Inflammation, nutrition and risk of dementia

The Rotterdam Study
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Inflammation, nutrition and risk of dementia
The Rotterdam Study

Ontsteking, voeding en het risico op dementie
Het Erasmus Rotterdam Gezondheid en Ouderen
(ERGO) Onderzoek

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Chapter 1

Introduction

Brains of patients with Alzheimer's disease are characterized by the presence of beta-amyloid containing plaques and neurofibrillary tangles. In addition, signs of inflammation and oxidative stress have been found, including activated microglia and inflammatory proteins as well as lesions that are typically associated with oxidative stress.

Several findings suggest that inflammation may play an important role in the pathogenesis of Alzheimer's disease. First, use of anti-inflammatory medication, such as NSAIDs, has been associated with reduced risk of dementia. Second, some inflammatory proteins including alpha-1-antichymotrypsin are known to reinforce the formation of beta-amyloid deposits, which is thought to be crucial in Alzheimer pathogenesis. Peripheral inflammation may also contribute to Alzheimer pathogenesis, because inflammatory proteins from the blood may pass the blood-brain barrier. However, the relation between inflammatory proteins in plasma and risk of dementia has not yet been studied.

Oxidative stress may also contribute to Alzheimer pathogenesis as suggested by a previous randomized controlled trial. That study found that Alzheimer patients taking supplements of the antioxidant vitamin E had a slower progression of the disease than patients taking placebo. However, results from studies that assessed antioxidant plasma levels in patients with Alzheimer's disease and healthy controls have been inconsistent. Furthermore, it is not known whether high dietary intake of antioxidants from food is associated with reduced risk of Alzheimer's disease.

In addition to inflammation and oxidative stress, lipids may be involved in Alzheimer pathogenesis. This view is supported by animal studies showing that higher dietary intake of cholesterol is associated with increased beta-amyloid deposits in the brain. Moreover, use of lipid-lowering statins has been suggested to possibly reduce risk of Alzheimer's disease. Other lipids including cis n-3 polyunsaturated fatty acids (n-3 PUFA) may affect inflammatory processes because of their anti-inflammatory properties. In humans the association between dietary intake of cholesterol and risk of dementia has not been extensively studied. In addition, no definite conclusions can be drawn from the previous studies on the relation between statin use and risk of dementia, because they were cross-sectional or based on medical records from general practitioners.
Furthermore, very few studies investigated the association between n-3 PUFA and dementia.

The relation between inflammation, oxidative stress, lipids and Alzheimer's disease may be through atherosclerosis that has been associated with dementia. Inflammation may contribute to atherogenesis, because some inflammatory proteins, such as C-reactive protein, activate the complement system, which is an important step in the development of atherosclerosis. Oxidative stress may increase atherosclerosis through the oxidation of LDL-cholesterol. Lipids may be involved in atherogenesis, because high levels of cholesterol, saturated fatty acids and cis monosaturated fatty acids and low levels of PUFA are associated with more atherosclerosis.

The aim of the research described in this thesis was to further investigate the relation between inflammation, oxidative stress and lipids and dementia and to examine whether these associations could be explained by atherosclerosis.

I investigated whether levels of inflammatory factors in plasma were associated with increased risk of dementia or its subtypes (chapter 2.1 and 2.2). Because inflammation may induce oxidative stress that may in turn activate inflammatory processes, I examined whether the relation between inflammatory cells, such as leucocytes, and Alzheimer's disease was modified by intake of antioxidants that reduce oxidative stress (chapter 2.2). In chapter 3.1.1 I reported on plasma levels of antioxidants in Alzheimer patients, subjects with cognitive decline and healthy controls. Furthermore, I examined the relation between dietary intake of antioxidants and risk of Alzheimer's disease (chapter 3.1.2). Finally, I further explored the relation between lipids and dementia and cognitive function. I investigated the effect of levels of fatty acids in plasma phospholipids (chapter 3.2.1), dietary fat intake (chapter 3.2.2), and use of lipid-lowering medication (chapter 3.2.3) on risk and presence of dementia. In chapter 4, I review the main results of this thesis in the light of current insights with respect to dementia pathogenesis, I comment on methodological issues and clinical relevance and I conclude with recommendations for future research.

References


Chapter 2.1

Inflammatory proteins in plasma and risk of dementia

Peripheral inflammation may occur in patients with Alzheimer's disease, possibly even prior to clinical onset of dementia. We investigated whether high plasma levels of inflammatory proteins are associated with an increased risk of dementia.

We performed a case-cohort study within the Rotterdam Study. The source population consisted of 6713 subjects who, at baseline (1990-1993), were free of dementia and underwent venipuncture. From these, we selected both a random subcohort of 727 subjects and 188 cases who had developed dementia at follow-up. The associations between plasma levels of alpha1-antichymotrypsin (ACT), C-reactive protein (CRP), interleukin-6 (IL6), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) and risk of dementia were examined using Cox proportional hazards models.

High levels of ACT, IL6 and to a lesser extent CRP were associated with an increased risk of dementia; rate ratios per standard deviation increase were 1.49 (95% confidence interval (CI) 1.23-1.81), 1.28 (95% CI 1.06-1.55) and 1.12 (95% CI 0.99-1.25), respectively. Similar associations were observed for Alzheimer's disease, whereas rate ratios of vascular dementia were higher for ACT and CRP. Levels of sICAM-1 and sVCAM-1 were not associated with risk of dementia or its subtypes.

Our findings suggest that high plasma levels of inflammatory proteins are associated with an increased risk of dementia, Alzheimer’s disease and vascular dementia.

Neuritic plaques and neurofibrillary tangles are the classical neuropathological features in brains of patients with Alzheimer’s disease. Furthermore, inflammation has been demonstrated in Alzheimer brains as is indicated by the presence of activated microglia and inflammatory proteins. In addition to an immunological response within the brain, the peripheral immune system of dementia patients may be activated as well. This is suggested by cross-sectional studies that reported
elevated blood levels of the acute phase proteins alpha1-antichymotrypsin (ACT), C-reactive protein (CRP) and their regulator, the cytokine interleukin-6 (IL6) in Alzheimer patients as compared to controls. Whether ACT, CRP and IL6 are increased before clinical onset of dementia is not yet known as the relation between inflammatory proteins and risk of dementia has not been studied in a prospective way. As a result, it is unknown whether peripheral inflammation is a cause or a consequence of the pathogenetic process of dementia. Furthermore, no studies examined the association between ACT, CRP and IL6 and dementia in a population-based setting, nor has the association been studied between other inflammatory proteins, such as cell adhesion molecules (CAMs), and dementia. We investigated the relation between plasma levels of ACT, CRP, IL6, soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) and the risk of dementia in a large population-based prospective study.

Methods and materials

Source population
The Rotterdam Study is a population-based prospective cohort study among persons living in Ommoord, a suburb of the city of Rotterdam, the Netherlands. The study was approved by the medical ethics committee of the Erasmus University Rotterdam. The objective was to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor and ophthalmologic diseases in the elderly.

All 10,275 residents of Ommoord who were aged 55 years and over in 1990, were invited to participate. Of these, 7,983 (78%) gave their written informed consent and took part in the baseline examination. The baseline examination took place between 1990 and 1993 and consisted of a home interview and 2 clinical examinations at the research center. At the clinical examination blood samples were drawn from 7,050 subjects (88% of total cohort), of whom cognitive status was assessed in 7,047 subjects. Dementia was diagnosed in 334 participants resulting in a source population of 6,713 subjects who both were at risk for dementia and had venipuncture at baseline. Follow-up examinations took place in 1993-1994 and 1997-1999. In addition, the total cohort was continuously monitored for major disease outcomes.

Study design
We used a case-cohort design in order to use the data from the source population most efficiently. In this design, a random sample, or a 'subcohort', is drawn from the source population that has been followed up for disease outcome. In addition, subjects from the source population, who
Inflammatory proteins in plasma and risk of dementia

Baseline exposure is measured in all members of the subcohort and all incident cases outside the subcohort. The statistical analyses that can be applied in a case-cohort study are similar to that of a cohort study after modification of standard errors.

Study population
The study population for the present study consisted of a subcohort of 727 subjects who were randomly drawn in July 1998 from all 6713 subjects who were dementia-free and had venipuncture at baseline. At that time, we additionally selected all 146 incident dementia cases that had occurred outside the subcohort. We followed the subcohort until December 31, 1999. By then, 42 subjects of the subcohort had developed dementia. The extension of follow-up period within the subcohort is valid, because it is independent of the occurrence of dementia as the subcohort is randomly drawn and only the subcohort contributes to follow-up time. Cases of the present study comprised 188 (42+146) incident dementia cases, of whom 140 were diagnosed with Alzheimer’s disease (119 without cerebrovascular disease, 21 with cerebrovascular disease), 23 subjects with vascular dementia and 25 had other dementias.

Diagnosis of dementia
During baseline and follow-up examinations the diagnosis of dementia followed a similar 3-step protocol, which has been described in detail elsewhere. In short, 2 brief tests of cognition (Mini-Mental State Examination (MMSE) and the Geriatric Mental State schedule (GMS) organic level) were used to screen all subjects. Screen positives (MMSE score < 26 or GMS organic level > 0) underwent further cognitive testing using the Cambridge examination for mental disorders of the elderly (CAMDEX). The CAMDEX also included an informant interview. Subjects who were suspected of having dementia were examined by a neurologist, a neuropsychologist and, if possible, had a brain scan made by magnetic resonance imaging. In addition, the total cohort was continuously monitored for incident dementia cases through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. Dementia diagnoses were made by a panel of a neurologist, neuropsychologist and research physician in accordance with internationally accepted criteria for dementia (DSM-III-R), Alzheimer’s disease (NINCDS-ADRDA) and vascular dementia (NINDS-AIREN). By use of these criteria, subjects were classified as having Alzheimer’s disease in case they had a gradually progressive global decline of cognitive functions.
for which no other medical cause was found. If the ongoing gradual progressive decline had abruptly been worsened by the occurrence of an incident stroke, we subclassified Alzheimer’s disease as Alzheimer’s disease with cerebrovascular disease. Subjects with onset of dementia within 3 months following a recognized incident stroke, a history of abrupt deterioration in cognitive functions, or stepwise progression of cognitive deficits were classified as vascular dementia.

Assessment of plasma level of ACT, CRP, IL6, sICAM-1 and sVCAM-1
Levels of inflammatory proteins were assessed in baseline plasma from the subcohort and the cases. At the research center a venipuncture was performed with minimal stasis and a 21 gauge Butterfly needle with tube (Terumo Surflo® winged infusion set). Blood was collected in tubes containing 0.5 ml sodium citrate solution. All tubes were stored on ice before and after blood sampling. Platelet-free plasma was obtained by 2-stage centrifugation at 4 °C, firstly for 10 minutes at 1,600 g and subsequently for 30 minutes at 7,000 g. Platelet-free plasma was immediately frozen in liquid nitrogen, transferred to the laboratory and stored at −80 °C. Median storage time was 6.5 years (interquartile range 4.7-7.6 years).

Measurements of plasma levels of inflammatory proteins were performed without knowledge of the dementia diagnosis. Plasma levels of ACT and CRP were measured by kinetic nephelometry (Behring Nephelometer BN200®) after a 5 times dilution by use of Behrings N-dilutent®. Levels of IL6, sICAM-1 and sVCAM-1 were determined by means of commercially available ELISA (IL6: Quantikine HS®; sICAM-1 and sVCAM-1: Parameter®). Interassay coefficients of variations for ACT, CRP, IL6, sICAM-1 and sVCAM-1 were 2.8%, 4.4%, 8.7%, 6.9% and 5.0%, respectively. Due to insufficient plasma ACT, CRP, IL6, sICAM-1 and sVCAM-1 could not be determined in 17, 17, 6, 3 and 3 subjects, respectively.

Covariates
During the baseline home interview information on level of education and smoking habits was obtained. Data on medication use at time of venipuncture were obtained from the baseline interview if venipuncture had taken place before December 31, 1990 (n=226, 25.9%). After this date, data on medication use were obtained directly from a pharmacy database that was linked to the study database. At the research center height and weight were measured and blood samples were drawn. Systolic blood pressure at the ankle and the arm was measured by means of a random zero sphygmomanometer. Also, ultrasonography of both carotid arteries was performed.
Highest level of education was categorized in 4 groups: completed primary education, lower vocational training or general education, intermediate vocational training or intermediate and higher general education, and higher vocational training, college or university. Smoking habits were dichotomized in current versus former or never smoking. All drugs were classified according to their corresponding Anatomical-Therapeutical-Chemical-code (ATC-code). Participants who used systemic non-steroidal anti-inflammatory drugs (NSAID) (ATC-code m01a), systemic corticosteroids (ATC-codes h02a or h02b), or inhalation corticosteroids (ATC-code r03ba) were classified as users of systemic NSAID or users of systemic, or inhalation corticosteroids, respectively. Body mass index was calculated in kilograms per meter squared. Diabetes was considered present if the subject was taking oral diabetics or insulin (ATC-code a10) or if the random or post-load serum glucose level was equal to or higher than 11.1 mmol/l (WHO-criteria for epidemiological studies of diabetes).

As indicators of atherosclerosis, we used 3 measures. First, peripheral arterial disease was used, which was defined by the ankle-to-brachial ratio of the systolic blood pressure. This ratio was computed using the average systolic blood pressure of 2 measurements at the arm and the lowest value of 2 legs. Peripheral arterial disease was considered present if the ankle-to-brachial ratio was below 0.9 and absent if the ratio was equal to or above 0.9. Second, atherosclerotic carotid plaques were determined at 6 different locations in the carotid arteries: common carotid artery, carotid bifurcation, and internal carotid artery at both left and right side. Four categories were made according to number of locations with plaques: 0, 1-2, 3-4 and 5-6 locations. Third, the intima media thickness was defined as the average of near-wall and far-wall measurements of the common carotid artery. For the analyses, we computed the average of left and right common carotid intima media thickness.

Apolipoprotein E (APOE) genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis. The APOE genotype was dichotomized in presence or absence of the apolipoprotein E*4 (APOE*4) allele.

**Data analysis**

Differences between incident dementia cases within the subcohort (n=42) and incident cases outside the subcohort (n=146) were tested by means of Mann-Whitney test for baseline age, MMSE-score, ACT, CRP, IL6, sICAM-1 and sVCAM-1. Differences between gender and educational level were tested using the Chi-square test.

The association between plasma levels of inflammatory proteins (ACT, CRP, IL6, sICAM-1 and sVCAM-1, respectively) and incident dementia and
its subtypes was evaluated in a case-cohort design by use of standard Cox proportional hazards models with modification of standard errors based on robust variance estimates.\textsuperscript{16, 17} We used the method according to Barlow in which the subcohort is weighted by the inverse of the sampling fraction from the source population.\textsuperscript{16} In a case-cohort analysis only subjects from the subcohort contribute to follow-up time.

Analyses were based on subjects with complete data on inflammatory protein. Plasma levels of inflammatory proteins were first entered into the model as quintiles in order to explore any deviations from linearity in the relation between inflammatory proteins and risk of dementia. In case of a non-linear association or a threshold-effect all analyses were performed using quintiles of inflammatory proteins. Otherwise, plasma levels of inflammatory proteins were entered into the model through a linear term, in which the regression coefficient was expressed per standard deviation increase. Quintiles and standard deviations of the respective inflammatory proteins were based on the distribution of the subcohort. We additionally performed analyses in which level of inflammatory proteins were logtransformed in order to check whether associations were driven by extreme values. In all analyses adjustments were made for age, gender and education. To adjust most efficiently for age, we used age as timescale in the model. Entry time was defined as age at study entry. Participants from the subcohort were followed until the age of onset of dementia, death or end of study, whichever came first. The proportional hazard assumption was checked for each inflammatory protein and each covariate by adding the time-dependent interaction of the variable with age to all models.

We first analyzed the relation between inflammatory proteins and risk of dementia, Alzheimer's disease and vascular dementia. Subsequently, in order to investigate the relation between inflammatory proteins and Alzheimer's disease without cerebrovascular disease, we excluded subjects with the diagnosis 'Alzheimer's disease with cerebrovascular disease' (n=21). To examine whether current smoking, body mass index, diabetes and the use of anti-inflammatory medication confounded the association between inflammatory proteins and dementia, we additionally entered these variables into the model. Missing values on categorical variables, such as education (n=24) and smoking habits (n=23), were replaced by a missing indicator. No missing values were present for age, gender, body mass index, diabetes and use of anti-inflammatory medication. Because dementia is associated both with cardiovascular risk factors\textsuperscript{31} and with atherosclerosis,\textsuperscript{26} and because atherogenesis includes inflammatory mechanisms,\textsuperscript{32} the following indicators of atherosclerosis were additionally added to the model: peripheral arterial disease, carotid plaques and intima media thickness, respectively. Missing values on peripheral arterial disease (n=81) and
carotid plaques (n=53) were replaced by a missing indicator; missing values on intima media thickness (n=69) were replaced by the mean of either cases or subcohort, depending on subject status.

Furthermore, we performed the analyses of Alzheimer’s disease within strata of APOE*4 allele. In this latter analysis, 20 subjects, of whom no APOE genotype was available, were excluded. Statistical interactions were tested by adding a product term to the unstratified model.

In addition, the analyses were repeated after exclusion of all subjects with anti-inflammatory medication (n=93, 10.7%). Finally, the analyses for dementia were performed after exclusion of subjects with possible acute inflammation as is indicated by CRP levels over 15 mg/l (n=53, 6.1%). All data analyses were performed using SAS statistical software.

**Results**

Table 1 presents the baseline characteristics of the subcohort. The mean age was 71.7 years (SD 9.0), a small majority were women, one-third had

<table>
<thead>
<tr>
<th>Table 1 Baseline characteristics of the subcohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcohort (n=727)</td>
</tr>
<tr>
<td>Mean age (y) (SD)*</td>
</tr>
<tr>
<td>Women (%)</td>
</tr>
<tr>
<td>Primary education only (%)</td>
</tr>
<tr>
<td>Current smoking (%)</td>
</tr>
<tr>
<td>Mean body mass index (kg/m²) (SD)*</td>
</tr>
<tr>
<td>Diabetes (%)</td>
</tr>
<tr>
<td>Systemic use of NSAID (%)</td>
</tr>
<tr>
<td>Systemic use of corticosteroids (%)</td>
</tr>
<tr>
<td>Inhalation use of corticosteroids (%)</td>
</tr>
<tr>
<td>Presence of peripheral arterial disease (%)</td>
</tr>
<tr>
<td>5-6 Locations with carotid plaques (%)</td>
</tr>
<tr>
<td>Median intima media thickness (mm) (range)†</td>
</tr>
<tr>
<td>Carrier of APOE*4 allele (%)</td>
</tr>
<tr>
<td>Median ACT (mg/dl) (range)†</td>
</tr>
<tr>
<td>Median CRP (mg/l) (range)†</td>
</tr>
<tr>
<td>Median IL6 (pg/ml) (range)†</td>
</tr>
<tr>
<td>Median sICAM-1 (ng/ml) (range)†</td>
</tr>
<tr>
<td>Median sVCAM-1 (ng/ml) (range)†</td>
</tr>
</tbody>
</table>

*standard deviation
† interquartile range
Figure 1 Adjusted* rate ratios of dementia across quintiles of inflammatory proteins†
Inflammatory proteins in plasma and risk of dementia

primary education only and 28.6% carried at least one APOE*4 allele.

As expected, we found no statistical differences between incident dementia cases within the subcohort and incident dementia cases outside the subcohort in terms of baseline age, MMSE-score, ACT, CRP, IL6, sICAM-1 and sVCAM-1 (p-values were all above 0.10). Also, gender and educational level were similar between the 2 groups (p-values above 0.20).

The proportional hazards assumption was not violated for any of the inflammatory proteins or covariates in the subsequent analyses. In Figure 1 the median level of the respective inflammatory proteins within the quintiles was plotted against the rate ratios of dementia on a logscale. From this figure, we saw no reason to assume that the associations could not be described by a linear term, in particular given the wide and overlapping

Table 2 Adjusted* rate ratios of dementia and its subtypes per standard deviation (SD) increase in plasma level of inflammatory protein

<table>
<thead>
<tr>
<th></th>
<th>SD</th>
<th>Dementia n=188</th>
<th>Alzheimer's disease n=140</th>
<th>Vascular dementia n=23</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)†</td>
<td>RR (95% CI)†</td>
<td>RR (95% CI)†</td>
<td></td>
</tr>
<tr>
<td>ACT</td>
<td>16.2 mg/dl</td>
<td>1.49 (1.23-1.81)</td>
<td>1.35 (1.09-1.67)</td>
<td>2.48 (1.67-3.67)</td>
</tr>
<tr>
<td>CRP</td>
<td>5.87 mg/l</td>
<td>1.12 (0.99-1.25)</td>
<td>1.10 (0.96-1.26)</td>
<td>1.31 (1.06-1.61)</td>
</tr>
<tr>
<td>IL6</td>
<td>4.93 pg/ml</td>
<td>1.28 (1.06-1.55)</td>
<td>1.30 (1.05-1.61)</td>
<td>1.26 (0.97-1.65)</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>68.3 ng/ml</td>
<td>0.97 (0.74-1.27)</td>
<td>0.87 (0.63-1.21)</td>
<td>1.46 (0.82-2.60)</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>189.9 ng/ml</td>
<td>0.98 (0.75-1.28)</td>
<td>1.02 (0.75-1.38)</td>
<td>0.66 (0.32-1.38)</td>
</tr>
</tbody>
</table>

* adjusted for age, gender and education
† rate ratio (95% confidence interval)
95% confidence intervals of the point estimates within quintiles. Therefore, we further analyzed the data entering inflammatory protein as a linear term in our models.

Table 2 presents the rate ratios of dementia and its subtypes per standard deviation increase in level of inflammatory protein, adjusted for age, gender and education. Higher levels of ACT, CRP and IL6 were associated with an increased risk of dementia. With every increase of 16.2 mg/dl (standard deviation) in ACT level, the risk of dementia increased with 49% (rate ratio (RR) 1.49 (95% CI 1.23-1.81)). The rate ratio for IL6 per standard deviation increase in plasma level was 1.28 (95% CI 1.06-1.55). The relation between CRP and dementia was borderline significant (RR 1.12 (95% CI 0.99-1.25). The associations were not clearly driven by extreme values as shown by the analyses of logtransformed levels of inflammatory proteins: the association of logtransformed ACT with dementia was strongest (RR 3.40 (95% CI 1.61-7.19) p=0.001), the association of logtransformed IL6 was of intermediate strength (RR 1.36 (95% CI 0.98-1.88) p=0.06) and was weakest for logtransformed CRP (RR 1.12 (95% CI 0.91-1.37) p=0.29). Higher levels of ACT, CRP and IL6 were also associated with an increased risk of Alzheimer's disease, although high levels of ACT and CRP were most strongly associated with an increased risk of vascular dementia. IL6 was similarly associated with Alzheimer's disease and vascular dementia. Plasma levels of sICAM-1 and sVCAM-1 were not associated with risk of dementia and its subtypes. Rate ratios of dementia for logtransformed sICAM-1 and sVCAM-1 were 0.78 (95% CI 0.32-1.93) (p=0.60) and 0.78 (95% CI 0.35-1.75) (p=0.55), respectively. When Alzheimer cases with cerebrovascular disease were excluded the relations between the respective inflammatory proteins and Alzheimer's disease were very similar.

Table 3 shows the relation between inflammatory proteins and risk of dementia with additional adjustments for potential confounders. Adjustments for current smoking, body mass index, diabetes and use of anti-inflammatory medication did not change the results substantially. Also, similar results were observed when adjustments were made for measures of atherosclerosis.

Table 4 presents the association between inflammatory proteins and risk of Alzheimer's disease across strata of APOE genotype. For all inflammatory proteins the association with dementia was similar for subjects with or without at least one APOE*4 allele. None of the statistical interactions were statistically significant.

When the analyses of the relation between inflammatory proteins and risk of dementia were performed after exclusion of subjects who used anti-inflammatory medication, the results remained similar. Finally, separate
<table>
<thead>
<tr>
<th>Plasma Level</th>
<th>Model 1*</th>
<th>Adjusted for peripheral arterial disease†</th>
<th>Adjusted for carotid plaques‡</th>
<th>Adjusted for intima media thickness‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard deviation</td>
<td>RR (95% CI)†</td>
<td>RR (95% CI)†</td>
<td>RR (95% CI)†</td>
</tr>
<tr>
<td>ACT 16.2 mg/dl</td>
<td>1.52 (1.25-1.84)</td>
<td>1.47 (1.19-1.82)</td>
<td>1.67 (1.36-2.05)</td>
<td>1.49 (1.36-2.05)</td>
</tr>
<tr>
<td>CRP 5.87 mg/l</td>
<td>1.11 (0.99-1.26)</td>
<td>1.05 (0.92-1.21)</td>
<td>1.16 (1.03-1.31)</td>
<td>1.10 (0.98-1.25)</td>
</tr>
<tr>
<td>IL6 4.93 pg/ml</td>
<td>1.27 (1.06-1.54)</td>
<td>1.25 (1.01-1.55)</td>
<td>1.31 (1.05-1.63)</td>
<td>1.27 (1.05-1.55)</td>
</tr>
<tr>
<td>sICAM-1 68.3 ng/ml</td>
<td>0.88 (0.64-1.21)</td>
<td>0.86 (0.62-1.19)</td>
<td>0.80 (0.54-1.20)</td>
<td>0.89 (0.66-1.21)</td>
</tr>
<tr>
<td>sVCAM-1 189.9 ng/ml</td>
<td>0.96 (0.71-1.29)</td>
<td>0.94 (0.70-1.27)</td>
<td>0.89 (0.62-1.26)</td>
<td>0.97 (0.72-1.31)</td>
</tr>
</tbody>
</table>

*adjusted for age, gender, education, current smoking, body mass index, diabetes, use of anti-inflammatory medication
† additional to model 1
‡ rate ratio (95% confidence interval)
analyses after exclusion of subjects with CRP levels over 15 mg/l also did not change the results substantially.

**Discussion**

We observed that elevated plasma levels of ACT and IL6 were associated with an increased risk of dementia and its subtypes Alzheimer's disease and vascular dementia. This association was less clear for CRP and was absent for sICAM-1 and sVCAM-1. The associations were not confounded or modified by atherosclerosis and APOE genotype, respectively.

The present study has several advantages. First, our study is the first to prospectively examine the relation between inflammatory proteins and risk of dementia in a population-based study with a large number of subjects. Second, complete follow-up with respect to dementia diagnosis was obtained. As a result, selection bias as a consequence of loss to follow-up could not have occurred. Third, differential misclassification of level of inflammatory proteins was eliminated by assessing the levels blinded for dementia status. Finally, in contrast to most cross-sectional studies on inflammatory proteins and dementia, adjustments were made for an extensive number of potential confounders.

A limitation of the present study is that regression dilution may have occurred due to measurement error and within-person variability in plasma level of inflammatory protein. Unfortunately, we did not have repeated measurements of plasma levels of inflammatory proteins. Therefore, we may have underestimated the strength of the true association between inflammatory proteins and risk of dementia. Another limitation may be that

**Table 4** Adjusted* rate ratios of Alzheimer's disease per standard deviation (SD) increase in plasma level of inflammatory protein across strata of APOE*4 allele

<table>
<thead>
<tr>
<th></th>
<th>No APOE*4 allele</th>
<th>At least one APOE*4 allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cases n=80</td>
<td>cases n=56</td>
</tr>
<tr>
<td></td>
<td>RR (95% CI)†</td>
<td>RR (95% CI)†</td>
</tr>
<tr>
<td>ACT</td>
<td>16.2 mg/dl</td>
<td>1.35 (1.02-1.80)</td>
</tr>
<tr>
<td>CRP</td>
<td>5.87 mg/l</td>
<td>1.13 (0.98-1.32)</td>
</tr>
<tr>
<td>IL6</td>
<td>4.93 pg/ml</td>
<td>1.27 (0.98-1.64)</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>68.3 ng/ml</td>
<td>0.94 (0.68-1.30)</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>189.9 ng/ml</td>
<td>1.00 (0.68-1.46)</td>
</tr>
</tbody>
</table>

* adjusted for age, gender and education
† rate ratios (95% confidence interval)
‡ statistical interaction of plasma level of inflammatory protein with APOE genotype
none of the subjects who were screen negative (MMSE score >= 26 and GMS organic level = 0) were further examined. As a result, we may have missed some mild dementia cases. However, because this will only be a very small percentage of all cases and because we used identical procedures at baseline and follow-up, it is unlikely that our results have been affected by misclassification of cases.

Our findings are in line with many cross-sectional studies showing that plasma or serum levels of ACT and IL6 are higher in dementia patients compared to control subjects, although some studies did not observe this association. Furthermore, our findings are in accordance with studies in molecular-biological research that showed presence of ACT and IL6 in early and late stages of neuritic plaque formation. Although the relation between CRP and dementia has been examined in previous studies, only one study observed similar results as we did, showing that CRP levels in dementia patients were higher compared to controls. Blood levels of CAMs in relation to dementia have not been studied before. Our observations on sICAM-1 and sVCAM-1 and risk of dementia are in line with neuropathological findings showing no expression of these adhesion molecules on endothelial cells of capillaries in Alzheimer brains.

The observed elevations in plasma levels of inflammatory proteins prior to dementia diagnosis may either be derived from increased production in cerebro or by cells from the peripheral immune system. However, because levels of acute phase proteins (ACT, CRP) in brains are generally more than 100 times lower than in plasma, plasma elevations of acute phase proteins more likely originate from an increased production in the peripheral immune system. In contrast, levels of cytokines, such as IL6, are similar in brain and plasma, thus plasma elevations may either be derived from increased production in the brain or the periphery.

Elevated plasma levels of inflammatory proteins prior to clinical onset of dementia may support the view that inflammation is involved in the pathogenetic process that leads to dementia. This view is supported by several observations. First, anti-inflammatory medication use, such as NSAID use, is associated with a decreased risk of dementia. Although NSAIDs can have effects within the brain, the effect of NSAIDs on dementia risk may also act by means of suppressing the peripheral immune system. Second, acute phase proteins, such as ACT, are known to reinforce the formation of beta-amyloid deposits, which is thought to be crucial in the pathogenesis of Alzheimer's disease. Because peripheral proteins are able to cross the blood-brain barrier and because the permeability of the blood-brain barrier may increase with rising plasma levels of inflammatory proteins such as IL6, peripherally produced inflammatory proteins might also amplify formation of cerebral beta-amyloid deposits. Third, interleukin-
1 (IL1) has already been found elevated in brains of newborns with Down syndrome, a disease that is associated with beta-amyloid deposition and dementia from the age of 40 years. Finally, atherosclerosis, which is an inflammatory process, may be associated with increased risk of dementia. The idea of atherosclerosis being a link between inflammation and dementia is supported by our observation of relatively strong associations of ACT and CRP with vascular dementia. However, several other observations in our study suggest that atherosclerosis does not fully explain the relation between inflammatory proteins and risk of dementia. First, when we adjusted for measures of atherosclerosis the relation between inflammatory proteins and dementia did not change. Also, because atherosclerosis may be associated with increased plasma levels of CAMs, one would also have expected an association between sICAM-1, sVCAM-1 and risk of dementia if atherosclerosis was to contribute to the pathogenesis of dementia. Finally, the risk associated with IL6 was similar for Alzheimer's disease and vascular dementia.

On the other hand it can be hypothesized that elevation in plasma levels of inflammatory proteins is a consequence of the pathophysiological process of dementia. Elevated peripheral inflammatory proteins may be the result of the cerebral beta-amyloid deposition that induces the local production of inflammatory proteins, such as interleukin-1 (IL1) and IL6. These cerebrally produced cytokines may increase peripheral levels of inflammatory proteins, either because they pass the blood-brain barrier or through stimulation of peripheral production of inflammatory proteins as shown in animal models. If the activation of the peripheral immune system is both a cause and a consequence of the pathogenetic process of dementia, a self-enhancing cascade will take place. This cascade includes beta-amyloid deposit formation that leads to local inflammation within the brain resulting in the activation of the peripheral immune system that leads to increased beta-amyloid deposit formation.

In summary, we observed that high plasma levels of ACT and IL6 were associated with an increased risk of dementia, Alzheimer's disease and vascular dementia. Higher levels of CRP were less strongly associated with risk of dementia and Alzheimer's disease. These results suggest that a peripheral immunological response occurs prior to clinical expression of dementia. Whether peripheral inflammation contributes to the pathogenesis of dementia remains to be elucidated.

References

Inflammatory proteins in plasma and risk of dementia

Chapter 2.2

The association between leucocyte count and risk of Alzheimer’s disease is modified by intake of antioxidants

Background Inflammation may induce oxidative stress that possibly leads to increased inflammation. The relation between inflammation and Alzheimer’s disease may therefore be modified by oxidative stress and antioxidants that counteract oxidative stress.

Objective To examine whether the relation between plasma leucocyte count as indicator of inflammation, and risk of Alzheimer’s disease is modified by intake of antioxidants.

Methods We used data from the Rotterdam Study, a prospective cohort study among elderly. At baseline (1990-1993), plasma leucocyte count and dietary intake were both assessed in 5093 dementia-free subjects. The cohort was reexamined in 1993-1994 and 1997-1999 and in addition continuously monitored for incident dementia. The association between leucocyte count and incident Alzheimer’s disease was examined by Cox proportional hazards models, both unstratified and across strata of intake of the antioxidants beta carotene, flavonoids, vitamin C and vitamin E.

Results After a mean follow-up of 6.0 years, 183 subjects developed dementia, of whom 136 had Alzheimer’s disease. High leucocyte count was associated with increased risk of Alzheimer’s disease (rate ratio per standard deviation increase was 1.10 (95% confidence interval 1.02-1.19)). This relation was significantly modified by intake of antioxidants and was particularly present in subjects with low intake. The interaction between leucocyte count and antioxidant intake was most pronounced in current smokers.

Conclusion Our findings suggest that the association between high leucocyte count and increased risk of Alzheimer’s disease is amplified by low intake of antioxidants.

Inflammation and oxidative stress may both contribute to the etiology of Alzheimer’s disease.\(^1\)\(^-\)\(^3\) This is supported by studies showing that plasma levels of inflammatory proteins are elevated prior to clinical onset of dementia\(^4\) and by studies reporting lower risk of Alzheimer’s disease with higher dietary intake of antioxidants.\(^5\)\(^,\)\(^6\)
Inflammation and oxidative stress are related phenomena that may enhance each other in Alzheimer pathogenesis. On the one hand, inflammation may induce oxidative stress by the activation of microglia that generate free radicals and thus oxidative stress. On the other hand, inflammation may be amplified by free radicals, because free radicals may activate pro-inflammatory genes. We hypothesized that the relation between inflammation and dementia is modified by antioxidants that counteract oxidative stress.

We examined whether the association between activation of the peripheral inflammation system and risk of Alzheimer's disease is modified by dietary intake of antioxidants in the Rotterdam Study.

Methods and materials

The Rotterdam Study
The Rotterdam Study is a population-based prospective cohort study that investigates determinants of chronic and disabling diseases in the elderly. The study was approved by the medical ethics committee of the Erasmus University Rotterdam. In total, 7983 subjects (response rate 78%) who were 55 years and above and lived in Ommoord, a suburb of Rotterdam, the Netherlands, agreed to participate and gave their written informed consent.

The baseline examination took place between 1990 and 1993 and follow-up examinations were in 1993-1994 and in 1997-1999. In addition, the total cohort was continuously monitored for major disease outcomes.

Diagnosis of dementia
During baseline and follow-up examinations the diagnosis of dementia followed a similar 3-step protocol, which has been described in detail elsewhere. In brief, participants were first cognitively screened. Screen-positives underwent further cognitive testing. Subjects who were suspected of having dementia were subsequently examined by a neurologist, a neuropsychologist and, if possible, had a brain scan made by magnetic resonance imaging. In addition, the total cohort was continuously monitored for incident dementia through computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnoses of dementia and Alzheimer's disease were made in accordance with internationally accepted criteria for dementia (DSM-III-R) and Alzheimer's disease (NINCDS-ADRDA) by a panel of a neurologist, neuropsychologist and research physician that reviewed all existing information.
Assessment of peripheral inflammation

As an indicator of activation of the peripheral immune system we used leucocyte count in plasma, which is commonly used in clinical practice to detect inflammation. Leucocyte count was available at baseline for a large number of participants of the Rotterdam Study. A venipuncture was performed with minimal stasis and a 21 gauge Butterfly needle with tube (Terumo Surflo® winged infusion set). Immediately after blood sampling, leucocyte count was assessed in citrate plasma using a Coulter Counter T540® (Coulter electronics, Luton, England), which has a coefficient of variation of less than 2.0%. Quality of assessments was continuously monitored by Instruchemie® (Hilversum, the Netherlands).

Dietary assessment

Dietary intake of the participants was assessed at baseline in 2 stages. First, the participants indicated on a checklist all foods and drinks they had consumed at least twice a month during the preceding year. The checklist also contained questions on use of supplements. Second, on the basis of the completed checklist the participants were interviewed by a trained dietician, who used an extensive, validated semiquantitative food-frequency questionnaire. The food-frequency data were converted to energy intake and nutrient intake using the computerized Dutch Food Composition Table. For the present study we used data on intake of the antioxidants beta carotene, flavonoids, vitamin C, and vitamin E. Daily dietary intake of antioxidants from food was calculated in milligrams.

Covariates

During the baseline home interview information on level of education and smoking habits was obtained. Data on medication use at time of venipuncture were obtained from the baseline interview and from a pharmacy database that was linked to the study database. Participants who used systemic non-steroidal anti-inflammatory drugs (NSAID), systemic corticosteroids, inhalation corticosteroids, nasal corticosteroids or ophthalmologic corticosteroids were classified as users of anti-inflammatory medication.

At the baseline visit to the research center, height and weight were measured, and intake of total energy, alcohol and antioxidative supplements were indicated on the semiquantitative food-frequency questionnaire. Subjects who used supplement preparations that contained at least 1 of the antioxidants beta carotene, flavonoids, vitamin C or vitamin E, were classified as users of antioxidative supplements. Furthermore, ultrasonography of both carotid arteries was performed. We used presence of carotid plaques as indicator of atherosclerosis. Carotid plaques were
determined at 6 different locations in the carotid arteries: common carotid artery, carotid bifurcation, and internal carotid artery at both left and right side. Four categories were made according to number of locations with plaques: 0, 1-2, 3-4 and 5-6 locations.

Study population
At baseline, 7,525 participants of the Rotterdam Study underwent extensive screening for dementia. Dementia was diagnosed in 482 subjects resulting in 7,043 subjects who were free of dementia at baseline. Of these, dietary intake was assessed in 5,395 subjects who had normal cognition, lived independently, and had reliable dietary assessment at baseline. Of the subjects with dietary data, 5,093 subjects had also baseline assessment of leucocyte count. In 302 subjects with dietary data leucocyte count was not assessed because of failed venipuncture (n=16), missing citrate plasma (n=20), missing Coulter Counter® assessment (n=102) or unknown reasons (n=96). Thus, the study population of the present study consisted of 5,093 subjects who were at risk for dementia and had baseline assessment of both dietary intake and leucocyte count in blood.

Data analysis
The association between leucocyte count and risk of Alzheimer’s disease was evaluated using Cox proportional hazards models. Leucocyte count was represented in the model either categorized in quartiles or by a linear term (per standard deviation). In all analyses adjustments were made for age, gender and education.

To assess whether smoking habits, packyears of smoking, body mass index and use of anti-inflammatory medication confounded the relation between leucocyte count and risk of Alzheimer’s disease, these variables were simultaneously added to the model. Furthermore, because dementia may be associated with atherosclerosis, which is an inflammatory process, we separately adjusted for carotid plaques as a measure of atherosclerosis. In this analysis a missing indicator was used for all subjects with missing data on carotid plaques (n=766).

To examine whether intake of antioxidants modified the relation between leucocyte count and Alzheimer’s disease, we performed the analyses within strata of intake of beta carotene, flavonoids, vitamin C, and vitamin E, respectively. In these analyses rate ratios were expressed per standard deviation increase in leucocyte count. For each antioxidant, we made 2 strata by dichotomizing at the median intake of the study population. Statistical interactions were tested by adding a product term of the linear terms leucocyte count and antioxidant intake to the unstratified model.
Furthermore, we examined the interaction between leucocyte count and antioxidant intake across strata of smoking habits.

To investigate the combined effect of leucocyte count and antioxidant intake on risk of Alzheimer's disease, we created 4 new categorical variables. We first dichotomized leucocyte count and antioxidant intake into high and low level according to the median of the study population. Subsequently, for each variable 4 categories were made: low leucocyte count/high antioxidant intake (reference category), low leucocyte count/low antioxidant intake, high leucocyte count/high antioxidant intake and high leucocyte count/low antioxidant intake. Rate ratios of Alzheimer's disease were calculated for each of the categories adjusting for age, gender, and education. In additional analyses, we also corrected for smoking habits, packyears of smoking, body mass index, use of anti-inflammatory medication, total energy intake, alcohol intake, and use of antioxidative supplements. Separately, we additionally adjusted for presence of carotid plaques. Finally, the analyses were repeated after exclusion of all subjects using anti-inflammatory medication (n=736, 14%) or supplements (n=479, 9%).

**Table 1** Baseline characteristics of the study population (n=5093)*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>67.7 (7.8)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>23%</td>
</tr>
<tr>
<td>Former</td>
<td>43%</td>
</tr>
<tr>
<td>Never</td>
<td>34%</td>
</tr>
<tr>
<td>Packyears of smoking</td>
<td></td>
</tr>
<tr>
<td>Current smokers†</td>
<td>27.9 (13.6-42.4)</td>
</tr>
<tr>
<td>Former smokers†</td>
<td>17.5 (5.3-35.0)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.3 (3.7)</td>
</tr>
<tr>
<td>Use of anti-inflammatory medication</td>
<td>14%</td>
</tr>
<tr>
<td>Total energy intake (kJ/d)</td>
<td>8272 (2115)</td>
</tr>
<tr>
<td>Use of antioxidative supplements</td>
<td>12%</td>
</tr>
<tr>
<td>Beta carotene (mg/d)</td>
<td>1.53 (0.76)</td>
</tr>
<tr>
<td>Flavonoids (mg/d)</td>
<td>28.4 (12.2)</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>121 (54.1)</td>
</tr>
<tr>
<td>Vitamin E (mg/d)</td>
<td>13.8 (6.2)</td>
</tr>
<tr>
<td>Leucocyte count (10⁹/L)†</td>
<td>6.44 (5.44-7.57)</td>
</tr>
</tbody>
</table>

* values represent means (standard deviation) or percentages (%)
† median (interquartile range)
All data analyses were performed using SPSS statistical software, version 10 (SPSS Inc., Chicago, Illinois, USA).

Results

Table 1 shows the baseline characteristics of the study population. The mean age was 67.7 years (SD 7.8), 59% were women and one-third had primary education only. The median leucocyte count of the study population was $6.44 \times 10^9/L$.

During a mean follow-up of 6.0 years (SD 1.3) 183 subjects developed dementia, of whom 136 had Alzheimer's disease.

Table 2 presents the relation between leucocyte count and risk of Alzheimer's disease. Across quartiles, risk of Alzheimer's disease gradually increased with higher leucocyte count. When adjustments were made for age, gender, and education, the rate ratio of the highest quartile compared to the lowest quartile was 1.66 (95% CI 1.02-2.69)). Results were very similar after additional adjustments for smoking habits, packyears of smoking, body mass index and, use of anti-inflammatory medication, or presence of carotid plaques. Because the analyses across quartiles did not show deviations from linearity for the association between leucocyte count and risk of Alzheimer's disease, leucocyte count was added as a linear term per standard deviation (SD). With every increase of $2.1 \times 10^9/L$ (SD) in leucocyte count, the risk of Alzheimer's disease increased with 11% after

<table>
<thead>
<tr>
<th>Leucocyte count (10^9/L)</th>
<th>Model 1*</th>
<th>Model 2†</th>
<th>Model 3‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartiles</td>
<td>RR (95% CI)</td>
<td>RR (95% CI)</td>
<td>RR (95% CI)</td>
</tr>
<tr>
<td>&lt; 5.4</td>
<td>1 (reference)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5.4 - 6.4</td>
<td>0.99 (0.58-1.71)</td>
<td>0.99 (0.58-1.70)</td>
<td>0.99 (0.58-1.70)</td>
</tr>
<tr>
<td>6.4 - 7.7</td>
<td>1.31 (0.78-2.19)</td>
<td>1.27 (0.75-2.15)</td>
<td>1.30 (0.78-2.18)</td>
</tr>
<tr>
<td>&gt; 7.7</td>
<td>1.66 (1.02-2.69)</td>
<td>1.71 (1.04-2.81)</td>
<td>1.63 (1.00-2.65)</td>
</tr>
<tr>
<td>Per SD</td>
<td>2.1</td>
<td>1.10 (1.02-1.19)</td>
<td>1.10 (1.02-1.19)</td>
</tr>
</tbody>
</table>

* adjusted for age, gender and education  
† adjusted for age, gender, education, smoking habits, packyears of smoking, body mass index and use of anti-inflammatory medication  
‡ adjusted for age, gender, education and carotid plaques  
|| rate ratio (95% confidence interval)
Table 3 Adjusted* rate ratios of Alzheimer's disease per standard deviation increase in leucocyte count across strata of antioxidant intake (n=5093, 136 cases of Alzheimer's disease)

<table>
<thead>
<tr>
<th>Stratified by</th>
<th>Low intake†</th>
<th>High intake‡</th>
<th>p-value¶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)§</td>
<td>RR (95% CI)§</td>
<td></td>
</tr>
<tr>
<td>Beta carotene (1.43 mg/d)#</td>
<td>1.32 (1.11-1.58)</td>
<td>1.05 (0.90-1.21)</td>
<td>0.10</td>
</tr>
<tr>
<td>Flavonoids (27.4 mg/d)#</td>
<td>1.43 (1.16-1.77)</td>
<td>1.05 (0.91-1.21)</td>
<td>0.01</td>
</tr>
<tr>
<td>Vitamin C (113 mg/d)#</td>
<td>1.12 (0.96-1.31)</td>
<td>1.10 (1.00-1.20)</td>
<td>0.15</td>
</tr>
<tr>
<td>Vitamin E (12.9 mg/d)#</td>
<td>1.27 (1.09-1.49)</td>
<td>1.05 (0.92-1.21)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*adjusted for age, gender and education
† defined as lower than the median intake
‡ defined as equal to or higher than median intake
§ rate ratio (95% confidence interval)
¶ statistical interaction of the continuous variables leucocyte count and antioxidant intake
# median intake

extensive adjustments (rate ratio 1.10 (95% CI 1.02-1.19)).

Table 3 shows the association of leucocyte count with risk of Alzheimer's disease across strata of intake of antioxidants. The risk of Alzheimer's disease associated with higher leucocyte count was higher in subjects with low intake of any of the antioxidants compared to high intake. This was most striking across strata of flavonoids; the rate ratio of Alzheimer's disease was 1.43 (95% CI 1.16-1.77) for subjects with low intake of flavonoids and 1.05 (95% CI 0.91-1.21) for subjects with high intake. The statistical interaction between leucocyte count and antioxidant intake was significant for flavonoids and vitamin E. The interaction between leucocyte count and antioxidant intakes was most pronounced within current smokers; for beta carotene, flavonoids, vitamin C, and vitamin E the p-values of the statistical interactions were 0.02, 0.18, 0.28, and 0.003, respectively, for current smokers, whereas the p-values for never smokers were 0.63, 0.47, 0.15, and 0.30.

Figure 1 presents the relation of the combined effect of leucocyte count and antioxidant intake on risk of Alzheimer's disease when adjusted for age, gender, and education. Risk of Alzheimer's disease increased with higher leucocyte count and lower antioxidant intake and was thus highest for subjects with high leucocyte count and low intake of any of the antioxidants. For vitamin C, and vitamin E, rate ratios were significantly higher in the high count/low intake group when compared to the reference group (low count/high intake): rate ratios were 1.87 (95% CI 1.14-3.10) for vitamin C and 1.98 (95% CI 1.21-3.26) for vitamin E. When additional adjustments were made for smoking habits, packyears of smoking, body mass index, use
Figure 1 Adjusted* rate ratios (RR) of Alzheimer's disease for categories based on leucocyte count and antioxidant intake†

* adjusted for age, gender and education
† we made 4 categories on the basis of the median of leucocyte count and the median of the respective antioxidant intakes: low count/high intake (reference category), low count/low intake, high count/high intake and high count/low intake
of anti-inflammatory medication, total energy intake, alcohol intake, and use of antioxidative supplements, the results were very similar. Also, adjustment for presence of carotid plaques as a measure of atherosclerosis did not change the results, nor did exclusion of users of anti-inflammatory medication or antioxidative supplements.

Discussion

We observed that high leucocyte count was associated with increased risk of Alzheimer’s disease, in particular in persons with low dietary intake of antioxidants. Within current smokers the interaction between leucocyte count and antioxidant intake was most pronounced.

Before interpreting the results some methodological issues need to be discussed. First, leucocyte count is a rather aspecific indicator of inflammation, and it correlates with other inflammatory factors such as levels of inflammatory proteins. We are unable to conclude from our data whether the association of increased risk of Alzheimer’s disease with increased leucocyte count reflects a relation with increased inflammation in general or is due to specific inflammatory factors. Second, the interaction between leucocyte count and antioxidant intake may be confounded by smoking. Smoking is related to both inflammation and to intake of antioxidants. Although additional adjustment for smoking habits or packyears smoked did not change the results, confounding by smoking may still have occurred since the interaction between leucocyte count and antioxidants was particularly present within current smokers.

The strengths of this study are its prospective design, its size and its population-based setting. Furthermore, we achieved complete follow-up with regard to dementia status. As a result, selection bias as a consequence of loss to follow-up could not have occurred.

Several biological mechanisms may explain the observed modifying effect of dietary antioxidants on the relation between peripheral inflammation as indicated by leucocyte count and risk of Alzheimer’s disease. First, the interaction between dietary antioxidants and peripheral leucocyte count may be a reflection of an interaction between antioxidants and inflammation in the brain. This is suggested by animal studies showing that elevated inflammatory proteins in the brain may result in activation of the peripheral immune system and that antioxidant levels within the brain may be related to peripheral levels of antioxidants. Dietary antioxidants may thus block the interaction between inflammation and oxidative stress that may occur in Alzheimer brains. This interaction includes activation of microglia that leads to neuronal cell damage through the generation of free radicals, which in turn may amplify inflammation by the activation of pro-inflammatory genes. Second, the interaction between peripheral
inflammation and antioxidants in Alzheimer’s disease may be explained by atherosclerosis. In atherogenesis, inflammation is also suggested to interact with oxidative stress and thus antioxidants, because oxidative stress leads to LDL-oxidation, subsequent inflammation and atherosclerotic plaques and because inflammation is accompanied by increased count of leucocytes that produce free radicals in itself. However, because additional adjustment for carotid plaques as a measure of atherosclerosis did not change our results, we think that the interaction between leucocyte count and antioxidants in relation to risk of Alzheimer’s disease will not entirely be explained by atherosclerosis.

In summary, we observed that the relation between leucocyte count and risk of Alzheimer’s disease was modified by intake of antioxidants: higher leucocyte count was particularly associated with increased risk of Alzheimer’s disease in subjects with low antioxidant intake.

References

Chapter 3.1.1

Plasma levels of antioxidants are not associated with Alzheimer’s disease or cognitive decline

Antioxidants prevent oxidative stress that possibly causes neuronal loss in Alzheimer’s disease. We examined whether high plasma levels of the antioxidant vitamins A and E were associated with lower prevalence of Alzheimer’s disease or cognitive decline.

We performed a cross-sectional study based on 3717 participants from the Rotterdam Study who had blood samples drawn at second follow-up examinations. From these, we included 65 patients with Alzheimer’s disease and 293 dementia-free persons with cognitive decline. Controls were 437 randomly drawn subjects without dementia or cognitive decline. The association between vitamin A and E and Alzheimer’s disease and cognitive decline was examined by logistic regression.

In an univariate model higher levels of vitamin A and E were significantly associated with lower prevalence of Alzheimer’s disease. However, when additional adjustments were made for important confounders, such as age, gender and total cholesterol, the relation substantially weakened (odds ratios per standard deviation increase were for vitamin A 0.87 (95% confidence interval (95% CI) 0.64-1.19) and for vitamin E 0.94 (95% CI 0.60-1.48). Antioxidants were not related to cognitive decline.

Our findings suggest no association between plasma levels of vitamin A and E and Alzheimer’s disease or cognitive decline.

Signs of oxidative stress have been observed in brains of patients with Alzheimer’s disease. For instance, it has been found that DNA and membrane lipids are damaged by oxidation. This oxidation leads to neuronal cell loss, an important feature in Alzheimer brains. Because antioxidants can counteract oxidation and thus prevent neuronal cell death, high levels may lower risk of Alzheimer’s disease.

Recently, we and others reported that high dietary intake of vitamins C and E was associated with lower risk of Alzheimer’s disease. The relation between plasma levels of antioxidants, particularly vitamin A and E, and...
Alzheimer's disease has been examined in many cross-sectional studies, of which some observed an inverse relation\textsuperscript{4-8} whereas others did not find any association.\textsuperscript{9-14} However, all these studies were performed in hospital-based populations. Furthermore, many studies did not adjust for confounders,\textsuperscript{5, 6, 8, 10, 11, 13} whereas those that did make adjustments\textsuperscript{4, 7} only corrected for either age, gender or cholesterol. However, these covariates as well as education and smoking may be important confounders. We examined whether high plasma levels of vitamin A and E were associated with lower prevalence of Alzheimer's disease within a population-based study, adjusting for important confounders. In addition, we investigated whether high levels of antioxidants were related to an increased prevalence of cognitive decline, which may indicate imminent dementia.

**Subjects and methods**

** Eligible population**

We performed a cross-sectional study within the Rotterdam Study, a large population-based prospective cohort study that was designed to investigate determinants of chronic disabling diseases in the elderly.\textsuperscript{15} The Rotterdam Study was performed among inhabitants of a district of Rotterdam, the Netherlands, who were 55 years and over. The medical ethics committee of the Erasmus University Rotterdam approved the study and written informed consent was obtained from all 7983 participants.

The baseline, first follow-up and second follow-up examinations took place in 1990-1993, 1993-1994 and 1997-1999, respectively. In addition, the total cohort was continuously monitored for mortality and major morbidity.

At baseline, 7525 subjects were cognitively screened, of whom 5757 subjects were still alive at second follow-up. The 3717 participants (64.6% of the living) who had fasting blood samples drawn at second follow-up were eligible for the present study.

**Diagnosis of dementia**

Cognitive screening was performed by use of a 3-step protocol at both baseline and follow-up examinations.\textsuperscript{16} First, subjects underwent 2 brief tests of cognition, namely the Mini Mental State Examination (MMSE)\textsuperscript{17} and the Geriatric Mental State schedule (GMS) organic level.\textsuperscript{18} Screen positives (MMSE score < 26 or GMS organic level > 0) were further cognitively tested using the Cambridge examination for mental disorders of the elderly (CAMDEX).\textsuperscript{19} If a participant was suspected of having dementia, he or she was subsequently examined by a neurologist and a neuropsychologist and a magnetic resonance imaging scan of the brain was made. Diagnoses of dementia and Alzheimer's disease were made according
to criteria of DSM-III-R and NINCDS-ADRDA, respectively, by a panel of a neurologist, neuropsychologist and research physician.

Definition of cognitive decline
The definition of cognitive decline was based on MMSE-scores at baseline, first follow-up and second follow-up from all dementia-free participants in the eligible population (n=3633). For each participant the yearly decline in MMSE-score over a mean period of 6.5 years (SD 0.3) was calculated by means of a random effects model using SAS statistical software (SAS Institute Inc., Cary, NC, USA). We defined subjects as having cognitive decline in case their decline in MMSE-score fell within the highest 8th percentile of the distribution of MMSE-decline. This cut-off point corresponded to a decline in MMSE-score of 0.11 points per year or more.

Cases and controls
Cases and controls were selected from the eligible population (n=3717). Of the 84 dementia patients within this population, 65 subjects suffered from Alzheimer’s disease and served as Alzheimer cases in the present study. Cases of cognitive decline were all 293 dementia-free subjects from the eligible population, who had cognitive decline according to the definition given before. As controls we used 437 subjects who were randomly drawn from that part of the eligible population that was free of dementia and cognitive decline (n=3340).

Assessments of plasma levels of vitamin A and E
At the second follow-up examination blood samples were drawn from subjects who had fasted overnight. Obtained citrate plasma was immediately frozen in liquid nitrogen, transferred to the laboratory and stored at -80°C.

Measurements of vitamins were performed blindly for case/control status. We determined plasma levels of total vitamin A (retinol) and total vitamin E (alpha-tocopherol) using methods described elsewhere. In brief, reversed-phase HPLC with UV detection was applied using a RP C18 column 100 mm x 4.6 mm (Merck Lichrospher 100 RP-18e®). Vitamin A was detected at 324 nanometer (nm) and vitamin E at 288 nm. The interassay coefficient of variation was 4.0 % for vitamin A and 3.2 % for vitamin E.

Covariates
We assessed level of education and smoking habits. In addition, blood samples were drawn and weight and height were measured. For the present study, we categorized highest attained level of education in 2 groups: low
education versus high education. Low education was defined as completed primary education, lower vocational training or lower general education. High education was defined as intermediate or higher vocational training or education, college or university. Smoking habits were dichotomized into yes/no current smoking. Total cholesterol was determined in serum with an automated enzymatic procedure and body mass index was calculated by dividing the weight by the squared height.

Data analysis
The relation between plasma levels of vitamin A and E and Alzheimer's disease and cognitive decline was assessed using logistic regression analyses. Plasma levels of antioxidants were first entered into the model as a linear term. In this model, the regression coefficient was expressed per standard deviation increase in plasma level. In addition, we performed analyses using tertiles of antioxidant levels. Standard deviations and tertiles were based on the distribution of the controls (n=437). The analyses were firstly performed without adjustments for any covariate. Subsequently, adjustments were made for age and gender. In addition, total cholesterol was added to the model as well as education, current smoking and body mass index. Finally, we excluded current smokers from the fully adjusted model.

We used a significance level of 5% based on 2 sided testing. All data analyses were performed using SPSS statistical software, version 10 (SPSS Inc., Chicago, Illinois, USA).

<table>
<thead>
<tr>
<th>Table 1 Characteristics of the study population*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases of Alzheimer's disease (n=65)</td>
</tr>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>Women</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Low education†</td>
</tr>
<tr>
<td>Current smoking</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
</tr>
<tr>
<td>Vitamin A (μmol/L)</td>
</tr>
<tr>
<td>Vitamin E (μmol/L)</td>
</tr>
</tbody>
</table>

*values represent means (SD) or percentages (%)
† low education was defined as completed primary education, lower vocational training or lower general education
Results

Table 1 presents the characteristics of the study population. Cases of Alzheimer's disease and cognitive decline were older compared to control subjects and were more often women. Educational level was clearly lowest in cases with Alzheimer's disease compared to cases with cognitive decline and controls. Unadjusted plasma levels of vitamin A were lower for Alzheimer cases, whereas levels of vitamin E were lower for both cases of Alzheimer's disease and cognitive decline compared with controls.

Table 2 presents the odds ratios of Alzheimer's disease per standard deviation increase in plasma level of vitamin A and vitamin E. Unadjusted, the prevalence of Alzheimer's disease significantly decreased with 28% with every 0.40 μmol/L (SD) increase in vitamin A level (odds ratio (OR) 0.72 (95% confidence interval (95% CI) 0.55-0.96)). In addition, high level of vitamin E was associated with lower prevalence of Alzheimer's disease: the odds ratio per standard deviation increase was 0.58 (95% CI 0.43-0.80). However, when we entered age and gender into the models the relation substantially weakened both for vitamins A and E. The strength of the association further decreased after additional adjustment for total cholesterol as well as education, current smoking and body mass index; in the fully adjusted models the odds ratios were for vitamin A 0.98 (95% CI 0.71-1.37) and for vitamin E 0.86 (95% CI 0.53-1.40). The results were similar after exclusion of current smokers.

Table 3 shows the association between plasma levels of vitamins A and E and cognitive decline. Both antioxidants were not associated with presence of cognitive decline either in the univariate or in the multivariate model; when fully adjusted, odds ratios were 1.05 (95% CI 0.89-1.24) for vitamin A

Table 2 Adjusted odds ratios of Alzheimer's disease per standard deviation increase in plasma levels of vitamin A and E*

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted for age and gender</th>
<th>Adjusted for age, gender and total cholesterol</th>
<th>Fully adjusted†</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD‡</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0.40</td>
<td>0.72 (0.55-0.96)</td>
<td>0.84 (0.62-1.13)</td>
<td>0.87 (0.64-1.19)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>6.6</td>
<td>0.58 (0.43-0.80)</td>
<td>0.82 (0.57-1.18)</td>
<td>0.94 (0.60-1.48)</td>
</tr>
</tbody>
</table>

* 65 cases of Alzheimer's disease, 437 controls
† adjusted for age, gender, total cholesterol, education, current smoking and body mass index
‡ standard deviation (μmol/L)
¶ odds ratio (95% confidence interval)
Table 3 Adjusted odds ratios of cognitive decline per standard deviation increase in plasma levels of vitamins A and E*

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted for age and gender</th>
<th>Adjusted for age, gender and total cholesterol</th>
<th>Fully adjusted†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>0.40</td>
<td>1.00 (0.86-1.16)</td>
<td>1.02 (0.87-1.16)</td>
<td>1.04 (0.89-1.22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.05 (0.89-1.24)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>6.6</td>
<td>0.93 (0.80-1.07)</td>
<td>0.98 (0.84-1.15)</td>
<td>1.04 (0.85-1.28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.03 (0.84-1.27)</td>
</tr>
</tbody>
</table>

*293 cases with cognitive decline, 437 controls
† adjusted for age, gender, total cholesterol, education, current smoking and body mass index
‡ standard deviation (μmol/L)
¶ odds ratio (95% confidence interval)

and 1.03 (95% CI 0.84-1.27) for vitamin E. Exclusion of current smokers did not change the results.

Figure 1 presents the relation between tertiles of antioxidants and Alzheimer's disease with adjustments for age, gender, total cholesterol, education, current smoking and body mass index. Also across tertiles, levels of vitamins A and E were not associated with Alzheimer’s disease.

Figure 2 shows that tertiles of vitamins A and E were not related to cognitive decline.

Discussion

After adequate adjustment for confounding, we observed no association between plasma levels of the antioxidant vitamins A and E and presence of Alzheimer's disease. Furthermore, no relation was found between antioxidants and cognitive decline in dementia-free subjects.

The present study has several advantages. First, our study examined the relation between antioxidant levels and Alzheimer's disease and cognitive decline in a population-based study. Second, antioxidant assessments were performed blinded for case/control status. As a result, differential misclassification could not have occurred. Finally, adjustments were made not only for age and gender, but also for important other confounders such as total cholesterol, education, current smoking and body mass index.

A limitation of the study is the cross-sectional design that makes it impossible to determine whether antioxidants are etiologically involved in Alzheimer pathogenesis. Levels of antioxidants may have altered due to changing dietary intake as a result of cognitive dysfunction. Another limitation may be our definition of cognitive decline as a proxy for preclinical stages of Alzheimer’s disease. This definition was based on the
Figure 1 Adjusted odds ratios (OR) of Alzheimer's disease plotted on a logarithmic scale against median level of vitamins A and E within tertiles*

![Graph showing OR against median level of vitamin A and E within tertiles.]

* adjusted for age, gender, total cholesterol, education, current smoking and body mass index; whiskers indicate 95% CI

Figure 2 Adjusted odds ratios (OR) of cognitive decline plotted on a log-scale against median level of vitamins A and E within tertiles*

![Graph showing OR against median level of vitamin A and E within tertiles.]

* adjusted for age, gender, total cholesterol, education, current smoking and body mass index; whiskers indicate 95% CI

MMSE that may have been too crude to accurately classify dementia-free subjects in terms of cognitive decline. This random misclassification may have resulted in an underestimation of the true association of antioxidants with cognitive decline.

Numerous hospital-based studies examined the relation between blood levels of antioxidants and the prevalence of Alzheimer's disease. For vitamin E, the results were conflicting: 5 studies observed higher prevalence of Alzheimer's disease with lower levels of vitamin E, whereas 5 other studies did not find any association. For vitamin A, 5 previous studies showed significantly higher prevalence of Alzheimer's disease with lower levels. However, the studies on vitamin A and E and...
Alzheimer's disease either did not adjust for confounders\textsuperscript{5, 6, 9, 12, 14} or only adjusted for age and gender\textsuperscript{7, 8, 10, 11} or lipid levels.\textsuperscript{13} Therefore, results of these studies are difficult to interpret since we showed that the association between vitamin A and E and prevalence of Alzheimer's disease disappeared when adjustments were made for age, gender, total cholesterol, education, current smoking and body mass index. Our results on cognitive decline are in accordance with the findings of a previous population-based study that adequately adjusted for confounding.\textsuperscript{25} This French study also observed no association of vitamin E with cognitive decline 4 years later.

The absent association between plasma level of vitamin E and Alzheimer's disease in the present study is in contrast with previous findings of lower risk of Alzheimer's disease with higher vitamin E intake.\textsuperscript{2, 3} This discrepancy may be explained in 2 ways. First, the present study on plasma levels was cross-sectional, whereas the intake study was longitudinal. In cross-sectional studies rather than in longitudinal studies (absence of) associations may be due to changing antioxidant status as a result of cognitive dysfunction. Second, studies on plasma levels and intake may not be comparable because plasma levels are not only determined by intake, but also by absorption, usage and storage. As a result, the correlation between plasma levels of vitamin E and dietary intake is not very high ($r=0.34$).\textsuperscript{26}

In summary, this population-based study showed that the cross-sectional association between plasma levels of vitamin A and E and Alzheimer's disease was largely due to confounding factors. Vitamin A and E were not related to cognitive decline either. Prospective studies are needed to elucidate whether plasma levels of antioxidants are reduced prior to clinical onset of dementia.

References


Chapter 3.1.2

Dietary intake of antioxidants and risk of Alzheimer’s disease

Context Laboratory findings have suggested that oxidative stress may contribute to the pathogenesis of Alzheimer’s disease. Therefore, the risk of Alzheimer’s disease might be reduced by intake of antioxidants that counteract the detrimental effects of oxidative stress.

Objective To determine whether dietary intake of antioxidants is related to risk of Alzheimer’s disease.

Design and Setting The Rotterdam Study, a population-based, prospective cohort study conducted in the Netherlands.

Participants A total of 5395 participants who, at baseline (1990-1993), were aged at least 55 years, free of dementia, and noninstitutionalized and had reliable dietary assessment. Participants were reexamined in 1993-1994 and 1997-1999 and were continuously monitored for incident dementia.

Main Outcome Measures Incidence of Alzheimer’s disease, based on Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition (DSM-III-R) criteria and National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria, associated with dietary intake of beta carotene, flavonoids, vitamin C, and vitamin E.

Results After a mean follow-up of 6 years, 197 participants developed dementia, of whom 146 had Alzheimer’s disease. When adjustments were made for age, sex, baseline Mini-Mental State Examination score, alcohol intake, education, smoking habits, packyears of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements, high intake of vitamin C and vitamin E was associated with lower risk of Alzheimer’s disease (rate ratios (RRs) per standard deviation increase in intake were 0.82 (95% confidence interval (CI) 0.68-0.99) and 0.82 (95% CI 0.66-1.00), respectively. Among current smokers, this relationship was most pronounced (RRs 0.65 (95% CI 0.37-1.14) and 0.58 (95% CI 0.30-1.12), respectively) and also was present for intake of beta carotene (RR 0.49 (95% CI 0.27-0.92) and flavonoids (RR 0.54 (95% CI 0.31-0.96)). The associations did not vary by education or apolipoprotein E genotype.

Conclusion High dietary intake of vitamin C and vitamin E may lower the risk of Alzheimer’s disease.
Several findings suggest that oxidative stress may play an important role in the pathogenesis of Alzheimer's disease. First, the brains of patients with Alzheimer's disease contain lesions that are typically associated with exposure to free radicals. In addition, oxidative stress in brains of Alzheimer patients is indicated by elevated cerebral levels of endogenous antioxidants that scavenge free radicals. Moreover, in vitro studies suggest that exogenous antioxidants reduce the toxicity of beta-amyloid in brains of Alzheimer patients. Based on these findings, it has been hypothesized that antioxidants from food may reduce the risk of Alzheimer's disease.

A previous randomized controlled trial found that patients taking vitamin E supplement had a slower progression of the disease than patients taking placebo. It is thus possible that high intake of antioxidants may also prevent the onset of dementia, because antioxidants may reduce neuronal loss due to oxidative damage.

Two studies examined the longitudinal relationship between antioxidants from supplements and risk of Alzheimer's disease. These studies found conflicting results: vitamin C supplement use was related to lower risk of Alzheimer's disease in 1 study, whereas the other found no association for combined use of vitamin C and vitamin E supplements. However, studies on supplement use are prone to bias, because people who use supplements may also have more health problems and more health-seeking behavior. In addition, use of supplements is generally of short duration.

To date, only 1 study has prospectively examined the association between dietary antioxidants and risk of dementia, and found a reduced risk of dementia associated with increased intake of flavonoids. We investigated whether intake of a range of antioxidants from food, namely beta carotene, flavonoids, vitamin C, and vitamin E, was associated with the risk of Alzheimer's disease, using data from a population-based cohort study.

Methods

The Rotterdam Study

The Rotterdam Study is a population-based, prospective cohort study of the frequency and determinants of neurological, cardiovascular, locomotor, and ophthalmologic diseases in elderly persons. The medical ethics committee of the Erasmus University Rotterdam approved the study. The eligible population comprised all inhabitants of a suburb in Rotterdam, the Netherlands, who were aged at least 55 years (n=10 275). Of these, 7963 subjects (response rate 78%) gave their written informed consent and participated in the study.

During the baseline examination (1990-1993), a research assistant interviewed participants in their homes and obtained information on
current and past health, medication, lifestyle, and risk factors for chronic diseases. In addition, participants visited the research center twice for baseline clinical examinations. Follow-up examinations took place in 1993-1994 and 1997-1999. The total cohort was continuously monitored for mortality and major morbidity.

**Diagnosis of dementia and Alzheimer’s disease**
Case-finding and diagnostic procedures for dementia and Alzheimer’s disease have been described in detail. At baseline visit and both follow-up examinations, a 3-stage protocol was used. Participants were cognitively screened with the Mini-Mental State Examination (MMSE) and the Geriatric Mental State schedule (GMS) organic level. If subjects scored lower than 26 on the MMSE or higher than 0 on the GMS organic level, the Cambridge Examination of Mental Disorders in the Elderly (CAMDEX) was administered. The CAMDEX also included an informant interview. Finally, participants in whom dementia was suspected were examined by a neurologist and neuropsychologist and, if possible, had magnetic resonance imaging of the brain. In addition, the total cohort was continuously monitored for incident dementia cases through computerized linkage between the study database and computerized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnoses of dementia and Alzheimer’s disease were based on Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition (DSM-III-R) criteria and the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria, respectively, and were made by a panel of a neurologist, neuropsychologist, and research physician that reviewed all existing information. Follow-up with respect to dementia was virtually complete (99.9%).

**Dietary assessment**
Dietary intake was assessed at baseline by means of a 2-stage protocol. Participants first indicated on a checklist all foods and drinks they had consumed at least twice a month during the preceding year. The checklist also contained questions on dietary habits, use of supplements, and prescribed diets. At the second baseline visit to the research center, the participants were interviewed on the basis of the completed checklist. This interview was performed by a dietician, who used an extensive, validated semiquantitative food-frequency questionnaire (SFFQ). The SFFQ data were converted to energy intake and nutrient intake using the computerized Dutch Food Composition Table. We used data on intake of the antioxidants beta carotene, flavonoids, vitamin C, and vitamin E. Important
sources of beta carotene are kale, carrots, broccoli, and spinach. Flavonoids are found in cranberries, green and black tea, and pulses. Vitamin C is mainly found in citrus fruits, kiwi, sprouts, broccoli, and cabbage. Important sources of vitamin E are grain, nuts, milk, and egg yolk. Daily dietary intake of the antioxidants from food was calculated in milligrams.

Other variables
During the baseline home interview, participants were asked about their highest attained level of education and their smoking habits. At the visits to the research center, which were part of the baseline clinical examination, the MMSE was performed, height and weight were measured, and intake of alcohol, total energy, antioxidative supplements, total fat, and saturated fat were indicated on the SFFQ. Furthermore, ultrasonography of the carotid arteries was performed and blood samples were drawn.

Level of education was categorized in 3 groups: low (primary education only); intermediate (lower vocational or general education); and high (intermediate or higher vocational or general education, college, or university). Smoking habits were categorized as never, former, and current smoking. For former and current smokers, the number of packyears was defined as the number of years of smoking times the number of cigarettes smoked daily divided by 20. Alcohol intake was categorized in 5 groups: no alcohol intake, less than 1 drink per week, between 1 drink per week and 1 drink per day, between 1 and 4 drinks per day, and 4 drinks per day or more. Subjects who used beta carotene supplement, flavonoid supplement, vitamin C supplement, vitamin E supplement, or multivitamin supplement were classified as users of antioxidative supplements; all others were classified as nonusers. Intake of total and saturated fat was expressed in grams per day. Artherosclerotic plaques in the carotid arteries were defined as a focal widening relative to adjacent segments, with the protrusion into the lumen composed of either only calcified deposits or a combination of calcification and noncalcified material. The presence of carotid plaques was assessed at 6 different locations: the common carotid artery, carotid bifurcation, and internal carotid artery at both left and right side. Subsequently, according to the number of locations with plaques, 4 categories were made: plaques at 0, 1 to 2, 3 to 4, and 5 to 6 locations. Apolipoprotein E (APOE) genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis. We dichotomized APOE genotype into presence or absence of the apolipoprotein E*4 (APOE*4) allele.
Dietary intake of antioxidants and risk of Alzheimer's disease

Study sample
At the baseline clinical examination, 7525 participants of the Rotterdam Study were screened for dementia. Dementia was diagnosed in 482 participants, resulting in 7043 participants who were free of dementia at baseline. Of these, we excluded 602 participants from dietary assessment for 2 reasons. First, dietary intake was not assessed in 125 participants who had questionable cognitive status (<80 CAMDEX score), because the participants might provide unreliable answers regarding their food patterns. Second, we excluded nursing home residents (n=477), because their current diet may not reflect dietary habits in the past. Thus, 6441 participants were eligible for dietary assessment. Of these, reliable dietary data were missing in 1046 participants (16%) for several reasons. First, due to logical inconsistencies in dietary interviews, 212 participants were excluded. Second, because the SFFQ was administered at the second baseline visit to the research center, participants who did not complete the second visit did not have dietary assessment (n=192). Finally, 642 participants did not have dietary data due to logistic reasons. Thus, the sample comprised 5395 participants who had normal cognition, lived independently, and had reliable dietary assessment at baseline.

Eligible participants without dietary data were somewhat older (2.6 years) compared with participants from the study population, a somewhat lower percentage (4%) were women and a higher percentage had only primary education (7%). Smoking habits and body mass index were similar across the 2 groups.

Data analysis
To assess the relationship between intake of antioxidants from food and cognitive function at baseline, we performed linear regression analysis with antioxidant intake as the dependent variable and baseline MMSE score as independent variable. We adjusted for age, sex, alcohol intake, education, smoking habits, packyears of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements.

To determine whether the incidences of Alzheimer's disease differed between the sample and the eligible population with missing dietary data, we performed a Cox proportional hazards regression analysis with adjustment for age, sex, and education.

We evaluated the associations of daily dietary intake of antioxidants with risk of Alzheimer's disease using Cox proportional hazards regression analysis. Intake of antioxidant was represented in the model either by a linear term or by 2 dummy variables representing the 2 highest tertiles. In the first case, the regression coefficient was expressed per SD increase. Standard deviations and tertiles of the respective intake of antioxidants
were based on the distribution of the complete sample (n=5395). All analyses were initially adjusted for age and sex. Subsequently, additional adjustments were made for baseline MMSE score and alcohol intake, respectively. In another model, adjustments were simultaneously made for the following confounders: age, sex, baseline MMSE score, alcohol intake, education, smoking habits, packyears of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements. Missing values were indicated by a missing indicator for categorical variables. For continuous variables, we replaced missing values by the mean or median of the study population, depending on the distribution. Age was used as the timescale in the model. Entry time was defined as age at study entry. Participants were followed until onset of Alzheimer’s disease, onset of other types of dementia, death, or end of study, whichever came first. Age at onset of Alzheimer’s disease and other types of dementia was determined as the midpoint between the age of participant last known to be at risk of dementia and age at diagnosis of dementia.

To avoid confounding by supplement use, we also performed the analyses excluding users of antioxidative supplements (n=639). We investigated the combined effect of antioxidant intake from food and from supplements in an analysis in which users of an antioxidative supplement were added to the highest tertile of the corresponding antioxidant intake from food. For instance, users of beta carotene supplements were added to the highest tertile of beta carotene intake from food. Users of multivitamins were added to the highest tertile of each of the 4 antioxidant intakes from food, because multivitamins can contain more than 1 antioxidant and because multivitamins generally contain higher amounts of antioxidants than antioxidant intake from food.

All rate ratios in the subsequent analyses were calculated per standard deviation increase in intake of antioxidants after adjustment for age, sex, baseline MMSE score, alcohol intake, education, smoking habits, packyears of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements. Because low intake of total fat and saturated fat is related to both high intake of antioxidants\(^2^3\) and risk of dementia,\(^2^4\) the analyses were repeated with additional adjustments for total fat intake and saturated fat intake.

To examine possible effect modification by education, we performed stratified analysis by educational level. Furthermore, because smoking increases the load of free radicals and thus the extent of oxidative stress,\(^2^5\) we also performed the analyses within strata of smoking habits. Because the APOE*4 allele is an important risk factor for Alzheimer’s disease\(^2^6\) and is related to oxidative stress,\(^2\) we also performed the analyses within strata
Dietary intake of antioxidants and risk of Alzheimer's disease

Table 1 Baseline characteristics of the study population (n=5395)*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), (y)</td>
<td>67.7 (7.8)</td>
</tr>
<tr>
<td>Women</td>
<td>3183 (59)</td>
</tr>
<tr>
<td>Baseline MMSE score†</td>
<td>28 (27-29)</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1113 (21)</td>
</tr>
<tr>
<td>&lt; 1 drink/w</td>
<td>1156 (21)</td>
</tr>
<tr>
<td>1 drink/w-1 drink/d</td>
<td>1518 (28)</td>
</tr>
<tr>
<td>1-4 drinks/d</td>
<td>1443 (27)</td>
</tr>
<tr>
<td>≥ 4 drinks/d</td>
<td>165 (3)</td>
</tr>
<tr>
<td>Educational level†‡†</td>
<td></td>
</tr>
<tr>
<td>Low education</td>
<td>1854 (35)</td>
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<tr>
<td>Intermediate education</td>
<td>1538 (28)</td>
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<tr>
<td>High education</td>
<td>2003 (37)</td>
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<tr>
<td>Smoking</td>
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<tr>
<td>Current</td>
<td>1257 (23)</td>
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<tr>
<td>Former</td>
<td>2305 (43)</td>
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<tr>
<td>Never</td>
<td>1808 (34)</td>
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<td>Packyears of smoking</td>
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<tr>
<td>Current smokers†</td>
<td>28 (14-42)</td>
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<tr>
<td>Former smokers†</td>
<td>18 (5-35)</td>
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<td>Body mass index, mean (kg/m²)</td>
<td>26.3 (3.7)</td>
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<tr>
<td>Total energy intake (kJ/d)</td>
<td>8264 (2105)</td>
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<td>Use of antioxidative supplements</td>
<td>639 (12)</td>
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<td>Total fat intake (g/d)</td>
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<td>Saturated fat intake (g/d)</td>
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<td>Number of locations of carotid plaques</td>
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<tr>
<td>0</td>
<td>1930 (43)</td>
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<tr>
<td>1-2</td>
<td>1551 (34)</td>
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<tr>
<td>3-4</td>
<td>782 (17)</td>
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<tr>
<td>5-6</td>
<td>284 (6)</td>
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<tr>
<td>Carrier of at least 1 APOE*4 allele†</td>
<td>1426 (28)</td>
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<tr>
<td>Beta carotene (mg/d)</td>
<td>1.53 (0.75)</td>
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<tr>
<td>Flavonoids (mg/d)</td>
<td>28.5 (12.2)</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>121 (54)</td>
</tr>
<tr>
<td>Vitamin E (mg/d)</td>
<td>13.8 (6.2)</td>
</tr>
</tbody>
</table>

* values represent means (standard deviation) or percentages (%)
† median (interquartile range)
‡ low education represents primary education only; intermediate education represents lower vocational or general education; high education represents intermediate or higher vocational or general education, college or university
¶ proportion is based on the actual number of individuals with data on this variable
Table 2 Adjusted rate ratios of Alzheimer's disease per standard deviation increase in dietary antioxidant intake from food

<table>
<thead>
<tr>
<th></th>
<th>Adjusted for age and gender</th>
<th>Adjusted for age, gender and baseline MMSE score</th>
<th>Adjusted for age, gender and alcohol intake</th>
<th>Fully adjusted model*</th>
<th>Supplement users excluded†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)†</td>
<td>RR (95% CI)‡</td>
<td>RR (95% CI)‡</td>
<td>RR (95% CI)‡</td>
<td>n=4756</td>
</tr>
<tr>
<td>Beta carotene</td>
<td>0.88 (0.72-1.08)</td>
<td>0.88 (0.71-1.08)</td>
<td>0.87 (0.70-1.09)</td>
<td>0.81 (0.63-1.03)</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.98 (0.83-1.16)</td>
<td>0.98 (0.82-1.15)</td>
<td>0.99 (0.83-1.18)</td>
<td>0.98 (0.81-1.19)</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.85 (0.71-1.01)</td>
<td>0.85 (0.71-1.01)</td>
<td>0.82 (0.68-0.99)</td>
<td>0.83 (0.68-1.01)</td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.90 (0.76-1.08)</td>
<td>0.90 (0.75-1.07)</td>
<td>0.82 (0.66-1.00)</td>
<td>0.84 (0.68-1.05)</td>
<td></td>
</tr>
</tbody>
</table>

* adjusted for age, gender, baseline MMSE score, alcohol intake, education, smoking habits, packyears of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements

† adjusted for age, gender, baseline MMSE score, alcohol intake, education, smoking habits, packyears of smoking, body mass index, total energy intake, and presence of carotid plaques

†† standard deviation (mg/d)

‡ rate ratio (95% confidence interval)
Dietary intake of antioxidants and risk of Alzheimer's disease

of APOE*4 allele. In this latter analysis, 226 subjects, of whom no APOE genotype was available, were excluded.

Finally, to ensure that observed associations were not the result of changing dietary habits due to subclinical dementia, we excluded all subjects with less than 2 years of follow-up (n=212). Statistical interactions were tested by adding a product term to the unstratified model. Because the antioxidative effects of vitamin C and vitamin E might be synergistic, statistical interaction was also tested between vitamin C and vitamin E intake.

All data analyses were performed using SAS statistical software version 6.12 (SAS Institute Inc, Cary, NC). We used a significance level of 0.05 based on 2 sided test.

Results

Table 1 presents the baseline characteristics of the sample. At baseline, the mean age was 67.7 years, the majority (59%) were women (n=3183), 23% (n=1257) were current smokers, 12% (n=639) used antioxidative supplements, and 28% (n=1426) carried at least 1 APOE*4 allele.

Intake of flavonoids was significantly greater with a higher baseline MMSE score; with every point increase on the MMSE score intake of flavonoids increase with 0.24 mg/d (regression coefficient 0.24 (95% confidence interval (CI) 0.031-0.45). Intake of beta carotene, vitamin C, and vitamin E were not associated with baseline MMSE score (regression coefficient 0.009 (95% CI -0.004 to 0.022) for beta carotene; -0.19 (95% CI -1.12 to 0.75) for vitamin C; and 0.059 (95% CI -0.034 to 0.15) for vitamin E).

After baseline dietary assessment, participants were followed up for an average of 6 years (32,341 person-years of follow-up). During this period, 197 participants developed dementia, of whom 146 had Alzheimer's disease (134 without and 12 with cerebrovascular disease). The incidence of Alzheimer's disease did not differ between the sample and the eligible population with missing data on dietary intake; when adjustments were made for age, sex, and education, the rate ratio for subjects with dietary data compared with subjects without dietary data was 0.75 (95% CI 0.54-1.05).

Table 2 shows the rate ratios of Alzheimer's disease associated with intake of antioxidants per SD increase. When adjustments were made for age and sex; age, sex, and baseline MMSE score; or age, sex, and alcohol intake, intake of beta carotene, flavonoids, or vitamin E was not related to risk of Alzheimer's disease. High intake of vitamin C had a borderline significant association with risk of Alzheimer's disease in all models. When additional adjustments were made for education, smoking habits, packyears of smoking, body mass index, total energy intake, presence of carotid
plagues, and use of antioxidative supplements, high intake of vitamin C was significantly related to reduced risk of Alzheimer’s disease: the rate ratio (RR) per standard deviation increase was 0.82 (95% CI 0.68-0.99). For vitamin E, the inverse relationship was of borderline significance (RR 0.82 (95% CI 0.66-1.00)). The results for beta carotene and flavonoids did not change after extensive adjustment. Exclusion of supplement users did not substantially alter the results (Table 2).

Table 3 presents rate ratios of Alzheimer’s disease across tertiles of antioxidant intake. When adjustments were made for age and sex only, antioxidant intake was not related to Alzheimer’s disease. However, in the fully adjusted model, higher intake of vitamin E was significantly associated with lower risk of Alzheimer’s disease, and higher intake of vitamin C had a borderline significant association with lower risk of Alzheimer’s disease. For vitamin C, the rate ratio of highest compared with lowest tertile was 0.66 (95% CI 0.44-1.00) and for vitamin E, it was 0.57 (95% CI 0.35-0.91). Beta carotene and flavonoids were not associated with Alzheimer’s disease across tertiles of intake. Adding supplement users to the highest tertile of dietary intake did not change the results for any of the 4 antioxidants. When we performed the analyses with additional adjustments for total fat intake or saturated fat intake, respectively, the results were similar.

**Table 3 Adjusted rate ratios of Alzheimer’s disease across tertiles of antioxidant intake**

<table>
<thead>
<tr>
<th></th>
<th>Model 1*</th>
<th>Model 2†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg/d</td>
<td>Cases</td>
</tr>
<tr>
<td>Beta carotene</td>
<td>&lt; 1.22</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>1.22 - 1.67</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>&gt; 1.67</td>
<td>35</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>&lt; 22.6</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>22.6 - 32.7</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>&gt; 32.7</td>
<td>55</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>&lt; 95</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>95 - 133</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>&gt; 133</td>
<td>41</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>&lt; 10.5</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>10.5 - 15.5</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>&gt; 15.5</td>
<td>35</td>
</tr>
</tbody>
</table>

* adjusted for age and gender  
† adjusted for age, gender, baseline MMSE score, alcohol intake, education, smoking habits, packyears of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements  
‡ rate ratio (95% confidence interval)
Table 4 Adjusted* rate ratios of Alzheimer’s disease per standard deviation increase in dietary antioxidant intake from food across strata of education

<table>
<thead>
<tr>
<th></th>
<th>Low education† n=1854 (82 cases)</th>
<th>Intermediate education† n=1538 (26 cases)</th>
<th>High education† n=2003 (38 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta carotene</td>
<td>SD (mg/d) RR (95% CI)§</td>
<td>RR (95% CI)§</td>
<td>RR (95% CI)§</td>
</tr>
<tr>
<td></td>
<td>0.75 (0.66-1.19)</td>
<td>0.98 (0.66-1.44)</td>
<td>0.70 (0.44-1.13)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>12.2 (0.85-1.36)</td>
<td>0.91 (0.58-1.36)</td>
<td>0.91 (0.65-1.28)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>54 (0.73-1.16)</td>
<td>0.50 (0.30-0.85)</td>
<td>0.80 (0.55-1.16)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>6.2 (0.61-1.09)</td>
<td>0.88 (0.52-1.42)</td>
<td>0.76 (0.51-1.14)</td>
</tr>
</tbody>
</table>

* adjusted for age, gender, baseline MMSE-score, alcohol intake, smoking habits, packyears of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements
† low education represents primary education only; intermediate education represents lower vocational or general education; high education represents intermediate or higher vocational or general education, college or university
‡ standard deviation (mg/d)
§ rate ratio (95% confidence interval)
†† statistical interaction of intake with education

Table 4 shows the relationship between antioxidant intake and risk of Alzheimer’s disease across strata of education. The risk of Alzheimer’s disease for beta carotene, flavonoids, and vitamin E did not significantly differ across strata of education and none of the statistical interaction terms was significant. High vitamin C intake was related to a lower risk of Alzheimer’s disease within intermediate level of education. However, the statistical interaction was not significant.

Table 5 presents associations of antioxidants with risk of Alzheimer’s disease across strata of smoking habits. The risk of Alzheimer’s disease associated with higher intake of vitamin C and vitamin E was lower in current smokers compared with former and never smokers, but the respective statistical interaction terms were not significant. For beta carotene, statistical interaction with smoking habits was significant and of borderline significance for flavonoids: high intake of beta carotene and flavonoids was associated with reduced risk of Alzheimer’s disease in current smokers.

Table 6 shows the relationship between intake of antioxidants and risk of Alzheimer’s disease across strata of APOE*4 allele. In participants with at least 1 APOE*4 allele, higher intake of 3 of the 4 antioxidants (except for flavonoids) was associated with somewhat lower risk of Alzheimer’s disease compared with the risk of Alzheimer’s disease in participants without an APOE*4 allele. However, statistical interactions of intake with APOE
Table 5 Adjusted* rate ratios of Alzheimer’s disease per standard deviation increase in dietary antioxidant intake from food across strata of smoking habits

<table>
<thead>
<tr>
<th></th>
<th>Never smokers</th>
<th>Former smokers</th>
<th>Current smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=1808 (64 cases)</td>
<td>n=2305 (58 cases)</td>
<td>n=1257 (22 cases)</td>
</tr>
<tr>
<td>Beta carotene</td>
<td>0.75</td>
<td>0.77 (0.53-1.11)</td>
<td>1.09 (0.87-1.38)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>12.2</td>
<td>1.04 (0.80-1.36)</td>
<td>1.07 (0.81-1.42)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>54.1</td>
<td>0.83 (0.62-1.10)</td>
<td>0.91 (0.69-1.19)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>6.2</td>
<td>0.98 (0.71-1.35)</td>
<td>0.77 (0.56-1.06)</td>
</tr>
</tbody>
</table>

* adjusted for age, sex, baseline MMSE score, alcohol intake, education, smoking habits, pack-years of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements
† standard deviation (mg/d)
‡ rate ratio (95% confidence interval)
§ statistical interaction of intake with smoking habits

We observed no statistical interaction between vitamin C intake and vitamin E intake with Alzheimer’s disease. Finally, restriction of the analyses to participants with at least 2 years of follow-up did not substantially change the results: rate ratios per standard deviation increase in intake were 0.80 (95% CI 0.61-1.06) for beta carotene, 0.95 (95% CI 0.77-1.18) for flavonoids, 0.82 (95% CI 0.66-1.03) for vitamin C, and 0.85 (95% CI 0.66-1.08) for vitamin E.

Discussion

We found that high intake of vitamin C and vitamin E from food may be associated with a lower incidence of Alzheimer’s disease after a mean follow-up period of 6 years. The risk reduction associated with intake of all 4 antioxidants was consistently largest for current smokers, although the differences in rate ratios for beta carotene and flavonoids between smokers and nonsmokers were of marginal statistical significance, while those for vitamin C and vitamin E were not significant. Nonetheless, these associations persisted after controlling for a number of potentially confounding variables, such as use of vitamin supplements, education, and alcohol use.

Before interpreting the results, some methodological issues should be considered. First, although we adjusted for a large number of potential confounding factors such as age, sex, alcohol intake, education, smoking
habits, and use of supplements, the possibility of residual confounding can never be completely excluded from an observational study. Second, we cannot completely exclude the possibility of subclinical dementia at time of dietary assessment, which may have led to changes in dietary reporting or dietary habits. To minimize this potential source of confounding, we excluded cognitively impaired subjects and adjusted for baseline MMSE score. In addition, we also recomputed the results after excluding the first 2 years of follow-up, which did not alter the results. Thus, we do not think that our results were affected by the presence by subclinical dementia. Third, because dietary assessment was performed only once, it may not have precisely reflected the participants' long-term dietary habits, which are more likely to influence disease risk. However, this may have led to dilution and thus an underestimation of the associations of antioxidants with risk of Alzheimer's disease. Finally, we cannot completely rule out the possibility of confounding by use of dietary supplements. Although only a small number of participants reported supplement use, we do not have data on duration of use and dosage of the antioxidative supplements. Nonetheless, our results were unchanged after either excluding supplement users from the analysis or after controlling for supplement use, suggesting that our results are not confounded by supplement use.

The strengths of our study are its prospective design and the population-based setting. Another important feature is that follow-up with respect to dementia diagnosis was virtually complete, and thus there was no resulting selection bias.

Table 6 Adjusted* rate ratios of Alzheimer's disease per standard deviation increase in dietary antioxidant intake from food according to APOE genotype

<table>
<thead>
<tr>
<th></th>
<th>No APOE*4 allele</th>
<th>At least one APOE*4 allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=3743 (75 cases)</td>
<td>n=1426 (68 cases)</td>
</tr>
<tr>
<td><strong>Beta carotene</strong></td>
<td>0.75</td>
<td>0.95 (0.72-1.25)</td>
</tr>
<tr>
<td><strong>Flavonoids</strong></td>
<td>12.2</td>
<td>0.95 (0.73-1.23)</td>
</tr>
<tr>
<td><strong>Vitamin C</strong></td>
<td>54.1</td>
<td>0.89 (0.69-1.15)</td>
</tr>
<tr>
<td><strong>Vitamin E</strong></td>
<td>6.2</td>
<td>0.82 (0.61-1.09)</td>
</tr>
</tbody>
</table>

* adjusted for age, sex, baseline MMSE score, alcohol intake, education, smoking habits, packyears of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements
† standard deviation (mg/d)
‡ rate ratio (95% confidence interval)
§ statistical interaction of intake with APOE genotype
Several studies have examined the relationship between Alzheimer's disease and intake of vitamin C and vitamin E from supplements.\textsuperscript{4,6,28,29} A case-control study of Broe et al\textsuperscript{28} and a prospective study in men\textsuperscript{6} showed no association between supplement intake and Alzheimer's disease. Another prospective study found that use of supplements, in particular of vitamin C but not vitamin E, was associated with a lower risk of Alzheimer's disease.\textsuperscript{5} The only controlled trial of supplemented antioxidant intake and Alzheimer's disease was performed within patients who were already diagnosed with Alzheimer's disease.\textsuperscript{4} This study reported that patients who took vitamin E supplements had a slower progression of the disease than patients who took placebo.

Results on supplement use and risk of dementia, however, may not be comparable with results on intake from food for several reasons. First, supplement users are generally a selected group of persons with either health problems\textsuperscript{7} or more health-seeking behaviors.\textsuperscript{8} Therefore, associations between supplement use and Alzheimer's disease may be biased. Second, intake of antioxidants from food reflects long-term intake, whereas supplement intake is generally of shorter duration. If duration of antioxidant intake is more important than the dose, high-lifetime intake from food would more likely be related to Alzheimer's disease than short-term high intake by supplements. Finally, antioxidants from food are always simultaneously consumed with other nutrients in a certain proportion, whereas antioxidants from supplements are consumed in a very high dose either with or without other substances. This might lead to differences in absorption or biological activity between antioxidants from food and antioxidants from supplements, though little is yet known on these issues.\textsuperscript{30}

Previously, the relationship between intake of flavonoids from food and risk of dementia has been studied.\textsuperscript{9} This prospective study found that high flavonoid intake was significantly associated with a lower risk of dementia. However, the response rate for dietary assessment was relatively low, only a small part of the study population underwent a detailed dietary assessment, and confounding or effect modification by smoking was not examined.

In our study, risk of Alzheimer's disease associated with vitamin C and vitamin E was lowest in current smokers and beta carotene, and flavonoids seemed inversely related to Alzheimer's disease in current smokers only. Because smoking itself is associated with increased risk of Alzheimer's disease,\textsuperscript{31} high antioxidant intake may partly counteract the excess risk of Alzheimer's disease for smokers. This is supported by the finding of smokers' increased load of free radicals,\textsuperscript{25} which may be reduced by antioxidants.
Several biological mechanisms could explain a possible relationship between antioxidants from food and Alzheimer’s disease. First, antioxidants may decrease the level of oxidative stress in the brain. Antioxidants may thereby reduce the amount of DNA damage, neuronal cell death, and the aggregation of beta-amyloid within the brain. These phenomena are all important neuropathological features in Alzheimer’s disease; by preventing the genesis of these features, the risk of dementia might be reduced. Second, because Alzheimer’s disease is both associated with cardiovascular risk factors and atherosclerosis and oxidative processes are involved in atherogenesis, high intake of antioxidants could also decrease the risk of dementia by reducing the risk of atherosclerosis. However, because additional adjustment for carotid plaques as a measure of atherosclerosis did not change our results, we doubt that atherosclerosis is an important intermediary in the relationship between antioxidants and risk of Alzheimer’s disease.

In conclusion, our results suggest that higher intake of vitamin C and vitamin E from food may be associated with a lower risk of Alzheimer’s disease. Whether this reflects a causal association remains to be elucidated. Randomized controlled trials can help evaluate a possible causal relationship between antioxidant intake from supplements and risk of Alzheimer’s disease. However, the effect of short-term supplement use in clinical trials may not be comparable with long-term intake from dietary sources. Therefore, more cohort studies are needed to further investigate the relationship between dietary antioxidant intake and risk of Alzheimer’s disease.

References


Chapter 3.2.1

Fatty acid composition in plasma phospholipids and cognitive function

Background N-3 polyunsaturated fatty acids (n-3 PUFA) have anti-inflammatory properties. Other fatty acids, such as saturated fatty acids, are associated with increased atherosclerosis. Because inflammation and atherosclerosis may be involved in dementia pathogenesis, fatty acids may also be related to dementia as well as to cognitive decline.

Objective To examine whether fatty acids in plasma phospholipids were associated with cognitive decline in dementia-free subjects.

Methods The study was performed within the Rotterdam Study, a population-based study among elderly. Baseline, first follow-up and second follow-up examinations took place in 1990-1993, 1993-1994 and 1997-1999, respectively, and included the Mini Mental State Examination (MMSE). The eligible population for the present study consisted of all 5487 subjects who were alive and dementia-free at second follow-up. For each subject, cognitive change between baseline and second follow-up was calculated on the basis of the subsequent MMSE-scores. Fatty acids were assessed at second follow-up in plasma phospholipids of 731 subjects and were expressed as percentage of total fatty acids. The association between fatty acids and cognitive change was examined by linear regression.

Results After adjustments for age, gender and education, higher levels of saturated fatty acids, lower levels of PUFA and n-6 PUFA and a lower ratio of PUFA to saturated fatty acids were significantly associated with more cognitive decline. No association was found between trans fatty acids, monounsaturated fatty acids, n-3 PUFA, 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3, 22:6n-3 and the ratio of n-6 PUFA to n-3 PUFA and cognitive change.

Conclusion Our findings suggest that saturated fatty acids are positively and unsaturated fatty acids are inversely related to cognitive decline.

Several observations suggest that fatty acids may play a role in dementia and cognitive function. First, some fatty acids, such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are known to inhibit inflammatory responses, which have been found present in Alzheimer's disease. Second, high levels of saturated
fatty acids and cis monounsaturated fatty acids (MUFA) and low levels of cis polyunsaturated fatty acids (PUFA) are related to increased atherosclerosis, which may be associated with dementia and cognitive decline. Finally, the fatty acid composition of brain cell membranes differs between Alzheimer patients and control subjects. For instance, cell membranes within the (para)hippocampal region of Alzheimer brains contained less n-6 PUFA and less n-3 PUFA compared to membranes in the same region of control brains.

Previously, 2 cross-sectional studies examined the relation between fatty acids in plasma phospholipids and dementia and showed that levels of saturated fatty acids were higher and levels of EPA and DHA were lower within Alzheimer patients as compared to control subjects. Prospectively, DHA and EPA were studied in relation to Alzheimer’s disease showing lower risk with higher levels of DHA only. Whether fatty acids are related to cognitive function within dementia-free subjects is not yet known. We examined the relation between various fatty acids in plasma phospholipids and change in cognitive function within dementia-free subjects over a mean period of 6.5 years.

Methods

Eligible population
The present study was performed within the Rotterdam Study, a large population-based prospective cohort study designed to investigate determinants of chronic disabling diseases in the elderly. Participants of the Rotterdam Study were 7983 inhabitants (response rate 78%) of a suburb of Rotterdam, the Netherlands, aged 55 years and over. The medical ethics committee of the Erasmus Medical Center Rotterdam approved the study and written informed consent was obtained from all participants.

At present, 3 examination rounds have taken place, which all included a home interview and clinical examinations at the research center. The baseline examinations were performed in 1990-1993, first follow-up examinations took place in 1993-1994 and second follow-up examinations were in 1997-1999.

Cognitive examinations within the Rotterdam Study included the Mini Mental State Examination (MMSE) and the Geriatric Mental State schedule (GMS) organic level at both baseline and follow-up. In case of low scores on these tests (MMSE-score < 26 or GMS organic level > 0), additional cognitive testing was performed. If suspected of dementia, subjects were subsequently examined by a neurologist, a neuropsychologist and underwent magnetic resonance brain imaging. In addition, subjects were continously monitored for incident dementia. Diagnosis of dementia was based on DSM-III-R criteria.
The eligible population for the present study comprised 5463 persons, who were alive and still free of dementia at time of second follow-up examinations.

Assessment of change in cognitive function
In the eligible population, change of cognitive function over time was determined on the basis of subsequent MMSE-scores at baseline, first follow-up and second follow-up. We used a random effects model (SAS statistical software, SAS Institute Inc., Cary, NC, USA) to calculate yearly change in MMSE-score for each participant. Subsequently, yearly MMSE-change was multiplied by 6.5 in order to obtain change in cognitive function over the mean follow-up period of 6.5 years (SD 0.3).

Study population
The study population of the present study was selected from the eligible population (n=5463). For efficiency reasons, we oversampled subjects with severe decline in cognitive function. We therefore selected all 439 subjects who had a decline in MMSE-score that fell within the lowest 8% of the distribution of MMSE-change. This corresponded to a decline in MMSE-score of 2.4 or more over 6.5 years (MMSE-change <= -2.4). In addition, we randomly selected 650 subjects from the remaining part of the eligible population (n=5024). Two-third of both selected groups (293 and 438, respectively) had blood samples drawn at second follow-up; these subjects were included in the study. Thus, the study population consisted of 731 subjects who were dementia-free and underwent venipuncture at second follow-up.

Assessments of fatty acids in plasma phospholipids
At the second follow-up examination blood samples were drawn from subjects who had fasted overnight. Obtained citrate plasma was immediately frozen in liquid nitrogen, transferred to the laboratory and stored at -80°C.

Measurements of fatty acids in plasma phospholipids were performed blinded for cognitive status. The assessments of fatty acids followed a 5-stage protocol. First, lipids were extracted from plasma according to the methods used by Bligh and Dyer. Second, phospholipids were separated from other lipids by means of solid phase extraction. Third, phospholipids were methylated via reaction with 14% boron trifluoride in methanol at 100 °C for 1 hour. Fourth, butylated hydroxytoluene was added as antioxidant to all organic solvents. Finally, the fatty acid composition of the phospholipids was analysed using a gas chromatograph (Shimadzu® GC-17A) with a FID detector and a CP-Sil 88 column (50 m x 0.25 mm id. 0.22 μm film thickness,
Varian®). Fatty acids in the phospholipid fraction were quantified using 19:0 fatty acid methyl ester as an internal standard.

For the present study we classified fatty acids into various subgroups: saturated fatty acids, trans unsaturated fatty acids (trans fatty acids), cis monounsaturated fatty acids (MUFA), cis polyunsaturated fatty acids (PUFA), n-6 PUFA and n-3 PUFA. Furthermore, we examined the following separate fatty acids: the essential fatty acids linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3), the pro-inflammatory arachidonic acid (AA, 20:4n-6), and the anti-inflammatory n-3 PUFA eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). All fatty acids were expressed as percentages of total fatty acids in plasma phospholipids. In addition, we calculated ratios of PUFA to saturated fatty acids (P/S ratio) and n-6 PUFA to n-3 PUFA (n-6/n-3 ratio).

**Covariates**

Level of education and apolipoprotein E (APOE) genotype were assessed at baseline. Data on myocardial infarction was obtained at baseline and during follow-up. Depressive symptoms, functional status, medication use, fasting level of glucose and vitamin E, and presence of carotid plaques were assessed at the second follow-up examinations.

Level of education was categorized in 4 groups: completed primary education, lower vocational training or general education, intermediate vocational training or intermediate and higher general education, and higher vocational training, college or university. APOE genotype was assessed on coded DNA samples using polymerase chain reaction. We dichotomized APOE genotype into presence or absence of the apolipoprotein E*4 (APOE*4) allele. Incident myocardial infarction was reported by the general practitioners by means of a computerized system. Myocardial infarctions reported by the participant or the general practitioner were verified and defined as code I21 according to the International Classification of Diseases, 10th edition. Depressive symptoms were assessed in the interview using the Dutch version of the original Center for Epidemiology Studies Depression scale (CES-D). Functional status was also determined in the interview by means of the Disability Index of the Stanford Health Assessment Questionnaire. Data on medication use were obtained from the interview and a pharmacy database that was linked to the study database. Subjects who used lipid-lowering medication (Anatomical-Therapeutical-Chemical-code (ATC-code) C10) were classified accordingly. Diabetes was considered present if the subject was taking oral diabetics or insulin (ATC-code A10) or if the fasting serum glucose level was equal to or higher than 7.8 mmol/l (WHO-criteria for epidemiological studies of diabetes). Level of vitamin E (α-tocopherol) was determined in plasma by
means of reversed-phase HPLC with UV detection. We used ultrasonography of the carotid arteries to assess presence of carotid plaques at 6 different locations: the common carotid artery, carotid bifurcation and internal carotid artery at both left and right side. Subsequently, 4 categories were made according to the number of locations with plaques: plaques at 0, 1-2, 3-4 and 5-6 locations.

**Data analysis**

The association between fatty acids, fatty acid ratios and cognitive change over 6.5 years (mean follow-up of the study population) was firstly assessed by means of linear regression analyses. In these analyses, fatty acid or fatty acid ratio was entered as independent variable and change in MMSE-score as dependent variable. Regression coefficients were expressed per standard deviation increase in fatty acid or fatty acid ratio. In addition, we performed analyses of covariance in which mean change in MMSE-score was calculated across tertiles of fatty acid or fatty acid ratio. Tertiles and standard deviations were based on the distribution of the complete study population (n=731). In all analyses adjustments were made for age, gender and education.

Because comorbidity may be associated both with fatty acid level through diet and with cognitive function, we performed additional analyses in which we simultaneously adjusted for myocardial infarction, depressive symptoms and functional status. In addition, because diabetes changes fat metabolism and is associated with increased risk of dementia, we controlled for presence of diabetes in a separate analysis. Furthermore, in order to check whether atherosclerosis was an intermediate or confounder of the relation between fatty acids and cognitive change we adjusted for presence of carotid plaques. In this analysis a missing indicator was used for the 44 missing values on carotid plaques.

Because the APOE*4 genotype both is an important risk factor for Alzheimer's disease and is involved in fat metabolism, we repeated the analyses within strata of APOE*4 genotype. In this latter analysis, 36 subjects, of whom no APOE genotype was available, were excluded. Statistical interactions were tested by adding a product term to the unstratified model. Furthermore, because vitamin E may reduce detrimental oxidation of unsaturated fatty acids, we tested statistical interaction between unsaturated fatty acids and vitamin E in relation to MMSE-change. Finally, because the relation between fatty acid levels and cognitive change may be affected by use of lipid-lowering medication, we performed a separate analysis in which we excluded users (n=92, 12.6%).

All data analyses were performed using SPSS statistical software, version 10 (SPSS Inc., Chicago, Illinois, USA).
Results

Table 1 shows the characteristics of the study population. The mean age was 73.3 years, 61% of the subjects was female and 29% was carrier of at least 1 APOE*4 allele. The median change in MMSE-score over 6.5 years was -1.3 points. Figure 1 presents the median fatty acid levels in plasma phospholipids in a flow chart. The majority of fatty acids from phospholipids were unsaturated fatty acids (53.9%), to which cis fatty acids contributed most. The main contributor to cis fatty acids is n-6 PUFA (35.6%). Relative amounts of LA and ALA in phospholipids were 21.8% and 0.2%, respectively. The long-chain n-3 PUFA EPA and DHA contributed for 0.8% and 3.5%, respectively, to fatty acids in plasma phospholipids.

Table 2 presents the association of fatty acid composition in plasma phospholipids with change in MMSE-score over a period of 6.5 years. With

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)†</td>
<td>73.3 (7.5)</td>
</tr>
<tr>
<td>Women (%)</td>
<td>61.0</td>
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<tr>
<td>Low education (%)</td>
<td>37.9</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>15.0</td>
</tr>
<tr>
<td>Carrier of APOE*4 allele (%)</td>
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</tr>
<tr>
<td>5-6 Locations with carotid plaques (%)</td>
<td>6.7</td>
</tr>
<tr>
<td>Saturated fatty acids (% of total)‡</td>
<td>46.0 (45.2; 46.8)</td>
</tr>
<tr>
<td>Trans fatty acids (% of total)</td>
<td>0.9 (0.8; 1.1)</td>
</tr>
<tr>
<td>MUFA (% of total)</td>
<td>11.6 (10.5; 12.8)</td>
</tr>
<tr>
<td>PUFA (% of total)</td>
<td>41.3 (40.0; 42.7)</td>
</tr>
<tr>
<td>N-6 PUFA (% of total)</td>
<td>35.4 (33.7; 37.3)</td>
</tr>
<tr>
<td>N-3 PUFA (% of total)</td>
<td>5.46 (4.68; 6.53)</td>
</tr>
<tr>
<td>LA (18:2n6) (% of total)</td>
<td>21.8 (20.1; 23.6)</td>
</tr>
<tr>
<td>ALA (18:3n3) (% of total)</td>
<td>0.2 (0.1; 0.2)</td>
</tr>
<tr>
<td>AA (20:4n6) (% of total)</td>
<td>8.9 (7.7; 10.2)</td>
</tr>
<tr>
<td>EPA (20:5n3) (% of total)</td>
<td>0.8 (0.6; 1.0)</td>
</tr>
<tr>
<td>DHA (22:6n3) (% of total)</td>
<td>3.5 (2.8; 4.3)</td>
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<tr>
<td>P/S ratio</td>
<td>0.9 (0.86; 0.94)</td>
</tr>
<tr>
<td>N-6/n-3 ratio</td>
<td>6.5 (5.3; 7.9)</td>
</tr>
<tr>
<td>Change in MMSE-score in 6.5 yrs</td>
<td></td>
</tr>
</tbody>
</table>

*values represent medians (interquartile ranges) or percentages (%)
†mean (standard deviation)
‡percentage of total fatty acids in plasma phospholipids
§6.5 years corresponds to mean follow-up in the study population
every 1.4% (standard deviation) increase in saturated fatty acids, the MMSE-score significantly declined with 0.14 points (regression coefficient (RC) -0.14 (95% confidence interval (95% CI) -0.27; -0.010)). In contrast, higher percentages of PUFA and n-6 PUFA and higher P/S ratio were significantly associated with an increase in MMSE-score (RC was 0.16 (95% CI 0.030; 0.29)) for PUFA, 0.13 (95% CI 0.006; 0.26) for n-6 PUFA and 2.69 (95% CI 0.71; 4.67) for P/S ratio). Trans fatty acids, MUFA, n-3 PUFA, LA, ALA, AA, EPA, DHA and n-6/n-3 ratio were not associated with change in MMSE-score.

**Figure 1** Median percentages of various fatty acids in plasma phospholipids of the study population

\[\text{MUFA} = \text{cis monounsaturated fatty acids}\]
\[\text{PUFA} = \text{cis polyunsaturated fatty acids}\]
When we calculated mean changes in MMSE-score within tertiles of fatty acids and fatty acid ratios, we did not find any indication for non-linear associations. Figure 2 shows the mean change in MMSE-score according to tertiles of saturated fatty acids, PUFA, n-6 PUFA and P/S ratio. Increasing percentage of saturated fatty acids in plasma phospholipids across tertiles was associated with more decline in cognitive function: the mean change in MMSE-score for the highest tertile was -1.8 (95% CI -2.0; -1.6) versus -1.5 (95% CI -1.7; -1.3) for the lowest tertile. Higher PUFA, n-6 PUFA or P/S ratio were associated with less decline in cognitive function. Mean MMSE-changes in the highest versus the lowest tertile were for PUFA -1.6 (95% CI -1.8; -1.4) and -1.9 (95% CI -2.1; -1.7), for n-6 PUFA -1.6 (95% CI -1.8; -1.4) and -1.8 (95% CI -2.0; -1.6), and for the P/S ratio -1.5 (95% CI -1.7; -1.3) and -1.9 (95% CI -2.1; -1.7).

When additional adjustments were simultaneously made for myocardial infarction, depressive symptoms and functional status, the results were very similar. Also, additional adjustment for diabetes and presence of carotid plaques, respectively, did not change the results. Furthermore, the associations between fatty acids and fatty acid ratios and cognitive change were similar across strata of APOE genotype and none of the interactions

Table 2 Adjusted* regression coefficients describing the change in MMSE-score per standard deviation increase in percentage of total fatty acids or per 1 unit increase in fatty acid ratio

<table>
<thead>
<tr>
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<th>SD (%)†</th>
<th>Regression coefficient (95% CI)‡</th>
<th>p-value</th>
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<tr>
<td>Saturated fatty acids</td>
<td>1.4</td>
<td>-0.14 (-0.27; -0.010)</td>
<td>0.03</td>
</tr>
<tr>
<td>Trans fatty acids</td>
<td>0.3</td>
<td>0.032 (-0.098; 0.16)</td>
<td>0.63</td>
</tr>
<tr>
<td>MUFA</td>
<td>1.9</td>
<td>-0.081 (-0.21; 0.048)</td>
<td>0.22</td>
</tr>
<tr>
<td>PUFA</td>
<td>2.2</td>
<td>0.16 (0.030; 0.29)</td>
<td>0.02</td>
</tr>
<tr>
<td>N-6 PUFA</td>
<td>2.9</td>
<td>0.13 (0.006; 0.26)</td>
<td>0.04</td>
</tr>
<tr>
<td>N-3 PUFA</td>
<td>1.6</td>
<td>-0.027 (-0.16; 0.10)</td>
<td>0.68</td>
</tr>
<tr>
<td>LA</td>
<td>2.9</td>
<td>0.045 (-0.084; 0.17)</td>
<td>0.50</td>
</tr>
<tr>
<td>ALA</td>
<td>0.07</td>
<td>0.055 (-0.074; 0.19)</td>
<td>0.40</td>
</tr>
<tr>
<td>AA</td>
<td>2.0</td>
<td>0.096 (-0.032; 0.22)</td>
<td>0.14</td>
</tr>
<tr>
<td>EPA</td>
<td>0.6</td>
<td>-0.075 (-0.20; 0.053)</td>
<td>0.25</td>
</tr>
<tr>
<td>DHA</td>
<td>1.1</td>
<td>-0.020 (-0.15; 0.11)</td>
<td>0.76</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>2.69</td>
<td>(0.71; 4.67)</td>
<td>0.01</td>
</tr>
<tr>
<td>N-6/n-3 ratio</td>
<td>0.0078</td>
<td>(-0.053; 0.069)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*adjusted for age, gender and education
† standard deviation of the percentage of total fatty acids
‡ 95% confidence interval
Figure 2 Adjusted* mean change in MMSE-score across tertiles of saturated fatty acids, PUFA, n-6 PUFA and P/S ratio in plasma phospholipids

* adjusted for age, gender and education
between fatty acids and APOE genotype were statistically significant (p-values all above 0.10). In addition, we observed no statistical interaction between trans fatty acids, MUFA, PUFA, n-6 PUFA, n-3 PUFA, LA, ALA, AA, EPA or DHA and level of vitamin E in relation to cognitive change (p-values were all above 0.10). Finally, associations were very similar after exclusions of users of lipid-lowering medication.

**Discussion**

In this study we observed that high levels of saturated fatty acids in plasma phospholipids, low levels of PUFA and n-6 PUFA and a low ratio of PUFA to saturated fatty acids were associated with more cognitive decline. Trans fatty acids, MUFA, n-3 PUFA, LA, ALA, AA, EPA and DHA, and the ratio of n-6 PUFA to n-3 PUFA were not related to change in cognitive function. The associations were not confounded or modified by comorbidity and APOE genotype, respectively.

Before interpreting the results, some methodological issues need to be discussed. First, the design of the study was cross-sectional. We therefore cannot exclude that fatty acid levels are the consequence rather than the cause of change in cognitive function. Second, no data were available on dietary intake of fatty acids at time of the assessment of phospholipid levels. As a result, we could not determine whether observed associations were the consequence of differences in dietary intake or differences in fatty acid metabolism.

This study has several advantages. First, change in cognitive function was defined on the basis of several measurements over time. This will have reduced the disturbing effect of within-person variability and therefore improved the ranking of persons in terms of cognitive decline, even though using the MMSE to test cognitive function is rather crude. Other advantages of the study are its size and its population-based setting.

Previously, fatty acids in phospholipids have been studied in relation to cognitive impairment showing that levels of EPA, DHA and total n-3 PUFA were lower and the n-6/n-3 ratio was higher in cognitively impaired subjects compared to control subjects. Levels of saturated fatty acids, PUFA and n-6 PUFA were similar in cognitively impaired subjects compared to control subjects.

Three studies examined the relation between fatty acids in plasma phospholipids and Alzheimer's disease. Two of these were cross-sectional and observed that levels of EPA and DHA were lower in Alzheimer patients compared to control subjects. Also, levels of the main saturated fatty acids 16:0 and 18:0 were higher in Alzheimer patients compared to control subjects in one study, whereas the other study showed higher levels of n-6 PUFA in Alzheimer patients. A longitudinal study found that lower levels
of DHA, but not EPA, were associated with increased risk of Alzheimer's disease. A neuropathological study showed more saturated fatty acids in phospholipids within the hippocampus and frontal grey matter of Alzheimer patients when compared to control subjects. It has also been found that the (para)hippocampal region of Alzheimer brains contained less n-6 PUFA and less n-3 PUFA compared to membranes in the same region of control brains. However, results of previous studies on the relation between fatty acids and cognitive impairment or Alzheimer's disease are difficult to compare with our findings, because most of them did not adjust for important confounders, such as age, gender and education.

The relation between plasma phospholipid levels of saturated fatty acids, PUFA, n-6 PUFA, P/S ratio and cognitive decline may be etiological through several mechanisms. First, atherosclerosis may explain the observed relation, because increased atherosclerosis is associated both with increased prevalence of dementia and cognitive decline, and with high phospholipid levels of saturated fatty acids, low levels of PUFA and a low P/S ratio. However, additional adjustment for carotid plaques as a measure of atherosclerosis did not change our results. Also, MUFA was not associated with cognitive decline, whereas increased MUFA is related to increased atherosclerosis. Therefore, we think that atherosclerosis does not fully explain our findings. Second, peripheral levels of fatty acids may determine the fatty acid composition of brain cell membranes that is involved in optimal cell functioning. This is suggested by findings in both animals and humans. In animals, fatty acid composition within the brain can be altered by changing dietary intake, probably through changing blood levels of fatty acids. In human infants, higher DHA levels in erythrocytes were associated with a higher amount of DHA within the cortex. Thus, plasma levels of fatty acids may reflect fatty acid composition within the brain. Because fatty acid composition within the brain can affect functioning of neuronal cells, it might also alter cognitive function.

In conclusion, we observed that high phospholipid levels of saturated fatty acids, low levels of PUFA and n-6 PUFA and a low ratio of PUFA to saturated fatty acids were associated with increased cognitive decline. Whether fatty acid composition in plasma changes prior to onset of cognitive decline remains to be elucidated.

References
3. Ma J, Folsom AR, Lewis L, Eckfeldt JH. Relation of plasma phospholipid and cholesterol ester fatty acid composition to carotid artery intima-media
Chapter 3.2.2

Diet and risk of dementia: does fat matter?

Objective To examine whether high intake of total fat, saturated fatty acids (saturated fat), trans fatty acids (trans fat) and cholesterol and low intake of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-6 PUFA and n-3 PUFA are associated with increased risk of dementia and its subtypes.

Methods We used data from the Rotterdam Study, a prospective cohort study among elderly. At baseline (1990-1993), 5395 subjects had normal cognition, were non-institutionalized and underwent complete dietary assessment by a semiquantitative food-frequency questionnaire. We continuously monitored the cohort for incident dementia and performed re-examinations in 1993-1994 and 1997-1999. We examined the association between fat intake and incident dementia by Cox proportional hazards models.

Results After a mean follow-up of 6.0 years 197 subjects developed dementia (146 Alzheimer’s disease, 29 vascular dementia). High intake of total, saturated, trans fat and cholesterol and low intake of MUFA, PUFA, n-6 PUFA and n-3 PUFA were not associated with increased risk of dementia or its subtypes. Rate ratios of dementia per SD increase in intake were for total fat 0.93 (95% CI 0.81-1.07), for saturated fat 0.91 (95% CI 0.79-1.05), for trans fat 0.90 (95% CI 0.77-1.06), for cholesterol 0.93 (95% CI 0.80-1.08), for MUFA 0.96 (95% CI 0.84-1.10), for PUFA 1.05 (95% CI 0.80-1.38), for n-6 PUFA 1.03 (95% CI 0.77-1.36) and for n-3 PUFA 1.07 (95% CI 0.94-1.22).

Conclusions High intake of total, saturated and trans fat and cholesterol and low intake of MUFA, PUFA, n-6 PUFA and n-3 PUFA were not associated with increased risk of dementia or its subtypes.

Several findings in various research areas suggest that fat may be involved in the etiology of dementia. First, animal studies show that cerebral beta-amyloid deposition, assumed to be crucial in the pathogenesis of Alzheimer’s disease,1 is increased by higher dietary intake of cholesterol.2 Second, pharmaco-epidemiological studies in humans observed that use of medication that lowers serum cholesterol, such as statins, may be related to lower prevalence of Alzheimer’s disease.3·5 Third, other epidemiological studies found that higher serum levels of cholesterol are
associated with increased risk of dementia.\textsuperscript{6, 7} Finally, biochemical studies showed that specific fatty acids, such as cis n-3 polyunsaturated fatty acids (n-3 PUFA), have anti-inflammatory properties.\textsuperscript{8} Because inflammation may be associated with increased risk of dementia,\textsuperscript{9} high levels of n-3 PUFA may reduce dementia risk.

Based on these observations, we hypothesize that cholesterol increases and n-3 PUFA reduce dementia risk. Cholesterol levels are increased by a diet high in total fat, saturated fatty acids (saturated fat), trans unsaturated fatty acids (trans fat) and cholesterol and low in cis unsaturated fatty acids.\textsuperscript{10, 11} Likewise, n-3 PUFA levels are increased with increasing dietary intake of n-3 PUFA. We examined whether high dietary intake of total fat, saturated fat, trans fat and cholesterol and low intake of cis monounsaturated fatty acids (MUFA), PUFA, n-6 PUFA and n-3 PUFA were associated with an increased risk of dementia and its subtypes. We performed the study within the Rotterdam Study, a population-based cohort study with a mean follow-up of 6.0 years.

Methods

The Rotterdam Study

The Rotterdam Study is a large population-based prospective cohort study that examines risk factors for neurological, cardiovascular, locomotor and ophthalmologic diseases in the elderly population.\textsuperscript{12} The conduct of the study was approved by the medical ethics committee of the Erasmus University Rotterdam. All 10,275 inhabitants from Ommoord, a suburb of Rotterdam, the Netherlands, who were 55 years and over, were invited to participate in the study. Of these, 7983 subjects (response rate 78\%) gave their written informed consent and participated in the study.\textsuperscript{12}

The baseline examination (1990-1993) consisted of an interview at home and 2 visits at the research center. The interview was performed by a trained research assistant who obtained information on lifestyle, current and past health, medication and determinants for chronic diseases. Clinical examinations were carried out at the research center. Follow-up examinations took place in 1993-1994 and 1997-1999. In addition, the cohort was continuously monitored for major disease outcomes.

Diagnosis of dementia

Subjects were screened for dementia in a 3-step procedure that was similar at baseline and follow-up examinations.\textsuperscript{13} In brief, participants were cognitively screened with the Mini Mental State Examination (MMSE)\textsuperscript{14} and the Geriatric Mental State schedule (GMS), organic level.\textsuperscript{15} Subjects with a MMSE score below 26 or a GMS score above 0 underwent further neuropsychological testing by the Cambridge Examination of Mental
Disorders in the Elderly (CAMDEX). The CAMDEX also included an informant interview. Persons who were suspected of having dementia were examined by a neurologist and a neuropsychologist and, if possible, had a brain MRI. In addition, the cohort was continuously monitored by computerized linkage of the study database with general practitioners' medical records and the database of the Regional Institute for Outpatient Mental Health Care (RIAGG). Dementia diagnoses were based on DSM-III-R criteria, diagnoses of Alzheimer's disease on NINCDS-ADRDA, and diagnoses of vascular dementia on NINDS-AIREN criteria. All diagnoses were made on the basis of all existing information by a panel that consisted of the neurologist, the neuropsychologist and the research physician.

Dietary assessment
Dietary assessment was performed in 2 stages at baseline. First, participants completed a checklist at home, on which they indicated all foods and drinks they had consumed at least twice a month during the preceding year. The checklist also contained questions on use of supplements. Subsequently, the participants were interviewed by a trained dietician at the research center. An extensive, validated semiquantitative food-frequency questionnaire was used to quantify the amounts and frequencies of food and drink intake that had been indicated on the checklist. The data were converted to energy intake and nutrient intake using the computerized Dutch Food Composition Table. Intake of specific fatty acids was based on a food composition database derived from the TRANSFAIR study. For this database, the 100 food items that contribute most to fat intake in the Dutch dietary pattern were sampled and analyzed as methyl esters of the fatty acids present in the food. For the present study we used data on intake of total fat, saturated fat, trans fat, cholesterol, MUFA, PUFA, n-6 PUFA and n-3 PUFA. Total fat intake comprises all intake of fatty acids and cholesterol. Important sources of saturated fat are meat, dairy and cookies. Trans fat is partially hydrogenated fat and can be found in margarines and salad and cooking oils. Cholesterol is present in animal products only. Large amounts of MUFA are found in olive oil. Most PUFA intake is derived from intake of n-6 PUFA and n-3 PUFA. N-6 PUFA is particularly found in vegetable oil, and n-3 PUFA is present in fatty fish, such as salmon, tuna and mackerel. All fat intakes were calculated in grams per day. The amount of energy from fat intake was expressed as a percentage of total energy intake.

Other variables
The baseline home interview contained questions on highest attained level of education, smoking habits and prevalent diseases, such as myocardial
infarction. Data on medication use at time of the baseline examination and second follow-up examination were obtained from both the interview and a pharmacy database that was linked to the study database from December 31, 1990 onwards. At the baseline visit to the research center we assessed intake of total energy, vitamin E, alcohol, fruit and vegetables as well as use of dietary supplements on the basis of the food-frequency questionnaire. Also, height and weight were measured and blood samples were drawn.

Level of education was categorized in 4 groups: completed primary education, lower vocational training or general education, intermediate vocational training or intermediate and higher general education, and higher vocational training, college or university. Smoking habits were classified as current, former and never smoking. A history of myocardial infarction was considered present if any event that was reported by the subject could be verified in medical records. All drugs were classified according to their corresponding Anatomical-Therapeutical-Chemical-code (ATC-code). Subjects who used lipid-lowering medication (ATC-code c10) according to the interview data or the pharmacy data were classified accordingly. Daily intake of total energy was expressed in kilojoules and intake of vitamin E in milligrams. Intake of alcohol, fruit and vegetables were expressed as grams per day. Subjects who reported use of supplements were classified accordingly. Body mass index was expressed in kilograms per meter squared. Diabetes was considered present if the subject was taking oral antidiabetics or insulin (ATC-code a10), or if the random or post-load serum glucose level was equal to or higher than 11.1 mmol/L (WHO-criteria for epidemiological studies of diabetes).

**Study population**

At the baseline clinical examination 7525 participants of the Rotterdam Study underwent extensive screening for dementia. Dementia was diagnosed in 482 subjects resulting in 7043 subjects who were free of dementia at baseline. Of these, we excluded 602 subjects from dietary assessment for 2 reasons. First, dietary intake was not assessed in 125 subjects who had questionable cognitive status (CAMDEX-score lower than 80), because they might provide unreliable answers regarding their food patterns. Second, we excluded nursing home residents (n=477), because their current diet will not reflect dietary habits in the past as food will be prepared by nursing home staff. Thus, 6441 subjects were eligible for dietary assessment. Of these, reliable dietary data were missing in 1046 subjects (16%) for several reasons. First, due to logical inconsistencies in dietary interviews, 212 subjects were excluded. Second, because the food-frequency questionnaire was administered at the second visit to the research center, subjects who missed the second visit did not have dietary assessment (n=192). Finally, 642
subjects did not have dietary data due to logistic reasons. Thus, the study population for this study comprised 5395 participants who had normal cognition, lived independently and had reliable dietary assessment.

Eligible subjects without dietary data were somewhat older (2.6 years) compared to subjects from the study population, a somewhat lower percentage (4% lower) were women and a higher percentage had only primary education (7% higher). Smoking habits and body mass index were similar across the 2 groups.

Data analysis
Because of the high correlation between fat intake and total energy intake we calculated energy-adjusted intake of fat. Energy-adjusted intake was obtained by use of the residual method that consisted of a 3-step procedure. First, we performed a linear regression analysis with fat intake as the dependent and total energy intake as the independent variable. Second, we used this regression equation to calculate the expected mean fat intake of the study population for the mean total energy intake of the study population. Third, the energy-adjusted intake of each individual was calculated by adding the expected mean fat intake of the study population to the individual residual that was derived from the regression analysis. These 3 steps were repeated for each type of fat intake separately to obtain energy-adjusted intake for all eight fat variables. Additional to the residual method, we also applied the standard multivariate method and the nutrient density method to adjust for total energy intake.

To evaluate whether the incidence of dementia or its subtypes differed between the study population and the eligible population without dietary data we used a Cox proportional hazards regression model with adjustment for age, gender and education.

To assess whether energy-adjusted intake of fatty acids and cholesterol were associated with risk of dementia, Alzheimer's disease or vascular dementia, we performed Cox proportional hazards regression analyses. Intake of fat was first expressed as categorical variable (tertiles) in order to check whether the relation between fat intake and risk of dementia was linear. Because the relations did not show large deviations from linearity, all associations were analyzed using a linear term for fat intake. In this analysis, the regression coefficient was expressed per SD increase in intake to allow comparison of strengths of associations across the different fat intakes. The SDs of the respective intake of fatty acids and cholesterol were based on the distribution of the study population (n=5395). All models were adjusted for age, gender, education and intake of vitamin E. To adjust most efficiently for age, age was used as the timescale in the model. Entry time was defined as age at study entry. Participants were followed until onset of
dementia, death or end of study, whichever came first. Age at onset of dementia was determined as the midpoint between the age of subject last known to be at risk of dementia and age at diagnosis of dementia.

In additional analyses of the relation between fat intake and risk of dementia and Alzheimer's disease, adjustments were additionally made for lifestyle associated factors, such as smoking habits, intake of alcohol, fruit and vegetables, use of dietary supplements and body mass index. Because of the few cases with vascular dementia, no additional analyses were performed for vascular dementia.

Because the relation between fat intake and incident dementia and Alzheimer's disease may be disturbed by use of lipid-lowering medication, all analyses were repeated after exclusion of subjects who used lipid-lowering medication at baseline (n=145, 2.7%) or at second follow-up (n=490, 9.1%). Furthermore, because myocardial infarction, as a proxy for atherosclerosis, and diabetes may both be related to dietary intake and risk of dementia, we repeated the analyses after exclusion of subjects with one of these conditions (n=796, 14.8%). Finally, in order to minimize the possibility that the observed associations were the result of changing dietary habits due to subclinical dementia, we also performed the analysis after exclusion of subjects with less than 2 years of follow-up (n=212, 3.9%).

All analyses were performed using SAS statistical software (SAS Institute Inc., Cary, NC, USA).

Results

At baseline, the mean age of the study population was 68 years (SD 8 years), most participants were women (59%) and 35% had primary education only (Table 1). The mean vitamin E intake of the study population exceeded the recommended daily amount (RDA) of 10 mg/day for men and 8 mg/day for women. The rather high mean daily intake of 349 grams of vegetables can partly be attributed to the consumption of potatoes. One-third of the study population used dietary supplements, of which only 1% contained fatty acids. The study population consumed on average 81 grams of fat per day.

Thirty-seven percent of all energy intake from the study population was provided by fat intake (Figure 1). Fatty acids are the main contributor to energy from fat. Most of the energy from fatty acids was derived from unsaturated fat to which cis fat contributed most. The most important cis fat in terms of energy was MUFA and the most important PUFA was n-6 PUFA.

During a mean follow-up of 6.0 years (SD 1.3 years), 197 (3.7%) participants became demented. Of these, 146 (74.1%) were diagnosed as having Alzheimer's disease, 29 (14.7%) had vascular dementia and 22 (11.2%) had other types of dementia. The incidence of dementia, Alzheimer's
Diet and risk of dementia: does fat matter?

Table 1 Baseline characteristics of the study population (n=5395)*

<table>
<thead>
<tr>
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<th></th>
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<tbody>
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<td>Women</td>
<td>59%</td>
</tr>
<tr>
<td>Primary education only</td>
<td>35%</td>
</tr>
<tr>
<td>Total energy intake (kJ/d)</td>
<td>8264 (2105)</td>
</tr>
<tr>
<td>Vitamin E intake (mg/d)</td>
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</tr>
<tr>
<td>Smoking</td>
<td></td>
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<td>Current</td>
<td>23%</td>
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<td>43%</td>
</tr>
<tr>
<td>never</td>
<td>34%</td>
</tr>
<tr>
<td>Alcohol intake (g/d)</td>
<td>3.4 (0.2-14.9)†</td>
</tr>
<tr>
<td>Fruit intake (g/d)</td>
<td>228 (132)</td>
</tr>
<tr>
<td>Vegetable intake (g/d)</td>
<td>349 (137)</td>
</tr>
<tr>
<td>Use of supplements</td>
<td></td>
</tr>
<tr>
<td>with fatty acids</td>
<td>1%</td>
</tr>
<tr>
<td>without fatty acids</td>
<td>33%</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.3 (3.7)</td>
</tr>
<tr>
<td>Energy-adjusted intake of</td>
<td></td>
</tr>
<tr>
<td>total fat (g/d)</td>
<td>80.7 (13.5)</td>
</tr>
<tr>
<td>saturated fat (g/d)</td>
<td>34.4 (7.2)</td>
</tr>
<tr>
<td>trans fat (g/d)</td>
<td>2.7 (1.0)</td>
</tr>
<tr>
<td>cholesterol (g/d)</td>
<td>0.23 (0.062)</td>
</tr>
<tr>
<td>MUFA (g/d)</td>
<td>24.3 (5.4)</td>
</tr>
<tr>
<td>PUFA (g/d)</td>
<td>17.4 (6.7)</td>
</tr>
<tr>
<td>n-6 PUFA (g/d)</td>
<td>16.0 (6.6)</td>
</tr>
<tr>
<td>n-3 PUFA (g/d)</td>
<td>1.3 (0.60)</td>
</tr>
</tbody>
</table>

*values represent means (SD) or percentages (%)
† median (interquartile range)

disease and vascular dementia did not significantly differ between the study population and the eligible subjects without dietary data; when adjustments were made for age, gender and education the rate ratio (RR) for subjects with dietary data compared with subjects without dietary data was 0.76 (95% CI 0.57-1.01) for dementia, 0.75 (95% CI 0.54-1.05) for Alzheimer’s disease and 0.70 (95% CI 0.33-1.48) for vascular dementia.

When adjustments were made for age, gender, education, total energy intake and intake of vitamin E, intake of the various energy-adjusted fatty acids or cholesterol were not associated with risk of dementia or vascular
dementia (Table 2). After similar adjustments, we observed for Alzheimer's disease that higher intake of total fat, saturated fat, trans fat and cholesterol was associated with lower risk. With every increase of 13.5 g/day (SD) in intake of total fat, the risk of Alzheimer's disease decreased by 14% (RR 0.86 (95% CI 0.73-1.01)). Rate ratios per SD increase in intake were for saturated fat 0.83 (95% CI 0.70-0.98), for trans fat 0.80 (95% CI 0.65-0.97) and for cholesterol 0.86 (95% CI 0.72-1.02). Intake of MUFA, PUFA, n-6 PUFA and n-3 PUFA were not associated with Alzheimer's disease. The associations did not change when we applied the standard multivariate method or the nutrient density method instead of the residual method to

**Figure 1** Mean fat intake of the study population as percentage of total energy intake

* cis monounsaturated fatty acids
# cis polyunsaturated fatty acids
Table 2 Adjusted* rate ratios of dementia, Alzheimer's disease and vascular dementia per standard deviation increase in intake of energy-adjusted fat (g/d)

<table>
<thead>
<tr>
<th></th>
<th>Total dementia</th>
<th>Alzheimer's disease</th>
<th>Vascular dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=197</td>
<td>n=146</td>
<td>n=29</td>
</tr>
<tr>
<td>SD</td>
<td>RR (95% CI)†</td>
<td>RR (95% CI)†</td>
<td>RR (95% CI)†</td>
</tr>
<tr>
<td>Total fat</td>
<td>13.5</td>
<td>0.93 (0.81-1.07)</td>
<td>0.86 (0.73-1.01)</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>7.2</td>
<td>0.91 (0.79-1.05)</td>
<td>0.83 (0.70-0.98)</td>
</tr>
<tr>
<td>Trans fat</td>
<td>1.0</td>
<td>0.90 (0.77-1.06)</td>
<td>0.80 (0.65-0.97)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.062</td>
<td>0.93 (0.80-1.08)</td>
<td>0.86 (0.72-1.02)</td>
</tr>
<tr>
<td>MUFA</td>
<td>5.4</td>
<td>0.96 (0.84-1.10)</td>
<td>0.91 (0.77-1.07)</td>
</tr>
<tr>
<td>PUFA</td>
<td>6.7</td>
<td>1.05 (0.80-1.38)</td>
<td>1.09 (0.79-1.50)</td>
</tr>
<tr>
<td>N-6 PUFA</td>
<td>6.6</td>
<td>1.03 (0.77-1.36)</td>
<td>1.07 (0.77-1.49)</td>
</tr>
<tr>
<td>N-3 PUFA</td>
<td>0.60</td>
<td>1.07 (0.94-1.22)</td>
<td>1.07 (0.91-1.25)</td>
</tr>
</tbody>
</table>

*adjusted for age, gender, education, total energy intake and vitamin E intake
† rate ratio (95% confidence interval)

adjust for total energy intake. When additional adjustments were made for lifestyle associated factors (i.e. smoking habits, intake of alcohol, fruit and vegetables, use of dietary supplements and body mass index), the results were very similar both for dementia and Alzheimer's disease. Furthermore, restriction of the analyses to subjects without lipid-lowering medication did not substantially change the results of dementia nor did exclusion of subjects with myocardial infarction or diabetes. Also, results on dementia did not change after exclusion of subjects with less than 2 years of follow-up. For Alzheimer's disease the results after the various exclusions also remained very similar as is shown in Table 3.

Discussion

In this population-based study high dietary intake of total fat, saturated fat, trans fat and cholesterol and low intake of MUFA, PUFA, n-6 PUFA and n-3 PUFA were not related to increased risk of dementia, Alzheimer's disease or vascular dementia. Additional adjustment for lifestyle associated confounders did not change the results, nor did exclusion of users of lipid-lowering medication or subjects with a history of myocardial infarction or diabetes.

Advantages of the present study are the population-based and longitudinal design with long duration of follow-up. Furthermore, we achieved complete follow-up with respect to dementia diagnosis. Also,
Table 3 Adjusted* rate ratios of Alzheimer's disease per standard deviation (SD) increase in intake of energy-adjusted fat (g/d) in complete study population and in subpopulations

<table>
<thead>
<tr>
<th></th>
<th>Complete study population</th>
<th>Subjects without lipid-lowering medication, 142 cases</th>
<th>Subjects without MI†; or diabetes 117 cases</th>
<th>Subjects with at least 2 years of follow-up, 106 cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)†</td>
<td>RR (95% CI)†</td>
<td>RR (95% CI)†</td>
<td>RR (95% CI)†</td>
</tr>
<tr>
<td>SD</td>
<td>13.5</td>
<td>0.86 (0.73-1.01)</td>
<td>0.85 (0.72-1.01)</td>
<td>0.81 (0.68-0.98)</td>
</tr>
<tr>
<td>Total fat</td>
<td></td>
<td></td>
<td></td>
<td>0.85 (0.66-1.08)</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>7.2</td>
<td>0.83 (0.70-0.98)</td>
<td>0.83 (0.70-0.98)</td>
<td>0.81 (0.63-1.04)</td>
</tr>
<tr>
<td>Trans fat</td>
<td>1.0</td>
<td>0.80 (0.65-0.97)</td>
<td>0.79 (0.65-0.97)</td>
<td>0.81 (0.61-1.08)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.062</td>
<td>0.86 (0.72-1.02)</td>
<td>0.84 (0.70-1.01)</td>
<td>0.83 (0.63-1.08)</td>
</tr>
<tr>
<td>MUFA</td>
<td>5.4</td>
<td>0.91 (0.77-1.07)</td>
<td>0.90 (0.76-1.07)</td>
<td>0.91 (0.71-1.16)</td>
</tr>
<tr>
<td>PUFA</td>
<td>6.7</td>
<td>1.09 (0.79-1.50)</td>
<td>1.07 (0.77-1.48)</td>
<td>1.07 (0.66-1.71)</td>
</tr>
<tr>
<td>N-6 PUFA</td>
<td>6.6</td>
<td>1.07 (0.77-1.49)</td>
<td>1.06 (0.76-1.48)</td>
<td>1.01 (0.61-1.66)</td>
</tr>
<tr>
<td>N-3 PUFA</td>
<td>0.60</td>
<td>1.07 (0.91-1.25)</td>
<td>1.04 (0.89-1.22)</td>
<td>1.15 (0.93-1.42)</td>
</tr>
</tbody>
</table>

* adjusted for age, gender, education, total energy intake and vitamin E intake
† myocardial infarction
‡ rate ratio (95% confidence interval)
dietary data were available for specific groups of fat, such as trans fat, n-6 PUFA and n-3 PUFA. Finally, we made adjustments for an extensive number of potential confounders.

Some methodological issues of the present study have to be discussed. First, bias may have occurred because of conditions that are associated both with fat intake and with dementia. These conditions include cardiovascular disease indicating atherosclerosis, and diabetes. Although exclusion of participants with one of these conditions did not alter the results substantially, bias may still have occurred due to selective survival. If subjects with high intake of, for instance, saturated fat did not die from cardiovascular disease because of insensitivity to the effect of fat, these subjects will also less likely develop dementia. This would then lead to the spurious result of high intake of saturated fat being associated with reduced risk of dementia. Second, a single assessment of fat intake may not precisely reflect subjects' long-term intake, which is more likely to influence disease risk. According to Dutch dietary surveys, total fat intake as percentage of total energy intake in persons older than 65 years decreased by 3% from 40% to 37% over the period 1998-1998. The percentage of saturated fat slightly decreased by 0.7% and the percentage of PUFA increased by 0.6%. The change of fat intake over time may particularly have occurred in health-minded subjects and subjects at high cardiovascular risk. Because the first group may be at low risk of dementia and the latter group at high risk, the effect of misclassification, if any, on the association between fat intake and risk of dementia is hard to predict. Third, bias may have occurred due to the presence of subclinical dementia at baseline that may have led to changes in dietary habits or to less reliable dietary reporting. We tried to minimize this bias by excluding subjects who either were cognitively impaired at baseline or had logical inconsistencies in the food-frequency questionnaire. Also, we excluded the first 2 years of follow-up in an additional analysis. Because this analysis did not change the results, we think that bias due to subclinical dementia, if any, is limited. Finally, the number of cases with vascular dementia was small. As a result, we cannot draw firm conclusions on the relation between fat intake and vascular dementia.

Saturated fat intake as percentage of total energy intake in our study (15.6%) was similar to the intake reported in the Dutch Zutphen Elderly Study (16.3%) and 2 American studies: the Framingham Heart Study (15.0%) and the Nurses' Health Study (15.6%). In Finland, saturated fat intake was higher (19.8%), whereas in Italy it was lower (8.6%). MUFA intake was low (11.0%) in our study compared with other studies; the Zutphen study reported 13.8%, the American studies 15.8% and 16.0%, a Finnish cohort 12.3% and an Italian study 18.0%. PUFA intake was highest in the Netherlands (7.9% in our study and 7.0% in the Zutphen Study).
compared with the USA (5.4% and 4.3%), Finland (5.0%) and Italy (3.6%). However, intake differences in these studies are difficult to interpret due to large differences in subject characteristics such as age and gender. Also, intake may differ because of large differences in timing of dietary assessments that varied from 1966-1969 to 1990-1993. This may have led to differences in report of fat intake due to the general decrease in fat intake over time.

In the present study, we could not confirm that high intake of total fat, saturated fat, trans fat and cholesterol and low intake of MUFA, PUFA, n-6 PUFA and n-3 PUFA were associated with increased risk of dementia or its subtypes. In the literature, several findings suggest that high levels of cholesterol increase risk of dementia, however, evidence is not unambiguous. Some prospective studies on cholesterol levels in serum and risk of Alzheimer's disease show that high levels are associated with increased risk of dementia, whereas others report no association or even an inverse association. Also, increased cerebral beta-amyloid deposition with higher dietary intake of cholesterol has been observed in animals, but may not directly be translated to humans. Furthermore, although a relation between statin use and lower prevalence of Alzheimer's disease has been found, this may not be through the cholesterol-lowering effect of statins.

Previously, we examined the relation between fat intake and dementia within the Rotterdam Study after a follow-up period of 2 years on average and observed that high intake of total fat, and also low intake of fish as an indicator of n-3 PUFA, were significantly associated with an increased risk of dementia. High intake of total fat as well as high intake of saturated fat were particularly associated with an increased risk of dementia with a vascular component, whereas low intake of fish was particularly associated with an increased risk of Alzheimer's disease without cerebrovascular disease. No significant relations were found between either cholesterol and risk of dementia or linoleic acid (n-6 PUFA) and risk of dementia. The discrepancy between the results of that study and our present findings may be explained by the short follow-up in the previous study and a smaller number of incident dementia cases. Therefore, the previous study may have been more prone to bias due to subclinical dementia and observed estimates may not have been very precise.

Two cross-sectional studies previously reported on the association between intake of total fat and dementia, one of which found higher prevalence of Alzheimer's disease with higher intake, whereas the other study observed no association. Furthermore, a small randomized controlled trial found that Alzheimer patients who took PUFA supplements for 4 weeks improved in short-term memory compared to patients who took
placebo.\textsuperscript{42} Given the limited number of studies on fat intake and risk of dementia, we think it is premature to conclude from our observational study that cholesterol or cholesterol affecting fats are not associated with risk of dementia or its subtypes. More large prospective studies with long follow-up are needed to confirm our findings.

References

Chapter 3.2.2


Chapter 3.2.3

Statins and risk of dementia

Background Previous studies reported that use of statins was associated with lower prevalence of dementia. However, confounding by indication and misclassification of drug use may have played an important role in all of these studies.

Objective To examine prospectively whether statin use as reported by pharmacy records are associated with lower risk of dementia and Alzheimer’s disease.

Methods We used data from the Rotterdam Study, a prospective, population-based cohort study among elderly aged 55 years and above. The study population included 6983 subjects who were free of dementia at baseline (1990-1993). Information on drug use was derived from computerized pharmacy records. The cohort was continuously monitored for incident dementia and re-examined in 1993-1994 and 1997-1999. To examine the relation between statin use and risk of dementia and Alzheimer’s disease we used Cox proportional hazards models, in which statin use was entered as a time-varying variable.

Results During an average follow-up period of 6.8 years, 394 subjects became demented (293 Alzheimer’s disease, and 101 other types of dementia). Any use of statins was not associated with lower risk of dementia; when adjustments were made for age and gender the rate ratio was 0.72 (95% confidence interval (CI) 0.34-1.35) for statin use compared to nonuse. Also, longer duration or higher dosage of statin use were not associated with lower risk of dementia. Additional adjustment for level of education or vascular risk factors did not change the results. Results were similar for Alzheimer’s disease.

Conclusion The present study does not support the hypothesis that statin use is associated with a lower risk of dementia or Alzheimer’s disease.

To date, 3 studies reported on the relation between statin use and dementia.1-3 These studies observed that statin use was related to a lower prevalence or risk of dementia. However, these studies were cross-sectional1,3 or based on medical records of general practitioners2 and confounding by indication most likely influenced the results. Moreover, no adjustments were made for several possible confounders, such as educational level1,2 and some vascular risk factors.2,3 Finally, the relation
between duration and dosage of statin use and risk of dementia has not been studied.

We examined whether any statin use, duration of statin use and dosage of statin use were associated with lower risk of dementia and Alzheimer's disease in a prospective, population-based cohort study. We used computerized pharmacy records in order to obtain complete information on statins that were used a few years before dementia diagnosis. We controlled for educational level and vascular risk factors.

Methods

The Rotterdam Study

The Rotterdam Study is a large population-based prospective cohort study that examines risk factors for neurological, cardiovascular, locomotor and ophthalmologic diseases in the elderly population. The study was approved by the Medical Ethics Committee of the Erasmus University. All 10,275 inhabitants from Ommoord, a suburb of Rotterdam, the Netherlands, who were 55 years and over, were invited to participate in the study. Of these, 7,983 subjects (response rate 78%) gave their written informed consent and participated in the study.

The baseline examination of the Rotterdam Study was conducted in 1990-1993. The follow-up examinations were done in 1993-1994 and 1997-1999. During both baseline and follow-up examinations participants were interviewed at home and subsequently clinically examined at the research center. In addition, the cohort was continuously monitored for major disease outcomes.

Use of statins and other lipid-lowering drugs

Data on medication use were derived from the pharmacies in Ommoord. These pharmacies were fully automated and registered all prescriptions on drug use from January 1, 1991 through December 31, 1998. Prescriptions included the product name of the drug, the generic name, the Anatomical Therapeutical Chemical (ATC) code, the number of tablets, capsules or other vehicles in the filled prescription, the date of delivery of the product, the prescribed daily number of tablets to be taken, the daily drug dosage, and the duration of the prescription. Thus, for all statin prescriptions we have data on date of delivery of statins, duration and dosage of statin use as well as type of statin.

Statin use was expressed in 3 ways. First, any use of statins was defined as at least one prescription for statins during the study period (1991-1998). Second, duration of statin use was defined as the cumulative number of days for which statins were prescribed. Third, dosage of statins was defined by means of defined daily doses (DDD). The DDD is referred to as the
average dosage of a drug used by an adult for the main indication.\textsuperscript{5} We calculated the average DDD for the total duration for which statins were used.

Use of other lipid-lowering drugs was defined as at least one prescription for fibrates, colestipol, cholestyramine, acipimox or niacin/nicotinic acid during the study period (1991-1998).

**Other variables**

During the baseline home interview information on level of education, smoking and medical history was obtained. Data on co-medication at baseline were obtained from both the interview and the pharmacy database. At the research center dietary intake was assessed by means of a semi-quantitative food frequency questionnaire.\textsuperscript{6} Also, height and weight were measured, blood samples were drawn and blood pressure of the arm was measured using a random sphygmomanometer. Finally, ultrasonography of both carotid arteries was performed.\textsuperscript{7}

Level of education was categorized in 4 groups: completed primary education, lower vocational training or general education, intermediate vocational training or intermediate and higher general education, and higher vocational training, college or university. Smoking was classified as current, former and never smoking. Packyears of smoking were calculated as the average daily number of cigarettes divided by 20 and multiplied with the number of years smoked. A history of myocardial infarction or stroke was considered present if any event that was reported by the subject could be verified in medical records.\textsuperscript{7} Alcohol intake was expressed in grams per day and body mass index in kilograms per meter squared. Diabetes was considered present if the subject was taking oral antidiabetics or insulin at baseline (ATC-code a10), or if the random or post-load serum glucose level was equal to or higher than 11.1 mmol/L (WHO-criteria for epidemiological studies of diabetes).\textsuperscript{5} Total cholesterol concentrations were determined in serum by an automated enzymatic procedure.\textsuperscript{9} Hypercholesterolaemia was defined as a cholesterol level above 6.5 mmol/L. Apolipoprotein E (APOE) genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis.\textsuperscript{10} The APOE genotype was dichotomized in presence or absence of at least one APOE*4 allele. Hypertension at baseline was considered present if the systolic blood pressure was 160 mm Hg or above, if the diastolic blood pressure was 95 mm Hg or above, or if a subject used antihypertensive drugs (ATC-code c02). As an indicator of atherosclerosis we used presence of carotid plaques. Carotid plaques were detected in 6 different locations: common carotid artery, carotid bifurcation, and internal carotid artery at both left and right
Four categories were made according to number of locations with plaques: 0, 1-2, 3-4 and 5-6 locations.

**Diagnosis of dementia**

Dementia was assessed by a 3-phase approach. Firstly, all participants were screened for dementia using 2 tests: the Mini Mental State Examination (MMSE) and the Geriatric Mental State schedule, organic level (GMS). Secondly, participants scoring below 26 on the MMSE or more than 0 on the GMS were further neuropsychologically tested by a research physician using the Cambridge examination for mental disorders of the elderly (CAMDEX). Finally, persons suspected of dementia were examined by a neurologist and a neuropsychologist and, if possible, had a magnetic resonance imaging (MRI) scan of the brain.

In addition, the cohort was continuously monitored by means of computerized linkage of the study database with general practitioners' medical records and the database of the Regional Institute for Outpatient Mental Health Care (RIAGG). Diagnoses of dementia and Alzheimer's disease were based on DSM-III-R and NINCDS-ADRDA criteria, respectively, using all existing information that was reviewed by a panel that consisted of the neurologist, the neuropsychologist and the research physician.

**Study population**

At the baseline clinical examination 7525 participants of the Rotterdam Study underwent screening for dementia. Dementia was diagnosed in 482 subjects resulting in 7043 subjects who were free of dementia at baseline. Of these, we excluded 60 subjects for whom follow-up ended before July 1, 1991, because they had data on medication use for less than 6 months. Thus, the study population of the present study consisted of 6983 subjects.

**Data analysis**

The relation between statin use and dementia was assessed by means of Cox proportional hazards regression analyses in which statin use was added to the model as a time-varying variable. Statin use was firstly entered into the model as any use. Secondly, to examine the effect of duration of statin use, we created 3 categories based on the median duration of use in incident dementia cases: nonuse (reference), use of statin for 2.8 years or less and statin use for more than 2.8 years. Thirdly, to examine the relation between dosage of statins and risk of dementia we made 3 categories on the basis of the median dosage of statin use in incident dementia cases: nonuse (reference), use of statins in a dosage of 0.66 DDD or less and statin use in a dosage of more than 0.66 DDD.
Table 1 Baseline characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Entire cohort</th>
<th>Statin use‡</th>
<th>No statin use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 6983</td>
<td>n = 776 (11%)</td>
<td>n = 6207 (89%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>69.4 (9.1)</td>
<td>65.2 (5.9)</td>
<td>69.9 (9.2)</td>
</tr>
<tr>
<td>Women</td>
<td>60%</td>
<td>58%</td>
<td>60%</td>
</tr>
<tr>
<td>Primary education only</td>
<td>37%</td>
<td>33%</td>
<td>37%</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>22%</td>
<td>25%</td>
<td>22%</td>
</tr>
<tr>
<td>Former</td>
<td>41%</td>
<td>47%</td>
<td>37%</td>
</tr>
<tr>
<td>Never</td>
<td>35%</td>
<td>27%</td>
<td>36%</td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>12%</td>
<td>19%</td>
<td>11%</td>
</tr>
<tr>
<td>History of stroke</td>
<td>3%</td>
<td>2%</td>
<td>3%</td>
</tr>
<tr>
<td>Alcohol intake (g/d)†</td>
<td>9.0 (0.7-10.6)</td>
<td>7.4 (0.4-14.5)</td>
<td>9.0 (0.7-10.5)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.3 (3.6)</td>
<td>26.6 (3.4)</td>
<td>26.3 (3.7)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>10%</td>
<td>11%</td>
<td>10%</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>6.6 (1.2)</td>
<td>7.6 (1.4)</td>
<td>6.5 (1.1)</td>
</tr>
<tr>
<td>Carrier of at least 1 APOE*4 allele</td>
<td>26%</td>
<td>30%</td>
<td>25%</td>
</tr>
<tr>
<td>Hypertension†</td>
<td>33%</td>
<td>43%</td>
<td>32%</td>
</tr>
<tr>
<td>5-6 Locations with carotid plaques</td>
<td>5%</td>
<td>8%</td>
<td>5%</td>
</tr>
<tr>
<td>Statin use‡</td>
<td>11%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (y)†</td>
<td>2.1 (0.9-4.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dosage (DDD)</td>
<td>0.7 (0.6-1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of other lipid-lowering drugs‡</td>
<td>9%</td>
<td>26%</td>
<td>7%</td>
</tr>
</tbody>
</table>

* values represent means (standard deviation) or percentages (%)
† median (interquartile range)
‡ any use within the study period (1991-1998)
¶ defined as a systolic blood pressure of 160 and above, a diastolic blood pressure of 95 or above, or the use of antihypertensive drugs.

All analyses were adjusted for age and gender. To adjust most efficiently for age, we used age as the timescale in the model. Participants were followed until onset of dementia, death or end of study, whichever came first. Age at onset of dementia was determined as the midpoint between the age of subject last known to be at risk of dementia and age at diagnosis of dementia. Age at study end was defined as age at which second follow-up examination was performed unless this examination was performed after 1998. In this case study end was defined as December 31, 1998, because it was the last date on which data on statin use were available.

To examine whether level of education confounded the association between statins and dementia, we additionally adjusted for level of
education. Subsequently, we added the following possible vascular confounders to the model: smoking, packyears of smoking, history of myocardial infarction, history of stroke, alcohol intake, body mass index, diabetes, cholesterol, presence of APOE*4 allele, hypertension and carotid plaques. Missing values on continuous variables were replaced by the mean or the median, depending on distribution of the variable. Missing values on categorical variables were replaced by a missing indicator. To study the relationship between statin use and Alzheimer's disease we repeated all analyses.

We also repeated the analyses of dementia within strata of age (younger than 75 years versus 75 years and above), level of education (primary education only versus more than primary education), hypercholesterolaemia (yes/no), and presence of APOE*4 allele (yes/no), respectively. Statistical interactions were tested by adding a product term to the unstratified model.

In addition, we examined the relation between use of other lipid-lowering drugs and risk of dementia. Finally, we studied the relation between hypercholesterolaemia at baseline and risk of dementia within subjects who did not use any lipid-lowering drugs (n=5761). For all analyses we used SAS software package (version 6.12, SAS Institute, Cary, NC, USA).

Results

Table 1 presents the baseline characteristics of the study population. The mean age of the cohort was 69.4 years (SD 9.1), 60% of the participants were women and 37% had primary education only. The mean cholesterol level of was 6.6 mmol/L (SD 1.2). In total, 11% of the subjects had used statins within the study period and 9% had used other lipid-lowering drugs. Statin users had higher baseline prevalence of vascular risk factors, such as smoking, history of myocardial infarction, diabetes, hypertension and carotid plaques, compared with nonusers.

During a mean follow-up time of 6.8 years, 394 subjects became

<table>
<thead>
<tr>
<th>Type of statin use</th>
<th>Prescriptions (n=14 038)†</th>
<th>Defined daily dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>10 276 (73%)</td>
<td>15</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>2420 (17%)</td>
<td>20</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>566 (4%)</td>
<td>10</td>
</tr>
<tr>
<td>Fluvastatin</td>
<td>483 (3%)</td>
<td>40</td>
</tr>
</tbody>
</table>

† type of statin was unknown in 3% of prescriptions
Table 3 Adjusted rate ratios of dementia associated with use of statins (n=6983)

<table>
<thead>
<tr>
<th>Cases</th>
<th>RR (95% CI)†</th>
<th>RR (95% CI)‡</th>
<th>RR (95% CI)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any use of statins</td>
<td>7</td>
<td>0.72 (0.34-1.35)</td>
<td>0.73 (0.34-1.55)</td>
</tr>
<tr>
<td>Duration of use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nonuse</td>
<td>387</td>
<td>1 (reference)</td>
<td>1</td>
</tr>
<tr>
<td>&lt;=2.8 y</td>
<td>4</td>
<td>0.60 (0.22-1.62)</td>
<td>0.61 (0.22-1.64)</td>
</tr>
<tr>
<td>&gt; 2.8 y</td>
<td>3</td>
<td>0.98 (0.31-3.08)</td>
<td>0.99 (0.31-3.11)</td>
</tr>
<tr>
<td>Dosage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nonuse</td>
<td>387</td>
<td>1 (reference)</td>
<td>1</td>
</tr>
<tr>
<td>&lt;=0.66 DDD*</td>
<td>4</td>
<td>0.88 (0.33-2.39)</td>
<td>0.89 (0.33-2.41)</td>
</tr>
<tr>
<td>&gt; 0.66 DDD*</td>
<td>3</td>
<td>0.57 (0.18-1.80)</td>
<td>0.58 (0.19-1.83)</td>
</tr>
</tbody>
</table>

† adjusted for age, gender, level of education, smoking, packyears of smoking, history of myocardial infarction, history of stroke, alcohol intake, body mass index, diabetes, cholesterol, presence of APOE*4 allele, hypertension and carotid plaques
‡ rate ratio (95% confidence interval)
* DDD is defined as the average dosage of a drug used by an adult for the main indication

Table 3 shows the association of statin use with risk of dementia. When adjustments were made for age and gender, any use was associated with a lower risk of dementia. However, the association was not statistically significant; the rate ratio of any use compared with nonuse was 0.72 (95% confidence interval (CI) 0.34-1.35). Additional adjustment for level of education did not change the results, nor did adjustment for vascular risk factors in the fully adjusted model. Also, longer duration of statin use was not associated with lower risk of dementia; with nonuse as the reference rate ratios were 0.60 (95% CI 0.22-1.62) for statin use of 2.8 years or less, and 0.98 (95% CI 0.31-3.08) for use of more than 2.8 years. Again, results were very similar after additional adjustments. Risk of dementia was lower with higher dosage of statins, although associations were not statistically significant. Compared with nonusers rate ratios were 0.88 (95% CI 0.33-2.39) for users of a statin dosage of 0.66 DDD or less and 0.57 (95% CI 0.18-
Table 4 Adjusted rate ratios of Alzheimer's disease associated with use of statins (n=6983)

<table>
<thead>
<tr>
<th>Duration of use</th>
<th>Cases</th>
<th>Adjusted for age and gender</th>
<th>Adjusted for age, gender and level of education</th>
<th>Fully adjusted†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any use of statins</td>
<td>6</td>
<td>1.28 (0.55-2.98)</td>
<td>1.31 (0.56-3.06)</td>
<td>0.99 (0.40-2.49)</td>
</tr>
<tr>
<td>Duration of use</td>
<td>287</td>
<td>1 (reference)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&lt;=2.8 y</td>
<td>4</td>
<td>1.18 (0.42-3.30)</td>
<td>1.21 (0.43-3.49)</td>
<td>1.01 (0.34-3.01)</td>
</tr>
<tr>
<td>&gt; 2.8 y</td>
<td>2</td>
<td>1.53 (0.36-6.61)</td>
<td>1.55 (0.36-6.75)</td>
<td>1.01 (0.21-4.82)</td>
</tr>
<tr>
<td>Dosage</td>
<td>287</td>
<td>1 (reference)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>nonuse</td>
<td>4</td>
<td>1.03 (0.37-2.87)</td>
<td>1.06 (0.34-2.97)</td>
<td>0.77 (0.26-2.27)</td>
</tr>
<tr>
<td>&gt; 0.66 DDD*</td>
<td>2</td>
<td>2.53 (0.54-11.92)</td>
<td>2.47 (0.52-11.70)</td>
<td>2.57 (0.45-14.73)</td>
</tr>
</tbody>
</table>

† adjusted for age, gender, level of education, smoking, packyears of smoking, history of myocardial infarction, history of stroke, alcohol intake, body mass index, diabetes, cholesterol, presence of APOE*4 allele, hypertension and carotid plaques
‡ rate ratio (95% confidence interval)
* DDD is defined as the average dosage of a drug used by an adult for the main indication

1.80) for users of a dosage of more than 0.66 DDD. Similar results were obtained in the fully adjusted model.

Table 4 presents the association between statin use and risk of Alzheimer's disease. Any use of statins was not associated with lower risk of Alzheimer's disease; when adjustments were made for all confounders the rate ratio was 0.99 (95% CI 0.40-2.49). Also, risk of Alzheimer's disease did not decrease with longer duration of statin use or higher dosage.

Table 5 presents rate ratios of dementia associated with use of statins across strata of age, education, cholesterol and APOE*4 allele, respectively. The rate ratio of dementia associated with statin use was lower for participants younger than 75 years compared with those aged 75 years and above. Also, the rate ratio was lower in subjects with high education, in subjects with high cholesterol (≥6.5 mmol/L) and in subjects with at least one APOE*4 allele compared with subjects with lower education, subjects with lower cholesterol levels (<6.5 mmol/L) and subjects with no APOE*4 allele, respectively. However, none of the interactions were statistically significant.

Use of other lipid-lowering drugs was also not significantly associated with lower risk of dementia; when adjustments were made for age and
gender the rate ratio was 0.63 (95% CI 0.26-1.54). Furthermore, hypercholesterolaemia at baseline was not associated with risk of dementia within nonusers of any lipid-lowering drugs; when adjustments were made for age and gender the rate ratio was 1.17 (95% CI 0.93-1.46).

Discussion

In this prospective cohort study we observed no significant association between any use of statins and risk of dementia or Alzheimer’s disease. In addition, long duration of statin use or high dosage of statins were not related to reduced risk of dementia or Alzheimer’s disease. Use of other lipid-lowering medication was also not related to lower dementia risk. The results did not change after extensive adjustment for educational level and vascular factors.

Statistical power was low in our study as indicated by the broad confidence intervals of the rate ratios. This may have led to nonsignificant findings even if statin use is truly related to lower dementia risk. However, given our study sample we had a power of 80% to detect a true risk reduction associated with statin use of 50% or more and a power of 90% to detect a true risk reduction larger than 56%. Thus, our findings are in

| Table 5 Adjusted* rate ratios of dementia associated with any use of statins across strata of age, level of education, cholesterol and APOE*4 allele, respectively |
|---------------------------------|---------|-----------------|--------|
| Age <75 y                       | 1872    | 0.57 (0.23-1.41)| 0.52   |
| Age >=75 y                      | 5111    | 1.01 (0.25-4.10)| 0.52   |
| Low education#                  | 2574    | 0.99 (0.36-2.72)|        |
| High education#                 | 4211    | 0.56 (0.18-1.76)| 0.52   |
| Cholesterol < 6.5 (mmol/L)      | 3102    | 1.03 (0.25-4.20)|        |
| Cholesterol >= 6.5 (mmol/L)     | 3881    | 0.62 (0.25-1.52)| 0.55   |
| No APOE*4 allele                | 4657    | 0.75 (0.24-2.39)|        |
| At least 1 APOE*4 allele        | 1792    | 0.51 (0.16-1.62)| 0.08   |

* adjusted for age and gender
† total number of subjects; subjects with missing values were excluded
‡ rate ratios for any use compared with nonuse (95% confidence interval)
¶ statistical interaction
# low education was defined as primary education only; high education was defined as lower vocational training or general education, intermediate vocational training or intermediats and higher general education, and higher vocational training, college or university
contrast with results from previous studies\textsuperscript{1-3} that reported that use of statins was associated with a reduction in dementia risk of 60-73\%,\textsuperscript{1} 71\%,\textsuperscript{2} and 74\%,\textsuperscript{3} respectively.

The discrepancy in results between our study and the 3 previous studies may be explained by methodological differences. First, 2 of the previous studies were cross-sectional\textsuperscript{1, 3} and the third study obtained data on dementia from general practitioners,\textsuperscript{2} who generally diagnose dementia in a late stage. As a result, in all 3 studies statin use in dementia cases may have differed as a result of rather than preceding the dementia. In our study, we diagnosed dementia earlier in the disease course through repeatedly screening all subjects in the cohort for dementia. Nevertheless, there may still have been some residual confounding by indication in our study as is suggested by our observation that risk of dementia was lower for short-term statin users only. Another methodological difference between previous studies and our study is that previous studies obtained information on statin use from either medical records\textsuperscript{1, 2} or informants,\textsuperscript{3} which are both vulnerable methods for misclassification of drug use. In contrast, we used computerized pharmacy records that are complete sources of information on the delivery of prescribed drugs including statins in the Netherlands. Finally, the previously reported associations may be due to confounding by unmeasured factors, such as level of education\textsuperscript{1, 2} or diabetes,\textsuperscript{2, 3} although adjustment for these factors did not change the results in our study.

An advantage of the present study is that diagnoses of dementia were made by a specialized panel of a neurologist, neuropsychologist and research physician. As a result, classification of dementia and Alzheimer's disease will have been rather accurate. In addition, complete follow-up with respect to diagnosis of dementia was achieved and thus selection bias as a consequence of loss to follow-up could not have occurred.

We could not confirm that use of statins was related to a lower risk of dementia or Alzheimer's disease. This is in contrast with several observations that indicate that statins may prevent or delay dementia pathogenesis. For instance, statins reduce cholesterol levels that may result in lower brain levels of beta-amyloid,\textsuperscript{20} which is thought to cause neurodegeneration in patients with Alzheimer's disease.\textsuperscript{21} Furthermore, statins may increase blood flow,\textsuperscript{22, 23} which is reduced in Alzheimer brains.\textsuperscript{24} Finally, statins may reduce inflammation\textsuperscript{26, 27} that may contribute to the pathophysiology of Alzheimer's disease.\textsuperscript{28} Other findings, however, dispute a possible protective effect of statins on dementia. Some studies suggest that statins may damage neurons, because they found that long-term statin use was related to an increased risk of polyneuropathy.\textsuperscript{29, 30} Statins may also damage neurons in the brain, because some statins, such as simvastatin, cross the blood-brain barrier.\textsuperscript{31} Furthermore, results on the association
between cholesterol levels and risk of dementia were conflicting: some studies showed that high cholesterol levels are associated with increased risk of dementia, whereas others demonstrated no relation or even an inverse association. In addition, high dietary intake of fats that increase cholesterol levels is not associated with increased risk of Alzheimer’s disease. Finally, although one study observed lower risk of dementia with use of non-statin lipid-lowering medication, another study as well as our study showed no association.

In conclusion, this prospective cohort study does not support the hypothesis that statin use is associated with a lower risk of dementia or Alzheimer’s disease. However, because of limited power we cannot exclude a beneficial effect of statins on dementia. If anything, the risk reduction associated with statin use is less than the 70% previously reported. Larger prospective cohort studies are needed in order to examine whether statin use is moderately associated with lower risk of dementia.

References


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Chapter 4

General discussion

The aim of the research described in this thesis was to further investigate the relation between inflammatory factors, antioxidants and lipids, and dementia and to examine whether these associations could be explained by atherosclerosis.

All studies in this thesis were based on the Rotterdam Study, an ongoing prospective population-based study among 7983 subjects of 55 years and over, who were living in Ommoord, a suburb in Rotterdam, the Netherlands. At baseline (1990-1993), 7525 participants of the Rotterdam Study underwent extensive screening for dementia, of whom 482 were diagnosed as having dementia. Of these, 353 (73%) suffered from Alzheimer’s disease and 72 from vascular dementia (15%). The 7043 subjects who were dementia-free at baseline were followed up for 5.7 years on average. During this period, 395 participants developed dementia, of whom 293 (74%) had Alzheimer’s disease and 57 (14%) vascular dementia.

In this general discussion, I will give an overview of the main findings in this thesis in the light of current knowledge regarding dementia pathogenesis. In addition, some methodological issues will be discussed as well as the clinical relevance of our findings. Finally, I will give some recommendations for future research.

Main findings and mechanisms

Inflammatory factors and dementia

To date, it is generally accepted that inflammation occurs within brains of patients with Alzheimer’s disease. In the periphery, inflammation may also occur as is suggested by elevated inflammatory proteins in blood samples of dementia patients.

We studied whether blood levels of inflammatory proteins were already elevated prior to clinical onset of dementia, Alzheimer’s disease and vascular dementia (chapter 2.1). We showed that higher levels of alpha1-antichymotrypsin (ACT), interleukin-6 (IL6) and to a lesser extent C-reactive protein (CRP) were associated with increased risk of dementia, Alzheimer’s disease and particularly vascular dementia. No association was found for soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1. Increased plasma levels of inflammatory cells, such as
leucocytes, were also associated with higher risk of Alzheimer's disease (chapter 2.2). None of these associations could be explained by atherosclerosis.

Elevated plasma levels of inflammatory factors in dementia may be derived from the brain or from the peripheral immune system. However, plasma levels of inflammatory proteins, such as ACT and CRP, are generally more than 100 times higher compared to brain levels. Therefore, the observed plasma elevations of ACT and CRP are probably derived from the peripheral immune system rather than from the brain. In contrast, levels of cytokines, such as IL6, are similar in brain and plasma, thus plasma elevations of IL6 may either be derived from increased production in the brain or the periphery.

Increased plasma levels of inflammatory factors before clinical dementia indicate that the peripheral immune system contributes to the pathogenetic process that leads to dementia. This view is supported by the observation that inflammatory proteins, such as ACT, are known to reinforce the formation of beta-amyloid. Because ACT is elevated in the periphery and because peripheral proteins may be able to cross the blood-brain barrier, peripherally produced inflammatory proteins may also amplify formation of cerebral beta-amyloid deposits. In addition, inflammatory proteins in plasma, particularly CRP, may increase the amount of atherosclerosis. Atherosclerosis in arteries that supply the brain may in turn lead to cerebral ischemia, which may result in oxidative stress and abnormal protein processing and thus increased beta-amyloid production and deposition. However, the relation between inflammatory proteins and dementia may not entirely be explained by atherosclerosis, because adjustment for atherosclerotic measures did not change our results.

Alternatively, elevated inflammatory proteins in plasma prior to clinical dementia onset may be the result of the beta-amyloid deposits within the brain. Beta-amyloid deposition may trigger the occurrence of local inflammation and the production of cytokines, which may subsequently stimulate the peripheral production of inflammatory proteins as shown in animal models. Furthermore, elevated inflammatory proteins may be a consequence of atherosclerosis that is both related to higher plasma levels of inflammatory proteins and to dementia. However, this explanation is less likely, because our results were similar after adjustment for atherosclerosis.

If inflammation is both a cause and a consequence of the pathogenetic process of dementia, a self-enhancing cascade will take place. This cascade includes beta-amyloid deposit formation that leads to local inflammation within the brain and inflammation in the periphery, which both result in increased beta-amyloid deposit formation.
Antioxidants and dementia

Oxidative stress within the brain may lead to neuronal cell death, which is thought to result in clinical symptoms of Alzheimer's disease. Because oxidative stress is reduced by antioxidants, high levels of antioxidants may also be associated with lower risk of Alzheimer's disease.

Previous hospital-based studies examined the relationship between plasma levels of antioxidants and prevalence of Alzheimer's disease. These studies showed inconsistent results, but did not adequately adjust for confounders. In our population-based study we showed that the cross-sectional association between plasma levels of the antioxidant vitamins A and E and Alzheimer's disease was largely due to confounding factors, such as age, gender, total cholesterol and smoking (chapter 3.1.1). In addition, no relation was observed between vitamin A and vitamin E and cognitive decline (chapter 3.1.1). Because levels of antioxidants may have changed as a result of dementia or cognitive decline, prospective studies are needed to elucidate the longitudinal relation between antioxidants and risk of dementia.

We performed a prospective study to examine the association between dietary intake of antioxidants from food and risk of Alzheimer's disease. We found that higher intake of vitamin C and vitamin E were associated with a lower risk of Alzheimer's disease (chapter 3.1.2). Within current smokers, this relationship seemed also present for intake of beta carotene and flavonoids. The associations could not be explained by the presence of atherosclerosis.

The discrepancy in results of the study on antioxidant plasma levels and antioxidant intake may be explained by the difference in study design, i.e. cross-sectional versus longitudinal. Another explanation for the discrepancy may be the relatively low correlation between blood levels and intake of vitamin A and vitamin E.\(^{13}\)

Antioxidants from food may be associated with lower risk of dementia, because they may reduce the level of oxidative stress within the brain. This may occur if antioxidants pass the blood-brain barrier, which has been suggested by animal studies. These studies showed that higher antioxidant intake or antioxidant blood levels were associated with increased brain levels for vitamin A, vitamin C and vitamin E.\(^{14,16}\) In addition, high intake of antioxidants may decrease dementia risk by reducing the risk of atherosclerosis, because atherosclerosis has both been associated with oxidative stress\(^ {17}\) and with dementia.\(^ {12,18}\) However, because additional adjustment for carotid plaques as a measure of atherosclerosis did not change our results, I doubt that atherosclerosis is an important intermediary in the relationship between antioxidants and risk of Alzheimer's disease.
Inflammatory factors, antioxidants and dementia

Inflammation and oxidative stress may interact with each other in Alzheimer pathogenesis. On the one hand, inflammation may induce oxidative stress by the activation of microglia that generate free radicals within the brain. On the other hand, inflammation within the brain may be amplified by oxidative stress, because the accompanying free radicals may activate pro-inflammatory genes in neurons. Because inflammation in the brain may result in activation of the peripheral immune system and because peripheral antioxidants may be associated with antioxidant levels within the brain, peripheral antioxidants may interact with inflammation as reflected in peripheral inflammation in Alzheimer pathogenesis.

We examined whether the association between peripheral inflammation, as indicated by plasma leucocyte count, and risk of Alzheimer’s disease was modified by dietary intake of the antioxidants beta carotene, flavonoids, vitamin C and vitamin E (chapter 2.2). We found this interaction and showed that risk of Alzheimer’s disease was higher with higher leucocyte count, particularly when dietary intake of antioxidants was low.

The interaction between peripheral inflammation and antioxidants in Alzheimer’s disease may also be explained by atherosclerosis. In atherogenesis, inflammation is also suggested to interact with oxidative stress and thus antioxidants, because oxidative stress leads to LDL-oxidation, subsequent inflammation and atherosclerotic plaques and because inflammation is accompanied by increased count of leucocytes that produce free radicals and oxidative stress in itself. However, we think that the interaction between peripheral inflammation and antioxidants cannot entirely be explained by atherosclerosis, because additional adjustment for carotid plaques as a measure of atherosclerosis did not change our results.

Lipids and dementia

Risk of Alzheimer’s disease may be increased with higher levels of cholesterol for 2 reasons. First, high levels of cholesterol have been associated with more beta-amyloid deposition in animal models. Second, high cholesterol levels are associated with more atherosclerosis. Other lipids including saturated fatty acids (saturated fat), trans fatty acids (trans fat), cis monounsaturated fatty acids (MUFA) and cis polyunsaturated fatty acids (PUFA) have also been associated with cholesterol levels and atherosclerosis and may thus also be related to dementia risk. The n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may be associated with dementia risk because of their anti-inflammatory properties.
Previously, 2 cross-sectional studies examined the relation between fatty acids in plasma phospholipids and dementia and showed that levels of saturated fat were higher and levels of EPA and DHA were lower within Alzheimer patients as compared to control subjects. Whether fatty acids are related to cognitive decline, which precedes dementia diagnosis, had not yet been studied.

We examined the relation between fatty acids in plasma phospholipids and cognitive change in dementia-free subjects and observed that high levels of saturated fat, low levels of PUFA and n-6 PUFA and a low ratio of PUFA to saturated fat were significantly associated with increased cognitive decline (chapter 3.2.1). These relations could not be explained by atherosclerosis. Cognitive decline was not associated with levels of trans fat, MUFA, n-3 PUFA, EPA, DHA, linoleic acid, alpha-linolenic acid or arachidonic acid or with the ratio of n-6 PUFA to n-3 PUFA. However, because fatty acid levels were assessed after the occurrence of cognitive decline, we cannot exclude that the (absence of) associations are the consequence rather than the cause of change in cognitive function. Therefore, prospective studies are needed.

We prospectively examined the relation between lipids and dementia using data on dietary intake (chapter 3.2.2). Our hypothesis was that high intake of total fat, saturated fat and trans fat, and low intake of MUFA, PUFA, n-6 PUFA and n-3 PUFA were associated with higher risk of dementia and its subtypes. However, we could not confirm this hypothesis for any of the lipids.

The discrepancy in results for fatty acids in plasma and fatty acids from intake may be explained in several ways. First, the plasma study was cross-sectional, whereas the intake study was longitudinal. Second, because correlations between plasma phospholipid levels and dietary intake of fatty acids are generally low, results may not be comparable. Third, results of studies on cognitive decline may not necessarily be comparable with results of studies on dementia, as will be later discussed in this chapter.

If saturated fat and n-6 PUFA are etiologically related to cognitive decline, it may be through atherosclerosis, although atherosclerosis was no intermediary factor in our analysis. Another possible explanation for the association may be that peripheral levels of fatty acids may determine the fatty acid composition of brain cell membranes that is involved in optimal cell functioning. However, not much is known on this topic and the latter mechanism would particularly hold for DHA for which no association was found.

Based on the cholesterol-lowering properties of statin medications, it has been hypothesized that statin use may be associated with lower risk of dementia. Previously, 3 studies reported that use of statins was related to
an approximately 70% reduction in risk of Alzheimer's disease. However, these studies may have been biased because of confounding by indication and misclassification of drug use. In our study, we could not confirm that statin use was associated with a dementia risk reduction of 70% (chapter 3.2.3). If anything, the risk reduction associated with statin use is less than 50%. Whether an inverse association of modest strength is present remains to be elucidated, because the power to detect such a relationship was insufficient in our study.

**Inflammation, antioxidants and lipids and Alzheimer's disease**

In summary, inflammatory proteins from plasma, and antioxidants and lipids from plasma or diet may etiologically be related to Alzheimer's disease in several ways. Inflammatory proteins in plasma may increase the amount of beta-amyloid formation and deposition within the brain and thereby increase neuronal cell loss either directly or through local inflammation and subsequent oxidative stress. In addition, inflammatory proteins may increase the amount of atherosclerosis, which might result in more oxidative stress, beta-amyloid formation, and neuronal cell loss.

Antioxidants in plasma or from food may be related to a lower risk of Alzheimer pathogenesis in 2 ways. First, antioxidants may reduce oxidative stress within the brain and thereby reduce inflammation, beta-amyloid formation and neuronal cell death. Second, antioxidants may be associated with lower risk of Alzheimer's disease through the inverse relationship with atherosclerosis. If atherosclerosis is reduced, oxidative stress, beta-amyloid formation and neuronal cell death will also be reduced.

Cholesterol may be related to Alzheimer's disease through its possible association with beta-amyloid formation or atherosclerosis. However, our findings did not support a relation between cholesterol and Alzheimer's disease as we saw no association between Alzheimer's disease and high intake of fats that increase levels of cholesterol and low intake of fats that reduce cholesterol. Also, we could not confirm that use of cholesterol-lowering medication, such as statins, is associated with risk of dementia or Alzheimer's disease. On the other hand, we did observe that high levels of saturated fat and low levels of n-6 PUFA in plasma phospholipids that are related to more atherosclerosis, were associated with increased cognitive decline.

N-3 PUFA, particularly EPA and DHA, would be related to dementia because of their anti-inflammatory properties. However, even though inflammatory processes seem to play a role in Alzheimer pathogenesis, we did not find an association between plasma levels of n-3 PUFA and cognitive decline or between intake of n-3 PUFA and risk of dementia.
Methodological considerations

Nutrient intake versus nutrient blood levels
The relationship between nutrient and disease can be examined by assessment of dietary intake or by measurement of nutrient level in blood or tissue. For the purpose of etiologic research, nutrient blood levels may generally be more appropriate for 2 reasons. First, nutrient levels will generally reflect nutrient status better, because nutrient levels are not only affected by intake but also by nutrient absorption, usage and storage. Second, measurement of nutrient level may be less susceptible to random misclassification particularly because dietary assessment is prone to inaccuracies. These inaccuracies are the result of error in dietary reporting and use of composition tables that assume constant nutrient content of foods and are based on chemical analyses with their own measurement error.31 However, if intake and blood levels of nutrients are highly correlated, both measures can be used as a proxy for nutrient status. If the nutrient level is extremely susceptible to short-term fluctuations in intake, assessment of dietary intake may be more appropriate than nutrient levels, because dietary assessment will then better reflect long-term nutrient status that is thought to influence disease risk rather than short-term status.

The extent to which intake and levels of antioxidants reflect long-term status considerably differs for the various antioxidants.13 For beta carotene both intake and blood levels would represent long-term status reasonably well. Vitamin C intake would better reflect long-term vitamin C status than blood levels, because vitamin C levels are very susceptible to short-term fluctuations of dietary intake. With respect to vitamin E, blood levels would be more appropriate as a marker of long-term status, because the correlation between intake and blood levels is low. The reflection of long-term vitamin A status by intake or blood levels is limited due to storage of large amounts of vitamin A in the liver that buffers blood levels to maintain constant levels.13 The correlation between intake and blood levels of flavonoids has not yet been studied.

Long-term status of fatty acids and cholesterol may generally be hard to predict, because fat intake may have substantially changed over the last decade due to health campaigns. Nevertheless, blood levels of fatty acids may be a better marker of fatty acid status than intake because of the weak correlation between intake and (fasting) blood levels.26 This also holds for cholesterol.

Blood levels versus brain levels
Because dementia pathology occurs within the brain, levels of inflammatory factors or nutrients within the brain are likely to be more closely related to
dementia pathogenesis than peripheral levels, such as plasma levels. However, obtaining a sample of cerebrospinal fluid (CSF) to assess brain levels is a large burden for people and is thus not feasible for large population-based studies. Instead, blood samples were drawn, although blood levels may not necessarily reflect levels within the brain because of the blood-brain barrier.

To date, correlations between inflammatory protein levels in plasma and CSF have hardly been reported. These correlations are also unknown for antioxidants, fatty acids and cholesterol. However, as previously discussed peripheral proteins, such as inflammatory proteins, as well as antioxidants may be able to cross the blood-brain barrier. Therefore, higher plasma levels of inflammatory proteins and antioxidants might also lead to higher levels within the brain. In addition, some PUFAs have been found to cross the blood-brain barrier by means of transporters. Saturated fat and cholesterol have not been found to pass the blood-brain barrier.

Cognitive decline versus dementia
In addition to dementia I examined risk factors for cognitive decline since dementia is preceded by decline in cognitive function. However, although previous studies showed at least a 3-fold increased risk of dementia for subjects with cognitive impairment compared with the general population, many subjects with cognitive decline do not develop dementia. Therefore, the use of cognitive decline as a proxy for preclinical dementia is susceptible to misclassification. In our studies on cognitive decline, misclassification may also have occurred, because decline was calculated on the basis of actual performed Mini Mental State Examinations (MMSE). This may have led to bias, because subjects with serious cognitive decline will have been less inclined to participate in follow-up examinations and thus in the MMSE. As a result, the distribution of cognitive decline in our study population will have been too small and the power to detect associations will therefore have been less. However, research on cognitive decline may still help to elucidate early risk factors of dementia, because appropriate (biological) markers of preclinical stages of dementia are not yet available. In addition, research on cognitive decline is important on its own, because cognitive decline reduces quality of life and should therefore be prevented.

Clinical relevance
The finding of higher risk of dementia with higher plasma levels of inflammatory factors may be of clinical relevance in 2 ways. First, if the relation is etiological, our finding may indicate that anti-inflammatory medication that reduces these levels would also reduce dementia risk. Although observational studies showed this association for NSAIDs,
randomized controlled trials should first confirm this finding before recommendations can be given for clinical practice. Second, increased inflammatory proteins may help to identify subjects who are at high risk of dementia. However, because dementia is not the only condition in which inflammatory factors are elevated, assessment of inflammatory factors as a biomarker for dementia may only be useful in combination with determination of other risk factors.

Our study on dietary intake of antioxidants and dementia suggests that subjects with high antioxidant intake are at lower risk of Alzheimer’s disease compared with subjects with low intake. If antioxidants truly reduce dementia risk, recommendations to increase antioxidant intake should be given, particularly to elderly at high risk of dementia. However, randomized controlled trials should first be performed before our finding can be applied in clinical practice.

Our observations with respect to fatty acids in phospholipids and change in cognitive function imply that low intake of saturated fatty acids and high intake of PUFA might limit decline in cognition. Even though this could not be confirmed in our prospective study of fat intake and dementia, such a diet might be worthwhile particularly in the light of reduction of cardiovascular disease.

Future research

We found that peripheral inflammatory factors are elevated prior to clinical onset of dementia. However, the impact of peripheral inflammation on dementia pathogenesis is yet unknown. To answer this question, studies are needed that examine the time-dependent relation between peripheral inflammatory factors and clinical symptoms as well as the association between peripheral inflammatory factors and the extent of cerebral pathology, such as beta-amyloid deposits, neuronal cell loss and neuroinflammation. To study the latter association, specific markers of cerebral pathology need to be developed. In this respect, the current possibility to depict activated microglia by PET-scans with labelling may be helpful, although this technique is not suitable for large population-based studies. In addition, genetic research should examine the association between genes or genetic profiles that are involved in responses to inflammation, and risk of dementia.

Our findings regarding the relation between diet and risk of dementia were based on a single dietary assessment. Although single dietary assessment reasonably reflects long-term intake, repeated measurements of dietary intake will be a more reliable marker. Therefore, and because long-term intake rather than short-term intake will affect dementia risk, the association between diet and dementia should be further examined using
repeated measurements of dietary intake. In addition, it should be further explored what factors determine the bioavailability of nutrients from food within plasma as well as within the brain. These factors may include genetic influences, lifestyle and intake of other nutrients.

Furthermore, because dementia is a multifactorial disease, effort should be put into research of mutual relationships between the different pathogenetic processes in Alzheimer's disease, such as beta-amyloid deposition, the formation of neurofibrillary tangles, inflammation, oxidative stress and atherosclerosis.

In parallel with research on mechanisms, preventive strategies should be developed and examined. In particular, randomized controlled trials should be performed to investigate whether anti-inflammatory medication, such as NSAIDs, and antioxidant supplements can prevent or slow down dementia onset. Preferably, the effect of NSAIDs and antioxidant supplements should be examined in a 4-arm-trial in order to additionally examine whether NSAIDs and antioxidant supplements interact with each other in relation to dementia. However, it should first be considered what the optimal timing and duration of use would be in order to obtain most benefit of treatment.

If a preventive effect of NSAIDs or antioxidant supplements is demonstrated, these medications may be recommended for elderly. However, considering costs and possible side-effects of treatment, recommendations should be selectively given to persons who are at high risk of dementia. To identify those persons, predictive models should be made on the basis of the risk factors that are thus far recognized.

References


Chapter 5.1

Summary

This thesis describes the relationship between inflammation, antioxidants and lipids and risk of dementia. The results were based on data from the Rotterdam Study, a large population-based cohort study among elderly inhabitants of the suburb Ommoord in Rotterdam, the Netherlands. In total, 7983 persons of 55 years and over (response rate 78%) participated in the study. Of these, 7525 participants underwent cognitive screening at baseline (1990-1993), of whom 482 persons were diagnosed with dementia. This resulted in 7043 dementia-free participants at baseline who were followed for incident dementia by means of follow-up examinations (1993-1994 and 1997-1999) and continuous monitoring of medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care (RIAGG). After a mean follow-up of 5.7 years 395 incident dementia cases were identified.

Chapter 2.1 described the relationship between inflammatory proteins in plasma and risk of dementia. I found that higher levels of alpha1-antichymotrypsin, interleukin-6 and to a lesser extent C-reactive protein were elevated prior to clinical onset of dementia, Alzheimer’s disease and particularly vascular dementia. Soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1 were not associated with dementia or its subtypes. The associations between inflammatory proteins and dementia could not be explained by atherosclerosis and were similar for subjects with and subjects without an apolipoprotein E (APOE)*4 allele.

In chapter 2.2 I examined whether leucocyte count in serum was associated with risk of Alzheimer’s disease and whether this relationship was modified by dietary intake of antioxidants. I observed that higher leucocyte count was related to a higher risk of Alzheimer’s disease, particularly within subjects with low antioxidant intake. The association was not confounded by smoking, atherosclerosis or supplement use.

In chapter 3.1.1 I studied the cross-sectional relationship between plasma levels of the antioxidant vitamins A and E and Alzheimer’s disease and cognitive decline. I showed that the inverse association between antioxidant levels and Alzheimer’s disease was largely due to confounding factors including age, gender and total cholesterol level. Plasma levels of vitamin A and E were not related to cognitive decline.
Chapter 3.1.2 presented the longitudinal association between dietary intake of antioxidants and risk of Alzheimer's disease. High antioxidant intake, particularly of vitamin C and vitamin E, was related to a reduced risk of Alzheimer's disease. Within current smokers, this relationship was most pronounced and was also present for beta carotene and flavonoids. The relationship was not confounded by education or atherosclerosis. In addition, APOE genotype did not modify the relationship between antioxidant intake and Alzheimer's disease.

In chapter 3.2.1 I studied the relationship between fatty acid levels in plasma phospholipids and change in cognitive function that occurred in dementia-free subjects over a mean period of 6.5 years. I observed that higher percentage of saturated fatty acids in plasma phospholipids was associated with more decline in cognitive function. In addition, decline in cognitive function was increased with lower percentage of cis polyunsaturated fatty acids (PUFA) and n-6 PUFA and a lower ratio of PUFA to saturated fatty acids. I did not observe that the anti-inflammatory n-3 PUFA were associated with less cognitive decline. Also, I found no association between cognitive change and trans fatty acids, cis monounsaturated fatty acids (MUFA), the essential fatty acids linoleic acid and alpha-linolenic acid, arachidonic acid and the n-6 PUFA to n-3 PUFA ratio.

In chapter 3.2.2 I reported findings about dietary intake of fat and risk of dementia and its subtypes. I could not confirm the a priori hypothesis that high intake of total fat, saturated fat, trans fat and cholesterol and low intake of MUFA, PUFA, n-6 PUFA and n-3 PUFA, were associated with increased risk of dementia, Alzheimer's disease or vascular dementia. The results did not change when lifestyle-associated factors, use of lipid-lowering medication or cardiovascular disease were taken into account.

Chapter 3.2.3 showed the longitudinal relationship between use of lipid-lowering medication, such as statins, and risk of dementia and Alzheimer's disease using time-varying exposure methods. I failed to show that statin use, either short-term or long-term or in low dose or high dose, was associated with reduced risk of dementia or Alzheimer's disease. Although the power of the study was low, I showed that, if anything, statin use is associated with a smaller reduction in dementia risk than the 70% previously reported in cross-sectional and GP register based case-control studies.

Finally, in chapter 4 I discussed our findings in the light of other studies. I gave an overview of possible mechanisms in which I showed that inflammation and antioxidants may be related to dementia through their effect on beta-amyloid deposition, on loss of neuronal cells or on atherosclerosis. In addition, inflammation and antioxidants may interact
with each other in relation to beta-amyloid deposition or atherosclerosis. Although findings in this thesis indicate no clear relationship between lipids and dementia, I also discussed possible mechanisms by which fat might play a role in dementia. Furthermore, I considered methodological issues of the studies in this thesis. In particular, I considered the relationship between intake and blood levels of nutrients, the relationship between blood levels and brain levels, and the relationship between dementia and cognitive decline. I also discussed shortly the possible clinical relevance of our results. Finally, I gave some recommendations for future research that should focus on further elucidating the importance of inflammation, both cerebral and peripheral, on dementia pathology. In addition, future research should aim to elucidate factors that determine bioavailability in blood and brain of nutrients that have been associated with lower risk of dementia, such as antioxidants. This may help to translate the potential beneficial effects of these nutrients into preventive interventions.

Hoofdstuk 2.1 beschrijft de relatie tussen ontstekingsewitten in plasma en het risico op dementie. Ik vond dat de concentraties van alpha-antichymotrypsine, interleukine-6 en in mindere mate C-reactief proteïne waren verhoogd voordat klinische symptomen van dementie, de ziekte van Alzheimer en vasculaire dementie aanwezig waren. Oplosbaar intercellulair adhesiemolecuul-1 en oplosbaar vasculaire cel adhesiemolecuul-1 waren niet geassocieerd met dementie of dementie subtypen. De associatie tussen ontstekingsewitten en dementie kon niet worden verklaard door atherosclerose en was gelijk voor personen met en personen zonder een apolipoproteïne E (APOE) *4 allel.

In hoofdstuk 2.2 onderzocht ik of het aantal witte bloedcellen in serum geassocieerd is met het risico op de ziekte van Alzheimer en of deze relatie werd gemodificeerd door inname van de antioxidanten beta caroteen, flavonoiden, vitamine C en vitamine E uit de voeding. Ik zag dat een hoger aantal witte bloedcellen was gerelateerd aan een hoger risico op de ziekte van Alzheimer, met name in personen met lage antioxidant inname. Dit verband werd niet verklaard door roken, atherosclerose of het gebruik van voedingssupplementen.

In hoofdstuk 3.1.1 onderzocht ik de relatie tussen de concentraties van de antioxidanten vitamine A en E in het bloed en de ziekte van Alzheimer en cognitieve achteruitgang. Dit dwarsdoorsnede onderzoek liet zien dat het omgekeerde verband tussen antioxidant concentraties en de ziekte van
Alzheimer met name te verklaren was door de confounders leeftijd, geslacht, en cholesterol concentratie in het bloed.

In hoofdstuk 3.1.2 presenteerde ik de associatie tussen inname van antioxidanten uit de voeding en het risico op de ziekte van Alzheimer. Hogere inname van antioxidanten, met name van vitamine C en vitamine E, bleek gerelateerd te zijn aan een lager risico op de ziekte van Alzheimer. Binnen rokers was deze relatie het duidelijkst en was dan ook aanwezig voor beta caroteen en flavonoiden. Het verband werd niet verklaard door opleidingsniveau of atherosclerose. Tevens werd de relatie tussen antioxidant inname en de ziekte van Alzheimer niet gemodificeerd door het APOE genotype.

In hoofdstuk 3.2.1 werd binnen dementie-vrije personen de relatie onderzocht tussen vetzuren in plasma fosfolipiden en verandering in cognitie over gemiddeld 6.5 jaar. Een hoger percentage van verzadigde vetzuren in plasma fosfolipiden bleek geassocieerd te zijn met meer cognitieve achteruitgang. Ook nam de cognitieve achteruitgang toe met lagere percentages van meervoudig onverzadigde vetzuren (PUFA) en een lagere ratio van PUFA en verzadigde vetzuren. De ontstekingsremmende n-3 PUFA waren niet geassocieerd met minder cognitieve achteruitgang. Ook vond ik geen verband tussen cognitieve verandering en het percentage van trans vetzuren, enkelvoudig onverzadigde vetzuren (MUFA), de essentiële vetzuren linolzuur en alpha-linoleenzuur, arachidonzuur en de n-6 PUFA/n-3 PUFA ratio.

In hoofdstuk 3.2.2. rapporteer ik onze bevindingen over de inname van vetten en het risico op dementie en dementie subtypen. Mijn a priori hypothese kon niet bevestigd worden, want hogere inname van totaal vet, verzadigd vet, trans fat en cholesterol en een lagere inname van MUFA, PUFA, n-6 PUFA en n-3 PUFA waren niet geassocieerd met een hoger risico op dementie, de ziekte van Alzheimer of vasculaire dementie. Deze bevindingen veranderden niet als rekening gehouden werd met het gebruik van cholesterol-verlagende medicatie, de aanwezigheid van hart/vaatziekten of factoren die gerelateerd zijn aan levensstijl.

Hoofdstuk 3.2.3 liet de longitudinale relatie zien tussen het gebruik van cholesterol-verlagende medicatie (statines) en het risico op dementie en de ziekte van Alzheimer. Dit verband werd geanalyseerd door middel van methoden die rekening houden met verandering in statine gebruik over de tijd (time-varying exposure methoden). Ik kon niet bevestigen dat korte of langdurig gebruik van statines in lage of hoge dosering geassocieerd was met een lager risico op dementie of de ziekte van Alzheimer. Alhoewel de power van de studie laag was, liet ik zien dat als statines al geassocieerd zijn met een lager risico op dementie, de risico
reductie in ieder geval kleiner is dan de 70% dat gerapporteerd was in eerder dwarsdoorsnede en patiënt-controle onderzoek.

Tot slot besprak ik in hoofdstuk 4 de bevindingen in het licht van andere studies. Ik gaf een overzicht van mogelijke mechanismen, waarbij ik laat zien dat ontsteking en antioxidanten aan dementie gerelateerd zouden kunnen zijn via hun effect op de neerslag van beta-amyloid, op het verlies van neuronen of op atherosclerose. Tevens besprak ik mogelijke mechanismen waarop lipiden een rol zouden kunnen spelen bij dementie, ook al laat het onderzoek in dit proefschrift geen duidelijk verband zien tussen lipiden en dementie. Ook maakte ik methodologische kanttekeningen bij het proefschrift en besprak ik met name het verband tussen inname van nutriënten en hun concentraties in het bloed, het verband tussen concentraties in het bloed en in de hersenen en de relatie tussen dementie en cognitieve achteruitgang. Daarna besprak ik kort de mogelijke klinische relevantie van onze resultaten. Tot slot gaf ik wat aanbevelingen voor toekomstig onderzoek dat zich zou moeten richten op het verder ophelderen van de rol die ontsteking speelt in zowel de hersenen als daarbuiten bij het ontstaan van dementie pathologie. Daarnaast zou toekomstig onderzoek zich tot doel moeten stellen factoren te achterhalen die de beschikbaarheid van nutriënten in het bloed en de hersenen bepalen. Als deze factoren bekend zijn voor nutriënten die samen hangen met dementie, zoals mogelijk de antioxidanten, dan zou dit kunnen bijdragen aan het vertalen van de resultaten van ons onderzoek naar eventuele preventieve interventies.
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Marianne Engelhart was born on April 7th, 1973 in Haren (Noord-Brabant). She graduated in 1991 at the “Maasland College” in Oss, and studied for one year Health Sciences at the University of Nijmegen. At the same university, she started her medical study in 1992. During this period, she participated in a research project at the Department of Neurology (head: Prof. dr G.W. Padberg) on hereditary motor and sensory neuropathies. She graduated from medical school in December 1998. In January 1999, she started the work described in this thesis at the Department of Epidemiology & Biostatistics (head: Prof. dr A. Hofman) of the Erasmus Medical Center Rotterdam. In 2001, she obtained a Master of Science in Clinical Epidemiology at the Netherlands Institute for Health Sciences in Rotterdam. She plans to start a training in family medicine.