RISK FACTORS FOR COGNITIVE DECLINE

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RISK FACTORS FOR COGNITIVE DECLINE

Risicofactoren voor cognitieve achteruitgang

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Voor Jacob

Chapter

1

INTRODUCTION

Cognitive impairment is one of the major symptoms of dementia. The main cognitive functions are orientation to time and place, recall and memory, attention, language, calculation, and visual construction. Impairment of cognitive functions influences the ability of an individual to live independently, and it diminishes the quality of life. In addition to the consequences for an individual, cognitive impairment imposes a major burden on the health care system because it induces an increased risk of institutionalization and hospitalization.

Although cognitive impairment is a less severe disorder than dementia, it is much more common. In a representative population of subjects over 65 years of age, the prevalence of cognitive impairment was 15.8%, whereas the prevalence of dementia was 4.2%.[1] The risk of cognitive impairment rises exponentially with age. Therefore, we may expect an increase in the number of people with cognitive impairment in our aging society. At present, there are a number of medications that can delay the progression of dementia and that can stabilize cognitive function. However, no cure or prevention for these disorders has been found yet. Therefore, it is important to identify modifiable risk factors for cognitive impairment and dementia. If these risk factors can be found, preventive intervention may become feasible.

In the past decade, knowledge on risk factors for cognitive impairment and dementia has accumulated. The most commonly mentioned risk factors include age, sex, education, head trauma, smoking, family history and the apolipoprotein E gene. Recently, new risk factors have emerged, such as atherosclerosis (i.e. plaques in the carotid arteries, intima-media wall thickness of the carotid artery, and ankle-brachial index) and risk factors for cardiovascular diseases. These new risk factors increase the risk of stroke, lacunar infarcts, and white matter lesions, which in turn can lead to cognitive impairment and vascular dementia, and perhaps even to Alzheimer's disease.[2-5] In addition, experimental, clinical and epidemiological data suggest that other processes, such as

oxidative stress[6], inflammation[7], and hypothalamic-pituitary-adrenal axis overactivity[8], may also be involved in the pathogenesis of cognitive impairment and dementia. We thus focused on nutritional and metabolic factors that are involved in these processes. Most of the risk factors we studied have not been linked to cognitive decline or dementia in previous studies.

It is well known that specific nutrients influence the risk of coronary heart disease, certain types of cancer, and all-cause mortality. Diet has, however, not received much attention as a risk factor for cognitive decline and dementia. In Part I the results of studies on nutritional risk factors are reported. Chapter 2 focuses on nutrients that are related to oxidation. Polyunsaturated fatty acids increase the susceptibility of LDL cholesterol to oxidation, rendering it more atherogenic. Antioxidants decrease this susceptibility. Apart from the oxidation of LDL cholesterol, oxidation in itself may induce damage to neurons, especially after brain infarction. In Chapter 3 established dietary risk factors for coronary heart disease, i.e. fat intake and fish consumption, are examined as risk factors for the incidence of dementia.

The results of studies on metabolic risk factors are described in Part II. In Chapter 4 the relation of diabetes, and pre-clinical stages of diabetes, i.e. hyperinsulinemia and impaired glucose tolerance, to cognitive impairment is reported. Diabetes is considered a major risk factor for cardiovascular diseases, and the pre-clinical stages have also been related to coronary heart disease and stroke. The association between a relatively new cardiovascular risk factor, serum homocysteine and cognitive decline is examined in Chapter 5. Chapter 6 describes the association between two adrenal steroids, cortisol and dehydroepiandrosterone, and cognitive decline. The concentrations of these steroids are influenced by the hypothalamic-pituitary-adrenal axis activity and they have different effects on the hippocampus, which is involved in memory and learning.

Furthermore, to gain better insight in the role of risk factors in the pathogenesis of cognitive decline, we examined the combined effect of two risk factors and the major susceptibility gene for Alzheimer's disease, the apolipoprotein E gene. In Chapter 7 the interaction between education and the apolipoprotein E gene is described. In Chapter 8 we studied the combined effect of a history of cardiovascular diseases and the apolipoprotein E gene on cognitive decline.

It is known that subjects with dementia have a higher mortality risk, but cause-specific mortality has not been investigated yet in a population-based follow-up study. Therefore, in Chapter 9 we investigated total and cause-specific mortality in subjects with (subtypes of) dementia. It is important for relatives and caregivers of demented subjects, and for clinicians and planners of health care facilities to have detailed information on the prognosis of dementia.

In the general discussion the results described in this thesis are briefly summarized. Some methodological problems are discussed. The hypotheses on the etiology of cognitive decline and dementia are addressed in the light of our findings, and finally, some suggestions for future research are given.

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Part I

Nutrition and cognition

Chapter

2

POLYUNSATURATED FATTY ACIDS, ANTIOXIDANTS, AND COGNITIVE FUNCTION

The Zutphen Elderly Study

Abstract

Atherosclerosis and thrombosis may lead to cognitive impairment through cerebral infarcts or white matter hyperintensities. Oxidative stress is now seen as a major contributor to the process of atherogenesis. High intake of polyunsaturated fatty acids (PUFA), e.g. linoleic acid, or low intake of antioxidants can increase oxidative stress. High intake of n-3 PUFAs and its main source fish may reduce the risk of thrombosis. Little is known, however, about the relation between these dietary factors and cognitive function. The authors investigated this relation with data derived from a cohort of men, aged 69 to 89, who were participants in the Zutphen Elderly Study. The 30-point Mini-Mental State Examination was used to assess cognitive impairment in 1990 (score ≤ 25 in 153/476 men, 32%) and cognitive decline from 1990 to 1993 (drop>2 points in 51/342 men, 15%). Food intake was estimated in 1985 and 1990 by the cross-check dietary history method. High linoleic acid intake was associated with cognitive impairment, after adjustment for age, education, cigarette smoking, alcohol consumption, and energy intake (odds ratio (OR) highest versus lowest tertile = 1.76, 95% confidence interval (CI) 1.04-3.01). Intake of n-3 PUFAs was not associated with cognitive impairment, whereas high fish consumption tended to be inversely associated with cognitive impairment (OR=0.63, 95%CI 0.33-1.21) and cognitive decline (OR=0.45, 95%CI 0.17-1.16). Intakes of beta-carotene, vitamin C and E and flavonoids were not inversely associated with cognitive impairment or decline. In conclusion, this study raises the possibility that high linoleic acid intake is positively and high fish consumption inversely associated with cognitive impairment.

Introduction

Cognitive performance decreases with increasing age. Cognitive impairment is a major component of dementia and influences the individual's ability to function independently. Due to aging of the population, the prevalence of cognitive impairment is expected to increase. Therefore, it is important to elucidate possible modifiable risk factors for impaired cognitive function, such as diet.

Polyunsaturated fatty acids (PUFA) and antioxidants may affect the development of cognitive impairment through their impact on atherosclerosis and thrombosis. These processes have been associated with an increased risk of stroke, lacunar infarcts, and white matter changes, as seen on neuroimaging, which can subsequently lead to cognitive impairment.[1-4] Oxidative stress is now seen as an important contributor to the process of atherogenesis.[5] PUFAs are highly susceptible to oxidation, and may increase the oxidative modification of low density lipoprotein (LDL) cholesterol, making it more atherogenic.[6,7] Since linoleic acid, which is an n-6 PUFA, constitutes nearly 90% of the PUFAs in LDL cholesterol, it is the major substrate of LDL oxidation.[6] Dietary antioxidants, such as vitamin E, beta-carotene and flavonoids, may protect against oxidative damage and thus reduce the risk of atherosclerosis.[8-10]

PUFAs may also influence the risk of thrombosis. N-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), compete with linoleic acid in the eicosanoid metabolism.[11] A high linoleic acid intake may lead to increased production of the proaggregatory thromboxane A_2. N-3 PUFAs, on the other hand, reduce the production of thromboxane A_2 and increase the production of the antiaggregatory prostacyclin PGI_3, consequently reducing the risk of thrombosis.[11] Therefore, we examined the association of PUFAs and antioxidants with cognitive function. We also investigated the association between fish consumption and cognitive function, because fish is the main source of n-3 PUFAs. We investigated these associations in a community-based longitudinal study of very old men.

Methods

Study population

The Zutphen Elderly Study is a longitudinal study on risk factors for chronic diseases in men who live in Zutphen, a town in the eastern part of the Netherlands.[12] It is the continuation of the Zutphen Study, which was initiated in 1960 as the Dutch contribution to the Seven Countries Study.[13] In 1985, 555 subjects from the original cohort, born between 1900 and 1920, were still alive. In addition, 711 men of the

same age (64-84 years) were randomly selected from all other men living in Zutphen. This resulted in a population of 1,266 men, of which 939 participated (response rate, 74%). In 1990, 560 of 718 surviving men (78%) were reexamined. The examinations were repeated in 1993 on 390 of 553 surviving men (71%). The study has been approved by the medical ethics committee of the University of Leiden, the Netherlands, and informed consent was obtained from all participants.

Examinations

In the spring of 1985 and 1990, interviews and examinations were carried out at home and in a study center. In the spring of 1993, interviews were conducted at home. Dietary intake was assessed in 1985 and 1990 and cognitive function was tested in 1990 and 1993. For the analyses on prevalent cognitive impairment, information on cognitive function in 1990 was used; complete information on dietary and other risk factors was available for 476 men. For the longitudinal analyses on cognitive decline, information on cognitive function in 1990 and 1993 was used; complete information was available for 342 men.

Food and beverage intake was estimated by the cross-check dietary history method[14], adapted to the Dutch situation.[15] This method provides information about the usual food consumption pattern during the 2-4 weeks preceding the interview. The interviews were carried out by well-trained dietitians in 1985 and 1990, using the same methodology in both years. All subjects were interviewed at home, preferably in the presence of the partner. First the usual food consumption pattern of a person was assessed during weekdays and weekends. Thereafter, a checklist with an extensive number of foods was reviewed, and the frequencies and amounts consumed were recorded. Portion sizes were estimated by a portable scale. The results were checked by comparing the average consumption of foods during a week with the quantities of food purchased for the family during a week. At the same time, the use of prescribed diets, diet products, vitamins and health food products was assessed by a standardized questionnaire. The whole interview took approximately one and a half hours.

Food intake data were coded and converted into energy and nutrients using a computerized version of the Dutch food table (Uniform Food Encoding food table 1984 and NEVO table 1989), updated with information on beta-carotene, vitamin E and flavonoids[10], and with additional information on the n-3 PUFAs EPA and DHA.[16] The flavonoid content was defined as the sum of quercetin, kaempferol, myricetin, apigenin, and luteolin, the five major antioxidant food flavonoids. The average daily intake of 1985 and 1990 combined was calculated for all dietary factors of interest and used for the analyses, because this gives a more reliable estimate of a subject's true intake than only one measurement.[17] The average intake was then categorized into tertiles, and the

first tertile, which corresponded to the lowest intake, used as the reference. Fish consumption was divided into three categories: 0 grams/day, > 0-20 grams/day, and > 20 grams/day. We defined use of vitamin C or E supplements as use in 1985 or 1990, either during the whole year or during winter time only. Users of vitamin C and E supplements were assigned to the highest vitamin C and E tertiles.

Global cognitive function was tested with the Dutch version of the 30-point Mini-Mental State Examination (MMSE).[18,19] The MMSE includes questions on orientation to time and place, registration, attention and calculation, recall, language and visual construction. This screening test was originally created for a clinical setting[18], and is extensively used in epidemiologic studies.[20] Although it tests a limited set of cognitive functions, these are important to daily functioning and severely affected in dementia. In 1990, it was administered in a controlled hospital setting, while in 1993, it was administered at the subject's homes. Research assistants were uniformly trained to administer the MMSE and the scoring was checked by the same person in 1990 and 1993 with strict scoring criteria. If less than four individual items (out of a total of 20 items) were not answered by the subject, these were rated as errors.[21] If items could not be performed because of severe physical disability a weighted total score was given. If a subject did not answer four or more individual items the total MMSE score was considered missing. We used a score of ≤ 25 as the cut-off point, because this is indicative of cognitive impairment.[22] Cognitive decline was defined as a drop of more than two points in the MMSE over a 3-year period (> 1 SD, which corresponds to the 15th percentile of change).

We considered the following potentially confounding variables in our analyses: age (continuous); education, obtained from a self-administered questionnaire ($\leq$ 6 years, 7-12 years and > 12 years of education); cigarette smoking in 1990 (current: yes/no) assessed by a trained physician, with the use of a standardized questionnaire[23]; usual alcohol consumption in 1990, obtained from the cross-check dietary history (none, <1 drink/day (<13.2 grams), $\geq$1 drink/day)[23]; and energy intake (continuous).

Statistical methods

Univariate associations were tested with the nonparametric Mann-Whitney test for continuous variables. We used logistic regression with cognitive impairment (MMSE score > 25 as reference) and cognitive decline (change in MMSE score $\leq$ 2 points as reference) as dependent variables and the following dietary factors as independent variables: PUFAs, which were divided into the n-6 fatty acid linoleic acid and the n-3 fatty acids EPA and DHA; fish; and the antioxidants beta-carotene, vitamin C, vitamin E and flavonoids. Age, education, current cigarette smoking, alcohol and energy intake were added to the model as poten-

tial confounding factors. In the analyses on cognitive decline, we also adjusted for baseline MMSE score. When analyzing intake of vitamin E, we in addition adjusted for PUFA intake, because these variables were highly correlated (r=0.6).

Since high antioxidant intake may diminish the oxidation of LDL induced by linoleic acid[6], and thereby may attenuate the relation between linoleic acid and cognitive impairment, we examined the interaction between intake of linoleic acid and antioxidants. This was done by including the product terms of the linoleic acid and antioxidant dummy variables into the adjusted model. We investigated the interaction between antioxidants and smoking in the same manner to see whether antioxidants were more strongly associated with cognition in smokers, in whom the increased free radical load may lead to an increased need for antioxidants.[24] All tests were two-sided and a p-value of less than 0.05 was considered to be statistically significant. We used SAS-program version 6.09 software in the analyses.[25]

Results

Description of the sample

The median MMSE score was 27 (10th centile: 22, 90th centile: 29). Twenty-two percent of subjects currently smoked. Mean energy intake decreased from 9,668 ± 2,078 kJ in 1985 to 8,858 ± 1,951 kJ in 1990. Fifty-two subjects (11%) used vitamin C supplements in 1985 or 1990, and seven subjects (1%) used vitamin E supplements. Eighty-six percent of the men consumed fish.

Cognitive impairment

Thirty-two percent of the subjects were cognitively impaired in 1990 (MMSE score ≤ 25). Subjects with cognitive impairment were older[19] and concluded less years of education (data not shown). To determine whether cognitive impairment altered dietary intake, we investigated the change in nutrients of interest from 1985 to 1990 in subjects who were cognitively impaired in 1990 and in those who were unimpaired. We found no significant differences in change in total energy and PUFA intake between these two groups (table 1) or in change in antioxidant intake (data not shown). The 1985-1990 average intakes of total fat, PUFAs and linoleic acid were higher in subjects with cognitive impairment, while total energy, fish, EPA, and DHA intakes were lower (table 2).

Cognitive impairment was associated with high linoleic acid intake, after adjustment for potential confounding (table 3). The intake of n-3 PUFAs was not independently related to cognitive impairment. Com-

Table 1
Mean change (SD) in daily intake of selected nutrients and fish from 1985 to 1990 by level of cognitive function.

	Cognitive function in 1990		
	Normal* (n = 323)	Impaired† (n = 153)	p‡
Total energy (kJ)	-196.7 (447.9)	-172.9 (427.8)	0.7
n-6 PUFA:			
Linoleic acid (en%)	0.65 (2.77)	1.02 (3.36)	0.3
n-3 PUFA:			
EPA (mg)	-15.4 (189.0)	10.5 (149.8)	0.6
DHA (mg)	-19.6 (224.2)	10.7 (173.5)	0.7
Fish (g)	-1.54 (21.66)	-3.13 (22.61)	0.8

PUFA = polyunsaturated fatty acids
EPA = eicosapentaenoic acid
DHA = docosahexaenoic acid

* MMSE score > 25
† MMSE score ≤ 25
‡ Mann-Whitney test

pared with no fish consumption, fish consumption of more than 20 grams per day was inversely associated with cognitive impairment in the crude analysis (odds ratio (OR) = 0.43, 95% confidence interval (CI) 0.23-0.78, p-trend = 0.004). This association was weakened after adjustment (OR = 0.63, 95% CI 0.33-1.21, p-trend = 0.13). There was no relationship between cognitive function and intake of any of the antioxidants. The results reported above did not change essentially after exclusion of subjects with stroke or myocardial infarction (n=83). Likewise, the results did not change either when we excluded subjects who were on a prescribed diet in 1990 (18%). There were no significant interactions between linoleic acid intake and any of the antioxidants, nor between antioxidants and smoking (p > 0.1).

Cognitive decline

The 3-yr decline in MMSE score was studied in 342 men who took part in both examination years. Participants did not differ from nonparticipants in nutrients of interest, except in vitamin C intake in 1990, which was lower in the 1993 nonparticipants (mean intake 87.3 mg versus 98.6 mg, p-value = 0.03). The baseline MMSE score was lower in the nonparticipants as well (median MMSE score 26 versus 27, p-value = 0.005). The associations between the nutrients of interest and cogni-

Table 2
Mean (SD) daily intake* of selected nutrients and fish by level of cognitive function.

	Cognitive function in 1990		
	Normal † (n = 323)	Impaired ‡ (n = 153)	p§
Energy (kJ)	9,364 (1,774)	8,975 (1,799)	0.03
Total fat (en%)	38.8 (5.6)	40.0 (5.1)	0.02
Saturated fat (en%)	16.7 (3.1)	16.8 (2.5)	0.6
MUFA (en%)	14.3 (2.8)	14.7 (2.6)	0.2
PUFA (en%)	6.5 (2.5)	7.3 (2.7)	0.002
n-6 PUFA:			
Linoleic acid (en%)	5.4 (2.5)	6.1 (2.8)	0.006
n-3 PUFA:			
EPA (mg)	88.2 (137.2)	78.6 (129.6)	0.1
DHA (mg)	103.0 (156.8)	89.5 (147.6)	0.05
Fish (g)	18.8 (18.4)	14.9 (15.7)	0.02
Antioxidants:			
Beta-carotene (mg)	1.42 (0.52)	1.42 (0.61)	0.6
Vitamin C (mg)	97.3 (40.4)	96.9 (38.9)	0.9
Vitamin E (mg)	8.49 (2.23)	8.44 (2.03)	0.7
Flavonoids (mg)	26.8 (12.9)	26.6 (10.8)	0.6

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid

* Average of intake in 1985 and 1990
† MMSE score > 25
‡ MMSE score ≤ 25
§ Mann-Whitney test

tive impairment in 1990 were not different for the nonparticipants compared with the participants.

Mean 3-yr decline in MMSE score was 0.27 (standard deviation = 2.62). Fifteen percent of the men (n=51) showed a decline of more than two points. After adjustment for possible confounding factors there was no association between linoleic acid intake and cognitive decline (table 4). Fish consumption was inversely but not significantly associated with cognitive decline (adjusted OR = 0.45, 95% CI 0.17-1.16, p-trend = 0.09). Vitamin C intake was positively related to decline of cognitive function (table 4). All results were essentially the same when subjects who were on a prescribed diet (18%) were excluded from the analyses. To examine whether the mechanism behind the observed associations was more acute (i.e. thrombosis) we performed an analysis with 1990

Table 3
Adjusted odds ratios for the association between nutrient intake* and prevalent cognitive impairment† in 476 men.

Daily intake		Tertiles Low	Medium	High	p-trend
Linoleic acid (g)	Range	2.8-9.3	9.3-15.3	15.3-48.7	
	OR ‡	1.00	1.23 (0.73-2.07)	1.76 (1.04-3.01)	0.04
N-3 Fatty acid§ (mg)	Range	0-37.5	37.5-155.5	155.5-2110.5	
	OR	1.00	1.09 (0.65-1.80)	0.96 (0.57-1.62)	0.9
Beta-carotene (mg)	Range	0.55-1.14	1.14-1.54	1.54-4.81	
	OR	1.00	0.87 (0.52-1.47)	1.26 (0.76-2.09)	0.4
Vitamin C (mg)✠	Range	18.0-74.4	74.4-108.8	108.8-295.6	
	OR	1.00	1.00 (0.60-1.65)	1.19 (0.71-1.97)	0.8
Vitamin E (mg)✠¶	Range	3.3-7.5	7.5-9.1	9.1-17.5	
	OR	1.00	1.19 (0.69-2.07)	1.40 (0.71-2.77)	0.3
Flavonoids (mg)	Range	2.4-20.7	20.7-30.0	30.0-96.1	
	OR	1.00	1.50 (0.90-2.48)	1.44 (0.86-2.41)	0.2

* Average of intake in 1985 and 1990, categorized into tertiles
† MMSE 1990 with score > 25 as reference
‡ Odds ratio (95% CI), adjusted for age, education, cigarette smoking, alcohol and energy intake
§ Eicosapentaenoic acid and docosahexaenoic acid
✠ Vitamin C and E supplement users were assigned to the highest tertile
¶ Also adjusted for polyunsaturated fatty acids

diet and cognitive decline from 1990 to 1993. The results for fish or for the other nutrients did not change.

In addition, we investigated the incidence of cognitive impairment at three years of follow-up by excluding subjects with cognitive impairment at baseline (1990, n = 105/342). High linoleic acid intake was associated with an increased risk of becoming cognitively impaired (adjusted OR = 2.57, 95% CI 1.05-6.27, p-trend = 0.04). For fish the risk was similar to the risk of cognitive decline.

Table 4
Adjusted* odds ratios (95% confidence interval) for the association between nutrient intake† and risk of cognitive decline‡ (1990-1993) in 342 men.

	Tertiles			
Daily intake	Low	Medium	High	p-trend
Linoleic acid (g)	1.00	0.95 (0.45-2.05)	1.05 (0.49-2.27)	0.9
N-3 Fatty acid§ (mg)	1.00	0.85 (0.40-1.82)	0.78 (0.35-1.73)	0.5
Beta-carotene (mg)	1.00	0.83 (0.39-1.77)	0.67 (0.30-1.48)	0.3
Vitamin C⌘ (mg)	1.00	1.42 (0.62-3.25)	2.02 (0.90-4.52)	0.09
Vitamin E⌘¶ (mg)	1.00	1.13 (0.48-2.62)	1.20 (0.43-3.31)	0.7
Flavonoids (mg)	1.00	0.89 (0.41-1.93)	0.86 (0.39-1.89)	0.7

* Adjusted for age, education, cigarette smoking, alcohol, energy intake and baseline MMSE score
† Average of intake in 1985 and 1990, categorized into tertiles
‡ >2 points decline in the MMSE score
§ Eicosapentaenoic acid and docosahexaenoic acid
⌘ Vitamin C and E supplement users were assigned to the highest tertile
¶ Also adjusted for polyunsaturated fatty acids

Discussion

Our data suggested that high linoleic acid intake was associated with cognitive impairment. High fish consumption was inversely associated with cognitive impairment and decline in the univariate analysis, although this was weakened after adjustment for confounding. None of the antioxidants we investigated were inversely related to cognitive impairment. These results could not be explained by differences in age, education, smoking behavior, alcohol or energy intake.

Linoleic acid and fish

We found a positive association between intake of linoleic acid and cognitive impairment. The most important food groups that predicted absolute intake of linoleic acid in this population were margarines (with

>40 gr linoleic acid added), butter, baking fats, sauces, and cheese. Studies have shown that replacement of saturated fatty acid intake by linoleic acid decreases serum total and LDL cholesterol concentrations[26] and that linoleic acid intake is inversely related to coronary heart disease.[27] On the other hand, high linoleic acid intake may increase the susceptibility of LDL to oxidation, which makes it more atherogenic.[5-7] However, the association between linoleic acid and atherosclerosis is not consistent. Some studies show an inverse association[28], some a positive association[29-31], and others no association.[32,33] Our findings are consistent with the hypothesis that linoleic acid is atherogenic. Apart from the role of oxidation in atherogenesis, several in vitro and in vivo studies have shown that free radicals may increase vasogenic edema after cerebral ischemia and that they may aggravate the neurological consequences of ischemia, which could increase the risk of vascular related cognitive impairment.[34,35] The association between linoleic acid and cognitive impairment was not modified by any of the antioxidants, including the relatively high correlated vitamin E (r=0.6).

We did not observe an association between linoleic acid intake and cognitive decline. This may be due to regression toward the mean[36]; subjects with the lowest MMSE scores, and also the highest linoleic acid intake, in our sample will on average have a smaller decline. Furthermore, the mechanisms by which linoleic acid may affect cognitive function could be more chronic, in which case a follow-up period of three years would be too short. Theoretically, cognitive impairment could have led to a high linoleic acid intake. It is conceivable that subjects with cognitive impairment have a higher prevalence of cardiovascular disease and therefore might have changed their diet, favoring linoleic acid above saturated fatty acids. However, exclusion of subjects who were on a prescribed diet or of subjects with a history of stroke or myocardial infarction did not change the results. Moreover, confining the analyses to subjects with normal cognitive function at baseline (1990) showed a significantly increased risk of becoming cognitively impaired for subjects with high linoleic acid intake, which is consistent with the analyses on cognitive impairment.

A diet with a high intake of n-3 PUFAs or fish may reduce the risk of thrombosis.[11] In addition, n-3 PUFAs are important for the development of the brain and play a role in the functioning of cerebral membranes. Several studies on infants have suggested that breastfeeding, which leads to higher DHA concentrations in the brain, or supplementation with DHA, is related to better cognitive performance at later age.[37-39] An inverse association between fish consumption and cognitive impairment and decline was suggested by our results. However, we found no association between the n-3 PUFAs, EPA and DHA, and cognitive function, despite the fact that consumption of even small amounts of fish has been shown to be reflected in the concentration of

plasma phospholipid EPA and DHA.[40] Possibly, other nutrients than the n-3 PUFAs in fish play a role in the protection against cognitive impairment, such as selenium, which has antioxidant properties.[41] Or maybe non-fish eaters are different in factors that are related to cognitive impairment.

Antioxidants

We observed no protective effect from any of the antioxidants on cognitive impairment or decline. In contrast, vitamin C was associated with a higher risk of cognitive decline. We found no interaction between antioxidants and smoking. This study may be too small to examine interaction, and we suggest that larger studies examine this issue. Most studies that have investigated the association between antioxidants and cognition have been cross-sectional. They found no clear association of vitamin E and C to dementia[42,43] and an inverse association between a high intake of beta-carotene and cognitive impairment.[44] In the cohort of the Honolulu Heart Program users of vitamin E, C and multivitamin supplements four years prior performed better on cognitive tests.[45] Two intervention studies on geriatric patients found improvement of cognitive capacities after one year of supplementation with a mixture of antioxidants.[41,46]

In the present study the intake of antioxidants was relatively low and only a few people used supplements. The positive association between vitamin C and cognitive decline did not change appreciably after exclusion of subjects who used a prescribed diet. Stroke and myocardial infarction were more prevalent in subjects with the highest vitamin C intake, but exclusion of these subjects did not alter the results. Perhaps unknown and unmeasured confounding factors account for this association. However, it is possible that vitamin C behaves as a pro-oxidant in the presence of free iron[47], which is highly concentrated in the brain.

Methodological considerations

Habitual food intake of the participants was assessed with the cross-check dietary history method. The validity and reproducibility of this method have been well described.[15,48] The average intake between 1985 and 1990 was used to obtain a more reliable estimate of the diet during the five years prior to measurement of cognitive function. Nevertheless, differential misclassification could have influenced the results, because subjects with poor cognitive function might give less precise information on their food intake.[49] This could either lead to overestimation or underestimation of the association between diet and cognitive function. Yet, we found that change in dietary intake from 1985 to 1990 was not different in subjects who were cognitively impaired in 1990 compared with those who were unimpaired. Still, we can not be completely sure

that cognitive impairment did not alter (the report of) dietary intake, because subjects could already have been impaired in 1985.

The Mini-Mental State Examination was used to assess cognitive function. It has proven to be a reliable and valid indicator of cognitive impairment, with a test-retest reliability generally between 0.80 and 0.95.[22] The MMSE can measure a substantial decline in cognitive function that may result from a strong risk factor, such as the apolipoprotein e4 allele.[50] Nevertheless, dietary risk factors may be associated with small effects on cognitive decline, which may not be detected with the MMSE.[51]

It could be argued that selective participation may have affected the validity of our results. Subjects who did not participate in 1993 had a significantly lower baseline MMSE score and a lower vitamin C intake than subjects who did participate. However, the cross-sectional association between cognitive function and intake of vitamin C or other nutrients in 1990 was essentially the same for the nonparticipants and the participants. Thus, it is unlikely that selection bias accounted for our results.

In conclusion, this study raises the possibility that high linoleic acid intake is positively and high fish consumption inversely associated to cognitive impairment in elderly men. None of the antioxidants were protective. Because this is one of the first studies on this subject caution is called for in the interpretation of our findings. Modest associations are difficult to detect with dietary data in a relatively small study. Our findings therefore need confirmation in large prospective studies, preferably with a longer follow-up period, or using a more sensitive measure of cognitive decline.

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Chapter

3

DIETARY FAT INTAKE AND THE RISK OF DEMENTIA

The Rotterdam Study

Abstract

A high intake of saturated fat and cholesterol and a low intake of polyunsaturated fatty acids have been related to an increased risk of cardiovascular disease. Cardiovascular disease has been associated with dementia. We investigated the association between fat intake and incident dementia among participants, age 55 years or older, from the population-based prospective Rotterdam Study. Food intake of 5,386 nondemented participants was assessed at baseline with a semi-quantitative food-frequency questionnaire. At baseline and after an average of 2.1 years of follow-up, we screened for dementia with a three-step protocol that included a clinical examination. The risk of dementia at follow-up (RR (95% CI)) was assessed with logistic regression. After adjustment for age, sex, education, and energy intake, high intakes of the following nutrients were associated with an increased risk of dementia: total fat (RR=2.4 (1.1-5.2)), saturated fat (RR=1.9 (0.9-4.0)), and cholesterol (RR=1.7 (0.9-3.2)). Dementia with a vascular component was most strongly related to total fat (p-trend=0.02) and saturated fat (p-trend=0.01). Fish consumption, an important source of n-3 polyunsaturated fatty acids, was inversely related to incident dementia (RR=0.4 (0.2-0.9)), and in particular to Alzheimer's disease (RR=0.3 (0.1-0.9)). This study suggests that a high saturated fat and cholesterol intake increases the risk of dementia, whereas fish consumption may decrease this risk.

Introduction

As the population includes a growing proportion of older people, dementia will become an increasing burden to society and the individual. Therefore, research on potentially modifiable risk factors, such as diet, is of enormous relevance. Few studies have reported on the relation between diet and dementia.[1-3] Because the study designs were cross-sectional and the demented subjects were usually nursing-home residents, it was difficult to ascertain whether their diet was the cause or consequence of dementia.

In this study, we focused on different components of fat intake, including total fat, saturated fat, cholesterol and polyunsaturated fatty acids (PUFAs). Saturated fat and cholesterol may increase the risk of dementia, because they are associated with adverse cardiovascular events.[4-6] These events, in turn, have been related to both vascular dementia and Alzheimer's disease.[7-10] On the other hand, n-3 PUFAs, which have antithrombotic effects[11], and n-6 PUFAs, which have a beneficial effect on the lipid profile[12,13], may reduce the risk of dementia. We examined the association between fat intake and (subtypes of) dementia in a prospective population-based study, in which dietary data were collected at baseline before the onset of dementia.

Methods

Study population

We used data from the Rotterdam Study, which is a single-center prospective population-based study, designed to investigate determinants of selected chronic diseases and disabilities in older persons.[14] The conduct of the study was approved by the Medical Ethics Committee of the Erasmus University and written consent was obtained from all participants. All residents of a suburb in Rotterdam, age 55 years or older, were invited and of the 10,275 eligible subjects, 7,983 (78%) agreed to participate. During a home visit, trained interviewers administered a questionnaire, covering, among other areas, sociodemographic background, medical history, and medication use. This was followed by two clinical examinations at the research center. Subjects living in nursing homes were visited at home. The food-frequency questionnaire was included in the baseline examinations after the pilot phase, and offered to independently living subjects, who underwent additional clinical examinations (n=6,250). As part of the protocol, subjects who scored below 80 points on the CAMCOG[15] (n=122), which is the neuropsychological test administered in the case-finding procedure for dementia, were excluded because they were thought to be unable to provide reliable answers re-

garding their food patterns. Furthermore, 482 unselected subjects did not receive a dietary questionnaire due to logistic reasons, and 212 respondents were excluded from the sample because of logical inconsistencies in the dietary interviews, resulting in 5,434 completed questionnaires. Baseline data were collected from May 1990 to June 1993. Follow-up data were obtained until December 1994. Incident dementia was ascertained in two ways; i.e., on the basis of a study examination in those attending the follow-up visit, or from medical records if the subject refused reexamination or had died before reexamination.

Dietary intake

Dietary intake was assessed with a 170-item semiquantitative food-frequency questionnaire, which is a modified version of a reliable and valid questionnaire.[16,17] Modifications of the original questionnaire included a different mode of administration and the addition of more detailed questions on vegetable, fruit, and meat consumption. The questionnaire was administered in two stages. In the first stage, respondents were asked to report foods consumed regularly (at least twice a month) in the last year. This took place at home. The second stage was completed during the second visit to the research center. After a review of the checked food items, a trained dietician asked the respondent how much and how often each food item was consumed. The whole procedure was completed in 40 minutes. Average daily nutrient intake was calculated by multiplying the frequency and amount consumed for each food item by its nutrient content listed in an automated version of the Dutch Food Composition Table.[18] We used linoleic acid intake as a marker of n-6 PUFA intake, because it is the most important n-6 PUFA in Western populations.[13] The intake of the n-3 PUFAs eicosapentaenoic acid and docosahexaenoic acid was estimated by fish consumption, since fish is their main source.

Dementia casefinding

Case-finding procedures for dementia have been described elsewhere.[19] As in the baseline examination, the follow-up study examination consisted of a three-stage procedure. First, all participants were screened with the 30-point Mini-Mental State Examination (MMSE)[20] and the short version of the Geriatric Mental State schedule (GMS-A, organic level).[21] Subjects with an MMSE score below 26, or a GMS score greater than 0 were considered screenpositive. They were subsequently examined with the Cambridge Mental Disorders of the Elderly Examination (CAMDEX), which included an informant interview.[15] Participants who were judged to be demented or suspected of dementia after the CAMDEX were examined by a neurologist and tested by a neuropsychologist; a part also underwent magnetic resonance imaging of the

brain. Additional information for those who did not complete the diagnostic phase was obtained from the general practitioner, the regional institute for outpatient mental health care, and specialist medical files. Diagnosis of Alzheimer's disease (AD) was based on NINCDS-ADRDA criteria.[22] Patients with this diagnosis had a gradually progressive global decline of cognitive functions for which no medical cause was found. Dementia patients with a history of stroke, who otherwise fulfilled AD criteria, were subclassified as AD with cerebrovascular disease. Vascular dementia was diagnosed in accordance with NINDS-AIREN criteria.[23] In these patients the onset of dementia was related to cerebrovascular disease. They generally had an acute onset of dementia symptoms with stepwise progression of the disease. DSM-III-R criteria were used to classify other dementias.[24]

Other measurements

The following variables were considered as possible confounding variables: age, sex, total energy intake, cigarette smoking (current, former, or never), alcohol consumption (none, <1 drink (13.2g), 1-2 drinks, or ≥3 drinks per day), fiber consumption, antioxidant intake, and level of education (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) (UNESCO).

A history of cardiovascular disease at baseline, indicators of atherosclerosis, and total and high-density lipoprotein cholesterol were considered as possible mediators of the association between dietary factors and dementia. Data on medical history were obtained at the home interview. A history of myocardial infarction was considered present if the subject reported an event that was diagnosed by a physician and led to hospitalization. A history of stroke was considered present if a self-reported event was confirmed by either a detailed history, neuroimaging, or discharge reports collected from the general practitioner or neurologist.[25] The following indicators of atherosclerosis were used: plaques in the carotid arteries, intima-media wall thickness of the carotid artery, and ankle-brachial index of systolic blood pressure. Measurement methods are extensively described elsewhere.[26] Wall thickness was measured in a relatively small but random subset of subjects (n=1935), and we restricted the analyses on atherosclerosis to this subset.

Statistical analysis

Complete information on diet, incidence of dementia, and confounding factors was available for 5,386 respondents. Mean daily nutrient intake in incident demented and non-demented subjects, and the difference (95% confidence intervals (CI)) between the two groups, was obtained

Table 1
Baseline characteristics of participants who completed the food-frequency questionnaire (n=5386): the Rotterdam Study

Characteristics	
Mean age (yrs)	67.7 (7.8)*
Sex (men/women)	2204/3182
Primary education only (%)	1871 (34.7%)
Median baseline MMSE score (range)	28 (16-30)
Mean alcohol consumption (≥1 drink/day) (%)	1518 (28.2%)
Current smokers (%)†	1256 (23.4%)
Former smokers (%)†	2305 (42.9%)
History of stroke (%)‡	97 (1.8%)
History of myocardial infarction (%)§	380 (7.1%)

MMSE = Mini-Mental State Examination
* Standard deviation between parentheses
† Information available for 5377 subjects
‡ Information available for 5290 subjects
§ Information available for 5321 subjects

from analysis of covariance, controlling for age. Because of the high correlation between fat and total energy intake, energy-adjusted fat and fish intakes were calculated with the residual method.[27] This method gives an estimation of the regression coefficient which is independent of total energy intake. Energy-adjusted intake of, for example, saturated fat was calculated as the summation of the residuals from regressing saturated fat on total energy intake and a constant equal to the expected saturated fat intake at the level of the mean total energy intake. Energy-adjusted intakes of the nutrients of interest were grouped into tertiles, with the lowest tertile as the reference group.

We used multiple logistic regression analysis to estimate the relative risks (RR) for the associations between fat intake, fish consumption and incident dementia. We included confounding variables in the model. The p-value for linear trend was assessed by putting the categorical variable as a continuous variable in the model. We also investigated whether there was effect modification by sex or age by including the product terms of the nutrients with sex or age in the model.

Results

Baseline characteristics are given in Table 1. Fifty-eight (1.1%) of the 5,386 subjects became demented during follow-up (mean = 2.1 years (SD = 0.8)); 42 (72%) were classified as having AD (including five with

Table 2
Age-adjusted mean daily intake of selected nutrients and fish according to incidence of dementia: the Rotterdam Study

Daily intake	Dementia at follow-up		Difference (95% CI)
	No (5328)	Yes (58)	
Total energy (kJ)	8254 (28.8)*	8763 (278.5)	509 (-40, 1058)
Total fat (en%)	36.5 (0.08)	38.2 (0.82)	1.7 (0.1, 3.3)
Saturated fat (en%)	14.4 (0.04)	15.5 (0.42)	1.1 (0.3, 1.9)
Cholesterol (mg/MJ)	28.5 (0.10)	30.1 (0.99)	1.6 (-0.53, 3.44)
Linoleic acid (en%)	5.7 (0.04)	5.3 (0.38)	-0.4 (-1.2, 0.3)
Fish (g)	15.8 (0.26)	10.9 (2.48)	-4.8 (-9.7, 0.1)

CI = confidence interval
en% = intake as percentage of total energy intake
* Standard error between parentheses

AD and cerebrovascular disease), seven (12%) as having vascular dementia, and nine (16%) as having other types of dementia. Age-adjusted mean intake of total and saturated fat was higher, and fish consumption lower, in subjects with incident dementia (Table 2).

Subjects in the highest tertiles of total fat, saturated fat and cholesterol intake had an increased risk of dementia (RR = 2.4, 95%CI: 1.1-5.2; RR = 1.9, 95%CI: 0.9-4.0; and RR = 1.7, 95%CI: 0.9-3.2, respectively) (Table 3).

High total and saturated fat intakes were most strongly associated with an increased risk of dementia with a vascular component (vascular dementia and AD with cerebrovascular disease). Fish consumption was associated with a reduced risk of dementia (RR = 0.4, 95%CI: 0.2-0.9) and especially of AD without cerebrovascular disease (Table 4).

Linoleic acid was also associated with a lower risk of dementia, although not significantly (RR = 0.6, 95%CI: 0.3-1.2). These associations did not change after additional adjustment for cigarette smoking, alcohol consumption, fiber consumption, antioxidant intake, or serum total and high-density lipoprotein cholesterol. Entering the indicators of atherosclerosis into the model did not change the associations, compared with the associations in the same subsample, but without atherosclerosis in the model (data not shown). Adjustment for stroke or myocardial infarction did not alter the results; neither did exclusion of subjects with a history of stroke or myocardial infarction or exclusion of subjects who were using a prescribed diet.

Table 3. Risk of dementia according to tertiles of energy-adjusted fat intake estimated by multiple logistic regression: the Rotterdam Study

	n	Total dementia (n=58)		AD without cerebrovascular disease (n=37)		Dementia with a vascular component* (n=12)	
		RR (95% CI) †	p-trend	RR (95% CI) †	p-trend	RR (95% CI) †	p-trend
Total fat (g/d)							
≤75.5	1795	1.0 (ref)		1.0 (ref)		1.0 (ref)	
75.5-85.5	1768	2.1 (0.9-4.7)		1.8 (0.7-4.5)		1.7 (0.3-10.3)	
>85.5	1823	2.4 (1.1-5.2)	0.02	1.6 (0.6-3.9)	0.25	3.0 (0.6-14.7)	0.02
Saturated fat (g/d)							
≤29.0	1873	1.0 (ref)		1.0 (ref)		1.0 (ref)	
29.0-34.0	1653	1.8 (0.8-4.1)		1.8 (0.7-4.7)		1.0 (0.1-7.2)	
>34.0	1860	1.9 (0.9-4.0)	0.12	1.3 (0.5-3.3)	0.66	2.9 (0.6-13.8)	0.01
Cholesterol (mg/d)							
≤208.5	1808	1.0 (ref)		1.0 (ref)		1.0 (ref)	
208.5-254.5	1785	1.1 (0.5-2.4)		1.2 (0.5-3.0)		1.1 (0.3-4.6)	
>254.5	1793	1.7 (0.9-3.2)	0.11	1.3 (0.6-3.1)	0.40	0.9 (0.2-3.7)	0.80

RR = relative risk; CI = confidence interval; ref.= reference category; AD = Alzheimer's disease

* Includes vascular dementia and Alzheimer's disease with cerebrovascular disease; other dementias are excluded from these analyses

† Adjusted for age, sex, education, and total energy intake

Table 4. Risk of dementia according to tertiles of energy-adjusted linoleic acid and fish intake estimated by multiple logistic regression: the Rotterdam Study

		Total dementia (n=58)		AD without cerebrovascular disease (n=37)		Dementia with a vascular component* (n=12)	
	n	RR (95% CI) †	p-trend	RR (95% CI) †	p-trend	RR (95% CI) †	p-trend
Linoleic acid (g/d)							
≤9.5	1871	1.0 (ref)		1.0 (ref)		1.0 (ref)	
9.58-15.0	1775	1.2 (0.7-2.3)		0.9 (0.4-1.9)		1.0 (0.3-3.6	
>15.0	1740	0.6 (0.3-1.2)	0.23	0.7 (0.3-1.5)	0.17	0.4 (0.1-1.9)	0.09
Fish (g/d)							
≤3.0	1807	1.0 (ref)		1.0 (ref)		1.0 (ref)	
3.0-18.5	1773	0.8 (0.4-1.4)		0.9 (0.4-1.8)		0.6 (0.2-2.5)	
>18.5	1806	0.4 (0.2-0.9)	0.03	0.3 (0.1-0.9)	0.005	0.7 (0.2-2.8)	0.39

RR = relative risk; CI = confidence interval; ref = reference category; AD = Alzheimer's disease

* Includes vascular dementia and Alzheimer's disease with cerebrovascular disease; other dementias are excluded from these analyses

† Adjusted for age, sex, education, and total energy intake

Discussion

This large follow-up study showed an association between dietary factors and incidence of dementia. High intakes of total fat, saturated fat, and cholesterol were related to an increased risk of dementia, and total and saturated fat in particular to dementia with a vascular component. Fish consumption was associated with a reduced risk of dementia, and most strongly with Alzheimer's disease. To our knowledge, this is the first prospective cohort study that investigated the association between fat intake and incident dementia.

Some methodological problems of this study must be discussed. Dietary data collected from subjects who are cognitively impaired may be less reliable, because they may under- or overestimate their dietary intake, leading to differential misclassification.[28] Subjects with dementia at follow-up may more often be cognitively impaired at baseline than those who were not demented at follow-up. It does not appear very likely, however, that they consistently overreported the consumption of foods with a high saturated fat and cholesterol content and underreported the consumption of fish. In addition, we attempted to reduce systematic reporting error by a priori excluding nursing-home residents, individuals with low baseline scores on the CAMCOG, and questionnaires with logical inconsistencies. This exclusion, on the other hand, increases the possibility of response bias. The incidence of dementia is more likely to have been higher in those who did not complete the dietary questionnaire, but it is not very likely that they also had a lower fat intake and a higher fish consumption. Therefore, response bias is not a plausible explanation for these findings. Another methodological problem would occur when subjects with incident dementia altered their food pattern at baseline, because of behavioral changes in pre-clinical stages of dementia. In our sample, however, subjects with incident dementia performed relatively well at the baseline cognitive screening tests. Still, we can not fully exclude the possibility that these subjects changed their food pattern towards a higher consumption of high-fat foods and a lower consumption of fish. Finally, persons with prevalent cardiovascular disease may have altered their diet towards lower intake of saturated fat and cholesterol. Since they might also have a higher risk of dementia, this could have resulted in an underestimation of our estimates. However, the association between saturated fat or cholesterol and dementia did not change when we excluded subjects with cardiovascular disease or subjects using a prescribed diet.

We found a borderline significant association of saturated fat and cholesterol intake with incident dementia. This association may have been mediated by cardiovascular disease. A high intake of saturated fat and cholesterol has been consistently associated with an increased risk of coronary heart disease[4,5], and there are some data showing an asso-

ciation between dietary saturated fat and cerebrovascular disease.[6] Cardiovascular disease has subsequently been related to dementia, especially vascular dementia.[7-9] The hypothesis of a vascular mechanism is consistent with the finding that a high saturated fat intake was most strongly related to dementia with a vascular component. Therefore, we expected that adjustment for cardiovascular disease would have attenuated our estimates, but it did not. The reason for this may be that we adjusted for cardiovascular disease at baseline, whereas it would have been optimal to adjust for cardiovascular disease that developed during follow-up and before onset of dementia. Furthermore, only few subjects suffered from clinical stroke.[25] Silent cerebrovascular disease, which may be an important intermediate as well, is thought to be at least four times more frequent than clinically recognized strokes.[29] We had information on indicators of atherosclerosis, but only in a relatively small subset of subjects, and adjustment for them did not change the results either. This may have been due to measurement error, lack of power, or the fact that these indicators of atherosclerosis might not correlate exactly with subclinical lesions of the brain.

The relation of a high-fat diet to dementia may also be related to other factors associated with socioeconomic status, such as lower education, smoking, and alcohol consumption. Adjustment for these factors did not change our results, but we cannot exclude residual confounding. There may be alternative mechanisms; for instance, cholesterol may have a more direct effect on the brain. An experimental study in rabbits showed that -amyloid accumulated in a dose-dependent way in brains of rabbits fed a high-cholesterol diet.[30]

In the present study, a high linoleic acid intake was slightly, but not significantly, associated with a lower risk of dementia. Although some studies have suggested that linoleic acid may be atherogenic by increasing the oxidative modification of low-density lipoprotein cholesterol[31], our result is compatible with a beneficial effect of linoleic acid on the lipid profile.[12]

Fish, as a marker of n-3 PUFAs in fish, was associated with a reduced risk of dementia, primarily of Alzheimer's disease, even at relatively low levels of fish consumption. The results of the present study are similar to another study, in which we found an inverse association between a comparable fish consumption and the risk of cognitive impairment and decline.[32] A cross-sectional study in Norway indicated that increased plasma phospholipid levels of n-3 PUFAs were observed with a usual intake of one to two servings of fish per week.[33] There are several biologically plausible mechanisms for the association between fish and Alzheimer's disease. First, fish may protect against dementia by reducing the risk of cardiovascular disease.[34,35] Among subjects with neuropathological brain lesions typical for Alzheimer's disease, brain infarcts, and especially lacunar infarcts, more often resulted in clinical demen-

tia.[10] In addition, results from the Rotterdam Study indicate that atherosclerosis is related to an increased risk of Alzheimer's disease.[9] Our finding of a particular association with pure Alzheimer's disease may suggest a different mechanism as well. The n-3 PUFAs in fish have antiinflammatory properties; that is, they can decrease the production of proinflammatory cytokines in humans.[36] Immune processes are believed to be involved in the pathogenesis of Alzheimer's disease. Increased concentrations of acute phase reactants and other markers of immune processes, such as cytokines, have been found in brain tissue of patients with Alzheimer's disease.[37] In addition, interleukin-1 may regulate the amyloid precursor protein synthesis, resulting in amyloid deposits.[38] Epidemiological studies have suggested that the use of nonsteroidal antiinflammatory drugs reduced the risk of Alzheimer's disease.[39] Finally, n-3 PUFAs also play a role in brain development and the functioning of nerve membranes[40], and they can still be incorporated in the brains of animals after the period of brain development.[41] They may therefore be of importance in the regeneration of nerve cells.[42] A human autopsy study showed that the PUFA content, including the n-3 PUFA docosahexaenoic acid, was decreased in the hippocampus and frontal grey matter of Alzheimer's disease brains, whereas the fatty acid composition of normal aged brains was not altered.[43]

In conclusion, this study suggests that a high saturated fat and cholesterol intake raises the risk of dementia, in particular of dementia with a vascular component. In addition, fish consumption may reduce the risk of dementia, and primarily of Alzheimer's disease. The associations we report here are biologically plausible. However, since this is the first prospective study examining the relation of dietary fat intake to the risk of dementia, additional epidemiological studies with a longer follow-up period are needed to address this issue. If these findings are replicated, they could have important implications for reducing the risk of dementia.

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Part II

Metabolism and cognition

Chapter

4

GLUCOSE INTOLERANCE, HYPERINSULINAEMIA, AND COGNITIVE IMPAIRMENT

The Zutphen Elderly Study

Abstract

Cognitive impairment is highly prevalent among the elderly. Subjects with disturbances in glucose metabolism may be at risk for impaired cognitive function, as these disturbances can influence cognition through atherosclerosis, thrombosis and hypertension. We therefore studied the cross-sectional association of cognitive function to hyperinsulinaemia, impaired glucose tolerance and diabetes in a population-based cohort of 462 men, aged 69 to 89 years. Cognitive function was measured by the 30-point Mini-Mental State Examination. Results were expressed as the rate ratio (95% confidence interval) of the number of erroneous answers given on the Mini-Mental State Examination by the index compared to the reference group. Compared to subjects with normal glucose tolerance, known diabetic patients had a rate ratio of 1.23 (1.04-1.46), newly diagnosed diabetic patients of 1.16 (0.91-1.48) and subjects with impaired glucose tolerance of 1.18 (0.98-1.41), after adjustment for confounding due to age, occupation and cigarette smoking (p-trend=0.01). Non-diabetic subjects in the highest compared to the lowest quartile of the area under the insulin curve had a rate ratio of 1.24 (1.03-1.50), after adjustment for confounding (p-trend=0.02). The results did not change appreciably when potentially mediating factors, including cardiovascular diseases and risk factors associated with the insulin resistance syndrome, were taken into account. These results suggest that diabetes mellitus, as well as impaired glucose tolerance and hyperinsulinaemia in non-diabetic subjects are associated with cognitive impairment.

Introduction

Cognitive impairment is an important component of dementia, and a major determinant of the quality of life. It is important to identify risk factors for cognitive impairment that are potentially modifiable. There is now substantial evidence that in addition to diabetes, impaired glucose tolerance and hyperinsulinaemia are also associated with atherosclerosis, thrombosis, and abnormal haemodynamic processes.[1-4] All these processes may have an etiologic role in stroke and lacunar infarcts, which can subsequently lead to cognitive impairment and dementia. In addition, they are risk factors for white matter changes as seen on neuroimaging, which are associated with cognitive impairment.[5]

Several clinical studies support the hypothesis that diabetes and hyperglycaemia may be related to reduced cognitive function.[6-8] However, little is known about the role of insulin in processes that may influence cognitive function. One study suggested, that among hypertensives, hyperinsulinaemia increased the risk of cognitive impairment.[9] To our knowledge, no community-based studies have examined the relation of cognitive function to diabetes mellitus, impaired glucose tolerance and hyperinsulinaemia. Therefore we examined these relations with data from the Zutphen Elderly Study. In addition we also assessed the role of possible mediators of this relation, including cardiovascular disease and risk factors associated with the insulin resistance syndrome.

Methods

Subjects

The Zutphen Elderly Study is a longitudinal study of risk factors for chronic diseases in men. It is a continuation of the Zutphen Study, which was initiated in 1960 as the Dutch contribution to the Seven Countries Study.[10] Zutphen is a small industrial town located in the eastern part of the Netherlands. Of the targeted 1266 men, 555 of whom were from the original cohort, born between 1900 and 1920, and 711 of whom were randomly selected from all other men in the same age range living in Zutphen, 939 participated in 1985. In the Spring of 1990, 544 of 718 (76%) surviving men were reexamined. Complete information on risk factors was available for 462 men in 1990, who formed the sample for the present cross-sectional analysis.

Examinations

An oral glucose tolerance test was performed according to World Health Organization guidelines[11] in subjects without known diabetes. In the morning, after an overnight fast, a blood sample was obtained and then

a glucose load of 75 g given. Blood samples were again taken after 1 and 2 hours. Fasting glucose concentration was determined for subjects known to have diabetes mellitus. Samples were collected in tubes with sodium fluoride. Plasma glucose was determined with the hexokinase method. Serum insulin was measured with a radio-immune assay (Pharmacia Diagnostics, Uppsala, Sweden). Within- and between-run coefficients of variation ranged from 6 to 7%. The men were classified into three groups of glucose tolerance using the WHO criteria for diabetes and impaired glucose tolerance.[11] Diabetes was defined as a fasting glucose concentration ≥7.8 mmol/L or a 2-hour post-load glucose concentration ≥11.1 mmol/L. Impaired glucose tolerance was defined as a fasting glucose concentration <7.8 mmol/L and a 2-hour post-load concentration between 7.8 and 11.1 mmol/L. Normal glucose tolerance was defined as both concentrations <7.8 mmol/L. In addition, a fourth group of known diabetic subjects was created on the basis of the medical interview, independent of their fasting glucose measure. This group included subjects who were treated with insulin, oral hypoglycaemic agents and diet. The area under the post-load glucose and the insulin curve was calculated with the trapezoidal rule ((fasting concentration*30 min) + (1-h concentration*60 min) + (2-h concentration*30 min)). The area under the insulin curve correlated most strongly with 1-hour post-load insulin concentration (1-h: r=0.97, 2-h: r=0.71, fasting: r=0.71).

Global cognitive function was tested with the Dutch version of the 30-point Mini-Mental State Examination (MMSE).[12] The MMSE includes questions on orientation to time and place, registration, attention and calculation, recall, language and visual construction. Although this screening test was originally created for a clinical setting, it is extensively used in epidemiological studies, and has proven to be a reliable and valid indicator of cognitive impairment.[13,14] It was administered by two trained nurses in a controlled hospital setting. If the subject did not answer fewer than four individual items (of a total of 20) these were rated as errors (n=22)[15], except for items which could not be performed because of physical disability, in which case a weighted total score was given (n=10). If a subject did not answer four or more individual items the total MMSE score was considered missing (n=5). We used a score of ≥26 as cut-off point for unimpaired cognitive function, since cognitive impairment is not very likely above this point. We used a score of ≤23 as an indicator of poor cognitive function, since this cut-off point has been shown to be indicative of dementia.[14] A score of either 24 or 25 was called borderline cognitive function.

Cardiovascular risk factors were obtained from a physical examination and a questionnaire, carried out by trained physicians. Body Mass Index (weight/height^2) was calculated from height, measured to the nearest mm and body weight, recorded to the nearest 0.5 kg while the men were in underwear. Systolic and diastolic (fifth Korotkoff phase)

blood pressure were measured with a random zero sphygmanometer in duplicate in the right arm in supine position. The mean of the two blood pressure values was used in the analyses. Hypertension was defined as a systolic blood pressure greater than or equal to 160 mmHg or a diastolic blood pressure greater than or equal to 95 mmHg or the use of anti-hypertensive medication, regardless of blood pressure level.[16]

Fasting and non-fasting blood was obtained to determine the concentrations of lipoproteins and haemostatic factors. Non-fasting serum HDL cholesterol was determined enzymatically after precipitation of apo B containing particles by dextran sulphate-Mg^{2+}[17] by the standardized Lipid Laboratory at the Dept. of Human Nutrition, Wageningen Agricultural University, Netherlands. Fasting triglyceride was determined enzymatically by the same laboratory using a test kit (Boehringer, Mannheim).[18] Fibrinogen concentration was determined by the method of Clauss[19], in which the clotting time is measured in a solution of thrombin in constantly high concentration, added to diluted plasma. A standard curve is used to read the concentration of fibrinogen. Analyses were carried out at the Laboratory of the Dept. of Human Biology, University of Limburg, Maastricht, Netherlands.

History of myocardial infarction, angina pectoris and intermittent claudication was obtained from the Dutch translation of a questionnaire developed at the London School of Hygiene and Tropical Medicine.[20] A standardized history of stroke, transient ischemic attack and diabetes mellitus was also obtained. Medical records on all subjects, including ECGs, hospital discharge data and written information from general practitioners, were collected to verify diagnoses. Occupation was used as an indicator of intellectual capacity in this elderly Dutch population, that had a reduced access to education. Lifetime occupation was obtained from a self-administered questionnaire, and coded from class one (professionals, managers and teachers) to class four (manual workers). Finally, smoking habits were assessed by the physician, on the basis of a standardized questionnaire.

Statistical analysis

Nonparametric tests (Kruskall-Wallis) were used to compare continuous skewed variables and chi-square tests to compare categorical variables. The number of erroneous answers on the MMSE, defined as 30 minus the MMSE score, followed a Poisson distribution. Therefore, multiple Poisson regression was used to estimate the rate ratio (RR) of the number of erroneous answers on the MMSE in the index versus the reference group. Thus, an RR of 1.29 means that the number of erroneous answers on the MMSE in that group is on average 29% higher than in the reference group. Because of extra Poisson variation, the standard errors of the estimated betas were computed using the robust (i.e. independent of the Poisson assumption) method described by

Royall[21], and used to calculate the 95% confidence intervals (CI). The SAS-program, version 6.09, was used.[22]

To examine the relation between glucose tolerance and cognitive function, we categorized subjects into normal and impaired glucose tolerance, and newly diagnosed and known diabetes, according to the WHO categories. For the analyses on insulin, subjects were categorized into quartiles of the area under the insulin curve, after excluding known and newly diagnosed diabetic patients, because insulin concentrations usually decline when a person becomes diabetic, which makes insulin levels difficult to interpret. Age, occupation, and cigarette smoking (current: yes/no) were added to the model to adjust for potential confounding. We examined whether the association changed after adjusting for possible mediating factors: those associated with the insulin resistance syndrome (Body Mass Index, hypertension, HDL cholesterol, triglycerides, fibrinogen), and cardiovascular diseases, including stroke, transient ischemic attack, myocardial infarction, angina pectoris and intermittent claudication. To examine whether glucose tolerance and insulin were independently related to cognitive function, we additionally adjusted for fasting insulin in the analysis on glucose tolerance, and for glucose tolerance in the analysis on hyperinsulinaemia. We also performed a joint analysis in which the 2-hr glucose and the area under the insulin curve were both added to one model as continuous variables. As with the other analysis on hyperinsulinaemia, these analyses exclude known and newly diagnosed subjects. Interactions between age and insulin or glucose concentrations, and between insulin and glucose concentrations, were investigated by including interaction terms in the models. All p-values are based on two-sided tests of statistical significance.

Results

The participants were aged 69 to 89 years, with a mean age of 75 ± 4.6 (SD). The median MMSE score was 26. Sixteen percent had a poor cognitive function (MMSE score ≤23), and 31% had a MMSE score of 24 or 25, indicating borderline cognitive performance. The subjects with the lowest scores were older and belonged more frequently to the manual occupation group (Table 1). Other characteristics were not significantly different across levels of cognitive function.

Cognitive function in diabetes mellitus and impaired glucose tolerance

Of the 37 known diabetic patients with complete information on risk factors, 8 were insulin treated. The subjects with impaired glucose tol-

Table 1
Selected characteristics of elderly men by level of cognitive function: the Zutphen Elderly Study, 1990.

Characteristics	Cognitive function			All
	Poor MMSE ≤23 (*n*=74)	Borderline MMSE 24-25 (*n*=76)	Unimpaired MMSE ≥26 (*n*=312)	(*n*=462)
Mean age (yrs)	77.5* (4.8)†	75.1 (4.7)	74.4 (4.3)	75.0 (4.6)
Manual occupation (%)	36‡	26	21	25
Current cigarette smokers (%)	26	22	21	22
Mean Body Mass Index (kg/m^2)	25.6 (3.6)	25.8 (2.6)	25.5 (3.0)	25.6 (3.1)
Mean HDL cholesterol (mmol/L)	1.11 (0.28)	1.14 (0.27)	1.17 (0.30)	1.15 (0.30)
Mean triglycerides (mmol/L)	1.38 (0.70)	1.52 (0.82)	1.44 (0.76)	1.44 (0.76)
Mean fibrinogen (g/L)	3.75 (0.34)	3.72 (0.28)	3.68 (0.33)	3.69 (0.32)
Presence of (%):				
• Stroke	5	4	4	4
• Transient ischemic attack	5	5	7	6
• Myocardial infarction	14	13	14	14
• Angina pectoris	20	14	13	15
• Intermittent claudication	8	14	9	10
• Hypertension	34	42	41	40

* overall chi-square test: p≤0.0001
† (SD)
‡ overall chi-square test: p≤0.001

erance were older than the other subjects (Table 2). Occupational history did not vary significantly by level of glucose tolerance.

As expected, the mean area under the insulin curve was highest in the subjects with impaired glucose tolerance and lowest in the subjects with normal glucose tolerance. However, the correlation between the area under the insulin curve and 2-hr glucose concentration in non-

Table 2.
Selected characteristics and rate ratios (95% confidence interval) of the number of incorrect responses to the MMSE by glucose tolerance status: the Zutphen Elderly Study, 1990.

	Normal (n=340)	IGT (n=47)	New diabetes (n=38)	Known diabetes (n=37)	
Characteristics					p*
• Mean age (yrs)	74.8 (4.5)†	77.0 (5.1)	73.7 (4.2)	75.8 (4.8)	0.01
• Manual occupation %)	26	19	16	27	0.41
• Presence of stroke (%)	3	13	5	3	0.02
• Mean insulin AUC (pmol/L.min)	37274 (16696)	50799 (21095)	38020 (23764)	-	0.0001
• MMSE score ≤25 %)	28	41	37	52	0.02
• Mean number of incorrect responses on the MMSE‡	3.7 (2.7)	4.7 (3.2)	4.0 (2.8)	4.9 (2.9)	0.01
Rate ratios					p-trend
• Unadjusted	1.00	1.26 (1.02-1.55)	1.08 (0.86-1.36)	1.31 (1.07-1.60)	0.01
• Adjusted§	1.00	1.18 (0.98-1.41)	1.16 (0.91-1.48)	1.23 (1.04-1.46)	0.01

IGT = impaired glucose tolerance; AUC = area under the curve
* Kruskall-Wallis test for continuous variables and chi-square test for categorical variables
† (SD)
‡ MMSE score = 30 - mean number of incorrect responses
§ adjusted for age, occupation and cigarette smoking

diabetic subjects was low (r=0.19, p<0.001). Stroke was considerably more frequent in subjects with impaired glucose tolerance.

The prevalence of poor cognitive function (MMSE score ≤23) was highest in subjects with impaired glucose tolerance. After correction for confounding factors, including age, the number of erroneous answers on the MMSE increased with increasing glucose intolerance level (p-trend=0.01) (Table 2). Known diabetic subjects made 23% more errors in the MMSE, newly diagnosed diabetic subjects 16%, and subjects with impaired glucose tolerance 18%, compared with subjects with normal glucose tolerance. The results did not change when potentially mediating factors such as stroke, other cardiovascular diseases and variables associated with the insulin resistance syndrome, were taken

into account. There were no significant interactions between age and levels of glucose tolerance ($p>0.1$).

When the analyses were confined to known and newly diagnosed diabetic patients (n=75) only, fasting glucose concentration was positively associated with the number of erroneous answers on the MMSE. Median fasting glucose concentration of the subjects with diabetes was 8.0 mmol/L (10th centile: 6.3 mmol/L, 90th centile: 12.4 mmol/L). For each increase of 2 mmol/L subjects made 5% more errors in the MMSE, after adjustment for age and occupation (RR=1.05, 95% CI:0.98-1.19). Eight of the 37 known diabetic patients were using insulin. Their mean age at clinical diagnosis of diabetes mellitus was 61.5 years (range 40-84 years). Exclusion of these eight subjects did not change the results.

Cognitive function and hyperinsulinaemia

Among the 386 non-diabetic subjects age, occupation, and presence of stroke was comparable across the quartiles of the area under the insulin curve (Table 3). The percentage with poor cognitive function (MMSE score ≤ 23) as well as the percentage with borderline cognitive function (MMSE score 24-25) increased from the lowest to the highest quartile of the area under the insulin curve. The mean MMSE score decreased from 26.5 in the lowest quartile to 25.5 in the highest quartile ($p=0.01$).

After adjustment for possible confounding effects of age, occupation and cigarette smoking, the subjects in the highest insulin quartile gave 25% more erroneous answers on the MMSE items compared with subjects in the lowest quartile (Table 3). Adjustment for potentially mediating factors, including cardiovascular diseases and factors associated with the insulin resistance syndrome, did not change the coefficient.

Analyses pointed to a possible interaction between age and insulin quartiles ($p=0.09$), suggesting that the association between hyperinsulinaemia and cognitive impairment was stronger in the older (>75 years) than in the younger (≤ 75 years) age group. Persons older than 75 years in the highest insulin quartile made 45% more errors in the MMSE compared with those in the lowest quartile after adjustment for potential confounders (RR=1.45, 95% CI: 1.14-1.85). Cognitive impairment was more strongly associated with the area under the insulin curve than with the fasting or post-load insulin concentrations. Comparing the highest to the lowest quartiles of hourly insulin measures, the RR for 1-hour and 2-hour insulin concentrations was 1.16 (95% CI:0.96-1.39) and 1.11 (95% CI:0.92-1.33) respectively. The lowest RR was found for the fasting insulin concentrations (RR=1.08, 95% CI:0.89-1.32).

The glucose tolerance and hyperinsulinaemia results were essentially the same when subjects with stroke were excluded from the analyses (results not shown). Additional adjustment for fasting insulin in the analysis on glucose tolerance did not change the results (p-trend=0.01),

Table 3
Selected characteristics and rate ratios (95% confidence interval) of the number of incorrect responses to the MMSE by the level of area under the insulin curve in men free of diabetes mellitus: the Zutphen Elderly Study, 1990.

	Quartiles of area under the insulin curve*				
	First (n=97)	Second (n=96)	Third (n=96)	Fourth (n=97)	
Characteristics					p†
• Mean fasting insulin (pmol/L)	42.5 (11.7)‡	58.9 (16.5)	71.8 (21.5)	96.9 (38.8)	-
• Mean 1-hr post-load insulin (pmol/L)	239.6 (65.1)	373.1 (54.9)	512.3 (78.9)	761.3 (183.7)	-
• Mean 2-hr post-load insulin (pmol/L)	150.7 (70.5)	221.7 (85.4)	307.8 (113.4)	491.5 (233.2)	-
• Mean age (years)	74.8 (4.8)	75.3 (4.7)	75.0 (4.3)	75.1 (4.6)	0.81
• Manual occupation (%)	27	25	24	25	0.95
• Presence of stroke (%)	4	4	3	5	0.93
• MMSE score ≤25 (%)	24	27	30	40	0.1
• Mean number of incorrect responses on the MMSE§	3.5 (2.8)	3.8 (2.6)	3.7 (2.7)	4.5 (2.8)	0.01
Rate ratios					p-trend
• Unadjusted	1.00	1.08 (0.88-1.33)	1.06 (0.85-1.31)	1.29 (1.05-1.57)	0.02
• Adjusted¶	1.00	1.06 (0.87-1.29)	1.05 (0.85-1.30)	1.25 (1.04-1.50)	0.02

* First quartile: area under the insulin curve (pmol/L.min) ≤26347, second quartile: >26347 to 35660, third quartile: >35660 to 48065, fourth quartile: >48065
† Kruskall-Wallis test for continuous variables and chi-square test for categorical variables
‡ (SD)
§ MMSE score = 30 - mean number of incorrect responses
¶ Adjusted for age, occupation and cigarette smoking

neither did adjustment for glucose tolerance in the analyses on hyperinsulinaemia (p-trend=0.02).

In the joint analysis in non-diabetic subjects with the 2-hr glucose and the area under the insulin curve as continuous measures, the rate ratio for 2-hr glucose was 1.01 per mmol/L (95% CI: 0.97-1.05) and 1.04

per 10 nmol/L.min (95% CI: 1.00-1.07) for the area under the insulin curve. These rate ratio's were not different from those obtained when each variable was entered seperately in the model. There were no significant interactions between levels of glucose tolerance and insulin quartiles ($p>0.1$), meaning that the relation between glucose tolerance and cognitive function did not vary across the quartiles of area under the insulin curve and vice versa.

Discussion

We found that non-diabetic subjects with impaired glucose tolerance and hyperinsulinaemia had impaired cognitive function as measured by the MMSE. Among diabetic patients cognitive function was even more impaired and decreased with increasing concentrations of fasting plasma glucose, which is an index of short-term glycaemic control. These results could not be explained by differences in age, occupation or smoking behaviour. Nor were they altered when subjects with stroke or diabetic patients using insulin were excluded. Joint analysis showed that hyperinsulinaemia was related to cognitive impairment, independent of glucose tolerance. Furthermore, diabetes and impaired glucose tolerance were related to cognitive impairment, independent of fasting insulin. Among non-diabetic subjects, the relation of cognitive function to 2-hr glucose and area under the insulin curve did not change when both glucose levels and area under the insulin curve were entered into the model.

There are a few methodological issues that should be taken into account when interpreting our results. First, as our study was cross-sectional, caution is needed regarding the direction of causality. Possibly, severe cognitive impairment leads to a deterioration of glycaemic control in diabetic patients. It is however not likely that cognitive impairment leads to glucose intolerance (i.e. diabetes mellitus or impaired glucose tolerance) or hyperinsulinaemia. Furthermore, selective non-response or survival may have affected the validity of this study. In another study, cognitive impairment and diabetes mellitus were shown to be more frequent in non-responders than responders.[23] It is also possible that persons in whom diabetes and cognitive impairment are both present, have relatively higher mortality rates. If these selection biases apply to our study, it would probably result in an underestimation of the association between cognitive impairment and glucose intolerance or hyperinsulinaemia. We used the area under the insulin curve as indicator of hyperinsulinaemia, rather than a single measurement of insulin. This area under the curve was a better predictor of cognitive function. This might be so because the within-person variation of the area under the insulin curve is smaller than that of the hourly insulin measures.[24] Finally, serum insulin was measured with a conventional immunoassay, which is

known to cross-react with proinsulin. Although proinsulin is known to be increased in diabetes and impaired glucose tolerance, it constitutes only a minor part of the total insulin measured[25] and is therefore probably not responsible for the observed association with cognitive function.

Our findings of impaired cognitive function in diabetic patients agree with earlier clinical studies showing first, that elderly patients with non-insulin-dependent diabetes mellitus (NIDDM) had poorer cognitive function compared with normal controls[6-8,26], and secondly, that increasing levels of glycosylated haemoglobin in NIDDM patients were associated with poorer performance.[6,7] Meneilly et al.[26] showed that improvement of glycaemic control in patients with NIDDM increased selected aspects of cognition. In contrast, a longitudinal study found no significant relation between NIDDM and cognitive impairment.[27] However, the subjects with NIDDM were young and had a high level of formal education. U'ren et al.[8] found that persons not known to have diabetes but who had raised glycosylated haemoglobin (>10%), showed levels of cognitive performance intermediate between normal and diabetic subjects. This study is consistent with our finding that subjects with impaired glucose tolerance perform more poorly on the cognitive test compared to normoglycaemic subjects. Our finding of an association between hyperinsulinaemia and cognitive impairment is consistent with that of Kuusisto et al.[9], who found that hyperinsulinaemia identified a subgroup of hypertensive subjects with particularly impaired cognition.

We examined some potential explanatory mechanisms for the relation between glucose intolerance, hyperinsulinaemia and cognitive function. First, we investigated risk factors associated with the insulin resistance syndrome (hypertension, obesity, decreased HDL cholesterol, increased triglyceride concentrations and increased fibrinogen)[2-4] as mediating factors. The inclusion of these factors did not alter the results. Second, our adjustment for cardiovascular disease, as clinical manifestations of atherosclerosis, did not change the outcome. Moreover, exclusion of subjects with stroke did not alter the results. There is substantial evidence that glucose intolerance and hyperinsulinaemia are associated with atherosclerosis, thrombosis and hypertension[1,4,28], all of which may lead to cognitive impairment through cerebral infarcts or white matter disease.[5] Since atherosclerosis was not directly measured and we had no information on the severity of the cardiovascular disease, we cannot exclude this process as a mechanism explaining the association between glucose intolerance, hyperinsulinaemia and cognitive impairment. Furthermore, other mechanisms may be responsible for the poorer cognitive function in subjects with glucose intolerance and hyperinsulinaemia. The cognitive impairment of diabetic patients may be due to hypoglycaemia[29], increased free radical generation[30], or the same axonal damage as occurs in peripheral neuropathy.[31] Hyperinsulinaemia might affect cogni-

tive function through vascular processes regulated by the sympathetic nervous system[32] or by influencing synaptic activity in the brain.[33] In future studies, information on the degree of atherosclerosis, the presence of cerebral infarcts or white matter disease, and the activity of the sympathetic nervous system, by measurement of plasma norepinephrine, would help to clarify the mechanisms through which insulin can affect cognitive function. Also important is to test aspects of cognitive function not measured by the MMSE, but that can be affected in diabetes, such as complex psychomotor function.[34] Further studies may also help to explain why dose-response was not strong and why the association between insulin and cognition varied by age.

In conclusion, cognitive function might not only be impaired in diabetic patients, but also in elderly subjects with impaired glucose tolerance and hyperinsulinaemia. In addition, poorer glycaemic control was associated with poorer cognitive function in diabetic patients. Clinicians should be alert of the possibility that patients with diabetes mellitus, impaired glucose tolerance or hyperinsulinaemia may be at risk for cognitive impairment.

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Chapter

5

HOMOCYSTEINE AND COGNITIVE DECLINE

The Rotterdam Study

Abstract

Objective – *To investigate the association of homocysteine to cognitive decline.*

Design – *A prospective cohort study with a mean duration of follow-up of 2.9 years.*

Setting and Participants – *472 Community-dwelling respondents of the Rotterdam Study, aged 55 years or over.*

Main Outcome Measure – *Cognitive decline, estimated with the Mini-Mental State Examination (MMSE), defined as a drop in the MMSE score of >1 point/year.*

Results – *Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) of the association between elevated total homocysteine levels (≥15.8 μmol/l, i.e. 66th percentile) and cognitive decline, adjusted for age, sex, education, alcohol consumption, smoking, and baseline MMSE score. There was a small nonsignificant relationship between total homocysteine and cognitive decline in the total group (adjusted OR=1.4, 95%CI: 0.7-2.8). In subjects <75 years and in women, high homocysteine level was more strongly associated with cognitive decline (OR=2.3, 95%CI: 1.0-5.6 and OR=2.2, 95%CI: 0.9-5.5, respectively). The associations did not change after additional adjustment for indicators of atherosclerosis.*

Conclusions – *Elevated total homocysteine levels may be associated with cognitive decline, particularly in women and subjects under 75 years of age. Since supplementation with folate is a safe way to reduce homocysteine levels, these results, when confirmed by other prospective studies, may have important implications to individuals at risk for cognitive decline.*

Introduction

The prevalence and incidence of dementia increases dramatically with age. Cognitive decline is one of the major symptoms of dementia. As the proportion of older people in our society increases, we can expect a rise in the number of people with cognitive decline. Therefore, it is important to search for modifiable risk factors. The amino acid, homocysteine, may be such a risk factor.[1] Several cross-sectional and longitudinal studies have shown that homocysteine is associated with an increased risk of stroke[2-5], coronary heart disease[2,6,7] and carotid artery atherosclerosis.[2,8,9] Both cardiovascular disease and carotid atherosclerosis have been related to cognitive impairment and dementia.[10-12] Homocysteine levels can be modified, as several intervention studies have shown a decrease in homocysteine concentration after supplementation with folate.[13] There have been two small cross-sectional studies that found an association between homocysteine and cognitive function.[14,15]

We investigated the association between non-fasting total homocysteine levels and cognitive decline in a random sample of 472 Dutch subjects from a prospective population-based study of older persons. In addition, we examined whether atherosclerosis was a possible mediating factor in the association between total homocysteine and cognitive decline.

Methods

Study population

Data are from the Rotterdam Study, which is a single-center prospective population-based study, designed to investigate determinants of selected chronic diseases and disabilities in older persons.[16] The conduct of the study was approved by the Medical Ethics Committee of Erasmus University and written informed consent was obtained from all participants. All residents of a suburb of Rotterdam, aged 55 years or over, were invited to participate. The baseline examination took place between 1990 and 1993. Of the 10,275 eligible subjects, 7,983 (78%) agreed to participate. During a home visit, trained interviewers administered a questionnaire covering, among other areas, sociodemographic background, medical history, and medication use. This was followed by two visits to the research center, where subjects underwent clinical examinations, including neuropsychological testing. Subjects living in homes for the elderly were visited at home. The follow-up examination took place in 1993 and 1994. Of the 7,215 subjects who were still alive, 6,315 (88%) participated in the follow-up examination.

We randomly selected 630 subjects from all those who performed a Mini-Mental State Examination (MMSE) at baseline and at follow-up (n=5535) for determination of serum total homocysteine. This sample did not differ from the population from which they were selected in age, sex or education. To increase power, we additionally selected a sample of subjects with a decline in the MMSE score of >1 point/year (n=75), resulting in a total sample size of 705 subjects. Our analyses were performed on 472 of the 705 respondents for whom complete information on total homocysteine, cognitive decline and all confounding and mediating factors was available. Indicators of atherosclerosis were missing for 231 subjects. These subjects were older (68.9 vs 67.4, p=0.04), had a lower median baseline MMSE score (28 vs 29, p=0.01), and less often consumed alcohol than subjects included in our analyses. Total homocysteine level, educational attainment, sex ratio, smoking habits, and prevalence of stroke did not differ between these groups (results not shown).

Based on the findings of a previous study[2], we assessed whether our results were affected by selective mortality and non-response. In order to do this, we randomly selected an additional sample, proportional to the percentage in the total study population, of subjects who had died during follow-up (n=56) or who for other reasons did not participate at the follow-up examination (n=67).

Homocysteine determination

At baseline, non-fasting serum samples were obtained. They were put on ice directly and were processed within sixty minutes, which has been shown to be sufficient to prevent increases in total homocysteine concentration due to ex vivo generation.[17] Serum was kept frozen at -20°C until determination of total homocysteine in 1995 and 1996. Average storage duration was 4.3 (SD=0.4) years for subjects without cognitive decline and 4.5 (SD=0.6) years for those with cognitive decline (p=0.01). Total homocysteine was determined at the clinical chemistry laboratory of the University Hospital Rotterdam, the Netherlands, as a fluorescence derivative, using HPLC according to Araki and Sako[18], as modified by Ubbink and co-workers.[19] A number of quality control samples was incorporated into runs. The estimation of the total homocysteine concentration of these samples had to be within two standard deviations of the level of the control serum. The within-run coefficient of variation ranged from 2.3% to 4.0% and the day-to-day coefficient of variation from 3.2% to 4.0%, for elevated and normal total homocysteine concentrations, respectively. A total homocysteine level of ≥15.8 μmol/l[7] (66th percentile) was a priori considered as an elevated level.

Mini-Mental State Examination

Global cognitive function was tested with the Netherlands version of the 30-point MMSE during the visit to the research center[20], as described earlier.[10] The test was administered by specially trained research assistants. The MMSE includes questions on orientation to time and place, registration, attention and calculation, recall, language and visual construction. This screening test was originally created for a clinical setting[20], and is extensively used in epidemiologic studies.[21] If less than four individual items (out of 20) were not answered by the subject, these were rated as errors.[22] If a subject did not answer four or more individual items, the total MMSE score was considered missing. Cognitive decline was defined as a drop in the MMSE score of >1 point/year (approximately >1 SD). Mean follow-up duration between the first and second MMSE was 2.9 years (SD = 0.4).

Other measurements

The following variables were considered as possible confounding variables: age (continuous); sex; cigarette smoking (current, former, never); alcohol consumption (none, <1 drink (13.2 g), 1-2 drinks, ≥3 drinks per day), assessed with a semi-quantitative food frequency questionnaire[23]; baseline MMSE score (continuous); level of education, grouped into four levels (completed primary education, lower vocational or general education, intermediate vocational or general education, and higher vocational training, college or university (Unesco, Paris, 1976)); and hypertension, which was defined in accordance with the WHO[24] as a systolic blood pressure of 160 mmHg or more, or a diastolic blood pressure of 95 mmHg or more, or the use of anti-hypertensive medication.

Given previous studies[8,9,11], atherosclerosis was considered as a possible intermediate of the association between total homocysteine and cognitive decline. Three indicators of atherosclerosis measured at baseline were used: thickening of the carotid artery intima-media wall (≥0.9mm), presence of plaques in the carotid arteries, and presence of peripheral arterial disease (ankle-brachial index <0.90). Plaques and wall thickness were assessed by ultrasonography of both carotid arteries using a 7.5 MHz linear array transducer and an ATL UM4 Duplex scanner, as described in detail elsewhere.[25] An average of three frozen images of both the left and the right near and far wall of the common carotid arteries was calculated. The common carotid artery and the carotid bifurcation were evaluated for the presence (yes/no) of atherosclerotic plaques, defined as any focal widening relative to adjacent segments with protrusion into the lumen.[26] The ankle-brachial index was obtained by dividing the systolic blood pressure of the posterial tibial artery by the brachial pressure on each side (the lowest value was used). The ankle pressure was measured with a Doppler probe (Huntleigh 500 D,

Huntleigh Technology, Bedfordshire, UK) and a random-zero sphygmomanometer.[25] To obtain a measure of total atherosclerotic burden a score was developed by assigning one point to the presence of each of the indicators of atherosclerosis.[2] The categories with two and three points were grouped together. We also examined whether stroke mediated the association between total homocysteine and cognitive decline. A history of stroke was considered present if a self-reported event was confirmed by either a detailed history, neuroimaging, or discharge reports collected from the general practitioner or neurologist.[27]

Statistical analysis

Differences in baseline characteristics, adjusted for age and sex, were tested with analysis of covariance. We used logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between total homocysteine and cognitive decline, adjusting for confounding variables. We investigated whether atherosclerosis or stroke were intermediates in the association between total homocysteine and cognitive decline, by including these variables separately as covariates in the model. To test for interaction between total homocysteine and sex, age, or hypertension their product terms were put in the model.[2,4,8]

To investigate selective mortality and non-response, we performed a sensitivity analysis in which we included the sample of those who were lost to follow-up in our analysis. The following two extreme options were examined: first, we assumed that those lost to follow-up would all have shown cognitive decline; secondly, we assumed that none of them would have shown cognitive decline. Since we expected selective loss to follow-up to be present in the older age group, we performed the sensitivity analyses in two age strata (<75 years and ≥75 years). All tests were two-sided and a p-value of less than 0.05 was considered to be statistically significant. Data analyses were performed using BMDP statistical software.

Results

The mean age of the participants at baseline was 67.4 years (SD = 7.0). Sixty-three percent of the sample was female. The median baseline MMSE score was 29 (range: 16 to 30) and the mean total homocysteine concentration was 15.4 μmol/l (SD = 9.7). After adjustment for sex, the average age was higher in subjects with elevated total homocysteine levels and after adjustment for age, the percentage of women was lower in these subjects (Table 1). A history of stroke was more frequent in subjects with hyperhomocysteinemia. Subjects with a drop in the MMSE score of >1 point/year more often had a primary education as the highest attained level. In addition, they less often consumed alcohol,

Table 1
Age and sex adjusted baseline characteristics and cognitive function according to total homocysteine level.

	Total homocysteine level		
	<15.8 µmol/L (n=310)	≥15.8 µmol/L (n=162)	p-value*
Mean MMSE score	28.2 (0.10)†	28.2 (0.14)	0.91
Mean age (yrs)	66.1 (0.39)	69.9 (0.54)	<0.001
Sex (% female)	68%	52%	0.001
Primary education (%)	38%	32%	0.23
Current smokers (%)	24%	29%	0.27
Alcohol consumers (%)	69%	70%	0.85
Mean alcohol consumption (g/day)‡	14.7 (1.22)	17.0 (1.69)	0.27
Intima-media thickness ≥0.9 mm (%)	13%	18%	0.17
Plaques in carotid arteries (%)	48%	47%	0.84
Peripheral arterial disease (%)	7%	9%	0.47
History of stroke (%)	3%	6%	0.09

MMSE = Mini-Mental State Examination
* Analysis of covariance
† Standard error between parentheses
‡ Among alcohol consumers (n=329)

and plaques in the carotid arteries were slightly more frequent (data not shown).

After adjustment for age, the association between high total homocysteine levels and cognitive decline was non-significant (OR=1.2, 95% CI:0.6-2.2). After additional adjustment for sex, education, alcohol consumption, smoking, and baseline MMSE score, the OR was 1.4 (95% CI: 0.7-2.8). In stratified analyses, high total homocysteine levels were more strongly associated with an increased risk of cognitive decline in subjects under 75 years of age and in women (Table 2). The p-values for interaction with age and sex were not significant, however (p=0.4 and p=0.2, respectively). There was no interaction with hypertension (p=0.9). When the atherosclerosis score was included in the model none of the above results essentially changed (Table 2). Neither did they change when the indicators were included in the model separately (results not shown). Adjustment for a history of stroke did not alter the associations either.

Sensitivity analyses

Subjects who died during follow-up had a higher age-adjusted total homocysteine concentration than those who participated at follow-up (18.6

Table 2
Adjusted odds ratios (95% confidence intervals) for cognitive decline* according to total homocysteine level.

Strata	Homo-cysteine	N	% Cognitive decline	OR (95% CI)	
				Model I	Model II
< 75 years	normal	272	6.6%	1 (ref)	1 (ref)
	high	123	9.8%	2.3 (1.0-5.6)	2.3 (0.9-5.5)
≥ 75 years	normal	38	23.7%	1 (ref)	1 (ref)
	high	39	15.4%	0.4 (0.1-2.3)	0.5 (0.1-2.7)
Men	normal	98	8.2%	1 (ref)	1 (ref)
	high	78	7.7%	0.7 (0.2-2.3)	0.5 (0.1-2.0)
Women	normal	212	9.0%	1 (ref)	1 (ref)
	high	84	14.3%	2.2 (0.9-5.5)	2.6 (1.1-6.5)

OR = odds ratio; CI = confidence interval
* Defined as a drop in the Mini-Mental State Examination score of >1 point/year
Model I: adjusted for age, sex, education, alcohol consumption, smoking, baseline Mini-Mental State Examination score
Model II: adjusted for age, sex, education, alcohol consumption, smoking, baseline Mini-Mental State Examination score, atherosclerosis score (thickening of the intima-media carotid artery wall, carotid plaques in either common carotid artery or carotid bifurcation, and presence of peripheral arterial disease)

vs 15.6 μmol/l, p=0.07), and a lower median baseline MMSE score (26 vs 29, p<0.01). Non-respondents at the follow-up examination had a lower median baseline MMSE score (26 vs 29, p<0.01), but no higher age-adjusted total homocysteine concentration (16.1 vs 15.6 μmol/l, p=0.7). Twenty-five (20%) of the subjects who died or did not respond at follow-up were younger than 75 years and had normal total homocysteine levels, 20 (16%) were younger than 75 years and had elevated total homocysteine levels, 29 (24%) were older than 75 years and had normal total homocysteine levels, and 49 (40%) were older than 75 years and had elevated total homocysteine levels.

If we placed all those who did not attend the follow-up examination in the cognitive decline groups, the ORs were 1.7 (0.9-3.1) for subjects <75 years, and 1.2 (0.4-3.0) for those ≥75 years (Table 3). If we placed them in the groups without cognitive decline, these ORs were similar to the results without the sensitivity analyses, 2.3 (1.0-5.3) for those <75 years and 0.3 (0.1-1.4) for those ≥75 years.

Table 3
Sensitivity analysis: adjusted odds ratios (95% confidence intervals)* for cognitive decline† according to total homocysteine level, with those lost to follow-up in the cognitive decline group.

Strata	Homo-cysteine	N	% Cognitive decline	OR (95% CI)
< 75 years	normal	297	14.5%	1 (ref)
	high	143	22.4%	1.7 (0.9-3.1)
≥ 75 years	normal	67	56.7%	1 (ref)
	high	88	62.5%	1.2 (0.4-3.0)
Men	normal	116	22.4%	1 (ref)
	high	102	29.4%	0.9 (0.4-2.1)
Women	normal	247	21.9%	1 (ref)
	high	130	43.8%	2.0 (1.1-3.8)

OR = odds ratio; CI = confidence interval
* Adjusted for age, sex, education, alcohol consumption, smoking, and baseline Mini-Mental State Examination score
† Defined as a drop in the Mini-Mental State Examination score of >1 point/year

Discussion

In this prospective population-based study of elderly subjects, there was a small nonsignificant association between elevated total homocysteine levels and subsequent cognitive decline. In women and in subjects under 75 years of age, total homocysteine was more strongly related to cognitive decline. These results were independent of differences in education, alcohol consumption and cigarette smoking.

There are a number of methodological issues that need to be discussed before we can interpret our findings. Total homocysteine was measured in non-fasting serum that had been stored for approximately four years. Serum total homocysteine is stable for at least ten years when stored at -20°C.[28] Still, differences in food intake before blood was obtained and reduction of total homocysteine levels over time stored can lead to random misclassification[17,28], which would dilute our estimates. The average storage duration was slightly longer in subjects with cognitive decline compared with those without. But the same technique and protocol were used for the estimation of total homocysteine during the whole study period and the day-to-day variation of the estimation was small. If anything, the longer storage duration would

have led to lower total homocysteine levels in subjects with cognitive decline, in which case our results would have been attenuated.

Another methodological issue is that the MMSE was not created to estimate change in cognitive function. One study examined the reliability of change in the MMSE in patients with dementia. They found that for a time interval between the MMSE's of one year or more the reliability was around 0.74, which is reasonable.[29] No such studies were performed in a general population, though. We defined cognitive decline as a drop in the MMSE of >1 point/year, which is on average >2.9 points for the total follow-up period. This change would be significant at the 5% level (one-tailed) for an individual, based on the standard error of measurement (1.96*SEM=2.6). If misclassification occurred, it probably has been random, leading to dilution of our results.

This study has a number of strong points. It is a prospective study, in which total homocysteine levels are measured at baseline and thus are not influenced by the outcome, cognitive decline. Furthermore, we took a random sample of subjects participating at baseline and at follow-up. And, we were able to investigate whether selective loss to follow-up biased our findings.

Subjects who died after the baseline examination had higher total homocysteine levels. Both subjects who died or who for other reasons did not participate at the follow-up examination were older and had a lower baseline MMSE score. Since we found no association between total homocysteine and cognitive decline in subjects over 75 years of age we were especially interested whether this might have been the result of selective loss to follow-up. Perhaps older subjects with elevated total homocysteine levels who died were more susceptible to the harmful effects of total homocysteine and those who were still alive less susceptible. When we included our random sample of those who were lost to follow-up in the cognitive decline group, the non-significant inverse association became a non-significant small positive association in subjects over 75 years of age. In those under 75 years the association was not slightly reduced, but still borderline significant. This suggests that especially in the older age group there may have been selective loss to follow-up, leading to alteration of our estimates.

Next to the stronger association between total homocysteine and cognitive decline in those under 75 years of age, we found a stronger relation among women. This modification is not likely to be due to an age difference of the sexes, since the proportion of subjects under 75 years of age did not differ between men and women. When we included the sample of those who were lost to follow-up in the cognitive decline group, the estimates for men did not change, suggesting that selective loss to follow-up is not a likely explanation for the lack of an association in men. Some other studies[8,30] and a meta-analysis[31] found that the association between homocysteine and cardiovascular events was

stronger in women than in men as well. Reasons for this are not clear. It was found that homocysteine levels were lower in healthy premenopausal women than in men of the same age and that homocysteine levels in women abruptly increased after menopause.[32] So men were exposed to elevated homocysteine levels for a longer period than women.

One of the mechanisms for an association between total homocysteine and cognitive decline may be atherosclerosis.[33,34] We investigated whether indicators of atherosclerosis were mediating factors in the association between total homocysteine and cognitive decline. But, the associations did not change when we entered these indicators into the model. The reason for this may be that in this sample the association between atherosclerosis and total homocysteine levels or between atherosclerosis and cognitive decline was not very strong, which may be due to the relatively small sample size. Another possible explanation for the fact that atherosclerosis did not attenuate our results may be that we used atherosclerosis data measured at the same time as the total homocysteine levels, instead of in between the total homocysteine and cognitive decline measurement.

Total homocysteine could be associated with cognitive decline through other mechanisms than atherosclerosis, such as thrombosis[35] or a direct neurotoxic effect.[36] Furthermore, elevated total homocysteine levels might be seen as an indicator of vitamin B12 and folate deficiency, which have been related to cognitive impairment and dementia in a number of studies[37-39], although the evidence of an association between these vitamins and cognitive impairment is not consistent.[14,37,40]

In summary, total homocysteine did not appear to be a major risk factor for cognitive decline in a general population of elderly. Total homocysteine seemed, however, to increase the risk of cognitive decline in women and subjects under 75 years of age. The non-significant inverse association in those over 75 years of age may be the result of selective survival. Since homocysteine is a modifiable risk factor, it is important that other larger prospective studies examine whether elevated homocysteine levels increase the risk of cognitive decline.

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Chapter

6

CORTISOL, DEHYDROEPI-ANDROSTERONE SULFATE, AND COGNITIVE FUNCTION

The Rotterdam Study

Abstract

Objective – *To investigate the relation between the peripheral concentrations of the adrenal steroid hormones cortisol and dehydroepiandrosterone sulfate (DHEAS) and cognitive impairment and decline.*
Design – *A prospective population-based study.*
Setting – *A suburb of Rotterdam, the Netherlands.*
Population – *A random sample of 189 participants from the Rotterdam Study, aged 55-80 years, who were invited for an additional examination at baseline. Follow-up examinations took place after an average of 1.9 years.*
Measurements – *Fasting blood levels of cortisol and corticosteroid-binding-globulin (CBG) before and after 1 mg dexamethasone overnight, and of DHEAS were obtained. The 30-point Mini-Mental State Examination (MMSE) was used to assess cognition. The association with cognitive impairment (MMSE score <26, 6% of the population) and the risk of cognitive decline (drop in MMSE score >1 point/yr, 24%) was estimated using logistic regression, with adjustment for age, sex, and education.*
Results – *The OR for the association with cognitive impairment according to one standard deviation (SD) increase in the cortisol/CBG ratio (SD=0.21), a measure of the 'free' cortisol level, was 1.7 (95%CI: 0.9-3.1). One standard deviation increase in the natural logarithm of cortisol after 1 mg of dexamethasone (SD=0.68) was associated with an increased risk of cognitive decline (OR=1.5 (95%CI:1.0-2.3)). One SD increase in DHEAS (SD=2.10 umol/l) was inversely but nonsignificantly related to cognitive impairment (OR=0.5 (95%CI:0.2-1.1)) and cognitive decline (OR=0.6 (95%CI:0.4-1.1)). The ratio of cortisol over DHEAS was significantly related to cognitive impairment (OR=1.8 (95%CI:1.2-2.8)).*
Conclusion – *This longitudinal study among elderly subjects suggests that there is a positive association between cortisol and cognitive impairment, and that high cortisol after dexamethason increases the risk of cognitive decline. There seemed to be an inverse association between DHEAS and cognitive impairment and decline.*

Introduction

Cortisol and dehydroepiandrosterone (DHEA) are adrenal steroid hormones with multiple effects on the function of the hippocampus, which is involved in learning and memory processes.[1,2] These adrenal steroids may thus be associated with cognitive impairment, which is a major symptom of dementia.

Hypothalamo-pituitary-adrenal (HPA) axis overactivity, which is related to stress and possibly also to aging, leads to increased cortisol levels.[3] Sustained cortisol exposure in rodents and primates results in damage of the hippocampus.[4] In human studies, increased cortisol levels and HPA axis overactivity have been associated with cognitive impairment and dementia.[3,5-11] These studies were, however, mostly cross-sectional, making it impossible to determine the direction of the association.

DHEA and its sulfate DHEAS are regarded as markers of aging.[12] DHEAS may affect the excitability and plasticity of hippocampal neurons.[2] In animal experiments DHEAS has been shown to enhance neuronal and glial survival and differentiation in culture[13,14], and injection of DHEAS into the brains of mice improved long-term memory.[13] There are a number of case-control studies on the association between DHEAS and Alzheimer's disease; however, these yielded conflicting results.[15-21] In one prospective population-based study there was no consistent association between DHEAS and subsequent cognitive function.[22]

In this prospective population-based study we examined whether peripheral levels of the steroid hormones cortisol and DHEAS are related to cognitive impairment and decline.

Methods

Study population

The Rotterdam Study is a single-centre prospective population-based study[23], designed to investigate determinants of chronic disabling diseases in the elderly. The conduct of the study was approved by the Medical Ethics Committee of the Erasmus University and written consent was obtained from all participants. All residents of Ommoord, a suburb in Rotterdam, aged 55 years or over, including those living in homes for the elderly, were invited to participate. The baseline examinations started in May 1990 and continued until June 1993. Of the 10,275 eligible subjects, 7983 (78%) agreed to participate. During a home visit, trained interviewers administered a questionnaire, covering, among other areas, sociodemographic background, medical history, and

medication use. This was followed by two clinical examinations at the research centre, including neuropsychological testing. The follow-up examination started in September 1993 and lasted until December 1994. Of the 7215 subjects who were still alive, 6315 (88%) agreed to participate.

For the present additional examination, a random sample (n=219) was taken of participants from the Rotterdam Study, who had completed the baseline examination in the preceding six months, were between 55 and 80 years old, had no history of psychiatric or endocrine diseases, including diabetes mellitus treated with medication. One subject who was using hydrocortisone and one subject who had a greatly elevated DHEAS level (15.1 umol/l) were excluded from the analyses. There were no differences in age, sex, or education between our sample and the other participants of the Rotterdam Study in the same age range and without dementia or known diabetes mellitus. The mean baseline Mini-Mental State Examination (MMSE) score was higher in our sample (28.1 (SD=1.6) vs 27.6 (SD=1.9), p=0.002). Complete information on cognitive function at baseline was available for 189 subjects. There were no differences in age, sex, cortisol or DHEAS levels between subjects with and subjects without complete information on cognitive function. Follow-up data on cognitive decline were available for 169 subjects. Compared with subjects who attended the follow-up examination (n=169), those who did not (n=20) were older (71.1 vs 66.9 years, p=0.002) and had a lower median MMSE score (27 vs 28, p=0.001). There were no differences in the cortisol/corticosteroid-binding-globulin (CBG) ratio (0.69 vs 0.72, p=0.70) or DHEAS levels (3.29 vs 3.38, p=0.75) between these groups.

Blood measurements

Blood was obtained after an overnight fast at the research centre between 8.00 and 9.00 AM, and allowed to coagulate for 30 minutes. Serum was separated by centrifugation and quickly frozen in liquid nitrogen. In addition, an overnight dexamethasone suppression test was performed.[24] Participants were given a tablet of 1 mg dexamethasone and were instructed to take this at 11.00 PM. Next morning, at the same time as the previous day, a fasting blood sample was obtained in the same manner. Measurements included cortisol and CBG before and after dexamethasone, and DHEAS before dexamethasone. Radioimmunoassays were used for determination of CBG (Medgenix Diagnostics, Brussels, Belgium) and of cortisol and DHEAS (Diagnostic Product Corporation, Los Angeles, CA, USA). The coefficients of variation of these assays were less than 12.0%, 12.5%, and 6.0%, respectively. To estimate the level of free circulating cortisol, the ratio of cortisol over CBG was used. Diminished suppression of cortisol after dexamethasone can be regarded as an indicator of HPA axis overactivity. Because the cortisol

level after dexamethasone was not normally distributed, we took the natural logarithm.

Mini-Mental State Examination

Global cognitive function was tested with the Dutch version of the 30-point MMSE during the (first) visit to the research centre.[25] It was administered by specially trained research assistants. The MMSE includes questions on orientation to time and place, registration, attention and calculation, recall, language and visual construction. This screening test was originally created for a clinical setting[25], and is extensively used in epidemiological studies.[26] Although it tests mainly cortical functions, these are important to daily functioning and severely affected in dementia. If less than four individual items (out of 20) were not answered by the subject, these were rated as errors.[27] If a subject did not answer four or more individual items the total MMSE score was considered missing. Cognitive impairment was defined as a score of <26[28], and cognitive decline as a drop in the MMSE score of >1 point/year (approximately >1 SD). Mean follow-up time between the first and second MMSE was 1.9 years (SD = 0.23).

Other measurements

A number of factors were considered as possible confounding variables: age, sex, and education, classified into four levels: completed primary education; lower vocational or general education; intermediate vocational or general education; and higher vocational training, college or university (Unesco, Paris, 1976). Body mass index (weight/height^2) was considered as a possible confounding factor, because low food intake and low body weight might induce relative resistance to the dexamethasone suppression test. Symptoms of depression were assessed at the follow-up examination with translated versions of the Center for Epidemiologic Studies Depression Scale (CES-D)[29] in the first 112 participants and the Hospital Anxiety and Depression Scale (HADS)[30] in the last 43 participants. In 14 participants, there was no depression scale available. We used the standard cut-off of ≥ 16 for the CES-D[29] and a cut-off of ≥ 8 for the HADS (after exclusion of the anxiety items)[30] to identify subjects with depressive symptoms. For the analyses on cortisol, estrogen users (n=2) and users of anti-epileptics (n=4) were excluded, because these medications can influence cortisol and/or CBG levels and the sensitivity to dexamethasone. Subjects were asked to show all vials of the medication they were currently using during the home visit. In the analyses on cognitive decline we additionally adjusted for baseline MMSE score.

Statistical analysis

Differences in baseline characteristics according to cognitive impairment and according to steroid hormone levels above and below the median were tested with the Mann-Whitney test for continuous and the Chi-square test for categorical variables. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the risk of cognitive impairment and decline. The independent variables of interest were total cortisol, the cortisol/CBG ratio, the natural logarithm of cortisol after dexamethasone, and DHEAS. We also investigated the ratio of cortisol over DHEAS, because DHEAS may antagonize the actions of cortisol.[31-33] We included confounding variables in the model. We also investigated whether there was effect modification by gender[34] by including the product term as covariate in the model. All tests were two-sided and a p-value of less than 0.05 was considered statistically significant. Data analyses were performed using BMDP statistical software.

Results

The mean age of the participants at baseline was 67.3 years (SD = 5.7). Fifty percent of the sample was female. The median baseline MMSE score was 28 (range=20-30) and 6.3% of the subjects was cognitively impaired. The mean drop in the MMSE per year was 0.22 (SD = 0.95) and 24% showed a drop in the MMSE score >1 point/year. Subjects with cognitive impairment were older, more often had symptoms of depression at follow-up, and had a lower DHEAS concentration (Table 1). Subjects with a total cortisol level above the median (506.5 nmol/l) had a lower body mass index than those below the median (25.8 (SD=3.8) vs 27.1 (SD=3.4), p=0.01). Subjects with a cortisol/CBG ratio above the median were older and less often female, which is the result of a higher CBG level in women (Table 2). Participants with DHEAS levels below the median were more often female (66% vs 34%, p<0.001).

Multiple logistic regression analyses showed that total cortisol levels were not related to cognitive impairment or decline (Table 3). Subjects with a higher cortisol/CBG ratio more often seemed to be cognitively impaired. CBG levels were not associated with cognitive function (data not shown). The natural logarithm of cortisol after dexamethasone was associated with an increased risk of cognitive decline. Subjects with high DHEAS levels less often seemed to be cognitively impaired. For one standard deviation increase in DHEAS level there was a 40% reduced risk of cognitive decline, although this was not significant. The ratio of cortisol over DHEAS was significantly associated with cognitive impairment (OR=1.8, 95%CI: 1.2-2.8). Adjustment for body mass index or symptoms of depression at follow-up did not change the results. Exclu-

Table 1
Baseline characteristics according to cognitive impairment.*
The Rotterdam Study.

	Cognitive impairment		
	Absent (n=177)	Present (n=12)	p-value†
Mean age (yrs)	67.1 (5.7)‡	71.3 (4.0)	0.01
Sex (% female)	50%	58%	0.56
Only primary education (%)	32%	42%	0.72
Body mass index (kg/m^2)	26.4 (3.7)	26.2 (3.7)	0.86
Depressive symptoms at follow-up (%)	8%	17%	0.03
Mean cortisol (nmol/l)§	512 (134)	544 (142)	0.45
Mean cortisol/CBG ratio§¶	0.70 (0.20)	0.80 (0.27)	0.16
Mean logarithm of cortisol after dexamethasone (nmol/l)§	3.12 (0.68)	3.32 (0.61)	0.29
Mean dehydroepiandrosterone sulfate (umol/l)	3.36 (2.09)	2.25 (2.00)	0.03
Mean cortisol/dehydroepiandrosterone sulfate ratio§	242 (238)	543 (645)	0.03

CBG = corticosteroid-binding-globulin
* Defined as a Mini-Mental State Examination score of <26 at baseline
† Mann-Whitney or Chi-square test
‡ Standard deviation in parentheses
§ Six subjects were excluded because they were using estrogens or anti-epileptics
¶ Used as an estimate of the free cortisol level

sion of subjects with depressive symptoms (n=16) essentially yielded the same estimates, but confidence intervals were wider because of the smaller sample size (results not shown). There were no significant interactions between the variables of interest and sex.

Additionally, we investigated the association with the memory item of the MMSE[35], since cortisol and DHEAS are believed to influence the function of the hippocampus, which is involved in memory processes. Fifty-three percent of the subjects scored less than three points on the recall of three words. There was no significant association of the cortisol/CBG ratio or DHEAS with memory impairment (OR=1.0, 95%CI: 0.7-1.4 and OR=1.2, 95%CI: 0.9-1.7, respectively).

Discussion

In this prospective population-based study we investigated the association between the peripheral concentrations of two adrenal steroid hor-

Table 2
Characteristics according to the cortisol/CBG ratio* above and below the median. The Rotterdam Study.

	Cortisol/CBG ratio		
	< 0.68 (n=89)†	≥ 0.68 (n=94)†	p-value‡
Mean age (yrs)	66.2 (5.8)§	68.3 (5.5)	0.04
Sex (% female)	58%	40%	0.01
Only primary education (%)	34%	29%	0.20
Body mass index (kg/m^2)	26.3 (3.5)	26.6 (3.9)	0.96
Depressive symptoms at follow-up (%)	5%	13%	0.11

CBG = corticosteroid-binding-globulin
* Used as an estimate of the free cortisol level
† In total, six subjects were excluded because they were using estrogens or anti-epileptics
‡ Mann-Whitney or Chi-square test
§ Standard deviation in parentheses

Table 3
Adjusted* odds ratios (95% confidence intervals) for the risk of cognitive impairment and decline† according to one standard deviation increase in total cortisol, the cortisol/CBG ratio, cortisol after dexamethasone, and dehydroepiandrosterone sulfate.

		Risk of	
	SD	Cognitive impairment (n=12/189)	Cognitive decline (n=40/169)
Total cortisol (nmol/l)	140.1	1.3 (0.7-2.3)‡	0.9 (0.6-1.3)‡
Cortisol/CBG ratio§	0.21	1.7 (0.9-3.1)‡	0.9 (0.6-1.3)‡
Cortisol after dexamethasone (nmol/l)¶	0.68	1.2 (0.7-2.1)‡	1.5 (1.0-2.3)‡
Dehydroepiandrosterone sulfate (umol/l)	2.10	0.5 (0.2-1.1)	0.6 (0.4-1.1)
Cortisol/dehydroepiandrosterone sulfate ratio	295	1.7 (1.1-2.6)‡	1.0 (0.6-1.6)‡

CBG = corticosteroid-binding-globulin; SD = standard deviation
* Adjusted for age, sex, and education (and baseline Mini-Mental State Examination score in the analyses on cognitive decline)
† Cognitive impairment: Mini-Mental State Examination score of <26 at baseline; and cognitive decline: drop in the Mini-Mental State Examination score of >1 point/year
‡ Six subjects were excluded because they were using estrogens or anti-epileptics
§ Used as an estimate of the free cortisol level
¶ After logarithmic transformation

mones and cognitive function. The results suggest that a high cortisol/CBG ratio, as an estimate of free cortisol, is related to cognitive impairment. The cortisol level after dexamethasone, as an indicator of HPA axis activity, was associated with an increased risk of cognitive decline. DHEAS, which may antagonize the actions of glucocorticoid, appears to be inversely related to cognitive impairment and decline. The ratio of cortisol over DHEAS was significantly related to cognitive impairment. These associations could not be explained by differences in age, sex, education, body mass index, or depressive symptomatology at follow-up.

Methodological issues

It could be argued that selection bias may have affected the validity of our results. Subjects were healthy and relatively young and follow-up duration was short, leading on average to only a small drop in the MMSE score. Combined with the small sample size, this would only impede the detection of a significant modest association. Furthermore, subjects who were lost to follow-up (due to death or non-response) had a significantly lower baseline MMSE score and were older than subjects who were not lost to follow-up. However, there were no differences in cortisol or DHEAS levels, making selective loss to follow-up less likely.

Another methodological issue is that we may not have adequately adjusted for depression. Depression is strongly associated with cognitive impairment[36] and also with increased cortisol levels and diminished suppression of cortisol after dexamethasone.[37] Thus, depression is potentially an important confounder. The use of two different questionnaires might not have been optimal. The CES-D has been designed for epidemiologic research, whereas the HADS has been designed for a clinical setting. Both have been validated, but the CES-D is probably more reliable, especially in this setting.[29] Furthermore, we adjusted for symptoms of depression at follow-up and not at baseline. Thus, there may be residual confounding in our results on cognitive impairment, but less in our results on cognitive decline.

Cortisol and HPA axis overactivity

This study represents the only prospective population-based study that we are aware of that investigated the association between cortisol and cognition. Our results confirm and extend the findings of previous studies. Increasing cortisol levels and diminished suppression of cortisol to dexamethasone have been associated with cognitive impairment and decline in small samples of healthy elderly subjects.[3,5,8] A number of case-control studies showed that HPA axis overactivity is more frequent and that cortisol levels are higher among patients with Alzheimer's disease.[5,11,38] In addition, cortisol has been associated with severity of

dementia and cognitive decline in demented patients.[9,10] Furthermore, patients with Cushing's syndrome more often present with cognitive impairment[39], whereas their memory deficits tend to recover after cure.[39] Finally, one of the side effects of treatment with synthetic corticosteroids has been cognitive deterioration.[40]

In experimental animal studies increased cortisol levels have been found to be neurotoxic to certain vulnerable nerve cells, e.g. those in the hippocampus.[4] The hippocampal neurons modulate HPA axis activity, i.e. they are involved in the negative feedback of glucocorticoid secretion. Damage to hippocampal cells therefore might lead to HPA axis overactivity and subsequently to increased cortisol levels. This is described as the 'glucocorticoid cascade' of aging.[41] Since hippocampal damage is one of the main neuropathological findings in Alzheimer's disease, it may be possible that Alzheimer's disease leads to HPA axis overactivity. It could however also be that stress-induced HPA axis overactivity and increased cortisol levels cause hippocampal damage and, subsequently, cognitive decline. But of course, both sequences of events may proceed at the same time. Our observation of an association between cortisol after dexamethasone and subsequent cognitive decline may support the latter pathway, but cortisol after dexamethasone may also be an early marker of the pathological processes behind Alzheimer's disease. For the cortisol/CBG ratio, which is considered to be representative of the biologically active free cortisol levels, we found an association with cognitive impairment, but not with cognitive decline.

Dehydroepiandrosterone sulfate

There has been one previous prospective population-based study that investigated the relationship between DHEAS and subsequent cognitive impairment, in which no consistent association was found.[22] Follow-up duration was quite long (16 years) and subjects with lower DHEAS levels at baseline had a higher mortality, suggesting there may have been selective survival bias. There have been a number of case-control studies on DHEAS levels and Alzheimer's disease. Some found that Alzheimer's disease patients had lower DHEAS levels than controls[15,16,42], but this could not be confirmed by others.[17-21] These studies were mostly based on small numbers of patients and controls, thereby increasing the possibility of false negative results. In the present study we found an association between DHEAS levels and cognitive decline, although not significantly, suggesting that decreased DHEAS levels may precede changes in cognition. This is in agreement with a small pilot study among patients with major depression, who showed improvement of selective memory functions after DHEA administration.[43]

Serum levels of DHEAS are the highest of all steroids in human beings, but the physiological function of DHEAS remains unknown. Cerebrospinal fluid levels of DHEA and DHEAS correlated significantly

with peripheral concentrations.[44] Animal experiments showed that DHEAS can enhance neuronal and glial survival and differentiation in culture[13,14], and that it can improve memory in mice.[13] The memory improvement may be explained by the fact that DHEAS affects the excitability and plasticity of the hippocampal neurons[2], possibly through induction of changes in GABAergic transmission.[45] Another explanation for the association with cognition may be that DHEA can be transformed into estrogens in peripheral tissues.[46] The risk of Alzheimer's disease has been shown to be reduced in women using estrogen compared with those who did not.[47] Furthermore, DHEA may act as a glucocorticoid antagonist, as described for a number of systems.[31-33] So a high ratio of cortisol over DHEAS may especially lead to hippocampal damage[16], and thus to cognitive impairment. Indeed, the present study showed a significant association between the cortisol/DHEAS ratio and cognitive impairment. Finally, DHEAS may be regarded as a marker of general health status and could therefore be related with cognitive function.

Conclusion

In conclusion, this small population-based prospective study indicates that there may be an association between adrenal steroid hormones and cognitive function. The cortisol/CBG ratio seemed to be associated with cognitive impairment and cortisol after dexamethasone increased the risk of cognitive decline. DHEAS was inversely, but not significantly, associated with cognitive impairment and decline. However, these results are preliminary and larger follow-up studies of longer duration are needed to verify these findings. It remains unclear if the observed cognitive impairment and decline are the direct result of the changing levels of the peripheral steroids, or vice versa, or if another factor influences both the adrenal steroid biosynthesis and the processes leading to reduced cognitive function.

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Part III

Genetics and cognition

Chapter 7

THE APOLIPOPROTEIN E4 ALLELE, EDUCATION, AND COGNITIVE DECLINE

The Zutphen Elderly Study

Introduction

A number of studies have shown that less educated subjects have an increased risk of cognitive decline.[1] Several biological mechanism have been postulated to explain these findings. Some have suggested that the activation of nerve cells in higher educated subjects protects these cells against degeneration[2], thereby delaying the pathological process leading to cognitive decline. Higher education could also lead to an increased brain reserve capacity, so that other neurons can take over the tasks of dead neurons.[3] This would retard the onset of cognitive decline. However, the association could be due to confounding by factors related to lifestyle.

The apolipoprotein e4 allele on chromosome 19 is an important risk factor for cognitive decline, as shown previously.[4] In the present study, we examine the association between education and cognitive decline in elderly men with and without the e4 allele, to see whether this genetic risk factor modifies the association between education and cognition.

Methods and results

The Zutphen Elderly Study is a longitudinal study on risk factors for chronic diseases in men living in Zutphen, the Netherlands (4). In 1985 939 men participated, and in 1990, 560 of 718 (78%) surviving men were re-examined. The examinations were repeated in 1993 on 390 of 553 (71%) surviving men. Complete information was available for 356 men participating both in 1990 and 1993.

Global cognitive function was tested with the Dutch version of the 30-point Mini-Mental State Examination. Cognitive decline was defined

Table.
Adjusted odds ratios for cognitive decline* according to level of education in the total group and in apolipoprotein e4 allele strata. The Zutphen Elderly Study, 1990-1993.

Education	n	Total population (n=356)	Carrier of the e4 allele	
			No (n=272)	Yes (n=84)
Adjustment for age				
> 6 yrs	313	1†	1†	1†
≤ 6 yrs	43	1.8 (0.8-4.0)‡	2.6 (1.0-7.1)	0.7 (0.2-3.1)
Adjustment for age and baseline MMSE				
> 6 yrs	313	1†	1†	1†
≤ 6 yrs	43	2.1 (0.9-4.9)	3.1 (1.1-8.8)	0.9 (0.2-3.8)

MMSE = Mini-Mental State Examination
* Defined as a drop of > 2 points on the Mini-Mental State Examination
† Reference
‡ 95% confidence interval between parentheses

as a drop of more than two points (>1 standard deviation) from 1990 to 1993, which corresponded to the 14th percentile of change. Years of formal education was obtained in 1990 and divided into two categories ≤ 6 and > 6 years). Serum samples were obtained in 1990 and frozen at -20°C until determination of the apolipoprotein E phenotype in 1993. The phenotype was determined by isoelectric focusing of delipidated plasma samples followed by immunoblotting. Logistic regression was used to examine the association between education and cognitive decline in the total group and the two apolipoprotein e4 strata, adjusting for age and baseline Mini-Mental State Examination score.

The mean age of the participants in 1990 was 74.6 (standard deviation = 4.2) years. The median Mini-Mental State Examination score was 27 (10th centile: 23, 90th centile: 29). The association between education and cognitive decline was strong in non-carriers of e4, but absent in carriers of e4 (table). The test for interaction was not significant (p-value = 0.11). Additional adjustment for a history of cardiovascular diseases did not change these results. The association between education and cognitive impairment in 1990 was similar in 1993 non-participants and 1993 participants, within strata of the e4 allele (results not shown).

Discussion

We observed a significant increased risk of cognitive decline associated with a lower level of education in subjects without an apolipoprotein e4 allele. In contrast, there was no association between education and cognitive decline in carriers of the e4 allele.

Several explanations can be offered for the lack of an association with three-year cognitive decline in subjects carrying an e4 allele. It may be due to selective survival and non-response. However, our data do not suggest that lower educated people with e4 who did not participate in 1993 had a different risk of cognitive impairment than those who participated. We do not know though, whether the risk of cognitive decline differed between the groups. Furthermore, the e4 allele may be such a strong risk factor for cognitive decline, that cognitive performance in carriers of an e4 allele will deteriorate, regardless of educational level. Alternatively, apolipoprotein e4 may play a role in inhibiting neuronal growth[5], which in turn can play a role in the development of Alzheimer's disease[2]; e4 may thus block the putative stimulating effect of education on neuronal growth. Finally, we cannot exclude the possibility that our results are a chance finding.

In conclusion, the results of the present study suggest that the apolipoprotein e4 allele may modify the association between education and cognitive decline. Since this study was relatively small, our findings need to be confirmed by larger studies.

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Chapter

8

THE APOLIPOPROTEIN E4 ALLELE, CEREBROVASCULAR DISEASE, AND COGNITIVE DECLINE

The Zutphen Elderly Study

Abstract

Background and purpose. *Cerebrovascular disease and the apoliprotein e4 (APOE*4) allele are both important risk factors for cognitive decline. We investigated the combined effect of APOE*4 and cerebrovascular disease on cognitive decline.*

Methods. *Data are from a cohort of 353 men, aged 69-89 years at baseline, living in Zutphen, the Netherlands. The 30-point Mini-Mental State Examination (MMSE) was used to measure cognitive decline (drop of >2 points) from 1990 to 1993 (14% of the sample). Odds ratios (OR (95% confidence interval)) for cognitive decline were adjusted for age, education and baseline MMSE score.*

Results. *Compared with those without APOE*4 and without a history of cerebrovascular disease, the adjusted OR was 4.7 (1.7-12.7) for subjects without APOE*4 but with cerebrovascular disease, 3.3 (1.6-6.8) for those with APOE*4 and no cerebrovascular disease, and 17.2 (2.7-110.0) for those with both risk factors. The risk for cerebrovascular disease and APOE*4 combined was more than expected from the separate effects. The combined risk of coronary heart disease and APOE*4 was 6.1 (1.7-22.3). The analysis of cardiovascular risk factors showed that the risk of cognitive decline was highest in subjects with both APOE*4 and a high cholesterol level, high fibrinogen level, normal blood pressure, or diabetes mellitus.*

Conclusions. *Cerebrovascular disease and APOE*4 may have a synergistic effect on cognitive decline.*

Introduction

Cerebrovascular diseases have been associated with an increased risk of cognitive impairment and vascular dementia.[1,2] Other cardiovascular diseases, such as coronary heart disease and peripheral arterial disease, have also been related to cognitive impairment[2] or vascular dementia.[3] Furthermore, several studies observed an association of cognitive impairment to cardiovascular risk factors, such as hypertension, diabetes mellitus, total cholesterol level, and fibrinogen level.[4-8]

In addition, several studies have shown that the apolipoprotein e4 allele (APOE*4) on chromosome 19 is an important risk factor for Alzheimer's disease.[9-11] This was also demonstrated for cognitive impairment, which is a major component of dementia.[12-14] Feskens et al.[12] estimated that 22% of the incident cases of cognitive impairment in a community-based sample of elderly men were attributable to the APOE*4 allele and that subjects with APOE*4 were at a two fold increased risk of developing impaired cognitive function. Furthermore, APOE*4 has been related to cardiovascular diseases and their risk factors. Apolipoprotein E is a constituent of plasma chylomicrons, lipoproteins and their remnants. It serves as a ligand for their receptor mediated uptake. Total and LDL cholesterol concentrations are higher in subjects carrying the APOE*4 allele.[15] Carriers of APOE*4 may also have an increased risk of coronary heart disease[15-17] and cerebrovascular disease.[18]

In this study we examined whether the combined effect of cardiovascular diseases and the APOE*4 allele may lead to a higher risk of cognitive decline than would be expected from the separate effects. The existence of subgroups with an especially high risk may throw light on the etiology of cognitive impairment and could have important implications for preventive intervention. These relations were examined in the context of a community-based longitudinal study of elderly men.

Methods

Study population

The Zutphen Elderly Study is a longitudinal study of risk factors for chronic diseases in men living in Zutphen, a town in the eastern part of the Netherlands.[19] It is the continuation of the Zutphen Study, which was initiated in 1960 as the Dutch contribution to the Seven Countries Study.[20] In 1985, 1266 men were asked to participate, 555 from the original cohort and 711 from a randomly selected sample from all other men living in Zutphen in the same age range. In 1985, 939 agreed to participate, and in 1990, 560 of 718 surviving men (78%) were

re-examined. The examinations were repeated in 1993 on 390 of 553 surviving men (71%). Complete information on all risk factors was available for 353 men participating in both 1990 and 1993. The study has been approved by the medical ethics committee of the University of Leiden, the Netherlands and informed consent was obtained from all participants.

Mini-Mental State Examination

Global cognitive function was tested with the Dutch version of the 30-point Mini-Mental State Examination (MMSE).[21] The MMSE includes questions on orientation to time and place, registration, attention and calculation, recall, language and visual construction. This screening test was originally created for a clinical setting[21], and is extensively used in epidemiological studies.[22] Although it tests a limited set of cognitive functions, these are important to daily functioning and severely affected in dementia. In 1990, it was administered in a controlled hospital setting, and in 1993 it was administered at the subject's homes. If fewer than four individual items (of a total of 20 items) were not answered by the subject, these were rated as errors[23], unless items could not be performed because of severe physical disability, in which case a weighted total score was given. If a subject did not answer four or more individual items the total MMSE score was considered missing. Before we performed the analyses, we defined the cut-off point for cognitive decline as a drop in the MMSE of >2 points (>1 SD) from 1990 to 1993, which corresponded to the 14th percentile of change. In test-retest studies it was found that the maximum difference between two mean measurements was 2.1 points with a standard error varying between 0.4 and 0.7.[24]

Apolipoprotein E phenotype

During the examination in 1990 serum samples were obtained and frozen at -20°C until determination of phenotype in 1993. For 19 subjects samples stored at the same temperature from 1985 were used. The apolipoprotein E phenotype was determined by isoelectric focusing of delipidated plasma samples followed by immunoblotting.[25] The use of stored serum for this purpose has been judged to be valid.[25] Because of a relatively small sample size, homozygotes (2.5%) and heterozygotes (20.7%) for the APOE*4 allele were considered together.

Cardiovascular disease

In the Spring of 1990 interviews and physical examinations by trained physicians were performed at home and in a study center. History of myocardial infarction, angina pectoris and intermittent claudication was obtained from the Dutch translation of a questionnaire developed at the

London School of Hygiene and Tropical Medicine.[26] A history of stroke, transient ischemic attack and diabetes was assessed with a standardized questionnaire. Medical records, including electrocardiograms, hospital discharge data and written information from general practitioners, were collected to verify diagnoses. All information was uniformly coded by two trained medical staff members. Cerebrovascular disease was considered present when either stroke or transient ischemic attack was diagnosed, and coronary heart disease was considered present when either myocardial infarction or angina pectoris was diagnosed. Diabetes mellitus was defined as known or newly diagnosed diabetes, as defined by a fasting glucose concentration >7.8 mmol/l or a 2-hour post-load glucose concentration >11.1 mmol/l.[27]

Cardiovascular risk factors

Systolic and diastolic (fifth Korotkoff phase) blood pressure were measured with a random zero sphygmomanometer in duplicate on the right arm with the subject in supine position at the end of the physical examination. The mean of the two blood pressure values was used in the analyses. Hypertension was defined as a systolic blood pressure ≥160 mmHg or a diastolic blood pressure ≥95 mmHg or the use of anti-hypertensive medication, regardless of blood pressure level.[28] Blood was taken to determine the concentrations of lipids and hemostatic factors. Total and HDL cholesterol were analyzed in non-fasting blood samples by the standardized Lipid Laboratory at the Department of Human Nutrition, Wageningen Agricultural University, The Netherlands. Serum cholesterol was determined enzymatically with the CHOD-PAP monotestkit from Boehringer Mannheim.[29] It was dichotomized and a concentration of ≤6.5 mmol/l was taken as the reference category. Serum HDL cholesterol was determined enzymatically after precipitation of apolipoprotein B containing particles by dextran sulphate-Mg^{2+}.[30] HDL cholesterol was dichotomized and a concentration of ≤0.9 mmol/l was used as the reference category. Fibrinogen concentration was determined in citrate plasma by the method of Clauss.[31] Analyses were performed at the Laboratory of the Department of Human Biology, University of Limburg, Maastricht, The Netherlands. We used the upper tertile of fibrinogen (3.8 g/l) as cut-off point, because there is no consensus on this matter. Age (continuous) and education (≤6 years, 7-12 years and >12 years of education) were considered as confounding variables.

Statistical analysis

Differences in baseline characteristics were evaluated between carriers, defined as either homozygote or heterozygote for the APOE*4 allele, and non-carriers. We used the nonparametric Mann-Whitney test for continuous variables, and the Chi-square test for categorical variables.

The proportion of men with cognitive decline according to the presence or absence of cardiovascular factors was calculated for the total group and for the two APOE*4 strata separately. These percentages were adjusted for age and education by multiple linear regression analysis.

Next, we investigated whether the combined effect of the APOE*4 allele and cardiovascular factors on cognitive decline was different from the separate effects.[32] We categorized subjects into four groups: subjects without the APOE*4 allele and the risk factor (reference group); subjects without APOE*4 and with the risk factor; subjects with APOE*4 and without the risk factor; and subjects in whom both APOE*4 and the risk factor were present. These groups were entered into a multiple logistic regression model as dummy variables. We adjusted for age, education, and baseline MMSE score. We additionally adjusted for the score on the self-rating depression scale that was available for a subgroup. Since the results did not essentially change we decided not to present the results with adjustment for depression.

We examined whether subjects who did and did not participate in 1993 differed at baseline (1990). In addition, we investigated whether the relation between cardiovascular diseases, APOE*4, and cognitive impairment at baseline was different for those who dropped out of our study population compared with those who did not. All tests were two-sided and a value of $p<0.05$ was considered statistically significant. The SAS-program, version 6.10, was used.

Results

The mean age of the participants at baseline (1990) was 74.6 (SD = 4.2) years. The median MMSE score was 27 (10th centile: 23, 90th centile: 29). Fourteen percent (n=51) showed a drop in the MMSE of >2 points. Carriers of the APOE*4 allele more often showed cognitive decline and had a higher serum cholesterol concentration (Table 1).

In the total group, a history of cerebrovascular disease was significantly associated with cognitive decline, after adjustment for age, education and baseline MMSE score (OR=4.3, 95% confidence interval (CI): 1.8-9.9). A history of coronary heart disease slightly increased the risk of cognitive decline (OR=1.7, 95% CI: 0.8-3.5). Intermittent claudication and cardiovascular risk factors were not significantly associated with cognitive decline in the total group.

After adjustment for age and education, the proportion of subjects with cognitive decline in those without APOE*4 and without a history of cerebrovascular disease was 9%, and in those without APOE*4 and with cerebrovascular disease it was 32% (Table 2). Therefore, among non-carriers, the difference in the percentage of men with cognitive decline in those with cerebrovascular disease and those without was

Table 1
Baseline characteristics in 1990 according to presence of the APOE*4 allele. The Zutphen Elderly Study (1990-1993).

Variable	APOE*4 allele		p-value
	Absent (n=271)	Present (n=82)	
Baseline MMSE score	26.5 (2.5)*	26.1 (2.6)	0.2
Age (yrs)	74.7 (4.2)	74.5 (4.3)	0.7
Higher education (>12 yrs)	25%	20%	0.3
History of:			
• Cerebrovascular disease	9 %	7 %	0.7
• Coronary heart disease	19 %	15 %	0.3
• Intermittent claudication	9 %	6 %	0.4
Hypertension†	40 %	34 %	0.4
Diabetes mellitus‡	13 %	18 %	0.3
Total cholesterol (mmol/l)	6.05 (1.08)	6.42 (1.17)	0.01
HDL cholesterol (mmol/l)	1.15 (0.28)	1.13 (0.27)	0.7
Fibrinogen (g/l)	3.7 (0.4)	3.7 (0.3)	0.7

MMSE = Mini-Mental State Examination; APOE*4 = apolipoprotein e4 allele
* Standard deviation between parentheses
† Defined as diastolic blood pressure ≥95 mmHg and/or systolic blood pressure ≥160 mmHg or use of antihypertensive medication
‡ Known and newly diagnosed diabetes

23%. In the stratum of APOE*4 carriers, the proportion of subjects with cognitive decline in those without cerebrovascular disease was 22%, and in those with cerebrovascular disease it was 66%

Thus among APOE*4 carriers, the difference in the percentage of men with a decline in those with cerebrovascular disease and those without was 44%. This percentage (44%) was approximately twice as high as in the non-carriers (23%). The difference in percentage of men with cognitive decline in those with and without coronary heart disease was also two times higher in carriers of APOE*4 compared with the non-carriers (13% vs 6%). Intermittent claudication was not associated with cognitive decline among non-carriers, but 11% more subjects experienced cognitive decline among APOE*4 carriers, which was not significant.

The results of the logistic regression analysis, in which subjects without the cardiovascular disease and without the APOE*4 allele were taken as the reference group, are shown in Figure 1. The presence of both the APOE*4 allele and cerebrovascular disease increased the risk of cognitive decline substantially to 17.2 (95% CI: 2.7-110.0) (Figure

Table 2

Proportion of men with cognitive decline* according to the history of cardiovascular diseases in the total group and APOE*4 strata, adjusted for age and education. The Zutphen Elderly Study.

	Total population (n=353)			Carrier of the APOE*4 allele					
				No (n=271)			Yes (n=82)		
History of disease:	No	Yes	Difference†	No	Yes	Difference	No	Yes	Difference
Cerebrovascular disease	12% (323)‡	38% (30)	26%§	9% (247)	32% (24)	23%§	22% (76)	66% (6)	44%¶
Coronary heart disease	13% (289)	20% (64)	7%	10% (219)	16% (52)	6%	24% (70)	37% (12)	13%
Intermittent claudication	14% (323)	14% (30)	0%	11% (246)	11% (25)	0%	25% (77)	36% (5)	11%

APOE*4 = apolipoprotein E e4 allele

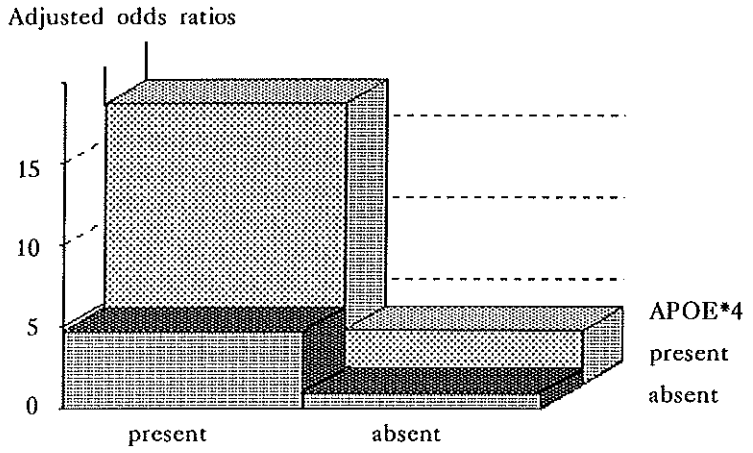
* Defined as a drop of >2 points on the Mini-Mental State Examination

† Difference in the proportion of men with cognitive decline between those with and without the cardiovascular disease

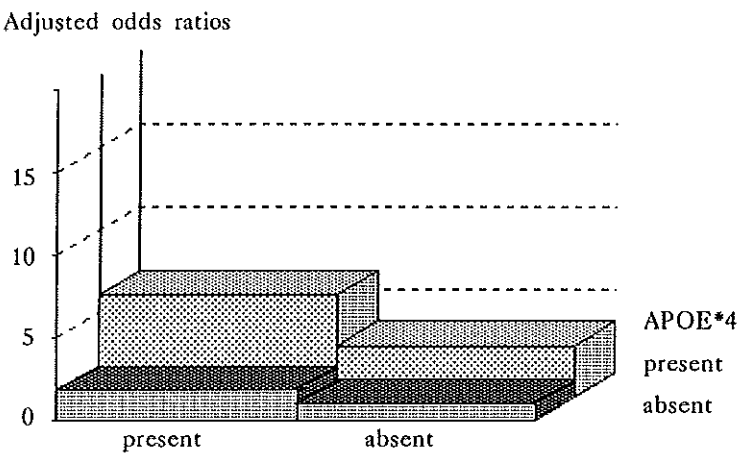
‡ Number of men in this group

§ $p<0.001$, for the difference in proportion of cognitive decline comparing subjects with and without disease

¶ $p<0.05$, idem



A. Cerebrovascular disease



B. Coronary heart disease

Figure 1
Interaction between APOE*4 and cardiovascular diseases – The Zutphen Elderly Study.
ORs are adjusted for age, education and baseline Mini-Mental State Examination score; no APOE*4 and no disease = reference. APOE*4 = apolipoprotein E e4 allele.

1A). This OR was more than would be expected from the sum of the separate ORs (4.7 and 3.3), suggesting that the effect of the two factors together on cognitive decline was synergistic.[33] The same synergy, although not as strong, may be present for coronary heart disease and the APOE*4 allele (Figure 1B). The OR for subjects with APOE*4 and coronary heart disease was 6.1 (95% CI: 1.7-22.3). Intermittent claudication did not seem to be related to cognitive decline, whether APOE*4 was present or not (results not shown).

The analysis of cardiovascular risk factors showed that in the APOE*4 stratum, there were proportionally more men with cognitive decline in the high than in the low category of total cholesterol, HDL cholesterol and fibrinogen, although these differences were not significant (Table 3). None of the ORs of the cardiovascular risk factors were significantly increased in the absence of APOE*4 (Table 4). The risk of cognitive decline was highest in subjects with both APOE*4 and a high cholesterol or high fibrinogen level or diabetes mellitus. On the other hand, the risk of cognitive decline appeared to be lower in subjects with APOE*4 and hypertension than in those with APOE*4 and without hypertension.

Cognitive impairment in 1990 (MMSE score <26) and a history of major cardiovascular diseases (stroke or myocardial infarction) was more frequent in those who did not participate in 1993 than in those who did (results not shown). The APOE*4 frequency was not significantly different between these groups. In both non-participants and participants the percentage of men with cognitive impairment at baseline tended to be higher in those with an APOE*4 allele than in those without (42% vs 36% and 38% vs 27%, respectively). In both non-participants and participants the percentage of men with cognitive impairment did not differ significantly between those with a history of cardiovascular disease and those without (45% vs 39% and 26% vs 30%, respectively). There was no significant interaction between participation status and one of these risk factors ($p>0.1$).

Discussion

Our study was a community-based prospective study in which the change in cognitive function was measured. To our knowledge, this is one of the first studies that investigated the combined effect of cardiovascular diseases and APOE*4 on cognitive decline. We found that APOE*4 carriers who suffered a stroke or transient ischemic attack had a particularly increased risk of cognitive decline, which was more than could be expected from the separate effects of these risk factors combined. This suggests synergism.[33] The same synergy may be present between coronary heart disease and APOE*4, although this was less

Table 3
Proportion of men with cognitive decline* according to the presence of cardiovascular risk factors in the total group and APOE*4 strata, adjusted for age and education. The Zutphen Elderly Study.

	Total population (n=353)			Carrier of the APOE*4 allele					
				No (n=271)			Yes (n=82)		
Risk factor present:	No	Yes	Difference†	No	Yes	Difference	No	Yes	Difference
Hypertension‡	14% (217)§	15% (136)	1%	10% (163)	13% (108)	3%	27% (54)	22% (28)	-5%
High total cholesterol	13% (237)	17% (116)	4%	11% (191)	12% (80)	1%	21% (46)	31% (36)	10%
High HDL cholesterol	11% (73)	15% (280)	4%	8% (52)	12% (219)	4%	20% (21)	28% (61)	8%
High fibrinogen	15% (235)	14% (118)	-1%	12% (178)	10% (93)	-2%	23% (57)	32% (25)	9%
Diabetes mellitus¶	14% (282)	11% (47)	-3	12% (216)	3% (33)	-9%	24% (66)	29% (14)	5%

APOE*4 = apolipoprotein E e4 allele
* Defined as a drop of >2 points on the Mini-Mental State Examination
† Difference in the proportion of men with cognitive decline comparing those with and without the cardiovascular risk factor
‡ Defined as diastolic blood pressure ≥95 mmHg and/or systolic blood pressure ≥160 mmHg or use of antihypertensive medication
§ Number of men in this group
¶ Known and newly diagnosed diabetes; analyses on 329 men due to missing values

Table 4
Odds ratios for cognitive decline* according to presence of cardiovascular risk factors and APOE*4, adjusted for age, education, and baseline Mini-Mental State Examination score. The Zutphen Elderly Study.

	Carrier of the APOE*4 allele			
	No (n=271)		Yes (n=82)	
Risk factor present:	No	Yes	No	Yes
Hypertension†	1.0	1.3 (0.6-2.8)‡	3.7 (1.6-8.6)	2.7 (0.9-8.3)
High total cholesterol	1.0	1.0 (0.4-2.6)	2.5 (1.0-6.0)	4.0 (1.6-10.0)
High HDL cholesterol	1.0	2.0 (0.6-7.1)	3.7 (0.7-19.2)	6.2 (1.6-23.6)
High fibrinogen	1.0	0.9 (0.4-2.1)	2.6 (1.2-6.0)	3.6 (1.3-10.4)
Diabetes mellitus§	1.0	0.2 (0.03-1.9)	2.7 (1.3-5.7)	4.0 (1.0-15.8)

APOE*4 = apolipoprotein E e4 allele
* Defined as a drop of >2 points on the Mini-Mental State Examination
† Defined as diastolic blood pressure ≥95 mmHg and/or systolic blood pressure ≥160 mmHg or use of antihypertensive medication
‡ 95% confidence interval between parentheses
§ Known and newly diagnosed diabetes; analyses on 329 men due to missing values

clear. These results were independent of age, education, baseline MMSE score and depression. However, there is still a possibility that our results are due to residual confounding.

The MMSE is a reliable and valid indicator of cognitive impairment.[34] We used the MMSE to assess change in cognitive function, for which it was not originally created. One study examined the reliability of change in the MMSE in patients with dementia. They found that for a time interval between the MMSE's of one year or more the reliability was approximately 0.74, which is reasonable.[35] We chose a cut-off point of a drop in the MMSE of >2 points, which may not be pathologically significant on an individual level, but can be of major importance on a population level.

The synergism we observed may suggest that APOE*4 potentiates the effect of cerebrovascular disease on cognitive decline. Frisoni et al.[36] hypothesized that different insults, either degenerative or vascular, might result in greater damage when a particular apolipoprotein E isoform allele is present. The possibility that cerebrovascular disease is more harmful in carriers of APOE*4 is suggested in a study showing that after

hemorrhagic stroke the functional neurological outcome in subjects with an APOE*4 allele was worse and survival reduced, compared with subjects with no APOE*4 allele.[37] This may result from reduced neuronal repair in APOE*4 carriers.[38] Alternatively, cerebrovascular disease may potentiate the effect of APOE*4 on cognitive decline by increasing the susceptibility of the brain to the effects of APOE*4. Coronary heart disease and peripheral arterial disease are associated with an increased risk of cerebral damage. Thus, the same mechanisms may account for the association between these diseases, APOE*4 and cognitive decline. However, in this study there was no obvious synergism between coronary heart disease and APOE*4. In addition, intermittent claudication was not clearly associated with cognitive decline, irrespective of APOE*4. This may be due to the relatively small number of subjects with intermittent claudication in our study and to the fact that our diagnosis was based on typical symptoms and information on peripheral atherosclerosis was lacking.

Total cholesterol, fibrinogen, hypertension and diabetes mellitus were not independently associated with cognitive decline. However, the data suggest that the risk of cognitive decline was increased in subjects with APOE*4 and one of these cardiovascular risk factors, except for hypertension. Perhaps these risk factors have an effect on cognition only when the brain tissue is altered by the effects of APOE*4. Our results on total cholesterol correspond to a recent case-control study, which suggested that the association between apolipoprotein E genotype and Alzheimer's disease was stronger when cholesterol levels were higher.[39] We found that high HDL cholesterol was insignificantly associated with an increased risk of cognitive decline, irrespective of APOE*4. Additional adjustment for cigarette smoking and alcohol consumption did not essentially alter this association, while additional adjustment for cholesterol reduced it only slightly. Several other studies have not found an association between cognitive function and HDL cholesterol.[14,40] We can not exclude the possibility that this is a chance finding.

We investigated the possibility of selection bias due to non-participation. The association between APOE*4 and cognitive impairment in 1990 was not different for those who participated in 1993 compared with those who did not, nor was the association between a history of cardiovascular disease and cognitive impairment. This suggests that our results were probably not biased by selective non-participation.

From this study we conclude that cerebrovascular disease and the APOE*4 allele may have a synergistic effect on cognitive decline. In addition, coronary heart disease and cardiovascular risk factors may interact with APOE*4 to increase the risk of cognitive decline above and beyond the effect of APOE*4 alone. This study suggests that genetic risk factors are important to consider when studying the association between cardiovascular diseases and cognitive function. Additional studies

will be needed to determine whether synergism is also suggested when the risk of dementing disorders such as Alzheimer's disease and vascular dementia is examined.

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Part IV

Survival and cognition

Chapter

9

DEMENTIA AND CAUSE-SPECIFIC MORTALITY

The Rotterdam Study

Abstract

Background – *Dementia has been associated with an increased mortality, but few studies investigated cause-specific mortality. We investigated the association between subtypes of dementia and total and cause-specific mortality.*

Methods – *Data are from the population-based Rotterdam Study. Participants were aged 55 years or over and living in a suburb of Rotterdam. At baseline we screened for dementia with a three step protocol, that included a clinical examination. Subdiagnoses were made according to international criteria. Causes of death were obtained from general practitioners and medical records. The risk for mortality (RR (95% CI)) was assessed with proportional hazards analysis, adjusting for age, sex, education, smoking, and alcohol consumption. After a mean follow-up time of four years (SD=1.2), 751 (10.8%) of the 6985 subjects with information on prevalent dementia and with complete follow-up had died.*

Results – *Subjects with dementia had an increased risk of dying (RR=2.0 (1.6-2.4)). This risk was 2.9 (1.9-4.3) for subjects with vascular dementia, and 1.8 (1.5-2.3) for subjects with Alzheimer's disease. In addition, the age-adjusted percentage of subjects that died from cancer was lower among demented than non-demented subjects (13.3% vs 24.9%, p<0.01), whereas the percentage that died from noncardiovascular-noncancerous causes was higher among demented subjects (49.2% vs 31.3%, p<0.001).*

Conclusion – *This study suggests that there is a shift in the distribution of the causes of death among demented subjects towards less deaths from malignancies and more deaths from noncardiovascular-noncancerous causes.*

Introduction

Dementing diseases are associated with an increased mortality[1-5], with mortality risks varying from 1.4 to 5.4 for Alzheimer's disease.[3-5] The number of life years lost due to dementia is considerable.[6] For the next 50 years, the number of deaths attributable to dementia in the United States is expected to increase by 180% as a result of demographic changes.[7]

For relatives and caregivers of demented subjects and for clinicians and planners of health care facilities it is important to have detailed information on the prognosis of dementia. To shed more light on the increased mortality of demented subjects, we examined the association between (subtypes of) dementia and cause-specific mortality. These relations were studied in a population-based follow-up study of elderly subjects.

Methods

Study population

We used data from the Rotterdam Study, a single-centre prospective population-based study, designed to investigate determinants of selected chronic diseases and disabilities in older persons.[8] The conduct of the study was approved by the Medical Ethics Committee of Erasmus University and written consent was obtained from all participants. All residents of the Ommoord suburb of Rotterdam, the Netherlands, aged 55 years or over, were invited and of the 10,275 eligible subjects, 7,983 (78%) agreed to participate. Baseline data were collected from May 1990 to June 1993. During a home visit, trained interviewers administered a questionnaire, covering, among other areas, sociodemographic background, medical history, and medication use. This was followed by two clinical examinations at the research centre. Subjects living in nursing homes were visited at home.

Dementia case-finding

Prevalence of dementia was ascertained on the basis of a study examination, in which 7528 subjects participated. Screening consisted of a three stage procedure.[9] First, all participants were screened with the 30-point Mini-Mental State Examination[10] and the short version of the Geriatric Mental State schedule (GMS-A, organic level).[11] Subjects with an MMSE score below 26, or a GMS score greater than 0 were considered screen-positive. They were subsequently examined with the Cambridge Examination of Mental Disorders in the Elderly (CAMDEX), which included an informant interview.[12] Participants who were judged

to be demented or suspected of dementia after the CAMDEX, were examined by a neurologist and tested by a neuropsychologist; a part also underwent magnetic resonance imaging (MRI) of the brain. In addition, medical files were used to diagnose dementia. Dementia was diagnosed according to the American Psychiatric Association's criteria (DSM-III-R).[13] The subdiagnosis of Alzheimer's disease was based on NINCDS-ADRDA criteria.[14] The presence of cerebrovascular disorders did not prohibit a diagnosis of Alzheimer's disease. In accordance with above-mentioned criteria, a subdivision was made into Alzheimer's disease patients with and without cerebrovascular disease. In Alzheimer's disease patients with cerebrovascular disease, this disease was not considered to be directly etiologically related to the dementia. Vascular dementia was diagnosed conform NINDS-AIREN criteria.[15] In these patients the onset of dementia was related to cerebrovascular disease. They generally had an acute onset of dementia symptoms with stepwise progression of the disease. DSM-III-R criteria were used to classify other dementias, but they were not included in our analyses.

Other measurements

The following variables were considered as possible confounding variables: age; sex; cigarette smoking (never, former and current); alcohol consumption (none, <1 drink (13.2g), 1-2 drinks, ≥3 drinks per day), assessed with a semi-quantitative food frequency questionnaire[16]; level of education (completed primary education; lower vocational or general education; intermediate vocational or general education; and higher vocational training, college or university) (UNESCO); body mass index (weight/height^2); and hypertension, which was defined as a systolic blood pressure of 160 mmHg or over, or a diastolic blood pressure of 95 mmHg or over, or the use of anti-hypertensive medication.[17]

Follow-up

Vital status of the participants was obtained in several ways. Information on vital status was acquired at regular intervals from the municipal authorities of Rotterdam. In addition, general practitioners working in the study district of Ommoord regularly gave computerized reports on the deaths of all participants from the Rotterdam Study. For all reported deaths, study physicians investigated the causes of death using medical records from the general practitioners and hospital discharge records in case of admittance or referral. All general practitioners working in Rotterdam, but outside the district of Ommoord, who had patients who participated in the Rotterdam Study, were visited to obtain information on vital status, and if applicable, on the cause of death. Participants who had left Rotterdam (n=543, 6.8%) were not further tracked for this analysis. These subjects were older than the 6985 with information on

prevalent dementia and vital status (74.2 years (SD=11.7) vs 70.1 years (SD=9.5), p<0.001). In addition, they were more often female (68% vs 61%, p=0.002) and had a much higher prevalence of dementia (20% vs 5%, p<0.001). The end of the follow-up period was set at April 30, 1996. Mean duration of follow-up was 4.0 (SD=1.2) years.

The underlying causes of death were coded independently by two study physicians. Any discrepancy was discussed until consensus was reached. Cause of death was coded according to the 10th Revision of the International Classification of Diseases.[18] The end-points investigated were all-cause mortality, mortality from cardiovascular disease (codes I00-I99), from ischemic stroke (codes I63-I69), from coronary heart disease (codes I20-I25), from malignancies (codes C00-C97), and from noncardiovascular-noncancerous causes. The latter category was further subdivided in mortality from mental and behavioral diseases (codes F00-F99), from diseases of the respiratory system (codes J00-J99), from diseases of the digestive system (codes K00-K93), from diseases of the genitourinary system (codes N00-N99), from external causes (codes S00 to Y98), from unknown causes (codes R96, R98 and R99), and from other causes.

Statistical analysis

Complete information on baseline dementia and subsequent mortality was available for 6985 subjects. Baseline characteristics according to dementia and vital status after adjustment for age were tested with analysis of covariance. The frequency distribution of the causes of death among demented and nondemented subjects was tested with analysis of covariance as well. Cox's proportional hazards analysis was carried out to investigate the association between prevalent dementia (subtypes) and mortality. We included confounding variables in the model. Additional adjustment for age squared did not improve the model (likelihood ratio test: X^2=3.54 with 1df, p>0.05) and it did not change the estimate for dementia. Adjustment for 5-yr age groups instead of age as a continuous variable did not change the estimate either. For missing values of confounding variables, an indicator variable was created. Since we investigated the mortality risk of subjects with prevalent dementia, for whom the onset of dementia was not known, we additionally examined whether the severity of dementia, as a proxy for the duration of the disease, was associated with mortality. The severity of dementia was categorized into four groups according to the clinical dementia rating scale and the Mini-Mental State Examination score[19]: mild, minimal, moderate and severe dementia. The first two and the latter two groups were combined because of small numbers. All tests were two-sided and a p-value of less than 0.05 was considered to be statistically significant. Data analyses were performed using BMDP statistical software.

Table 1
Age-adjusted baseline characteristics according to vital status and dementia. The Rotterdam Study.

	Alive		Dead	
	No dementia (n=6028)	Dementia (n=206)	No dementia (n=585)	Dementia (n=166)
Mean age (yrs)	68.5 (8.5)*	82.7 (7.8)	77.7 (9.2)	85.7 (6.5)
Sex (% female)	62%	67%	47%	60%
Primary education only	38%	37%	39%	39%
Current smokers	21%	22%	30%	27%
Body mass index (kg/m^2)	26.4 (0.0)†	25.7 (0.4)	25.4 (0.2)	24.4 (0.3)
Hypertension	31%	32%	35%	13%

* Standard deviation in parentheses
† Standard error in parentheses

Results

The mean age of the participants at baseline was 70.1 years (SD = 9.5). Of the 6985 participants, 372 (5.3%) were demented at baseline; 245 (3.6%) were classified as having 'pure' Alzheimer's disease, 31 (0.4%) as having Alzheimer's disease with cerebrovascular disease, 54 (0.8%) as having vascular dementia, and 42 (0.6%) as having other types of dementia. After a mean follow-up period of four years (SD=1.2), 751 (10.8%) subjects had died. Age-adjusted baseline characteristics according to dementia and vital status are described in Table 1.

Total mortality risk was twice as high in demented subjects compared with non-demented subjects, after adjustment for age, sex, education, smoking, and alcohol consumption (RR=2.0, 95% CI: 1.6-2.4). The 5.5 year survival for a 75 year old man without dementia was 80%, and with dementia approximately 65% (Figure). A 75 year old woman without dementia had a 90% chance of surviving 5.5 years, a woman with dementia 80%. For subjects with Alzheimer's disease the risk of dying was 1.8 (95%CI: 1.5-2.3), for subjects with vascular dementia it was 2.9 (95%CI: 1.9-4.3). Additional adjustment for body mass index or hypertension did not essentially alter these results. Mortality risk for subjects with mild and minimal dementia (RR=1.9, 95% CI: 1.5-2.4)) was not essentially different from the mortality risk for those with moderate and severe dementia (RR=2.0 (95% CI: 1.5-2.7)).

To examine whether there was a shift in the distribution of the causes of death in subjects with dementia compared with those without dementia, we calculated the age-adjusted frequencies of the causes of death among all those who died. Approximately two-fifth of the non-

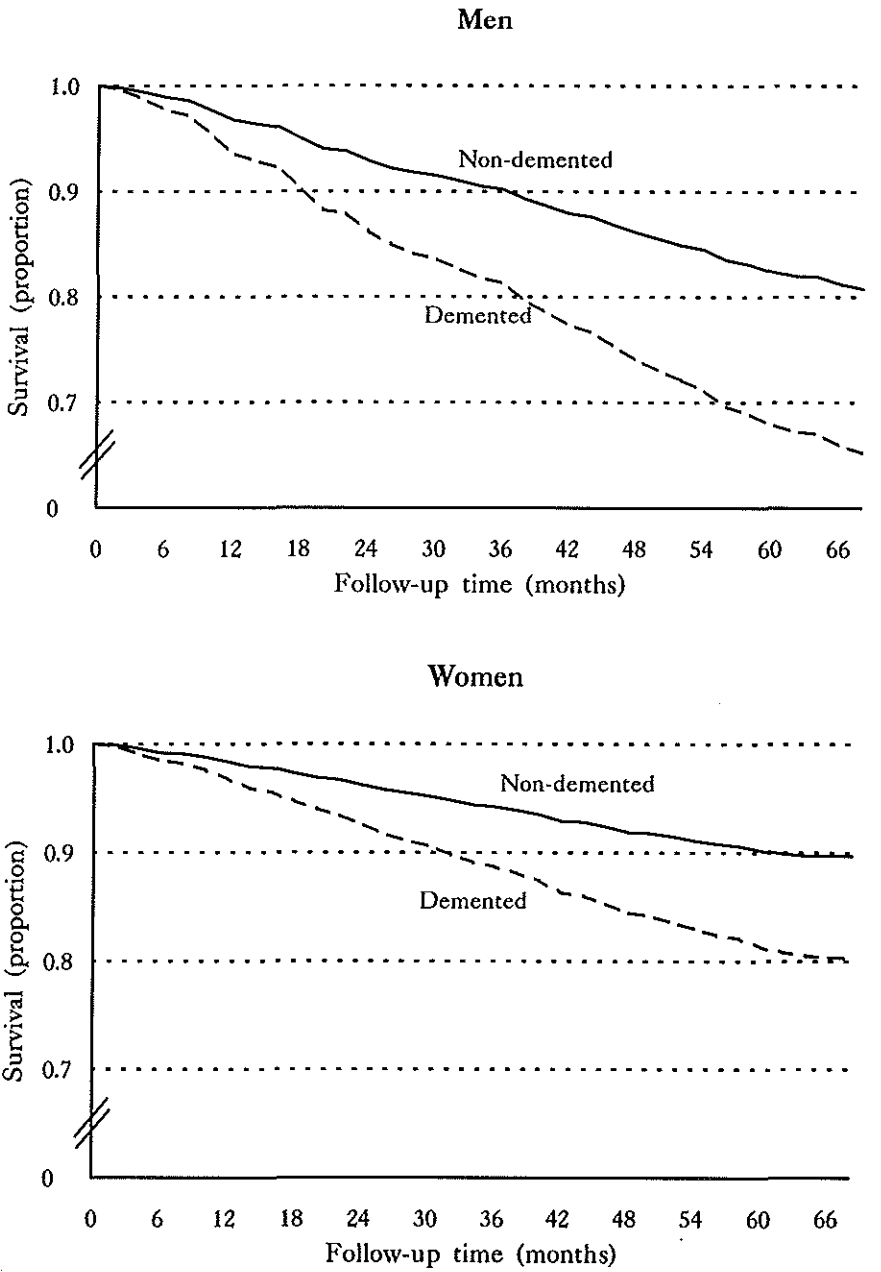

Figure
Survival curves for a 75 year old man and woman with or without dementia. The Rotterdam Study.

Table 2
Age-adjusted frequency distribution of the causes of death among demented and nondemented subjects who died. The Rotterdam Study.

Mortality from	No dementia (n=585)	Total dementia (n=166)	Alzheimer's disease (n=105)	Vascular dementia (n=44)
Cardiovascular disease	43.7%†	37.5%	34.8%	45.2%
• Ischemic stroke	11.4%	13.9%	12.9%	15.0%
• Coronary heart disease	12.7%	7.2%	7.1%	8.7%
Malignancies	24.9%	13.3%**	14.8%*	10.2%*
Noncardiovascular noncancerous causes‡	31.3%	49.2%***	50.4%***	44.6%

† Percentage of subjects of all those who died in this group
‡ Included are: mortality from mental and behavioural diseases, diseases of the respiratory system, diseases of the digestive system, diseases of the genitourinary system, external causes, cause unknown, and other causes
* $p<0.05$, ** $p<0.01$, and *** $p<0.001$ for the age-adjusted difference with nondemented subjects

demented subjects that died, died from cardiovascular disease (Table 2). This proportion was about the same among demented subjects, but somewhat lower among subjects with Alzheimer's disease and somewhat higher among subjects with vascular dementia, although the difference was not significant. The percentage of subjects that died from ischemic stroke was not significantly higher among participants with vascular dementia than among those without dementia. A quarter of the non-demented participants that died, died from malignancies, whereas this proportion was significantly lower among demented participants that died. Relatively more demented than non-demented participants died from noncardiovascular-noncancerous causes, including mental and behavioural diseases (5.5% vs 0.3%, $p<0.001$), diseases of the respiratory system (only significant for Alzheimer's disease patients (10.8% vs 5.1%, $p<0.05$)), and unknown causes (19.1% vs 11.5%, $p<0.05$). Additional adjustment for education did not essentially alter these results. Age at time of death was lower among subjects who died from cancer than among those who died from other causes (74.3 yrs (SD=8.8) vs 81.0 yrs (SD=8.8)).

Discussion

This large population-based prospective follow-up study suggested that demented subjects had an increased overall mortality compared with nondemented subjects. Mortality risk was higher for subjects with vascular dementia than for subjects with Alzheimer's disease. The results were independent of age, sex, education, smoking, alcohol consumption, body mass index, and hypertension. Furthermore, taking age into account, there was a shift in the distribution of the causes of death among demented subjects towards less deaths from malignancies and more deaths from noncardiovascular-noncancerous causes. There were no large differences in the frequencies of the cardiovascular causes of death.

This was a large study, allowing us to examine cause-specific mortality risks in subgroups. In addition, it was population-based, including demented subjects from institutions as well as those who lived independently, thereby increasing the generalizability of our results. The results were based on prevalent and not on incident dementia cases, which presumably resulted in a more heterogeneous sample of demented subjects, who were in different stages of the disease. Since the duration of dementia varies among subjects with prevalent dementia, the mortality risk will be different among prevalent compared with incident demented subjects. However, severity of dementia, as a proxy for the duration of the disease, did not influence mortality risks. But perhaps severity was not an appropriate proxy for duration of disease. Furthermore, the follow-up period in this study was relatively short, which may

have lead to an overrepresentation of rapidly progressive dementias among the demented subjects that died. This might have influenced our findings on cause-specific mortality. Finally, some subjects with vascular dementia may have been misclassified as having Alzheimer's disease, or vice versa. However, the criteria we used for the diagnosis of Alzheimer's disease have a high sensitivity and specificity.[20]

It could be argued that selective loss to follow-up has affected the validity of our results. Subjects who were lost to follow-up were older and more often demented at baseline. It is likely that the mortality rate was higher among those who were lost to follow-up. If anything, this would probably have attenuated our results on all-cause mortality. It is however not clear, how selective loss to follow-up may have influenced our findings on cause-specific mortality.

A number of community-based studies investigated the association of dementia and its subtypes with mortality.[1-5,21] They consistently found an increased mortality risk in demented compared with non-demented subjects, and a higher mortality for subjects with vascular dementia than for subjects with Alzheimer's disease[1,3,21], which is in agreement with our results. To our knowledge, three previous studies examined the causes of death in demented subjects, but these studies were not population-based and causes of death were obtained mostly from death certificates.[22-24] Kukull et al.[22] studied the causes of death among newly recognized dementia cases from a registry, and compared them with those who failed to meet the criteria for dementia. Dementia and pneumonia were more common causes of death in severe Alzheimer's disease patients, and stroke in mildly impaired Alzheimer patients. These results were crude and no significance was tested. Nielsen et al.[23] studied causes of death 15 years after a geronto-psychiatric prevalence study. Pneumonia was a more frequent cause of death among demented subjects compared with subjects without any mental illness. Again, results were not adjusted for age, or other covariates. In the study by Mölsa et al.[24], demented subjects from a community-based study had a lower frequency of deaths from malignancies and cardiovascular disease and a higher frequency of deaths from dementing diseases than subjects from a general population of 75 years or over. Cerebrovascular disease seemed to be a more common cause of death in patients with multi-infarct dementia. However, no significance was tested.

There was a shift in the distribution of the causes of death among demented participants towards less deaths from cancer. This may be due to the fact that subjects who died of cancer were on average younger at time of death than those who died of other causes, and therefore had a lower probability of being demented. Additionally, perhaps demented subjects are less able to recognize suspicious symptoms and signs, or they less often get a thorough diagnostic work-up in case

of suspicious symptoms and signs, thereby reducing the chance of being diagnosed with cancer.

There was a shift towards more deaths from noncardiovascular-noncancerous causes among demented persons. These causes included mental and behavioral diseases, diseases of the respiratory system and death from unknown causes (i.e. sudden death, cause unknown; unattended death; and other ill-defined causes of mortality). The increase in deaths from unknown causes may be explained by the fact that demented subjects more often die in nursing homes instead of in the hospital, where the cause of death is more easily assessed. There were no reports of suicide or euthanasia among the causes of death. Suicide and euthanasia are probably often under-reported causes of death. Suicide may occur more often in subjects with dementia, especially early dementia, than in those without.[25] There is considerable debate about active euthanasia of demented patients, and it is not clear how often this is performed.[26] Passive euthanasia probably occurs more often in demented than non-demented subjects. A study conducted in the Netherlands on the frequency of decisions to forgo (artificial) feeding and hydration with hastening of death as a possible result, showed that this had occurred in 23% of the deaths attended by nursing home physicians, compared with 4% of those attended by general practitioner or specialists. Most of the persons that died this way were incompetent and the reported cause of death was often a psychiatric disturbance.[27] It is possible that suicide and euthanasia were coded as death due to unknown cause, which would then partly explain the increased frequency of unknown causes of death among demented persons.

This large population-based follow-up study showed that subjects with dementia had an increased all-cause mortality, with mortality risk being higher in subjects with vascular dementia than in subjects with Alzheimer's disease. There was a shift in the distribution of the causes of death among demented subjects towards less deaths from malignancies and more deaths from noncardiovascular-noncancerous causes. Clinicians who are involved in the treatment of demented subjects and planners of health care facilities may use this information to improve the care of demented subjects.

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General discussion

Chapter

10

GENERAL DISCUSSION

Knowledge on risk factors for dementia has expanded considerably during the past decade, demonstrating important risk indicators and risk factors, such as age, sex, education, and family history.[1] In this thesis several new potential risk factors for cognitive decline and dementia have been identified. The rationale for investigating these risk factors is that vascular, oxidative, inflammatory, and stress-related processes may play a role in the pathogenesis of cognitive decline and dementia. Most of the risk factors we studied can be modified, which creates the possibility of delaying the onset of dementia. This could be of significant public health importance. In addition, our findings may give new clues to the etiology of dementia and provide ideas for further epidemiologic and basic research.

After a brief discussion of the main findings, some methodological problems specific for this study will be discussed. Furthermore, the hypotheses on the mechanisms behind the associations with cognitive decline and dementia will be addressed in the light of our findings. Finally, some ideas will be presented for future research on risk factors for cognitive decline and dementia.

Main findings

The main results on nutritional risk factors for cognitive decline and dementia described in this thesis are summarized in Table 1. In conclusion, we did not find a consistent inverse association between antioxidants and cognitive decline or dementia.[2] However, we did find evidence for a relationship between fatty acids and cognition or dementia. In addition, fish consumption was inversely associated with dementia, cognitive impairment and decline.

In our investigation of metabolic risk factors we found an association of diabetes mellitus, but also of impaired glucose tolerance and hyperinsulinemia, with cognitive impairment (Table 2). Diabetes mellitus

Table 1
Odds ratios (95% confidence interval) for the association between nutritional risk factors and cognition or dementia.

	Cognitive impairment†		Cognitive decline‡		Dementia
Risk factors*	ZES	RS	ZES	RS	RS
Antioxidants					
• Vitamin E	1.4 (0.7-2.8)	1.1§ (0.7-1.7)	1.2 (0.4-3.3)	-	-
• Beta-carotene	1.3 (0.8-2.1)	0.6§ (0.4-1.0)	0.7 (0.3-1.5)	-	-
• Vitamin C	1.2 (0.7-2.0)	0.8§ (0.6-1.3)	2.0 (0.9-4.5)	-	-
• Flavonoids	1.4 (0.9-2.4)	-	0.9 (0.4-1.9)	-	-
Fatty acids					
• Total fat	-	-	-	-	2.4 (1.1-5.2)
• Saturated fat	-	-	-	-	1.9 (0.9-4.0)
• Cholesterol	-	-	-	-	1.7 (0.9-3.2)
• Linoleic acid	1.8 (1.0-3.0)	-	1.1 (0.5-2.3)	-	0.6 (0.3-1.2)
• N-3 PUFA	1.0 (0.6-1.6)	-	0.8 (0.4-1.7)	-	-
Fish	0.6 (0.3-1.2)	-	0.5 (0.2-1.2)	-	0.4 (0.2-0.9)

ZES = the Zutphen Elderly Study; RS = the Rotterdam Study; PUFA = poly-unsaturated fatty acids
* Low intake is the reference category
† MMSE score <26
‡ >2 points decline in the MMSE score in 3 yrs
§ Based on findings not described in this thesis[2]

also increased the risk of dementia.[3] The adrenal steroid cortisol was positively associated with impaired cognitive performance, the adrenal steroid dehydroepiandrosterone sulfate was inversely associated with cognitive impairment and decline, and the amino acid homocysteine was related to cognitive decline among subjects under 75 years of age and among women.

For a better understanding of the etiology of cognitive decline and dementia, it is important to examine the interaction between genetic and environmental risk factors. Therefore, we examined the interaction

Table 2
Odds ratios (95% confidence interval) for the association between metabolic risk factors and cognition or dementia.

	Cognitive impairment		Cognitive decline		Dementia
Risk factors	ZES*	RS†	ZES	RS‡	RS
Diabetes mellitus	1.2 (1.0-1.5)	-	-	-	1.9§ (1.3-2.8)
IGT	1.2 (1.0-1.4)	-	-	-	-
Hyperinsulinemia	1.3 (1.0-1.5)	-	-	-	-
Serum cortisol	-	1.7 (0.9-1.3)	-	0.9 (0.6-1.3)	-
Serum DHEAS	-	0.5 (0.2-1.1)	-	0.6 (0.4-1.1)	-
Serum homocysteine					
< 75 years	-	-	-	2.3 (1.0-5.6)	-
≥ 75 years	-	-	-	0.4 (0.1-2.3)	-

ZES = the Zutphen Elderly Study; RS = the Rotterdam Study; IGT = impaired glucose tolerance; DHEAS = dehydroepiandrosterone sulfate
* Error rate ratio for number of erroneous answers on the MMSE
† MMSE score <26
‡ Drop in the MMSE score of >1 point/year
§ Not described in this thesis[3]

Table 3
Gene-environment interaction in relation to cognitive decline.* The Zutphen Elderly Study.

	Carrier of the APOE*4 allele			
	No		Yes	
Risk factor present	No	Yes	No	Yes
Education ≤6 years	ref	3.1 (1.1-8.8)	ref	0.9 (0.2-3.8)
History of cerebrovascular disease	ref	4.7 (1.7-12.7)	3.3 (1.6-6.8)	17.2 (2.7-110.0)
History of coronary heart disease	ref	1.8 (0.7-4.3)	3.1 (1.5-6.6)	6.1 (1.7-22.3)

* >2 points decline in the MMSE score in 3 yrs

between the e4 allele of the apolipoprotein E gene and some environmental risk factors for cognitive decline, i.e. education and a history of cardiovascular disease. Low education was associated with an increased risk of cognitive decline, only in subjects without an e4 allele. The combined presence of the e4 allele and a history of cerebrovascular disease increased the risk of cognitive decline dramatically, suggesting synergism between these two risk factors. Hence, an interaction could be found between important environmental risk factors and a major susceptibility gene for cognitive decline.

Finally, we examined the prognosis of dementia among participants from the Rotterdam Study. Subjects with dementia had an increased risk of dying (RR=2.0, 95%CI: 1.6-2.4). There was a shift in the distribution of the causes of death among demented persons towards less deaths from malignancies and more deaths from noncardiovascular-noncancerous causes.

Methodological considerations

Exposure measurement

Dietary assessment – Measurement error is a major problem in nutritional epidemiologic research. In general, two types of error exist: differential and non-differential. Differential error can lead to under- or overestimation of the true effect, while non-differential error usually leads to attenuation of the effect.

Differential error may arise when the error in estimating dietary intake is different in subjects with the disease than in those without the disease. In cross-sectional studies on cognitive impairment or dementia it is conceivable that subjects with memory problems give less reliable information about their usual diet than subjects without memory problems.[4] We dealt with this in various ways, which is discussed in Chapter 2. In longitudinal studies, in which the dietary intake is assessed before the disease (i.e. dementia) occurs, differential error is less likely.

Non-differential error is a problem especially when true associations are weak and contrast in dietary intake is small. It can lead to a type II error or a false negative finding. This may explain the finding in Chapter 2 of no association between antioxidants and cognitive impairment and decline. However, we used the average of two measurements, which reduces the effect of measurement error. Further reduction of the influence of measurement error could be obtained by using the combined information from dietary intake and biochemical markers, by increasing the contrast of the dietary intake by doing e.g. a multi-center study, or by estimating the amount of measurement error and adjust for it afterwards.

Metabolic factors – When studying metabolic risk factors, a number of practical issues should be considered. Generally, fasting blood levels are more reliable than non-fasting levels. It is, however, not always feasible to obtain fasting blood in a large epidemiologic study, and therefore, homocysteine levels were determined in non-fasting serum. Intake of food may effect the plasma homocysteine concentration[5] and may thus lead to measurement error, which however probably will be random. Furthermore, sometimes blood levels after a loading test gives valuable additional information. For example, data on homocysteine levels after a methionine loading test could classify a considerable additional proportion of subjects as having hyperhomocysteinemia.[6] A loading test is also necessary for measurement of cortisol suppression after dexamethasone and for the measurement of glucose intolerance. However, a loading test is generally only practical in smaller sample sizes. Misclassification due to altered concentrations because of prolonged storage of the blood can be a problem. Homocysteine determinations were performed in blood that was stored at -20°C for approximately four years. Serum homocysteine is stable for years when stored at -20°C[7], so this has probably not influenced our findings. Serum values of glucose, insulin, cortisol and DHEAS were determined in blood that not had been stored for longer than a few months. Finally, it is probably optimal to measure the cerebrospinal fluid level or the cerebral tissue level of the metabolic factors we studied, since the peripheral concentrations may not reflect the biologically relevant concentration in the brain. For example, there seems to be an upregulation of glucose transport across the blood-brain barrier in case of chronic hypoglycemia.[8] Measurement of the cerebrospinal fluid level however, is not possible in a population-based study.

Another issue when studying metabolic risk factors, as when studying dietary factors, is non-differential error because of the usually high intra-individual variation for these factors. This variation is due to measurement error, but also to real temporary biological variations from the 'usual' level. Repeated measurements will reduce non-differential error, but often only a single measurement is available, as in our studies on metabolic risk factors.

Outcome measurement

The Mini-Mental State Examination – The 30 point Mini-Mental State Examination (MMSE) was developed as a brief screening instrument for dementia.[9] It measures the following cognitive functions or domains: orientation to time and place, registration, attention and calculation, recall, language and visual construction. Although, it was originally meant to be used in a clinical setting[9], it has also been widely used in epidemiologic studies.[10] It is a valid and reliable test for measuring cognitive impairment.[11] The MMSE score correlates well with other cognitive tests, and with measures of instrumental activities of daily living.[11] Test-

retest reliability for normal and cognitively impaired subjects ranges from 0.80 to 0.95.[11] In a clinical setting a cut-off of <24 is most widely used. The positive predictive value for dementia when using this cut-off point was 79% in most studies. We used a score of <26 as cut-off point for cognitive impairment, since this cut-off has a higher sensitivity and the same specificity in detecting early stages of Alzheimer's disease than a cut-off of <24.[12,13] One of the drawbacks of the MMSE is that it is not very sensitive to mild cognitive impairment, and that it can not discriminate between mildly demented and normal subjects.

There are a number of different ways in which the analyses using the MMSE score, can be performed. Neither the MMSE score, nor its logarithmic transformation, are normally distributed. Therefore, linear regression analyses does not apply when the MMSE score is the outcome. The number of erroneous answers on the MMSE (i.e. 30 minus the MMSE score) follows a Poisson distribution. So Poisson regression can be performed to estimate the rate ratio of the number of erroneous answers. This was described in Chapter 4. Alternatively, the MMSE score can be dichotomized, in which case logistic regression analysis can be used. The latter estimates the odds ratio for being cognitively impaired, which is easier to interpret. This was done in Chapters 2, 5, and 6.

The change in the MMSE score is normally distributed. Therefore, linear regression analysis applies, yielding the change in the MMSE score per unit increase of the independent variable. The change in the MMSE score can also be dichotomized. By using logistic regression analysis, one can then estimate the odds ratio for a specific decline in the MMSE. Again, the latter analysis may be easier to interpret, and has therefore been used in this thesis.

The cut-off points we used to define cognitive decline (>2 points in 3 yrs and >1 point/yr) may not be pathologically and clinically significant on an individual level. But, a small decline in cognitive function of a large part of the population can increase the proportion of subjects with cognitive impairment to a large extent, and may therefore be of major public health importance. It is not clear whether a drop of one point in the MMSE is more clinically meaningful when the initial score is high than when the initial score is low. To minimize this effect we adjusted for initial score when analyzing cognitive decline.

Subtypes of dementia – Dementia and its major subtypes, i.e. Alzheimer's disease and vascular dementia, were diagnosed according to standard international criteria. The differentiation between Alzheimer's disease and vascular dementia is not straightforward, especially when a cerebrovascular event has occurred. If the onset of dementia was related to cerebrovascular disease, vascular dementia was diagnosed. Because in the Rotterdam Study no autopsy information was available, a definite diagnosis could not be made. However, the criteria we used have a high

sensitivity and specificity.[14] Furthermore, to reduce the chance of misclassifying vascular dementia as Alzheimer's disease, we grouped subjects with Alzheimer's disease with cerebrovascular disease together with subjects with vascular dementia in Chapter 3. Recently, it has been found that 47% of the demented subjects in the Nun Study had Alzheimer's disease and large or small brain infarcts.[15] In addition, a number of vascular risk factors have been associated with an increased risk of Alzheimer's disease.[3,16, this thesis] So, it may be questioned whether it is possible to make a clear distinction between vascular dementia and AD.

Confounding and mediating factors

Confounding factors – Not adequately adjusting for confounding is a major threat to the validity of the results of epidemiologic studies. For cognitive decline and dementia, age is probably the most important confounder, since age shows a strong exponential relation with cognitive decline, independent of other risk factors. Age is also related to most risk factors studied in this thesis. Education is a potentially important confounder as well. It may be a real risk factor, indicating a lower brain reserve, it may be a marker for other (unknown) risk factors, but it also may lead to psychometric bias, because subjects with a lower education are less able to perform the cognitive test. Considering our results on the interaction between education and the apolipoprotein E gene, the latter explanation is less likely. In the Netherlands most subjects had at least six years of education and illiteracy is rare. Still, there is a large variation in educational background and education is related to most risk factors studied, making it necessary to adjust for it. Another important confounder is gender. Women may be at greater risk of developing dementia, even when longevity is taken into account.[17] In addition, most risk factors vary according to gender.

Cigarette smoking is another potential confounder. The association between smoking and dementia or cognitive decline is not clear yet. A number of studies have found that smoking was inversely related to dementia, but these studies were mostly cross-sectional.[18] Results from the prospective Rotterdam Study and the prospective Honolulu-Asia Aging Study showed that smoking increased the risk of dementia and cognitive impairment, respectively.[19,20] Since smoking can be related to a number of other risk factors, such as diet, homocysteine, and diabetes, it may be a confounder when investigating these risk factors. Alcohol consumption may be a confounder as well. Recently, it was found that moderate alcohol consumption may protect against dementia.[21] In contrast, it is well known that heavy alcohol consumption is associated with an increased risk of dementia. Risk factors, as for example diet and homocysteine, may also be related to alcohol consumption. An important confounder in the relation between diet and dementia or cognitive decline is total energy intake. Demented subjects often have a lower body

mass index than non-demented subjects[22], probably because their total energy intake is lower. Nutrient intake is obviously related to total energy intake as well. Finally, hypertension may be a confounder. Hypertension at middle age has been associated with an increased risk of cognitive impairment at older age. A low blood pressure at older age seemed to be associated with an increased risk of cognitive impairment[23], perhaps because high blood pressures are needed to guarantee a sufficient perfusion of the atherosclerotic brain vessels. Hypertension may also be related to risk factors, as for example homocysteine.

Mediating factors – In this thesis we have also examined a number of mediating factors, e.g. a history of cardiovascular disease and atherosclerosis, measured as plaques in and intima-media thickness of the carotid arteries, and ankle-brachial index. These factors may have been intermediates in the associations between diabetes and cognition, homocysteine and cognition, and diet and cognition. In some contexts, these mediating factors may have been confounders as well. To judge whether a factor is a confounder or an intermediate one has to rely on the existing knowledge about the biologic mechanisms underlying the relationship between the exposure and the outcome. For instance, apart from being an intermediate, a history of cardiovascular disease may be a confounder in the association between diet and cognitive decline. Cardiovascular disease might be associated with the exposure, because it often leads to changes in dietary habits. Additionally, it may, independently of diet, be associated with the outcome, i.e. cognitive decline.[24] So the association between cognitive decline and the intake of linoleic acid, saturated fat, cholesterol, or fish, may have been confounded by a history of cardiovascular disease.

When a risk factor is a confounder, one should adjust for it in the analysis in order to get a valid estimate. When a factor is an intermediate, however, adjustment would lead to overadjustment, taking away most of the effect. A very rough method to deal with this problem is to compare the results with and without adjustment for this risk factor. There are also more sophisticated and complicated methods to deal with this, as for example G-estimation.[25] For this analysis, however, the confounding and mediating factor has to be measured twice at different points in time. In the studies described in this thesis, adjustment for the possible intermediates (cardiovascular disease and atherosclerosis) did not attenuate our estimates, which may suggest that these factors were neither confounders nor intermediates. But of course, other explanations can be offered for the lack of attenuation, such as measurement error, or wrong timing of the measurement. Additionally, these variables may not constitute the true intermediating factors.

Effect modification – The investigation of interaction may point at the existence of high risk groups for whom modification of risk factors may

especially be beneficial. We found an interaction between the e4 allele of the apolipoprotein E gene and some major risk factors, i.e. education and a history of stroke, in relation to cognitive decline. Statistical interaction is usually examined on a multiplicative level, i.e. with the cross-product of the risk factors as interaction term in the regression model. Biological interaction, however, is considered present when the joint effect of two risk factors is more, or less, than the sum of the effects of each risk factor acting without the other.[26] When biological interaction is present, it means that two risk factors are not acting independently, but have a synergistic or antagonistic effect on the outcome. To examine this, subjects should be categorized into four groups: none of the two risk factors present (reference group); one risk factor present; the other risk factor present; and both risk factors present.[27]

Differences in cross-sectional and longitudinal studies

In this thesis, two major types of study design are used to investigate risk factors for cognition and dementia: cross-sectional and longitudinal cohort analyses. The cross-sectional and longitudinal results were not always similar. This may be due to the fact that a different outcome was measured, or that cross-sectional and longitudinal studies may be prone to different types of bias.

Cognitive impairment versus cognitive decline – Some results of the cross-sectional analyses could not be confirmed in the longitudinal analyses. An explanation for this may be that the outcome in the longitudinal analyses, i.e. cognitive decline, is a less reliable measure than the outcome in the cross-sectional analysis, i.e. cognitive impairment. The MMSE was not originally created to measure cognitive decline. In a sample of healthy elderly or in relatively young subjects the MMSE has a ceiling effect. Therefore, the MMSE may not be very sensitive to small cognitive changes in subjects with a normal cognition. In a longitudinal study among cognitively intact elderly subjects the MMSE score declined only slightly after one to five years[28], which was similar in our studies. Furthermore, the MMSE is less sensitive to progressive decline in severe dementia. Another problem is that, as with any variable, the change in the MMSE score has a larger intra-individual variation than a single MMSE score. Additionally, the second MMSE measurement will show regression towards the mean. Moreover, a practice effect may occur, leading to a smaller drop in the MMSE. Different testing site may influence cognitive decline, since some studies reported that scores tended to be higher at home than in a clinical setting. In the Zutphen Elderly Study, the first MMSE was administered in a clinical setting, whereas the second one was administered at home. If follow-up duration is short, no cognitive decline may be present, but if the duration is too long, selective loss to follow-up will have a larger influence on the

estimation of cognitive decline. One of the difficulties to be tackled, is to make an assumption about the induction period. If this induction period is very long, a longitudinal study is not very efficient. If the follow-up period is too short, it will lead to underestimation of the exposure-outcome association. This may be one of the explanations for the finding of a different relation between linoleic acid and cognitive impairment (positive) as compared with the relation between linoleic acid and cognitive decline (absent). Follow-up duration may have been too short for an effect of linoleic acid on cognitive decline (Chapter 2).

Selection bias – Selection bias and information bias may either lead to an underestimation or an overestimation of the true association and one can not correct for their effect in the analysis.

In a cohort study, selection bias occurs when nonresponse is related to the exposure as well as to the outcome. Nonresponse may be a problem in both cross-sectional and longitudinal cohort studies, especially when response rates are low. Nonrespondents often differ with regard to motivation, attitude towards health, and risk factor status. Even if the estimates are not biased, nonresponse can reduce the generalizability of the results. A way to evaluate whether nonresponse may have biased the estimates is to investigate whether nonrespondents and respondents are different with regard to major baseline characteristics, such as age, gender and socioeconomic status, and to risk factors, if this information is present. This was done in most of our analyses and nonresponse probably has not influenced our results.

Information bias – Information bias, in the form of differential misclassification, is the result of a systematic error in the measurement of the outcome which differs according to exposure status, or of a systematic error in the measurement of the exposure, which differs according to the outcome.

In a cross-sectional study, the exposure and the outcome are measured at the same point in time. This does not present a problem when the exposure does not change over time, such as the apolipoprotein E genotype. A critical problem arises when the risk factor changes after (or perhaps even some time before) the development of the disease. This may lead to information bias. Risk factors for which we found a cross-sectional relation with cognitive impairment include diabetes, dietary factors and adrenal steroids. It is not very likely that the occurrence of diabetes is influenced by the presence of cognitive impairment. Information on diet, or the dietary intake itself, can change as a result of cognitive impairment. We dealt with this in various ways, as is discussed in Chapter 2. Furthermore, adrenal steroids can be influenced by cognitive impairment, because cognitive impairment is often associated with loss of hippocampal neurons, which control adrenal steroid biosyn-

thesis. Therefore, from our cross-sectional results we can not conclude that altered adrenal steroid levels preceded cognitive impairment.

In a prospective cohort study, one type of information bias that can occur is loss of subjects during follow-up, which is related to both the exposure and the outcome. There are different ways to deal with this problem. First of all, it is of course preferable to keep loss to follow-up to a minimum. Furthermore, one can examine whether the exposure or other risk factors at baseline are systematically different in those who are lost to follow-up compared to those who are not. This was examined in some of the analyses, in which selective loss to follow-up was considered not very likely. A more quantitative approach is to do a sensitivity analysis. In this analysis the estimates are calculated assuming the two most extreme situations: firstly, it is assumed that all those who were lost to follow-up developed the outcome of interest, and secondly, it is assumed that all those who were lost to follow-up did not develop the outcome of interest. The true association will lie somewhere in between these two estimates. In Chapter 5, sensitivity analyses were performed. The results suggested that selective loss to follow-up may have led to the nonsignificant inverse association between homocysteine and cognitive decline in subjects ≥75 years of age.

Etiology of cognitive decline and dementia

Vascular processes

We examined metabolic, nutritional and genetic risk factors for cognitive decline and dementia, including Alzheimer's disease. One of the main hypotheses behind the examined associations was that atherosclerosis and cerebrovascular disease may increase the risk of cognitive decline and dementia. We found a positive association of impaired cognitive function or dementia to a high total and saturated fat intake, fish consumption, diabetes, hyperinsulinemia, and, in women and those <75 years of age, to serum total homocysteine. These risk factors have all been associated with a higher risk of atherosclerosis and cerebrovascular disease. Therefore, the observed associations fit well with the hypothesis that cognitive impairment and dementia are partly caused by vascular processes.

Oxidation

It has been suggested that oxidation may play a role in the etiology of dementia.[29] This, however, could not be verified by our results on the association between antioxidants and cognition. Previous studies that investigated the association between intake of antioxidants and cognition were mostly cross-sectional, and results were inconsistent.[2,30-32] The only

controlled trial so far showed a delay in the progression of Alzheimer's disease in subjects receiving alpha-tocopherol and selegiline.[33] However, there was no improvement in cognitive function. Results of studies on the association between antioxidants and coronary heart disease have been contradictory as well.[34] The positive association between linoleic acid and cognitive impairment would fit in the oxidation hypothesis. But this association could not be confirmed in relation to cognitive decline and dementia.

Inflammation

The possible protective effect of fish consumption on cognitive decline and especially on Alzheimer's disease could be due to the anti-inflammatory properties of n-3 PUFAs, the main fatty acids in fish. These n-3 PUFAs can decrease the production of proinflammatory cytokines in humans.[35] Immune processes are believed to be involved in the pathogenesis of Alzheimer's disease. Increased concentrations of acute phase reactants and other markers of immune processes, such as cytokines, have been found in brain tissue of patients with Alzheimer's disease.[36] In addition, interleukin-1 may regulate the amyloid precursor protein synthesis, resulting in amyloid deposits.[37]

Stress-hypothesis

A higher level of stress leads to overactivity of the hypothalamic-pituitary-adrenal axis, which results in high cortisol concentrations. These high concentrations are toxic to neurons, especially those in the hippocampus. The hippocampal neurons are involved in memory processes, but also in inhibitory feedback to the hypothalamic-pituitary-adrenal axis. So damage of hippocampal neurons will lead to HPA-axis overactivity, keeping cortisol concentrations high, thereby damaging more hippocampal neurons, which will eventually lead to cognitive decline. This is described as the glucocorticoid cascade of aging.[38] Our results on the adrenal steroids were in agreement with this hypothesis.

Genetic factors

It is well known that genetic risk factors, e.g. the e4 allele of the apolipoprotein E gene, contribute to the occurrence of dementia and cognitive decline.[39,40] Examination of gene-environment interaction may give clues to the etiology of cognitive decline and dementia. We found an interaction with two environmental risk factors, i.e. education and a history of cerebrovascular disease. Education was only associated with cognitive decline in subjects without the e4 allele. An explanation for this may be that the e4 allele plays a role in inhibiting neuronal growth[41], thereby blocking the putative stimulating effect of a high education on neuronal growth. Furthermore, there seemed to be a syn-

ergistic effect of the e4 allele and a history of cerebrovascular disease on cognitive decline. This may result from reduced neuronal repair in e4 carriers[41], which could then lead to greater damage after cerebrovascular disease.

Cognitive impairment versus dementia

A question that arises when studying risk factors for cognitive impairment and dementia, is whether the pathological mechanisms behind dementia and cognitive impairment are the same? This would be the case if cognitive impairment represented the pre-clinical phase of dementia and if everybody with cognitive impairment eventually would develop dementia. We did not investigate this problem, but it may be important for the interpretation of our findings to know whether this is true or not.

There are a number of factors which indicate that cognitive impairment represents the pre-clinical phase of dementia. In a study on subjects with isolated memory loss, almost half of them developed dementia after a follow-up of two and a half years.[42] In another study, all subjects who became demented after a two year interval were cognitively impaired at baseline.[43] Senile plaques and neurofibrillary tangles, the major pathological findings in Alzheimer's disease, the extent of brain atrophy, and ventricular volume all correlate well with cognitive performance.[44] White matter lesions, which represent the pathological substrate of vascular dementia, are also more often present in subjects with cognitive impairment.[45] Furthermore, in a representative community sample of elderly women the variables that are associated with dementia, such as cognitive function, showed a continuous unimodal distribution, and not a bimodal distribution.[46] This does however not perse indicate that dementia is not distinct from cognitive impairment or normal aging.[47]

From these findings, one can conclude that a number of the processes that occur in dementia are also observed in cognitive impairment. On the other hand, a decline in cognitive functions can also be regarded as part of usual aging. The above arguments do not prove that everybody with cognitive impairment eventually, if they lived long enough, would develop dementia. Therefore, it is important to examine risk factors for cognitive impairment as well as for dementia.

Future research issues

Dementia and cognitive decline impose a major burden on individuals and on society. They limit the independent functioning of an individual and have a negative impact on the quality of life. Subjects with dementia often have to be institutionalized, which imposes a great demand

on health care costs. Therefore, it is important to search for possibly modifiable risk factors. Most of the risk factors examined in this thesis can be modified. It is however too early for preventive action. The results obtained, have to be confirmed by other large longitudinal population-based studies on cognitive decline and dementia.

Nutritional risk factors

Diet is one of the major risk factors for mortality, coronary heart disease, and some types of cancer, but it has not received a lot of attention as a risk factor for dementia and cognitive decline.[48] In the Report of a WHO Study Group on diet and the prevention of chronic diseases, dementia is not mentioned as being influenced by diet.[48] Results presented in this thesis suggest that diet may play an important role in the development of cognitive decline and dementia. More research is however needed to confirm this.

We did not find an association between antioxidant intake and cognitive decline or dementia. It could be that measurement error is responsible for these negative findings. It would therefore be preferable to use dietary intake and biochemical markers of antioxidants in combination. Furthermore, it may be useful to have information on the combination of the intake of different nutrients, because of its potential influence on the bioavailability. The time of the day and the frequency with which a nutrient is consumed might be important as well. To further elucidate the role of oxidation it may be preferable to examine the susceptibility of LDL to oxidation in combination with the antioxidant status of an individual. It would also be interesting to examine the effect of food groups instead of specific nutrients. Vegetables and fruits, which are the major sources of many antioxidants, have been related to lungcancer[49] and coronary heart disease.[50] The n-3 poly-unsaturated fatty acids in fish may exert a protective effect through their anti-inflammatory properties.[35] Markers of inflammation, such as interleukines[37] should be studied, to further clarify the role of inflammation in the pathogenesis of Alzheimer's disease.

Metabolic risk factors

Metabolic risk factors seem to play a role in the occurrence of cognitive decline and dementia as well. Future research on the role of homocysteine in cognitive decline should measure homocysteine in fasting blood and possibly after a methionine loading test. In addition, information on the intake of vitamin B6, vitamin B12 and folate, or on blood levels of these vitamins would be helpful for the interpretation of the findings. Instead of measuring insulin after an oral glucose load, insulin resistance could be measured quantitatively with a glucose clamp technique[51] or an intravenous glucose tolerance test, which is however

not applicable in epidemiologic studies. Repeated measures of cortisol and DHEAS are preferred for more precise information on cortisol and DHEAS metabolism in an individual subject.[52,53] Furthermore, interaction with genetic risk factors like the apolipoprotein E gene should be studied for a better understanding of the underlying pathogenesis of cognitive decline and dementia.

Clinical trials

Experimental research is the only way to definitely establish a causal relationship between a certain risk factor and dementia or cognitive decline. In my opinion, for most risk factors described in this thesis, it would be premature to start intervention research examining the effect of modification of these risk factors on cognitive decline. It may be of great value, though, to measure cognitive decline as an additional endpoint in future trials investigating the role of risk factors in cardiovascular disease (e.g. antioxidant intake, fish consumption, fatty acid intake, glucose tolerance, and homocysteine levels). Recently, a controlled trial with selegiline and a-tocopherol has been conducted in patients with Alzheimer's disease, showing a delay in the progression.[33]

Cognitive impairment versus dementia

In future research, it is important to investigate different end points, i.e. cognitive decline, total dementia, Alzheimer's disease and vascular dementia. The distinction between the latter two may not be very clear, especially in the oldest old.[15,54] Therefore it is important to investigate both total dementia as well as Alzheimer's disease and vascular dementia separately. Cognitive decline can be regarded as a pre-clinical stage of dementia, but it is an important end point perse. A disadvantage is that cognitive decline is a softer outcome than dementia, and perhaps also clinically less meaningful. There are however several advantages for taking cognitive decline as the outcome. It is easier to assess than dementia, because diagnosing dementia is difficult and time-consuming in large population-based studies. Since cognitive decline is a continuous measure, the study size needed will be smaller than for studying the risk of dementia. Especially in intervention studies the use of a continuous measure will shorten the necessary duration of follow-up considerably. Furthermore, there maybe an interest in the early phase of the disease process, when preventive action might still be possible. Investigators may also be especially interested in cognitive deterioration and not in the other, mainly behavioral, changes that occur in Alzheimer's disease. Finally, when studying cognitive function, more extensive domain-specific cognitive tests, such as memory tests, tests on sensorimotor speed and on complex information processing speed, should be used to be able to distinguish more subtle differences between

individuals and to obtain a more precise estimate of changes in cognitive performance.

In conclusion, the results described in this thesis show that there are many possible new risk factors for cognitive decline and dementia. Modification of these risk factors may delay the underlying disease processes of cognitive decline and dementia, and could therefore have important implications for public health. Our results are however preliminary, and the time for preventive action has not arrived yet.

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SUMMARY

In this thesis studies on risk factors for cognitive decline and dementia are described. Deterioration of cognitive functions is one of the most important symptoms of dementia. The main cognitive functions are orientation to time and place, memory and recall, attention and concentration, language and abstract thinking, calculation, and constructive abilities. Diminished cognitive functioning reduces the independence of an individual and diminishes the quality of life. Besides these disadvantages for the individual, cognitive decline and dementia impose a major burden on the health care system, because of the great risk of institutionalization. Therefore, it is important to search for possibly modifiable risk factors for these disorders. For this thesis, we studied the relation of nutritional and metabolic risk factors to cognitive decline and dementia.

For the results presented in this thesis, data were used from two population-based studies on the elderly: the Zutphen Elderly Study and the Rotterdam Study. The Zutphen Elderly Study is the continuation of the Zutphen Study, which was part of the Seven Countries Study, that started in 1960. In 1990, 560 of the 718 surviving men (78%), born between 1900 and 1920, participated in the Zutphen Elderly Study. They were all extensively interviewed and examined. The level of cognitive function was assessed with the Mini-Mental State Examination (MMSE), which is a valid and widely used test. In 1993, these men were again invited to participate in the Zutphen Elderly Study; 390 (71%) of the 553 participants who were still alive at the time agreed to participate.

The Rotterdam Study is a prospective study among all inhabitants of the Ommoord suburb of Rotterdam, who were 55 years or older at the start of the study in 1990. Seventy-eight percent (n=7983) of the eligible men and women agreed to participate in extensive interviews and physical examinations. Cognitive function was tested with the MMSE, and additionally, subjects were screened for dementia. Participants who were suspected of having dementia after the screening were subsequently examined by a neurologist and a neuropsychologist; a part

also underwent magnetic resonance imaging of the brain. The final diagnosis and subtyping of dementia was made according to international criteria. In 1993 and 1994, 6315 (88%) of the 7215 subjects still alive participated in the second examination round of the Rotterdam Study, in which they were, among other things, again screened for dementia.

We focused mainly on cardiovascular risk factors, because previous studies showed that atherosclerosis and cardiovascular diseases were associated with cognitive impairment and vascular dementia. Comprehensive information on dietary intake of the participants in the Zutphen Elderly Study was used to examine the relation of linoleic acid and antioxidant intake (vitamin E, beta-carotene, vitamin C, and flavonoids) to cognitive function disorders (Chapter 2). Results showed that a high intake of antioxidants was not associated with a reduced risk of cognitive impairment or decline. There was an association, however, between a high linoleic acid intake and cognitive impairment in 1990 (relative risk (RR) = 1.8), but not with cognitive decline from 1990 to 1993.

In the Rotterdam Study, we investigated the relationship between fat and fish consumption and the risk of incident dementia (Chapter 3). The advantage of this study is that dietary intake was assessed at baseline in subjects with normal cognition. A high fat intake, and especially a high saturated fat and cholesterol intake, increased the risk of dementia 2.4 fold, and the risk of vascular dementia 3 fold. Fish consumption on the other hand, decreased the risk of dementia by 60%, and the risk of Alzheimer's disease by 70%. The latter finding might be explained by the anti-inflammatory actions of the n-3 poly-unsaturated fatty acids in fish.

The first metabolic risk factors we examined were diabetes mellitus and preclinical stages of this disease, i.e. glucose intolerance and hyperinsulinemia (Chapter 4). These disorders increase the risk of cardiovascular diseases. In the Zutphen Elderly Study, men with diabetes were more often cognitively impaired than men without diabetes (RR = 1.2). Even in preclinical stages of the disease, there was a positive association with cognitive impairment.

The amino acid homocysteine is a relatively new risk factor for cardiovascular disease. The homocysteine level in the blood can be reduced by folate suppletion. In a subpopulation of the Rotterdam Study, we have studied the association between homocysteine and cognitive decline (Chapter 5). In subjects under 75 years of age there was a positive relation between a high homocysteine level and cognitive decline (RR = 2.3). Furthermore, the association between homocysteine and cognitive decline was present in women (RR = 2.2), but not in men.

Cortisol and dehydroepiandrosterone sulfate (DHEAS) are adrenal steroids with opposite effects on the hippocampus. The hippocampus plays an important role in memory processes and is one of the brain

areas that is damaged in Alzheimer's disease. Previous investigations showed an association between these hormones and Alzheimer's disease, but, except for one study on DHEAS and cognition, they were all cross-sectional and not conducted in a general population. A number of participants of the Rotterdam Study (n=189) participated in an extensive additional examination on glucose metabolism, in which also serum cortisol and DHEAS was measured. Our study showed a positive relation of serum cortisol to cognitive impairment (RR = 1.7) (Chapter 6). There was an inverse association between DHEAS and cognitive impairment (RR = 0.5), and between DHEAS and cognitive decline (RR = 0.6).

In Part III we studied the combined effect of the apolipoprotein E gene and environmental risk factors on the risk of cognitive decline. Previous studies showed that both the e4 allele of the apolipoprotein E gene and a low education were associated with cognitive decline and Alzheimer's disease. In the Zutphen Elderly Study, we found that a low education (only primary school) was only associated with cognitive decline in subjects without the e4 allele (RR = 3.1) (Chapter 7).

It is widely known that a history of stroke increases the risk of cognitive decline. In the Zutphen Elderly Study, we examined whether the combination of a history of stroke and the e4 allele resulted in an even higher risk of cognitive decline (Chapter 8). It was found that men with both risk factors had a 17.2 fold increased risk of cognitive decline compared with men without these risk factors. This relative risk was much higher than the sum of separate relative risks for stroke and the e4 allele (RR = 4.7 and 3.3, respectively), which suggests a synergistic effect of these two factors on cognitive decline.

Finally, we investigated the prognosis of dementia in the Rotterdam Study (Chapter 9). It is known that subjects with dementia have an increased mortality. Cause-specific mortality was not examined yet in a general population, though. Our results showed that subjects with dementia have a 2.0 times increased risk of dying compared with subjects without dementia. There was a shift in the distribution of the causes of death. The percentage of subjects that died from cancer was lower among demented than among non-demented subjects (13.3% vs 24.9%), whereas the percentage that died from noncardiovascular-noncancerous causes was higher among demented subjects (49.2% vs 31.3%).

In Chapter 10 our results are briefly summarized and some methodological problems are discussed. The hypotheses on the etiology of cognitive decline and dementia are addressed in the light of our findings, and finally, some ideas for future research are given.

Until recently, not much was known on the relation between diet and cognition. This thesis shows that fatty acids may influence the occurrence of cognitive decline and dementia. Metabolic factors also seem to play a role in the underlying processes leading to these disorders. Our findings suggest that vascular processes and inflammation

are involved in the etiology of cognitive decline and dementia. Future research on risk factors should focus, among other things, on these processes. Although the investigated risk factors can be modified, our results should first be confirmed in other large population-based studies, before preventive measures can be taken.

SAMENVATTING

In dit proefschrift wordt onderzoek beschreven naar risicofactoren voor cognitieve achteruitgang en dementie. Achteruitgang in cognitieve functies is één van de belangrijkste symptomen van dementie. De voornaamste cognitieve functies zijn oriëntatie in tijd en plaats, inprenting en geheugen, waarneming, aandacht, taalgebruik en -begrip, rekenen, constructieve vaardigheden en ruimtelijke oriëntatie. Een slechter cognitief functioneren leidt tot een verminderde zelfstandigheid en tot een achteruitgang in de kwaliteit van leven. Naast deze nadelige consequenties voor het individu, leggen cognitieve achteruitgang en dementie een niet gering beslag op de gezondheidszorg door de hogere kans op institutionalisering. Derhalve is het van belang te zoeken naar risicofactoren voor deze aandoeningen die mogelijkerwijs gemodificeerd kunnen worden. In dit proefschrift wordt onderzocht of bepaalde voedingsfactoren en metabole parameters geassocieerd zijn met cognitieve achteruitgang en dementie.

Voor het in dit proefschrift beschreven onderzoek hebben we gegevens gebruikt van twee bevolkingsonderzoeken bij ouderen: de Zutphen Ouderen Studie en het Erasmus Rotterdam Gezondheid en Ouderen (ERGO) onderzoek. De Zutphen Ouderen Studie is de voortzetting van de Zutphen Studie, dat deel uitmaakte van het in 1960 gestarte Zeven Landen Onderzoek. In 1990 namen 560 van de 718 nog in leven zijnde mannen (78%), allen geboren tussen 1900 en 1920, deel aan de Zutphen Ouderen Studie. Zij werden uitgebreid geïnterviewd en onderzocht. Een valide en veelgebruikte cognitieve functietest, de Mini-Mental State Examination (MMSE), werd bij hen afgenomen. Vervolgens werden deze mannen in 1993 opnieuw gevraagd deel te nemen aan de Zutphen Ouderen Studie; 390 (71%) van de 553 nog in leven zijnde mannen stemden daarin toe.

Het ERGO onderzoek is een prospectief onderzoek onder alle inwoners van de Rotterdamse wijk Ommoord, die ten tijde van het begin van het onderzoek in 1990 55 jaar of ouder waren. Achtenzeventig procent (n=7983) van de mensen die in aanmerking kwamen deed mee

aan de uitgebreide interviews en lichamelijke onderzoeken. Ook bij hen werd de MMSE afgenomen en daarnaast werden ze gescreend op dementie. Personen die naar aanleiding van deze screening verdacht werden van dementie zijn vervolgens uitgebreider onderzocht door een neuroloog en een neuropsycholoog. De uiteindelijke diagnose en subtypering van dementie werd gesteld volgens internationale criteria. In 1993 en 1994 namen 6315 (88%) van de 7215 nog in leven zijnde deelnemers deel aan het vervolg van het ERGO onderzoek, waarin zij ondermeer opnieuw gescreend werden op dementie.

Aangezien er een verband lijkt te bestaan tussen aderverkalking en hart- en vaatziekten enerzijds en een verminderd cognitief functioneren en vasculaire dementie anderzijds, richtten wij ons in dit onderzoek vooral op vasculaire risicofactoren. Met behulp van uitgebreide gegevens over de voedselinneming van de deelnemers aan de Zutphen Ouderen Studie werd het verband tussen inneming van linolzuur en antioxydanten (vitamine E, beta-caroteen, vitamine C en flavonoïden) en cognitieve functiestoornissen onderzocht (**hoofdstuk 2**). Hieruit bleek dat een hoge inneming van deze antioxydanten niet geassocieerd was met een verlaagd risico op cognitieve functiestoornissen. Wel bleek er een verband te bestaan tussen een hoge linolzuurinneming en een verminderd cognitief functioneren in 1990 (relatief risico (RR) = 1,8), maar niet met cognitieve achteruitgang tussen 1990 en 1993.

In het ERGO onderzoek werd gekeken naar de relatie tussen vet- en visconsumptie en het risico op het krijgen van dementie in de jaren daarna (**hoofdstuk 3**). Het voordeel van deze studie is dat de voedingsinneming werd bepaald bij mensen die op dat moment nog normaal cognitief functioneerden. Een hoge vetinneming, en vooral een hoge inneming van verzadigd vet en cholesterol, leidde tot een 2,4 maal verhoogd risico op het krijgen van dementie in het algemeen, en vasculaire dementie in het bijzonder (RR = 3.0). Consumptie van vis daarentegen, verlaagde het risico op dementie met 60%, en het risico op de ziekte van Alzheimer met 70%. Dit laatste zou verklaard kunnen worden door de ontstekingsremmende werking van de n-3 meervoudig onverzadigde vetzuren in vis.

De eerste metabole risicofactoren die we onderzochten waren suikerziekte en voorstadia van suikerziekte, d.w.z. een gestoorde glucosetolerantie en hyperinsulinemie (**hoofdstuk 4**). Deze aandoeningen verhogen het risico op hart- en vaatziekten. In de Zutphen Ouderen Studie hadden mannen met suikerziekte 1,2 maal zo vaak cognitieve functiestoornissen als mannen zonder suikerziekte. Zelfs in voorstadia van suikerziekte bleek er een verhoogd risico op cognitieve stoornissen te bestaan.

Het aminozuur homocysteïne is een betrekkelijk nieuwe risicofactor voor hart- en vaatziekten. Het homocysteïnegehalte in het bloed kan vrij gemakkelijk verlaagd worden door middel van foliumzuursuppletie.

Wij hebben in een subpopulatie (n=472) van het ERGO onderzoek onderzocht of homocysteïne ook gerelateerd was met cognitieve achteruitgang (hoofdstuk 5). In personen jonger dan 75 jaar was er een positieve associatie tussen een hoog serum homocysteïnegehalte en cognitieve achteruitgang (RR = 2,3). Verder was er een relatie tussen homocysteïne en cognitieve achteruitgang in vrouwen (RR = 2,2), maar niet in mannen.

Cortisol en dehydroepiandrosteronesulfaat (DHEAS) zijn bijnierschorshormonen die een effect hebben op de hippocampus. De hippocampus speelt een grote rol in geheugenprocessen en is één van de aangedane hersengebieden bij de ziekte van Alzheimer. Eerder onderzoek heeft een verband tussen deze hormonen en de ziekte van Alzheimer aangetoond, maar deze onderzoeken waren, op één onderzoek naar DHEAS en cognitie na, allen cross-sectioneel en niet uitgevoerd in een algemene populatie. Een kleine groep deelnemers (n=189) van het ERGO onderzoek heeft meegedaan aan een uitgebreider onderzoek naar de glucosestofwisseling, waarin onder meer het serum cortisol en DHEAS gehalte is gemeten. Ons onderzoek laat zien dat er een positieve associatie bestond tussen het serum cortisol gehalte en cognitieve functiestoornissen (RR = 1,7) (hoofdstuk 6). Er was een negatief verband tussen DHEAS en cognitieve functiestoornissen (RR = 0,5), en tussen DHEAS en cognitieve achteruitgang (RR = 0,6).

In Deel III wordt onderzocht hoe een mogelijke wisselwerking tussen het apolipoproteïne E gen en omgevingsfactoren het risico op cognitieve achteruitgang beïnvloedt. Voorgaand onderzoek heeft aangetoond dat zowel het e4 allel van het apolipoproteïne E gen, als een lager opleidingsniveau, geassocieerd zijn met cognitieve achteruitgang en de ziekte van Alzheimer. Wij vonden in de Zutphen Ouderen Studie dat een lager opleidingsniveau (alleen lagere school) slechts geassocieerd bleek te zijn met cognitieve achteruitgang bij mensen zonder het e4 allel van het apolipoproteïne E gen (RR = 3,1) (hoofdstuk 7).

Het is alom bekend dat het doormaken van een cerebrovasculair accident (CVA) het risico op cognitieve achteruitgang verhoogt. In de Zutphen Ouderen Studie is onderzocht of de combinatie van een CVA en het e4 allel een nog sterker verhoogd risico geeft op cognitieve achteruitgang (hoofdstuk 8). Het bleek dat mannen met beide risicofactoren een 17.2 maal verhoogde kans hadden op cognitieve achteruitgang dan mannen zonder deze risicofactoren. Dit relatieve risico was veel hoger dan de som van de afzonderlijke relatieve risico's behorende bij een CVA en het e4 allel (RR = 4,7 en 3,3, respectievelijk), wat suggereert dat deze factoren een synergistisch effect hebben op cognitie.

Als laatste hebben we de prognose van dementie onderzocht in het ERGO onderzoek (hoofdstuk 9). Het is bekend dat personen met dementie een verhoogde sterftekans hebben. Er is echter weinig bekend over oorzaakspecifieke sterfte bij personen met dementie. Het blijkt dat

personen met dementie 2 maal zoveel kans hebben om te overlijden dan mensen zonder dementie. Er was een verschuiving in de verdeling van de doodsoorzaken. Het percentage mensen dat overleed aan kanker was lager onder dementen dan onder niet-demente personen (respectievelijk 13.3% en 24.9%), terwijl het percentage dat overleed aan andere oorzaken dan kanker en hart- en vaatziekten hoger was onder demente personen (49.2% in vergelijking met 31.3%).

Hoofdstuk 10 gaat in op de resultaten van dit proefschrift en de problemen die zich bij dit onderzoek kunnen voordoen. Tevens wordt ingegaan op de hypotheses omtrent het ontstaan van cognitieve achteruitgang en dementie in het licht van onze bevindingen, en worden enkele ideeën gegeven voor toekomstig onderzoek.

Tot voor kort was nog weinig bekend over de relatie tussen voeding en cognitie. Dit proefschrift laat zien dat vetzuren van invloed kunnen zijn op het ontstaan van cognitieve achteruitgang en dementie. Ook metabole factoren lijken een rol te spelen in de onderliggende processen die leiden tot cognitieve achteruitgang en dementie. Deze bevindingen suggereren dat vasculaire processen en ontstekingsmechanismen betrokken zijn bij het ontstaan van deze aandoeningen. Toekomstig onderzoek naar risicofactoren voor cognitieve achteruitgang en dementie zou zich dan ook onder andere op deze processen moeten richten. Alhoewel de beschreven risicofactoren modificeerbaar zijn, zullen onze resultaten eerst bevestigd moeten worden in andere grote bevolkingsonderzoeken alvorens over te gaan tot preventieve maatregelen.

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About the author

Sandra Kalmijn was born on October 28th, 1965, in Brussels, Belgium. In 1984, she graduated from the "Herman Jordan Lyceum" (Gymnasium beta) in Zeist, the Netherlands. She lived for a year in Seattle, USA, where she studied at the University of Washington. In 1985 she returned to the Netherlands and started her medical studies at the University of Amsterdam, from which she graduated in 1990. During her study, she did research on polyneuropathy at the Department of Neurology, Academic Medical Center, Amsterdam, under the supervision of Prof. dr. J. Stam. In 1992, she conducted a study on autonomic dysfunction in Hereditary Motor and Sensory Neuropathies, at the Department of Neurophysiology, Academic Hospital Utrecht, under the supervision of dr. P. L. Oey and Prof. dr. A. C. van Huffelen. She also worked as a teacher in physiology at the Academy for Physiotherapy, Utrecht. After obtaining her Medical Degree in 1993, she started as a PhD student at the Netherlands Institute for Health Sciences (NIHES) in Rotterdam. For two years she worked at the Department of Chronic Diseases and Environmental Epidemiology of the National Institute of Public Health and the Environment (RIVM) in Bilthoven. She completed her graduate studies at the Department of Epidemiology & Biostatistics at Erasmus University in Rotterdam, where she obtained her Master of Science in Epidemiology in 1995. In January 1998, she will move to Charlottesville, USA, where she will continue doing research in epidemiology.

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