	Odds ratios (95% confidence interval)	
	Prevalent vascular brain disease	
Per 50 mg/day increase in:	Any microbleeds (n=808)	Any lacunar infarcts (n=293)
Total dietary nitrate intake	0.97 (0.91-1.05)	1.05 (0.94-1.16)
Vegetable dietary nitrate intake	0.98 (0.91-1.05)	1.04 (0.94-1.16)
Non-vegetable dietary nitrate intake	0.65 (0.33-1.26)	1.13 (0.43-2.99)
	Incident vascular brain disease	
Per 50 mg/day increase in:	Any microbleeds (n=279)	Any lacunar infarcts (n=115)
Total dietary nitrate intake	1.01 (0.91-1.13)	0.96 (0.81-1.15)
Vegetable dietary nitrate intake	1.01 (0.91-1.13)	0.97 (0.82-1.16)
Non-vegetable dietary nitrate intake	0.64 (0.21-1.95)	0.34 (0.06-1.93)

Logistic regression models were used to obtain odds ratios and 95% confidence intervals. Models are adjusted for age, sex, energy intake, educational attainment, diet quality, alcohol intake, physical activity, smoking status, body mass index and *APOE* ε4 status. In the models assessing incidence, time between dietary nitrate intake assessment and MRI scan on which the incidence was detected was added.

DISCUSSION

In this population-based cohort study, higher dietary nitrate intake from vegetable sources was associated with a lower risk of dementia, while no association was found for dietary nitrate intake from non-vegetable sources. Dietary nitrate intake was also not associated with cerebral perfusion or vascular pathology (i.e. white matter hyperintensity volume, microbleeds, and lacunar infarcts), but a higher dietary nitrate intake from non-vegetable sources was associated with a higher total brain tissue volume.

Although it has previously been hypothesized that dietary nitrate affects brain health,[11] the direct link between dietary nitrate intake and the risk of dementia has not been studied before. Few randomized controlled trials, however, have studied the effect of nitrate or nitrite supplementation on cognitive performance. Some studies found an improvement in certain cognitive test performances after supplementation,[13-15] but most studies found no effect.[16-20] These null findings may be explained by the relatively small sample sizes (i.e. maximum of 62 participants) and short study periods (i.e. less than 7 days in most studies). Especially the hypothesis that nitrate becomes effective after long-term consumption seems likely, as particularly the trail with the longest supplementation duration of 10 weeks reported robust improvement in executive functioning at the end of the trial.[14] We

extended this evidence in a long-term cohort study by showing that dietary nitrate intake is also associated with a lower risk of dementia.

Our findings of an association between a higher dietary nitrate intake and a lower risk of dementia could be explained by the free radical nitric oxide. Oral bacteria metabolize dietary nitrate to nitrite and in the acid environment of the stomach, nitrite can be metabolized further into nitric oxide. This will be absorbed in the small intestine and subsequently released in the blood circulation.[37] In the blood circulation, nitric oxide regulates vasodilation and platelet aggregation. This maintains vascular health,[1, 2] which is a key determinant in the prevention of dementia.[38] Yet, our null findings with brain imaging markers could not confirm that vascular brain health drives the association between dietary nitrate intake and dementia. In line with these findings, a recent meta-analysis of randomized controlled trails found no strong evidence for an acute effect of dietary nitrate intake on cerebral perfusion,[12] but other brain imaging markers have not previously been studied. An explanation for the observed null findings could be that nitric oxide mainly affects small blood vessels that cannot be detected on brain images. Alternative potential mechanisms underlying the association between dietary nitrate and dementia, include a protective effects of nitric oxide on metabolic functions [30, 39] and the regulation of reactive oxygen species homeostasis.[40]

In the present study, the association between dietary nitrate intake and the risk of dementia seems to be driven by dietary nitrate from vegetable sources. This may be explained by accompanying bioactive compounds in vegetables, like vitamin C and polyphenols, which enhance the formation of nitrate into nitric oxide.[6, 7] Yet, we found no evidence for interaction between dietary intake of nitrate and vitamin C, which could possibly suggest that enhancing effects require the presence of multiple bioactive compounds. It is, however, also possible that bioactive compounds common in nitrate rich vegetables drive the observed link between nitrate and dementia via pathways unrelated to nitrate.[41]

That dietary nitrate from non-vegetable sources was not associated with the risk of dementia may be explained by the absence of enhancing effects of bioactive compounds present in vegetables. Without enhancing stimulus, nitrate can be converted into nitrosamines,[42] chemical compounds that may adversely affect brain health.[43] Furthermore, nitrate from non-vegetable sources is mainly derived from animal-based foods that contain high levels of saturated fats and sodium, of which excessive consumption has been linked to an increased risk of dementia.[44] These adverse effects may have eclipsed the positive effects of dietary nitrate on brain health. Yet, observed null findings should be interpreted with caution, as on average only 19% of the consumed dietary nitrate was

derived from non-vegetable sources. Intake levels of non-vegetable dietary nitrate in the present study, which are similar to those reported in other populations,[26, 45] may be too low to identify meaningful associations. In addition, the database used to assess dietary nitrate from non-vegetable sources was less extensive and up-to-date than the database from vegetable sources. Consequently, the relatively low precision of the non-vegetable dietary nitrate consumption levels may have diluted effect estimates towards the null.

Certain methodological considerations need to be taken into account when interpreting the findings. First, the FFQs used to determine dietary intake were not originally compiled to assess dietary nitrate intake. Consequently, intake of some nitrate rich foods (i.e. radish and turnip) was not assessed in detail. Also, information on preparation methods was lacking for some foods, while most meal preparation methods reduce the nitrate content of the food.[28] Second, dietary nitrate concentrations in foods vary due to environmental factors, such as season and temperature.[26] Composition tables for the Netherlands specifically are therefore needed to precisely determine dietary nitrate intake, and although we had access to an extensive database, for some food items, dietary nitrate content was not known for the Netherlands. As a results, misclassification, which is presumably differential, persist and may have led to an underestimation of the true association. Third, nitrate concentrations in foods are slightly reduced over the past 30 years in the Netherlands.[46] Such temporal changes were not considered in the databases, which could have impact the precision of the calculated nitrate values. Fourth, data on food intake was self-reported and thereby sensitive to recall bias. Fifth, although we corrected for a wide range of potential confounders like total energy intake and diet quality, residual confounding may persist. Sixth, this study includes individuals living in the Netherlands, an economic well-developed county, in which average daily nitrate consumption levels are substantially lower compared to low economic developed countries.[30] Moreover, approximately 95% of our study population was of Caucasian ethnicity. This hampers generalizability of our results to study populations from lower economic developed counties and other ethnicities. Last, dietary habits most likely change over time, but our data was too limited to take this into account.

In conclusion, a higher dietary nitrate intake from vegetable sources was associated with a lower risk of dementia in the general population. We found no evidence for vascular brain health as an underlying mechanism. Further studies should verify our observations, elucidate the potential effects of non-vegetable dietary nitrate intake on brain health, and identify mechanisms underlying the association between dietary nitrate intake and the risk of dementia.

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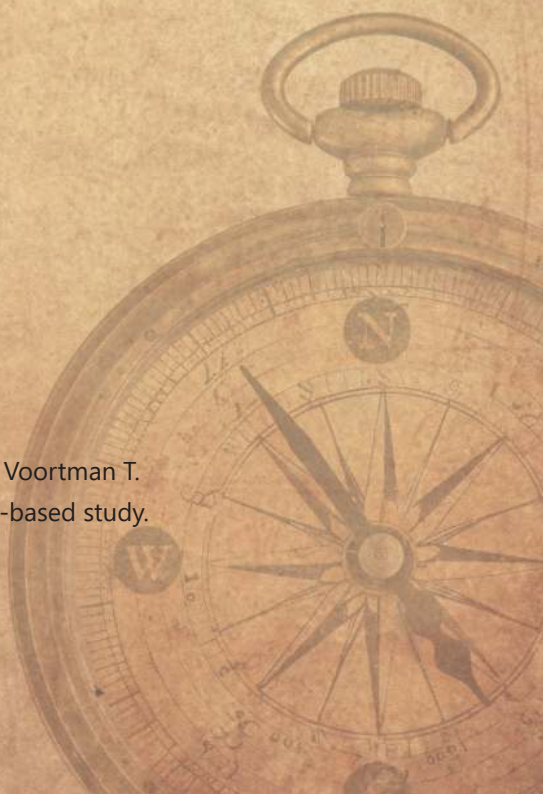
SUPPLEMENTARY MATERIAL

Chapter **2.2**

MIND diet and dementia



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Alzheimer's Research & Therapy. 2022;14(1):1-10.

ABSTRACT

Introduction: Adherence to the Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet has been linked to a decreased risk of dementia, but reverse causality and residual confounding by lifestyle may partly account for this link. We aimed to address these issues by studying associations over cumulative time-periods, which may provide insight into possible reverse causality; and by using both historical and more contemporary dietary data as this could give insight into confounding as historical data may be less affected by lifestyle factors.

Methods: In the population-based Rotterdam Study, dietary intake was assessed using validated food frequency questionnaires in 5,375 participants between 1989-1993 (baseline I); and in a largely non-overlapping sample in 2,861 participants between 2009-2013 (baseline II). We calculated the MIND diet score and studied its association with the risk of all-cause dementia, using Cox models. Incident all-cause dementia was recorded until 2018.

Results: During a mean follow-up of 15.6 years from baseline I, 1,188 participants developed dementia. A higher MIND diet score at baseline I was associated with a lower risk of dementia over the first 7 years of follow-up (hazard ratio (HR) [95% confidence interval (CI)] per standard deviation (SD) increase: 0.85 [0.74-0.98]), but associations disappeared over longer follow-up intervals. Mean follow-up from baseline II was 5.9 years during which 248 participants developed dementia. Higher MIND diet score at baseline II was associated with a lower risk of dementia over every follow-up interval, but associations slightly attenuated over time (HR [95% CI] for 7 years follow-up per SD increase: 0.76 [0.66-0.87]). The MIND diet score at baseline II was more strongly associated with the risk of dementia than the MIND diet score at baseline I.

Conclusion: Better adherence to the MIND diet is associated with a decreased risk of dementia within the first years of follow-up, but this may in part be explained by reverse causality and residual confounding by lifestyle. Further research is needed to unravel to which extend the MIND diet may affect the risk of dementia.

INTRODUCTION

Diet has gained increasing interest as a target for developing preventive opportunities against dementia, as it impacts several mechanisms underlying dementia, including oxidative stress, inflammation, and vascular abnormalities. Accordingly, various studies have linked adherence to healthy dietary patterns to a slower rate of cognitive decline[1] and a decreased risk of dementia.[2] Yet, such healthy dietary patterns may be sub-optimal for brain health. Therefore, the Mediterranean-Dietary Approaches to Stop Hypertension (DASH) Intervention for Neurodegenerative Delay (MIND) diet has been developed,[3] to uniquely emphasize foods linked to brain health, such as green leafy vegetables[4, 5] and berries.[6] Adherence to the MIND diet has indeed been linked to better cognitive performance,[7, 8] less cognitive decline,[3, 9-11] and a lower dementia risk.[12, 13] Nevertheless, in observational studies linking dietary patterns to dementia, two methodological issues remain challenging to address.

First, average follow-up time in previous studies was 4.5 to 6.6 years, which corresponds largely to the prodromal stage of dementia.[14] During this phase of incremental cognitive impairment, dietary habits may deteriorate,[15, 16] for instance due to depressive symptoms[17] and olfactory impairment.[18] This may introduce reverse causality in short-term associations between dietary patterns and incident dementia. Studies with long follow-up allowing for studying association over cumulative time-periods could provide insights into possible reverse causality.

Second, studying dietary patterns in observational studies is notoriously difficult as it invariably suffers from confounding by lifestyle. Although previous studies controlled for lifestyle factors in their analyses, residual confounding may persist.[19, 20] In this regard, it is important to realize that healthy diet and lifestyle awareness increased steeply over the past few decades and most likely thereby also the relation between diet quality and other lifestyle factors.[21] Meaning that those who care most about their health adhere to both a healthy diet and lifestyle, while those who care less about their health adhere to a less healthy diet and lifestyle. Using both historical and more contemporary data on dietary patterns in the same study population and compare their association with the risk of dementia may give insight in confounding since historical data may be less affected by lifestyle factors than more contemporary data.

The Rotterdam Study is a prospective population-based study, with dietary intake assessed in 1989-1993 and again in 2009-2013 in a largely non-overlapping sample. We

determined the association between adherence to the MIND diet and the risk of dementia across these two settings, two decades apart, over cumulative follow-up intervals. Moreover, to unravel whether the MIND diet is indeed more specific for brain health than other healthy diets, we also studied the association between adherence to two other healthy diets and the risk of dementia.

METHODS

Study population

This study was embedded within the first two sub-cohorts of the Rotterdam Study (RS), a prospective population-based cohort among inhabitants from the suburb Ommoord in Rotterdam, the Netherlands. Details regarding the study methodology have been published previously.[22] Briefly, the first sub-cohort (RS-I) was established in 1989 and consisted of 7,983 participants aged 55 years and older. In 1999, the study was expanded with the second sub-cohort (RS-II) consisting of 3,011 participants who had turned 55 years of age or moved into the study area. Extensive follow-up examination rounds take place every 3 to 5 years through home interviews and various physical and laboratory checks at the research center.

For the current study, we consider two different baselines. The periods considered as baselines were dependent on the examination rounds in which dietary intake was assessed: between 1989-1993 in the first cohort (RS-I-1), which forms baseline I in the current study, and between 2009-2012 in the first and second cohort (RS-I-5 and RS-II-3), which forms baseline II in the current study.

Of the 7,983 participants included in the study at baseline I, 5,435 participants had dietary data available. We excluded 3 participants who had unreliable dietary data (i.e. an estimated energy intake of <500 or >5000 kcal/day), 22 participants who had dementia at time of dietary assessment, and 35 participants who did not sign informed consent to link the study database to their medical records. This leaves a total of 5,375 participants eligible for follow-up from baseline I. Of the 4,040 participants who participated at baseline II, 2,998 participants had dietary data available. We excluded 110 participants who had unreliable dietary data, 23 participants who had dementia at time of dietary assessment, 1 participant who had insufficient cognitive screening to assess dementia, and 3 participants who did not sign informed consent to link the study database to their

medical records. This leaves a total of 2,861 participants eligible for follow-up from baseline II. A schematic overview of the study population is provided in **Figure 1**.

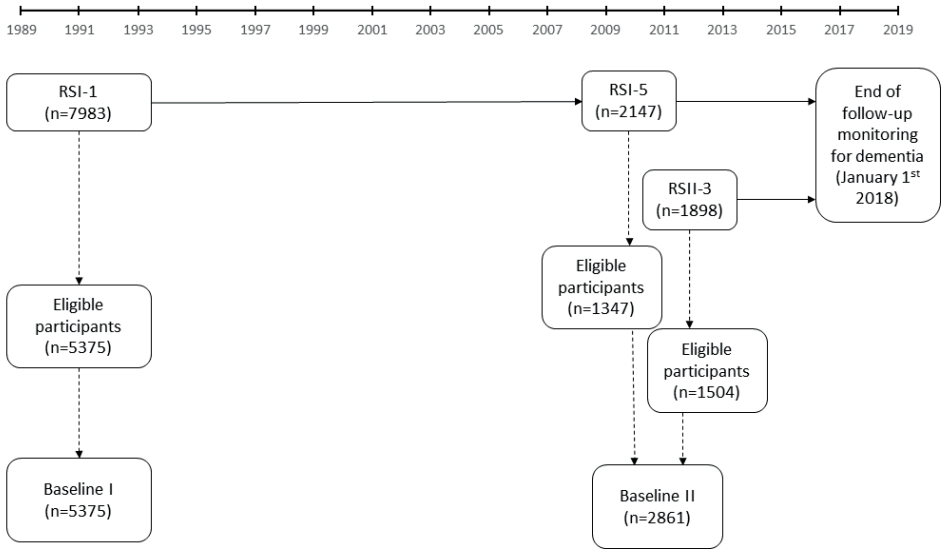

Figure 1. Schematic overview of eligible Rotterdam Study (RS) participants.

Dietary assessment

At baseline I, participants completed a 170-item food-frequency questionnaire (FFQ). They first completed a checklist on which food items they consumed at least twice a month in the preceding year, after which information on frequencies and portion sizes was obtained in an interview by a trained dietician. At baseline II, dietary intake was assessed with a self-administered 389-item FFQ including questions on frequency and portion sizes of food item consumption in the last month. Both FFQs have been validated against other dietary assessment methods which showed that based on these FFQs, participants can be adequately ranked according to their food and nutrient intake.[23-25] From the FFQ data, we derived adherence scores for the MIND diet, Dutch dietary guidelines, and Mediterranean diet, as outlined below.

MIND diet

The MIND diet as described by Morris et al.[3] contains recommendations regarding 15 food components, including 10 food components considered to be healthy for the brain (i.e. green leafy vegetables, other vegetables, nuts, berries, beans, whole grains, fish, poultry, olive oil, and wine) and five unhealthy food components (i.e. red meat, butter

and stick margarine, cheese, fast fried food, and pastries and sweets). An overview of food items on the different FFQs that summarizes these food components can be find in **Supplementary Table 1**. If participants used olive oil as primary cooking fat (>50%) a 1 was assigned and a 0 otherwise. For each other food component, a 0 was assigned if participants did not adhere to the recommendations, a 0.5 for moderate adherence, and a 1 for good adherence. Scores assigned to each food components were summed, obtaining a total score ranging from 0 to 15.

Dutch dietary guidelines

We used a previously defined score to assess adherence to Dutch dietary guidelines.[26] Briefly, participants received a score of 1 (adherence) or 0 (no adherence) for recommendations of 14 food components (i.e. vegetables, nuts, fruits, legumes, whole grains, whole grains of total grains, fish, dairy products, tea, coffee, unsaturated fats and oils of total fats, red and processed meat, sugar-containing beverages, alcoholic beverages, and salt). The sum score ranged from 0 to 14.

Mediterranean diet

The Mediterranean diet as described by Panagiotakos et al.[27] containing recommendations regarding 11 food components (i.e. vegetables, fruits, legumes, whole grains, fish, full fat dairy products, potatoes, olive oil, poultry, meat, and alcoholic beverages). Adherence was determined by assigning a score ranging from 0 to 5 to each food component with higher scores reflecting better adherence. The final sum score ranged from 0 to 55.

Dementia

Participants were screened for dementia at baseline and every 3 to 5 years during follow-up examinations using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Those with an MMSE score of <26 or a GMS organic level score of >0 were further examined using the Cambridge Examination for Mental Disorders in the Elderly diagnostic interview. Additionally, participants were continuously under surveillance for dementia through electronic linkage between the study database and medical records from general practitioners and the Regional Institute of Outpatients Mental health Care. The final diagnosis of dementia and its most common subtypes was made by a consensus panel led by a neurologist based on standard criteria for all-cause dementia (DSM-III-R), and for sub-diagnosis of Alzheimer's disease (NINCDS-ADRDA). Follow-up for dementia was completed until January 1st 2018.

Covariates

Data on relevant covariates were obtained at both baseline I and II. Trained interviewers obtained information regarding education attainment (primary, lower, intermediate, higher), smoking status (never, former, current), and use of medication. Height and weight were measured and body mass index (BMI) in kilogram per square meter was calculated. Physical activity was measured using a validated adapted version of the Zutphen Physical Activity Questionnaire at baseline I and the LASA Physical Activity Questionnaire at baseline II. Physical activity was expressed in Metabolic Equivalent of Task (MET)-hours per week. Daily energy intake in kcal was calculated from the FFQ data using the Dutch Food Composition Tables (NEVO). Diabetes mellitus was defined as having a fasting serum glucose of ≥7.0 mmol/L, a random serum glucose level of ≥11.1 mmol/L, or use of blood glucose lowering medication. Hypercholesterolemia was defined as a serum total cholesterol concentration ≥6.2 mmol/L or use of lipid-lowering medication. Systolic and diastolic blood pressure was measured twice on the right arm with the participant in a sitting position using a random zero sphygmomanometer of which the mean was used for analyses. Hypertension was defined as a systolic blood pressure of ≥140 mmHg, a diastolic blood pressure of ≥90 mmHg, or use of blood pressure-lowering medication. Depressive symptoms were considered as a score of ≥16 on the validated Center for Epidemiology Depression Scale. History of stroke was obtained from interviews and verified through medical records. *APOE* genotype was obtained using polymerase chain reaction of coded DNA samples for RS-I and with bi-allelic TaqMan assay for RS-II.

Statistical analysis

Cox proportional hazard models were used to determine the association between the different diet scores per standard deviation (SD) increase and incidence all-cause dementia. Analyses were conducted from baseline I and II separately. Participants were censored when they were diagnosed with dementia, died, were lost to follow-up, or at the end of the follow-up (January 1st 2018), whichever came first. To test for potential non-linear relationships, we added natural cubic splines with three knots to the diet scores in het model and tested whether this significantly improved the fit of the model using the analysis of variance test. To determine how associations changed over time, we performed analyses in cumulative follow-up intervals from the different baselines (i.e. performing analyses from the different baselines to 5 years, baselines to 7 years, etc.).[28] We constructed a basic model adjusted for sex, age, age^2, and educational attainment (model I). Subsequently, we further adjusted for smoking status, physical activity, and energy intake (model II). To minimize the risk of residual confounding, we considered an additional model in which we further adjusted for covariates that may act as confounder

and/or mediator, which include BMI, diabetes, hypercholesterolemia and hypertension (model III). Missing data on covariates (29% for physical activity and <5% for all other covariates) were imputed using five-fold multiple imputation. The distribution of the covariates in the imputed dataset was comparable to the original dataset (data not shown). Analyses were performed on each imputed dataset and results were presented as pooled hazard ratios (HRs) with 95% confidence intervals (95% CIs). All analyses are repeated considering Alzheimer's dementia as outcome variable. Possible effect modification by sex, educational attainment, smoking status, and *APOE* ε4 genotype (carrier vs. non-carrier) was investigated by including a multiplicative interaction terms to the MIND diet score in model II and if the interaction term was statistically significant ($p<0.05$), we performed stratified analyses.

To examine if single food components of the MIND diet drove the observed associations, we repeated analyses with versions of the MIND diet score for which each individual food component was one at a time excluded from the total score and included as covariate in het model. Furthermore, as the MIND diet covers five unhealthy food components which are not covered in the Dutch dietary guidelines and Mediterranean diet, we excluded all unhealthy food components all together from the total score and included these five components as covariate in the model to determine whether these unhealthy food components together drove the association.

To ensure the robustness of our findings, we conducted several sensitivity analyses. First, we repeated analyses for participants above and below the age of 75 years separately. Second, as cognitive impairment may have influenced the reliability of dietary recall, we repeated the analyses after excluding participants with an MMSE score of <26 at time of dietary assessment. Third, participants with a history of stroke at dietary assessment were excluded and censored at the date of incidence stroke. Fourth, because having depressive symptoms is an important confounder in the association between dietary intake and dementia risk, but for 58.2% of the participants we had no data on depressive symptoms, we repeated the analyses after excluding all participants with depressive symptoms or missing data on depressive symptoms. Finally, we repeated analyses after excluding the first 5 years of follow-up, to assess potential reverse causality.

All statistical analyses were conducted using R Statistical Software version 4.0.3.

RESULTS

Characteristics of the study population at baseline I and II are presented in **Table 1**. In **Supplementary Table 2**, characteristics of the study population are also presented stratified by tertiles of the MIND diet score. Participants at baseline II were on average older, higher educated, less often current smokers, and less physical active than participants at baseline I. **Supplementary Table 3** provides baseline characteristics stratified by age above and below 75 years and shows that differences in physical activity levels between the baselines in mainly attributable to age.

Table 1. Baseline characteristics of the study population.

	Baseline I (between 1989-1993) (N=5,375)	**Baseline II (between 2009-2012) (N=2,861)**
Sex (women)	3,169 (59.0)	1,643 (57.4)
Age (years)	67.7 ± 7.8	75.3 ± 5.9
Education attainment		
Primary	1,102 (20.6)	192 (6.8)
Lower	2,281 (42.6)	1,215 (43.3)
Intermediate	1,504 (28.1)	930 (33.1)
Higher	463 (8.7)	469 (16.7)
Smoking status		
Never	1,800 (33.7)	914 (32.0)
Former	2,296 (43.0)	1,667 (58.4)
Current	1,247 (23.3)	274 (9.6)
Body mass index (kg/m^2)	26.3 ± 3.6	27.4 ± 4.2
Physical activity (MET h/week)	83.4 ± 43.9	50.0 ± 45.4
Daily energy intake (kcal/day)	1,974 ± 499	1,994 ± 658
Diabetes (yes)	357 (6.9)	418 (15.2)
Hypercholesterolemia (yes)	3,602 (67.1)	1,549 (56.1)
Hypertension (yes)	3,202 (59.8)	2,461 (87.0)
Depressive symptoms (yes)	314 (18.7)	713 (15.7)
History of stroke (yes)	98 (1.8)	145 (5.1)
APOE ε4 carrier (yes)	1,416 (27.5)	703 (26.1)
Diet scores, mean ± standard deviation (range)		
MIND diet	5.9 ± 1.3 (2.0-11.5)	7.4 ± 1.6 (1.5-13.5)
Dutch dietary guidelines	6.8 ± 1.8 (1-13)	6.8 ± 1.8 (1-13)
Mediterranean diet	36.7 ± 3.3 (20-47)	37.1 ± 4.1 (19-51)

Data are shown for non-imputed data and are presented as mean ± standard deviation for continuous variables and number (percentages) for categorical variables unless stated.

Participants mean MIND diet score was 5.9 (SD: 1.3) at baseline I and 7.4 (SD: 1.6) at baseline II on a theoretical range from 0 (no adherence) to 15 (full adherence). Adherence scores of the individual food components are presented in **Supplementary Table 4**. Participants at baseline I had on average higher adherence scores for berries, beans, and fish and lower adherence scores for fried food than participants at baseline II. A total of 1,244 participants were both included in baseline I and II and therefore had two dietary assessments available. Pearson correlation coefficient between the first and second MIND diet score was 0.25. Moreover, the MIND diet score was moderately correlated with the Dutch dietary guidelines score (r: 0.42 at baseline I, and r: 0.51 at baseline II) and Mediterranean diet score (r: 0.46 at baseline I, and r: 0.55 at baseline II).

Table 2. Diet scores in association with the risk of all-cause dementia.

	Hazard ratio (95% confidence interval)		
	Model I	**Model II**	**Model III**
Baseline I (between 1989-1993)			
n/N: 1,188/5,375			
MIND diet score	0.99 (0.93-1.05)	1.00 (0.94-1.06)	0.99 (0.94-1.05)
Dutch dietary guidelines score	1.01 (0.95-1.07)	1.01 (0.96-1.07)	1.01 (0.96-1.07)
Mediterranean diet score	1.03 (0.97-1.10)	1.04 (0.98-1.10)	1.04 (0.97-1.10)
Baseline II (between 2009-2012)			
n/N: 248/2,861			
MIND diet score	0.79 (0.70-0.90)	0.80 (0.70-0.91)	0.79 (0.70-0.91)
Dutch dietary guidelines score	0.88 (0.78-1.00)	0.90 (0.79-1.02)	0.89 (0.78-1.02)
Mediterranean diet score	0.76 (0.67-0.86)	0.76 (0.66-0.86)	0.75 (0.66-0.86)

Hazard ratios are provided per standard deviation increase in diet score. Model I is adjusted for sex, age, age^2, and educational attainment. Model II is further adjusted for smoking status, physical activity, and daily energy intake. Model III is further adjusted body mass index, diabetes, hypercholesterolemia and hypertension. Abbreviation: MIND, Mediterranean- Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay; n, number of participants with incident all-cause dementia; N, total number of participants.

Mean follow-up from baseline I was 15.6 years (range: 0.0-27.7), during which 1,188 participants developed all-cause dementia (incidence rate: 14 per 1,000 person-years). When considering overall follow-up time, the MIND diet score at baseline I was not associated with the risk of dementia (model II adjusted HR [95% CI] per SD increase: 1.00 [0.94-1.06]), neither were the Dutch dietary guidelines and Mediterranean diet score (**Table 2**). Mean follow-up from baseline 2 was 5.9 years (range: 0.0-9.1), during which 248 participants developed dementia (incidence rate: 15 per 1,000 person-years). A higher MIND diet score at baseline II was associated with a lower risk of dementia (model II adjusted HR [95% CI] per SD increase: 0.80 [0.70-0.91]), and so were the Dutch dietary

guidelines (model II adjusted HR [95% CI] per SD increase: 0.90 [0.79-1.02]) and Mediterranean diet score (model II adjusted HR [95% CI] per SD increase: 0.76 [0.66-0.86]). Additional adjustment for covariates that may be confounders or mediators in this association (model III) did not substantially alter the risk estimates. No evidence was found for non-linear associations between the diet scores at either baseline I or II and the risk of dementia (p >0.05).

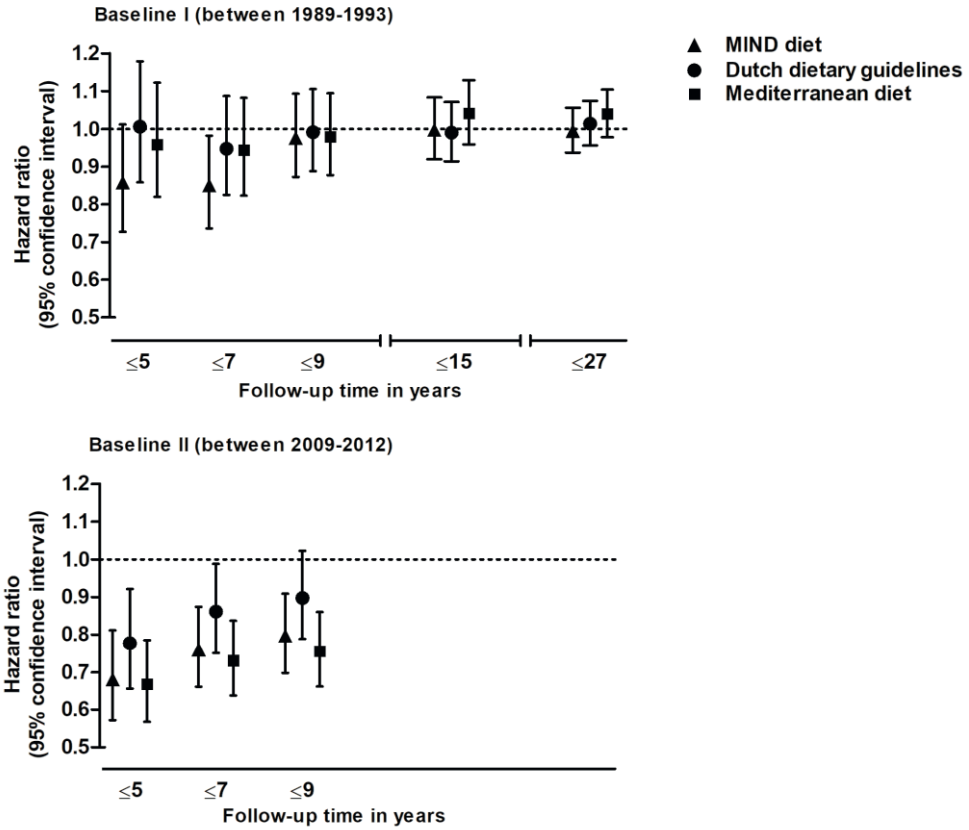

Figure 2. Diet scores in association with the risk of all-cause dementia per cumulative follow-up interval. Hazard ratios are provided per standard deviation increase in diet score and adjusted for sex, age, age^2, educational attainment, smoking status, physical activity, and daily energy intake. Abbreviations: MIND, Mediterranean- Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay.

When analyzing cumulative follow-up intervals from baseline I, a higher MIND diet score was associated with a lower dementia risk over 5 and 7 years of follow-up, but associations were no longer present over longer follow-up periods (**Figure 2**). The Dutch

dietary guidelines and Mediterranean diet score at baseline I were not associated with the risk of dementia during any cumulative follow-up interval. From baseline II, a higher MIND diet score was associated with a lower risk of dementia during each cumulative follow-up interval, but risk estimates were strongest within the first 5 years of follow-up and slightly attenuated over longer follow-up periods. Higher Dutch dietary guidelines and Mediterranean diet scores at baseline II were also associated with a lower risk of dementia during every cumulative follow-up interval, and risk estimates also slightly attenuated over time.

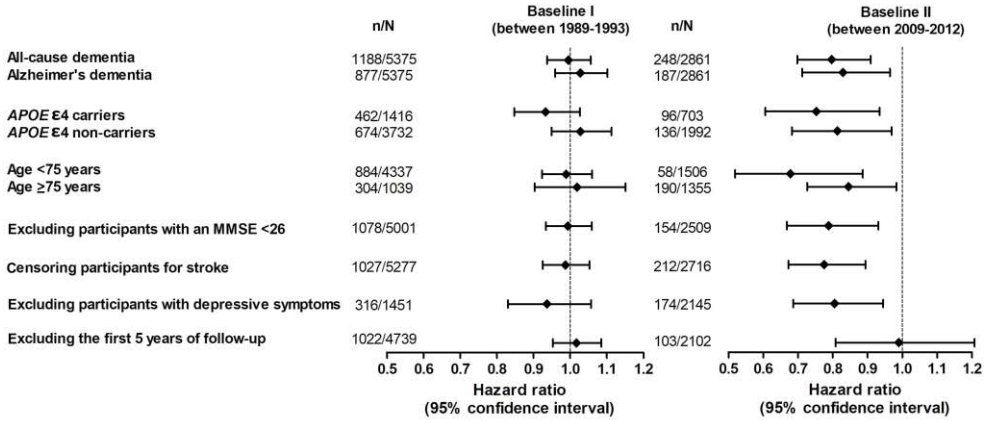

Figure 3. Subgroup and sensitivity analyses for the MIND diet score in association with the risk of all-cause dementia. Hazard ratios are provided per standard deviation increase in diet score and adjusted for sex, age, age^2, educational attainment, smoking status, physical activity, and daily energy intake. P for *APOE* ε4 carrier status interaction: 0.11 for baseline I and 0.01 for baseline II. Abbreviations: *APOE*, apolipoprotein ε; MIND, Mediterranean- Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay; MMSE, Mini-Mental State Examination; n, number of participants with incident dementia; N, total number of participants.

Associations between the MIND diet score and Alzheimer's dementia were similar as for all-cause dementia (**Figure 3**). Effect estimates were somewhat larger in *APOE* ε4 carriers compared to non-carriers (p-value for interaction: 0.11 for baseline I and 0.01 for baseline II). From baseline II, effect estimates were slightly larger in participants aged <75 years compared to those aged ≥75 years, but associations from baseline I were similar between these age groups. No meaningful differences in risk estimates were observed after excluding participants with an MMSE score of <26, after excluding participants with a history of stroke and while censoring during follow-up at the data of incidence stroke, and after excluding participants who had depressive symptoms or missing data on depressive symptoms. In line with our findings stratified by follow-up time, associations

were no longer present after excluding the first 5 years of follow-up. Moreover, associations were not driven by one individual food component or by the five unhealthy food components all together (data not shown) and there was no evidence for effect modification by sex, educational attainment or smoking status (p for interaction: >0.05).

DISCUSSION

In this population-based study, better adherence to the MIND diet between 1989-1993 was associated with a lower risk of dementia over the first 7 years of follow-up, but associations disappeared after longer follow-up periods. Better adherence to the MIND diet between 2009-2021 was associated with a lower risk of dementia over every cumulative follow-up period (maximum of 9 years), but risk estimates slightly attenuated over time. Besides, MIND diet adherence between 2009-2012 was, when comparing similar follow-up intervals, more strongly associated with the risk of all-cause dementia than MIND diet adherence between 1989-1993. Compared to other healthy diets, associations of the MIND diet were stronger or similar.

Few previous longitudinal studies reported on MIND diet adherence in association with cognitive performance,[7, 8] cognitive decline,[3, 7-11] or dementia risk.[12, 13] While some studies found an association between better MIND diet adherence and less cognitive decline,[3, 9-11] others only observed an association with cognitive performance at baseline.[7, 8] Furthermore, the Memory and Aging project found that better MIND diet adherence was associated with a lower risk of Alzheimer's dementia over an average period of 4.5 years (maximum of 9 years).[12] In the Personality and Total Health Through Life Study, persons who better adhered to the MIND diet were less likely to have all-cause dementia or mild cognitive impairment 12 years after baseline.[13] Our findings of an association within the first years of follow-up build on results from these previous studies, but associations in our study attenuated over time and disappeared after long-term follow-up periods. Studies have shown that dietary habits deteriorate up to 5 years before dementia diagnosis[15, 16] due to prodromal dementia symptoms such as depressive symptoms[17] and olfactory impairment.[18] This implies that when restricting follow-up time to 5 years, incident cases underwent dietary assessment while dietary habits have deteriorate, which could suggest that our findings of attenuating effect estimates may in part be explained by reverse causality.

Observational studies on diet and dementia risk are generally susceptible to residual confounding from lifestyle factors. More specifically, individuals with a healthy diet probably also lead an overall healthy lifestyle,[19, 20] encompassing meeting recommendations of physical activity and sleep, having lifelong cognitive training and sufficient levels of social contact, and timely visiting general practitioners by adverse health outcomes. Such behavioral factors are challenging to fully control for as data on such confounders is often not collected, impossible to measure or imprecisely measured (i.e. self-reported or categorized).[19, 20] As research and communication on the importance of healthy nutrition and lifestyle has strongly increased over the past few decades and thereby healthy diet and lifestyle awareness in the general population,[21] we speculate that the relation between adherence to the MIND diet and an overall healthy lifestyle has become stronger over time. More specific, individuals who care most about their health adhere to a healthy diet and lifestyle and those who care less about their health adhere to a less healthy diet and lifestyle. Against this background, we used historical and more contemporary dietary data to determine the risk of dementia and found a substantially stronger association when considering MIND diet adherence measured in more contemporary years (1989-1993 vs 2009-2012). This may suggest that residual confounding by lifestyle explains these discrepancies.

We do not rule out that observed attenuating risk estimates and discrepancies in the strength of associations across different baselines can be explained by other factors than reverse causality and residual confounding. A potential alternative explanation may be changes in dietary habits independent of the prodromal dementia phase. This could for instance be due to greater diversity and more affordable prices of both healthy and unhealthy products, and less seasonal dependence.[29] Indeed, the correlation of the MIND diet score after 20 years of follow-up was relatively low (r=0.25). Besides, participants had between 1989-1993 an average MIND diet score of 5.9, which is relatively low compared to the average score of 7.4 that participants had between 2009-2012. This difference in mainly attributable to a higher consumption of berries, beans, fish, and fried food between 2009-2012. The average MIND diet score between 1989-1993 was also relatively low compared to average scores from previous studies that ranged from 6.3 to 9.4. Yet, most previous studies did not report on average individual food components scores, limiting direct comparisons with our study. Another alternative explanation for discrepancies in strength of associations across baselines can be differences in population characteristics. Participants between 2009-2012 were generally older, higher educated, less often smokers, and less physical active than participants between 1989-1993. However, differences in physical activity levels are mainly attributable to the difference in age and we did not observe major differences in effect estimates after

repeating the analyses for participants above and below the age of 75 years. Moreover, we did not find evidence for effect modification by educational attainment or smoking status. We therefore assume that observed discrepancies across baselines cannot be explained by differences in these lifestyle factors.

In line with other studies,[3, 10, 12, 13] we found that associations for the MIND diet were stronger or similar compared to associations for other healthy diets, which support an effect of the MIND diet on the risk of dementia. The MIND diet emphasizes several food components, among which uniquely green leafy vegetables and berries, that could protect against dementia through its anti-inflammatory and ant-oxidative capacity.[4-6] Moreover, the MIND diet may protect against dementia through its favorable effects on cardiovascular risk factors such as obesity, insulin resistance, hypercholesterolemia, and hypertension.[30, 31] To further elucidate to what extent the MIND diet is accountable for the risk of dementia, we encourage future studies to compare long-term trajectories of adherence to the MIND diet before dementia diagnoses to trajectories of healthy controls, to link long-term MIND diet adherence to pre-clinical markers of dementia, and to conduct intervention studies.

Strengths of our study include its prospective population-based design with dietary intake measurements between 1989-1993 as well as between 2009-2013, and the long follow-up for incidence dementia. This unique combination of data allowed us to link MIND diet adherence at both periods to the risk of dementia over cumulative follow-up periods. We could thereby create more insight in whether reverse causality and residual confounding by lifestyle modify the association. However, there are also some limitations that should be taken into account when interpreting our results. First, different FFQs that varied in number of items were used to determine dietary intake at baseline I and II, which complicates direct comparison. However, the main differences between the FFQs was the level of detail on the food items, rather than the food items itself. Only the items that summarize the components “fried foods” and “pastries and sweets” varied substantially between baseline I and II, but we found no major differences in effect estimates after repeating the analyses with versions of the MIND diet score from which these food groups were excluded. Also, validation studies have shown that both FFQs can be used to rank participants adequately according to their dietary intake.[23-25] We therefore believe that comparisons of associations between the baselines are reliable. Second, strawberries were the only berries specified under fruit intake in the FFQs, while other berries such as blueberries, blackberries and raspberries are also included in the MIND diet. Last, dietary habits were self-reported based on FFQs, while participants with cognitive impairment as a result of prodromal dementia may have not been able to recall their dietary habits

accurately. Yet, we did not observe meaningful differences in risk estimates after excluding participants with an MMSE score of <26.

In conclusion, better adherence to the MIND diet is associated with a decreased risk of dementia within the first years of follow-up, but this may in part be explained by reverse causality and residual confounding by lifestyle. Further research is needed to unravel to which extend the MIND diet may affect the risk of dementia by for instance focusing on MIND diet adherence trajectories before dementia diagnosis, studying MIND diet adherence in association with pre-clinical markers of dementia, and by conducting intervention studies.

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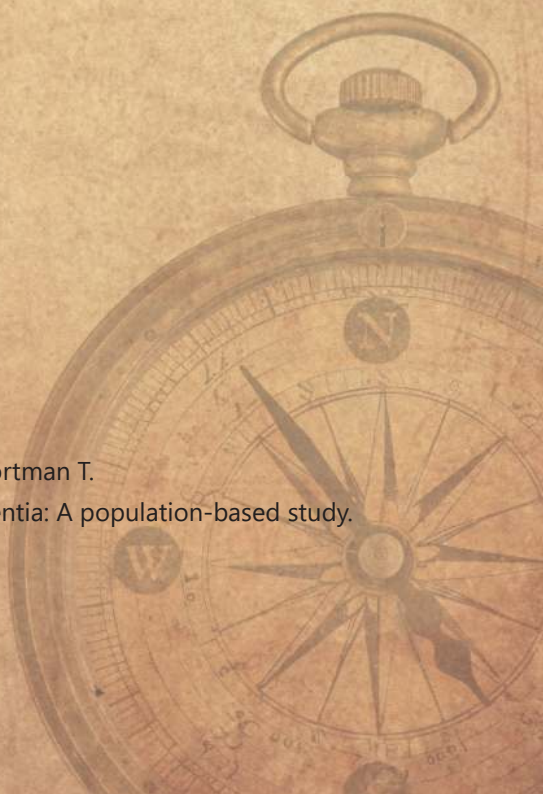
SUPPLEMENTARY MATERIAL

Chapter **2.3**

Plant-based diet and dementia



de Crom TOE, Steur M, Ikram MK, Ikram MA, Voortman T.
Plant-based dietary patterns and the risk of dementia: A population-based study.

Age and Ageing. 2023;52(9):178.

ABSTRACT

Introduction: Plant-based dietary patterns are increasingly popular in Western counties and are supported by many governments and health organizations for their potential beneficial role in the prevention of chronic diseases. Yet, the potential role of plant-based dietary patterns in the development of dementia remains unclear. We therefore evaluated the association between plant-based dietary patterns and the risk of dementia.

Methods: Dietary intake was measured at baseline in 9,543 dementia-free participants (mean age 64 years, 58% women) of the prospective population-based Rotterdam Study, using food frequency questionnaires. Based on these questionnaires, we calculated an overall plant-based dietary index (PDI), healthy PDI (hPDI), and unhealthy PDI (uPDI), with higher scores reflecting higher consumption of (any, healthy and unhealthy, respectively) plant-based foods and lower consumption of animal-based foods. We analyzed the association of the PDIs with incident dementia using Cox proportional hazard models.

Results: During a mean follow-up of 14.5 years, 1,472 participants developed dementia. Overall, the PDIs were not associated with the risk of dementia (hazard ratio [95% confidence interval] per 10 points increase: 0.99 [0.91-1.08] for PDI, 0.93 [0.86-1.01] for hPDI, 1.02 [0.94-1.10] for uPDI). However, among men and *APOE* ε4 carriers, the hPDI was linearly associated with a lower risk of dementia (0.86 [0.75-0.99] and 0.83 [0.73-0.95], respectively), while this association was U-shaped among *APOE* ε4 non-carriers (p-value for non-linearity: 0.01).

Conclusions: We found no strong evidence for an overall association between plant-based eating and the risk of dementia. Our findings in stratified analyses warranted further investigation.

INTRODUCTION

Plant-based dietary patterns are increasingly popular in Western counties and are supported by many governments and health organizations for their potential beneficial role in the prevention of chronic diseases.[1, 2] Indeed, diets with relatively more plant- and fewer animal-based foods have been linked to a lower risk of type 2 diabetes [3, 4] and cardiovascular diseases,[5, 6] which are in turn important risk factors for dementia.[7-9] Yet, the potential role of plant-based dietary patterns in the development of dementia remains unclear.

Healthy plant-based foods like vegetables, whole grains, fruits, nuts, and legumes, are sources of various health promoting nutrients that may affect the brain through their anti-inflammatory and anti-oxidative capacities,[10] along with their protective properties against metabolic abnormalities.[4, 11, 12] Moreover, although animal-based foods contain essential nutrients that may support brain health,[13, 14] various animal-based foods, including meat and full-fat dairy, are also sources of saturated fatty acids, of which excessive consumption has been linked to poor brain health.[15, 16]

Against this background, the Mediterranean diet has extensively been studied in relation to the risk of dementia, with most studies finding an inverse link.[17, 18] The Mediterranean diet mainly emphasizes plant-based foods, but in contrast to plant-based dietary patterns, moderate consumption of fish, poultry and dairy products is also incorporated. Given these distinct characteristics, one prospective cohort study has investigated the association of plant-based eating, in the form of a vegetarian diet (i.e. consuming no meat and fish), with the risk of dementia.[19] In this Taiwanese population, vegetarians were at a lower risk of dementia compared to omnivores. Moreover, two observational studies found that diets with relatively more healthy plant- and fewer animal-based foods were associated with a slower decline in cognitive functioning.[20, 21] Yet, to date, no published studies have investigated the relative contribution of plant- and animal-based foods to the diet in relation to the risk of dementia.

We therefore aimed to evaluate the association between the degree of adhering to an overall, healthy and unhealthy plant-based dietary pattern and the risk of dementia.

METHODS

Study population

This study was embedded within the Rotterdam Study, a prospective population-based cohort study in the Netherlands, of which details have been described previously.[22] Briefly, the original study (RS-I) started in 1990 with 7,983 participants aged ≥55 years (birth years: 1897-1938), residing in the district Ommoord, a suburb of Rotterdam. The study was extended in 2000 (RS-II), with 3,011 participants who moved into the study area or reached the age of 55 years (birth years: 1906-1944). In 2006, the study was further enlarged (RS-III), with 3,932 participants aged ≥45 years (birth years: 1918-1960). Participants are invited for follow-up examination rounds every 3-6 years.

Participants from all three sub-cohorts contributed to the current analysis. However, out of the 14,926 participants, 5,189 lacked data on dietary habits at study entry due to the implementation of dietary assessment after a pilot round (n=271), prevalent dementia in combination with the lack of caregiver assistance to recall dietary habits (n=393) or undocumented reasons (n=4,525). We further excluded participants who had unreliable dietary data (i.e. energy intake of <500 or >5000 kcal/day) (n=38), had dementia at baseline (n=38), were not sufficiently screened for dementia (n=58) or did not sign informed consent for follow-up monitoring (n=60), leaving 9,543 participants eligible for the current study.

Dietary assessment

Dietary intake was assessed at baseline using validated food frequency questionnaires (FFQ). The approach used to quantify dietary intake for sub-cohort RS-I and RS-II was slightly different from sub-cohort RS-III. For sub-cohorts RS-I and RS-II, participants first completed a checklist in which they indicated whether they consumed any of the 170 pre-defined food items at least twice a month within the past year. Next, participants underwent an structured interview conducted by trained dieticians to specify food items and identified in which frequencies and amounts the food items were consumed. For sub-cohort RS-III, responses to a self-administered FFQ including questions on the consumption of 389 food items over the past month was used to assess dietary intake. Both FFQs have been validated against other dietary assessment methods which showed that based on these FFQs, participants can be ranked adequately according to their food and nutrient intake.[23-25]

Plant-based dietary index

From the obtained dietary data, we derived an overall plant-based dietary index (PDI), a healthy PDI (hPDI), and an unhealthy PDI (uPDI). These indices are based on previously created indices,[3] and slightly modified to reflect Dutch dietary habits.[4] Food items were divided into 22 food categories, among which 7 healthy plant-based (fruits, vegetables, whole grains, nuts, legumes, tea and coffee, vegetable oils), 4 unhealthy plant-based (refined grains, potatoes, sugary beverages, sweets) and 11 animal-based (low-fat milk, low-fat yogurt, full-fat milk, full-fat yogurt, cheese, fish, eggs, animal fat, unprocessed lean meat, processed and red meat, dessert and sugary dairy). Food items that were not clearly animal- or plant-based were categorized in a miscellaneous category, which was similar to alcohol consumption not included in the PDIs, but included in the statistical models to account for potential confounding. Participants' intake for each food category were scored 0-4, based on cohort-specific distribution in quintiles, with 0 representing the lowest and 4 representing the highest consumption levels of the respective food group. The PDI was created by summing scores of both healthy and unhealthy plant-based food groups and inverse scores of animal-based food groups (**Supplementary Table 1**). The hPDI was calculated by summing scores of healthy plant-based food groups and inverse scores of unhealthy plant-based and animal-based food groups. The uPDI was calculated by summing scores of unhealthy plant-based food groups and inverse scores of healthy plant-based and animal-based food groups. This resulted in a final score theoretically ranging from 0 to 88, with higher scores reflecting better adherence to the respective PDI.

Dementia

Participants were screened for dementia at baseline and every 3-6 years during follow-up examinations using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Those with an MMSE <26 or a GMS >0 were further examined using the Cambridge Examination for Mental Disorders in the Elderly diagnostic interview. Participants were also monitored for dementia on a continuous basis through an electronic link between the study database and medical records from general practitioners and the regional institute of outpatients mental health care. The final diagnosis was established by a consensus panel led by a neurologist, according to standard criteria for dementia (DSM-III-R), and for sub-diagnosis of Alzheimer's disease (NINCDS-ADRDA). Follow-up was completed until January 1st 2020 for 96% of the potential person-years.

Covariates

During home interviews, information on educational attainment (i.e. primary, lower, intermediate, higher), smoking status (i.e. never, former, current) and alcohol intake

(gram/day) was assessed using questionnaires. Energy intake in kcal/day was calculated from the FFQ data using the Dutch Food Composition Tables (NEVO). Physical activity was measured using a validated adapted version of the Zutphen Physical Activity Questionnaire for RS-I-3 and RS-II-1,[26] and the LASA Physical Activity Questionnaire for RS-III-1.[27] From this data, the Metabolic Equivalent of Task (MET)-hours per week were calculated. *APOE* genotype (i.e. no allele, 1 allele, 2 alleles) was obtained using polymerase chain reaction of coded DNA samples for RS-I and with bi-allelic TaqMan assay for RS-II and RS-III. Height and weight were obtained during a research center visit and body mass index (kg/m2) was calculated. Diabetes mellitus was defined as having a fasting serum glucose of ≥7.0 mmol/L, a random serum glucose level of ≥11.1 mmol/L, or use of blood glucose lowering medication. Serum concentrations of total cholesterol and high-density lipoprotein (HDL) cholesterol in mmol/L were measured in fasting blood samples and use of blood lipid-lowering medication was obtained from interviews. Systolic and diastolic blood pressure in mmHg was measured twice on the right arm with the participant in a sitting position using a random zero sphygmomanometer. The average of the two measurements was used for analysis and use of blood pressure lowering medication was obtained from interviews.

Statistical analysis

We investigated the association of the PDI, hPDI and uPDI per 10 points increase with incident dementia, using Cox proportional hazard models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). We confirmed that the proportional hazard assumption was not violated by visual inspection of the Schoenfeld residuals. To test for potential non-linear associations, we included splines with 2 knots to the indices and tested whether this improved the fit of the model using ANOVA and studied the association per quintile of the dietary indices. We used follow-up time in years as time scale and repeated analyses using age as time scale to verify that this did not affect our results. In order to obtain cause-specific HRs, participants were censored when they were diagnosed with dementia, died, were lost to follow-up or at the end of follow-up, whichever came first. All analyses were adjusted for sub-cohort, age, sex, and energy intake (model I) and further for educational attainment, alcohol intake, the miscellaneous food category, smoking status, physical activity, and *APOE* ε4 status (model II, main model). To reduce the risk of residual confounding, we further adjusted in an additional model for cardiometabolic risk factors that could act as a confounder or mediator, namely body mass index, diabetes, total cholesterol, high-density lipoprotein cholesterol, systolic and diastolic blood pressure, and use of blood pressure and lipid lowering medication (model III). We repeated the analyses considering Alzheimer's disease as only outcome. To explore effect modification by sex, age and *APOE* ε4 status, we included an interaction term between each of these covariates

and the PDIs, and we performed stratified analyses (i.e. women versus men, <70 versus ≥70 years, and *APOE* ε4 carrier versus non-carrier). Moreover, we repeated the analyses after excluding participants with an MMSE <26 to address potential recall bias due to cognitive impairment, and after excluding the first 5 years of follow-up to address potential reverse causality. In addition, we repeated the analyses considering the healthy plant-based, unhealthy plant-based and animal-based food category scores individually as exposure to explore if one of those individual categories explains potential associations. To provide insight into the role of competing risk by mortality, we visually assessed Kaplan-Meier survival curves with age as underlying timescale, per age, sex and sub-cohort specific quintile of the PDIs.

Missing data on covariates (18.6% for physical activity, 5.3% for *APOE* ε4 status, and <5% for all other covariates) were imputed using five-fold multiple imputation. Analyses were performed on the five datasets and pooled HRs with their corresponding 95% CIs are provided. Statistical analyses were conducted using the "survival", "splines" and "mice" packages from R Statistical Software version 4.0.3.

RESULTS

Baseline characteristics of the study population are provided in **Table 1**. Participants were on average 64.1 years old (standard deviation: 8.6) and 58% of the participants were women. Participants with a higher PDI were less likely to be women, had a higher daily energy and lower alcohol intake (**Supplementary Table 2**), while participants with a higher hPDI and uPDI were more likely to be women and had a lower energy intake (**Supplementary Table 3 and 4).** The uPDI was higher among lower educated and older participants. As expected, participants with a higher PDI consumed more healthy and unhealthy plant-based foods and less animal-based foods (**Supplementary Table 5)**. Moreover, participants with missing data on dietary intake for reasons unrelated to dementia were somewhat older and lower educated compared to those with data on dietary intake (**Supplementary Table 6**).

The PDI was positively correlated with the hPDI (r: 0.56) and uPDI (r: 0.25), while no correlation between the hPDI and uPDI was observed (r: 0.06).

Table 1. Baseline characteristics of the study population.

Characteristics	Study population (N=9,543)
Women	5,530 (58)
Age, years	64.1 (8.6)
Education attainment	
Primary	1,463 (15)
Lower	3,900 (41)
Intermediate	2,662 (28)
Higher	1,463 (15)
Energy intake, kcal/day	2,099 (595)
Alcohol intake, grams/day	11.9 (16.2)
Miscellaneous food intake, grams/day	59.2 (64.9)
Smoking status	
Never	2,231 (23)
Former	4,152 (44)
Current	3,114 (33)
Physical activity, MET-hours/week	75.9 (49.4)
APOE ε4 status	
No allele	6,482 (72)
1 allele	2,353 (26)
2 alleles	205 (2)
Body mass index, kg/m^2	26.8 (4.0)
Diabetes	728 (8)
Systolic blood pressure, mmHg	137.7 (21.4)
Diastolic blood pressure, mmHg	76.9 (11.7)
Use of blood pressure lowering medication	2,754 (29)
Total cholesterol, mmol/L	6.3 (1.2)
High-density lipoprotein cholesterol, mmol/L	1.4 (0.4)
Use of lipid lowering medication	911 (10)
Overall plant-based dietary index (PDI)	44.7 (6.5)
Healthy plant-based dietary index (hPDI)	45.1 (7.0)
Unhealthy plant-based dietary index (uPDI)	46.9 (7.4)

Data are shown for non-imputed data and are presented as mean (standard deviation) for continuous variables and number (percentages) for categorical variables. Abbreviations: MET, Metabolic Equivalent of Task; N, number of participants.

During a mean follow-up of 14.5 years (range: 0.0-29.7), 1,472 participants developed dementia (incidence rate 10.6 per 1,000 person years). Overall, the PDI, hPDI and uPDI were not significantly associated with the risk of dementia (HR [95% CI] per 10 points increase: 0.99 [0.91-1.08] for PDI, 0.93 [0.86-1.01] for hPDI, and 1.02 [0.94-1.10] for uPDI; **Table 2**). Analyzing the PDIs in quintiles, showed that the association for the hPDI was suggestively U-shaped, but this was not confirmed by non-linear analysis (p-value: 0.08; **Figure 1**). No

evidence for a non-linear association was observed for the PDI and uPDI (p-value: 0.90 and 0.37, respectively).

Table 2. Plant-based dietary indices and the risk of dementia.

		Hazard ratio (95% confidence interval)		
	n/N	Model I	Model II	Model III
Overall plant-based dietary index				
Per 10 points increase	1,472/9,543	1.04 (0.96-1.13)	0.99 (0.91-1.08)	0.99 (0.91-1.08)
Per quintile:				
Quintile 1 (22-39)	283/2,084	1.00	1.00	1.00
Quintile 2 (39-43)	295/2,093	0.95 (0.80-1.11)	0.99 (0.84-1.17)	1.01 (0.85-1.19)
Quintile 3 (43-46)	272/1,711	1.03 (0.87-1.22)	1.07 (0.90-1.27)	1.09 (0.92-1.30)
Quintile 4 (46-50)	314/1,902	1.04 (0.88-1.22)	0.99 (0.84-1.17)	1.01 (0.85-1.20)
Quintile 5 (50-73)	308/1,753	1.12 (0.95-1.33)	1.03 (0.86-1.22)	1.04 (0.87-1.24)
Healthy plant-based dietary index				
Per 10 points increase	1,472/9,543	0.94 (0.87-1.02)	0.93 (0.86-1.01)	0.93 (0.75-1.01)
Per quintile:				
Quintile 1 (16-39)	280/2,059	1.00	1.00	1.00
Quintile 2 (39-43)	293/1,866	0.91 (0.77-1.08)	0.94 (0.80-1.12)	0.94 (0.80-1.12)
Quintile 3 (43-47)	326/2,117	0.85 (0.73-1.00)	0.84 (0.71-0.99)	0.84 (0.71-1.00)
Quintile 4 (47-51)	274/1,755	0.79 (0.66-0.93)	0.78 (0.65-0.93)	0.77 (0.65-0.92)
Quintile 5 (51-73)	299/1,746	0.90 (0.76-1.07)	0.90 (0.75-1.07)	0.89 (0.74-1.06)
Unhealthy plant-based dietary index				
Per 10 points increase	1,472/9,543	1.03 (0.95-1.11)	1.02 (0.94-1.10)	1.02 (0.94-1.10)
Per quintile:				
Quintile 1 (19-41)	266/2,248	1.00	1.00	1.00
Quintile 2 (41-45)	276/1,868	1.09 (0.92-1.30)	1.13 (0.95-1.35)	1.15 (0.96-1.37)
Quintile 3 (45-49)	321/1,966	1.08 (0.92-1.28)	1.12 (0.94-1.33)	1.13 (0.95-1.34)
Quintile 4 (49-53)	304/1,656	1.23 (1.03-1.45)	1.23 (1.03-1.46)	1.25 (1.05-1.50)
Quintile 5 (53-74)	305/1,805	1.05 (0.88-1.25)	1.04 (0.86-1.24)	1.05 (0.87-1.26)

Model I is adjusted for sub-cohort, age, sex, and energy intake. Model II is further adjusted for educational attainment, alcohol intake, miscellaneous food intake, smoking status, physical activity, and *APOE* ε4 status. Model III is further adjusted for body mass index, diabetes, total cholesterol, high-density lipoprotein cholesterol, use of lipid lowering medication, systolic blood pressure, diastolic blood pressure, and use of blood pressure lowering medication. Abbreviations: n, participants with incident dementia; N, participants at risk of dementia at baseline.

Risk estimates were similar when considering Alzheimer's disease as only outcome (**Figure 2**). In analyses stratified by sex, age, and *APOE* ε4 carriership, the hPDI was linearly associated with a lower risk of dementia among men (HR [95% CI]: 0.86 [0.75-0.99]) and *APOE* ε4 carriers (HR [95% CI]: 0.83 [0.73-0.95]). Among *APOE* ε4 non-carriers, a U-shaped

association between the hPDI and the risk of dementia was observed (p-value for non-linearity: 0.01; **Figure 1**). Nevertheless, none of the interactions investigated were significant (p-values for interaction >0.05).

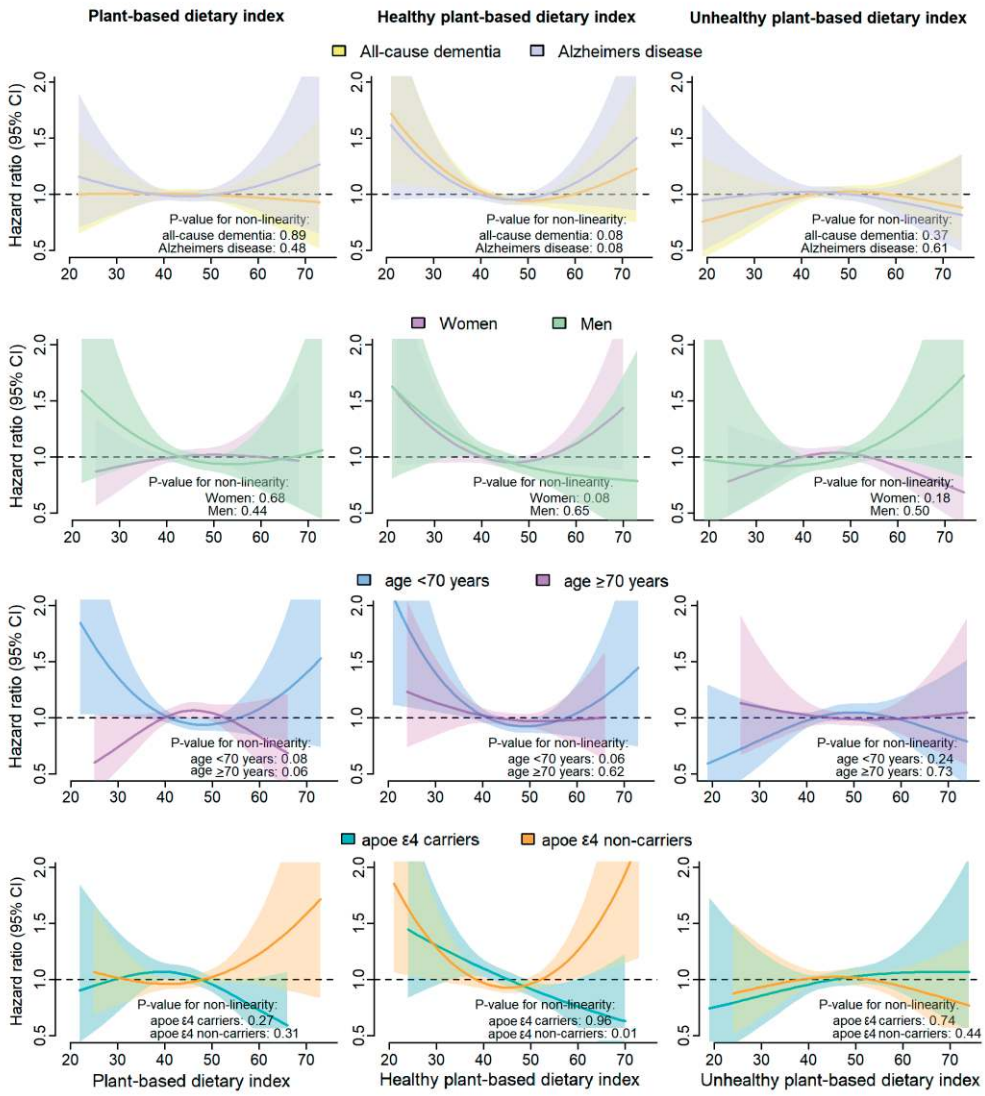


Figure 1. Subgroup analyses of non-linear association between the plant-based dietary indices and the risk of dementia. Splines with 2 knots are added in the model. The model is adjusted for sub-cohort, age, sex, energy intake, educational attainment, alcohol intake, miscellaneous food intake, smoking status, physical activity, and *APOE* ε4 status (corresponding to model II in Table 2). Abbreviations: CI, confidence.

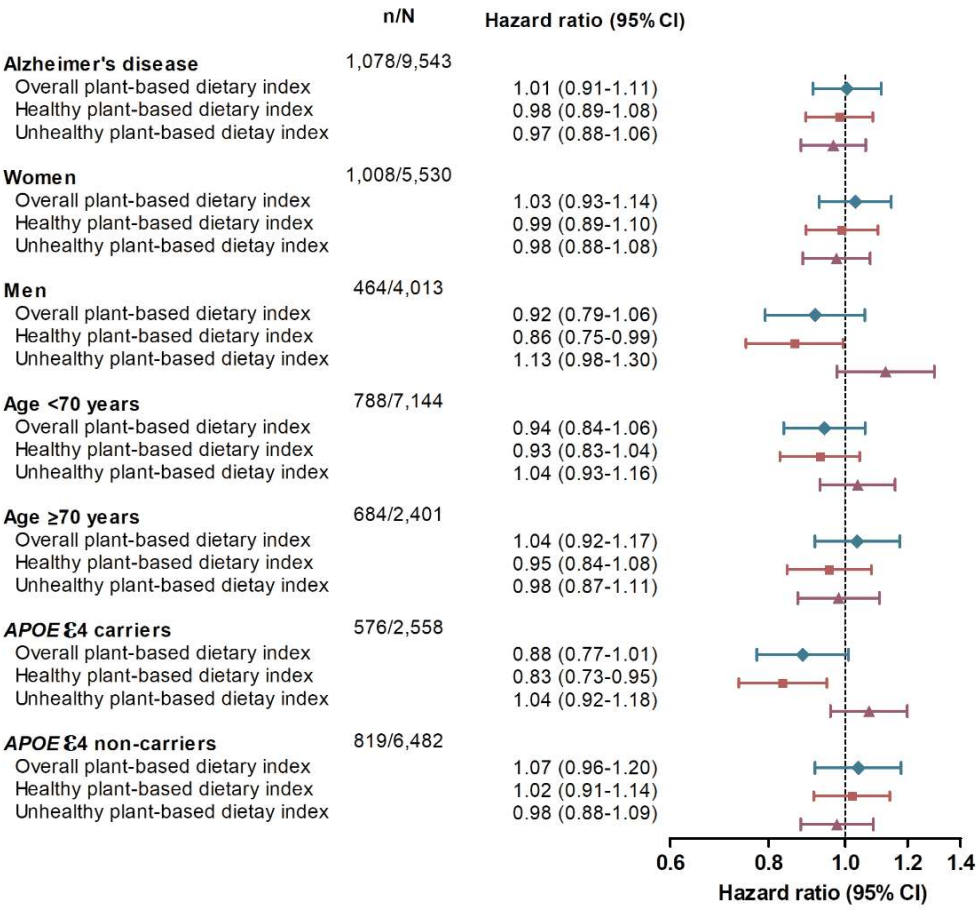


Figure 2. Subgroup analyses for the association of the plant-based dietary indices with the risk of dementia. Hazard ratios represent the association between the plant-based dietary indices per 10 points increase and the risk of dementia. Models are adjusted for sub-cohort, age, sex, energy intake, educational attainment, alcohol intake, miscellaneous food intake, smoking status, physical activity, and *APOE* ε4 status (corresponding to model II in table 2). None of the interaction terms reached statistically significance (p-value<0.05). Abbreviations: CI, confidence interval; n, participants with incident dementia; N, participants at risk of dementia at baseline.

Risk estimates for the PDI, hPDI, and uPDI remained similar after excluding participants with an MMSE score <26, and after excluding the first 5 years of follow-up (data not shown). Furthermore, the healthy plant-based, unhealthy plant-based and animal-based food categories were not associated with the risk of dementia (**Supplementary Table 7**). Finally, individuals in the lowest quantile of the PDI and hPDI had a somewhat lower survival than those in the highest quintile, while participants in the lowest quantile of the

uPDI had a higher survival compared to those in the highest quintile (**Supplementary Figure 1**).

DISCUSSION

In this population-based study, we found no association between the PDI – regardless of whether an overall, healthy or unhealthy PDI was defined – and the risk of dementia. Our findings in stratified analyses, including an inverse association between the hPDI and the risk of dementia among men and *APOE* ε4 carriers and the suggestion of a non-linear association among *APOE* ε4 non-carriers, warrants further investigation in future studies.

To our knowledge, we are the first to report on the relative contribution of plant- and animal based foods to the diet in relation to the risk of dementia. Previous studies, however, investigated the risk of dementia by various dietary patterns that are dominated by plant-based foods, such as the Mediterranean diet [17, 18, 28] and Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND) diet.[29-31] While most studies, including one in the Rotterdam Study,[29] have reported a positive association,[17, 18, 28, 30, 31] others reported no link.[17, 18] Moreover, a study among 5,710 Taiwanese participants found that vegetarians were at a lower risk of dementia compared to omnivores.[19] These findings support a role of (healthy) plant-based eating in the development of dementia, which contrasts the overall null findings observed in our study. Our findings also contradict a previous study among Chinese participants that linked a hPDI to a lower odds of mild cognitive impairment (defined as an MMSE <24).[20] Another study found a link between the hPDI and a slower rate of cognitive decline among African American, but not among white participants from the United States.[21] Notably, the composition of the diets substantially differed between African Americans and white participants, despite similar hPDI scores. For instance, compared to white participants, African Americans consumed more whole grains and sugar sweetened beverages, and fewer animal fat and dairy products. This may suggest that differences in diet composition explain contrasting findings across studies, but genetic variability and differences in lifestyle factors may also play a role.

Whilst we did not find statistically significant evidence to support an association of consuming relatively more plant- and fewer animal-based foods with the risk of dementia, our HR of 0.93 (95% CI: 0.86-1.01) for the hPDI cannot rule out a subtle beneficial effect of healthy plant-based eating on the brain. A subtle effect seems especially feasible as we

found that individuals with a lower hPDI had a somewhat lower survival rate, suggesting that mortality as competing event may has diluted the association towards the null.

Several mechanisms have been proposed that may explain a potential beneficial effect of healthy plant-based eating on dementia. Healthy plant-based diets consist of foods rich in various health promoting nutrients, such as fibers, antioxidants, and unsaturated fatty acids.[1] These nutrients may affect the brain through their anti-inflammatory and anti-oxidative capacities,[10] and favorable effects on metabolic functions.[3, 4, 12]

Although not confirmed based on non-linear analysis, we found that the association between healthy plant-based eating and the risk of dementia is somewhat U-shaped. This may suggest that an excessive reduction of animal-based products from the diet may no longer be beneficial, possibly as a result of deficiencies.[32] Animal-based foods are major sources of certain macro- and micronutrients, including vitamin B12, iron, long-chain n-3 fatty acids and proteins, which are crucial for brain health.[13, 14] Adequate consumption of these nutrients is particularly important for older adults, as requirements of nutrients increase with age, while energy needs decrease.[33, 34] Given that women in the present study generally had a higher hPDI, deficiencies may also explain our findings of a linear inverse association for the hPDI among men, which tended to be U-shaped among women.

That we found a linear inverse association among carriers of the *APOE* ε4 allele, but not among *APOE* ε4 non-carriers, suggest that healthy plant-based eating may counteract with pathological changes associated with the *APOE* gene. The *APOE* gene is a key regulator of lipid metabolism and the *APOE* ε4 allele has been linked to an unfavorable lipid profile,[35] which has subsequently been linked to an increased risk of dementia.[36] *APOE* ε4 carriers may benefit more from consuming predominantly healthy plant-based products which contain limited amounts of saturated fatty acids. Moreover, the *APOE* ε4 allele is mainly thought to affect the risk of dementia through impaired amyloid-β clearance, but also via susceptibility to neuro-inflammation, mitochondrial dysfunction and blood-brain barrier permeability.[34] Anti-inflammatory and anti-oxidative capacities of a healthy plant-based dietary pattern may diminish these effects.[10]

Strengths of this study include the population-based setting, large sample size and long follow-up for incident dementia. Moreover, the use of indices to determine the relative contribution of plant- and animal-based foods to the diet allowed us to study potential health effects on the brain beyond adopting a-priori defined vegetarian diets that are based on exclusion of specific animal-based food groups from the diet.

Certain limitations also need to be considered. First, none of the participants in this study were vegan and the majority of the participants in the highest PDI quintile consumed animal-based foods on a daily basis. This makes it impossible to extrapolate our findings to higher levels of plant-based eating. Second, dietary habits may change over time, but our data were too limited to take this into account. Long-term exposure could thus not be assured, which may have biased our findings. Third, information on supplementation use of certain vitamins and minerals that are mainly present in animal-based products was inadequate and could thus not be taken into account. Last, participants excluded from the study population due to missing data on dietary intake were somewhat older and lower educated. With both factors being related to poor diet quality and a higher risk of dementia,[37] this could have caused an underestimation of the true association on population level.

In conclusion, we found no strong evidence for an association between plant-based eating and the risk of dementia, regardless of whether the diet was dominated by healthy or unhealthy plant-based foods. Further studies that include participants with a greater variety in the proportion of plant- and animal-based foods in their diets are warranted to further evaluate the association of plant-based diets with the risk of dementia, and to disentangle the role of sex and the *APOE* gene in this association.

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SUPPLEMENTARY MATERIAL

Chapter **2.4**

Barriers and facilitators to adopt a plant-based diet

ABSTRACT

Introduction: Dietary patterns dominated by plant-based foods are generally more environmentally sustainable and have a beneficial impact on health. Nonetheless, the majority of the Western population, still consume substantial amounts of animal-based foods on a daily basis. We therefore aimed to identify barriers and facilitators to adopt a more plant-based dietary pattern through the use of focus groups among older adults from the Netherlands.

Methods: To optimize the design of the focus group sessions, a pilot study was performed. The pilot focus group was semi-structured and conducted among five participants from a pre-defined panel of the ongoing prospective population-based Rotterdam Study. The objectives were to identify potential logistical and methodological challenges for the focus group sessions and to obtain preliminary insights into barriers and facilitators to adopt a more plant-based dietary pattern.

Results: The pilot session unfolded smoothly, with active participant engagement. None of the questions asked were misinterpreted, but participants easily digressed off-topic. Participants were well-aware of the meaning of plant-based eating and its advantages. Accordingly, all participants reported that they had reduced their meat consumption over the years, but not their consumption of dairy, fish or eggs. They were also willing to consume fewer animal-based foods, as long as nobody forced them. The most important barrier identified was their taste preference for meat. Enhancing awareness of the advantages of plant-based eating on the environment and health was seen as the most effective motivation for adopting a more plant-based dietary pattern.

Discussion: This pilot study has demonstrated the effectiveness of a focus group session for the identification of both common and unique barriers and facilitators to adopt a more plant-based dietary pattern, while it simultaneously provided preliminary results.

INTRODUCTION

The current global food system is a major contributor to biodiversity loss, water pollution and greenhouse gas emission, which is mainly attributable to the production of animal-based foods.[1, 2] Plant-based foods are generally more sustainable as the production requires fewer resources and generates less greenhouse gas emissions.[1, 3] Moreover, dietary patterns with more plant-based and fewer animal-based foods have been linked to lower risks of several adverse health outcomes, including cancer,[4] diabetes,[5, 6] and cardiovascular diseases.[7] These beneficial effects have led to a widespread call from governments and health organizations to increase plant-based and reduce animal-based food consumption.[8, 9] Although dietary patterns dominated by plant-based foods are gaining popularity in Western countries, the majority of the Western population still consumes substantial amounts of animal-based foods on a daily basis.[10-12]

Several studies have investigated potential barriers and facilitators to adopt a more plant-based dietary pattern, with the most prominent barriers identified being a lack of knowledge on the advantages of plant-based eating, high costs, and taste preference for animal-based foods.[13-15] However, given the rising attention for climate change, health and animal well-being, the consumption pattern of plant- versus animal-based foods has changed rapidly over the past few years.[10, 16] Consequently, the previously mentioned barriers and facilitators to adopt a more plant-based dietary pattern may be outdated. Furthermore, barriers and facilitators to adopt a more plant-based dietary pattern vary among populations.[8] The lack of prior studies conducted in the Netherlands, highlight the need for investigations among its residents to develop interventions addressing considerations of the Dutch population in adopting a more plant-based dietary pattern.

Both common and unique barriers and facilitators for shifting towards a more plant-based dietary pattern can be identified using focus groups among diverse samples of individuals. To optimally design such focus groups, we conducted a pilot focus group among older adults from the Netherlands, aiming to identify potential logistical and methodological issues in the design of the focus groups and to obtain preliminary results on barriers and facilitators to adopt a more plant-based dietary pattern.

METHODS

Participants

For the upcoming focus group sessions, participants will be recruited from the Rotterdam Study, an ongoing prospective population-based cohort study including 17,931 participants aged 40 years and older.[17] For this pilot study, participants were recruited from a pre-defined panel of Rotterdam Study participants. This panel consists of 25 individuals from different age categories who volunteered to give their feedback and perspective on, for instance, the Rotterdam Study design, ethical considerations, and ideas for future research directions.

Procedure

The pilot focus group session was held at the research center of the Rotterdam Study. At the start of the session, we confirmed that participants did not know each other because if they did, they may have felt uncomfortable talking in each other's presence. The focus group was semi-structured and led by a moderator. At the beginning of the session, the moderator welcomed all participants and provided a brief overview of the aim of the focus group. Importantly, the moderator explicitly stated that everyone's point of view was of interest, even if it differed from what other participants say. Thereafter, the moderates started leading the discussion according to an a priori defined topic list, consisting of open-ended questions, and possible follow-up questions (**Table 1**). These questions were formulated by two researchers, among whom a sociologist who has experience with focus groups. Whether follow-up questions were asked depended on answers of the participants and the progress of the discussion. As the goal of the focus group was to generate discussion among participants, the moderator kept her participation in the discussion to a minimum in order to facilitate an open and unbiased discussion that allowed participants to freely express their perspectives.

RESULTS

Logistic and methodological insights

From the 25 invited participants, six individuals expressed interest in participating. Five participants attended the discussion, as one individual who expressed interest forgot about the scheduled session. The five participating participants were all retired men.

Overall, the pilot focus group discussion unfolded smoothly, characterized by a high level of participant engagement and interaction. Also, participants' responses indicated that they understood all of the main questions that were asked. Most follow-up questions were unnecessary, as participants often proactively addressed these points in response to the main questions. However, participants easily digressed off-topic.

Preliminary results

After asking participants to share their initial thoughts about plant-based eating, they unanimously referred to vegetarian and later on vegan dietary patterns. However, as the discussion progressed, they recognized the idea that individuals can adopt a more or less plant-based dietary pattern. All participants revealed that they were consuming less meat than 10 years ago, when they were all eating meat on a daily basis. Their current meat consumption varied between 4 to 6 days a week. Dairy products were consumed on a daily basis by all participants, while consumption levels of fish and eggs varied from once a month to multiple times a week. None of the participants reported changes in consumption levels of dairy, fish and eggs in recent years.

By asking participants about the pros and cons of plant- and animal-based foods, environmental sustainability emerged as a prominent topic. Participants generally believed that plant-based foods are more environmentally sustainable than animal-based foods. Moreover, participants widely perceived plant-based foods as healthier alternatives compared to those derived from animals. Animal welfare was also mentioned as a motivating factor for considering plant-based foods. Nevertheless, they did express their concerns about pesticides on fruits and vegetables, and the potential for high salt content in processed plant-based foods.

Overall, participants expressed a desire to consume fewer animal-based products, when asked about their dietary preferences, as long as nobody forced them to adjust their dietary habits by for instance limiting the availability of animal-based foods in restaurants and grocery stores. Also, participants expressed discomfort with judgments and criticisms from individuals who follow a vegetarian or vegan dietary pattern. The most important barrier to consume less animal-based foods was for all participants their taste preference for meat. Interestingly, almost all individuals mentioned that they were willing to spend more money on meat alternatives. Participants believed that enhancing awareness of the advantages of a more plant-based dietary pattern was the most effective way to motivate them to reduce their consumption of animal-based foods. They explicitly referred to advantages in terms of the environment and health, but they also suggested to show slaughterhouse footages on television to generate awareness of animal suffering.

Table 1. Topic list with question and possible follow-up questions

Subject	Question	Possible follow-up question(s)	Aim of question(s)
Knowledge	Wat is volgens u dierlijke voeding?	• Als vis, eieren en/of zuivel niet genoemd worden: Wat denkt u van vis, eieren en/of zuivel?	Whether the participants are aware of what plant- and animal-based foods are.
	Wat is volgens u plantaardige voeding?	• Als mensen niet door hebben wat niet dierlijk is, plantaardige voeding is: Wat denk u van bijvoorbeeld snoepjes, frietjes en een glas cola? • Vervolgvraag: Als iets niet dierlijk is, zou u dan zeggen dat het per definitie plantaardig is?	
Behavior	Wat is volgens u een plantaardig eetpatroon?	• In hoeverre komt dit eetpatroon overeen met uw eigen eetpatroon? • In hoeverre verschilt uw eetpatroon per dag in de week? • Hoe stabiel is uw eetpatroon de afgelopen jaren geweest? Bent u mogelijk meer of minder dierlijke of plantaardige voeding gaan eten?	Create insight in participants' dietary habits.
Attitude	Wat zijn volgens u de voor en nadelen van het eten van plantaardige voeding ten opzichte van dierlijke voeding?	• Ik hoor duurzaamheid/gezondheid. Kunt u daar wat meer over vertellen? En wat denken de andere daarvan?	Create insight into the attitude of the participants towards plant- and animal-based eating and thereby identify possible barriers and facilitators of adopting a more plant-based dietary pattern.
	Zou u meer of minder plantaardig of dierlijke voeding willen eten? (mogelijk wordt dit al genoemd, dan doorgaan met follow-up vragen)	• Waarom wel of niet? • Als duurzaamheid/gezondheid niet genoemd is in vorige vraag: Er wordt weleens gezegd dat plantaardig eten duurzaamheid bevorderd/goed is voor je gezondheid. Zou u meer plantaardig willen eten om duurzaamheid te bevorderen/gezonder te zijn. Follow-up: waarom wel of niet?	

		• Als duurzaamheid/gezondheid wel genoemd is, maar dat komt niet naar de voorgrond. Ik hoorde net ook iets over duurzaamheid/gezondheid. Hoe speelt dit een rol? • Wat zou voor u een reden zijn om uw voedingspatroon aan te passen? • Bij het noemen van barrières als kosten, niet zelf koken, stigma etc..: Als het goedkoper zou zijn, u zelf zou koken, stigma er niet zou zijn, zou u dan wel meer of minder dierlijke of plantaardige voeding eten? • *Toegevoegd voor aanstaande focusgroepen: Hoe zou u zich voelen als u geen dierlijke producten meer zou kunnen verkrijgen in restaurants, op uw werkplek of in de supermarkt? Wat denkt u dat de impact zou zijn op uw dagelijkse eetgewoonten en levensstijl?*	
Final remarks	Is er iets anders wat u zou willen zeggen over plantaardig of dierlijke eten en u voedselkeuzes?		Check if somebody wants to say something about plant- and/or animal-based eating

DISCUSSION

This pilot study demonstrate the effectiveness of a focus group session for the identification of a wide range of perspectives on both common and unique barriers and facilitators to adopt a more plant-based dietary pattern. Simultaneously, it provides preliminary insights on potential barriers and facilitators.

Although, the Rotterdam Study panel comprises a representative sample of participants from the entire cohort, those participating were exclusively retired men. This limits the generalizability of our findings to a more diverse population. For the upcoming focus groups, we should strive to recruit a more diverse group of participants by for instance offering flexible scheduling options. Moreover, of the six participants who expressed their interest in the study, five participants actually showed up, suggesting the importance of oversampling to ensure a sufficiently sized group of participants.

The focus group unfolded smoothly, with all participants proactively participating in the discussion. Participants understood all the questions, meaning that there is no need for any rephrasing. Nonetheless, participants easily digressed off-topic, indicating that the moderator should be proactive to keep discussions on track during the upcoming focus group sessions.

Preliminary results showed that enhancing knowledge on the advantages of plant-based eating was seen as the most promising facilitator to adopt a more plant-based dietary pattern. Nevertheless, participants were already well-aware of advantages like sustainability, health benefits, and considerations for animal well-being. Accordingly, participants reported that they had already reduced their meat consumption over the years, but not their consumption of dairy, fish or eggs.

Concerns associated with plant-based eating were also pointed out, regarding pesticides and the salt content of processed plant-based foods. This highlighted a potential misconception regarding salt content, as plant-based foods do not necessarily contain higher levels of salt compared to their animal-based counterparts.[18] Such misconception presents an opportunity for education and intervention.

Taste preference for meat consumption was identified as an important barrier in our study and thereby corroborates previous research findings.[13-15] This presents opportunities for the advancement of better tasting meat alternatives and possibly of cultured meat.

An interesting finding from this study was the participants' resistance to the idea of being forced to reduce their consumption of animal-based foods. This is a perspective that has, to the best of our knowledge, not been prominently identified in previous research.[13-15] In response to this finding, a follow-up question covering this topic has been incorporated into the topic list for future focus group sessions (**Table 1**). If these findings also emerge in the upcoming focus group sessions, it could open new avenues for further research.

In conclusion, this pilot study has demonstrated the effectiveness of using focus groups to identify barriers and facilitators to adopt a more plant-based dietary pattern. It has also offered crucial insights into both logistical and methodological challenges, and is thereby the first step towards conducting well-designed focus group sessions. Furthermore, preliminary findings concerning barriers and facilitators to adopt a more plant-based dietary pattern have been obtained.

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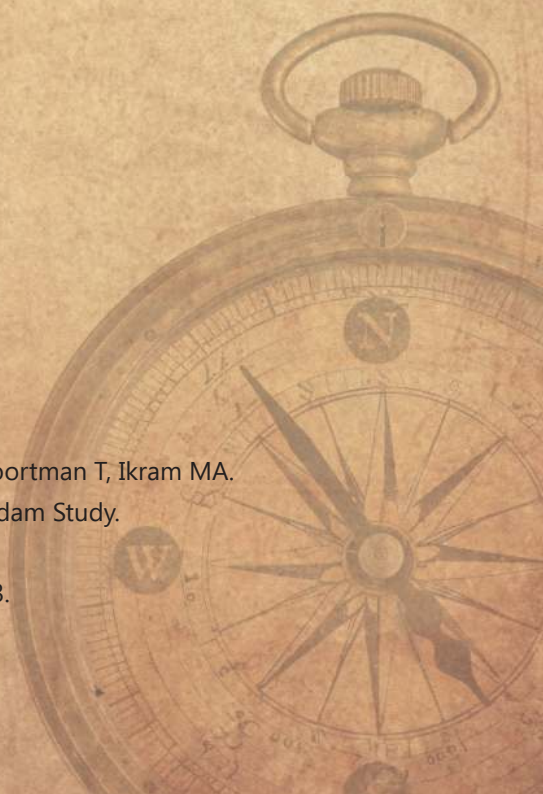
Chapter 3

Air pollution

Chapter **3.1**

Air pollution and dementia



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ABSTRACT

Introduction: Exposure to air pollution has been suggested to increase the risk of dementia, but studies on this link often lack a detailed screening for dementia and data on important confounders. We therefore determined the association of exposure to air pollution with the risk of dementia and cognitive decline in the population-based Rotterdam Study.

Methods: Between 2009 and 2010, we determined air pollutant concentrations at participants residential addresses using land use regression models. Determined air pollutants include particulate matter <10 μm (PM_{10}) and <2.5 μm ($PM_{2.5}$), a proxy of elemental carbon ($PM_{2.5}$ absorbance), nitrogen oxide (NO_x), and nitrogen dioxide (NO_2). As the individual air pollutant levels were highly correlated (r = 0.71-0.98), we computed a general marker covering all air pollutants based on the first unrotated component of a principal component analysis. We followed participants up for dementia until 2018, and determined cognitive performance during two subsequent examination rounds. Using Cox and linear mixed models, we related air pollution to the risk of dementia and cognitive decline.

Results: Of the 7,511 non-demented participants at baseline, 545 developed dementia during a median follow-up of 7 years. The general marker of all air pollutants was not associated with the risk of dementia (hazard ratio [95% confidence interval]: 1.04 [0.95-1.15]), neither were the individual air pollutants. Moreover, the general markers of all air pollutants or the individual air pollutant levels were not associated with cognitive decline.

Conclusion: In this study, we found no clear evidence for an association between exposure to air pollution and the risk of dementia or cognitive decline.

INTRODUCTION

Dementia is a multifactorial disease that currently affects over 55 million people worldwide, and this number is expected to triple by 2050 due to an aging population.[1] Consequently, the enormous burden on societies will increase further, which emphasizes the urgent need to identify modifiable risk factors to prevent dementia.

In this regard, air pollution could be of interest, as various studies have shown that individuals residing in highly air polluted areas are at an increased risk of dementia.[2, 3] Air pollution is a mixture of gases and particles that can enter the body after inhalation through the lungs and the smallest particles may reach the brain directly through the olfactory system.[4] This could affect the brain by triggering inflammation, oxidative stress, and cerebrovascular damage.[5-7]

Nevertheless, a recent meta-analysis [8] and systematic review [2] on the link between air pollution and the risk of dementia highlighted that most previous studies obtained incident dementia cases from healthcare administrative databases, such as insurance claims and medical records. A strength of these studies includes the large sample size that adequately represent the target population, but the documentation of dementia diagnosis is insufficient and data on important confounders are lacking.[9, 10] This may have led to an underestimation of the true association, for instance because individuals with a lower socioeconomic status are more often underdiagnosed for dementia,[10, 11] while they also reside more often in higher polluted areas than those with a higher socioeconomic status.[12] The scarcity of population-based cohort studies on the association between air pollution and the risk of dementia that used a valid approach to diagnose dementia and extensive data on confounding factors, encouraged the authors of the meta-analysis to conclude that replication is warranted.[8]

We therefore studied the association of exposure to air pollution with the risk of dementia and cognitive decline in a large population-based cohort study in the Netherlands.

METHODS

Study setting and population

This study was embedded within the Rotterdam Study, a prospective population-based cohort among inhabitants of Ommoord, a small, densely populated and well-defined

suburb of approximately 4.5 km^2 in Rotterdam, the Netherlands.[13] The original cohort (RS-I) started in 1990. All inhabitants of Ommoord who were 55 years and older were invited to participate and 7,983 agreed (response rate 78%). The study was enlarged with a second cohort (RS-II) in 2000 including 3,011 additional participants (response rate 67%) who had turned 55 years or moved into the study area, followed by a third cohort (RS-III) in 2006 with 3,932 additional participants (response rate 65%) aged 45 years and older. Every 3 to 5 years, participants are invited for a follow-up examination.

Between February 17th 2009 and February 19th 2010, annual mean air pollutant levels were determined in Ommoord. February 19th 2010 was therefore considered as baseline in the current study. Of the 9,241 participants who were still alive and participating in the study at baseline, 8,053 were still living in Ommoord and had reported their residential address. We excluded 275 participants with prevalent dementia, 227 participants who were insufficiently screened for dementia, and 40 participants who did not sign informed consent for follow-up data monitoring, resulting in a study population of 7,511 participants to assess the association between exposure to air pollution and the risk of dementia. Moreover, on average 1.8 years after baseline of the current study, 5,969 participants from all three cohorts of the Rotterdam Study visited the research center to undergo cognitive performance assessment and on average 4.7 years later 2,268 participants of the first and second cohort (RS-I and RS-II) visited the research center again to undergo a cognitive reexamination. Cognitive reexaminations for participants of the third cohort (RS-III) are currently taking place. Reexamination data from participants of RS-III, who are relatively younger than participants of RS-I and RS-II, could thus not been taken into account.

Air pollution

Annual air pollutant concentrations, including particulate matter of less than 10 μm (PM_{10}) and 2.5 μm ($PM_{2.5}$) in diameter, a proxy of elemental carbon ($PM_{2.5}$ absorbance), nitrogen oxide (NO_x), and nitrogen dioxide (NO_2), were calculated at participants geocoded residential address using land use regression (LUR) models. Geocoding was performed using the Google Maps geocoder in OGIS. LUR models were developed within the European Study of Cohorts for Air Pollution Effects (ESCAPE) project, as described in detail elsewhere.[14, 15] In brief, particulate matter was measured at 40 locations and nitrogen oxides at 80 locations in The Netherlands/Belgium (including one in Rotterdam) between February 17th 2009 and February 19th 2010. This was done in a cold, intermediate and warm temperature season, each for 14 days. Obtained concentrations of the individual air pollutants were averaged, adjusted for temporal variation as derived from a centrally located background reference site, and considered as the annual averages. LUR models were developed using linear regression with the annual average air pollutant

concentrations as outcome variable. Potential predictor variables considered included traffic, land use, population density and altitude data. Derived from European-wide geographic information system (GIS) databases, traffic was calculated for circular buffers with radii of 25, 50, 100, 300, 500, and 1000 meter and land use on buffers of 100, 300, 500, 1000, and 5000 meter.[14, 15] In addition, local GIS databases were used to obtain population density and altitude data on a special resolution of at least 100 meter. A stepwise selection procedure was used to obtain a model including a selection of predictor variables that minimized the model explained variance (R^2). The final LUR models are provided in **Supplementary Table 1** and had a R^2 of 68% for PM_{10}, 67% for $PM_{2.5}$, 92% for $PM_{2.5}$ absorbance, 86% for NO_2, and 87% for NO_x.

Variation in air pollutant concentrations in the current study was substantial, but the individual air pollutants were strongly correlated with each other (Spearman's correlation coefficients varying between 0.71 and 0.98, **Supplementary Figure 1**). We therefore computed a general marker covering all air pollutants based on the first unrotated component of a principal component analysis, explaining 82.7 % of the variance. Factor loadings of the individual air pollutants were all positive and ranged from 0.83 to 0.97 (**Supplementary Table 2**), indicating that the correlation between the individual air pollutants and the general marker was strong. Nevertheless, as the strength of the factor loadings varies between air pollutants, we also report results from the individual air pollutants.

The modeled air pollutant concentrations can be considered as long-term average values as the district of Ommoord is fairly stable with little environmental changes taking place in the last two decades (i.e. no major construction, no new highways etc.).

Dementia

Participants were screened for dementia at Rotterdam Study baseline and every 3 to 5 years during follow-up examinations using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level.[16] Those with an MMSE score less than 26 or a GMS organic level score more than 0 were further screened using the Cambridge Examination for Mental Disorders in the Elderly diagnostic interview. Participants were also monitored for dementia on a continuous basis through an electronic link between the study database and medical records from general practitioners and the Regional Institute of Outpatients Mental health Care. The final diagnosis was established by a consensus panel led by a neurologist, according to standard criteria for dementia (DSM-III-R), and for sub-diagnosis of Alzheimer's disease (NINCDS-ADRDA). Follow-up for dementia was conducted until January 1st 2018 and was completed for 96% of the potential person-years.

Cognition

Cognitive function was assessed using a neuropsychological test battery, consisting of the MMSE, letter-digit substitution test, word fluency test, Stroop test,15-word learning test, and the Purdue Pegboard test. Detailed information on the individual tests can be found elsewhere.[17] To enable comparisons between the different tests, we inverted the three Stroop subtask scores so that higher scores for each cognitive test indicate better performance. We calculated the G-factor as measure of general cognitive function based on the first unrotated component of a principal component analysis including a subset of the cognitive tests, namely the letter-digit substitution test, the word fluency test, inference subtask of the Stroop test, delayed recall subtask of the 15-word learning test, and the sum-score of the Purdue Pegboard test. This analysis was performed on test scores from both the first and second examination round together and explained 48% of the variance among the included test scores.

Covariates

Based on home interviews, we collected data on level of education (classified as primary, lower, intermediate, higher), smoking status (classified as current, former, never), monthly household income in euros per month (classified as ≤1050, 1050-1500, 1500-2100, 2100-2900, >2900), alcohol intake in grams per day, and working hours per day. Physical activity was measured using the validated LASA Physical Activity Questionnaire and obtained data was recalculated into metabolic equivalent of task (MET)-hours per week. Hours from home per day, as measure of mobility, was calculated by summing working hours and hours of outdoor physical activity per day. Body mass index (BMI) was computed from measurements of height and weight (kg/m^2). Depressive symptoms were evaluated with the validated Center for Epidemiology Depression Scale (CES-D), which was converted to a sum-score. *APOE* genotype was obtained using polymerase chain reaction of coded DNA samples for sub-cohort RS-I and with bi-allelic TaqMan assay for sub-cohort RS-II and RS-III.

Statistical analysis

We determined the association between the general marker of all air pollutants and the incidence of dementia, using Cox proportional hazard models. The general marker was analyzed continuously per standard deviation increase and per quartile. We repeated the analyses for each individual air pollutant. Based on plots of Schoenfeld residuals, we confirmed that the proportional hazard assumptions were not violated. Age in years was used as time scale and participants were censored when they were diagnosed with dementia, died, were lost to follow-up or at the end of the follow-up (January 1st 2018),

whichever came first. All models were adjusted for age at baseline, sex, and level of education (model I). We further adjusted in an additional model for smoking status, monthly household income, alcohol intake, physical activity, hours from home, BMI, and depressive symptoms (model II). We stratified for *APOE* genotype (ε4 carrier vs no ε4 carrier) and for age below and above 70 years at baseline to assess effect modification. To minimize the risk of exposure misclassification, we excluded all participants who changed residential address during follow-up. Also, we subsequently excluded participants who were Ommoord resident for less than 10 and 25 years before baseline. To create insights in the potential role of competing risk by mortality, we visualized Kaplan-Meier survival curves with advancing age per quartile of the general marker of all air pollutants and per quartile of the individual air pollutants.

We further assessed the association of the general marker of all air pollutants with performance on the cognitive test scores and change in the cognitive test scores during follow-up, using linear mixed models with a random intercept and slope. For certain cognitive test scores, models did not convert with a random slope. In these cases the random slope was removed from the model (i.e. for the reading and interference subtasks of the Stroop test, and the delayed recall and recognition subtasks of the word learning test). We again repeated the analysis for each individual air pollutant. The time interval between the first and second cognitive performance assessments was added as variable to the model and an interaction term between time and air pollutant levels was added, reflecting whether change in cognitive test scores differ among air pollution levels. We additionally added all covariates of model II as described above to the models. We also added a non-linear term for age (i.e. cubic splines including three knots) as this significantly ($p < 0.05$) improved the fit of the model according to the likelihood ratio test with the models fitted under the maximum likelihood procedure. We performed natural log transformation on cognitive test scores with a skewed distribution (i.e. MMSE, all Stroop tests, and Word Learning Test: Recognition), but given that this did not affect our results, we reported results based on non-transformed cognitive scores. To evaluate whether potential effects of air pollution differ by age, we repeated the analyses after replacing the time variable by age at cognitive assessment, while adjusting for date of birth.

Missing data on covariates (23.7% for physical activity, 10.5% for monthly household income, 9.9% for working hours, 5.4% for BMI <2% for all other covariates) were imputed using the mean of five-fold multiple imputation. All statistical analyses were conducted using R Statistical Software version 4.0.3.

RESULTS

Characteristics of the participants per quartile of the general marker of all air pollutants are presented in **Table 1**. Characteristics were not uniformly distributed, but there was also no clear trend across the different quartiles. **Figure 1** represents descriptive statistics of all modeled ambient air pollutants and the general marker of all air pollutants at participants' residential addresses.

Table 1. Characteristics of the study population per quartile of a general marker of all air pollutants.

	General marker of all air pollutants			
	Quartile 1 (n=1,879)	**Quartile 2 (n=1,879)**	**Quartile 3 (n=1,875)**	**Quartile 4 (n=1,878)**
Age, years	66.4 (10.3)	70.8 (10.9)	71.2 (11.3)	66.2 (10.2)
Female sex	1,026 (54.6)	1,190 (63.3)	1,177 (62.8)	1,008 (53.7)
Level of education				
Primary	122 (6.6)	265 (14.2)	229 (12.3)	129 (6.9)
Lower	676 (36.4)	856 (45.9)	803 (43.3)	672 (36.1)
Intermediate	544 (29.3)	520 (27.9)	540 (29.1)	572 (30.8)
Higher	517 (27.8)	223 (12.0)	284 (15.3)	486 (26.1)
Smoking status				
Never	613 (32.8)	555 (29.6)	638 (34.1)	584 (31.2)
Former	942 (50.4)	923 (49.3)	879 (47.0)	895 (47.8)
Current	315 (16.8)	395 (21.1)	355 (19.0)	394 (21.0)
Household income, euros/month				
≤1050	64 (3.8)	224 (13.1)	150 (8.9)	68 (4.1)
1050-150	147 (8.7)	345 (20.2)	267 (15.9)	122 (7.3)
1500-210	310 (18.4)	504 (29.6)	441 (26.3)	298 (17.9)
2100-2900	439 (26.1)	375 (22.0)	462 (27.6)	562 (33.8)
>2900	721 (42.9)	256 (15.0)	356 (21.2)	612 (36.8)
Alcohol intake, grams/day	8.8 (9.3)	7.2 (9.2)	7.3 (9.1)	7.9 (9.2)
Physical activity, MET hours/week	61.7 (58.2)	54.6 (57.1)	50.1 (52.4)	55.6 (57.1)
Hours from home per week	18.0 (32.7)	12.4 (13.9)	13.8 (32.2)	16.7 (17.1)
Body mass index, kg/m^2	27.3 (4.2)	28.0 (4.7)	27.9 (4.5)	27.5 (4.2)
CES-D, score	5.6 (7.1)	7.2 (7.7)	7.3 (7.8)	6.1 (7.1)
APOE ε4 carrier	512 (33.8)	465 (27.2)	425 (24.7)	510 (29.9)

Data are shown for non-imputed data and are either presented as frequency (%) or mean (standard deviation). Abbreviations: *APOE*, Apolipoprotein; CES-D, Center for Epidemiologic Studies Depression Scale; MET h, Metabolic Equivalent of Task hours; PM, particulate matter; NO, nitrogen oxide; n, number of participants.

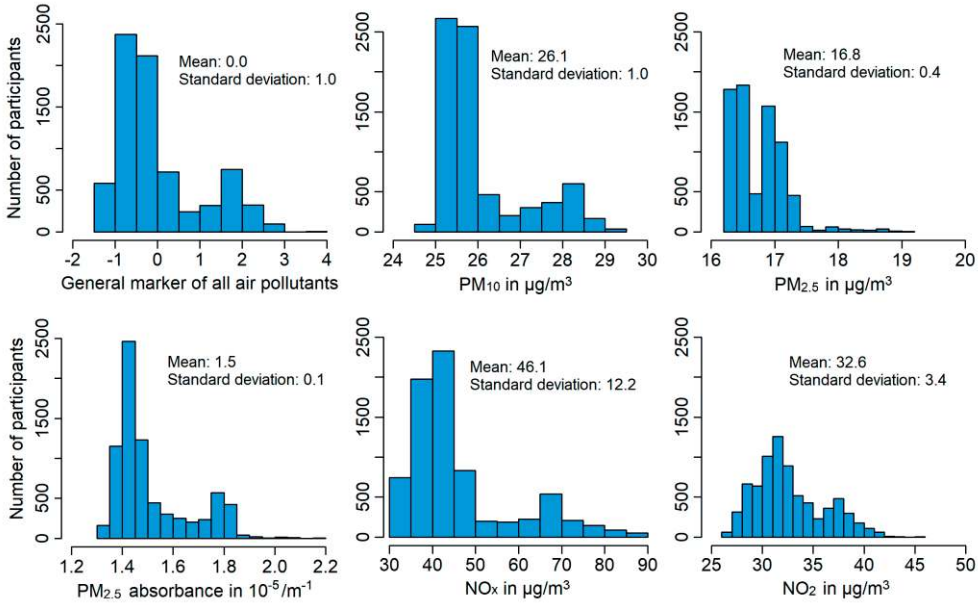

Figure 1. Descriptive and distributional information of residential air pollutant levels.
Abbreviations: NO, nitrogen oxide PM, particulate matter.

During a median follow-up of 7 years, 545 out of 7,511 participants developed dementia (incidence rate 11 per 1,000 person-years). The general marker of all air pollutants was not associated with the risk of dementia (hazard ratio (HR) [95% confidence interval (CI)] per standard deviation increase: 1.04 [0.95-1.15]), neither was exposure to PM_{10}, $PM_{2.5}$, $PM_{2.5}$ absorbance, NO_x, and NO_2 (**Table 2**). However, when categorizing $PM_{2.5}$ into quartiles, an increased risk of dementia was observed for participants in the second quartile (HR [95% CI]: 1.32 [1.02-1.70]) and last quartile (HR [95% CI]: 1.29 [0.97-1.71]), but not in the third quartile (HR [95% CI]: 1.13 [0.87-1.46]), compared to those in the first quartile (**Figure 2**). No clear linear or non-linear association was observed when categorizing the general marker of all air pollutants or the other individual air pollutants into quartiles.

Table 2. Exposure to air pollution in association with the risk of dementia.

	Dementia n/N = 545/7511 Hazard ratio (95% confidence interval)	
	Model I	**Model II**
General marker of air pollution	1.05 (0.96-1.16)	1.05 (0.95-1.16)
Individual air pollutants:		
PM_{10}	1.05 (0.95-1.15)	1.05 (0.95-1.16)
$PM_{2.5}$	1.02 (0.93-1.11)	1.01 (0.93-1.11)
$PM_{2.5}$ absorbance	1.05 (0.96-1.15)	1.04 (0.95-1.15)
NO_x	1.06 (0.96-1.17)	1.05 (0.95-1.17)
NO_2	1.05 (0.96-1.16)	1.05 (0.95-1.16)

Effect estimates are shown per standard deviation increase (1.02 for PM_{10}, 0.4 for $PM_{2.5}$, 0.145 for $PM_{2.5}$ absorbance, 12.18 for NO_x, and 3.38 for NO_2). Model I is adjusted for age and sex. Model II is further adjusted for smoking status, monthly household income, alcohol intake, physical activity, hours from home, body mass index, and depressive symptoms. Abbreviations: n, number of participants with incident dementia; N, total number of participants; NO, nitrogen oxide PM, particulate matter.

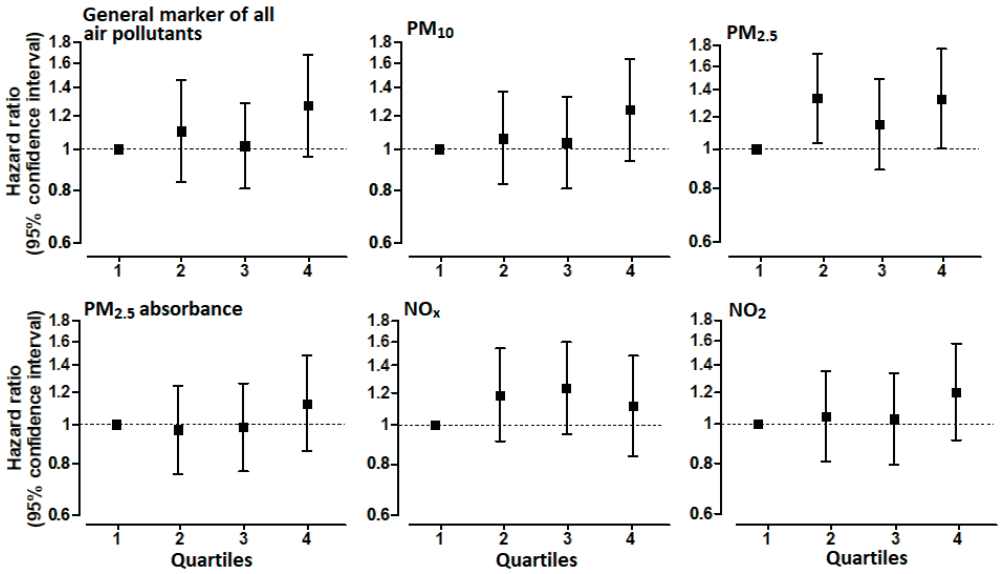

Figure 2. Exposure to air pollution per quartile in association with the risk of dementia. Models are adjusted for age, sex, level of education, smoking status, monthly household income, alcohol intake, physical activity, hours from home, body mass index, and depressive symptoms. Abbreviations: NO, nitrogen oxide PM, particulate matter.

Associations of exposure to the general marker of all air pollutants with the risk of dementia were similar for Alzheimer's disease (**Figure 3**). However, risk estimates were

slightly higher in *APOE* ε4 carriers compared to non-carriers, and in participants below compared to those above the age of 70 years. Moreover, risk estimates were similar after excluding participants who changed residential address during follow-up, and after subsequently excluding participants who were Ommoord residence for less than 10 and 25 years before baseline. When repeating these subgroup and sensitivity analyses considering individual air pollution concentrations as exposure, similar associations were observed as for the general marker of all air pollutants (**Supplementary Figure 2**). Survival curves showed that participants in the highest quartile of the general marker of all air pollutants and each individual air pollutant had a lower survival rate compared to participants in the other quartiles (**Supplementary Figure 3**).

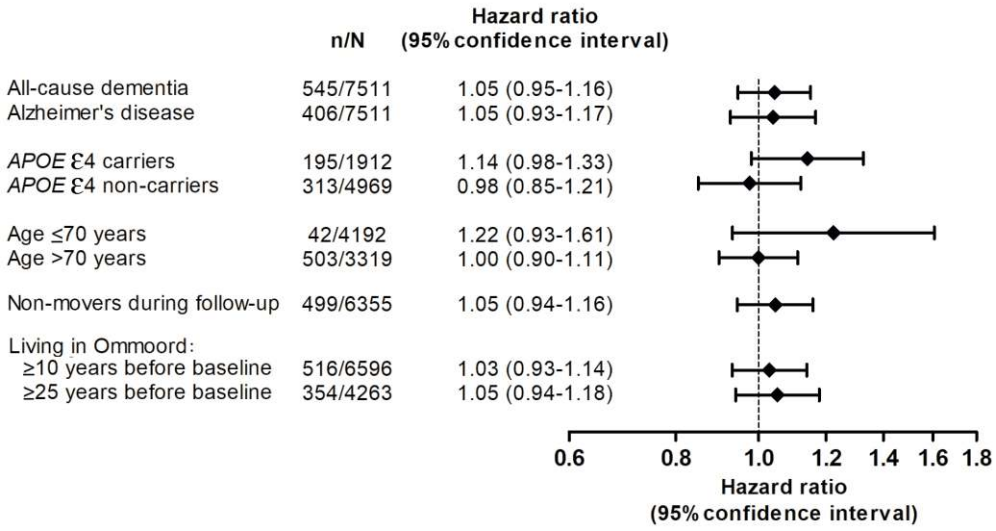

Figure 3. Subgroup and sensitivity analyses for the association between air pollutants and the risk of dementia. Effect estimates are shown per standard deviation increase and adjusted for age, sex, level of education, smoking status, monthly household income, alcohol intake, physical activity, hours from home, body mass index, and depressive symptoms. Abbreviations: n, number of participants with incident dementia; N, total number of participants; *APOE*, Apolipoprotein E; NO, nitrogen oxide PM, particulate matter.

No major difference in characteristics between the total sample and the sample with cognitive performance data was observed (**Supplementary Table 3**), but those with cognitive reexamination data were considerably older (mean age 74.1 years) compared to those with any data on cognitive performance (mean age 67.8 years). Trajectories of cognitive test performance scores over time did not differ per quartile of the general marker of all air pollutants (**Figure 4**), nor per quartile of PM_{10}, $PM_{2.5}$, $PM_{2.5}$ absorbance, NO_x, and NO_2 levels (data not shown). Main effect and slope differences for continues air

pollutant levels are presented in **Supplementary Table 4 and 5**. Trajectories of cognitive test performance scores with advancing age did also not differ per quartile of the general marker of all air pollutants (**Supplementary Figure 4**).

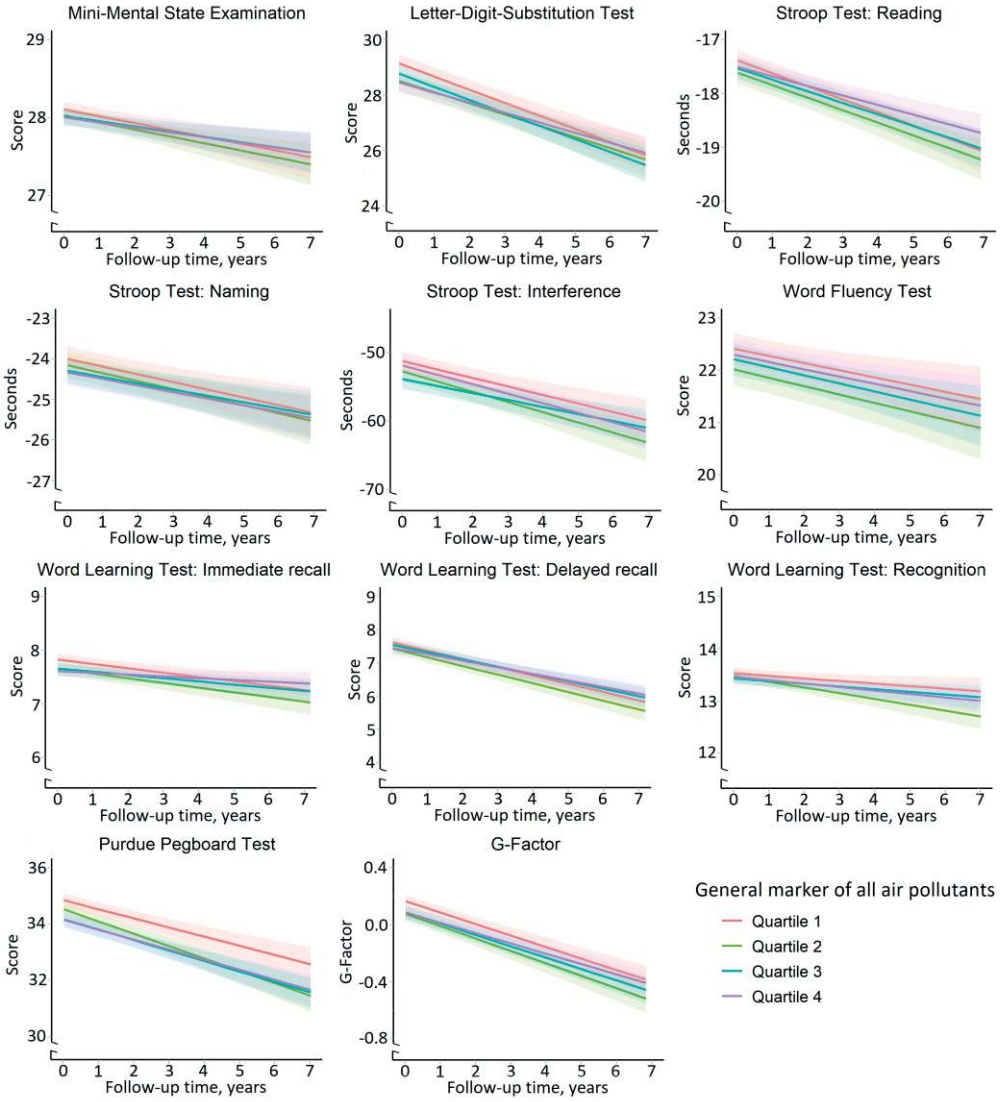

Figure 4. Cognitive performance trajectories over time per quartile of the general marker of all air pollutants. Models are adjusted for age, sex, level of education, smoking status, monthly household income, alcohol intake, physical activity, hours from home, body mass index, and depressive symptoms. Stroop scores are inversely transformed, meaning that higher scores for all cognitive tests indicate better cognitive performance.

DISCUSSION

In this large population-based study of middle-aged and elderly individuals from a well-defined suburb of approximately 4.5 km^2, we found that exposure to air pollution expressed as a general marker was not associated with the risk of dementia or cognitive decline. Also, exposure levels of individual air pollutants, namely PM_{10}, $PM_{2.5}$, $PM_{2.5}$ absorbance, NO_x, and NO_2, were not associated with either dementia or cognitive decline.

Previous population-based cohort studies determined the link between $PM_{2.5}$ and the risk of dementia and found almost consistently that exposure to higher levels of $PM_{2.5}$ was associated with an increased dementia risk.[18-23] Moreover, a study on the association of $PM_{2.5}$ absorbance and NO_2 with the risk of dementia did not observe a link,[19] while three other studies found that exposure to higher levels of NO_2 was associated with an increased risk of dementia.[21, 23, 24] Although we found in contrast to most of these previous studies no clear evidence for an association between air pollution and dementia, all observed effect estimates were in the hypothesized direction. That the effect estimates were small and not statistically significant may be explained by the fact that our study was conducted within a relatively small study area (4.5 km^2) in which variability in pollutant levels may have been too limited to identify meaningful associations. Moreover, pollutant levels were determined at participants residential addresses using LUR models, which had an explained variance ranging from 67-95% throughout the Netherlands. Yet, performance of the LUR models within Ommoord specifically was not determined, while Ommoord only covers a small area in the Netherlands. Explained variances may therefore not directly translate. Further, while we attempt to minimize exposure misclassification by correcting for the mobility of the participants, total mobility is presumably not fully covered. Consequently, exposure misclassification persists and may have diluted our effect estimates towards the null. This dilution may have been strengthened by the fact that exposure throughout lifetime could not be taken into account and by the relatively short follow-up period. In addition, although no clear disturbing pattern in general characteristics was observed across the different exposure levels, characteristics were also not uniformly distributed. Hence, confounding may has contributed to the dilution of the risk estimates.

Apart from methodological issues, non-linearity could be an alternative explanation for our null findings, because non-linear inverse associations between air pollution and dementia were observed in two prior studies that used similar methods as the current study.[21, 24] Also the variability in air pollutant concentrations was similar, but background levels in the present study were up to 3 times higher. Specifically, the first study was

conducted among 2,927 participants residing in Stockholm. For every 0.88 μg/m^3 in $PM_{2.5}$ increase a 54% increased risk of dementia was observed and for every 8.35 μg/m^3 increase in NO_x a 14% increased dementia risk.[21] Yet, these associations were fully driven by $PM_{2.5}$ levels below circa 8.5 μg/m^3 and NO_x levels below circa 25 μg/m^3. The second study was conducted among 1,806 participants residing in North Sweden. NO_x was not significantly associated with the risk of dementia when considering NO_x as a continuous variable, but compared to participants in the first quartile (4.8-9 μg/m^3), those in the third quartile (17-26 μg/m^3) were at a 48% higher risk and those in the fourth quartile (>26 μg/m^3) at a 43% higher risk of dementia.[24] In the present study, such non-linear associations were not clearly visible, suggesting that air pollutant concentrations in the exposure range of this study are not differentially harmful for brain health. To further unravel the shape of the association, we encourage future studies to focus on larger study areas with more variation in air pollutant concentrations.

Competing risk could also explain in part our null findings, as exposure to air pollution has been linked to increased risks of several adverse health outcomes, including cardiovascular disease[7, 25] and poor lung function,[26, 27] which subsequently leads to a higher risk of mortality.[7, 25] Indeed, we found that participants in the highest exposure quartiles of the pollutant concentrations had a lower survival rate compared to participants in the lower quartiles, suggesting that mortality precludes dementia onset.

Other factors should be considered that may explain our null findings, which are in contrast to most previous studies. For instance, particulate matter originate from more than hundred different sources, including traffic, industry, and agriculture.[28] Variation in air pollutant levels in our study originates mainly from traffic. Our null findings might indicate that traffic related air pollution is less harmful for the brain than particles from other sources. Also, some of the discrepancies between studies may be explained by differences in methods to determine dementia or air pollution, follow-up periods and population characteristics.

Several mechanisms through which air pollution potentially affect brain health have been proposed. $PM_{2.5}$ may enters the brain directly through the olfactory system after inhalation[4] or through the circulatory system after crossing the blood-brain barrier,[29] where it may induce oxidative stress and inflammatory responses such as microglial activation.[5, 30] Moreover, inhalation of air pollutants in the form of both particulate matter and nitrogen oxide via de lungs could cause systematic inflammation and oxidative stress, as well as cardiovascular abnormalities which may indirectly affect the brain.[5-7]

Strengths of this study include the large sample size, the extensive estimation approach that has been used to diagnose dementia and the availability of important confounders. Moreover, air pollutant levels were determined between 2009 and 2010, but the study area is fairly stable with little environmental changes over the last two decades. Modeled air pollutant levels can therefore be considered as long-term annual air pollutant levels. Nevertheless, few limitations of our study also need to be addressed. First, no information was available about small environmental changes in the study area that may have affected air pollutant levels. Second, given that air pollutant levels were highly correlated with each other, effects of individual air pollutants should be interpreted with caution. Third, we only examined ambient air pollution, while indoor air pollution from burning of biomass, as well as noise and green space may interact.

In conclusion, in the current study we found no clear evidence for an association between exposure to air pollution and the risk of dementia or cognitive decline.

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SUPPLEMENTARY MATERIAL

Chapter **3.2**

Air pollution and metabolomics

de Crom TOE*, Ginos BNR*, Ghanbari M#, Voortman T#.
Long-term air pollution exposure and the blood metabolome: The Rotterdam Study.

Under review.

*These first authors contributed equally to the respective manuscript

#These second authors contributed equally to the respective manuscript

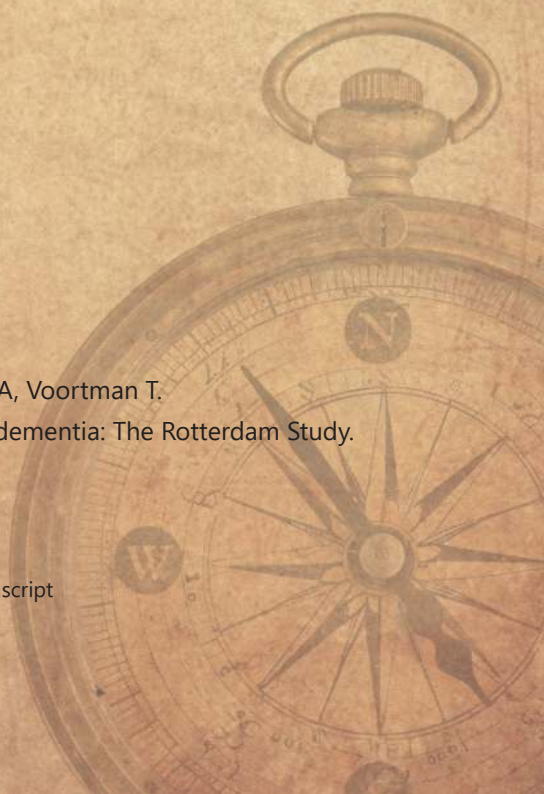
SUPPLEMENTARY MATERIAL

Chapter 4

Body composition and dementia

Chapter **4.1**

Adiposity and dementia



de Crom TOE*, Mooldijk SS*, Ikram MK, Ikram MA, Voortman T.
Adiposity in the older population and the risk of dementia: The Rotterdam Study.

Alzheimer's & Dementia. 2023;19(5):2047-2055.

*These authors contributed equally to the respective manuscript

ABSTRACT

Introduction: We determined associations of total and regional adiposity with incident dementia among older adults.

Methods: Within the population-based Rotterdam Study, adiposity was measured as total, android and gynoid fat mass using dual X-ray absorptiometry in 3,408 men and 4,563 women, every 3-6 years between 2002 and 2016. Incident dementia was recorded until 2020.

Results: Higher adiposity measures were associated with a decreased risk of dementia in both sexes. After excluding the first 5 years of follow-up, only the association of gynoid fat among women remained significant (hazard ratio [95% confidence interval] per standard deviation increase: 0.85 [0.75-0.97]). No major differences in trajectories of adiposity measures were observed between dementia cases and dementia-free controls.

Discussion: Higher total and regional fat mass related to a decreased risk of dementia. These results may be explained by reverse causality and competing risks, although a protective effect of adiposity cannot be excluded.

INTRODUCTION

Obesity and dementia are both substantial public health problems worldwide.[1, 2] Obesity during mid-life is a well-established risk factor for dementia later in life,[3-5] which may be explained by excessive adipose tissue.[6] Especially visceral fat, located around the abdominal organs, is thought to underlie this via metabolic dysfunction, for example hypertension, insulin resistance and dyslipidemia.[7, 8]

Although visceral fat at older age likely affects the brain through similar metabolic dysfunctions, obesity at older age has consistently been linked to a decreased risk of dementia.[4, 9-12] This may be explained by reverse causality, i.e. weight loss caused by preclinical dementia symptoms,[13-15] but biological mechanisms for a protective effect of subcutaneous adipose tissue in the gynoid (i.e. hips) region have also been suggested.[16, 17] These different health effects of adipose tissue deposits highlight the need to differentiate between total and regional adipose tissue, particularly in older adults, as adipose tissue increases and the distribution changes during the aging process.[18]

Yet, existing literature on the link between obesity and the risk of dementia mostly used body mass index (BMI) or waist circumference as marker of obesity, which do not necessarily reflect the amount and location of adipose tissue.[19-22] Alternatively, total and regional fat mass can be obtained using dual-energy absorptiometry (DXA), which allows the quantification of fat in the android (i.e. abdominal) and gynoid region.[21] Android fat accumulation is typically seen in men and includes visceral fat, while gynoid fat is typically seen in women and comprises of subcutaneous fat.

To improve the understanding of the effects of adiposity on the risk of dementia among older adults, we examined associations of measures of adiposity derived from DXA scans, namely total body mass, total fat mass, android fat mass and gynoid fat mass, with the risk of dementia in men and women separately. In addition, to understand the potential role of reverse causality in this association, we determined trajectories of adiposity measures before dementia diagnosis and compared those to trajectories of dementia-free controls.

METHODS

Study setting and population

This study was embedded within the Rotterdam Study, a prospective population-based cohort study among individuals from the Ommoord district in Rotterdam, the Netherlands. Details regarding the design and rationale of the Rotterdam study have been described elsewhere.[23] Briefly, the initial study (RS-I) started in 1990 with 7,983 participants aged 55 years and older. The cohort was expanded in 2000 with 3,011 participants aged 55 years and older (RS-II) and again in 2006 with 3,932 participants aged 45 years and older (RS-III). All participants were invited to undergo an extensive follow-up examination every 4 to 6 years.

Adiposity using DXA scans was measured from 2002 (RS-I-4), 2004 (RS-II-2) and 2006 (RS-III-1) onwards. Of the 9,950 participants who were still alive and actively participating in the study by then, 8,188 had data on adiposity measures available. We excluded participants without informed consent (n=50), with prevalent dementia (n=88) or who were insufficiently screened for dementia (n=79) (**Figure 1**). Of the 7,971 participants remaining eligible for analyses, 3,408 were men and 4,563 women.

Measures of adiposity

Anthropometrics and adiposity were measured at the research center every 4 to 6 years between 2002 and 2016. Body weight in kilograms was measured using a digital scale and body height using a stadiometer, while participants were wearing indoor clothes without shoes. Dual X-ray Absorptiometry (DXA; Prodigy and iDXA devices, GE Healthcare, Chicago, United States) scans were performed to obtain adiposity measures. As main outcomes, we used total body mass, total fat mass, android fat mass, and gynoid fat mass in kilograms. From these data, we additionally calculated commonly used indices of adiposity, namely BMI as body weight in kilograms divided by height in meters squared, fat mass index as total fat mass in kilograms divided by height in meters squared and android and gynoid fat percentage by expressing fat mass in kilograms as a percentage of total android or gynoid mass in kilograms, respectively.

Dementia

Participants were screened for dementia at Rotterdam Study baseline and every 4 to 6 years during follow-up examinations using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Those with an MMSE score of <26 or a GMS organic level score of >0 were further examined using the Cambridge Examination

for Mental Disorders in the Elderly diagnostic interview. Additionally, participants were continuously under surveillance for dementia through electronic linkage between the study database and medical records from general practitioners and the Regional Institute of Outpatients Mental health Care. The final diagnosis of dementia and its most common subtypes was made by a consensus panel led by a neurologist based on standard criteria for dementia (DSM-III-R), and for sub-diagnosis of Alzheimer's disease (NINCDS-ADRDA). Follow-up for dementia was completed until January 1st 2020.

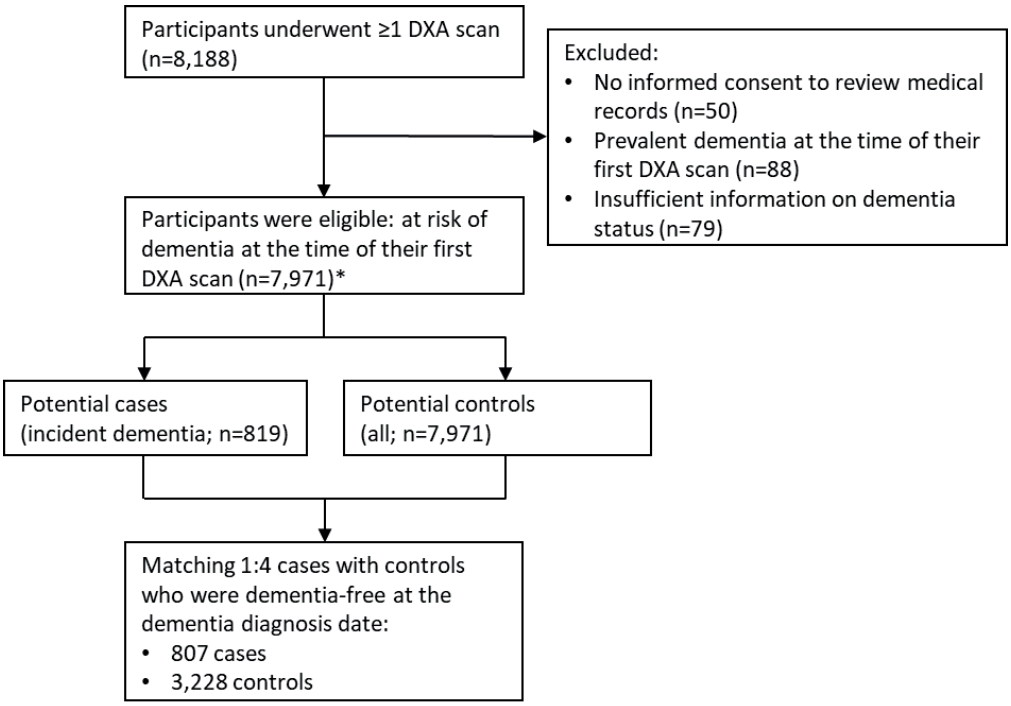


Figure 1. Flowchart of the total study population. *These 7,971 participants were at risk of dementia at the time of their first DXA scan and were included in the Cox proportional hazards analysis. Among them, 819 participants developed dementia during follow-up and were thus eligible as a case in the construction of the adiposity trajectories before their diagnosis. Cases that could not be matched (n=12) were excluded for the latter analysis, leaving 807 cases and 3228 controls.

Covariables

Covariables were determined at the round closest to the first available DXA scan. Education attainment, smoking status and alcohol intake were ascertained during home interviews. Physical activity was assessed using the LASA physical activity questionnaire and a modified version of the Zutphen Study Physical Activity Questionnaire and was expressed in metabolic equivalent of task (MET) hours per week. Depressive symptoms were

evaluated with the validated Center for Epidemiology Depression Scale, which was converted to a sum-score.[24] Diabetes mellitus was defined as having a fasting serum glucose of ≥7.0 mmol/L, use of blood glucose lowering medication, or being registered as having type 2 diabetes in records of general practitioners. Blood pressure was measured twice on the right arm with the participant in a sitting position using a random zero sphygmomanometer. The average of the two measurements was used. Total serum cholesterol and serum high-density lipoprotein (HDL) cholesterol were measured in fasting blood samples, collected at the research center. Dietary intake was determined using a validated 389-item food frequency questionnaire from which daily energy intake was determined using the Dutch Food Composition Tables (NEVO). A diet quality score reflecting adherence to the Dutch Dietary Guidelines was calculated by adding the adherence scores for 14 food components, as described in detail elsewhere.[25] *APOE* genotype was obtained using polymerase chain reaction of coded DNA samples for RS-I and with bi-allelic TaqMan assay for RS-II and RS-III.[26, 27]

Statistical analysis

The main analyses were conducted based on crude adiposity measures rather than their indices or percentages, since the use of indices may lead to spurious correlations.[28] To allow for comparison with existing literature that commonly used such indices, we repeated the analyses considering BMI, fat mass index, android fat percentage, and gynoid fat percentage.

We determined the associations between adiposity measures and the risk of dementia and Alzheimer's disease using Cox proportional hazard models. The proportional hazards assumption was assessed using Schoenfeld residuals. Participants were censored when they were diagnosed with dementia, died, were lost to follow-up or at the end of follow-up (January 1st 2020), whichever came first. All analyses were performed for men and women separately. We constructed three models. In model 1, we adjusted for height, age, and education attainment. In model 2, we further adjusted for smoking status, alcohol intake, physical activity, depressive symptoms, and *APOE* ε4 status. In model 3, we further adjusted for cardiovascular risk factors that are related to both adiposity and dementia, but are more likely to be mediators than confounders, namely systolic and diastolic blood pressure, diabetes, total cholesterol, and HDL cholesterol. Therefore, model 2 was considered the main model. Missing data on covariates (13% for physical activity, 7% for *APOE* ε4 status, 6% for smoking status, and <5% for all other covariates) were imputed using five-fold multiple imputation. Daily energy intake and diet quality are also potential confounders of the associations. However, the number of missing values for these variables was relatively high (34%), limiting the ability to reliably include them in the main models.

To explore whether diet explained the associations, we checked whether the results changed with further adjustment for daily energy intake and diet quality in addition to the covariates in model 2 as sensitivity analysis. The main analyses were repeated in subgroups for exploratory purposes: after stratifying for *APOE* ε4 status (carriers vs. non-carries), age (<70 vs. ≥70 years), and BMI (<25 vs. ≥25 kg/m^2). As suggested by previous literature, associations may differ by *APOE* ε4 status [29, 30] and age.[4, 9-12] Stratification by BMI was conducted to determine whether associations of adiposity with dementia are present, regardless of having a BMI considered as healthy. In addition, the main analyses were repeated after excluding the first 5 years of follow-up, to create insight in the potential impact of reverse causality. Moreover, the analyses were repeated with adiposity markers divided into tertiles to detect potential non-linear associations. To provide insight in the potential role of death as a competing risk for dementia, we repeated the main analyses with mortality as outcome. Furthermore, we visualized survival during follow-up by sex-, age- and height-specific tertiles of adiposity measures in Kaplan-Meier survival curves.

For the trajectories of adiposity measures, participants who developed dementia (cases) during follow-up were matched with four participants who were free of dementia (controls) at the diagnosis date of the case. Matching was performed based on sex and birth year, resulting in a maximum age difference of a year. We constructed linear mixed models with random intercepts and slopes, adjusted for age and height, to determine trajectories of adiposity measures before the index date. Differences in the trajectories of cases and controls were allowed by adding an interaction term between time and case/control status. We also added splines with two knots to the time variable as this significantly improved the fit of the model based on a likelihood ratio test with the models fitted under maximum likelihood procedure. In order to visualize whether differences in trajectories were statistically significant, we calculated 83.4% confidence intervals to archive a type 1 error probability of 0.05.[31]

All statistical analyses were conducted using R Statistical Software version 4.0.3. We considered results statistically significant when the p-value was below 0.05.

RESULTS

Baseline characteristics are shown in **Table 1**. The mean age was 66.4 years (standard deviation: 9.8) for men and 66.6 (standard deviation: 10.2) for women. During a mean follow-up of 10.3 years, 293 men and 526 women developed dementia (incidence rates

[95% confidence interval] per 1,000 person-years: 8.7 [7.7-9.7] and 10.9 [10.0-11.9], respectively).

Table 1. Baseline characteristics of the total study population.

Characteristics	Men (N=3,408)	Women (N=4,563)
Age, years	66.4 (9.8)	66.6 (10.2)
Education attainment		
Primary	256 (8)	545 (12)
Lower	924 (27)	2,282 (51)
Intermediate	1,272 (38)	1,073 (24)
Higher	930 (27)	608 (13)
Smoking status		
Never	655 (21)	1713 (40)
Former	1,996 (63)	1,876 (44)
Current	510 (16)	709 (16)
Energy intake, kcal/day	2,323 (734)	2,006 (642)
Dutch dietary guidelines, score	6.6 (1.8)	7.2 (1.9)
Alcohol intake, grams/day	13.6 (14.0)	6.9 (8.8)
Physical activity, MET hours/week	65.1 (51.0)	79.5 (53.1)
CES-D, score	4.2 (5.7)	6.7 (7.9)
Diabetes, yes	476 (14)	438 (10)
Systolic blood pressure, mmHg	143.7 (20.7)	142.9 (23.4)
Diastolic blood pressure, mmHg	82.5 (11.2)	81.1 (11.3)
Total cholesterol, mmol/L	5.3 (1.0)	5.8 (1.0)
High density lipoprotein cholesterol, mmol/L	1.3 (0.3)	1.6 (0.4)
APOE ε4 alleles		
No allele	2,318 (72)	3,030 (72)
1 allele	805 (25)	1,108 (26)
2 allele	79 (2)	71 (2)
General body composition measures		
Height, cm	176.1 (7.0)	162.6 (6.5)
Body mass, kg	85.4 (12.9)	73.0 (13.1)
Body mass index, kg/m^2	27.5 (3.6)	27.6 (4.7)
Fat mass, kg	25.0 (8.4)	29.1 (9.5)
Fat mass index, kg/m^2	8.0 (2.6)	11.0 (3.5)
Regional fat measures		
Android fat mass, kg	2.8 (1.0)	2.5 (1.0)
Android fat percentage, %*	40.1 (8.3)	45.7 (8.9)
Gynoid fat mass, kg	3.5 (1.1)	4.8 (1.5)
Gynoid fat percentage, %*	29.7 (6.1)	44.1 (6.5)

Data are shown for non-imputed data and are presented as mean (standard deviation) for continuous variables and number (percentages) for categorical variables. *Calculated as android or gynoid fat mass divided by total mass in android or gynoid region times 100%. Abbreviations: CES-D, Center for Epidemiologic Studies Depression Scale; MET, Metabolic Equivalent of Task; N, number of participants.

A higher body mass, fat mass, android fat mass and gynoid fat mass at baseline were associated with a decreased risk of dementia in both men and women, although not all statistically significant (**Table 2**).

Table 2. Adiposity at baseline in association with the risk of dementia

Adiposity measures	**Hazard ratio per SD increase (95% confidence interval)**		
	Model 1	**Model 2**	**Model 3**
Men (n/N = 293/3408)			
Body mass	0.91 (0.78-1.06)	0.89 (0.76-1.05)	0.85 (0.72-1.01)
Fat mass	0.89 (0.78-1.02)	0.87 (0.75-1.00)	0.84 (0.72-0.97)
Android fat mass	0.86 (0.75-0.98)	0.84 (0.73-0.97)	0.80 (0.69-0.93)
Gynoid fat mass	0.90 (0.79-1.04)	0.88 (0.76-1.01)	0.86 (0.74-1.00)
Women (n/N = 526/4563)			
Body mass	0.89 (0.81-0.99)	0.90 (0.82-1.00)	0.89 (0.80-0.99)
Fat mass	0.87 (0.79-0.97)	0.88 (0.80-0.98)	0.87 (0.78-0.97)
Android fat mass	0.86 (0.78-0.96)	0.87 (0.79-0.97)	0.84 (0.75-0.95)
Gynoid fat mass	0.83 (0.74-0.92)	0.84 (0.75-0.93)	0.84 (0.75-0.93)

Abbreviations: n, participants with incident dementia; N, participants at risk of dementia at baseline; SD, standard deviation. Hazard ratios per standard deviation increase in adiposity measures (based on the first available DXA scan). Model 1 is adjusted for age, height and education attainment. Model 2 is additionally adjusted for potential confounders (smoking status, alcohol intake, physical activity, depressive symptoms and *APOE* ε4 status). Model 3 is additionally adjusted for potential confounders that may also act as mediators (diabetes, systolic blood pressure, diastolic blood pressure, total cholesterol and high density lipoprotein cholesterol).

Risk estimates were similar when considering Alzheimer's disease as outcome (**Figure 2**). In men, associations were especially seen for *APOE* ε4 carriers and for men aged ≥70 years at adiposity measurement, but not in *APOE* ε4 non-carriers and in men aged <70 years. In women, the associations were consistent across the subgroups although the width of the confidence intervals differed. For both sexes, effect estimates for all adiposity markers attenuated after excluding the first 5 years of follow-up and only the association with gynoid fat in women remained significant (hazard ratio [95% confidence interval] per standard deviation increase: 0.84 [0.75-0.93]). We found no indications of non-linear associations when analyzing the adiposity markers in tertiles (**Supplementary Table 1**). Furthermore, additionally adjusting for daily energy intake and diet quality did not affect the association (data not shown), nor did repeating the analyses considering indices instead of crude adiposity measures as exposure (**Supplementary Table 2**).

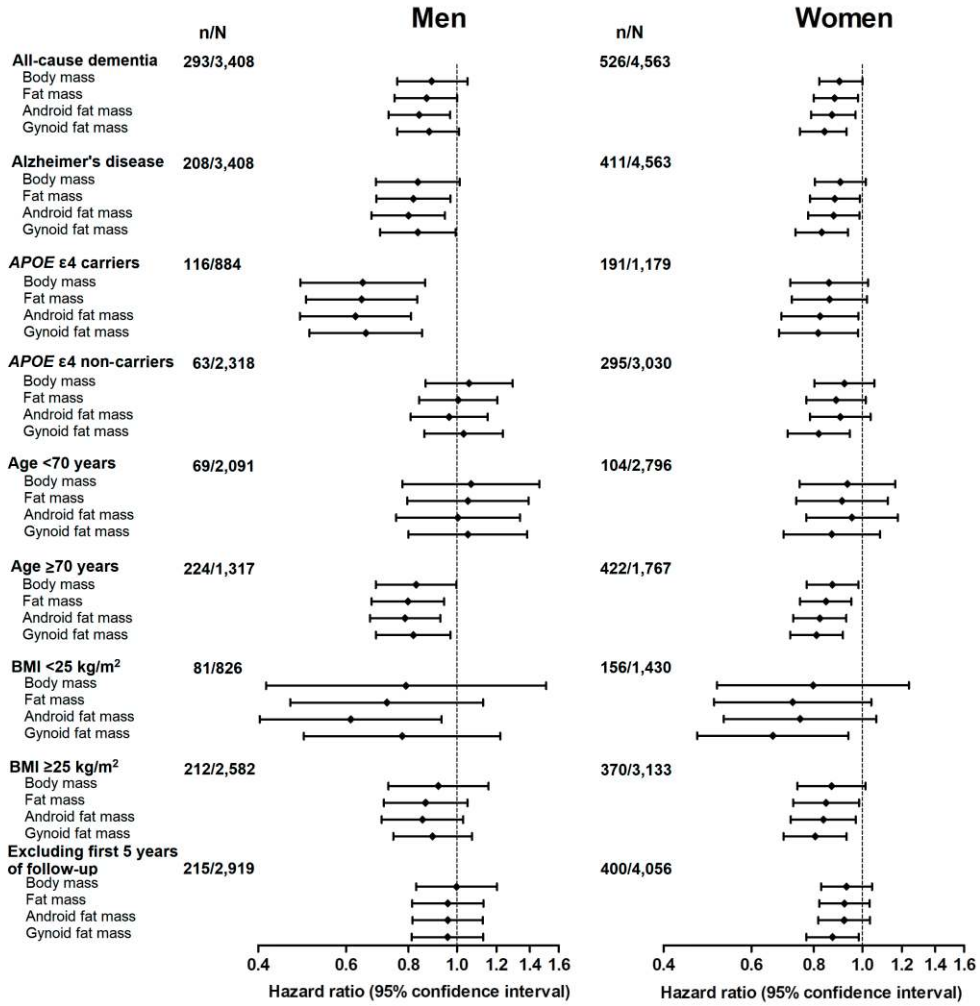


Figure 2. Subgroup and sensitivity analyses for adiposity at baseline in association with the risk of dementia. Hazard ratios per standard deviation increase in adiposity measure (based on the first available DXA scan), adjusted for age, height, education attainment, smoking status, alcohol intake, physical activity, depressive symptoms and *APOE* ε4 status. Abbreviations: BMI, body mass index; n, participants with incident dementia; N, participants at risk of dementia at baseline.

Survival curves showed subtle differences between tertiles of adiposity measures, namely a somewhat lower survival among men with higher total, android and gynoid fat mass (**Figure 3**). Among women, survival was lower in participants with lower total or gynoid fat mass. Yet, no statistically significant associations of adiposity measures with the risk of mortality were found. However, a non-significantly lower risk of mortality with higher gynoid fat mass was observed among women (**Supplementary Table 3**).

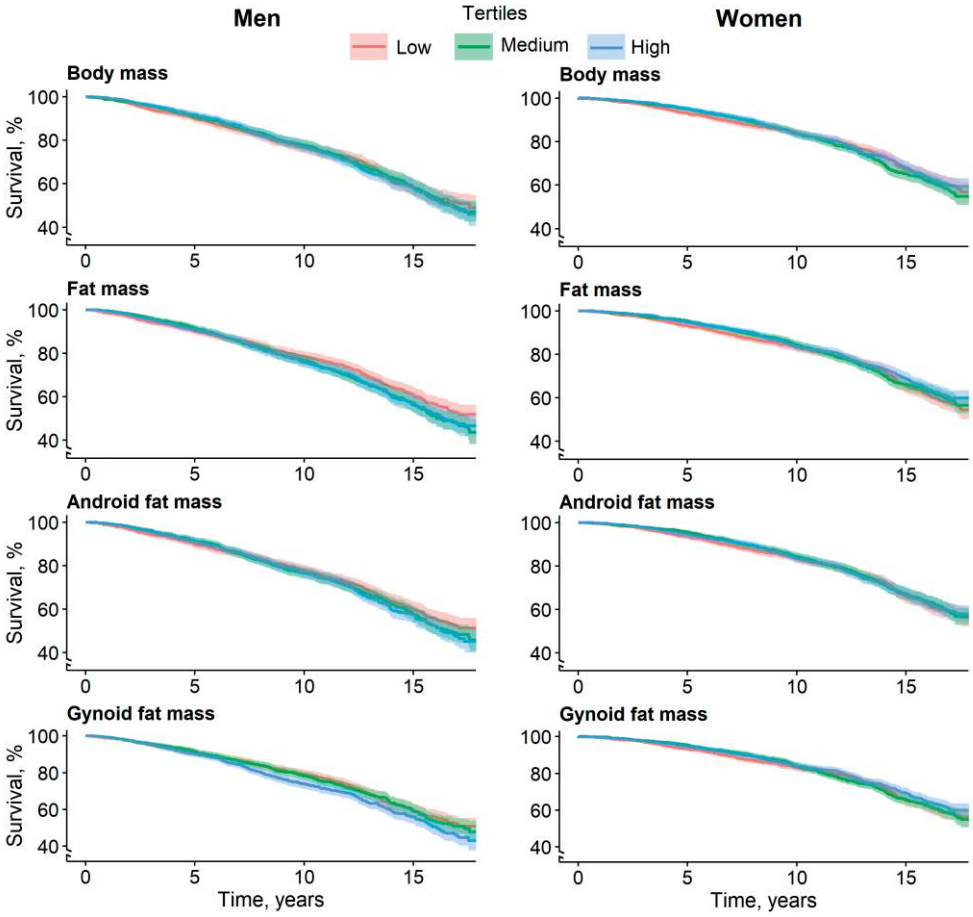


Figure 3. Survival by tertiles of adiposity measures. The tertiles were derived by regressing adiposity measures at time of the first available DXA scan on age and height for men and women separately, and categorizing the residuals of these regressions into tertiles.

Of the 293 men and 526 women who developed dementia during follow-up, 8 men and 4 women could not be matched with dementia-free controls (**Figure 1**). Matched cases had on average 1.6 adiposity measurements available (range 1-3), compared to 2.0 for controls (range 1-3). Cases were more often carriers of the *APOE* ε4 allele, but no other major differences in characteristics between cases and controls were observed (**Supplementary Table 4**).

Overall, trajectories of adiposity measures for cases and controls were similar, in both men and women (**Figure 4**). However, men who developed dementia tended to have higher

android and gynoid fat mass than controls 18 to 16 years before diagnosis, while women who developed dementia tended to have a slightly lower android and gynoid fat mass starting 4 years before diagnosis. Yet, these difference in trajectories were not statistically significant. Again, similar results were found when considering indices of the adiposity measures instead of crude measures (**Supplementary Figure 1**).

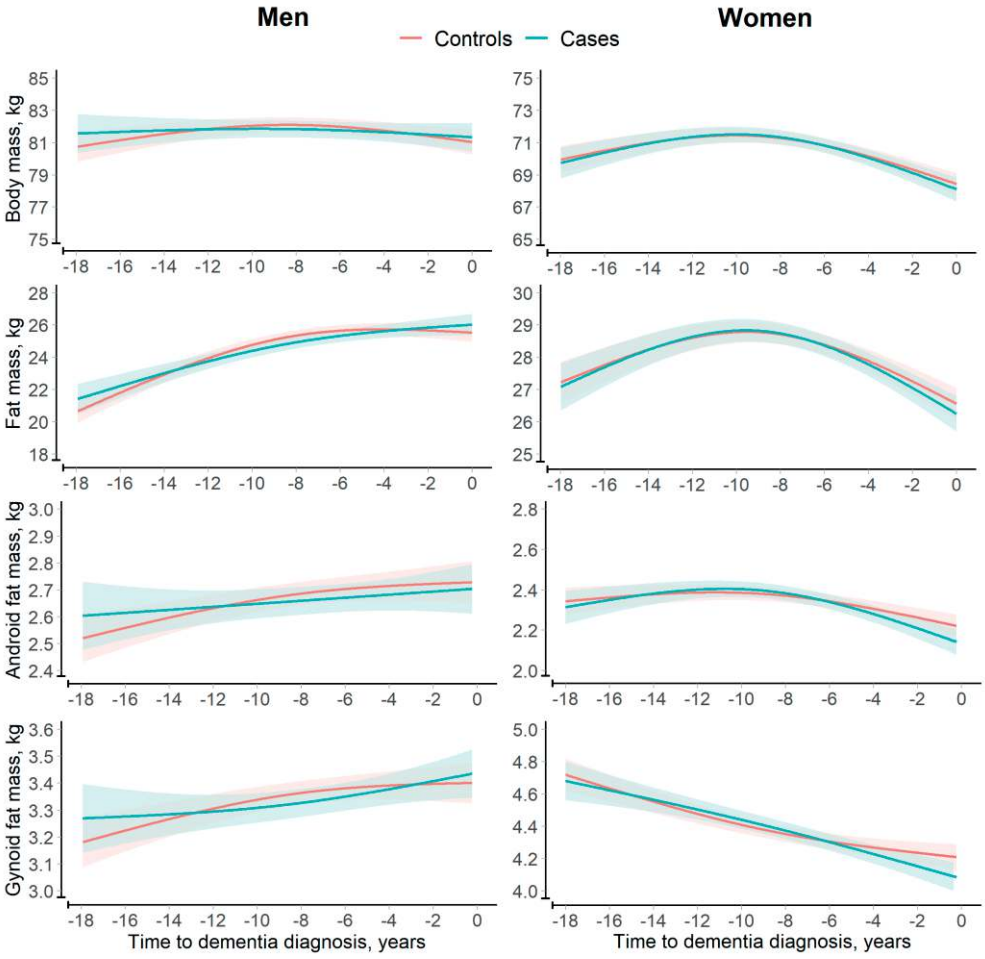


Figure 4. Change in adiposity for cases before the diagnosis of dementia and for matched controls based on repeated measurements. Trajectories are shown for a man and woman of average age (77.0 years for men and 78.5 years for women) and height (174 cm for men and 160 cm for women). The numbers of observations per 2-year time interval are provided in Supplementary Table 5.

DISCUSSION

In this population-based study, higher body mass, fat mass, android fat mass, and gynoid fat mass were associated with a decreased risk of dementia in both men and women. After excluding the first 5 years of follow-up, only the association between gynoid fat mass and the risk of dementia among women remained statistically significant. Trajectories of all adiposity measures up to 18 years before diagnoses of dementia in patients were comparable to those of dementia-free controls.

Various studies reported on the association between BMI and the risk of dementia and almost invariably found that a higher BMI during mid-life – generally defined as 50 years or younger – was associated with an increased risk of dementia, while a higher BMI during late-life was associated with a decreased risk of dementia.[3-5, 9-12] This is in line with a phenomenon that is also seen with cardiovascular diseases and mortality, known as the "obesity paradox"[32-34] and may in part be explained by failing to differentiate between fat mass and lean body mass.[35] However, studies that further investigated associations of different fat compartments with dementia in late-life are scarce. One previous longitudinal study among 344 older adults of the Cardiovascular Health Study with a mean age of 78 years also used DXA scans to distinguish fat from fat-free mass.[36] They observed no statistically significant associations of total and truncal fat mass with the risk of dementia, possibly because of the smaller sample size, but the effect estimates were in the same direction as in the current study (HRs [95% CI] for the highest truncal fat quartile versus the lowest: 0.69 [0.24-2.01] in men and 0.72 [0.37-1.39] in women). Previous studies have also highlighted the possibility of non-linear effects of weight on the risk of dementia as an explanation for the obesity paradox,[37, 38] but our results did not provide evidence for such effects.

Biological underpinnings of a relation between adiposity during mid-life and an increased risk of dementia is thought to include metabolic dysfunction such as hypertension, insulin resistance, dyslipidemia and inflammation. Dysregulation of adipokines, hormones released by adipose tissue, may also have a role.[39] At older age, adiposity likely confers similar metabolic consequences. Yet, we found a decreased risk of dementia in persons with a higher total or regional fat mass. Protective effects of adiposity in the older population on the risk of dementia have been suggested.[40] For instance, the adipokine leptin is thought to have neuroprotective effects by preventing neuronal death and improves cognitive performance in rodents.[41] High levels of leptin are seen in persons with adiposity, while their levels drop with weight loss. As such, decreased leptin levels in older

adults due to weight loss may contribute to their increased risk of dementia.[42] Estrogen levels may further explain protective effects of adiposity among older women.[16] In fact, in postmenopausal women, adipose tissue is the primary source of estrogen, which has been linked to brain health.[43] Potential alternative explanations for the association between higher adiposity markers and a decreased risk of dementia include reverse causality, i.e. decreasing body (fat) mass due to preclinical dementia,[13] or mortality as competing event.

Given that most of our associations attenuated after excluding the first five years of follow-up, reverse causality likely explains at least part of the associations. We further provided insight in the potential role of reverse causality by visualizing trajectories of adiposity measures in persons with dementia before diagnosis and in dementia-free controls. Surprisingly, those only showed a small deviation in android and gynoid fat among women up to 4 years before diagnosis. Besides, the trajectories did not reveal the expected higher initial mass and later reduction in mass in persons with dementia, as was previously found for BMI.[10, 44, 45] For instance, a decline in BMI was visible 7 years before dementia diagnosis (statistically significant 2.4 years before diagnosis) in the Three-City Study [44], 6 years before dementia diagnosis in the Honolulu-Asia Aging Study [45] and 8 years before diagnosis in the Whitehall II Study.[10] In the latter study, participants with dementia also had a higher BMI than dementia-free participants until 16 years before diagnosis. Such differences were not clearly seen in the current study, possibly due to the limited number of repeated measurements. For comparison, previous studies had up to 6 repeated measurements,[10, 44, 45] while the maximum in this study was 3. Especially amounts of DXA scans made more than 12 years before dementia diagnosis were relatively low among cases.

Competing risks could also explain seemingly protective effects of adiposity, namely if a higher body or fat mass relates to a higher risk of mortality as a competing event, which subsequently precludes a dementia diagnosis. In this study, no pronounced associations between adiposity measures and mortality were found, making it unlikely that the associations between adiposity and the risk of dementia are due to competing risks of mortality. In women, lower gynoid fat mass was related to a somewhat lower survival as well as a lower dementia risk, making a protective effect of gynoid fat more plausible.

Results from the stratified analyses by *APOE* ε4 carrier status suggest the *APOE* genotype modifies the association of adiposity with dementia, particularly in men. Prior studies have described similar synergistic effects of *APOE* ε4 and adiposity,[29, 30] for example as a result of their contributions to inflammation and metabolic disorders, although not all studies found this.[46] The stronger associations among *APOE* ε4 carriers could also be the result of

weight loss in the preclinical dementia phase,[47] since carriers are more likely to develop dementia than non-carriers.

Strengths of this study are the data derived from DXA scans in a large community-based study population and the meticulous collection of dementia data, also among participants who no longer visited the research center. Limitations include the small number of repeated DXA measurements (≤3), that were mainly derived from persons of older age. More repeated measurements, starting from mid-life, may be needed to reveal differences in trajectories. Second, visceral fat mass was not available and instead android fat mass was used as a proxy, which also includes abdominal subcutaneous fat mass. Third, subgroup analyses should be interpreted with caution because of limited numbers of cases in subgroups. Lastly, body composition and the related disease risks differ with race,[48, 49] thus translating these results derived from a predominantly white population to other populations should be done with caution.

In conclusion, higher total and regional fat mass were associated with a decreased risk of dementia. These results may be explained by reverse causality or competing risks, although a protective effect of adipose tissue, particularly in the gynoid region among women, cannot be excluded. To further clarify the effect of adiposity on the risk of dementia, we encourage further studies to investigated total and regional adiposity at older age in light of early and mid-life adiposity. Future studies are also needed to explore associations in populations with different racial background and to clarify potential interactions of adiposity and *APOE* ε4.

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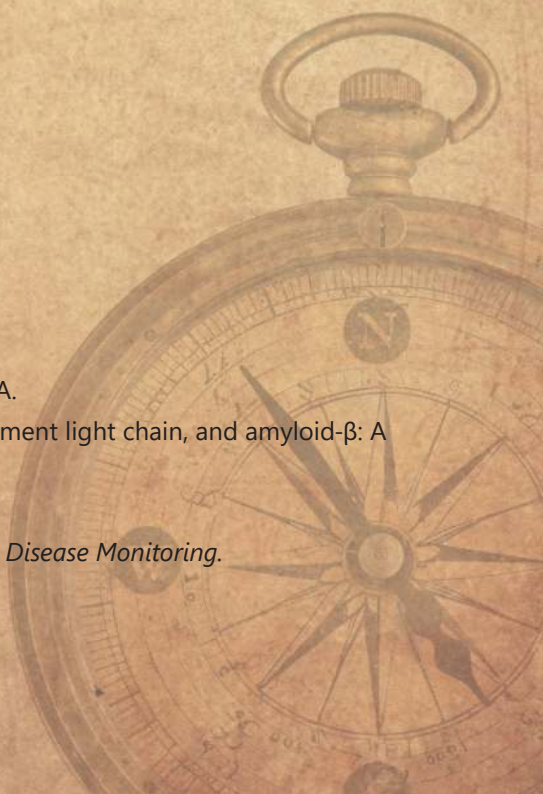
SUPPLEMENTARY MATERIAL

Chapter **4.2**

Body composition and pre-clinical marker of dementia



de Crom TOE, Ghanbari M, Voortman T, Ikram MA.
Body composition and plasma total-tau, neurofilament light chain, and amyloid-β: A population-based study.

Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring.
2023;16(1), e12519.

ABSTRACT

Introduction: A higher body mass at older age has been linked to a lower risk of dementia. This unexpected trend may be explained by age-related lean mass depletion, or methodological issues such as the long pre-clinical phase of dementia or competing risks. Focusing on pre-clinical markers of dementia may overcome these issues.

Methods: Between 2002 and 2005, body composition and plasma total-tau, neurofilament light chain (NfL), amyloid-β40 and amyloid-β42 were measured in 3,408 dementia-free participants from the population-based Rotterdam Study. The cross-sectional associations between body composition and plasma markers were determined using linear regression models.

Results: Whole body and fat mass, but not lean mass, were positively associated with total-tau, while all these measures were inversely associated with NfL. Apart from an inverse association between lean mass and amyloid-β40, body composition measures were not associated with plasma amyloid-β.

Discussion: Our findings suggest distinct effects of body composition on neurodegeneration.

INTRODUCTION

Obesity during mid-life is a key determinant of dementia,[1, 2] which could be explained by effects of adipose tissue on cardiometabolic health.[3] Although adiposity presumably confers such effects throughout the life course, a higher body mass at older age has been linked to a lower risk of dementia.[4] Given that the aging process is often accompanied by weight loss due to lean mass depletion,[5] failing to differentiate between fat and lean mass has been thought to explain at least part of this seemingly protective effect.[6, 7] Other explanations include methodological issues of studies showing these associations, such as weight loss caused by pre-clinical dementia or mortality precluding a dementia diagnosis.[8] Focusing on pre-clinical markers of dementia may overcome these issues.

Currently, the most accessible pre-clinical blood biomarkers of dementia are total-tau, neurofilament light chain (NfL) and amyloid-β.[9] Tau and NfL are building blocks of neurons and are thought to reflect neuronal breakdown when detected in plasma.[10] Amyloid plaques consist predominately of aggregated amyloid-β42 and to a lesser extend of amyloid-β40. In the early pre-clinical phase of dementia, amyloid-β production is thought to be increased, leading to elevated plasma concentrations. During later stages, amyloid-β42 plasma may decrease due to deposition.[11]

To further elucidate the complex link between body composition and dementia, we determined the association of body, fat and lean mass with plasma total-tau, NfL, amyloid-β40 and amyloid-β42 among older adults.

METHODS

Study setting and population

This study was conducted within the Rotterdam Study, a prospective population-based cohort in the Netherlands.[12] The original study was established in 1990 with 7,983 participants aged 55 years and older and expanded in the year 2000 with an additional cohort of 3,011 participants who had turned 55 years of age or moved into the study area. Extensive follow-up examination rounds take place every 4 to 5 years through home interviews and various physical and laboratory checks at the dedicated research center.

Between 2002 and 2005, corresponding to the fourth examination round of the original cohort and the second examination round of the second cohort, plasma samples were

collected from 5,069 participants (84.6% of the surviving participants) and stored at -80°C. In 2018, the plasma samples were utilized to assess total-tau, NfL, amyloid-β40 and amyloid-β42 concentrations. From these participants, 1,540 were excluded because they had missing data on body composition, primarily due to technical issues and time constraints. We further excluded 109 participants with invalid data on all plasma biomarkers, and 12 participants with prevalent dementia, leaving a total of 3,408 participants for analysis.

Body composition

Total body mass in kilograms was measured using a digital scale and body height using a stadiometer, while participants were wearing indoor clothes without shoes. Dual X-ray Absorptiometry (DXA; Prodigy and iDXA devices, GE Healthcare, Chicago, United States) scans were performed to determine fat and lean mass (excluding bone mineral content). From these data, we calculated body mass index as total body mass in kilograms divided by height in meters squared, fat mass index as total fat mass in kilograms divided by height in meters squared, and lean mass index as lean mass in kilograms divided by height in meters squared.

Assessment of plasma total-tau, NfL, amyloid-β40 and amyloid-β42

Plasma samples were collected in EDTA tubes, aliquoted and stored at -80°C according to standard procedures. Measurements were conducted in two separate batches at Quanterix (Lexington, MA, USA) on a Simoa HD-1 analyzer platform.[13] Total-tau, amyloid-β40 and amyloid-β42 were assessed using the Simoa Human Neurology 3-Plex A assay (N3PA) and NfL using the Simoa NF-light® advantage kit.[14] Samples were analyzed in duplicate and two quality control samples were run on each plate for each analyte. Data were considered as not valid if duplicates were missing, if the concentration coefficient of variation between the two measurement exceeded 20%, or if a control sample was out of range. Detailed descriptions of the concentration coefficient of variation are provided in **Supplementary Table 1**.

Covariables

Data on educational attainment, smoking status and alcohol consumption were collected through home interviews. *APOE* genotype was obtained using polymerase chain reaction of coded DNA samples [15] for the original cohort and with bi-allelic TaqMan assay for the second cohort.[16] Depressive symptoms were assessed using the validated Center for Epidemiology Depression Scale.[17] Physical activity in metabolic equivalent of task (MET) hours was assessed using a modified version of the Zutphen Study Physical Activity Questionnaire.[18] Estimated glomerular filtration rate was calculated with the chronic kidney

disease Epidemiology Collaboration formula using creatinine level, age, sex, and ethnicity.[19] Mild cognitive impairment (MCI) was defined as having self-reported subjective cognitive complaints in combination with having objective cognitive impairment as assessed using a cognitive test battery, comprising letter-digit substitution task, Stroop test, word fluency test, and 15-word learning test.[20] From the latter cognitive tests, a global cognitive factor was determined by taking the first unrotated component of a principal component analysis, explaining 55.7% of the variance in the cognitive test scores. Dementia diagnosis was established through a linkage of the study database with medical records from general practitioners and the regional institute for outpatient mental healthcare. Additionally, comprehensive screenings were conducted during research center visits.[21] For more detailed descriptions of MCI and dementia ascertainment, see **Supplementary Methods**. Serum concentrations of glucose, total cholesterol and high-density lipoprotein (HDL) cholesterol were measured in mmol/L using fasting blood samples. Systolic and diastolic blood pressure, measured in mmHg, were assessed by taking the average of two readings on the right arm while the participant was in a sitting position, utilizing a random zero sphygmomanometer. Information on the use of blood glucose-, lipid- and blood pressure-lowering medication was obtained through home interviews.

Statistical analysis

Total-tau, NfL, amyloid-β40 and amyloid-β42 plasma concentrations had a right-skewed distribution and were therefore $\log_2$ transformed to obtain an approximately normal distribution and standardized to facilitate comparison across the different markers.

Using multivariable linear regression models, we examined the cross-sectional association of each of the body composition measures per standard deviation increase with $\log_2$ concentrations of total-tau, NfL, amyloid-β40 and amyloid-β42. All models were adjusted for assay batch number, height in meters squared, age and sex (Model I) and further for education attainment, smoking status, *APOE* ε4 genotype, alcohol intake, depressive symptoms, physical activity, and the estimated glomerular filtration rate (Model II). In models considering amyloid-β40 as outcome, we further adjusted for amyloid-β42 and vice versa in an additional model (Model III). Missing values on covariables (8.7% for estimated glomerular filtration rate, 5.4% for *APOE* ε4 genotype, 5.3% for depressive symptoms, 3.6% for physical activity, and less than 2% for all other covariables) were handled using fivefold multiple imputation. Analyses were conducted using the five different datasets and pooled estimates are provided. To check whether associations deviate from linearity, we repeated the analyses after including splines to the body composition measures and tested whether this improved the fit of the model using

ANOVA. We confirmed that somewhat extreme body composition measures did not drive the associations by plotting body composition measures against corresponding residuals for visual inspection.

To investigate potential effect modification, we stratified the analyses for sex, age and *APOE* ε4 status. In sensitivity analyses, to minimize the risk of residual confounding, we repeated all analyses after excluding participants with MCI and while correcting for the global cognitive factor. To gain insights into the extent to which cardiometabolic health may contribute to this association, we also repeated all analyses while additionally adjusting for cardiometabolic markers, including serum concentrations of glucose, total cholesterol, and high-density lipoprotein cholesterol, systolic and diastolic blood pressure, and the use of blood glucose-, lipid-, and blood pressure-lowering medications. Moreover, in our main analyses, we refrained from utilizing ratios to mitigate the risk of generating spurious correlations.[22] However, to facilitate comparability with other studies, we repeated all analyses by first using relative body composition measures as exposure (i.e. body mass index, fat mass index and lean mass index) instead of the crude measures; and second, by using the ratio between amyloid-β42/40 as the outcome variable.

All analyses were performed using R statistical software 4.0.4.

RESULTS

Characteristics of the study population are provided in **Table 1**. Participants were on average 72.7 years old (standard deviation: 6.9) and 58% were women. Characteristics of the study population were similar to those of the excluded participants, regardless of whether the exclusion was due to missing body composition data or other factors (**Supplementary Table 2**). Body mass was positively correlated with fat (r: 0.68) and lean mass (r: 0.72), while no correlation was observed between fat and lean mass (r: -0.01). The body composition measures followed a somewhat normal distribution as depicted in **Supplementary Figure 1**.

Table 1. Characteristics of the study population.

	Total study population (n=3,408)
Age, years	72.7 ± 6.9
Sex, women	1,960 (58)
Education attainment	
Primary	391 (12)
Lower	1,487 (44)
Intermediate	1,040 (31)
Higher	433 (13)
Smoking status	
Never	982 (29)
Former	1,857 (56)
Current	501 (15)
APOE genotype	
Non-carriers	2,344 (73)
ε4 heterozygosity	826 (26)
ε4 homozygosity	55 (2)
Alcohol intake, grams/day	7.1 [19.3]
CES-D, score	3 [7]
Physical activity, MET-hours/week	81 [55]
Estimated glomerular filtration rate, mL/min/1.73 m^2	75.4 (13.5)
Mild cognitive impairment	314 (10)
Body composition measures	
Height, cm	166.8 ± 9.2
Body mass, kg	76.5 ± 13.3
Body mass index, kg/m^2	27.5 ± 4.0
Fat mass, kg	26.4 ± 8.7
Fat mass index, kg/m^2	9.6 ± 3.4
Lean mass, kg	47.1 ± 9.3
Lean mass index, kg/m^2	16.8 ± 2.1
Plasma biomarkers	
Total-tau, pg/mL	2.4 [1.1]
Neurofilament light chain, pg/mL	13.7 [8.4]
Amyloid-β40, pg/mL	261.3 [62.8]
Amyloid-β42, pg/mL	10.3 [4.2]

Data are shown for non-imputed data and are presented as mean ± standard deviation for normally distributed continuous variables, median [interquartile range] for skewed continuous variables, and number (percentages) for categorical variables. The data was missing for the following percentages of covariables: 8.7% for estimated glomerular filtration rate, 5.4% for APOE ε4 genotype, 5.3% for depressive symptoms, 3.6% for physical activity, and less than 2% for all other covariables. Abbreviations: CES-D, Center for Epidemiologic Studies Depression Scale; MET, Metabolic Equivalent of Task; n, number of participants.

A higher body and fat mass, but not lean mass, were associated with higher standardized log_2 total-tau levels (mean difference [95% confidence interval (CI)] per standard deviation increase: 0.11 [0.08;0.15], 0.14 [0.10;0.17] and -0.05 [-0.12;0.03], respectively; **Figure 1**). Including splines with 2 knots to log_2 levels of total-tau revealed that the associations with body and fat mass were non-linear (p-value: <0.00 and 0.02, respectively; **Figure 2**). Moreover, all body composition measures were inversely associated with standardized log_2 levels of NfL (mean difference [95% CI]:-0.22 [-0.25;-0.18] for body mass, -0.15 [-0.18;-0.12] for fat mass, and -0.40 [-0.46;-0.35] for lean mass; **Figure 1**). A higher lean mass was associated with lower standardized log_2 amyloid-β40 levels (mean difference [95% CI]: -0.11 [-0.17;-0.05]). All other body composition measures were not associated with either levels of amyloid-β40 or amyloid-β42. Associations were robust across all formulated statistical models, wherein adjustments were made for various covariables (**Supplementary Figure 2**).

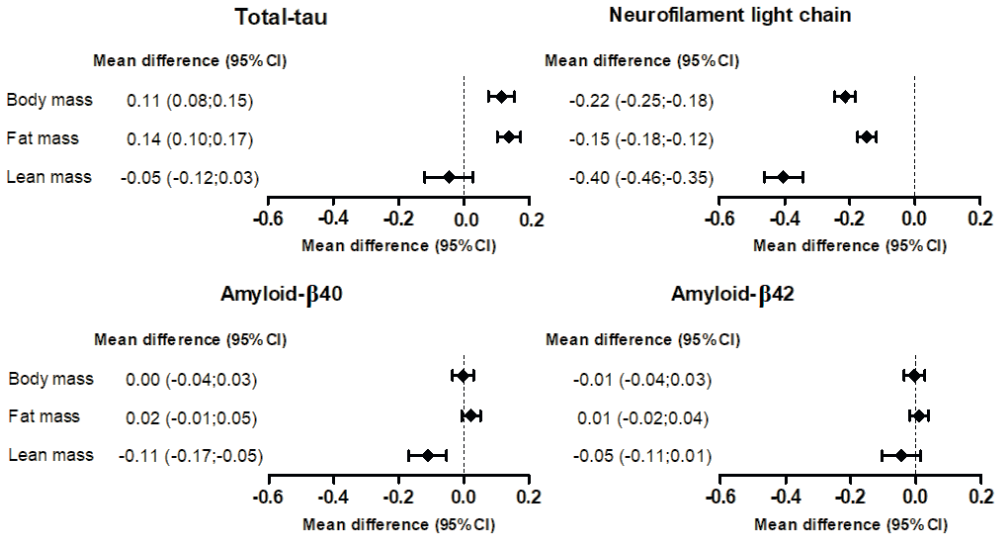


Figure 1. Body composition and plasma total-tau, neurofilament light chain and amyloid-β. Mean difference represents the association between body composition measures per standard deviation increase and standardized log_2 plasma levels. All models are adjusted for assay batch number, height in meters squared, age, sex, education attainment, smoking status, *APOE* ε4 status, alcohol intake, depressive symptoms, physical activity and estimated glomerular filtration rate. Models with amyloid-β40 as outcome are additionally adjusted for amyloid-β42 and vice versa. Abbreviations: CI; Confidence interval.

Stratified analyses uncovered effect modification by sex in the relationship between lean mass and total-tau (**Supplementary Figure 3**), primarily driven by a non-linear association with distinct inflection points occurring at circa 40 kg for women and 60 kg for men

(**Supplementary Figure 4**). No additional effect modification was observed for sex, *APOE* ε4 carriership or age. Body composition measures as well as total-tau and amyloid-β42 plasma concentrations were similar for participants with and without MCI, while NfL and amyloid-β40 concentrations were somewhat higher in participants with MCI (**Supplementary Table 3**). Excluding the 314 participants with MCI did not affect the results, neither did correcting for the global cognitive factor (**Supplementary Figure 5**). Results were also similar after correcting for cardiometabolic markers. Furthermore, repeating all analyses using relative body composition measures (i.e. body mass index, fat mass index and lean mass index) resulted in similar effect estimates as for absolute body composition measures (**Supplementary Figure 6 and 7**). Body composition measures were not associated with the amyloid-β42/40 ratio (**Supplementary Figure 8**).

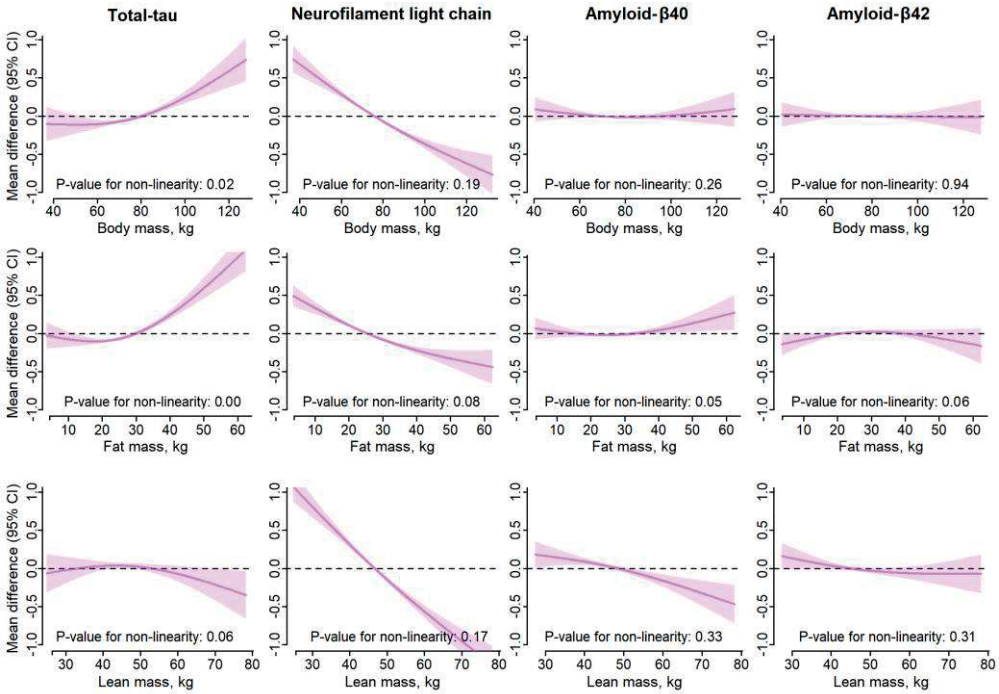


Figure 2. Non-linear associations of body composition with plasma total-tau, neurofilament light chain and amyloid-β. Mean differences represent the association between body composition measures and standardized log2 plasma levels. Splines with 2 knots are added to the body composition measures. All models are adjusted for assay batch number, height in meters squared, age, sex, education attainment, smoking status, APOE ε4 status, alcohol intake, depressive symptoms, physical activity and estimated glomerular filtration rate. Models with amyloid-β40 as outcome are additionally adjusted for amyloid-β42 and vice versa. Abbreviations: CI; Confidence interval.

DISCUSSION

In this population-based study, higher body and fat mass, but not lean mass, were associated with higher plasma levels of total-tau, which were driven by excessive adipose tissue as suggested by the non-linearity of the association. In contrast, a higher body, fat and lean mass were associated with lower NfL levels. Apart from an inverse association between lean mass and amyloid-β40, body composition measures were not associated with plasma levels of amyloid-β40 or amyloid-β42.

Given that plasma total-tau and NfL both reflect neurodegeneration,[10] our findings of higher total-tau and lower NfL levels in those with a higher body mass are contradicting, but corroborate previous studies.[23-28] More specific, previous cross-sectional studies consistently demonstrated an association between a higher body mass index and lower NfL concentrations, irrespective of the participants' health stage and age.[23-27] However, longitudinal research revealed a gradual weakening of this association over time among obese individuals.[28] Moreover, a prior cross-sectional study found a positive link between body mass index and total-tau concentrations,[23] whereas a longitudinal study showed an increase in total-tau levels among obese individuals during a 10-year follow-up period.[28] We extend these findings by differentiating between fat and lean mass, and showed that the positive link of a higher body mass with lower total-tau is driven by excessive adipose tissue, while the inverse association with NfL is driven by both fat and lean mass.

Cardiometabolic dysregulations may underlie the association between fat mass and total-tau levels.[3] More specific, adiposity is a well-established risk factor of insulin resistance, dyslipidemia, hypertension and inflammation,[29] which are in turn hallmarks of dementia.[30] Conversely, tau pathology may trigger insulin resistance,[31] suggesting that the association could be bidirectional. Nevertheless, we found that associations were independent of cardiometabolic health markers, which could imply the involvement of alternative pathways, such as inflammatory responses.[32]

The observed contrasting directions of effect estimates for total-tau and NfL may be explained by the affected brain regions. Although the clinical implication of total-tau and NfL remains largely unclear,[33] tau is most abundant in the cerebral cortex, while NfL is mainly present in the cerebral white matter.[34] White matter consists mainly of myelinated axons, a lipid-rich material, which could possibly be affected by adipose tissue depletion.[35]

Even though we studied pre-clinical markers of dementia, weight loss caused by neurodegeneration may also explain part of the inverse link between body composition measures and NfL. Increased plasma levels of NfL can, in contrast to total-tau, be detected up to 10 years before diagnosis and correlate with disease severity.[11, 36] Similarly, a decline in body mass index has been observed 6 to 8 years prior to a dementia diagnosis.[37-39]

With a higher body mass generally corresponding to more blood volume,[40] prior studies have suggested blood volume as alternative explanation for the inverse link between body mass and NfL through dilution.[24] Our findings that associations with NfL were most pronounced for lean mass may support this hypothesis, because muscle tissue is more perfused than adipose tissue.[40] Dilution may also explain that individuals with more lean mass had lower levels of amyloid-β40. Lean mass was not associated with total-tau and amyloid-β42, suggesting negligible dilution effects on these plasma levels, potentially attributed to their substantially lower concentrations compared to NfL and amyloid-β40.

A better physiological status reflected by higher lean mass may also explain part of the inverse link between lean mass and amyloid-β40 levels.[5] We found no association between other body composition measures and amyloid-β. This should, however, be interpreted with caution, as amyloid can also be produced by tissues of non-neurological origin, like platelets and vascular wall endothelial.[41] In addition, plasma concentrations of amyloid-β are highly dynamic across the different pre-clinical dementia stages, which presents a substantial challenge in detecting associations.[11]

Strengths of this study include the population-based design, use of DXA scans to differentiate between fat and lean mass, and the highly sensitive assay used to determine plasma biomarkers. Limitations are the cross-sectional design, hampering causal inference due to the potential for reverse causality and common causes; and the lack of data on biomarkers measured in cerebrospinal fluid, which provide a more accurate reflection of brain pathology.[10]

Taken together, these findings may suggest distinct effects of body composition on neurodegeneration, but factors of non-neurological origin may also explain part of the observed associations. This study could serve as a basis for the design of future studies to further elucidate the complex link between body composition at old age and brain health. More specifically, we encourage future research to validate these results using biomarkers measured in cerebral spinal fluid and to assess the temporality of these associations.

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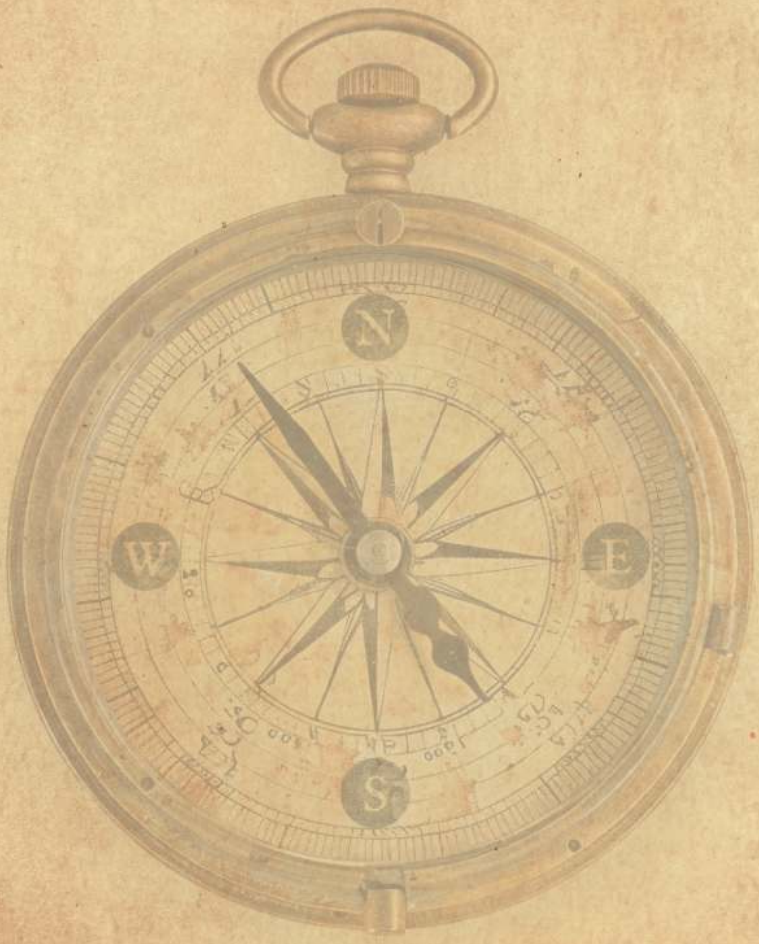
SUPPLEMENTARY MATERIAL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Voor mensen met dementie is de weg naar huis geen vertrouwde route meer. Het kompas, dat vroeger de weg wees, leidt nu naar alle kanten. Geliefden worden vreemden, en de realiteit lijkt steeds vager, waardoor ze verder afdwalen van wat eens vertrouwd was.

Het verlies van oriëntatie bij dementie is niet alleen fysiek, maar ook emotioneel ontwrichtend. Familie en vrienden voelen zich machteloos als ze zien dat hun geliefden verdwalen. Het kompas, dat vroeger een stabiele gids was, kan de kern van iemands identiteit niet meer vasthouden.

Chapter 5

General discussion

GENERAL DISCUSSION

The overall aim of this thesis was to evaluate the role of lifestyle and environmental factors in the risk of dementia, with the ultimate goal to enhance our understanding of possibilities to prevent dementia. More specifically, I have focused on the role of nutrition, air pollution and body composition. In this final section, I provide an overview of the main findings from all described studies, place these findings in the context of current knowledge, and discuss their implications. I also shed light on general methodological considerations, and provide suggestions for further research.

FINDINGS IN CONTEXT AND IMPLICATIONS

Chapter 2 – Nutrition

Nutrition is often referred to as the cornerstone of good health. Foods contain nutrients, vitamins and minerals necessary for growth, repair and maintenance. Furthermore, a healthy diet improves various health outcomes, including cognitive functioning.[1, 2] As such, nutrition has been suggested as a promising modifiable lifestyle factor for the prevention of dementia.

In **Chapter 2.1**, I investigated the association between dietary intake of nitrate and the risk of dementia. I found that a higher dietary nitrate consumption from vegetable sources was associated with a lower risk of dementia, but no such association was observed for dietary nitrate intake from non-vegetable sources. These contradicting findings for dietary nitrate from different sources may be explained by accompanying bioactive compounds in vegetables, like vitamin C and polyphenols. Such compounds enhance the formation of nitrate into nitric oxide,[3, 4] which may affect the risk of dementia by improving vascular function.[5, 6] To further test this hypothesis, I also explored the link between dietary nitrate intake and imaging markers of vascular brain health, but found no statistically significant association. An explanation for these null findings could be that nitric oxide mainly affects small blood vessels that cannot be detected on brain images. Alternatively, the impact of dietary nitrate on vascular functioning may occur predominantly at the peripheral level, which could improve metabolic outcomes and subsequently brain health.[7, 8]

Although studying individual nutrients is valuable, it also introduces complex challenges as nutrients are generally not consumed in isolation and rarely function independently. They mostly interact with each other, which may enhance or diminish potential beneficial

effects.[9, 10] To disentangle this challenge, researchers have increasingly turned to studying dietary patterns, capturing the collective intakes and effects of multiple nutrients.[11] In **Chapter 2.2** and **2.3**, I investigated the link between dietary patterns and the risk of dementia.

Chapter 2.2 focusses on the Mediterranean-Dietary Approaches to Stop Hypertension (DASH) Intervention for Neurodegenerative Delay (MIND) diet.[12] This diet is a hybrid of the Mediterranean and DASH diet, which uniquely emphasizes foods reported to be associated with brain health, among which in particular green leafy vegetables and berries.[13-15] I showed in **Chapter 2.2** that better adherence to the MIND diet was associated with a lower risk of dementia within the first years of follow-up, but that associations attenuated over time or completely disappeared. Since the publication of the study that forms the basis of this chapter, some additional observational studies have been published on this topic.[16-19] In the majority of these studies, an inverse link between MIND diet adherence and the risk of dementia was observed. However, mean follow-up periods in most studies do not exceed 5 years, a time span that aligns largely to the prodromal phase of dementia.[20] During this phase, characterized by initial cognitive impairment, dietary habits may deteriorate[21, 22] due to associated factors like depressive symptoms[23] and olfactory impairment.[24] The transient nature of the observed associations over extended follow-up periods could imply that changes in dietary habits, caused by preclinical dementia symptoms, underlie the observed association.

Another methodological issue that may lead to misleading results in observational studies when examining the adherence to health-conscious diets like the MIND diet includes residual confounding by lifestyle. Healthier dietary habits often coexist with other favorable lifestyle factors like regular exercise, lower stress, and a healthy sleep pattern,[25, 26] which can all independently impact the risk of dementia.[27-29] Fully accounting for such lifestyle factors presents challenges due to limited, unavailable, or imprecise data.[30] Research and communication on the importance of healthy nutrition and lifestyle have strongly increased over the past few decades and thereby healthy diet and lifestyle awareness in the general population.[31] Hence, it is conceivable that the connection between adhering to the MIND diet and maintaining an overall healthy lifestyle has strengthened over time, suggesting that the effect of residual confounding by lifestyle may be more profound in more contemporary years. Against this background, I used historical and more contemporary dietary data and found a substantially stronger association with a lower risk of dementia when considering MIND diet adherence measured in more contemporary years (1989–1993 vs. 2009–2012). These results lend support to the hypothesis that lifestyle-related confounding partly underpins the observed associations.

A recently published clinical trial provides backing for our nuanced conclusion on the link between the MIND diet and the risk of dementia.[32] In this trial, 604 cognitive unimpaired older individuals with a family history of dementia and a body mass index >25 kg/m^2 were randomized either to a "mild caloric restriction diet" or "the MIND diet in combination with mild caloric restriction". Participants were closely monitored for a duration of 3 years, after which no statistically significant differences in changes of cognitive performance or brain structures were observed between groups.

Although compelling evidence strongly suggest that diet plays a crucial role in the development of dementia[33], it is essential to approach the existing literature with a critical perspective and acknowledge that the literature underpinning the claims of the MIND diet might not be as robust as initially suggested. As such, the composition of the optimal diet to mitigate the risk of dementia remains unclear. This advocates for a broader scope of research that delves into dietary patterns beyond the extensively explored MIND diet.

In **Chapter 2.3**, the focus therefore lies on plant-based dietary patterns. In this study, I found no strong evidence for an overall association between plant-based dietary patterns and the risk of dementia. This was regardless of whether the diet was dominated by healthy or unhealthy plant-based foods. However, in stratified analyses, an inverse association between healthy plant-based eating and the risk of dementia was observed among *APOE* ε4 carriers. Among non-carriers of the *APOE* ε4 allele, the association was suggestively U-shaped, possibly as a result of deficiencies owing to an excessive reduction of animal-based foods from the diet.[34, 35] Furthermore, when the analyses were stratified by sex, an association between more healthy plant-based eating and a lower risk of dementia was observed in men, but not in women. Such dissimilarities among sexes could potentially stem from differences in eating habits and lifestyle factors.

Although the role of plant-based dietary patterns in the development of dementia remains largely uncertain, it is essential to recognize that plant-based eating offers broader positive implications, particularly in terms of sustainability.[36] In **Chapter 2.4**, I therefore studied barriers and facilitators to adopt a more plant-based dietary pattern, using a focus group of five participants from the Rotterdam Study. All participants indicated that they were willing to consume fewer animal-based foods, but they expressed discomfort to the idea of being forced to do so through for instance limiting the availability of animal-based foods. The primary barrier to adjust their dietary habits that emerged was their taste preference for meat. Enhancing awareness of the advantages of a more plant-based dietary

pattern was identified as the most effective strategy to inspire individuals to reduce their consumption of animal-based foods.

Taken together, nutritional factors may influence the risk of dementia, but this link is probably influenced by other lifestyle and genetic factors. Importantly, regardless of the conclusions drawn from this specific chapter, the broader importance of a healthy diet should be acknowledged, as a healthy diet is a fundamental component of a healthy lifestyle that beneficially impacts overall health and well-being. Furthermore, a shift towards a more plant-based dietary pattern is needed to mitigate environmental hazards, which has in turn implications for human health.

Chapter 3 – Air pollution

In recent years, a growing body of research has been aimed at understanding the complex role of environmental factors on human health and well-being.[37] Among these factors, air pollution has emerged as a topic of significant interest and concern.[38] With a long history of research on air quality and cardiovascular health, the adverse effects of air pollution on for instance the risk of hypertension and stroke are well-established.[39-41] Given that cardiovascular health plays a crucial role in maintaining optimal brain function,[42, 43] attention has aggregated towards the potential influence of air quality on cognitive function and the risk of dementia.[44]

In **Chapter 3.1**, I investigated whether individuals from the Rotterdam Study residing in more polluted areas were at a higher risk of dementia and cognitive decline than those residing in less polluted areas. Unlike most prior research,[45-47] I did not yield clear evidence for such a link, but all observed effect estimates aligned with our hypothesized direction. The fact that the effect estimates were small and not statistically significant may be explained by the relatively small variation in air pollutant levels within our study area. Nevertheless, variation was comparable in two previous studies in which non-linear association patterns with the risk of dementia were observed, such that strong associations were observed from low to mean pollutant levels and no association for mean to high levels.[48, 49] Given that background levels in these studies were up to three times lower compared to those in the Rotterdam Study, ceiling effects may be an alternative explanation for our observed null findings.

In **Chapter 3.2**, I further explored mechanisms through which air pollution may exert its detrimental health effect by linking different air pollutants to 940 metabolites measured in plasma. After correcting for multiple testing, several statistically significant associations between the air pollutants and metabolites were observed. Pathway analyses revealed that

particularly metabolites involved in the reproductive system and cell metabolism were related to the different air pollutants.

In summary, although this chapter may not support the hypothesis that limited variation in exposure to air pollution influences the risk of dementia, it does shed light on potential mechanisms through which air pollutants exert their detrimental effects on human health. This emphasizes the importance of focusing on improving air quality as part of broader efforts to keep the population healthy.

Chapter 4 – Body composition

Having studied nutrition and air pollution as potential determinants of dementia, I investigated in this chapter the potential role of body composition in the risk of dementia. Body composition refers to the proportion of different tissues that constitute an individual's body. Among the tissues, fat and muscle tissue are the most unstable components that can be shaped and modified through lifestyle choices and environmental factors.[50] Given that both components play a pivotal role in determining metabolic functioning, it is widely believed that the proportion and distribution of adipose and muscle tissue act as a mediator in the association of lifestyle and environmental factors with the risk of dementia.[28, 51]

Particularly adiposity, the measure of body fat, has widely been studied as a risk factor of dementia.[52] While adiposity during mid-life has consistently been linked to an increased risk of dementia,[53, 54] the opposite link is generally seen for adiposity later in life.[55] Proposed explanations for this counterintuitive pattern, known as the obesity paradox, include methodological issues. The most cited potential explanations are that weight loss is caused by pre-clinical dementia and that individuals decease due to excessive adipose tissue before developing dementia.[56] Another factor that may play a role is that the aging process is generally accompanied by a shift to more adipose tissue and a decrease in muscle mass.[57] Adiposity is mostly measured by body mass index, through which no distinction between fat and lean mass is made. As such, a decreased body mass index at older age may reflect lean mass depletion rather than adipose tissue depletion, especially considering that muscle mass carries relatively greater weight.[58]

To create more insight into the complex link between adiposity and brain health, I first studied the association of fat mass and the incidence of dementia among older adults over an average follow-up period of 10.3 years (**Chapter 4.1**). I found in line with prior research a seemingly protective effect of fat mass on the risk of dementia. However, after excluding the first 5 years of follow-up, which aligns largely with the pre-clinical dementia phase,[20]

most associations did not remain statistically significant. Next, I determined trajectories of fat mass before a dementia diagnosis and compared those to trajectories of individuals free of dementia at index date. Observed trajectories did not align with the expected pattern of an initially higher fat mass followed by later reductions in individuals who developed dementia. This may be explained by the relatively low number of repeated fat mass measurements per participant. Alternatively, this could support the speculative notion that more body fat may offer protective effects. This hypothesis stems from the recognition that adipose tissue is an endocrine organ that releases hormones called adipokines.[59] Such hormones have profound influences on various physiological processes, including insulin sensitivity, inflammation, and vascular function.[60, 61] It has been thought that hormones secreted from visceral fat, the type of fat located around the organs, mainly exhibit negative consequences. In contrast, hormones secreted by subcutaneous adipose tissue located around the gynoid region may have a positive effect on physical functioning.[62] Against this background, I linked apart from total fat, also regional fat (i.e. in the gynoid and abdominal region) to the risk of dementia and found indeed that after excluding the first 5 year of follow-up, only the link with gynoid fat among women remained statistically significant.

To further explore the complex relation between body composition and dementia, I linked in **Chapter 4.2** body composition measures to pre-clinical blood biomarkers of dementia, among which total-tau and neurofilament light chain. I found that more fat mass was associated with higher total-tau concentrations, while the opposite trend was observed for neurofilament light chain. Given that plasma total-tau and neurofilament light chain both reflect neurodegeneration,[63] observed directions are contradicting, but corroborate previous studies.[64-68] Affected brain regions may explain these findings as tau is most abundant in the cerebral cortex, while neurofilament light chain is mainly present in the cerebral white matter.[69] This may open new avenues for further studies to unravel pathways that underpin this relationship.

Taken together, the paradoxical relationship between body fat and the risk of dementia may in part be explained by methodological issues. Whether total and regional adipose tissue offers protective effects against dementia remains a topic of debate and should be studied in further detail before public health and clinical implications can be established.

METHODOLOGICAL CONSIDERATIONS

Studies that form the basis of this thesis are exclusively conducted within the prospective population-based Rotterdam Study.[70] Although population-based studies are commonly used in healthcare research, randomized controlled trials are typically considered as the gold standard to establish causality. However, for studying the role of lifestyle and environmental factors in the risk of dementia, ethical constraints and practical limitations often preclude the application of such trials. Therefore, prospective population-based studies play a pivotal role by improving our understanding in causal inference on determinants of dementia. Nevertheless, such studies are also subject to numerous methodological challenges. In the following section, I will elaborate on the challenges that are generally most applicable to the research described in this thesis.

Reverse causality

Reverse causality is a complex phenomenon that is a recurrent issue in observational studies on dementia risk. It refers to a scenario where the direction of the cause-and-effect is mistaken. In prospective population-based studies, time-to-event analyses are mostly used to study determinants of a certain incidental health outcome. One could argue that by studying incidence after a baseline assessment, temporality can be assured. Nevertheless, for several chronic diseases, among which particularly dementia, this presents complex challenges due to the stages preceding the disease. As shown in **Figure 1**, the preclinical stage of dementia emerges up to 20 years before diagnosis and is marked by neuropathological changes despite normal cognition.[71, 72] The prodromal stage, characterized by mild cognitive impairment, initiates within a timespan up to 7 years prior to a diagnosis.[20] During these stages, various physiological adaptations can occur, which probably influence behavioral patterns.[73-75]

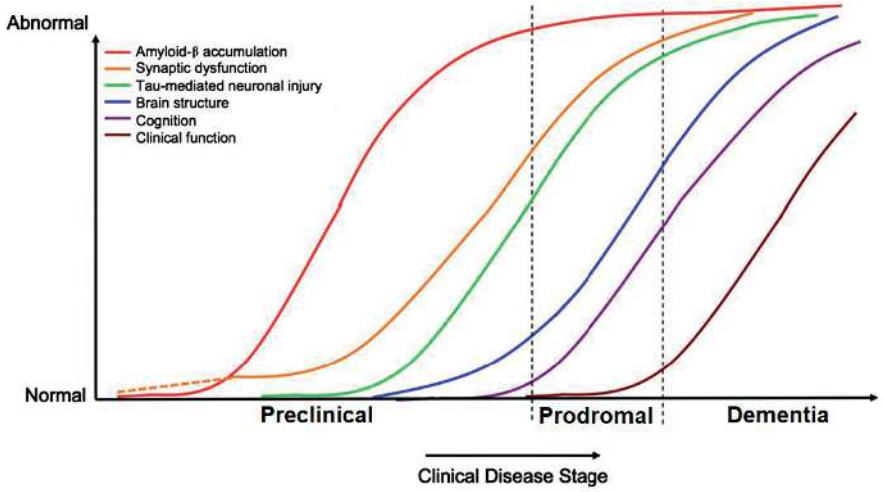

Figure 1. Stages preceding a dementia diagnosis along with hypothetical data on physiological changes associated with these stages. The figure represents physiological changes from normal to maximally abnormal (y-axis) as a function of the disease stage (x-axis). Figure adapted from Sperling et al.[76]

By considering the aspect of time, a better understanding of the sequence of events can be obtained. I showed in **Chapter 2.2** that by determining effect estimates over cumulative follow-up intervals, certain patterns can be identified. I further showed in **Chapter 4.1** differences in effect estimates after excluding the first 5 years of follow-up. In addition, I provided trajectories of the body composition measures before diagnosis and compared these trajectories to those of dementia-free controls. Here, I further explored this topic by using physical activity as an example. **Figure 2** provides the association between physical activity and the risk of dementia over cumulative follow-up intervals as well as per 4 years epoch after baseline. Overall, this figure shows that associations are driven by the first years of follow-up. The question remains whether based on such observations, we can state that individuals tend to be less physically active as a result of their pre-clinical dementia symptoms or whether such pattern rather explains during which stage of the preclinical disease the physical activity exhibits its influence. Still, mapping the sequence of such events can serve as a guide to identify areas that warrant further investigation through clinical trials. If conducting such trials may not be possible due to for instance ethical concerns or practical limitations, alternative methods as employing animal models can come into play.

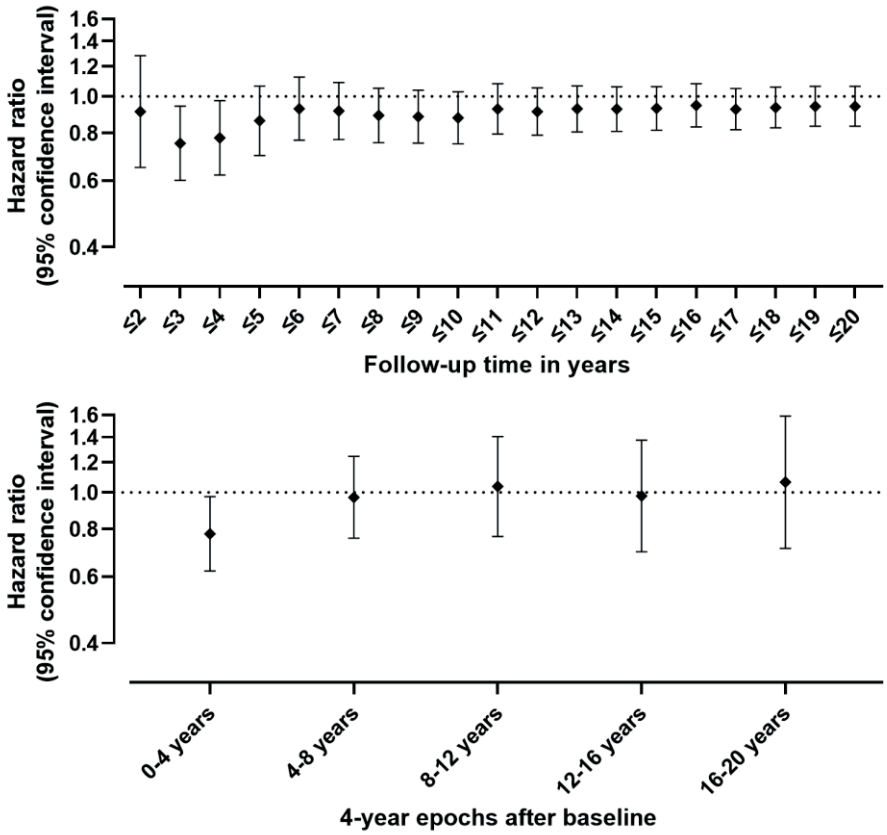

Figure 2. Physical activity and the risk of dementia. Cox proportional hazard models were used to determine the link between physical activity and the risk of dementia. Physical activity was assessed in 1,612 participants from the first sub-cohort of the Rotterdam Study (2000-2004), who were followed-up for incident dementia until 2020. Models were adjusted for age, sex, level of education, smoking status, body mass index, alcohol consumption and presence of depressive symptoms.

Competing risk by mortality

Another major methodical issue in dementia research is competing risk by mortality, meaning that mortality precludes an individual from developing dementia. More specifically, if a determinant is associated with the risk of death, mortality could conceal effects of the determinant on dementia risk. This is particularly problematic in dementia research as dementia is a disease that typically manifests at older age as shown in **Figure 3**. The figure also depicts that the distribution of mortality age is somewhat more widespread and has a slightly left-skewed nature. This indeed denotes that it is likely that individuals develop other diseases that can contribute to the risk of mortality before dementia onset.

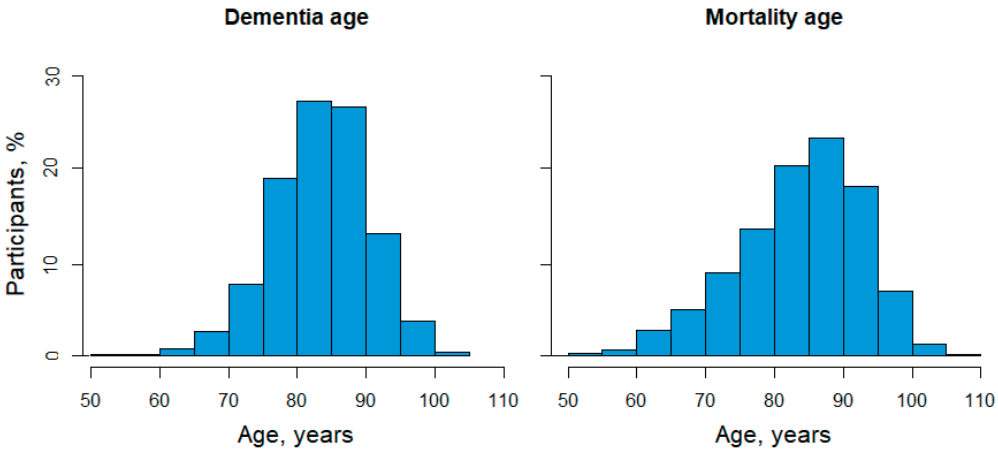

Figure 3. Age onset of dementia and mortality in the Rotterdam Study.

In time-to-event analyses, there are several options to deal with competing events, but given that studies presented in this thesis exclusively included etiological questions, participants were censored at the time of a competing event. By adopting this approach, cause-specific hazard ratios were obtained as hazard ratios are determined only among individuals who are at risk of dementia.[77] Nevertheless, one of the assumptions underlying this statistical model is that censoring is non-informative, meaning that censoring is independent of the prognostic value of the outcome of interest.[78, 79] If the determinant of interest is also related to the risk of mortality, this assumption is not met when censoring participants once they decease. As such, effects of a determinant of dementia can still be concealed. This makes it important to describe the occurrence of the competing event by for instance visualizing Kaplan-Meier survival curves per category or per covariate-adjusted quartile of the exposure as shown in **Figure 4**. The figure depicts that individuals in the lowest MIND diet score quartile have a somewhat higher survival rate compared to those in higher MIND diet score quartiles. Similarly, individuals residing in more polluted areas had a higher survival rate than those in less polluted areas. No clear differences in survival rates across quartiles of the healthy plant-based dietary index of body fat mass can be visualized. These findings suggest that the effect estimates obtained in the previous chapters, concerning the associations of the MIND diet and air pollution, with the risk of dementia, may deviate from the true effects due to the presence of competing risk by mortality.

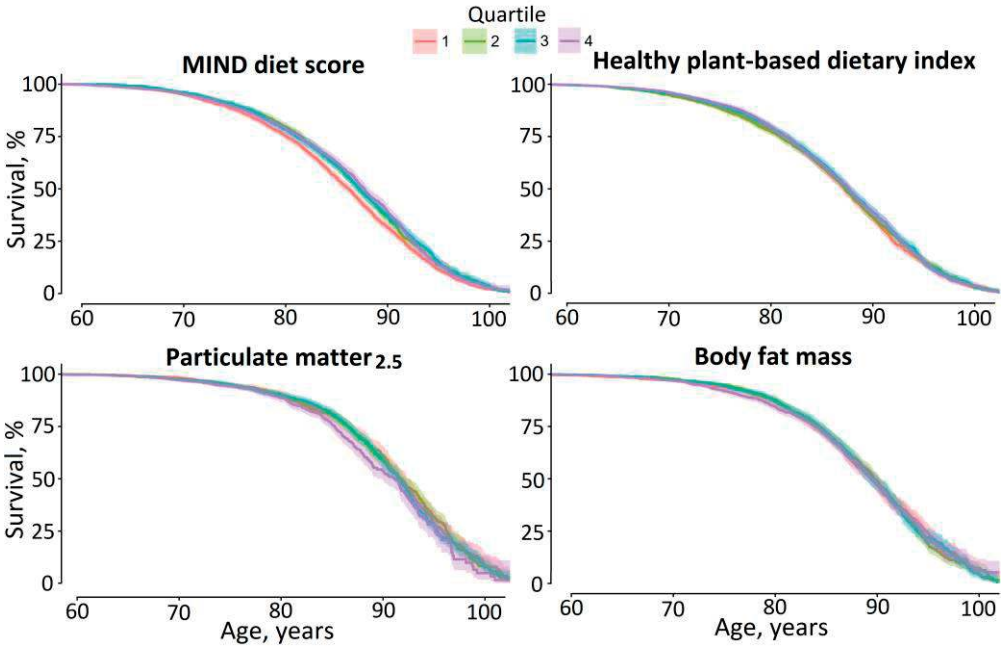

Figure 4. Kaplan-Meier survival curves by quartiles of potential modifiable risk factors from dementia. Quartiles of the diet scores are sex specific and those of body fat mass are sex and height specific. Quartiles were generated by regressing the exposure of interest against covariates (i.e. sex and height), and then categorizing the resulting residuals into quartiles.

Residual confounding by lifestyle

Residual confounding is a critical concern in research on behavioral factors, as individuals with certain healthy lifestyle behaviors are more likely to also adhere to an overall healthy lifestyle.[80] In the used statistical models, I corrected for a large set of potential confounding factors like educational attainment, physical activity and smoking status. Nevertheless, even after thoughtful attempts to control for such factors, confounding may persist and can lead to spurious associations.[81, 82] Such spurious associations may arise due to incomplete or imprecise data on confounding variables, making it challenging to fully account for their influence.[30, 83]

In **Chapter 2.2**, I described a method beyond adjusting for potential confounders through which the issue of residual confounding by lifestyle may in part be reduced. I used both historical and more contemporary data with the assumption that historical data is less affected by lifestyle confounding. This hypothesis stems from the fact that awareness on a healthy lifestyle has increased steeply over the past decades and that accordingly the correlation across different healthy lifestyle factors has increased as well.[31] Another method

that may overcome this issue in observational studies includes the use of instrumental variables as exposure.[84] Such a variable affects the exposure of interest, but is unrelated to potential confounding. Through for instance Mendelian randomization, a method that leverages genetic variants as instrumental variables, the causal impact of the exposure of interest can potentially be identified in a more robust and reliable manner.

Measurement bias

Measurement bias can arise from, for instance, inaccurate or invalid assessment tools, human errors or the complex nature of the variables being measured. Among the diverse domains studied in public health research, dietary habits and exposure to air pollution may be the factors most prone to measurement bias.

The complexity of nutritional assessments arises from the multidimensionality of the diet that consists of various foods as well as various macronutrients and micronutrients with different health effects.[85] Establishing the nutrient content of foods presents major challenges as this varies by geographic region and depends on factors like how they are stored, prepared and processed.[86] Besides, in the studies provided in this thesis, dietary habits were self-reported using food frequency questionnaires (FFQ). This method is subject to recall bias, where participants may overestimate or underestimate their actual dietary intake.[87] Yet, given that individuals were unaware of whether they would develop dementia or any other neurodegenerative disease during follow-up, this form of measurement bias probably has been non-differential, which generally leads to an underestimation of the true association. However, part of the measurement bias may also be differential as adiposity relates to brain health[52] and individuals with overweight are generally more likely to underreport their dietary intake compared to normal weight individuals.[87] To reduce this measurement error, I corrected for energy intake in the statistical models.[88] Moreover, it is important to recognize that the FFQs have been validated against various other dietary assessment methods, namely 3-day diet records,[89] dietary history[90] and urea samples.[91] All validation studies concluded that the FFQs can adequately rank participants based on their food and nutrient intake.

Challenges that arise when measuring exposure to air pollution mainly stem from the wide range of sources from which air pollutants are derived and the spatial and temporal variability in air pollutant levels.[92, 93] In the studies presented in this thesis, exposure to air pollution was determined as ambient air pollutant concentrations at participants' residential addresses based on pre-developed land use regression models.[94-96] Using data on traffic, population density and other meteorological factors, pollution levels at fine spatial scales can be calculated.[97] Although this is a sophisticated method to determine air

pollution, the models were not developed for our specific study area, limiting the precision and accuracy of the models. Moreover, ambient air pollutant concentrations were determined at participants' residential addresses, which introduces measurement bias due to the mobile nature of populations. Although I attempt to minimize measurement errors by correcting for several factors representing the mobility of the participants, including physical activity and occupational status, measurement errors cannot completely be filtered out. Besides, indoor air quality was not measured, which further limits the identification of full exposure patterns.

Selection bias

Selection bias should not be conflated with sampling bias. While sampling bias refers to a scenario where enrolled participants differ from the broader population they are meant to represent, selection bias is a type of bias where the exposure of interest is differentially related to the outcome in the study population than in the population that is theoretically eligible to participate. While sampling bias affects the generalizability of the results, selection bias affects the internal validity.

The Rotterdam Study has an overall response rate of 72%, which is relatively high and thereby minimizes the risk of selection bias. Probably even more important, the attrition rate of approximately 5% for incident disease is relatively low. This is due to the computerized linkage of the medical records from general practitioners and the regional institute for outpatient mental healthcare with the study database, through which participants are continuously under surveillance for incident disease. This relatively low attrition rate does, however, not apply to the follow-up research visits for which participants are invited every 3 to 6 years. Per round, the attrition rate was approximately 20%, which can pose challenges as older and less healthy participants are more likely to drop out of the study. Individuals who, for instance, experience substantial cognitive decline between examination rounds may be less inclined to visit the research center. In addition, those who experience mild cognitive impairment may not be willing to undergo cognitive examination, creating a bias where those with milder cognitive decline or no decline at all become overrepresented in the study. This form of differential attrition will lead to misleading results when studying changes in cognitive performance over time.

Generalizability

Participants from the population-based Rotterdam Study were residing in the Ommoord district at study entry and are predominantly of Caucasian ethnicity. Although such demographic homogeneity offers certain advantages by reducing potential confounding, it limits generalizability of our findings to other geographical areas and ethnic groups. This

may especially be an issue for studies on dietary habits, as diets vary widely across populations.[98] In the Netherlands, a traditional lunch for instance often consists of a sandwich while in most Asian countries, a traditional lunch meal includes rice with vegetables and meat or fish. Similarly, findings from our studies on air pollution may not be generalizable to other geographic areas due to distinct socio-economic, infrastructural, and regulatory landscapes that probably influence both the source and levels of different air pollutants. In the context of body composition, restricted generalizability primarily arises due to the variation in adipose tissue deposits among different ethnicities.[99]

DIRECTIONS FOR FURTHER RESEARCH

Research presented in this thesis has shed light on the role of certain lifestyle and environmental factors in the development of dementia. However, our journey towards a comprehensive understanding of dementia prevention is far from complete. To increase our understanding on this topic, further research should focus on potential determinants throughout the life course. Interactions across lifestyle, environmental and genetic factors should also be disentangled in further detail. Moreover, with the vast majority of research on dementia being conducted in Europe and North America,[100] further research should focus on underrepresented geographic areas and ethnicities to improve generalizability of the current knowledge on determinants. In this paragraph, I will propose specific recommendations on further research per potential determinant.

Nutrition

As described previously, measurement bias is a major challenge in studying nutrition. By combining nutritional intake assessed by food frequency questionnaires with objective measures such as nutrient biomarkers, a cross-verification of the findings can be obtained. The fact that diets are not static, but rather evolve over time, complicates research on nutrition further. Modelling trajectories of dietary habits throughout the lifespan could provide insights on long-term exposure patterns and their accumulating effects on dementia risk. In addition, it may inform about the causal direction of this link.

Further research is also warranted on the interactive effect of nutrition with genetic factors, among which particularly the *APOE* ε4 gene. This gene carries the strongest genetic risk of late-onset Alzheimer's disease, while it simultaneously plays a key role in the regulation of lipid metabolism.[101] It has been thought that carriers of the *APOE* ε4 genotype may be more susceptible to the detrimental effects of high saturated fat intake on cognitive

function.[102] Conversely, *APOE* ε4 carriers may benefit more from diets rich in antioxidants, omega-3 fatty acids, and other neuroprotective nutrients as this may counteract the genetic risks.[103] This highlights the need to consider genetic factors, among which particularly the *APOE* gene, for the selection of individuals eligible for nutrition interventions. By improving our understanding on this topic, more personalized dietary recommendations can be developed that match individual's unique genetic predispositions and metabolic responses.

Another pressing direction for further research on dementia includes timing of food consumption.[104] An eating pattern characterized by switching from fasting to eating on a regular schedule, without enduring nutrition deficiencies, may diminish oxidative stress and inflammatory responses and also improves insulin sensitivity.[105] Moreover, it has been thought that such eating pattern minimizes amyloid pathology[106] and improves cerebrovascular dynamics,[107] which are key hallmarks of dementia. Yet, direct evidence supporting this hypothesis remains scarce. Although strict adherence to such a dietary schedule may not be ideal for preventive purposes, it may be promising for individuals who already experience early dementia symptoms. This warrants advanced exploration in well-designed trials.

Air pollution

To better understand the role of air pollution in the risk of dementia, efforts should be made in determining exposure to air pollution in a more accurate and precise matter. This could be established using devices carried by the participants during their daily routine.[108] However, such method is cost, time and labor intensive. Alternative methods proposed therefore involve tracing an individual's location with a GPS device so that air pollutant levels can be modelled throughout the day.[109] With the addition of data on indoor pollution gathered through administering questionnaires, a more precise calculation of the total exposure patterns can probably be made. Furthermore, knowledge should be extended on the effect of different sources of pollutant particles in the air on brain health. By enhancing our understanding of these subjects, more targeted interventions can be developed aiming at minimizing cognitive impairment.

Body composition

A burning question remains whether moderate to high adipose tissue mass at older age has protective capacities against dementia. As described previously, the hypothesis that a higher adipose tissue mass has protective properties against dementia stems from its endocrine function.[62] Adipose tissue has the capacity to excrete bioactive compounds called adipokines, that exhibit effects on peripheral levels as well as in the central nervous

system. In recent years, more than a hundred different adipokines have been identified, which play vital roles in regulating various physiological processes including inflammation, metabolism and immune functions.[110] Also, interactive effects among adipokines have been identified, which highlights the need to study their combined effect on dementia risk.[111] To further elucidate the effect of adipokines on the brain, population-based cohort studies should track changes in body composition throughout the life course, while simultaneously measuring circulating adipokine levels. Given that the release of adipokine is fat deposit-specific, differentiations between total and regional fat mass should also be made.[112, 113]

CONCLUSION

The prevalence of dementia is on an alarming upward trajectory as projections indicate that the number of dementia cases worldwide will triple by 2050.[114] To combat this growing public health concern, it is imperative to identify modifiable risk factors for dementia. In this thesis, I have sought to extend our understanding on the potential modulating role of nutrition, air pollution and body composition in the development of dementia. Our journey towards unraveling the environmental and lifestyle factors contributing to the multifactorial etiology of dementia is still in its early stages, and warrants further research.

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Chapter 6

Summary

SUMMARY

With the projected increase in population ageing and growth, the prevalence of dementia is expected to increase rapidly in the coming years. Accordingly, the already enormous burden of dementia on patients, their family and society will increase further. This emphasizes the urgent need to identify strategies to reduce the prevalence of dementia. The overall aim of this thesis was therefore to evaluate the role of lifestyle and environmental factors in the risk of dementia, with the ultimate goal to enhance our understanding in opportunities to reduce or prevent dementia. More specifically, I examined the role of nutrition, air pollution and body composition.

The first part of this thesis focused on nutrition. In **Chapter 2.1**, I showed that a higher consumption of nitrate derived from vegetable sources was associated with a decreased risk of dementia, whereas nitrate derived from non-vegetable sources did not exhibit such an association. Moreover, associations could not be explained by vascular brain health. Although studying individual nutrients is valuable, it also presents complex challenges as nutrients are typically consumed in combination with other nutrients and rarely act independently. In **Chapter 2.2** and **Chapter 2.3**, I therefore shifted the focus to the role of dietary patterns in the development of dementia, capturing the collective intakes and effects of multiple nutrients. I first investigated the Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND) diet, a diet that has been specifically developed for dementia prevention. Individuals who adhered better to the MIND diet were at a lower risk of developing dementia within the first years of follow-up, but this may in part be explained by reverse causality and residual confounding by lifestyle. I further explored plant-based dietary patterns, but found no compelling evidence for an association with the risk of dementia in the total study population. Nevertheless, among *APOE* ε4 carriers and men, those who consumed more healthy plant-based foods were at a lower risk of dementia, while this relationship was somewhat U-shaped among *APOE* ε4 non-carriers and absent among women. Although the exact role of plant-based dietary patterns in the development of dementia remains unclear, it is important to acknowledge that plant-based eating offers broader positive implications, particularly in terms of sustainability. I therefore studied in **Chapter 2.4** barriers and facilitators to adopt a more plant-based dietary pattern, and found that taste preference for animal-based foods was the most important barrier individuals encountered. Enhancing awareness of advantages was generally seen as the most effective strategy to inspire individuals to reduce their consumption of animal-based foods.

Focusing on air pollution, I investigated in **Chapter 3.1** the link between exposure to air pollution and the risk of dementia and showed that individuals residing in relatively higher polluted areas were not at a higher risk of developing dementia than those residing in less polluted areas. The variations in air pollutant levels within the study area were relatively small, suggesting that minor fluctuations may not have a differential impact on brain health. Nonetheless, previous studies with comparable exposure level variations have observed associations, which could imply that our null findings are attributed to non-linear associations. In **Chapter 3.2**, I investigated mechanisms through which air pollutants exhibit their detrimental effects on human health by linking exposure to air pollution to circulating metabolites in plasma. I discovered that exposure to higher air pollutant levels was associated with differentially expressed metabolites related to the reproductive system and cell metabolism.

The studies in **Chapter 4** related to the role of body composition in the risk of dementia. In **Chapter 4.1** I first reported on the link between body composition and the risk of dementia and found that individuals with a higher body and fat mass were at a lower risk of dementia. However, these associations largely disappeared after excluding the first 5 years of follow-up, suggesting that the seemingly protective effect of more fat mass can be explained by reverse causality. Nevertheless, determining trajectories of fat mass before dementia onset did not reveal a higher initial mass and later reduction. This could support the hypothesis that more fat mass offers protective effects. I therefore further examined the potential effect of adipose tissue on the brain by linking body composition measures to pre-clinical plasma markers of dementia in **Chapter 4.2**. I discovered that a higher fat mass was related to higher total-tau concentrations, while the opposite trend was observed for neurofilament light chain levels. These contradicting findings may be explained by affected brain regions as tau is most abundant in the cerebral cortex, while neurofilament light chain is mainly present in the cerebral white matter.

In **Chapter 5**, I provided an overview of the main findings from all the described studies, placed these findings in the context of current knowledge, and discussed their implications. I also shed light on general methodological considerations, and provided suggestions for further research directions to unravel lifestyle and environmental factors contributing to the multifactorial etiology of dementia.

SAMENVATTING

Met de verwachte vergrijzing en bevolkingsgroei zal de prevalentie van dementie de komende jaren naar verwachting snel toenemen. Hierdoor zal de reeds enorme last van dementie op patiënten, hun familie en de maatschappij verder toenemen. Dit benadrukt de dringende noodzaak om strategieën te identificeren om de toename van dementie tegen te gaan. Het algemene doel van dit proefschrift was dan ook om de rol van levensstijl en omgevingsfactoren in het risico op dementie te evalueren, met als ultiem doel ons begrip van preventieve mogelijkheden tegen dementie te vergroten. Specifiek onderzocht ik de rol van voeding, luchtvervuiling en lichaamssamenstelling.

Het eerste deel van dit proefschrift richtte zich op voeding. In **Hoofdstuk 2.1** heb ik aangetoond dat een hogere consumptie van nitraat afkomstig van groente als voedingsbron geassocieerd was met een verminderd risico op dementie, terwijl nitraat afkomstig van andere voedingsbronnen dan groente geen dergelijke associatie liet zien. Bovendien konden de verbanden niet worden verklaard door vasculaire hersengezondheid. Hoewel het bestuderen van individuele nutriënten waardevol is, brengt het ook complexe uitdagingen met zich mee, aangezien nutriënten gewoonlijk in combinatie met andere nutriënten worden geconsumeerd en zelden onafhankelijk functioneren. In **Hoofdstuk 2.2** en **Hoofdstuk 2.3** verlegde ik daarom de focus naar de rol van voedingspatronen in de ontwikkeling van dementie, waarbij de gezamenlijke inname en effecten van meerdere nutriënten werd onderzocht. Ik onderzocht eerst het MIND (Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay) dieet, dat specifiek is ontwikkeld voor de preventie van dementie. Individuen die zich beter hielden aan het MIND dieet liepen een lager risico om binnen de eerste jaren van follow-up dementie te ontwikkelen, maar dit kan deels worden verklaard door omgekeerde causaliteit en resterende confounding door levensstijlfactoren. Verder onderzocht ik plantaardige voedingspatronen, maar vond geen overtuigend bewijs voor een associatie met het risico op dementie in de totale onderzoekspopulatie. Desalniettemin, dragers van het *APOE* ε4 gen en mannen die meer gezonde plantaardige voeding consumeerden liepen een lager risico op het ontwikkelen van dementie, terwijl deze relatie enigszins U-vormig was bij *APOE* ε4-niet-dragers en afwezig was bij vrouwen. Hoewel de exacte rol van plantaardige voedingspatronen in de ontwikkeling van dementie onduidelijk blijft, is het belangrijk om te erkennen dat plantaardig eten bredere positieve implicaties biedt, met name op het gebied van duurzaamheid. Daarom heb ik in **Hoofdstuk 2.4** onderzocht welke belemmeringen en stimulerende factoren er zijn om een meer plantaardig voedingspatroon aan te nemen. Ik

ontdekte dat smaakvoorkeur voor dierlijke voedingsmiddelen de belangrijkste belemmering was. Het vergroten van het bewustzijn van de voordelen werd over het algemeen gezien als de meest effectieve strategie om mensen te inspireren hun consumptie van dierlijke voedingsmiddelen te verminderen.

Met betrekking tot luchtvervuiling heb ik in **Hoofdstuk 3.1** onderzocht of er een verband bestaat tussen blootstelling aan luchtvervuiling en het risico op dementie. Hieruit bleek dat individuen die in relatief meer vervuilde gebieden wonen, niet een hoger risico op het ontwikkelen van dementie hebben in vergelijking met degenen die in minder vervuilde gebieden wonen. Echter, de variaties in luchtvervuiling binnen het onderzoeksgebied waren relatief klein, wat suggereert dat kleine schommelingen mogelijk geen differentiële invloed hebben op de gezondheid van de hersenen. Desalniettemin hebben eerdere studies met vergelijkbare variaties in blootstellingsniveaus associaties waargenomen, wat zou kunnen betekenen dat onze negatieve bevindingen kunnen worden toegeschreven aan niet-lineaire verbanden. In **Hoofdstuk 3.2** heb ik verder onderzocht via welke mechanismen luchtverontreinigende stoffen hun schadelijke effecten op de gezondheid van de mens uitoefenen door blootstelling aan luchtvervuiling te koppelen aan circulerende metabolieten in plasma. Hieruit bleek dat blootstelling aan hogere niveaus van luchtvervuiling geassocieerd was met differentieel tot expressie gebrachte metabolieten gerelateerd aan het voortplantingssysteem en celmetabolisme.

De studies die werden behandeld in **Hoofdstuk 4** draaiden om de rol van lichaamssamenstelling in het risico op dementie. In **Hoofdstuk 4.1** rapporteerde ik over de relatie tussen lichaamssamenstelling en het risico op dementie, waarbij ik ontdekte dat individuen met een hogere lichaamsmassa en vetmassa een lager risico op dementie hadden. Echter, deze verbanden verdwenen grotendeels nadat de eerste 5 jaar van follow-up werden uitgesloten, wat suggereert dat het schijnbaar beschermende effect van meer vetmassa verklaard kan worden door omgekeerde causaliteit. Desalniettemin toonde het bepalen van veranderingen van vetmassa vóór de diagnose van dementie geen hogere aanvankelijke massa en latere afname aan. Dit zou de hypothese kunnen ondersteunen dat meer vetmassa beschermende effecten heeft. Daarom deed ik verder onderzoek naar het mogelijke effect van vetweefsel op de hersenen door lichaamssamenstelling te koppelen aan preklinische plasma markers van dementie in **Hoofdstuk 4.2**. Ik ontdekte dat een hogere vetmassa geassocieerd was met hogere totaal-tau concentraties, terwijl de omgekeerde trend werd waargenomen voor de concentraties van neurofilament light chain. Deze tegenstrijdige bevindingen kunnen worden verklaard door beïnvloede hersengebieden, aangezien tau het meest overvloedig is in de hersenschors, terwijl neurofilament light chain voornamelijk aanwezig is in het cerebrale witte stof.

In **Hoofdstuk 5** heb ik een overzicht gegeven van de belangrijkste bevindingen uit alle beschreven studies, deze bevindingen heb ik in de context van de huidige kennis geplaatst en ik heb de implicaties ervan besproken. Ik heb ook algemene methodologische overwegingen belicht en suggesties gegeven voor verdere onderzoeksrichtingen om de levensstijl- en omgevingsfactoren te ontrafelen die bijdragen aan de multifactoriële etiologie van dementie


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APPENDICES

DANKWOORD

Met trots kijk ik terug op de afgelopen 3,5 jaar, wat heeft geresulteerd in dit proefschrift. Deze reis was intens, uitdagend, maar bovenal verrijkend. Ik wil mijn oprechte dank uitspreken voor de kansen die ik heb gekregen, maar vooral voor de onschatbare steun die ik mocht ontvangen van lieve mensen om me heen. Mijn collega's, vrienden en familie hebben me zowel inhoudelijk, als mentaal gesteund gedurende deze jaren.

Allereerst wil ik mijn promotor, **prof. dr. M. Arfan Ikram,** en co-promotor, **dr. ir. Trudy Voortman**, bedanken voor de geboden kansen en de vrijheid die jullie mij gaven om mezelf te ontwikkelen op gebieden die voor mij belangrijk waren en mijn aandacht trokken.

Beste **Arfan**, ik herinner me nog goed het moment waarop ik met trots de eerste versie van mijn eerste manuscript met jou deelde en jij tegen mij zei, zonder dat je er zelfs maar een blik op had geworpen, "Ik wil dat je deze versie verwijdert en het manuscript volledig opnieuw schrijft". Jij geloofde dat een tweede schrijfronde altijd beter zou zijn. Dit voorbeeld is illustratief voor de uitdagingen die je me herhaaldelijk liet aangaan. Ondanks dat ik deze uitdagingen aanvankelijk moeilijk vond, ben ik hier nu dankbaar voor omdat dit me in staat gesteld heeft het beste uit mezelf te halen. Ook jouw vertrouwen in mij, zoals je dat keer op keer naar mij uitsprak, heeft mij enorm geholpen om mezelf optimaal te ontwikkelen. Bedankt voor alles wat je me de afgelopen jaren hebt geleerd.

Beste **Trudy**, vooral in het eerste jaar van mijn PhD-traject was jouw begeleiding als copromotor absoluut onmisbaar. In de jaren die volgden, heb ik jouw vertrouwen in mij gewaardeerd en de ruimte die je me bood om te groeien als zelfstandig onderzoeker. Dank je wel voor het actief meedenken met projecten, het geven van feedback en de tijd die je hebt genomen om mij uitleg te geven en samen met mij te brainstormen. Met onze gedeelde achtergrond heb ik onze samenwerking niet alleen als zeer plezierig, maar ook inspirerend ervaren.

Ik wil ook graag de andere leden van de commissie bedanken, en in het bijzonder de leden van de leescommissie. Ik kijk ernaar uit om tijdens mijn verdediging de discussies aan te gaan.

Beste **prof. dr. M. Kamran Ikram,** beste **Kamran**, bedankt dat je me af en toe 'adopteerde' door de tijd te nemen om mijn vragen te beantwoorden, DEGs met me te tekenen en statistische formules aan me uit te leggen.

Mirjam, op mijn eerste werkdag stond je klaar om me rond te leiden op de afdeling en alles uit te leggen wat ik moest weten. Hierdoor voelde ik me meteen op mijn gemak. Ik waardeer je betrokkenheid, vriendelijkheid en behulpzaamheid enorm. **Gabrielle**, ook op jou kon ik altijd rekenen. Je wist steeds weer tijd voor mij vrij te maken in de drukke agenda van Arfan en was altijd bereid om te helpen bij administratieve zaken, maar ook bij het organiseren van activiteiten zoals de kerstborrel.

Beste **Frank**, **Jolande**, **Sylvia**, **Jacqueline**, **Nano** en **Brenda**, jullie vormen de essentiële schakel binnen ERGO. Ik blijf gefascineerd door jullie toewijding en optimisme. Jullie harde werk en positieve instelling zijn inspirerend.

Daarnaast wil ik alle deelnemers, huisartsen, apothekers en staf van ERGO hartelijk bedanken voor hun toewijding, inzet en bijdrage aan dit proefschrift.

Mijn lieve **Glitter** vriendinnen, mijn vriendschap met jullie is de voornaamste reden dat ik met pijn in mijn hart, mijn PhD-traject afsluit. **Ilse** en **Marije**, samen borrelen, een tripje naar Berlijn, en zelfs in het weekend kun je ons samen vinden. **Ilse**, of beter gezegd CB, als inventers van de kuikens hebben wij veel gelachen, wijn gedronken, gehuild, elkaar afgeleid, dexa-dagen georganiseerd en eindeloos gepraat over van alles en nog wat. **Marije**, fijne collega's die ook goede vriendinnen zijn, zijn er maar weinig. Hoewel mijn optimistische tijdsplanning roet in het eten heeft gegooid voor ons gezamenlijke project, beschouw ik onze samenwerking als een waardevolle en leerzame ervaring. **Jacqueline** en **Julianne**, of beter gezegd Shak en Huevitos. No sorry people here, because bts goes Colombia, Scandenevia, Dominicaanse Republic and many more countries to come, and no, these queens/barbies don't go by horse, lo siento. **Jacqueline**, als twee impulsieve en chaotische figuren vullen wij elkaar perfect aan bij het organiseren van activiteiten zoals year in review en de kerstborrel, maar ook bij het plannen van reisjes naar de meest random bestemmingen. Ik mis onze lachmomenten op kantoor. **Julianne**, met jou kan ik echt eindeloos blijven praten, maar ook lachen om jouw droge humor. Samen kennen we geen angst, maar gelukkig is Shak er altijd om ons op tijd terug te fluiten. Ik kijk nu al uit naar onze volgende trip, waar je weer mijn vaste kamergenoot zult zijn.

Een aantal andere collega's verdienen ook een speciaal plekje in mijn dankwoord. **Gina**, hoewel we als tegenpolen - 'chaoot' en 'perfectionist' - zouden moeten botsen, blijkt niets

minder waar te zijn. Met veel plezier kijk ik terug op onze samenwerking, waar we niet alleen het optimale in elkaar naar boven haalden, maar ook genoten van koffiemomenten vol interessante en leuke gesprekken. **Amber**, even bij jou langslopen om "hoi" te zeggen eindigde keer op keer in gesprekken van minstens een half uur. Ook konden we samen goed sparren over projecten. Onze reis naar Japan was een onvergetelijk hoogtepunt. **Rowina**, avondjes borrelen met jou lopen altijd uit in gezellige chaos. Ik ben dankbaar dat ik je heb leren kennen en dat ik jou ondertussen ook echt een vriendin kan noemen. **Brian**, met jou in de party room was het altijd feest. Bedankt voor de leuke gesprekken, borrelavondjes en niet te vergeten, dank voor de onmisbare hulp bij het verhuizen. **Sanne**, met onze overlappende PhD-onderwerpen, kon het niet anders dan dat we als twee blondines op hetzelfde moment aan hetzelfde project werkten. Gelukkig beseften we dit op tijd en konden we onze krachten bundelen, wat resulteerde in een gezamenlijk project. Het is een eer voor mij om jouw paranimf te mogen zijn. **Niels**, als mijn buddy was het jouw taak om me op weg te helpen, en ik maakte daar soms grondig gebruik van. Bedankt dat je altijd de tijd nam om mij te helpen, maar ook voor de leuke koffiemomentjes. **Joyce** en **Noor**, jullie enthousiasme en vrolijkheid maakten de COVID-periode een aangename tijd om op kantoor te werken. **Yuchan**, I had a wonderful time with you in Japan. Thanks for making this trip so much fun, and I feel honored to be your paranymph. **Yahong**, our collaborations were invaluable, and I am proud to witness your steep learning curve. I am looking forward to visit you in China. **Joelle**, onze samenwerking zie ik als een zeer waardevol onderdeel van mijn promotieproject. Het heeft me de mogelijkheid geboden om inzicht te krijgen in het perspectief van een andere onderzoeksgroep en om een ander ziektebeeld te leren kennen. Ik heb veel van jou geleerd. **Renate**, na samen onze scriptie te hebben geschreven aan de Wageningen Universiteit, hebben we allebei onze carrière voortgezet aan het Erasmus MC. Het was leuk om daar nog steeds koffiemomentjes met jou te hebben. **Annemarie**, bedankt voor onze leuke, inspirerende gesprekken. Deze hebben mij erg geholpen bij het maken van keuzes en het verkrijgen van inzichten. **Frank**, bedankt dat ik altijd op jouw expertise kon rekenen. Het is verbazingwekkend hoe diepgaand jouw kennis is. **Mohsen**, your insights have greatly enriched the quality of my work. Thank you for always making time for meetings to discuss the progress of my work and for your feedback.

Natuurlijk wil ik ook alle **kuikens** en andere **collega's van de 27ste en 28ste** verdieping bedanken voor de gezelligheid, waaronder **Eline**, **Anna**, **Sanne H**, **Mathijs**, **Tim**, **Camiel**, **Merel**, **Charlotte**, **Cevdet**, **Tian**, **Gena**, **Jeremy**, **Lana**, **Amy**, **Marlou**, **Janneke**, **Sjoerd**, **Xiangjun**, **Mojgan**, **Marianna**, **Hong**, en **Marinka**.

Larissa, met onze vergelijkbare achtergrond en gedeelde ambities is er geen vriendin met wie ik liever werkgerelateerde gesprekken voer. Ik wens je heel veel succes met je PhD aan Cornell University en kijk er nu al naar uit om je daar te bezoeken. Ik ben ervan overtuigd dat je fantastisch werk zult leveren. Bedankt dat je niet alleen een inspirerende gesprekspartner bent, maar ook een goede vriendin. Ik ben blij dat jij vandaag naast mij staat.

Lieve **Errol**, jouw onvoorwaardelijke liefde en het feit dat je altijd voor mij klaarstaat, betekenen heel veel voor me. Lieve **Anouk**, met jou als onderdeel van de familie heb ik eindelijk het zusje wat ik altijd al wilde hebben. Ik ben trots op jouw doorzettingsvermogen en kijk ernaar uit om jouw afstuderen te vieren.

Lieve **papa** en **mama**, jullie verdienen een bijzonder plekje in mijn dankwoord, want zonder jullie was ik niet geweest waar ik nu ben. Dank voor jullie onvoorwaardelijke steun in elke beslissing die ik in mijn leven heb genomen en bovenal voor de onvoorwaardelijke liefde die jullie mij hebben gegeven. Dit betekent heel veel voor me.

PHD PORTFOLIO

Name PhD student:	Tosca de Crom
Research school:	Netherlands Institute for Health Sciences (NIHES)
Erasmus MC department:	Epidemiology
PhD period:	March 2020 – October 2023
Supervisors:	Prof.dr. M.A. Ikram and dr.ir. T. Voortman

	Year	ECTS*
1. PhD training		
General courses		
Master of Science in Clinical Epidemiology (NIHES)	2020-2022	70.0
Scientific integrity (Erasmus MC)	2021	0.3
Basic course on R (MolMed)	2020	1.7
Conferences		
Alzheimer's Association International Conference, Virtual *Attendance*	2020	2.0
Alzheimer's Association International Conference, Virtual *Poster presentation: MIND diet and risk of dementia in a population-based cohort*	2021	2.0
Nutritional science day, Virtual *Oral presentation: MIND diet and risk of dementia in a population-based cohort*	2021	0.3
Alzheimer's Association International Conference, Virtual *Poster presentation: Air pollution and the risk of dementia: the Rotterdam Study* *Poster presentation: Adiposity in the older population and the risk of dementia: the Rotterdam Study*	2022	2.0
Nutrition science days, Heeze, the Netherlands *Oral presentation: Plant-based dietary pattern and the risk of dementia: a population-based study*	2022	0.6
European Public Health Conference, Berlin, Germany *Oral presentation: Plant-based dietary pattern and the risk of dementia: a population-based study*	2022	1.7
International Union of Nutritional Sciences International Congress of Nutrition, Tokyo, Japan *Oral presentation: Adiposity and plasma levels of amyloid-β, total-tau and neurofilament light chain*	2022	2.0
Alzheimer's Association International Conference, Virtual *Poster presentation: Dietary nitrate intake and the risk of dementia: a population-based study*	2023	2.0
Alzheimer Europe Conference, Helsinki, Finland *Oral presentation: Omics in Alzheimer's disease: the cross-roads of internal and external pathways*	2023	1.7

*1 ECTS (European Credit Transfer System) equals a workload of 28 hours

Workshops, seminars and symposia		
Journal club (Epidemiology)	2020-2023	2.0
Departmental seminars (Epidemiology)	2020-2023	2.0
Seminars ADAIR	2020-2023	1.7
ISTAART seminars	2020-2023	2.0
TUBE & ADAIR workshop and general assembly meeting, Venice, Italy	2022	1.7
General assembly meeting ADAIR, Helsinki, Finland	2023	0.6
Research visits		
Javeriana University, Bogota, Colombia	2023	1.7
2. Teaching activities		
Teaching assistance		
Practice of Epidemiologic Analysis	2021	0.6
Cardiovascular Epidemiology	2021	0.6
Master student supervision		
Nena Goossens: Dietary inflammatory index and the risk of dementia	2022	1.5
Emma Vieveen: Plant-based dietary patterns and MRI markers of brain health	2022-2023	1.5
3. Other activities		
Peer-review	2020-2023	2.0
Nutrition data cleaning and management	2020-2023	4.0
Cognition data cleaning and management	2020-2023	1.5
2020 meeting commission	2020-2023	2.0

*1 ECTS (European Credit Transfer System) equals a workload of 28 hours

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*These first or second authors contributed equally to the respective manuscript
#These last authors contributed equally to the respective manuscript

ABOUT THE AUTHOR

Tosca de Crom was born on the 27th of March 1994 in Bergen op Zoom, the Netherlands. She completed her secondary education at the Roncalli Scholengemeenschap in Bergen op Zoom, graduating in 2012. Following her graduation, she pursued a bachelor's program in Nutrition and Dietetics at the Hague University of Applied Science. Tosca developed a passion for research when she undertook, as part of her bachelor's program, an internship at the Center for Translational Research in Aging and Longevity in College Station, Texas, United States. She obtained her bachelor's degree in 2016, after which she continued her academic journey by enrolling in the master's program in Nutrition & Health at Wageningen University & Research. She undertook an internship at the Princess Alexandra Hospital in Brisbane, Australia and obtained her master's degree in 2018. After taking a gap year that involved traveling through Australia, Asia, and the United States, Tosca started her PhD trajectory at the department of Epidemiology, under the supervision of prof. dr. M. Arfan Ikram and dr. ir. Trudy Voortman. The outcome of this doctoral trajectory is the work described in this thesis. As part of her research training, she obtained a master's degree in Epidemiology at the Netherlands Institute for Health Sciences, which she completed in 2022 (cum laude). Further expanding her expertise, Tosca joined the Netherlands Organisation for Applied Scientific Researsh (TNO) in November 2023, where she works as an epidemiologist.