Determinants of Cognitive Decline and Dementia in the Very Old

Sjoerd Marijn Euser

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Determinants of Cognitive Decline and Dementia in the Very Old

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	Dr. E.J.G. Sijbrands
	Dr. A.J.M. de Craen

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1

Introduction

The gradual but increasing decline of cognitive abilities is arguably one of the most devastating features that accompany the aging process. This loss of cognitive function in the elderly is often considered intrinsic to "normal aging"; a disputable view as the regular occurrence of impaired cognitive function does not directly imply inevitability.

Especially in the very old, there is accumulating evidence that a variety of causes, rather than a single factor, result in impaired cognitive function and the clinical symptoms of dementia.[1] This "mixed pathology" was shown in post mortem studies and emphasizes the multifactorial and heterogeneous nature of the dementia syndrome.[1,2] Vascular factors, inflammation factors, endocrine factors and lifestyle factors have been shown to be associated with the risk of dementia in elderly populations.[3]

Many studies on the etiology of dementia have focused on the distinction between demented and non-demented persons. Although the choice for this clinically based definition has undoubtedly improved our understanding of the biological mechanisms that underlie the development of dementia, it is unlikely that the gradually progressing character of cognitive decline that often precedes clinical dementia is adequately reflected by this distinction. Furthermore, the complex diagnosis of the clinical syndrome of dementia, that is characterized by criteria including both decline in cognitive abilities and its interference with activities of daily living, might not always be specific enough.

Studying changes in cognitive function in the developmental stage of dementia may not only better represent the underlying biology of the disease but could possibly lead to the identification of risk factors in the early stages of the disease process.

One would expect that the occurrence of impaired cognitive function in predominantly the oldest old individuals, and the concurrently large potential gain in experienced health in this group, would have increased the focus on biological mechanisms involved in the development of cognitive impairment in especially these oldest old. In most studies however, there is a clear underrepresentation of oldest old individuals.

A particular research interest regarding the development of cognitive decline and dementia is the identification of potentially modifiable risk factors, including factors involved in glucose metabolism, vascular pathology and inflammation.

The relation between impaired glucose metabolism and cognitive impairment, in for instance diabetes mellitus patients, could be explained by concurrent cerebrovascular disease, accumulation of advance glycation end-products (AGE) or disruption of amyloid β clearance.[4-6] However, the relation between a pre-diabetic state, when impaired fasting glucose levels are present, and cognitive function and decline needs further clarification.

Furthermore, vascular pathology in general has been shown to play a prominent role in the development of dementia.[3,7] Cerebral small vessel disease, stroke and disturbed cerebral perfusion could underlie this association.[7,8] Several other lines of investigation have additionally suggested a role for inflammation in the development of cognitive impairment and

dementia.[9,10] How these vascular and inflammatory risk factors relate to cognitive function in the early stages of the disease remains to be investigated.

Next to the investigation of these potentially modifiable risk factors for cognitive impairment and dementia, there is an increasing interest in research on preclinical markers of dementia that could be used to identify people who are at increased risk of developing dementia. Serum levels of amyloid β peptides have already been suggested as biomarkers for dementia,[11] although the relation between the peptide levels and the risk of dementia and cognitive decline warrants further research.

Moreover, there have been studies that suggest that in general, possible risk factors that play a role at younger ages may not be important anymore when people survive until higher ages.[12,13] To examine the potential age-specific effects of these risk factors, studies with larger numbers of oldest old participants are needed. I was in the fortunate position to have the availability over several large prospective studies including the Rotterdam Study,[14] the Leiden 85-plus Study,[15] and the PROSPER study,[16] that were complementary with respect to the age of the participants and consequently provided me with the tools to study the association between several potentially modifiable risk factors and cognitive function and decline over a wide age range in the general population.

The Rotterdam Study is a large population-based prospective study that was conducted among 7,983 inhabitants of Ommoord, a district of Rotterdam, aged 55 years and older.[14] The study was designed to target cardiovascular, neurological, opthalmological and endocrine diseases and is ongoing since 1990. All participants underwent baseline examinations (1990-1993) and were invited to visit the research center during follow-up examinations (1993-1994, 1997-1999, 2002-2004) where cognitive function was assessed with a neuropsychological test battery.

The Leiden 85-plus Study is a prospective population-based study of 85-year-old inhabitants of Leiden.[15] Between September 1997 and September 1999, all inhabitants of Leiden born between 1912 and 1914 were contacted within a month of their 85th birthday, and 599 individuals agreed to participate. Annual follow-up examinations were performed until age 90, during which cognitive function was assessed.

The PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) is a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major cardiovascular events in the elderly.[16] Between December 1997 and May 1999, a total of 5,804 participants (aged 70-82 years) with an increased risk of cardiovascular disease were recruited in Scotland, Ireland and the Netherlands. Cognitive function was assessed at baseline, after 9, 18 and 30 months, and at the end of the study (between 36 and 48 months) with a dedicated neuropsychological test battery.

In this thesis, I first explored the measurement of cognitive function, with respect to the effect of selection by health and survival on the estimation of cognitive function and decline

in epidemiological studies. I used several study designs and methods to handle multiple and missing data to investigate how these affected the estimates of cognitive function and decline in our study populations. This work is described in chapter 2.

In chapter 3, I examined the relation between glucose metabolism and cognitive function and decline. First, I investigated the association between fasting glucose levels and cognitive function and decline in a combined study of data from the Rotterdam Study and the PROSPER study. Additionally, I studied the association between genetic variation in genes that influence insulin/IGF-1 signaling and cognitive function in the Leiden 85-plus Study. The insulin/IGF-1 signaling pathway has previously been shown to be involved in the regulation of health and lifespan in a number of model organisms and humans, although its relation with cognitive function has thus far not been extensively studied.

In chapter 4, I investigated the association between several vascular and inflammatory risk factors and cognitive function and decline. First, I investigated how age influences the relation between blood pressure and cognitive function, using pooled data from both the Rotterdam Study and the Leiden 85-plus Study which provided a large sample of participants over a broad age range. Second, I studied the association between circulating markers of inflammation and cognitive function and decline in a combined analysis of data from the Rotterdam Study and the Leiden 85-plus Study. Inflammation has been suggested to be involved in the pathogenesis of dementia by several lines of research, although the relation between inflammatory processes and cognitive function in the early stages of the development of the disease is not clear yet. Third, I described the association between serum uric acid and cognitive function and dementia. Serum uric acid is a well known risk factor for cardiovascular disease and stroke, but is also thought to possess beneficial properties, as it accounts for a substantial part of the antioxidative capacity of the plasma. These seemingly contradictory properties with respect to the development of cognitive impairment and dementia are addressed in this chapter.

In chapter 5, I examined potential biomarkers that are suggested for the identification of people who are at increased risk of developing dementia. Senile plaques in the brain of Alzheimer's disease patients are a characteristic hallmark of this disease and predominantly contain amyloid peptides. Serum levels of these amyloid β peptides have been associated with an increased risk of dementia and the plasma $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio has been suggested as a premorbid biomarker for dementia. I have further examined the relation between serum amyloid β peptides and cognitive decline in the early stages of the development of dementia.

Finally, in chapter 6, I acknowledged several methodological issues that were related to the work described in this thesis and discussed some suggestions for further research.

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2

Measuring cognitive function

2.1

Measuring Cognitive Function with Age: the Influence of Selection by Health and Survival

ABSTRACT

Background: Research into the pathophysiology of age-associated cognitive function and decline requires a valid estimate of cognitive function. However, this estimation can be grossly influenced by a selective loss to follow-up.

Methods: We investigated the influence of health selection on the estimated age-associated cognitive function and decline by studying the effect of study design and the handling of multiple and missing data on this estimation. We used linear regression analyses and linear mixed models to assess cognitive function from cross-sectional and longitudinal data. Repeated measures of cognitive function (assessed with dedicated neuropsychological tests) were carried out in two independent population-based cohort studies: the Rotterdam Study (3719 participants, mean age 71 years) and the Leiden 85-plus Study (369 participants, age 85 years).

Results: The effect of age on cognitive function was greater in cross-sectional analyses when all participants were included than when analyses were restricted to participants with repeated measurements. The decline in cognitive function over 4.6 years of follow-up was intermediate between the cross-sectional estimates from the total and from the restricted sample. Moreover, the estimated decline in cognitive function was larger when using a short follow-up than when using the complete follow-up over 5 years. The estimated decline using linear mixed models was similar to analyses including those with a complete follow-up over 5 years.

Conclusion: Selection for health and survival results in better age-specific cognitive test scores and less cognitive decline. Statistical methods handling multiple and missing data do not fully correct for this bias.

INTRODUCTION

Cognitive function decreases with age. The investigation of causes of age-associated cognitive function and decline requires a valid estimate of deterioration in cognitive function.

Previous population-based studies that estimated cognitive function over time have yielded diverse results. The variety in estimates has been ascribed to differences in study population, assessment of cognitive function and statistical analysis technique.[1-7] Another important contribution to these discrepancies could come from selection bias, which is a common problem in the study of longitudinal estimates in epidemiologic settings.[8-10]

The objective of the present study was to examine the effect of health selection on the estimated cognitive decline in a population-based setting. In particular, we examined the influence of cross-sectional versus longitudinal designs, and the influence of various ways to handle multiple and missing data. We examined these issues in two independent population-based studies: the Rotterdam Study and the Leiden 85-plus Study.

METHODS

Population

The Rotterdam Study is a large prospective population-based cohort study that is being conducted among all inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, The Netherlands.[11] The medical ethics committee of the Erasmus University of Rotterdam approved the study, and informed consent was obtained from all participants. A total of 7983 individuals (response rate 78 percent) participated in the baseline examinations between 1990-1993 (mean age 71, range 55-106 years). All participants were interviewed at home, and visited the research center for further examinations. In the 3rd survey (1997-1999) and the 4th survey (2002-2004) cognitive function was assessed more extensively with a dedicated neuropsychological test battery.

The Leiden 85-plus Study is a prospective population-based cohort study of inhabitants of Leiden, The Netherlands. The medical ethics committee of the Leiden University Medical Centre approved the study, and informed consent was obtained from all participants. Between September 1997 and September 1999, all inhabitants of Leiden born between 1912 and 1914 (n=705) were contacted within a month after their 85th birthday. A total of 599 individuals (response rate 87 percent) agreed to participate. Over the next 5 years (until the age of 90), annual neuropsychological tests were performed during home visits.

Study sample

In the Rotterdam Study, 4797 individuals participated in the 3rd survey, of whom 4206 underwent the neuropsychological test battery. Complete data on all tests in the neuropsychologi-

cal test battery were available for 3865 participants. Of these, 2470 also had complete data on all cognitive tests at the 4th survey. Of the remaining 1395 persons with no or incomplete data for the 4th survey, 31 percent had died, 40 percent declined to participate or were too ill to visit the research centre, 7 percent could not be contacted and 22 percent had incomplete data on the neuropsychological test battery. We restricted the analyses to participants younger than 85 years, because of the relatively limited number of participants above this age. This resulted in a study sample of 3719 participants at the 3rd survey available for cross-sectional analyses, and 2441 participants with repeated measurements available for longitudinal analyses. The proportion of participants without follow-up was especially high among those oldest at baseline (for baseline age 60-64 years, 22.4% had no follow-up information; 65-69 years, 22.4% ; 70-74 years, 33.2% ; 75-79 years, 48.2% ; 80-84 years, 65.7%).

In the Leiden 85-plus Study, 599 participants underwent neuropsychological testing at age 85, and 446 had complete data on all tests in the neuropsychological test battery. Of these, 369 also had complete data on the neuropsychological test battery at age 86 and were therefore available for comparison among the methods for handling missing data in a longitudinal study design. For 158 participants, annual data on the neuropsychological test battery during the entire 5-year follow-up period were available. Of the remaining 288 persons who did not participate, 59 percent died, 11 percent declined to participate and 30 percent had incomplete data on the neuropsychological tests.

Cognitive function

Global cognitive function was measured by use of the Mini-Mental State Examination in both cohorts. In addition, a dedicated neuropsychological test battery was used to assess executive function and memory function. Executive function was assessed by use of the abbreviated Stroop test trial 3 and the Letter Digit Substitution Task in both cohorts and by use of the Word Fluency Test in the Rotterdam Study. Memory function was assessed in the Leiden 85-plus Study only, by use of the 12-Picture Learning Test (immediate and delayed recall).

The Mini-Mental State Examination is a widely used test developed as a screening instrument for dementia; it provides a reliable measure of global cognitive function.[12-14] The Letter Digit Substitution Task is a modified version of the Symbol Digit Modalities Test, measuring processing speed.[15] Participants make as many letter-digit combinations as possible within 60 seconds, following an example that shows the correct combinations. The abbreviated Stroop test is a measure of attention, consisting of three trials in which the participant has to name 40 items shown on a card.[16,17] In trial 1 the card contains color-names printed in black and participants are asked to name the printed word. In trial 2 the card contains colored blocks and participants are asked to name the printed color. In trial 3 the card contains color-names printed in a different color than the color-name and participants are asked to name the color of the ink. The outcome variable is the time needed to finish trial 3. The Word Fluency Test is a test for verbal fluency as an element of executive function. Participants are asked to name

as many animals as possible within 60 seconds.[18] Memory function was assessed by the 12-Picture Learning Test, a measure of memory function with immediate- recall and delayed-recall components.[19] In the immediate recall portion, participants are shown pictures of 12 different objects and then asked to recall as many as possible. This is done three consecutive times with the total number of correct answers as the outcome variable. After 20 minutes the participants are asked again to recall the 12 objects. This number is the outcome variable for the delayed recall.

Additional measurements

In the Rotterdam Study, education was measured at the baseline examination (1990-1993), and dichotomized into more or less than primary education. In the Leiden 85-plus Study, education was assessed at age 85 years, and also dichotomized into more or less than primary education.

Statistical analyses

Estimates of cross-sectional and longitudinal analyses

Age-associated cognitive function was estimated from both cross-sectional and longitudinal analyses in the Rotterdam Study. In the cross-sectional analyses, we first calculated cognitive test scores at the 3rd survey in the total sample, in the sample of participants who were alive at follow-up regardless of whether they participated or not, and in the participants who participated at follow-up; we then compared those results, for consecutive 5-year age categories. In the longitudinal analyses, we compared cognitive test scores at the 3rd survey with those at the 4th survey within 5-year age categories.

The longitudinal decline in cognitive function over 1-year periods was examined in the sample of the Leiden 85-plus Study (from age 85 to 86, 86 to 87, 87 to 88, 88 to 89 and 89 to 90 years). For every 1-year period, we selected participants with complete data on cognitive tests at the beginning and the end of the one-year interval. The difference in cognitive function between those two examinations was compared.

Handling of multiple and missing data

We evaluated the effects of four different ways of handling multiple and missing data on the estimated rate of cognitive decline in the sample of the Leiden 85-plus Study.

First, we assessed the one-year change in cognitive function by comparing test scores at baseline (age 85 years) with test scores after one year of follow-up (age 86 years). We selected those participants with complete data at age 85 and at age 86 years, and calculated the difference in cognitive function between age 85 and 86 years. Second, we assessed the annual change in cognitive function by comparing test scores at baseline (85 years) with those at the last available follow-up visit. Annual change in cognitive function was calculated as the difference between test score at baseline and test score at the last available year of follow-

up, divided by the length of follow-up (1-5 years). Third, we assessed the annual change in cognitive function for participants with a complete annual follow-up from age 85 to 90 years, using a linear regression analysis with cognitive function as dependent and age as independent variable. Fourth, we assessed the annual change in cognitive function by use of a linear mixed model. The linear mixed model uses all available data during follow-up to assess the annual change in cognitive function. All participants with at least 1 year of follow-up were included in these analyses.

RESULTS

Table 1 shows the baseline characteristics of both study samples. In the Rotterdam Study at the 3rd survey (baseline for these analyses) the mean age of the participants was 71.4 years, 58 percent were women, and 28 percent had primary education at most. Mean follow-up was 4.6 years. Participants with a follow-up examination were slightly younger and had a better cognitive function at the 3rd survey (Mini-Mental State Examination-score [range]: 28.1 points [18-30]) compared with participants without at least one follow-up examination (Mini-Mental State Examination-score [range]: 27.8 [13-30]). In the Leiden 85-plus Study age at baseline was 85 years, 65 percent of participants were women and 56 percent had primary education only. Those participants who were followed for 5 years had a better cognitive function (Mini-Mental State Examination-score [range]: 27.4 points [20-30]) than participants in the total sample (Mini-Mental State Examination-score [range]: 26.7 points [19-30]). Additionally, in both the Rotterdam Study and the Leiden 85-plus Study, a smaller proportion of the participants with a follow-up examination had a history of myocardial infarction and cardiovascular accident compared to participants without at least one follow-up examination.

Estimates from cross-sectional and longitudinal analyses

Figure 1 presents the cross-sectional cognitive test scores in consecutive age categories at the 3rd survey in the Rotterdam Study for the total sample, for the sample of participants who were alive at follow-up regardless of whether they participated, and for the restricted sample of participants for whom repeated measurements were available. The latter group had better cognitive test scores in all age categories when compared with the total sample and the sample of participants who were alive at follow-up. The difference in cognitive test scores between the total and restricted samples increased with age, as is illustrated by the diverging dotted lines. Similar patterns were seen for all tests of cognitive function. These findings were confirmed by additional analyses, which showed an association between lower cognitive test scores and an increased risk of mortality (hazard ratios for mortality [95% confidence interval (CI)] per SD increase in cognitive test score were: Mini-Mental State Examination (0.81 [0.76-0.86]) ; Letter Digit Substitution Task (0.69 [0.63-0.75]) ; Stroop test trial 3 (1.23 [1.16-1.31]) and

Table 1. Participant characteristics at baseline (for the Rotterdam Study, at third survey, and for the Leiden 85-plus Study, at study entry [age 85])

Baseline characteristics	Rotterdam Study				Leiden 85-plus Study			
	Total sample	Complete information available at next (4th) survey			Total sample	Complete 5-year follow-ups		
		Yes	No			Yes	No	
			Deceased	Dropped out			Deceased	Dropped out
	(n=3719)	(n=2441)	(n=387)	(n=891)	(n=369)	(n=158	(n=113)	(n=98)
Age (years); mean (SD)	71.4 (5.9)	70.1 (5.4)	74.8 (6.0)	73.3 (6.2)	85	85	85	85
Female; %	58	58	42	64	65	76	50	64
Low level of education; %	28	26	28	36	56	58	54	56
Neuropsychological test scores; mean (SD)								
MMSE (points)	27.8 (1.9)	28.1 (1.6)	27.0 (2.6)	27.2 (2.2)	26.7 (2.5)	27.4 (2.1)	26.5 (2.5)	25.8 (2.9)
LDST (correct answers)	26.9 (6.9)	28.2 (6.4)	23.3 (7.2)	24.7 (7.2)	17.9 (7.0)	19.6 (6.8)	16.9 (7.4)	16.2 (6.3)
Stroop test trial 3 (seconds)	56.8 (20.6)	53.3 (17.3)	66.2 (27.4)	62.3 (22.7)	81.1 (34.4)	72.5 (26.5)	85.3 (36.4)	90.2 (40.0)
Word Fluency Test (words)	21.0 (5.5)	21.8 (5.3)	19.1 (5.7)	19.7 (5.3)	-	-	-	-
12-PLT immediate recall (pictures)	-	-	-	-	24.7 (5.3)	26.2 (4.6)	24.3 (4.9)	22.9 (6.0)
12-PLT delayed recall (pictures)	-	-	-	-	9.0 (2.4)	9.7 (2.1)	8.9 (2.3)	8.0 (2.7)
History of myocardial infarction; % (no.)	9 (327)	7 (183)	19 (74)	8 (70)	9 (33)	3 (5)	14 (16)	12 (12)
History of cardiovascular accident; % (no.)	1 (34)	1 (17)	2 (9)	1 (8)	6 (23)	4 (7)	7 (8)	8 (8)
Total cholesterol (mmol/L); mean (SD)	5.8 (1.0)	5.9(1.0)	5.7 (1.1)	5.9 (1.0)	5.7 (1.1)	5.8 (1.0)	5.6 (1.1)	5.7 (1.1)
HDL cholesterol (mmol/L); mean (SD)	1.4 (0.4)	1.4 (0.4)	1.3 (0.4)	1.4 (0.4)	1.3 (0.4)	1.4 (0.4)	1.3 (0.4)	1.3 (0.3)
Systolic blood pressure (mm Hg); mean (SD)	143 (21)	142 (21)	145 (22)	146 (22)	157 (18)	161 (18)	155 (18)	155 (16)
Diastolic blood pressure (mm Hg); mean (SD)	75 (11)	76 (11)	75 (11)	75 (11)	78 (9)	79 (9)	76 (10)	78 (8)

MMSE indicates Mini-Mental State Examination; LDST, Letter Digit Substitution Task; 12-PLT, 12-Picture Learning Test

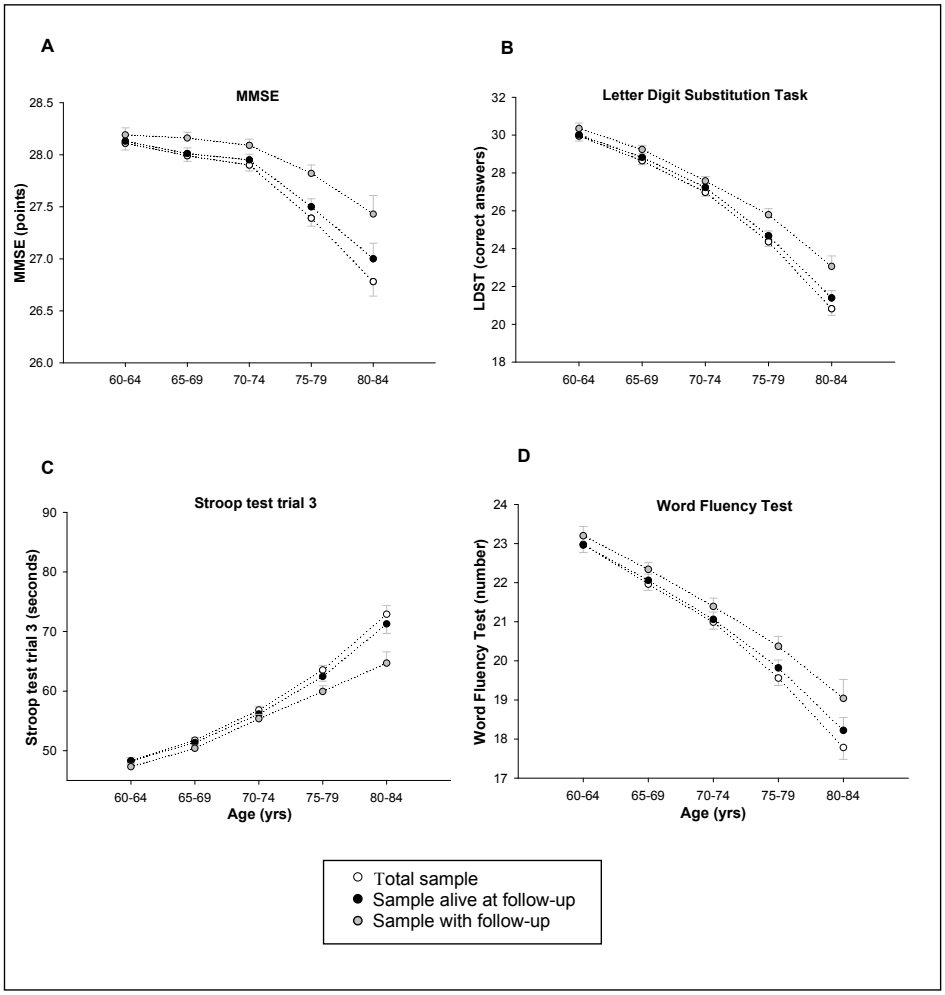

Figure 1. Results for (A) the Mini-Mental State Examination (MMSE), (B) the Letter Digit Substitution Task (LDST), (C) the Stroop test trial 3 and (D) the Word Fluency Test. Note that in the Stroop test trial 3 (C) a higher score indicates a worse cognitive function. Error bars represent standard errors of the mean.

Word Fluency Test (0.76 [0.70-0.82])) and an association between lower cognitive test scores and non-participation (odd ratios for non-participation [95%CI] per SD increase in cognitive test score were: Mini-Mental State Examination (0.69 [0.64-0.75]) ; Letter Digit Substitution Task (0.68 [0.63-0.75]) ; Stroop test trial 3 (1.35 [1.25-1.47]) and Word Fluency Test (0.76 [0.70-0.83])).

Figure 2 shows the longitudinal decline of cognitive test scores within 5-year age categories in the Rotterdam Study. The age-specific cognitive function is consistently better in participants at the 3rd survey than in participants of the same age at the 4th survey. The longitudinal decline in cognitive function over a 4.6-year follow-up period is stronger compared with the

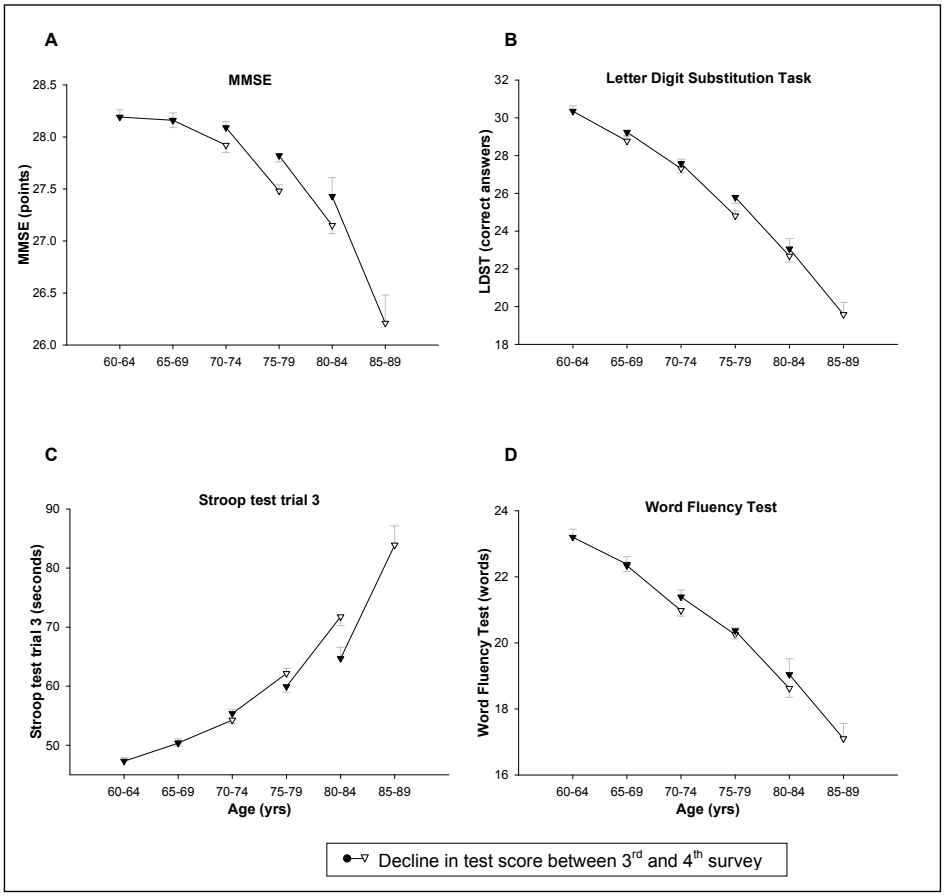

Figure 2. Results for (A) the Mini-Mental State Examination (MMSE), (B) the Letter Digit Substitution Task (LDST), (C) the Stroop test trial 3 and (D) the Word Fluency Test. The solid lines show the decline in cognition over 4.6 years per age category.

cross-sectional estimates of the same sample. This effect was more pronounced at older ages. All tests scores of cognitive function were similarly affected.

Figure 3 shows the longitudinal decline of cognitive test scores over 1-year periods in the Leiden 85-plus Study. This figure shows a similar (though more extreme) pattern compared with that in figure 2. The age-specific cognitive function at the beginning of the 1-year periods is better than in participants of the same age at the end of the 1-year periods. Again, for all tests of cognitive function, age-specific test scores were better when based solely on participants with follow-up examinations.

Handling of multiple and missing data

Table 2 shows the annual decline in cognitive function as estimated by different statistical handling of the data in the Leiden 85-plus Study. The estimated decline in cognitive function

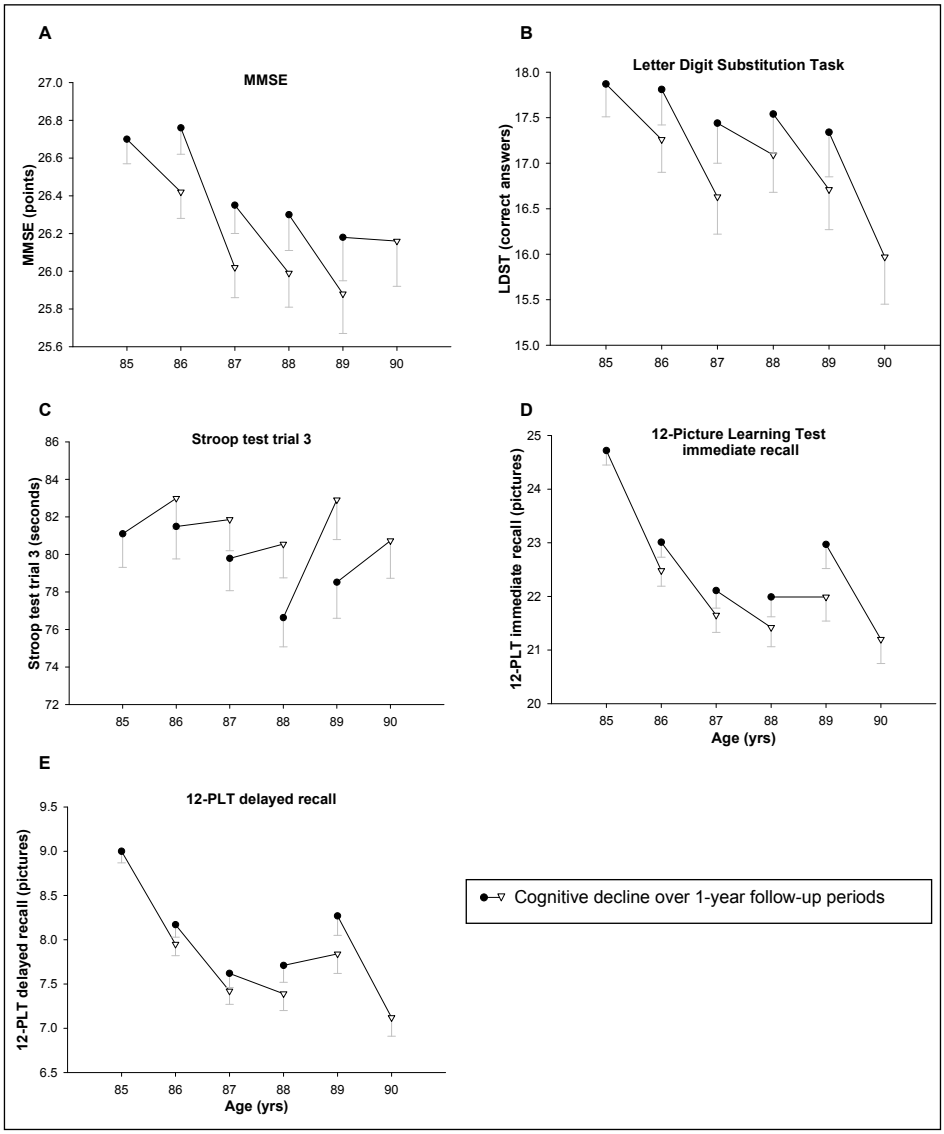

Figure 3. Results for (A) the Mini-Mental State Examination (MMSE), (B) the Letter Digit Substitution Task (LDST), (C) the Stroop test trial 3, (D) the 12-Picture Learning Test immediate recall (12-PLT immediate recall) and (E) the 12-Picture Learning Test delayed recall (12-PLT delayed recall). The number of participants in each 1-year period varies per year; age 85-86 (n=369), 86-87 (n=315), 87-88 (n=265), 88-89 (n=210), 89-90 (n=164).

was larger when using a shorter rather than a longer follow-up. Cognitive decline was also estimated to be larger when using only the data at baseline together with the latest follow-up data, compared with the decline estimated from the complete annual follow-up over 5 years. The decline in cognitive function when using linear mixed models was similar to that estimated by use of complete follow-up data.

Table 2. Estimates of annual change in cognitive function derived from different methods for handling multiple and missing data in the Leiden 85-plus Study.

Neuropsychological tests	Annual change in cognitive function (95% CI)							
	Short follow-up (1 year) (n=369)		Maximal follow-up (1-5 years) (n=369)		Complete follow-up (5 years) (n=158)		Mixed models (1-5 years) (n=369)	
MMSE (points)	-0.3	(-0.5, -0.1)	-0.4	(-0.6, -0.3)	-0.2	(-0.3, -0.2)	-0.3	(-0.4, -0.3)
LDST(correct answers)	-0.6	(-1.0, -0.2)	-0.9	(-1.1, -0.7)	-0.6	(-0.8, -0.5)	-0.7	(-0.8, -0.6)
Stroop test trial 3 (seconds)	1.9	(-0.8, 4.6)	1.4	(-0.4, 3.1)	1.5	(0.9, 2.1)	1.6	(1.0; 2.1)
12-PLT immediate recall (pictures)	-2.2	(-2.7, -1.8)	-1.6	(-1.9, -1.4)	-0.8	(-1.0, -0.7)	-1.0	(-1.1, -0.9)
12-PLT delayed recall (pictures)	-1.1	(-1.2, -0.9)	-0.8	(-0.9, -0.7)	-0.4	(-0.5, -0.3)	-0.5	(-0.5, -0.4)

DISCUSSION

Estimated cognitive function is better when the analyses are based on participants for whom follow-up examinations are available. Moreover, longer follow-up periods further diminish the rate of decline. Estimates from linear mixed models analyzing all individuals with different follow-up periods were not materially different from the estimates obtained when analyzing data from participants with complete follow-up data.

The strengths of our study include the use of two independent population-based studies. The age distributions of the 2 cohorts were complementary and allowed us to study the change in cognitive function from age 60 till 90 years. The dedicated neuropsychological tests used in both populations were comparable and assessed several cognitive domains. However, extensive data on cognitive function in the Rotterdam Study were available only for the 3rd and the 4th survey, resulting in a follow-up of just 4.6 years. A longer follow-up period could give more insight into the effect of health-selection on the estimated age-associated cognitive function in the different study designs.

Our data show that the various study designs resulted in different estimations of age-associated cognitive function. This is due to selective non-participation and drop-out of participants with lower levels of cognitive function. In a cross-sectional setting, difference in cognitive function in population-based studies depends highly on response rate. In a longitudinal setting, the estimated decline in cognitive function depends on response rate, drop-out during follow-up and the statistical method used to handle multiple and missing data. The selection for health and survival is the overriding phenomenon that results in the different estimates for the age-associated cognitive function in both study designs.

Sample selection has an important effect on the outcomes of a study. A cross-sectional design will estimate cognitive function in the relatively healthy part of the population; non-participants tend to have lower cognitive function and are less healthy than participants.[6,20,21] This effect was confirmed by our data that showed a smaller proportion of participants with

a history of myocardial infarction and cardiovascular accident, and a smaller difference in cognitive function between age categories in the sample with repeated measures compared with the total sample. Health related-selection affects not only cross-sectional study designs but also longitudinal designed studies. The drop-outs during follow-up were not random, but consisted predominantly of older participants with lower cognitive function. In a longitudinal study the participants at baseline are a relatively healthy selection of the total population and the participants who complete follow-up are a further selection of these participants.

Cross-sectional results are generally considered inferior to longitudinal ones, but this may not be the case if longitudinal data are distorted by deaths and drop-outs during follow-up.

The 4 ways we used to handle multiple and missing data resulted in different estimates of cognitive decline. Either a short or a maximal follow-up produced a higher estimated cognitive decline than a complete follow-up. This suggests that the participants who dropped out during follow-up have a larger decline in cognitive function than participants who completed the follow-up period. Advanced statistical models such as linear mixed models take missing data during follow-up into account in longitudinal data analysis.[22-24] However, linear mixed models assume that missing data are "missing at random" or "missing completely at random," which is not always true for longitudinal studies on cognitive function. Our data showed that the estimated cognitive decline assessed by a linear mixed model was similar to that based on the participants with a complete follow-up over 5 years. Thus, a linear mixed model underestimates the decline in cognitive function in this setting.

The problem of defining meaningful longitudinal estimates in the presence of "censoring by death" has been extensively studied in the statistical literature.[25-28] One of the suggested methods for dealing with missing longitudinal data due to death or drop-out is principal stratification – i.e., participants are stratified based on their potential outcome concerning death or missingness and the analyses take into account this stratification.[25] Another promising method to account for missing longitudinal data is "joint modeling," in which both the random effects in the longitudinal covariates and the survival components are simultaneously modeled.[29,30] Application of such methods may possibly help overcome some of the biases due to health selection in longitudinal epidemiologic studies.

In conclusion, health-related selection artificially elevated age-specific cognitive test scores and lowered the estimated slope of age-associated cognitive function, in both cross-sectional and longitudinal study designs. In addition, the length of follow-up can affect the estimation of cognitive decline, with a short follow-up resulting in a larger estimated rate of decline than a longer follow-up period. Even advanced statistical methods such as linear mixed models do not adequately account for selective attrition. Assessments of cognitive function in the elderly must take particular care in considering possible biases from health selection.

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3

Glucose metabolism and cognitive function

3.1

A Prospective Analysis of Elevated Fasting Glucose Levels and Cognitive Function in Older People: Results from the PROSPER and Rotterdam Studies.

ABSTRACT

Context: Diabetes mellitus is strongly associated with cognitive impairment in older people. However, the link between elevated fasting glucose levels and insulin resistance in non-diabetics and the risk of cognitive impairment is unclear.

Objective: To investigate the relation between fasting glucose levels, and insulin resistance, and cognitive impairment in old age.

Design, Setting, and Participants: We analyzed two independent prospective studies: the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER): 5536 participants, age 69-84 years, and the Rotterdam Study: 3547 participants, age 61-97 years. Fasting glucose levels were assessed at baseline in both studies, fasting insulin levels were assessed in the Rotterdam Study only. Cognitive function was assessed at baseline and during follow-up.

Main Outcome Measures: Neuropsychological test battery to assess executive function and memory.

Results: Subjects with diabetes had impaired cognitive function at baseline and over time. In contrast, there was no clear association between baseline fasting glucose levels and executive function and memory in people without a history of diabetes, neither in the PROSPER study nor in the Rotterdam Study. No consistent relation existed between elevated baseline fasting glucose levels and the rate of cognitive decline in either cohort. Insulin resistance (HOMA index) was also unrelated to cognitive function and decline. The results did not change after additional adjustment for BMI, systolic and diastolic blood pressure, HDL-cholesterol and APOE-genotype.

Conclusion: Elevated fasting glucose levels and insulin resistance are not associated with worse cognitive function in older people without a history of diabetes. These data suggest either a threshold for effects of dysglycaemia on cognitive function, or that factors other than hyperglycaemia impair cognition in individuals with frank diabetes.

INTRODUCTION

Diabetes has been shown to be associated with an increased risk of dementia and impaired cognitive function.[1-5] Suggested biological mechanisms that are involved in this relation are: an indirect relation through cerebrovascular disease,[2,5,6] accumulation of advanced glycation end-products (AGE),[7] and reduced amyloid β clearance through disturbing the role of the insulin-degrading enzyme.[8]

The accumulating evidence that diabetes mellitus is involved in a number of health problems ranging from retinopathy and cardiovascular symptoms to neurological complications, has started a discussion about the necessity to identify those people who are at increased risk for diabetes.[8-14]

Classifying people based on levels of fasting glucose to indicate "impaired fasting glucose" has been suggested as a possible tool for risk assessment of gestational diabetes mellitus.[11,13] However, the relation between the preceding stage of diabetes, when impaired fasting glucose levels are present, and cognitive function has not been comprehensively elucidated. A number of studies have investigated the relation between this "pre-diabetes" state and cognitive function but showed contradictory or inconclusive results,[4,15-17] possibly due to relatively small sample sizes and limited numbers of participants with an impaired fasting glucose level.[4] Alternatively, peripheral insulin resistance that could underlie the elevated fasting glucose levels in the "pre-diabetes" state may contribute to impaired cognitive function.[18,19]

Therefore, in this study we investigated the association between fasting glucose levels and cognitive function and decline in a large sample of 9083 participants for whom fasting glucose levels at baseline were available together with longitudinal data from a dedicated neuropsychological test battery. Additionally, we investigated the relation between insulin resistance (using the HOMA index) and cognitive function and decline in 3452 participants.

METHODS

Populations

The PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) was a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major cardiovascular events in the elderly.[20,21] Between December 1997 and May 1999, a total of 5804 participants (aged 70-82 years) with pre-existing vascular disease or increased risk of such disease due to a history of smoking, hypertension, or diabetes, were recruited in Scotland, Ireland and the Netherlands. The institutional ethics review boards of all centres approved the protocol, and all participants gave written informed

consent. Participants with very severe cognitive impairment (Mini-Mental State Examination score <24) were excluded for inclusion in the study.

The Rotterdam Study is a large prospective population-based cohort study that is conducted among all inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, the Netherlands.[22] The medical ethics committee of the Erasmus University of Rotterdam approved the study, and written informed consent was obtained from all participants. Of 10,275 eligible subjects, 7983 individuals (78%) participated in the baseline examinations between 1990-1993 (mean age 71±25, range 55-106). All participants were interviewed at home, and visited the research centre for further examinations.

Fasting glucose levels

In PROSPER, fasting glucose levels were assessed at baseline in 5599 of the 5804 participants. Of the 5599 participants, 63 did not have cognitive function tests available at baseline. All of the resulting 5536 participants had full data available for other cardiovascular risk factors including BMI, systolic and diastolic blood pressure and HDL-cholesterol levels at baseline. This resulted in a study sample of 5536 participants for PROSPER.

In the Rotterdam Study, fasting glucose levels were assessed at the 3rd survey (1997-1999) in 3795 participants. Of these participants, 3664 were free of dementia and 3550 had data available for other cardiovascular risk factors including BMI, systolic and diastolic blood pressure and HDL-cholesterol levels. Three participants did not have cognitive function tests available at the start of the 3rd survey. This resulted in a study sample of 3547 participants for the Rotterdam Study. Additionally, in 3452 of these 3547 participants, fasting insulin levels were assessed at the 3rd survey.

In both the PROSPER and the Rotterdam sample, fasting glucose levels were additionally measured during follow-up. 5156 of the 5536 participants in the PROSPER sample and 2414 of the 3547 participants in the Rotterdam sample underwent at least one additional measurement of fasting glucose level in addition to the baseline examination. These data were used to study the variability of the fasting glucose levels over time and to assess the appropriateness of using a single baseline fasting glucose measurement to assess the relation between fasting glucose and cognitive function and decline.

History of diabetes mellitus

At baselinebaseline (PROSPER) or at the 3rd survey (Rotterdam Study), history of diabetes mellitus (DM) was defined by self-reported history of diabetes (reporting the use of oral antidiabetes medication, the use of insulin, treatment by diet, or registration by a general practitioner as having diabetes) in both study samples.

Cognitive function

Global cognitive function was measured with the Mini-Mental State Examination (MMSE)[23] in both studies. In addition, a dedicated neuropsychological test battery was used to assess executive function and memory. Executive function was assessed with the Letter-Digit Substitution Task (LDST)[24] and the abbreviated Stroop test part 3[25] in both studies, and with the Word Fluency Test (WFT)[26] in the Rotterdam Study only. Memory was assessed with the 12-Picture Learning Test (12-PLT) immediate and delayed recall,[27] in PROSPER only.

In PROSPER, cognitive function was measured at six time points during the study; before randomization, at baseline, after 9, 18, and 30 months, and at the end of the study. The time point of the last measurement was different for the participants and ranged from 36 to 48 months. Therefore we performed the analyses with their individual varying time-point but report the results for the mean of these time points (at 42 months). The pre-randomized measurement was discarded in the analyses to preclude possible learning effects. This resulted in a mean follow-up of 3.2 years. Change in cognitive function could be assessed in 5245 participants for whom at least one follow-up examination of cognitive function was available.

In the Rotterdam Study, cognitive function was assessed at the 3rd survey (1997-1999) and additionally at the 4th survey (2002-2004). This resulted in a mean follow-up of 4.6 years. Of the 3547 participants of the Rotterdam sample who were present at the 3rd survey, 2657 remained in the study until the end of follow-up (4th survey) and were available for the assessment of change in cognitive function.

Additional assessments

In both samples, level of education, BMI, systolic and diastolic blood pressure, HDL-cholesterol level and APOE ε4 carrier ship were assessed at baseline (PROSPER) or at the 3rd survey (Rotterdam Study).

Statistical analyses

The relation between baseline fasting glucose levels and cognitive function and decline was assessed by use of linear mixed models. In PROSPER, all analyses were adjusted for age, sex, education, country, use of pravastatin, and where appropriate, test version. In the Rotterdam Study, all analyses were adjusted for age, sex, and education. Additionally, adjustments were made for systolic and diastolic blood pressure, HDL-cholesterol and APOE ε4 carrier ship, and stratified analyses were performed in strata of BMI. Analyses were carried out using the SPSS statistical package (release 12.0.1; SPSS inc., Chicago, Illinois).

In the Rotterdam Study sample, data on fasting glucose and fasting insulin levels were used to calculate the degree of insulin resistance according to the homeostasis model assessment (HOMA).[28] The HOMA index is calculated by dividing the product of fasting levels of glucose

and insulin by a constant, and has been shown to correlate well ($r = 0.82$, $p<0.0001$) with the euglycemic hyperinsulinemic clamp method.[29]

RESULTS

Table 1 shows the baseline characteristics of the total sample, and for participants with a history of diabetes mellitus at baseline (PROSPER) or at the 3rd survey (Rotterdam Study). In PROSPER, the fasting glucose levels (SD) differed between the three countries from which the participants were enrolled; Scotland 5.63 (1.28) mmol/L, Ireland 5.09 (1.39) mmol/L, and the Netherlands 5.76 (1.64) mmol/L. This resulted in a lower mean fasting glucose level for the PROSPER study sample compared to that of the Rotterdam Study sample. Participants with a history of diabetes had a higher fasting glucose level, BMI, systolic blood pressure and lower levels of HDL-cholesterol, compared to participants without a history of diabetes.

Table 1. Baseline Characteristics

	Total sample	History of diabetes	
		No	Yes
PROSPER			
Number	5536	4935	601
Age, years (SD)	75.3 (3.4)	75.4 (3.4)	75.2 (3.3)
Female, No (%)	2869 (52)	(53)	(44)
Age when leaving school, years (SD)	15.1 (2.1)	15.2 (2.1)	15.0 (2.0)
MMSE-score, points (SD)	28.0 (1.5)	28.0 (1.5)	27.9 (1.6)
Fasting glucose levels, mmol/L (SD)	5.45 (1.43)	5.13 (0.81)	8.03 (2.44)
BMI, kg/m^2 (SD)	26.9 (4.2)	26.7 (4.2)	27.9 (4.4)
Systolic blood pressure, mmHg (SD)	155 (22)	154 (22)	158 (22)
Diastolic blood pressure, mmHg (SD)	84 (11)	84 (11)	84 (12)
HDL-cholesterol, mmol/L (SD)	1.28 (0.35)	1.29 (0.35)	1.18 (0.31)
Rotterdam Study			
Number	3547	3225	322
Age, years (SD)	72.0 (6.8)	71.8 (6.7)	73.9 (6.9)
Female, No (%)	57	58	51
Low level of education, No (%)	29	29	27
MMSE-score, points (SD)	27.7 (1.9)	27.8 (1.9)	27.6 (1.9)
Fasting glucose levels, mmol/L (SD)	5.88 (1.34)	5.67 (0.93)	8.09 (2.42)
Fasting insulin levels, mU/L (SD)[a]	11.2 (7.8)	10.9 (7.1)	14.6 (12.3)
Insulin resistance, HOMA (SD)[a]	3.07 (2.92)	2.83 (2.25)	5.54 (6.10)
BMI, kg/m^2 (SD)	26.8 (3.9)	26.7 (3.9)	27.8 (4.4)
Systolic blood pressure, mmHg (SD)	143 (21)	143 (21)	149 (21)
Diastolic blood pressure, mmHg (SD)	75 (11)	75 (11)	74 (11)
HDL-cholesterol, mmol/L (SD)	1.40 (0.40)	1.41 (0.40)	1.26 (0.39)

Data are presented as mean (SD) or percentage.

[a] Fasting insulin levels were available for 3452 participants in the Rotterdam Study

In PROSPER, fasting glucose levels were assessed during follow-up after 3, 6, 12, 24 and 36 months, in addition to the baseline assessment. In 3821 participants without a history of diabetes, fasting glucose levels were available at baseline and after 36 months of follow-up. The glucose levels measured at baseline did not dramatically change during follow-up, although in general fasting glucose levels seemed to slightly increase over time. The mean baseline fasting glucose levels (SD) and fasting glucose levels (SD) after 36 months within the quintiles of the fasting glucose distribution were; 4.29 (0.20) mmol/L and 4.77 (0.65) mmol/L for the lowest quintile (Q1); 4.70 (0.08) mmol/L and 4.97 (0.50) mmol/L for Q2; 5.00 (0.08) mmol/L and 5.23 (0.68) mmol/L for Q3; 5.33 (0.11) mmol/L and 5.45 (0.69) mmol/L for Q4; and 6.25 (0.81) mmol/L and 6.28 (1.38) mmol/L for the highest quintile (Q5). In the Rotterdam sample, fasting glucose levels were assessed at the 3rd survey as well as at the end of follow-up (4th survey) in 2257 participants without a history of diabetes. The mean fasting glucose levels (SD) at the 3rd survey and fasting glucose levels (SD) at the end of follow-up within the quintiles of the fasting glucose distribution were comparable; 4.82 (0.24) mmol/L and 5.07 (0.39) mmol/L for the lowest quintile (Q1); 5.20 (0.08) mmol/L and 5.36 (0.42) mmol/L for Q2; 5.50 (0.08) mmol/L and 5.55 (0.50) mmol/L for Q3; 5.84 (0.11) mmol/L and 5.86 (0.57) mmol/L for Q4; and 6.74 (0.99) mmol/L and 6.77 (1.42) mmol/L for the highest quintile (Q5).

Of the 5536 participants in the PROPSER sample who were present at baseline, 4410 remained in the study sample until the end of follow-up, 565 withdrew from the study, and 561 died during follow-up. The 1126 participants who did not stay in the study sample were divided over the two groups: 20.0% of the participants without a history of diabetes and 23.3% of the participants with a history of diabetes dropped out during follow-up. Of the 3547 participants of the Rotterdam sample who were present at the 3rd survey, 2657 remained in the study until the end of follow-up, 520 refused to participate or were too ill to visit the research center, and 370 died during follow-up. In the group without a history of diabetes, 24.1% was not present at the end of follow-up compared to 34.4% of the participants with a history of diabetes.

Figure 1 and 2 show the relation between fasting glucose levels and cognitive function at baseline (PROSPER) and 3rd survey (Rotterdam Study). Cognitive test scores are shown for quintiles of the distribution of fasting glucose levels in participants without a history of diabetes, and for participants with a history of diabetes separately. These data indicate that in participants without a history of diabetes, a rise in fasting glucose levels in the non-diabetes range is not clearly associated with impairment in cognitive function in neither of the study samples. This was seen for all cognitive tests, except for the MMSE in the PROSPER sample where higher levels of fasting glucose were modestly associated with worse cognitive function (p-trend = 0.043). Additionally, we compared the cognitive test scores at baseline (PROSPER) or 3rd survey (Rotterdam Study) for participants with and without a history of diabetes (Figure 1 and 2) and showed that participants with a history of diabetes had worse cognitive

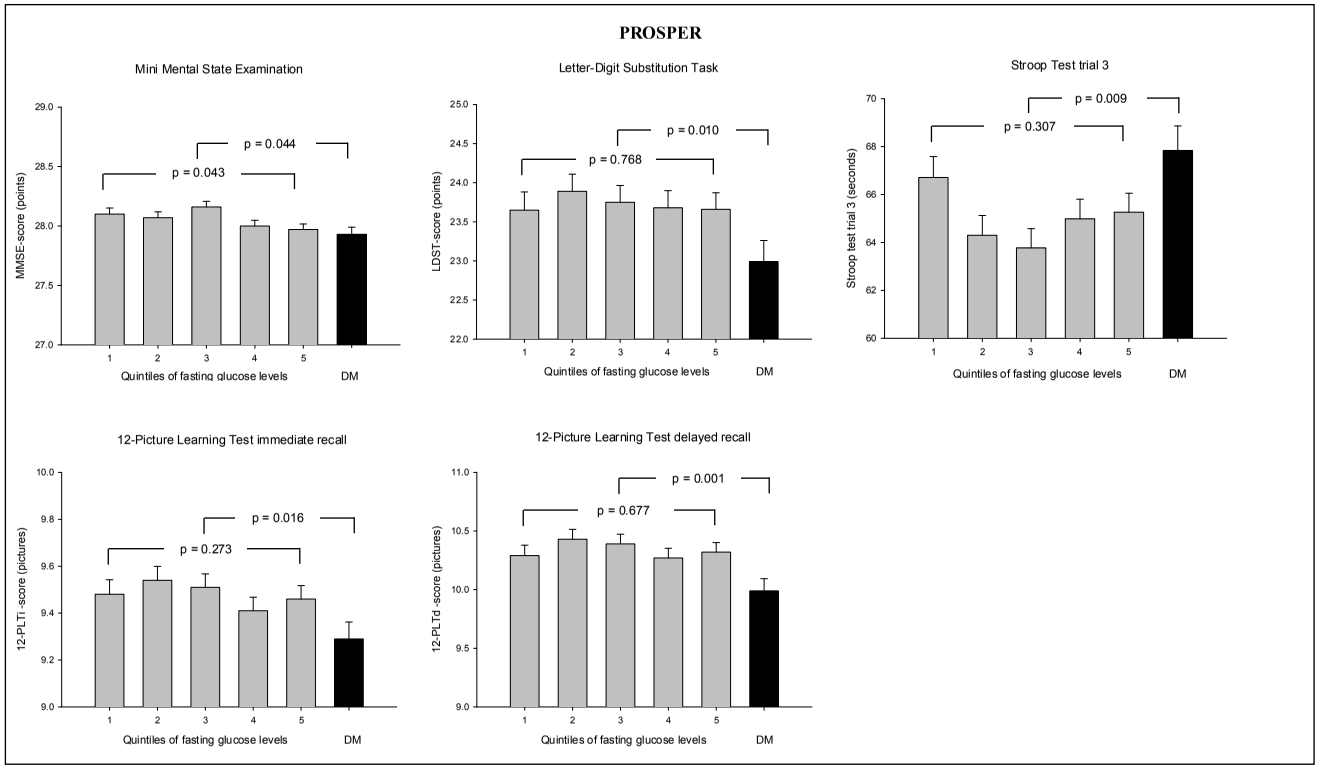

Figure 1. Fasting glucose levels and cognitive function in PROSPER.
Bars represent cognitive test-scores [SEM] for quintiles of fasting glucose levels in non diabetic participants (from lowest (Q1) to the highest (Q5) levels of fasting glucose), and for participants with a history of diabetes (DM). Linear mixed models were used, adjusted for age, sex, level of education, country, treatment group and test version where applicable; Estimates are based on the maximum number of participants available per cognitive test; MMSE (n = 5536), LDST (n = 5201), Stroop test trial 3 (n = 5168), 12-PLTi (n = 5223), 12-PLTd (n = 5223). P-values reflect the trend over the quintiles of fasting glucose levels, and the difference between participants without a history of diabetes (n = 4935) and participants with a history of diabetes (n = 601).

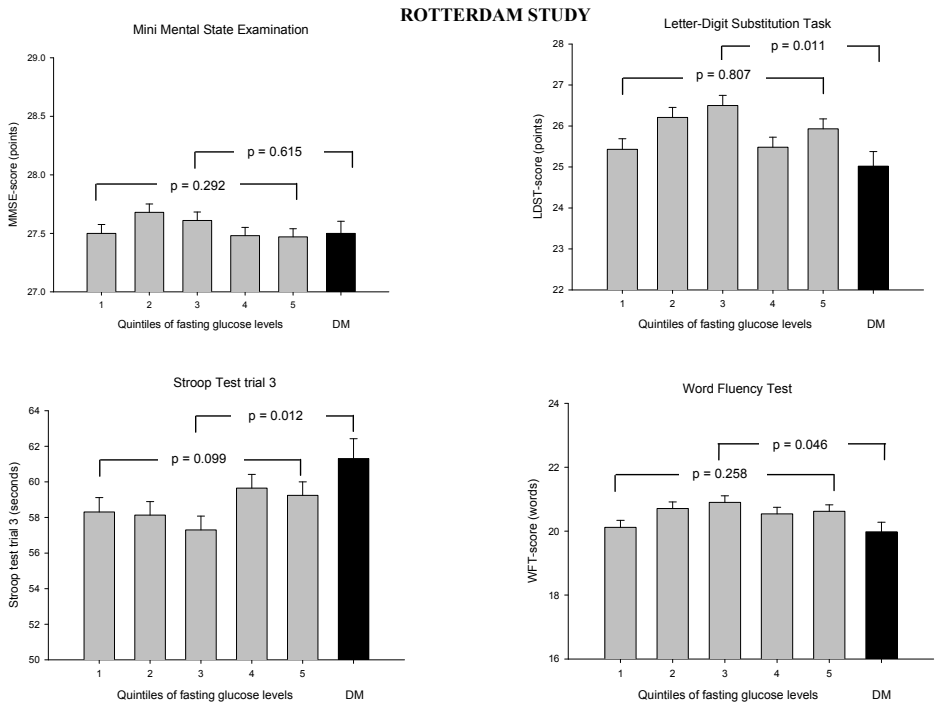

Figure 2. Fasting glucose levels and cognitive function in the Rotterdam Study.
Bars represent cognitive test-scores [SEM] for quintiles of fasting glucose levels in non diabetic participants (from lowest (Q1) to the highest (Q5) levels of fasting glucose), and for participants with a history of diabetes (DM). Linear mixed models were used, adjusted for age, sex and level of education; Estimates are based on the maximum number of participants available per cognitive test; MMSE (n = 3547), LDST (n = 3492), Stroop test trial 3 (n = 3449), WFT (n = 3518). P-values reflect the trend over the quintiles of fasting glucose levels, and the difference between participants without a history of diabetes (n = 3225) and participants with a history of diabetes (n = 322).

function across majority of tests at baseline compared to participants without a history of diabetes (p-value <0.05 for all tests except for the MMSE in the Rotterdam Study sample).

In both study samples, there was a limited number of participants (134 in PROSPER and 167 in the Rotterdam Study) without a history of diabetes who could have been allocated to the diabetes mellitus group based on their fasting glucose levels that were ≥ 7.0 mmol/L.[11-13] The choice to include these "new diabetic" participants in either the diabetes mellitus group or in the non-diabetic group could have affected our results. Therefore we did both. When these "new diabetic" participants were moved from the highest quintile of the fasting glucose distribution to the group with a history of diabetes, the trend over MMSE-scores in quintiles of fasting glucose in the PROSPER sample was no longer significant (p = 0.081). Moreover, the contrast in LDST-score, Stroop test trial 3-score and 12-PLT immediate and delayed recall in PROSPER, and the MMSE-score, the LDST-score and the WFT-score in the Rotterdam Study between the diabetes mellitus group and the participants without diabetes mellitus attenuated.

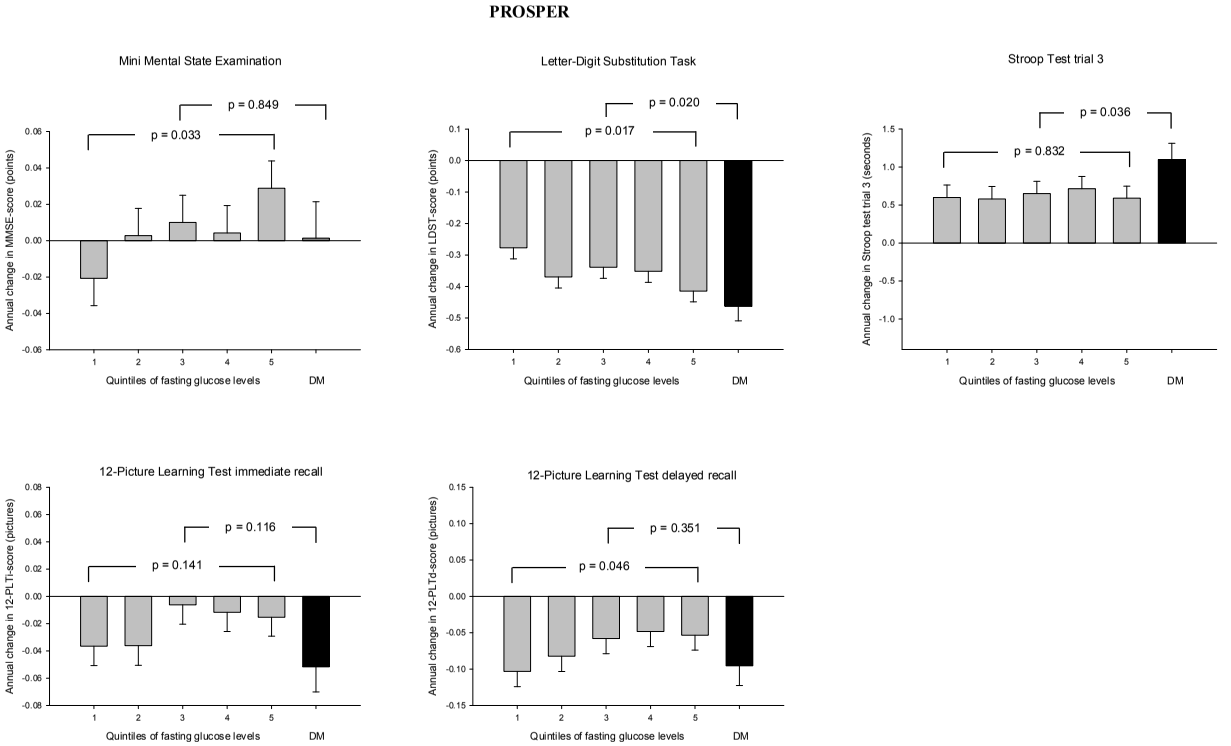

Figure 3. Fasting glucose levels and annual change in cognitive function in PROSPER.
Bars represent annual change in cognitive test-scores [SEM] for quintiles of fasting glucose levels in non diabetic participants (from lowest (Q1) to the highest (Q5) levels of fasting glucose), and for participants with a history of diabetes (DM). Linear mixed models were used, adjusted for age, sex, level of education, country, treatment group and test version where applicable; Estimates are based on the maximum number of participants available per cognitive test; MMSE (n = 5245), LDST (n = 4938), Stroop test trial 3 (n = 4904), 12-PLTi (n = 4960), 12-PLTd (n = 4960). P-values reflect the trend over the quintiles of fasting glucose levels, and the difference between participants without a history of diabetes (n = 4681) and participants with a history of diabetes (n = 564).

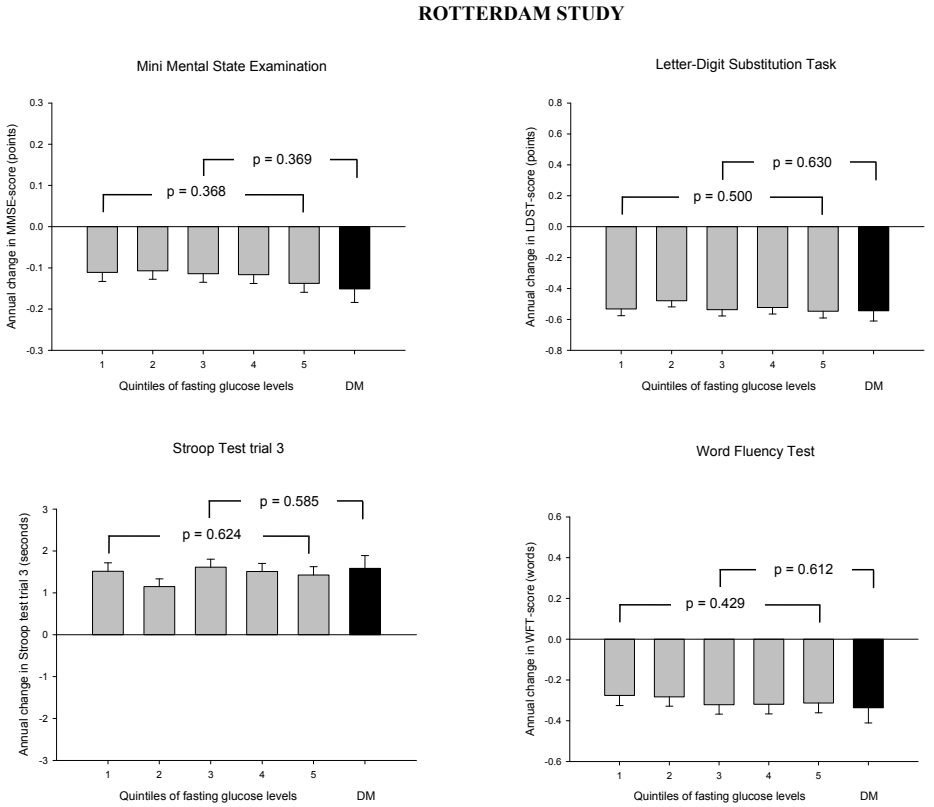

Figure 4. Fasting glucose levels and annual change in cognitive function in the Rotterdam Study. Bars represent annual change in cognitive test-scores [SEM] for quintiles of fasting glucose levels in non diabetic participants (from lowest (Q1) to the highest (Q5) levels of fasting glucose), and for participants with a history of diabetes (DM). Linear mixed models were used, adjusted for age, sex and level of education; Estimates are based on the maximum number of participants available per cognitive test; MMSE (n = 2657), LDST(n = 2632), Stroop test trial 3 (n = 2610), WFT (n = 2639). P-values reflect the trend over the quintiles of fasting glucose levels and the DM group and the difference between participants without a history of diabetes (n = 2449) and participants with a history of diabetes (n = 208).

Figure 3 and 4 show the results from the longitudinal analyses for both study samples. There was no clear association in either study between baseline or 3rd survey fasting glucose levels and change in cognitive function during follow-up in participants without a history of diabetes. Although in the PROSPER sample higher levels of fasting glucose were associated with an increased rate of decline on the Letter-Digit Substitution Task (p-trend = 0.017), this was not confirmed in the sample from the Rotterdam Study (p-trend = 0.500). In contrast, participants in the highest quintiles of the fasting glucose distribution in the PROSPER sample seemed to have a decreased rate of decline in MMSE-score and 12-Picture Learning Test delayed recall-score, although this was again, not confirmed in the Rotterdam Study sample. Participants with a history of diabetes showed an increased rate of decline on the

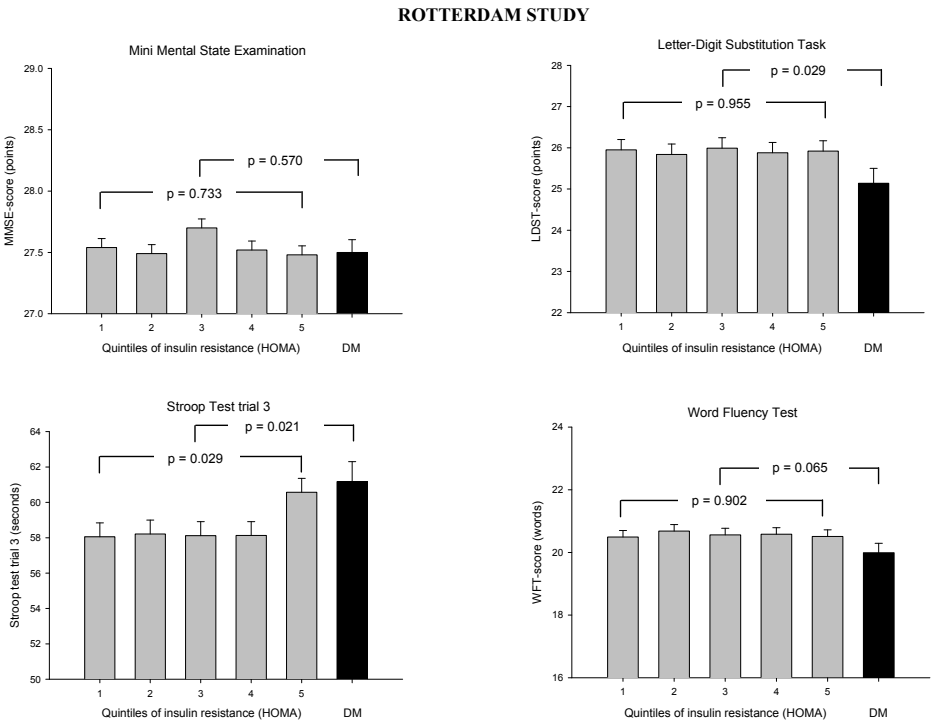

Figure 5. Insulin resistance (HOMA) and cognitive function in the Rotterdam Study.
Bars represent cognitive test-scores [SEM] for quintiles of insulin resistance (HOMA) in non diabetic participants (from lowest (Q1) to the highest (Q5) levels of insulin resistance), and for participants with a history of diabetes (DM). Linear mixed models were used, adjusted for age, sex and level of education; Estimates are based on the maximum number of participants available per cognitive test; MMSE (n = 3452), LDST (n = 3401), Stroop test trial 3 (n = 3360), WFT (n = 3424). P-values reflect the trend over the quintiles of insulin resistance (HOMA), and the difference between participants without a history of diabetes (n = 3139) and participants with a history of diabetes (n = 313).

Letter-Digit Substitution Task (p-value = 0.020) and the Stroop test trial 3 (p-value = 0.036), compared to participants without a history of diabetes in the PROSPER sample. All other tests in both study samples broadly indicated (albeit not significant) an increased rate of cognitive decline for participants with a history of diabetes. Relocating "new diabetic" participants from the highest quintile of the fasting glucose distribution to the diabetes mellitus group did not markedly change these results.

In Figure 5 and 6, the relation between insulin resistance and cognitive function and decline are shown for the 3452 participants of the Rotterdam Study sample for whom fasting insulin levels were available. The homeostasis model assessment (HOMA) that was calculated for these participants was correlated with the fasting glucose levels ($r = 0.54$, $p < 0.001$), although the overlap between quintiles of the HOMA index and quintiles of fasting glucose levels was limited: only 35% of the participants without a history of diabetes were in the same quintile of the distribution for both fasting glucose and HOMA index. The relation between

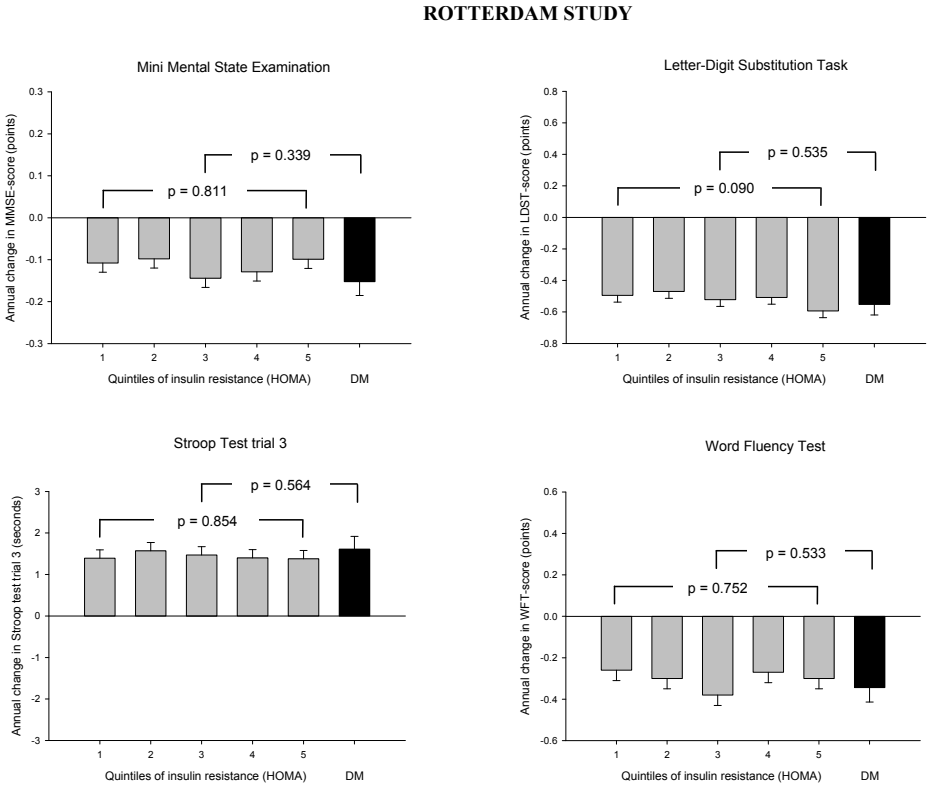

Figure 6. Insulin resistance (HOMA) and annual change in cognitive function in the Rotterdam Study. Bars represent annual change in cognitive test-scores [SEM] for quintiles of insulin resistance (HOMA) in non diabetic participants (from lowest (Q1) to the highest (Q5) levels of insulin resistance (HOMA)), and for participants with a history of diabetes (DM). Linear mixed models were used, adjusted for age, sex and level of education; Estimates are based on the maximum number of participants available per cognitive test; MMSE (n = 2584), LDST(n = 2562), Stroop test trial 3 (n = 2541), WFT (n = 2567). P-values reflect the trend over the quintiles of insulin resistance (HOMA), and the difference between participants without a history of diabetes (n = 2379) and participants with a history of diabetes (n = 205).

insulin resistance and cognitive function that is shown in Figure 5, is in accordance with findings on fasting glucose levels and cognitive function: in participants without a history of diabetes, rising insulin resistance was not associated with cognitive function. Similarly, there was no clear relation between levels of insulin resistance and change in cognitive function during follow-up (Figure 6).

Additional adjustment for systolic and diastolic blood pressure, HDL-cholesterol level and APOE ε4 carrier ship did not markedly change any of the associations linking fasting glucose / insulin resistance with measures of cognitive function / change. We additionally analyzed the relation between fasting glucose levels and cognitive function and decline in strata of BMI, to investigate possible effect modification. There were no differences seen in the association between fasting glucose levels and cognitive function in the different strata of BMI compared

to the total samples. Interaction-terms of BMI and fasting glucose levels were added to the linear mixed models, but were not significant.

All analyses were based on the maximum amount of cognitive data that were available for the different cognitive tests that were assessed in both cohorts. When we restricted our analyses to those participants who had complete data available on all the cognitive tests in the neuropsychological test battery (PROSPER; (n = 4857), Rotterdam Study; (n = 3413)), the results did not change (data not shown).

COMMENT

Our study shows that in two large independent prospective studies, higher levels of fasting glucose in absence of a history of diabetes mellitus are not associated with cognitive function or decline. Furthermore, there was no association between insulin resistance (HOMA index) and cognitive function and decline in people without a history of diabetes. Participants with a history of diabetes, however, did have clear evidence of worse cognitive function at baseline, and there was some evidence of an accelerated rate of decline in cognitive function during follow-up.

When we extended the diabetes group with "new diabetic" participants without a history of diabetes but who had a fasting glucose level ≥ 7.0 mmol/L, the already modest relation between fasting glucose levels and cognitive function in participants without diabetes became even less clear. Furthermore, the difference in cognitive function between this extended diabetes mellitus group and the non-diabetic participants was less apparent in these analyses. This supports the idea that there is no clear relation between fasting glucose levels and cognitive function in the absence of a history of diabetes mellitus, and that the "new diabetics" are on their way to become part of the group with a history of diabetes, with respect to cognitive function.

Together with diabetes or an impaired fasting glucose level, many other cardiovascular risk factors often co-occur in individual participants. The metabolic syndrome is seen as a clustering of a number of these risk factors (abdominal obesity, hypertriglyceridemia, low HDL-cholesterol, hypertension and hyperglycemia) and is the subject of an ongoing discussion on the clinical use of the syndrome and its individual components in establishing the risk of cardiovascular disease and diabetes.[30] Other studies have suggested a relation between the metabolic syndrome and the risk of cognitive impairment and dementia.[31-33] In our study, the clustering of these factors of the metabolic syndrome with the fasting glucose levels we assessed could have influenced our results. However, the results from the analyses that we stratified for BMI, and additionally adjusted for several of these factors (systolic and diastolic blood pressure, HDL-cholesterol) did not differ from the analyses that were unadjusted for these covariates.

We used fasting insulin levels that were available in almost the entire sample of the Rotterdam Study to calculate the HOMA index as a measure of insulin resistance to further investigate the relation between glucose metabolism and cognitive function. However, the relation between insulin resistance and cognitive function and decline showed similarities with the association between fasting glucose levels and cognitive function and decline: in participants without a history of diabetes, insulin resistance was not associated with cognitive function or decline.

The strengths of this study consist of the prospective design, the large amount of participants in both studies, and the dedicated neuropsychological test battery that was used in both samples. Furthermore, we had the possibility to study the variability of the fasting glucose levels during follow-up and to examine the appropriateness of using a single measurement of fasting glucose level to assess the association between fasting glucose levels and cognitive function and decline. A large variation in fasting glucose levels over time could have disturbed our analyses through the phenomenon of "regression-to-the-mean". However, the levels of fasting glucose during follow-up did not materially differ from the baseline or 3rd survey in both study samples. Therefore we decided to use the baseline or 3rd survey fasting glucose measurement in our analyses.

Some limitations need to be addressed. Participants who were present at baseline but dropped out of the study during follow-up were predominantly present in the group with a history of diabetes. They had worse cognitive function at baseline compared to the participants that stayed in the study until the end of follow-up. This selective drop-out of participants with relatively high levels of fasting glucose and concurrent low levels of cognitive function could have resulted in an underestimation of our estimates of cognitive decline for participants with a history of diabetes. We also recognize that some individuals with diabetes would have been missed because of lack of oral glucose tolerance testing (OGTT) – however, oral glucose tolerance testing on such a large number of individuals would have been onerous. More importantly, undiagnosed diabetes would be more prevalent in those in the higher quintiles for fasting glucose and would have biased the study towards an association of higher quintiles and cognitive decline, not the other way around. Thus, lack of OGTT does not negate our findings; rather it gives us added confidence that our observations are valid.

In conclusion, elevations in fasting glucose levels are not clearly associated with impaired cognitive function or with an accelerated rate of cognitive decline in participants without a history of diabetes. Furthermore, there was no clear relation between insulin resistance (HOMA index) and cognitive function and decline in participants without a history of diabetes. These data suggest that cognitive decline accelerates strongly once diabetic but not with lesser degrees of dysglycaemia. As a result, preventing individuals at risk from developing diabetes through lifestyle changes, may also lead to large societal gains by preventing such individuals from undergoing accelerated cognitive decline.

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3.2

Genetic Influence on Insulin/IGF-1 Signaling and Cognitive Function

ABSTRACT

Background: An accumulating body of evidence suggests the involvement of an evolutionary conserved insulin/insulin-like growth factor-1 (IGF-1) signaling (IIS) pathway in the regulation of the life and health span in nematodes, flies, rodents, and humans.

Methods: We studied the association between insulin/IGF-1 signaling and cognitive function among 1015 participants, 85 years old or older, of the population-based Leiden 85-Plus Study. A composite IIS6 score, based on expected effects (increased or decreased signaling) of selected variants in the IIS pathway, was calculated to estimate IIS pathway activity. Cognitive function was assessed at baseline and annually during a 5-year follow-up, using the Mini-Mental State Examination (MMSE).

Results: In women, but not in men, lower IIS6 scores (indicating decreased signaling) were associated with a lower risk of cognitive impairment (MMSE score ≤ 18) (p trend = .010). The IIS6 score was not associated with change in cognitive function.

Conclusion: In addition to old age survival, genetically reduced IIS seems to be beneficial for cognitive function in women.

INTRODUCTION

The involvement of the evolutionary conserved insulin/ insulin-like growth factor-1 (IGF-1) signaling (IIS) pathway in the hormonal regulation of aging has been shown in a number of model organisms like nematodes, flies, and rodents.[1-8] Genetic variation that causes a reduced IIS activation was associated with an increased life span in these organisms, with a stronger effect seen in the female sex.[6,9-11] In accordance with these data on model organisms, we previously showed an association between a genetically reduced IIS pathway and better survival in elderly women.[12]

Moreover, in model organisms, the IIS pathway has not only been implicated in the determination of life span but also in that of health span. At old age, there is a high prevalence of cognitive impairment, which for many is one of the most frightening prospects of old age. An accumulating body of evidence suggests the involvement of insulin and IGF-1 in the development of cognitive impairment.[13-15] Suggested biological mechanisms that underlie this relationship are inhibition of the amyloid β breakdown (through the insulin-degrading enzyme) and the involvement of insulin and IGF-1 in τ phosphorylation.[14,16,17] In humans, it is yet to be elucidated how the evolutionary conserved IIS pathway, which has been shown to be associated with better survival in old age, is related to cognitive function and decline.

Therefore, we studied the relationship between genetic variation in IIS pathway activation and cognitive function and decline in a prospective, population-based study among 1015 participants of the Leiden 85-Plus Study.

METHODS

Population

The Leiden 85-Plus Study is a prospective, population-based cohort study that consists of two cohorts of inhabitants of Leiden, The Netherlands.[18,19] For cohort '87, 977 inhabitants of Leiden, 85 years old or older, were enrolled between 1987 and 1989, and cognitive function was assessed at baseline. For cohort '97, 599 inhabitants of Leiden, 85 years old, were enrolled between 1997 and 1999. Cognitive function was assessed at baseline and annually during home visits, from age 85 until age 90 years. Of the 1576 participants in the two cohorts of the Leiden 85-Plus Study, DNA was available for 1245 participants, and IIS scores could be calculated for 1037 participants.[12] Twenty-two participants did not have cognitive data available at baseline, resulting in a study sample of 1015 participants.

IIS Score

To determine the genetic variation that influences IIS, we selected six polymorphisms in five genes from the IIS pathway (Growth Hormone Releasing Hormone Receptor (GHRHR);

Growth Hormone 1 (GH1); Insulin-like Growth Factor 1 (IGF1); Insulin (INS); and Insulin Receptor Substrate 1 (IRS1)) based on previous reports of their effects on gene expression or protein function. As the effect sizes of the six selected IIS polymorphisms were unknown, these were assumed to be equal, and a simple additive model (hereafter referred to as IIS6 score) was used to test for combined effects of the selected polymorphisms. Per individual, we calculated a composite IIS6 score by assessing a value of –1 for carriership of each variant predicted to cause reduced IIS activity (GHRHR rs4988496, GH1 rs2665802, IGF-1 CA repeat, INS Variable Number Tandem Repeats (VNTR), IRS1 rs1801278), and a value of +1 for carriership of the variant predicted to cause increased IIS activity (IGF1 CT repeat). In total, the IIS6 score could be calculated for 1037 of the 1245 participants for whom DNA was available. Because of the limited number of participants (n = 3) with an IIS6 score of –5, we included these participants in the IIS6 score –4 category. Likewise, the participants in the IIS6 score +1 category (n = 6) were included in the IIS6 score 0 category. A more detailed description of the selected polymorphisms and assessment of the IIS6 score have been published elsewhere.[12]

Cognitive Function

Cognitive function was assessed with the Mini-Mental State Examination (MMSE),[20] and was available for 1015 participants of both cohorts at baseline. The MMSE is a measure of global cognitive function and ranges from 0 to 30 points, with lower scores indicating worse cognitive function. Although the MMSE is a widely used test that was developed as a screening instrument for dementia and is a reliable measure of global cognitive function, it might lack reliability and validity in persons with severe cognitive impairment.[21] Therefore, we dichotomized the cognitive test scores and defined cognitive impairment as an MMSE-score ≤ 18 points. Additionally, 455 of the 511 participants of cohort '97 underwent at least one measurement of the MMSE during follow-up, resulting in a study sample of 455 participants available for the longitudinal analyses. Annual change in cognitive function was assessed by comparing test scores at baseline with those at the last available follow-up visit. Annual change in cognitive function was calculated as the difference between test score at baseline and test score at the last available year of follow-up, divided by the length of follow-up (1–5 years).

Statistical Analyses

The relationship between the IIS6 score and the risk of cognitive impairment was assessed with logistic regression models.

The relationship between the IIS6 score and the annual change in cognitive function during follow-up was assessed with analyses of covariance, with the annual change in cognitive function as the dependent variable, and the IIS6 score as the grouping variable. To assess the association between the 1-unit increase in IIS6 score and the annual change in cognitive function, we used linear regression models with the annual change in MMSE score as the dependent variable and the IIS6 score as the independent variable.

All analyses were adjusted for age and carried out using the SPSS statistical package (release 12.0.1; SPSS, Inc., Chicago, IL).

RESULTS

Table 1 shows the baseline characteristics of the study participants. The mean age of the 1015 participants from the combined cohorts of the Leiden 85-Plus Study was 87.4 years, and 68% were women. Mean MMSE score at baseline was 23.9 points and ranged from 0 to 30 points.

Table 2 shows the sex-specific relationship between the composite IIS6 score and the risk of cognitive impairment, adjusted for age. A higher IIS6 score was associated with a higher

Table 1. Baseline Characteristics

Characteristic	Combined Cohorts	Cohort '87	Cohort '97
Number	1015	504	511
Age, y (*SD*)	87.4 (3.1)	89.8 (2.9)	85 (0)
No. of women (%)	68	69	66
MMSE-score, points (*SD*)	23.9 (6.4)	23.9 (6.4)	23.8 (6.5)
IIS6-score, points (IQR)	−2 (−3 to −1)	−2 (−3 to −1)	−2 (−2 to −1)

Note: Data are presented as mean (standard deviation [*SD*]), percentage, or median (interquartile range [IQR]). MMSE, Mini-Mental State Examination, IIS6 score, insulin/insulin-like growth factor-1 signaling score.

Table 2. Association Between Insulin/IGF-1 Signaling Score and the Risk of Cognitive Impairment in the Leiden 85-Plus Study

	Women				Men			
IIS6 Score	*n*	MMSE Score	OR (95% CI)	p_{trend}	*n*	MMSE Score	OR (95% CI)	p_{trend}
−4 and −5	33	24.6 (1.2)	1 (Ref)	.010	13	22.8 (1.5)	1 (Ref)	.329
−3	139	24.0 (0.6)	0.80 (0.29-2.17)		73	24.3 (0.6)	0.55 (0.13-2.33)	
−2	259	23.8 (0.4)	1.09 (0.42-2.78)		120	25.1 (0.5)	0.32 (0.08-1.35)	
−1	193	22.5 (0.5)	1.65 (0.64-4.24)		89	25.7 (0.6)	0.30 (0.07-1.35)	
0 and +1	65	22.3 (0.8)	1.61 (0.56-4.57)		31	24.0 (1.0)	0.56 (0.11-2.87)	
Per IIS6 score	689		1.28 (1.06-1.53)		326		0.85 (0.60-1.19)	

Notes: Insulin/insulin-like growth factor-1 (IGF-1) signaling (IIS)6 scores were calculated for each participant by assigning a score of +1 for carriership of a gene variant causing increased signaling and a score of −1 for carriership of a gene variant causing decreased signaling. Because of the limited number of participants with an IIS6 score of −5 ($n = 3$) or an IIS6 score of +1 ($n = 6$), we combined these, respectively, with the IIS6 score −4 and IIS6 score 0 categories. Mini-Mental State Examination (MMSE) scores (standard error [*SE*]) are presented per IIS6 score category. Odds ratios (OR) represent the risk of cognitive impairment per IIS6 score category. In women, 146 cases of cognitive impairment were present, in men, 38 cases were present. Values of p_{trend} represent the linear trend for the risk of cognitive impairment per increase in IIS6 score. All analyses were adjusted for age. CI, confidence interval.

risk of cognitive impairment, but only for women (odds ratio [95% confidence interval] per 1-unit increase in IIS6 score was 1.28 [1.06-1.53], p = .010). In men, a higher IIS6 score was, if anything, associated with a lower risk of cognitive impairment (odds ratio [95% confidence interval] per 1-unit increase in IIS6 score was 0.85 [0.60-1.19], p = .329).

The longitudinal analyses, which were performed of 455 participants of cohort '97 only, showed no clear relationship between the IIS6 score and the annual change in cognitive function during follow-up. In both women (annual change in MMSE score [standard error {SE}]) per 1-unit increase in IIS6 score was –0.06 [0.12] points, p = .612) and men (annual change in MMSE-score [SE] per 1-unit increase in IIS6 score was –0.16 [0.15] points, p = .311) a higher IIS6 score was not associated with an accelerated or decelerated rate of decline in cognitive function during follow-up.

DISCUSSION

Our study showed that, in humans, a genetically reduced IIS pathway was associated with a lower risk of cognitive impairment in women, but not in men. There was no association between the IIS pathway and a change in cognitive function over time.

Together with our previous finding that showed an association between a genetically reduced IIS pathway with improved old age survival in women,[12] our current findings are in accordance with the available data from model organisms that showed favorable effects of genetically induced lower IIS activity on both life span and health span. In model organisms, such as Caenorhabditis elegans, down-regulation of the IIS pathway occurs in response to adverse environmental conditions, such as food shortage. Its main function is to coordinately adapt various aspects of the worm's physiology, so that priorities are changed from growth and reproduction to mere survival until conditions become more favorable. In C. elegans, environmentally, as well as genetically induced down-regulation of the IIS pathway can dramatically increase life span, with concurrent maintenance of functional abilities.[22] Furthermore, it was shown that the mutations in the IIS pathway are associated with associative learning behavior in C. elegans.[23]

At first glance, the beneficial effects of genetically induced down-regulation of the IIS pathway seem to conflict with the available data on the association of IGF-1 serum levels with the human health span. In humans, the activity of the hypothalamic–growth hormone (GH)–IGF-1 axis declines with age, and several aspects of functional decline, including cognitive decline, have been attributed to the somatopause. In several studies, higher levels of total circulating IGF-1 were associated with better cognitive function and less cognitive decline in elderly persons,[24,25] although in at least one study, free IGF-I levels were not associated with cognitive decline.[25] The a priori prediction based on the genetic data from long-lived worms would have predicted an association between low serum IGF-1 levels (instead of high

levels) and improved survival and better maintenance of functional abilities.[22] Recently, a similar paradox was observed in mice: decreased IGF-1 levels are not only a key feature of the serum profile of long-lived mice, such as calorically restricted wild-type mice, but also of mice that are short-lived due to mutations in DNA repair.[26,27] The picture that now emerges is that down-regulation of IIS is an ancient survival response that can occur both in response to sudden external stresses (such as food shortage) as well as in response to chronic internal stresses (such as the accumulation of damage with aging).[28] Likewise, in humans, the decline in GH/IGF-1 levels with age may be an adaptive response aimed to prolong survival. According to this hypothesis, higher serum IGF-1 levels in old age might be indicative of a lower degree of (age-associated) accumulated damage.

The observed sex-specific involvement of IIS pathway activity in the development of cognitive impairment that is presented here is supported by previous findings. In the first discovered long-lived C. elegans mutant that mapped to the IIS pathway, both male and hermaphrodite worms were shown to be affected by age.[1] Moreover, in female but not in male rats, heightened cognition, in addition to increased motor activity and reproductive shutdown, was observed as an important aspect of the survival response to caloric restriction.[29] Improved learning and memory may play important roles in the success of seeking food elsewhere, which is especially important for the female sex, as females must obtain sufficient energy to support the survival and development of their offspring as well as themselves. The possible, not mutually exclusive, mechanisms by which reduced IIS pathway activity may contribute to heightened cognition in elderly females, include increased stress resistance and reduced amyloid β–induced autophagosome accumulation. Recent data obtained in a C. elegans model suggest that reduced IIS activity promotes the maturation of autophagosomes into degradative autolysosomes, whereas amyloid β impairs this process.[30] Another study showed the involvement of IIS pathway mediation in the aggregation-mediated amyloid-β toxicity in C. elegans.[31]

The observed association between a lower IIS6 score and better cognitive function was not seen in the longitudinal analyses. A lower IIS6 score was not associated with an accelerated or decelerated rate of decline in cognitive function during follow-up. A possible explanation could be that there were only longitudinal data on cognitive function available for the participants of cohort '97, which reduced our sample size and concurrently the statistical power of our analyses. Furthermore, it has been suggested that in situations with substantial dropout during follow-up, cross-sectional analyses may provide the better estimates.[32,33] Therefore, cross-sectional estimates might have been the preferred choice here to study the relationship between IIS and cognition as we were testing the consequence of genetic variants that have accumulated over a lifetime. Alternatively, genetically determined lifelong lower IIS activity may mainly have an effect on the onset of cognitive decline (postponing it to a later age) and not on the rate of the decline.

In conclusion, we showed that genetically reduced IIS seems to be beneficial for cognitive function in women but not in men. These results are in line with our previous findings that showed associations between reduced IIS signaling and increased longevity in women, and further indicates the involvement of the IIS pathway in the regulation of health span.

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4

Vascular and inflammatory factors and cognitive function

4.1

The Effect of Age on the Association of Blood Pressure with Cognitive Function Later in Life

ABSTRACT

Background: High blood pressure at middle-age relates to worse cognitive function later in life. It is unclear whether this relation is similar in old age. Therefore we examined the relationship between baseline blood pressure and cognitive function later in life across age groups.

Methods: We used data from two independent population-based cohort studies: the Rotterdam Study (3078 participants, initial age 55-84 years, mean follow up 11 years) and the Leiden 85-plus Study (276 participants, initial age 85 years, follow-up 5 years). Systolic and diastolic blood pressure were measured at baseline, cognitive function was assessed at the end of follow-up with a dedicated neuropsychological test battery. The association between baseline blood pressure levels and cognitive function later in life was assessed in 10-year age groups in the Rotterdam Study and in the 85-year olds of the Leiden 85-plus Study.

Results: In the youngest participants (<65 years), systolic and diastolic blood pressure were not associated with cognitive function 11 years later. For persons aged 65-74 years, higher baseline systolic and diastolic blood pressure were related to worse cognitive function 11 years later. In contrast, in older age (over 75 years) higher systolic and diastolic blood pressure were related to a better cognitive function at the end of follow-up This effect appeared strongest in the highest age group (85 years).

Conclusions: High blood pressure increases the risk of cognitive impairment up to 75 years but is associated with better cognitive function thereafter. Age-specific guidelines for blood pressure management are needed, as the current directive that 'lower is better' may not apply to blood pressure levels in the very old.

INTRODUCTION

Data on the relation between blood pressure and cognitive function are not consistent, notwithstanding a large number of studies that investigated the relation between blood pressure and cognitive function.[1] Most studies report that higher blood pressure levels at middle-age relate to cognitive impairment later in life.[2-5] However, results on the relation of higher blood pressure in older age and later cognitive function are conflicting. Some studies showed a negative effect of higher blood pressure on cognitive function,[6,7] whereas others did not.[8,9] These findings may suggest that age influences the relation between blood pressure and cognitive function. When correct, this might have consequences for blood pressure management, especially in the very old. Earlier evidence for beneficial effects of blood pressure lowering on cognitive function in older people,[10] could not be replicated in recent studies,[11] despite the fact that stroke risk was significantly reduced in those studies. Therefore, we examined the effect of age on the relationship between baseline blood pressure and cognitive function later in life over a wide range of age groups in two prospective population-based studies, i.e. the Rotterdam Study and the Leiden 85-plus Study. We hypothesized that the relationship between blood pressure and cognitive function changes with age. Whereas high blood pressure is a risk factor for cognitive impairment at middle-age, it might also have beneficial effects in old age.

METHODS

Populations

The Rotterdam Study is a large prospective population-based cohort study that was conducted among all inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, The Netherlands.[12] The medical ethics committee of the Erasmus University of Rotterdam approved the study, and informed consent was obtained from all participants. Of 10,275 eligible subjects, 7983 individuals (78%) participated in the baseline examinations between 1990-1993 (mean age 71±25, range 55-106). All participants were interviewed at home, and visited the research center for further examinations. At the 4th survey (2002-2004) cognitive function was extensively assessed by use of a dedicated neuropsychological test battery.

The Leiden 85-plus Study is a prospective population-based cohort study of 85-year-old inhabitants of Leiden, The Netherlands. The medical ethics committee of the Leiden University Medical Centre approved the study, and informed consent was obtained from all participants. Between September 1997 and September 1999, all inhabitants of Leiden born between 1912 and 1914 (n=705) were contacted within a month after their 85th birthday. A total of 599 individuals (87%) agreed to participate. From age 85 till 90 years, annual neuropsychological tests were performed during home visits.

Study sample

In the Rotterdam Study, the sample for this study was restricted to participants aged 55-85 years and with blood pressure measurements at baseline (n=6502) due to the limited number of participants aged 85 years and over with follow-up examinations 11 years later (n=4). Of these 6502 participants, 3424 individuals (56%) did not participate in the 4th survey; 63% had died, 30% refused the in person examination or were too ill to visit the research center, and 7% could not be contacted. The proportion of participants who did not participate in the 4th survey increased with age from 31% for age group 55-64 years to 54% for age group 65-74 years and 88% for age group 75-84 years. The study sample therefore consisted of 3078 participants with both baseline blood pressure measurements and cognitive measurements 11 years later.

In the Leiden 85-plus Study, blood pressure was measured in 572 participants at age 85 years. Of these 572 participants, 276 participants underwent neuropsychological testing 5 years later at age 90 years. Of the remaining 296 individuals (52%) who did not participate at age 90 years, 88% had died, and 12% refused to participate.

Blood pressure

In both study samples, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice at baseline with a sphygmomanometer, after 5 minutes of seated rest. The averages of two measurements were used in the analyses. In the Rotterdam Study, the two measurements were separated by a count of the pulse rate. In the Leiden 85-plus Study the two measurements were two weeks apart.

Cognitive function

Global cognitive function was measured in both cohorts with the Mini-Mental State Examination (MMSE).[13] We used a serial 7's question only, not the WORLD-backward version. In addition, a dedicated neuropsychological test battery was used to assess executive function and memory. Executive function was assessed with the abbreviated Stroop test part 3[14] and the Letter Digit Substitution Task (LDST)[15] in both cohorts and with the Word Fluency Test (WFT)[16] in the Rotterdam Study only. Memory was assessed with the 15-Word Learning Test (15-WLT) immediate and delayed recall,[17] in the Rotterdam Study, and with the 12-Picture Learning Test (12-PLT) immediate and delayed recall,[18] in the Leiden 85-plus Study. Dutch translated and validated versions were used for all neuropsychological tests.

The MMSE-score was used as a measure of global cognitive function and ranges from 0 to 30 points, with lower scores indicating worse cognitive function. In the Leiden 85-plus Study, executive function and memory were not assessed in participants with a MMSE-score of 18 points or lower (n = 74) as it was assumed that the tests lack reliability and validity in subjects with severe cognitive impairment. These participants were assigned to the lowest quartile of the distribution to reflect their impaired state of cognitive function.

Additional measurements

In both study populations, education was measured at baseline and dichotomized into primary education or less, and more than primary education. Antihypertensive drug-use was determined at baseline during the home interview (Rotterdam Study) or through pharmacy records (Leiden 85-plus). Additionally, smoking status, alcohol intake, history of stroke, history of diabetes and history of cardiovascular disease were assessed at baseline.

Statistical analysis

The association between baseline blood pressure and cognitive function later in life was examined using linear regression models, with blood pressure as independent and cognitive test score as dependent variable. All analyses were adjusted for age (Rotterdam Study only), sex, and education level. Additional adjustments were made for the use of antihypertensive drugs, smoking status, alcohol intake, history of stroke, history of diabetes and history of cardiovascular disease. Analyses were carried out using the SPSS statistical package (release 11.1; SPSS inc., Chicago, Illinois).

RESULTS

Table 1 shows the baseline characteristics of participants who did and did not undergo cognitive testing at the follow-up examination for both cohorts. In both studies, the percentage of women was higher and their proportion increased with age. The level of education was lower in older age, i.e. among those who originate from earlier birth cohorts. Average SBP increased while DBP decreased with age. Among the oldest old however, DBP was highest. The percentage of participants with a history of stroke, diabetes mellitus, or cardiovascular disease increased with age, together with the use of antihypertensive treatment.

In the Rotterdam Study, participants without cognitive assessment at follow-up were older, less educated, had higher SBP and DBP, and a worse cognitive function at baseline compared to the participants included in the analyses. This was seen similarly for all age groups from 55 to 85 years. In the oldest old (Leiden 85-plus Study), the participants who did not undergo cognitive testing at the follow-up examination had lower SBP and DBP, and concurrent worse cognitive function at baseline compared to the participants included in the analyses. In both samples, the participants who were not included in the analyses more often had a history of stroke, diabetes mellitus, or cardiovascular disease compared to the participants in the study samples.

Figure 1 shows the effect of age on the association between baseline blood pressure and cognitive function later in life. Individuals up to 65 years of age showed in general little decline in cognitive function over the 11 year follow-up period, and neither baseline SBP nor DBP were related to cognitive function 11 years later. In individuals aged 65-74 years,

Table 1. Baseline Characteristics of Study Samples and Participants without Follow-up Examination

Characteristic	Rotterdam Study								Leiden 85-plus Study	
	Total sample		Age groups							
			55-64 yrs		65-74 yrs		75-84 yrs		85 years	
	Study Sample	No follow-up	Study Sample	No follow-up	Study Sample	No follow-up	Study Sample	No follow-up	Study Sample	No follow-up
Number	3078	3424	1772	791	1126	1341	180	1292	276	296
Age, mean ± SD	64.5 ± 6.0	71.6 ± 7.8	60.2 ± 2.8	60.7 ± 2.8	69.1 ± 2.7	70.3 ± 2.8	77.8 ± 2.3	79.6 ± 2.8	85	85
Female, %	59.1	58.3	58.4	54.9	58.8	54.4	67.8	64.4	72.1	61.8
Low level of education, %	27.9	44.1	23.8	32.9	31.6	40.4	44.9	55.3	62.0	68.3
Use of antihypertensive drugs, %	23.9	37.1	20.2	25.9	27.4	37.4	39.1	43.6	36.2	35.8
SBP, mmHg, mean ± SD	135 ± 20	143 ± 23	131 ± 20	135 ± 21	139 ± 20	144 ± 22	140 ± 19	147 ± 24	158 ± 18	153 ± 19
DBP, mmHg, mean ± SD	74 ± 11	74 ± 12	74 ± 11	76 ± 11	73 ± 11	74 ± 12	71 ± 11	72 ± 13	78 ± 9	75 ± 10
MMSE-score, mean ± SD	28.1 ± 1.5	26.9 ± 2.9	28.2 ± 1.4	27.7 ± 2.1	28.0 ± 1.5	27.4 ± 2.2	27.7 ± 1.6	26.0 ± 3.7	25.7 ± 4.3	22.4 ± 7.2
Ever smoking, %	67	66	69	78	65	70	50	54	43	52
Alcohol intake, units/day ± SD	1.3 ± 1.8	1.3 ± 2.0	1.4 ± 1.9	1.5 ± 2.2	1.3 ± 1.8	1.3 ± 2.0	0.8 ± 1.2	0.9 ± 1.6	0.8 ± 0.8	1.0 ± 0.9
History of stroke, %	1	4	1	2	1	4	3	7	7	13
History of diabetes mellitus, %	6	14	5	7	7	15	10	17	13	19
History of cardiovascular disease, %	18	40	15	25	21	36	25	52	56	68

SBP = systolic blood pressure; DBP = diastolic blood pressure; MMSE = Mini-Mental State Examination
SD = standard deviation

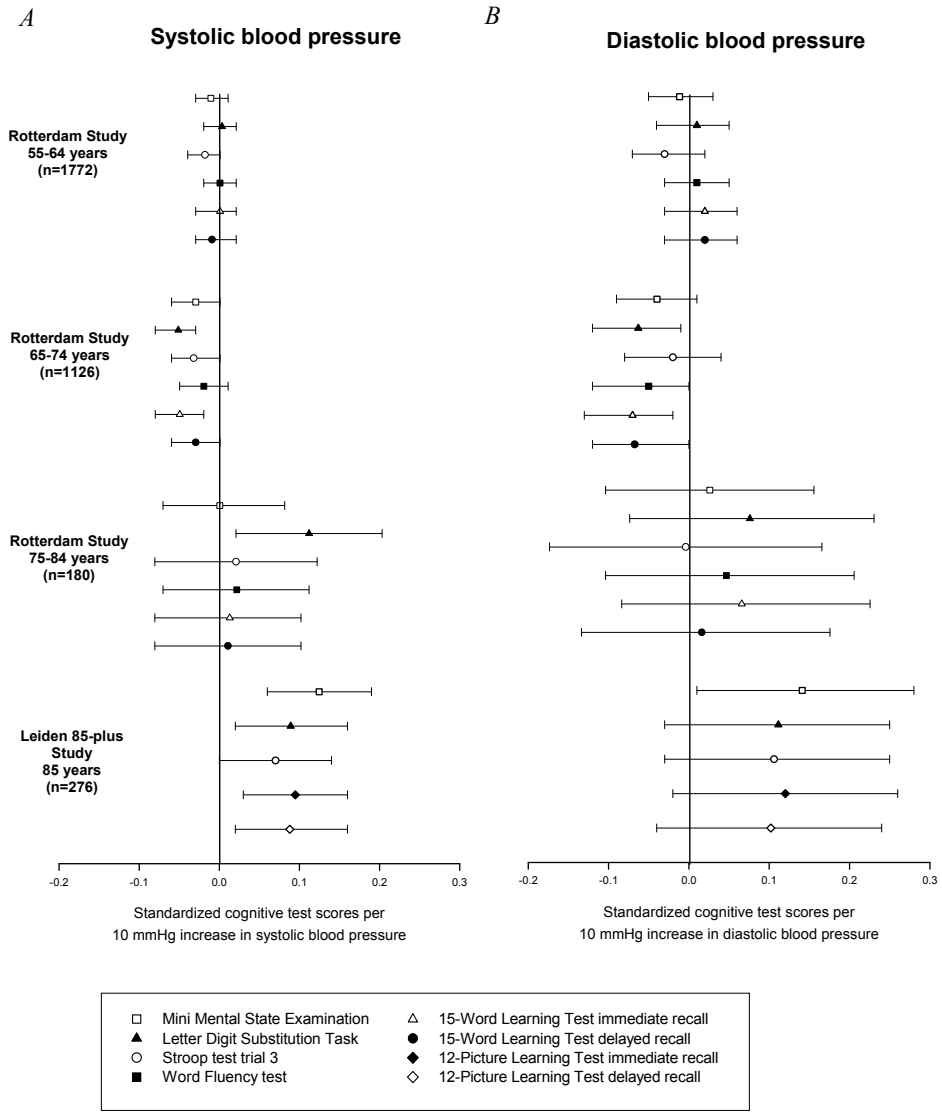

Figure 1. The effect of age on the association of baseline blood pressure and cognitive function later in life.
Symbols represent the mean standardized cognitive test scores and 95% confidence intervals per 10 mmHg increase in systolic (A) and diastolic (B) blood pressure. For graphical reasons, the estimates for the Stroop test were inversed as a higher Stroop score reflects worse cognitive function. Analyses were adjusted for age (Rotterdam Study only), sex and level of education.

higher SBP and DBP at baseline were related to worse cognitive function 11 years later. With increasing baseline age, the effect of blood pressure on cognitive function seemed to invert: In individuals over 75 years of age, higher SBP and DBP at baseline were related to a better cognitive function 11 years later, although in the Rotterdam Study the number of people

in this age group was rather small and consequently results not significant for the majority of tests. This effect was however even stronger in the highest age group (85 years, subjects from the Leiden 85-plus Study) where higher SBP and DBP were related to a better cognitive function 5 years later. The shift from risk to benefit of high blood pressure from age 65 to 85 years and over was observed for all neuropsychological tests (Figure 1).

Additional adjustments

Additional adjustments for the use of antihypertensive drugs, smoking status, alcohol intake, history of stroke, history of diabetes or history of cardiovascular disease did not markedly change any of these results (data not shown).

DISCUSSION

Our data show that age has an important effect on the relation between actual blood pressure and cognitive function later in life. In participants aged up to 74 years, both higher SBP and DBP were, if anything, associated with worse cognitive function 11 years later. This relation reversed in older participants, in whom higher SBP and DBP at baseline were associated with better cognitive function later in life.

The detrimental effect of higher blood pressure levels on cognitive performance in the middle-aged is well established,[2-5] and the results from our analyses in the 65-74 year age group in the Rotterdam Study sample were consistent with these previous findings. The mechanisms behind this association may involve atherosclerotic changes in large and hyaline degeneration in small cerebral vessels, ischemic brain lesions and disturbances in endothelial or brain cell permeability.[1-5] However, in the oldest-old participants in the Rotterdam Study high blood pressure at baseline was, if anything, related to better cognitive function later in life, and when we replicated our analyses in the 85 year old participants of the Leiden 85-plus Study sample we found similar results.

In our study, participants with a relatively low blood pressure and good cognitive function at baseline were more likely to be included in the study sample than participants with higher blood pressure and worse cognitive function at baseline. One might expect that this selective attrition could have influenced our results, especially in the oldest age group of the Rotterdam Study where a large proportion of the participants who were present at baseline were not available at follow-up anymore. Although a large proportion of participants in especially the oldest age group in the Rotterdam Study were not included in the study sample due to missing data, the selective drop out was seen across all age groups from 55 to 85 years. Furthermore, the participants in the oldest age group (Leiden 85-plus Study) for which we do not have follow-up examinations had predominantly lower blood pressure levels and worse cognitive function when compared to those who are included in the analyses. When

taken together, it is unlikely that selective attrition in itself can explain for our results, and this concurs with previous findings that high blood pressure is not a risk factor for mortality in the oldest old.[19,20]

The previous studies that associated higher blood pressure in middle-age with worse cognitive function later in life,[2-5] suggested that antihypertensive treatment might prevent or delay the onset of impaired cognitive function. However, data from randomized clinical trials on the beneficial effects of antihypertensive treatment on cognitive function are not consistent.[7,10,21-23] From these earlier studies, only the Syst-Eur trial[10] showed benefit that could not be replicated in the HYVET-study.[11] This large, double-blind, placebo-controlled trial included 3336 participants aged 80 years or over and showed that antihypertensive treatment did not reduce incidence of dementia.[11] Although this result could have been affected by the relatively short follow-up, owing to the early termination of the trial, the alternative explanation is that there is no clear benefit in correspondence with the observational data presented here. Alternatively, the use of the relatively insensitive Mini-Mental State Examination as an outcome measure for cognitive impairment or decline could also have contributed to the inconsistent findings in clinical trials. Another explanation is that the oldest old among whom impaired cognitive function is most prevalent were underrepresented in these studies.

It is tempting to speculate why blood pressure lowering is consistently associated with a lower risk of stroke whereas this benefit is not reflected in a consistent preservation of cognitive function. Although counter intuitive, the aggregated data from the observational and experimental studies suggest that in the oldest old a higher blood pressure may have also have a benefit with respect to cognitive function, possibly through the necessity to maintain adequate cerebral perfusion.[24-26] Cerebral perfusion is tightly regulated over a wide range of blood pressures by local regulation of cerebral blood flow.[27] This autoregulation of cerebral blood flow is mediated by a combination of myogenic and neurogenic mechanisms.[28] With increasing age, basal cerebral blood flow decreases, possibly caused by impaired cerebral autoregulation through atherosclerosis or endothelial dysfunction.[29,30] In the oldest old, a higher blood pressure may therefore be required to prevent cerebral hypoperfusion and preserve cognitive function. These individuals with increased risk of morbidity and mortality are present in the population based prospective studies but are less likely to be included in the randomized clinical trials. Participants of the HYVET-study,[11] who were randomized to placebo suffer a mortality risk less than half in comparison with the general population indicating the recruitment of relative healthy people into the trial.

Our study had several strengths. Data on blood pressure and cognitive function were available from two independent Dutch prospective population-based studies. These two cohorts are complementary and allowed us to examine the association between blood pressure level and cognitive function from age 55 years onwards. The dedicated neuropsychological test batteries that were used in both studies were comparable and assessed several cognitive domains, including global cognitive function, executive function, and memory. Some of the

participants in the oldest age group (Leiden 85-plus Study) could not undergo all the cognitive tests that were available, because of their severely impaired cognitive status (MMSE-score ≤ 18). Rather then excluding these persons from the analyses, we used the information on the cognitive status of these participants that was available (MMSE-score) to infer their scores on the other cognitive tests.

There were also some limitations. The associations of blood pressure and cognitive function were based on the assessment of blood pressure at baseline and the measurement of cognitive function 11 years (Rotterdam Study) or 5 years (Leiden 85-plus Study) later. The difference in follow-up length between the two study samples warrants some caution in the comparison and interpretation of the results, as the Rotterdam Study results are based on 11 years of follow-up compared to 5 years of follow-up in the Leiden 85-plus Study. Despite the relatively long follow-up periods, extension of these periods may have revealed even stronger associations between baseline blood pressure and cognitive function later in life, as the effect of blood pressure on cognitive function is thought to be long-term.[1] This may especially play a role up to age 65 years where the follow-up period may just have been too short. However, a longer follow-up, especially in older age, would also lead to a further drop out of participants and consequently to a limited statistical power.

CONCLUSION

In conclusion, this study shows that the relation between baseline blood pressure levels and cognitive function later in life differs across age groups. High blood pressure is a risk factor for cognitive impairment, especially up to age 75 years but may also help to preserve cognitive function in the oldest old as perfusion pressure is maintained. Further elucidation of risks and benefits of blood pressure lowering therapy in the oldest old have to be determined.

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4.2

Systemic Markers of Inflammation and Cognitive Decline in Old Age

ABSTRACT

Background: Several lines of investigation suggest that inflammation is involved in the pathogenesis of dementia. However, the role of inflammatory markers in the early stages of the disease process, where there is cognitive decline but no full-blown clinical dementia syndrome is unclear. Therefore we investigated whether higher circulating levels of C-reactive protein (CRP), interleukin-6 (IL-6), and α1-antichymotrypsin (ACT) are associated with worse cognitive function and decline in old age.

Methods: We used data from two independent population-based cohort studies: The Rotterdam Study (3874 individuals, mean age 72, mean follow-up 4.6 years) and the Leiden 85-plus Study (491 individuals, all aged 85, maximal follow-up 5 years).

In both studies inflammatory markers were measured at baseline, and global cognition, executive function and memory were assessed using a dedicated neuropsychological test battery.

Results: In the Rotterdam Study, higher levels of CRP and IL-6 were cross-sectionally associated with worse global cognition and executive function ($p<0.05$). ACT was not associated with cognitive function. In the Leiden 85-plus Study estimates were similar for CRP, though not statistically significant. In the longitudinal analysis, higher IL-6 levels were related to a steeper annual decline in memory function in the Leiden 85-plus Study ($p<0.05$). The effect of higher IL-6 levels on global and memory function decline was stronger in APOE-ε4 carriers than in non APOE-ε4 carriers (p-interaction 0.011 respectively 0.048). In the Rotterdam Study, higher IL-6 levels were related to a steeper annual decline in global cognition in APOE-ε4 carriers only.

Conclusion: Systemic markers of inflammation are only moderately associated with cognitive function and decline, and tend to be stronger in carriers of the APOE-ε4 allele. Systemic markers of inflammation are not suitable for risk stratification.

INTRODUCTION

Several lines of investigation suggest that inflammation is involved in the pathogenesis of dementia. Animal models expressing high levels of pro-inflammatory cytokines in the brain suffer from neurodegeneration,[1] whereas upregulation of pro-inflammatory cytokines in tissue cultures leads to microglial activation and neuronal damage.[2] Furthermore, markers of inflammation are found in and around condense plaques in the brain,[3] and several population-based studies show an association of plasma levels of inflammatory markers with the risk of dementia.[4,5] However, dementia develops over a long pre-clinical period, and its association with inflammatory markers may reflect a consequence of the disease process, rather than a causal association. In addition, elderly individuals with cognitive dysfunction often have co-morbidities, which might underlie their elevated inflammatory markers. Therefore, it is important to investigate the role of inflammatory markers in the early stages of the disease process, where there is cognitive decline but no full-blown clinical dementia syndrome yet.

Several studies have reported an association of inflammatory markers with cognitive decline. However, these studies were relatively small,[6-8] had a relatively short follow-up,[6,9] included only one marker of inflammation,[7] and typically did not include substantial numbers of individuals aged 80 years or over. Moreover, these studies, except for one,[6] did not account for the possible interrelationships between inflammatory markers, atherosclerosis, and cognitive decline. Many elderly individuals have atherosclerosis, which is a strong risk factor for the development of cognitive decline,[10] and which is strongly related to inflammatory markers such as C-reactive protein (CRP).[11] Therefore, the observed associations between inflammatory markers and cognitive decline may be non-specific, i.e., due to the presence of atherosclerosis.

In view of these considerations, we tested the hypothesis that higher levels of the inflammatory markers CRP, interleukin-6 (IL-6) and α1-antichymotrypsin (ACT) are associated with cognitive decline and investigated whether this association is mediated by atherosclerosis, in two large prospective population-based studies: the Rotterdam Study and the Leiden 85-plus Study. Both studies used a dedicated neuropsychological test battery to assess cognitive decline. As the APOE ε4 allele is an important risk factor for cognitive decline,[12,13] which may assorts its effects via inflammatory mechanisms, we also investigated the effect of APOE ε4 carriership on the association between inflammatory markers and cognitive decline.

METHODS

Population

Rotterdam Study

The Rotterdam Study is a large prospective population-based cohort study to which all inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, The Netherlands were invited.[14] The medical ethics committee of the Erasmus University of Rotterdam approved the study and informed consent was obtained from all participants. A total of 7983 individuals (response rate 78%) participated in the baseline examinations between 1990-1993 (mean age 71±25 years, range 55-106). All individuals were interviewed at home, and invited to visit the research centre for further examinations. In the 3rd (1997-1999) and 4th survey (2002-2004) cognitive function was assessed at the research centre by use of a dedicated neuropsychological test battery.

Source Population

The current analyses on inflammation and cognition concern participants who underwent the neuropsychological test battery and had blood drawn at the 3rd survey. In the 3rd survey 4797 individuals participated, of whom 4206 underwent neuropsychological testing. Blood samples were obtained in 3993 of these individuals. Individuals with dementia at the 3rd survey (n=119) were excluded, resulting in 3874 individuals available for analyses.

Of these 3874 individuals, 2433 individuals completed the neuropsychological test battery at the 4th survey, and thus were available for longitudinal analyses. Of the 1441 individuals who did not participate, 444 had died, 297 refused to participate, 193 were too ill to visit the research centre, 111 could not be contacted and 396 had incomplete data on cognitive function tests.

Additional Measurements

Education was measured at the baseline examination (1990-1993), and dichotomised more or less than 6 years of schooling. The following measures were all assessed at the 3rd survey (1997-1999). Depressive symptoms were assessed during the home interview, by use of a depression questionnaire (the 20-item version of the Center for Epidemiological Studies Depression Scale (CES-D)). The use of cardiovascular drugs or anti-inflammatory drugs was assessed by questionnaire during the home-interview. The presence of cardiovascular disease was defined as a positive history of myocardial infarction, percutaneous transluminal coronary angioplasty (PTCA), coronary artery bypass graft (CABG), stroke, and/or the presence of angina pectoris or intermittent claudication as assessed by the Rose questionnaire. Furthermore, carotid intima medial thickness above 1.0 mm and the presence of plaques in the carotid arteries as assessed by ultrasonography[15] were used as measures of atherosclero-

sis. APOE genotypes were assessed as previously described,[16] and were available in 3664 of the 3874 individuals.

The diagnosis of dementia was made following a three-step protocol.[17] In short, two brief cognition tests (Mini-Mental State Examination (MMSE)[18] and Geriatric Mental State schedule (GMS))[19] were used to screen all subjects. If persons screened positive (MMSE score<26 or GMS organic level>0) they were additionally examined with the Cambridge examination for mental disorders of the elderly (Camdex) by a physician who also obtained an interview with an informant.[20] Subjects who were suspected of having dementia were examined by a neuropsychologist if additional neuropsychological testing was required for diagnosis. Final diagnosis was made by an adjudication panel.

Leiden 85-plus Study

The Leiden 85-plus Study is a prospective population-based cohort study of inhabitants of Leiden, The Netherlands. The Medical Ethical Committee of the Leiden University Medical Centre approved the study, and informed consent was obtained from all participants. Between September 1997 and September 1999, all inhabitants of Leiden born between 1912 and 1914 (n=705) were contacted within a month after their 85th birthday. A total of 599 individuals (response rate 87%) agreed to participate. From age 85 till 90 years, annual neuropsychological tests were performed during home visits.

Source Population

The current analyses on inflammation and cognition concern participants that underwent the neuropsychological test battery and had blood samples drawn at age 85. Five hundred ninety nine individuals underwent neuropsychological testing of whom 563 had blood samples taken, 29 refused and 7 died before blood samples could be taken. Individuals with dementia at age 85, as defined by a clinical diagnosis of the treating physician (n=72), were excluded, resulting in 491 individuals available for analyses.

Data on cognitive function with at least one follow-up collection were available in 440 individuals. At age 86 cognitive function was assessed in 437 individuals, at age 87 in 392 individuals, at age 88 in 374 individuals, at age 89 in 299 individuals, and at age 90 in 255 individuals. Of the remaining 51 individuals without follow-up, 35 died before the age of 86 years, 13 refused to participate, and 3 had no data on cognitive function tests at follow-up visits.

Additional Measurements

The following measures were assessed at age 85 years. Education was dichotomised into more or less than 6 years of schooling. Depressive symptoms were assessed by use of the 15-item Geriatric Depression Scale (GDS-15)[21] during the home interview. The use of cardiovascular drugs or anti-inflammatory drugs was determined by pharmacy records. The presence

of cardiovascular disease was defined as a positive medical history of myocardial infarction, arterial surgery, stroke, angina pectoris, and/or intermittent claudication. APOE genotypes were assessed as previously described,[22] and were available in 479 of the 491 individuals.

Cognitive Function

Global cognitive function was measured by use of the Mini-Mental State Examination (MMSE) in both cohorts. In addition, a neuropsychological test battery was used to assess global cognitive function, executive function, and memory function. The test battery included the abbreviated Stroop test part 3 and the Letter Digit Substitution Task in both cohorts.[23]

In the Rotterdam Study, word fluency[24] was additionally assessed. Since at the third survey no separate test was administered to measure memory function, we used the items from the MMSE and Geriatric Mental State examination, (GMS-A organic level test)[25] that aim to assess memory. The thus constructed memory score had a range of 0 (worst score) to 4 points (best score).

In the Leiden 85-plus Study memory function (immediate and delayed recall) was assessed by use of the 12-Picture Learning Test.[26]

Compound cognitive test scores were constructed by transforming individual test scores into standardised Z-scores (Z-score = (individuals score – mean population score) / SD population score). Compound scores were estimated for global cognitive function and executive function. Global cognitive function was calculated by averaging the Z-scores of the Stroop test, the Letter Digit Substitution Task, the Word Fluency Test and the memory score (in the Rotterdam Study), or the Stroop test, the Letter Digit Substitution Task, and the 12-Picture Learning Test immediate and delayed recall (in the Leiden 85-plus Study). Executive function included the Z-scores of the Stroop test and the Letter Digit Substitution Task in both cohorts.[27]

Inflammatory Markers

High sensitivity CRP (hsCRP) was measured by use of a Rate Near Infrared Particle Immunoassay (IMMAGE®, Immunochemistry System, Beckman Coulter, USA, detection limit 0.2 mg/l, coefficient of variation (CV) 3.2%) in the Rotterdam Study. CRP was measured by use of a fully automated Hitachi 747 system (Hitachi, Tokyo, Japan, detection limit 1 mg/l, CV <5%) in the Leiden 85-plus Study.

IL-6 plasma levels were determined by a quantitative ELISA technique (Quantikine HS IL-6 kit, R&D Systems, Oxon, UK, detection limit 0.094 pg/ml, CV 8.7%) and ACT plasma levels by kinetic nephelometry (Behring Nephelometer BN200®, Marburg, Germany, detection limit 1.5 mg/dl, CV 2.8%) in a random sample of the Rotterdam Study (n=491). IL-6 levels in the Leiden 85-plus study were obtained from an ex vivo whole blood stimulation assay at age 85 years.[28] In short, after incubation of venous blood samples at 37°C and 5% CO_2 for 24 hours, supernatants were collected and stored at -80°C until measurement of IL-6 by use of

a standard ELISA (Sanquin, Amsterdam, the Netherlands, detection limit 4 pg/ml, CV 5-10%). We used the unstimulated IL-6 levels as an estimate of circulating IL-6 levels. In addition, we measured circulating IL-6 levels at age 86 years using the same ELISA.

Statistical Analyses

First, we investigated the cross-sectional association of systemic markers of inflammation with cognitive function, with linear regression analyses and with markers of inflammation both in categories and as continuous variables in the models. CRP and IL-6 were log-transformed because of their skewed distribution.

Second, we used linear regression to investigate the longitudinal association of systemic markers of inflammation with cognitive decline, with the annual change of cognitive function as dependent and inflammatory markers as independent variables. In the Rotterdam Study, annual cognitive decline was calculated as the difference between the test scores at the 4th and the 3rd survey divided by follow-up time (mean follow-up 4.6 ± 0.5 years). In the Leiden 85-plus Study annual cognitive decline was calculated by subtracting test scores at the latest follow-up examination from the test scores at age 85 and dividing them by the follow-up time (mean follow-up 3.4 ± 1.8 years).

All analyses were adjusted for age (Rotterdam Study only), sex, and education level, because these factors strongly relate to cognitive function. On further analysis, we adjusted for body mass index, diabetes and prevalent cardiovascular disease. Finally, we tested for interactions of inflammatory markers with APOE-ε4, cardiovascular disease and atherosclerosis in the association with cognitive function.

RESULTS

Table 1 gives principal features of both study populations. Table 2 shows the clinical characteristics of both study samples used for cross-sectional and longitudinal analyses. In the sample from the Rotterdam Study mean age was 72.1 years, 58% were women and 29% had primary education only. In the Leiden 85-plus Study mean age was 85 years, 65% were women and 62% had primary education only. Cognitive function in the Rotterdam Study was better than in the participants of the Leiden 85-plus Study, reflecting the younger age range and higher education level in the Rotterdam Study.

As expected, participants who were included in the follow-up examinations of the Rotterdam Study were younger and had a better cognitive function than the participants of the complete sample. Median follow-up was 4.5 years (range 1.5 to 7.1). In the Leiden 85-plus Study, individuals with at least 1 year of follow-up had only slightly better cognitive function than the participants of the complete sample. Median follow-up was 5.0 years (range 1.0 to 5.0).

Table 1. Overview of both study populations

	Rotterdam Study	Leiden 85-plus Study
Baseline investigations	1990-1993	1997-1999
Inclusion	All individuals aged 55 years and over, living in Ommoord, a district of Rotterdam	All individuals born between 1912-1914, living in Leiden
Age at baseline	55 years and over (range 55-99)	85 years
Cognitive function		
Measurements	Mini-Mental State Examination	Mini-Mental State Examination
	Stroop test	Stroop test
	Letter Digit Substitution Task	Letter Digit Substitution Task
	Memory score Word Fluency Test	Immediate and delayed recall of the 12-Picture Learning Test
Number of measurements	two	annual (max. 5)
Measurements used	3rd survey (1997-1999)	baseline (1997-1999)
	4th survey (2002-2004)	latest follow-up examination
Follow-up	4.6 years	max. 5 years
Markers of Inflammation		
Measurements	High sensitivity CRP	CRP
	IL-6	unstimulated IL-6
	ACT	
Measured at	3rd survey (1997-1999)	baseline
Number	3874 for hsCRP 491 for IL-6 / ACT	491 for CRP 491 for unstimulated IL-6

Inflammatory Markers and Cognitive Function

In the Rotterdam Study, higher CRP and IL-6 levels were associated with worse cognitive function (Table 3). Estimates were similar in the Leiden 85-plus Study for CRP, though not statistically significant (Table 3). ACT was not related to cognitive function (all $p>0.37$).

Additionally, we stratified the study populations for the presence or absence of an APOE-ε4 allele, and according to the presence or absence of cardiovascular disease or atherosclerosis at baseline. The association between inflammatory markers and cognitive function did not differ between those with or without an APOE-ε4 allele, neither between those with or without cardiovascular disease or atherosclerosis. This held true for both cohorts (data not shown).

Inflammatory Makers and Cognitive Decline

Table 4 shows the association of CRP and IL-6 with cognitive decline, as measured during follow-up. In both cohorts, nearly all point estimates of cognitive function were negative, indicating worse outcome, when levels of inflammatory markers at baseline were higher. However, there may be an association of IL-6 with memory decline in the Leiden 85-plus Study. Higher levels of ACT were not related to a steeper annual cognitive decline (all $p>0.19$).

Table 5 shows the associations of inflammatory markers with cognitive decline stratified for carriers and non-carriers of the APOE-ε4 allele. In the Rotterdam Study, IL-6 was associated

Table 2. Characteristics at the 3rd survey of the Rotterdam Study and at age 85 of the Leiden 85-plus Study

	Rotterdam Study		**Leiden 85-plus Study**	
	Study sample at 3rd survey (n = 3874)	Study sample at 3rd survey with 4.6 year follow-up (n = 2433)	Study sample at baseline (n = 491)	Study sample at baseline with at least 1 year follow-up (n = 440)
Clinical characteristics				
Age (yrs)	72.1 ± 6.9	70.2 ± 5.7	85	85
Women (%)	58	58	65	67
Low level of education (%)	29	26	62	60
Cardiovascular disease (%)	37	31	62	61
Stroke (%)	5	3	9	8
Present smoking (%)	18	17	15	14
Diabetes (%)	12	10	16	16
Medication use				
Use of cardiovascular drugs (%)	48	45	53	52
Use of NSAIDs (%)	8	8	28	27
Cognitive function				
MMSE (points)	27.7 ± 2.0	28.1 ± 1.6	25.4 ± 4.6	25.5 ± 4.4
CES-D (points)	1 (0 - 6)	1 (0 - 5)	-	-
GDS (points)	-	-	2 (1 - 3)	2 (1 - 3)
Stroop test part 3 (seconds)	57.5 ± 21.6	53.6 ± 17.6	81.5 ± 32.9	81.1 ± 33.3
Letter Digit Substitution Task (correct answers)	26.6 ± 7.1	28.1 ± 6.5	17.3 ± 7.0	17.6 ± 7.0
Word Fluency Test (words)	20.9 ± 5.5	21.8 ± 5.3	-	-
Memory Score delayed recall (words)	3.2 ± 0.9	3.3 ± 0.8	-	-
12-Picture Learning Test immediate recall (total pictures of 3 trials)	-	-	24.4 ± 5.4	24.4 ± 5.5
12-Picture Learning Test delayed recall (pictures)	-	-	8.9 ± 2.5	8.9 ± 2.5
Inflammatory markers				
hsCRP (mg/l)	2.42 (1.21 - 4.59)	2.18 (1.14 - 4.23)	-	-
CRP (mg/l)	-	-	4 (1-8)	3 (1 - 7)
IL-6 (pg/ml)	2.11 (1.44 - 3.32) [a]	1.96 (1.37 - 2.88) [b]	-	-
Unstimulated IL-6 (pg/ml)	-	-	11 (0 - 53)	10 (0 - 57)
ACT (mg/dl)	39.8 ± 11.3 [a]	38.9 ± 10.8 [b]	-	-

Results are presented as percentage, mean ± sd or median (interquartile range). a) In a random sample of 491 only. b) In a sample of 300. Results in the Rotterdam Study for neuropsychological tests and CRP were available in 3874 individuals for MMSE, 3720 for the Stroop test, 3766 for the Letter Digit Substitution Task, 3802 for Word Fluency, and, 3871 for the Memory Score delayed recall. Results for neuropsychological tests, IL-6 and ACT were available in 491 individuals for MMSE, 487 for the Stroop, 489 for the Letter Digit Substitution Task, 487 for Word Fluency and 491 for the Memory Score delayed recall. Results in the Leiden 85-plus Study for neuropsychological tests and inflammatory markers were available in 491 individuals for MMSE, 413 for the Stroop test, 408 for the Letter Digit Substitution Task, and, 426 and 425 for immediate and delayed recall of the 12-Picture Learning Test.

Table 3. Association of inflammatory markers with cognitive test performance

	Rotterdam Study					Leiden 85-plus Study				
	Difference in test performance (95%CI)		R	R^2	p-value	Difference in test performance (95% CI)		R	R^2	p-value
CRP* per SD	(n = 3874)					(n = 491)				
MMSE (points)	-0.04	(-0.10;0.02)	0.38	0.14	0.145	-0.14	(-0.52;0.23)	0.32	0.10	0.456
Global cognitive function (Z-score)	-0.03	(-0.05;-0.02)	0.53	0.29	<0.001	-0.05	(-0.11;0.02)	0.30	0.09	0.201
Executive function (Z-score)	-0.04	(-0.07;-0.02)	0.54	0.29	<0.001	-0.05	(-0.13;0.03)	0.40	0.16	0.230
Memory (delayed recall)	-0.03	(-0.05;0.001)	0.29	0.09	0.055	-0.15	(-0.39;0.09)	0.13	0.02	0.214
IL-6 per SD	(n = 491)					(n = 491)				
MMSE (points)	-0.22	(-0.41;-0.03)	0.38	0.15	0.025	0.16	(-0.22;0.53)	0.32	0.10	0.411
Global cognitive function (Z-score)	-0.08	(-0.14;-0.02)	0.56	0.31	0.009	0.01	(-0.06;0.07)	0.29	0.09	0.885
Executive function (Z-score)	-0.10	(-0.17;-0.03)	0.54	0.30	0.008	0.02	(-0.06;0.09)	0.40	0.16	0.661
Memory (delayed recall)	-0.05	(-0.14;0.04)	0.31	0.10	0.273	0.01	(-0.22;0.25)	0.11	0.01	0.920

CRP; C-reactive Protein, IL-6; interleukin-6. *Measured as hsCRP in the Rotterdam Study and as CRP in the Leiden 85-plus Study. All analyses were performed by use of linear regression, adjusted for age (Rotterdam Study only), sex, and education.

Table 4. Association of inflammatory markers with annual cognitive decline

	Rotterdam Study					Leiden 85-plus Study				
	Annual decline* (95% CI)		R	R^2	p-value	Annual decline* (95% CI)		R	R^2	p-value
CRP† per SD	(n = 2433)					(n = 440)				
MMSE (points)	0.001	(-0.016;0.019)	0.14	0.02	0.881	-0.100	(-0.290;0.090)	0.07	0.01	0.302
Global cognitive function (Z-score)	-0.002	(-0.006;0.002)	0.18	0.03	0.428	-0.005	(-0.032;0.021)	0.03	0.00	0.690
Executive function (Z-score)	-0.003	(-0.007;0.002)	0.22	0.05	0.205	-0.020	(-0.055;0.014)	0.13	0.02	0.244
Memory (delayed recall)	0.002	(-0.006;0.011)	0.06	0.00	0.589	-0.020	(-0.137;0.097)	0.10	0.01	0.737
IL-6 per SD	(n = 304)					(n = 440)				
MMSE (points)	-0.035	(-0.087;0.020)	0.19	0.04	0.219	-0.133	(-0.317;0.050)	0.08	0.01	0.154
Global cognitive function (Z-score)	-0.008	(-0.021;0.006)	0.14	0.03	0.256	-0.024	(-0.049;0.001)	0.11	0.01	0.055
Executive function (Z-score)	-0.005	(-0.019;0.009)	0.24	0.06	0.488	-0.011	(-0.043;0.022)	0.12	0.02	0.518
Memory (delayed recall)	-0.008	(-0.038;0.022)	0.08	0.01	0.598	-0.123	(-0.233;-0.013)	0.15	0.02	0.028

CRP; C-reactive Protein, IL-6; interleukin-6. *Estimates from a mean follow-up of 5 years. †Measured as hsCRP in the Rotterdam Study and as CRP in the Leiden 85-plus Study. All analyses were performed by use of linear regression, adjusted for age (Rotterdam Study only), sex and education level. In the Leiden 85-plus Study follow-up data on cognitive function were available in 440 individuals for MMSE, 350 for Global cognitive function, 328 for Executive function, and, 368 for Memory.

Table 5. Association of inflammatory markers with annual cognitive decline in carriers and non-carriers of the APOE-ε4 allele

	Rotterdam Study									Leiden 85-plus Study								
	APOE ε4 non-carrier				APOE ε4 carrier					APOE ε4 non-carrier				APOE ε4 carrier				
	Annual decline* (95% CI)		R	R^2	Annual decline* (95% CI)		R	R^2	p for interaction	Annual decline* (95% CI)		R	R^2	Annual decline* (95% CI)		R	R^2	p for interaction
CRP† per SD	(n = 1698)				(n = 621)					(n = 342)				(n = 85)				
MMSE (points)	-0.001	(-0.022; 0.019)	0.14	0.02	-0.007	(-0.045; 0.031)	0.13	0.02	0.783	-0.130	(-0.366; 0.106)	0.08	0.01	-0.021	(-0.336; 0.294)	0.07	0.01	0.585
Global cognitive function (Z-score)	-0.002	(-0.007; 0.003)	0.18	0.03	-0.004	(-0.012; 0.004)	0.18	0.03	0.719	0.017	(-0.010; 0.045)	0.09	0.01	-0.061	(-0.136; 0.015)	0.21	0.05	0.012
Executive function (Z-score)	-0.006	(-0.011; 0.000)	0.20	0.04	-0.001	(-0.010; 0.008)	0.26	0.07	0.321	-0.014	(-0.054; 0.026)	0.14	0.02	-0.024	(-0.101; 0.053)	0.09	0.01	0.754
Memory (delayed recall)	0.006	(-0.005; 0.016)	0.08	0.01	-0.007	(-0.025; 0.012)	0.05	0.00	0.219	0.054	(-0.076; 0.183)	0.10	0.01	-0.228	(-0.519; 0.063)	0.26	0.07	0.033
IL-6 per SD	(n = 204)				(n = 85)					(n = 342)				(n = 85)				
MMSE (points)	-0.013	(-0.077; 0.052)	0.22	0.05	-0.094	(-0.206; 0.018)	0.21	0.05	0.267	-0.094	(-0.306; 0.118)	0.07	0.01	-0.231	(-0.648; 0.187)	0.14	0.02	0.605
Global cognitive function (Z-score)	-0.002	(-0.018; 0.014)	0.20	0.04	-0.028	(-0.054; -0.001)	0.34	0.11	0.197	-0.016	(-0.040; 0.008)	0.10	0.01	-0.127	(-0.234; -0.021)	0.30	0.09	0.011
Executive function (Z-score)	-0.006	(-0.023; 0.010)	0.22	0.05	-0.014	(-0.040; 0.012)	0.31	0.09	0.550	-0.012	(-0.046; 0.023)	0.14	0.02	-0.018	(-0.129; 0.094)	0.05	0.00	0.976
Memory (delayed recall)	0.006	(-0.029; 0.041)	0.16	0.02	-0.042	(-0.106; 0.021)	0.21	0.04	0.262	-0.099	(-0.212; 0.014)	0.13	0.02	-0.486	(-0.892; -0.079)	0.33	0.11	0.048

CRP; C-reactive protein, IL-6; interleukin-6. †Measured as hsCRP in the Rotterdam Study and as CRP in the Leiden 85-plus Study. All analyses were performed by use of linear regression adjusted for age, sex and education level. *Estimates from a mean follow-up of 5 years. In the Leiden 85-plus Study, follow-up data on cognitive function were available in 440 individuals for MMSE, 346 for Global cognitive function, 348 for Executive function, and, 364 for Memory.

with a decline in global cognitive function in APOE- ε4 carriers, but not in APOE- ε4 non-carriers. In the Leiden 85-plus Study, the associations of CRP and IL-6 with global cognitive decline and memory decline were stronger in carriers of the APOE- ε4 allele compared to non-carriers (p-interaction<0.05). ACT was associated with a decline in executive function in APOE- ε4 carriers (annual decline -0.020 [95%CI, -0.039;-0.002]), but not in non-carriers (annual decline -0.001 [-0.017; 0.019], p-interaction 0.146).

The presence of cardiovascular disease or atherosclerosis at baseline did not consistently influence the relation between inflammatory markers and cognitive decline, in either of the two cohorts (data not shown).

Additional analyses

Additional adjustment for symptoms of depression, use of anti-inflammatory drugs, body mass index, cardiovascular disease or diabetes did not materially change our results (data not shown). Furthermore, in the Rotterdam sample we investigated whether the association of inflammatory markers with cognitive function and decline was stronger at old age. We could not demonstrate any consistent influence of age on this association.

In this follow-up study on inflammation contributing to cognitive decline, we have excluded those who suffered from dementia at baseline (Rotterdam Study: n=119, Leiden 85-plus Study: n=72). However, when we re-introduced these individuals with dementia at baseline into the analyses, the results did not materially change (data not shown).

Next to unstimulated production levels of IL-6 we also measured circulating IL-6 at age 86 in the Leiden 85-plus Study (n=427). In this sample we repeated all analyses on cognitive function and decline with circulating IL-6. The key question was whether we could replicate the association between higher unstimulated IL-6 production levels and an increased annual cognitive decline, especially in the memory domain. High compared to low circulating IL-6 levels were associated with an annual decline in MMSE of -0.418 points (-0.829;-0.133) and with an annual decline in memory function of -0.254 words (-0.488; -0.019). Corresponding estimates for unstimulated IL-6 levels were -0.352 points (95%CI, -0.756;0.061) for the MMSE and -0.334 words (-0.588;-0.080) for memory decline. The similar effect sizes suggest that the unstimulated production level of IL-6 in whole blood samples is a valid estimate of circulating IL-6.

DISCUSSION

We found that systemic levels of CRP and IL-6 were only moderately associated with estimates of global cognition and executive function in cross-sectional analyses. Systemic levels of IL-6 were related to a longitudinal decline in memory function in the Leiden 85-plus study only. When compared to non-carriers, in APOE- ε4 carriers increasing IL-6 levels tended to be

more strongly associated with the annual decline in global cognition and memory function. However, due to the large number of comparisons made and the large sample size, these results may be due to chance.

Previous studies also demonstrated minor associations between inflammatory markers and cognitive decline.[6-9,29] However, the effect size being modest does not imply that the involvement of systemic inflammation in the pathophysiology of cognitive decline is modest as well.[30] In fact, the consistent finding of this association in different populations may suggest that inflammation is involved in the pathophysiology of cognitive decline. However, we cannot rule out the possibility that both inflammatory activation and cognitive decline reflect the consequence of an underlying common disease process. In contrast, these findings do imply that measurement of inflammatory markers is not suitable for risk prediction of cognitive decline in clinical practice.

Dementia, the end stage of severe cognitive decline, develops over a long pre-clinical period. Therefore its association with inflammatory markers may reflect a consequence of the disease process, rather than a causal path. This may play a role both in cross-sectional studies and studies with a relatively short follow-up. In order to limit the possibility that individuals with dementia would drive the association between inflammatory markers and cognition, we vigorously excluded subjects with dementia from the analyses. It cannot be excluded that subjects who are early in the dementia process and do not fulfil the criteria for dementia yet, drive the associations as described here. However, when we repeated the analyses and included those with dementia at baseline, the observed associations did not change. We previously demonstrated in the Rotterdam Study that ACT was strongly related to incident dementia, while CRP and IL-6 were only moderately associated to dementia.[5] The discrepancy with our current finding suggest that timing is important in the association of inflammatory markers with cognitive decline and dementia. Possibly, inflammation can only predict the late stages of cognitive decline, that of dementia, while it is not suitable for risk stratification of early cognitive decline.

We found a trend towards a stronger association of inflammatory markers with cognitive decline in carriers of the APOE-ε4 allele. This is in agreement with a previous finding that the APOE- ε4 allele is associated with an impaired response to cerebral damage,[31] which may lead to a steeper decline of cognitive function. However, we only observed significant interactions in the Leiden 85-plus sample, which may imply an important influence of age on the relation between inflammatory markers and cognitive decline. However, formal testing of this suggestion did not demonstrate a stronger association of inflammatory markers with cognitive decline at old age. Previously, the LASA study investigated whether APOE- ε4 modulates the association between inflammatory markers and cognitive decline,[6] and did not find an interaction between APOE- ε4, inflammatory markers and cognitive decline. Taken together, the effect of the APOE- ε4 allele on the relation between inflammation and cognition needs further investigation.

We did not observe an interaction of atherosclerosis or cardiovascular disease in the association of inflammatory markers with cognitive function and decline. This is in contrast to previous results from the Leiden 85-plus Study, which showed that inflammatory markers interact with atherosclerosis in their association with cognitive decline.[32] However, these results were derived from cross-sectional data, and are therefore more difficult to interpret with respect to causality.

Our study had several strengths. First, we had data on inflammation and cognitive function in a large population-based sample of 3874 individuals from the Rotterdam Study over 4.6 years of follow-up, and of 491 individuals of the Leiden 85-plus Study, with annual follow-up measures over a period of 5 years. Second, we measured cognitive function by use of a neuropsychological test battery which measures separate cognitive domains -notably executive and memory function as well as global function-, and used cognitive decline as an early indicator of the dementia process. Third, by combining data from two population-based studies with a different age-range, we can conclude that the association of inflammatory markers with cognition is present over a large age range.

Our study had also several limitations. First, plasma levels of CRP, IL-6 and ACT were only measured once, which may have diluted the associations we found, and may thus have lead to an underestimation of the risk estimates. Second, different assays were used to determine CRP levels. The discriminative power of the Rotterdam study for the analyses including CRP was larger due to both the sample size and the use of a high sensitivity CRP assay. This may explain why we could not replicate significant findings in both study samples. Third, in the Leiden 85-plus Study we used unstimulated IL-6 production in a whole blood assay as an estimate of circulating plasma IL-6 levels. Though these production levels are clearly higher when compared to circulating plasma levels, we have demonstrated it to be a good estimate of plasma IL-6 levels. Fourth, we have performed a number of statistical analyses, which carries the possibility of significant findings by chance, i.e., caused by random type 2 errors produced by the large sample size and number of statistical test performed. Although, our main interest was to replicate our findings in two independent populations, which automatically doubled the number of analyses performed, and the analyses were based on an a priori hypotheses, a stricter p-value, for instance of <0.01, may be more appropriate to use for these analyses. With this stricter interpretation statistical significance in the prospective analyses would have been lost.

Although our cohorts were population-based and prospectively followed, selection bias may have occurred by selective non-response. Probably the participation rate was lowest among subjects with cognitive impairment. Individuals with follow-up data had a better cognitive performance than those who did not return for follow-up examinations, especially in the Rotterdam Study. This suggests that the observed cognitive decline is probably lower than the actual decline, and therefore, that we may have underestimated the associations with the inflammatory markers.

In conclusion, systemic markers of inflammation are only moderately associated with cognitive function and decline. Our data show some suggestion that these associations may be stronger in the presence of the APOE- ε4 allele. However, these results should be interpreted with care, and may be due to chance. Systemic markers of inflammation are not suitable to predict individual risk of cognitive decline.

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4.3

Serum Uric Acid and Cognitive Function and Dementia

ABSTRACT

Background: Uric acid is a risk factor of cardiovascular disease, as well as a major natural antioxidant, prohibiting the occurrence of cellular damage. The relation between uric acid and cognitive decline, in which both vascular mechanisms and oxidative stress are thought to play a role, is unknown.

Methods: We assessed the relation between serum uric acid levels and the risk of subsequent dementia in a prospective population-based cohort study among 4618 participants aged 55 years and over. Additionally, we investigated the relation between serum uric acid and cognitive function later in life (on average 11.1 years later) in a subsample of 1724 participants who remained free of dementia during follow-up. All analyses were adjusted for age, sex, and cardiovascular risk factors.

Results: Our data showed that only after correcting for several cardiovascular risk factors, higher serum uric acid levels were associated with a decreased risk of dementia (HR, adjusted for age, sex and cardiovascular risk factors, 0.89 (95% confidence interval (CI) 0.80-0.99) per standard deviation (SD) increase in uric acid). In participants who remained free of dementia, higher serum uric acid levels at baseline were associated with better cognitive function later in life, for all cognitive domains that were assessed (adjusted difference in z-score (95%CI) per SD increase in uric acid 0.04 (0.01;0.08) for global cognitive function; 0.03 (-0.01;0.07) for executive function; and 0.07 (0.02;0.12) for memory function), but again only after correcting for cardiovascular risk factors.

Conclusion: Notwithstanding the associated increased risk of cardiovascular disease, higher levels of uric acid are associated with a decreased risk of dementia and better cognitive function later in life.

INTRODUCTION

Uric acid is associated with an increased risk for myocardial infarction, stroke, and cardiovascular mortality.[1-3] Suggested mechanisms for this are a uric-acid-induced stimulation of vascular smooth muscle cell proliferation; the inflammatory properties of soluble uric acid; and the direct effect of uric acid on endothelial function by impairing nitric oxide production.[1,2,4] However, as a major natural antioxidant, uric acid also accounts for a substantial part of the antioxidative capacity of the plasma.[5] This beneficial property might reduce oxidative stress and protect against the detrimental effect of free radicals.

Both vascular pathology and oxidative stress have been associated with an increased risk of dementia and cognitive impairment.[6-8] Therefore, the different properties of uric acid might have contradictory effects on the risk of dementia and on cognitive function. Previous studies have shown that levels of serum uric acid in subjects with mild cognitive impairment (MCI) and in patients with Alzheimer's disease (AD) are lower than those in healthy controls, suggesting that uric acid may have a protective effect.[9,10] To our knowledge, no prospective, population-based studies have investigated the relation between serum uric acid and the risk of dementia or cognitive function later in life.

We hypothesized an association between serum uric acid and a decreased risk for dementia, and better cognitive function later in life based on the antioxidative properties of uric acid, possibly masked by the uric acid-related vascular pathology that is associated with worse cognitive function. Therefore, we investigated the association between serum uric acid levels and the risk of dementia as well as the relation between serum uric acid levels and several domains of cognitive function later in life, in the Rotterdam Study, a large prospective population-based cohort study in subjects aged 55 years and over, for whom several known cardiovascular risk factors were available.

METHODS

Population

The Rotterdam Study is a large ongoing prospective population-based cohort study that is being conducted among all inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, the Netherlands.[11] The study was conducted according to the Declaration of Helsinki, and the appropriate Medical Ethics Committees approved the study protocols. A written informed consent was obtained from all participants. Of 10,275 eligible subjects, 7983 individuals (78%) participated in the baseline examinations between 1990-1993 (mean age (SD) 71 (25) years, range 55-106 years). All participants were interviewed at home, and visited the research centre for further examinations. At the 4th survey (2002-2004) cognitive

function was more extensively assessed with a dedicated neuropsychological test battery. The entire cohort was continuously monitored for incident dementia.

Study population

Uric acid assessments were performed only until December 31, 1992, when they were stopped because of financial constraints, leaving 5150 participants for whom baseline uric acid levels were available.[3] Participants with dementia (n=278), stroke (n=126) or missing cognitive test data (n=128) at baseline were excluded. This resulted in a sample of 4618 participants available for the analyses on the relation between uric acid and the risk of dementia (Figure 1).

More than 10 years later, at the 4th survey in 2002-2004, we assessed cognitive function with a neuropsychological test battery in all surviving, non-demented, consenting participants. Of these 4618 participants with uric acid assessments at baseline, 457 had developed dementia during follow-up; 1315 had died; 846 refused the in person examination; 186 had

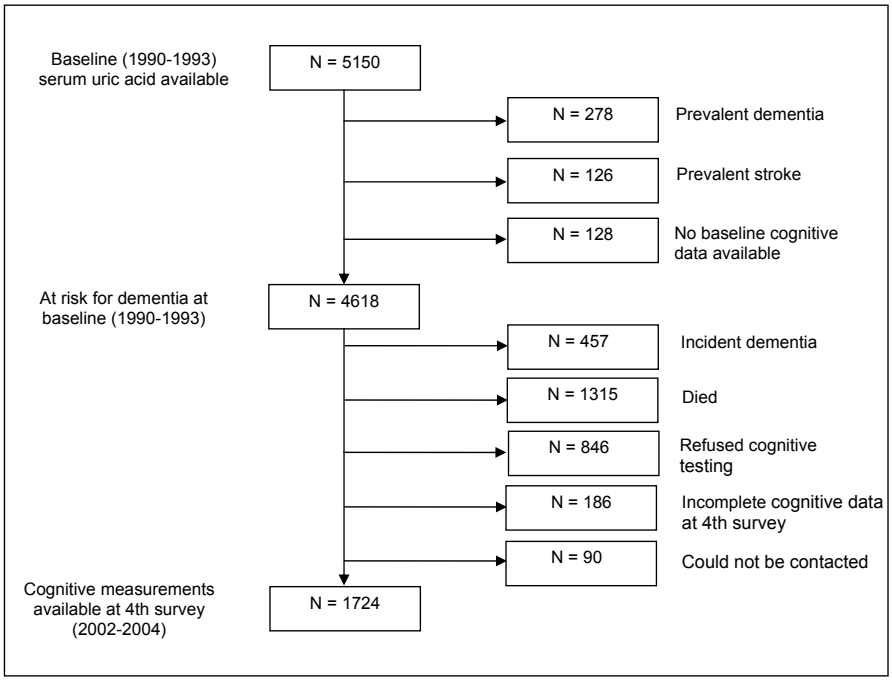

Figure 1. Description of the study population.
Of the 5150 participants whose serum uric acid measurements were available, 532 were excluded at baseline (1990-1993): 278 were diagnosed with dementia, and 126 with a stroke; 128 did not have baseline cognitive measurements available. This resulted in 4618 participants in the total sample who were at risk for dementia at baseline. Of these 4618 participants, 2894 did not participate in the 4th survey: 457 had developed dementia during follow-up, 1315 had died, 846 refused the in person examination, 186 did not have a complete cognitive test assessment, and 90 participants could not be contacted. This resulted in a subsample of 1724 participants who had complete data on the neuropsychological test battery available at the 4th survey.

an incomplete cognitive test assessment; and 90 participants could not be contacted. The subsample available for the analyses on the relation between uric acid levels and cognitive function later in life therefore consisted of 1724 participants who remained free of dementia during follow-up and had both baseline uric acid levels and cognitive test data available 11 years later (Figure 1).

Uric acid

Nonfasting blood was collected and centrifuged. Within 30 minutes, the blood was centrifuged for 10 minutes at 3000 rotations per minute. Subsequently, the serum was stored at -20ºC for one week until uric acid activity was determined with a Kone Diagnostica reagent kit and a Kone autoanalyzer.[12] To check calibration, after every 10 samples, three control samples were included; if the average values of the control samples of each run (100 samples) were not within 2.5% of the true value, the run was repeated. Day-by-day variation had to be within 5%. Finally, we compared the serum uric acid levels in our population with those in two other large population-based studies.[1,13]

Diagnosis of dementia

At baseline and during follow-up examinations, dementia was diagnosed similarly according to a three-step protocol.[14] The total cohort was continuously monitored for incident dementia through linkage between the study database and the digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnosis of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III) by a panel consisting of a neurologist, a neuropsychologist, and a research physician.

Cognitive function

Global cognitive function was measured with the Mini-Mental State Examination (MMSE) at baseline.[15] In addition, a dedicated neuropsychological test battery was used to assess global cognitive function, executive function, and memory function at the 4th survey (2002-2004). Compound cognitive test scores were constructed by transforming individual test scores into standardized Z-scores (Z-score = (individuals score – mean population score) / SD population score).[16] Global cognitive function was calculated by averaging the Z-scores of the Letter Digit Substitution Task (LDST),[17] the Word Fluency Test (WFT),[18] the Stroop test (sum of the reading, colour naming and interference subtask),[19] and the 15-Word Learning Test immediate and delayed recall.[20] Executive function included the Z-scores of the Stroop test (interference subtask), the LDST, and the WFT. Memory function included the Z-score of the 15-WLT immediate and delayed recall.[16]

Additional measurements

Blood pressure was measured twice at baseline with a sphygmomanometer after 5 minutes of seated rest. The average of these two measurements, separated by a count of the pulse rate, was used in the analyses. Total and high-density lipoprotein cholesterol levels were measured at baseline in nonfasting blood with an automated enzymatic procedure. During the home interview, smoking status (classified as current, former or never), history of diabetes mellitus and history of cardiovascular disease (defined as history of myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, peripheral artery disease, cerebrovascular accident or atrial fibrillation) were assessed. Level of education was measured at baseline and dichotomized into primary education or less, and more than primary education The waist/hip-ratio was calculated by dividing the waist circumference by the hip circumference.

Statistical analyses

We used Cox proportional hazard models to calculate hazard ratios with 95% confidence intervals (95%CI) for the association between uric acid and risk of dementia. Hazard ratios were calculated for quartiles of uric acid (with the lowest quartile as the reference) as well as continuously, per SD increase in uric acid level. Participants were followed from date of study entry until age of diagnosis of dementia, age at death or age at end of study, whichever came first. Because serum uric acid is associated with stroke,[3] and stroke is associated with risk of dementia,[21] we censored incident stroke cases. The relation between baseline uric acid levels and cognitive function later in life was assessed with linear regression models, with uric acid as an independent, and cognitive function as the dependent variable, and with analyses of covariance (ANCOVA). All analyses were adjusted for age, sex, level of education and additionally for several known cardiovascular risk factors. Analyses were carried out using the SPSS statistical package (release 11.1; SPSS inc., Chicago, Illinois).

RESULTS

The baseline characteristics of the total sample of 4618 participants and the subsample of 1724 participants for whom complete data on the neuropsychological test battery were available at the 4th survey, are shown in Table 1. The mean age of the total sample of 4618 participants was 69.4 years, 61 percent were female and the mean serum level of uric acid was 322.3 µmol/L. Thirty-two percent of these participants had a history of cardiovascular disease. During a total of 41,651 person-years of follow-up (mean (SD) 9.0 (3.5)), 457 new cases of dementia were detected. Not surprisingly, the 1724 non-demented participants with complete cognitive test data at the 4th survey were at baseline younger, had lower levels of uric acid, less often a history of cardiovascular disease and a better cognitive function

Table 1. Baseline characteristics (1990-1993)

	Total sample	Complete data of cognitive function available at 4th survey(2002-2004)					
		Yes	No (n = 2894)				
			Incident dementia	Died	Refused	Incomplete cognitive data	Could not be contacted
	(n = 4618)	(n = 1724)	(n = 457)	(n = 1315)	(n = 846)	(n = 186)	(n = 90)
Age (years)	69.4 (8.6)	64.1 (5.7)	76.2 (7.3)	74.5 (8.7)	68.5 (7.0)	67.6 (7.0)	73.5 (8.8)
Gender (% female)	61	61	70	51	69	61	80
Serum uric acid level (μmol/L)	322.3 (80.5)	312.4 (73.4)	317.4 (78.9)	342.1 (87.8)	316.2 (77.6)	315.9 (78.3)	315.7 (86.6)
MMSE-score (points)	27.7 (1.9)	28.2 (1.4)	26.7 (2.2)	27.3 (2.1)	27.7 (1.8)	28.0 (1.7)	27.5 (2.1)
Low level of education (%)	40	29	55	47	44	37	51
Creatinine plasma level (μmol/L)	82.8 (21.9)	80.6 (14.0)	83.7 (25.5)	88.0 (31.3)	79.6 (14.0)	79.6 (12.7)	81.4 (18.1)
Systolic blood pressure (mm Hg)	138.9 (21.8)	133.5 (19.8)	141.9 (21.7)	144.2 (23.8)	141.0 (20.5)	133.5 (19.4)	142.8 (21.2)
Total cholesterol (mmol/L)	6.6 (1.2)	6.7 (1.2)	6.5 (1.2)	6.4 (1.3)	6.9 (1.2)	6.7 (1.2)	6.6 (1.1)
High-density cholesterol (mmol/L)	1.3 (0.4)	1.4 (0.4)	1.3 (0.3)	1.3 (0.4)	1.4 (0.4)	1.4 (0.4)	1.3 (0.4)
Diabetes mellitus (%)	11	6	15	18	9	10	12
Smoking (% ever)	65	67	56	68	63	63	39
Waist/hip ratio	0.91 (0.09)	0.89 (0.09)	0.91 (0.09)	0.93 (0.09)	0.90 (0.09)	0.91 (0.09)	0.90 (0.09)
Cardiovascular disease (%)	32	19	42	48	29	27	39

Data are presented as means (SD) or percentages. The total sample represents the 4618 participants who were at risk of developing dementia. Of these 4618 participants, 1724 participated at the 4th survey (2002-2004) and underwent cognitive assessments during this follow-up examination. Of the 2894 participants who did not have cognitive data available at the 4th survey, 457 had developed dementia, 1315 had died, 846 refused the in person examination, 186 did not have a complete cognitive test assessment, and 90 participants could not be contacted. (MMSE = Mini-Mental State Examination).

compared to the remainder of the cohort (Table 1). However, after adjustment for several vascular risk factors and markers of vascular disease (serum creatinine levels, systolic blood pressure, ever smoking, total cholesterol and HDL-cholesterol levels, diabetes mellitus, waist/hip ratio and prevalent cardiovascular disease), there was no significant difference in baseline uric acid levels anymore between the 1724 participants with complete cognitive test data at the 4th survey and the rest of the cohort.

In the total sample of 4618 participants, higher baseline levels of uric acid were not associated with the risk of dementia in the analyses that were adjusted for age and sex only. However, when we accounted for vascular risk factors and markers of vascular disease (serum creatinine levels, systolic blood pressure, ever smoking, total cholesterol and HDL-cholesterol levels, diabetes mellitus, waist/hip ratio and prevalent cardiovascular disease) higher uric

Table 2. Uric acid and the risk of dementia

Uric acid	Model 1[a] (457 cases)	Model 2[b] (457 cases)
Per SD increase uric acid	0.93 (0.84-1.03)	0.89 (0.80-0.99)*
1st Quartile	1 (reference)	1 (reference)
2nd Quartile	0.97 (0.75-1.26)	0.95 (0.73-1.23)
3rd Quartile	0.94 (0.73-1.22)	0.90 (0.69-1.18)
4th Quartile	0.81 (0.62-1.05)	0.73 (0.55-0.97)*
p-trend	0.114	0.030

Estimates indicate hazard ratios with corresponding 95% confidence intervals.
[a]Adjusted for age, sex and level of education.
[b]Adjusted for age, sex, level of education serum creatinine levels, systolic blood pressure, ever smoking, total cholesterol and HDL-cholesterol levels, diabetes mellitus, waist/hip ratio, cardiovascular disease, all at baseline. * p< 0.05

acid levels were associated with a lower risk of dementia (hazard ratio (95%CI) for the highest versus the lowest quartile of uric acid 0.74 (0.56-0.99), Table 2).

In the subsample of 1724 participants, higher baseline levels of uric acid were associated with better cognitive function later in life (on average 11.1 years later), but only after adjustment for cardiovascular risk factors (Figure 2). The difference in z-score (95%CI) per SD increase in uric acid was 0.04 (0.00-0.07) for global cognition; 0.02 (-0.02-0.06) for executive function; and 0.06 (0.02-0.11) for memory function. There was a significant trend (p<0.05) over the quartiles of serum uric acid for global cognition and memory function, indicating better cognition for participants with higher levels of serum uric acid.

Furthermore, persons with higher levels of serum uric acid at baseline were more likely to have cardiovascular disease (age- and sex-adjusted odds ratio (95%CI) for cardiovascular disease for the highest versus the lowest quartile of uric acid 2.21 (1.82;2.67)), and had an increased risk to die from cardiovascular diseases (age- and sex-adjusted hazard ratio (95%CI) for cardiovascular mortality for the highest versus the lowest quartile of uric acid 1.73 (1.37;2.17)). These associations remained after adjusting for serum creatinine, systolic blood pressure, ever smoking, total cholesterol, HDL-cholesterol, diabetes mellitus, waist/hip ratio and (for the cardiovascular mortality analysis only) prevalent cardiovascular disease.

DISCUSSION

In this large, population-based cohort study, serum uric acid levels were not associated with the risk of dementia or with cognitive function later in life. However, after adjustment for several cardiovascular risk factors, higher serum uric acid levels were associated with a decreased risk of dementia and better cognitive function later in life. The age- and sex-adjusted analyses showed no clear association between serum uric acid levels and the risk of dementia, or cognitive function later in life. However, higher levels of uric acid were associated with an

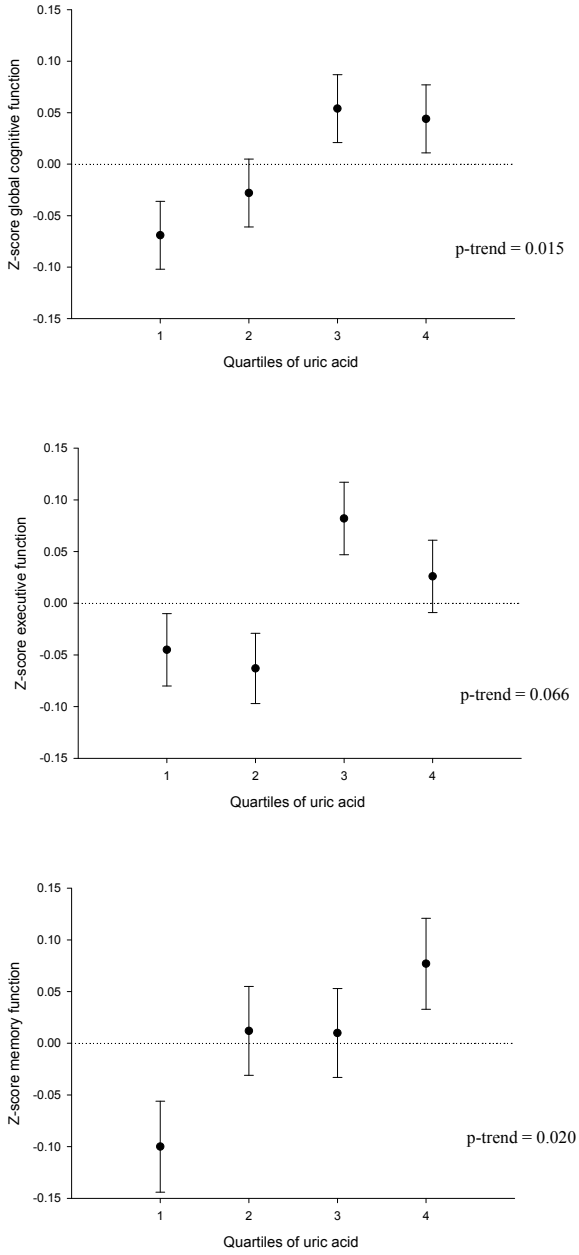

Figure 2. Relation between plasma uric acid levels in quartiles and cognitive function later in life (n= 1724).

Z-scores (standard error of the mean) for the different cognitive domains are plotted for the quartiles of plasma uric acid levels. Linear regression models and analyses of covariance (ANCOVA) were used, adjusted for age, sex, level of education, serum creatinine, systolic blood pressure, total cholesterol and high-density cholesterol levels, diabetes mellitus, ever smoking, waist/hip-ratio, prevalent cardiovascular disease.

increased risk of cardiovascular disease and mortality. After adjustment for several cardiovascular risk factors, the protective effect of uric acid was unmasked: higher levels of uric acid were associated with a lower risk of dementia and better cognitive function later in life.

We know of only one other population-based study that has examined the relation between serum uric acid and cognitive function. This study reported an association between higher levels of uric acid and impaired memory function in a sample of 96 community dwelling participants, which is not in line with our results.[22] The relatively small sample size and the limited number of cardiovascular risk factors for which the analyses were adjusted, may have made it hard to separate the contradictory properties of uric acid in relation to cognitive function in this study.

The strengths of our study include its prospective, population-based design with a large study population, the nearly complete dementia-follow-up, the dedicated neuropsychological test battery that was used and the large number of cardiovascular risk factors that were assessed.

Our study also had some limitations. First, the assessment of uric acid had been stopped before all participants could visit the research centre. However, because all participants were invited in random order, this is unlikely to have affected our results. Furthermore, the mean levels of serum uric acid measured in our study population were comparable with those in two other large population-based studies: NHANES I and the Cardiovascular Study in the Elderly.[1,13] Second, the association between uric acid and cognitive function later in life was based on the assessment of serum uric acid at baseline and the measurement of cognitive function more than 10 years later. Participants with relatively low levels of uric acid and good cognitive function at baseline were more likely to have complete data on the neuropsychological test battery at the 4th survey. The selective attrition of participants with relatively high levels of uric acid and concurrent worse cognitive function did not surprise us, as uric acid is an important risk factor for cardiovascular mortality, myocardial infarction and stroke,[1-3] but could have affected our results. However, after adjustment for several cardiovascular risk factors, there was no longer a significant difference in uric acid level between participants in the subsample and participants who did not have cognitive tests available at the 4th survey. Therefore, selective attrition is unlikely to be the only explanation for our results.

In conclusion, we found in a prospective population-based cohort study that higher serum uric acid levels are related to a decreased risk of dementia and better cognitive function later in life, but only after adjustment for several cardiovascular risk factors. This corroborates the notion that oxidative stress is involved in the pathogenesis of dementia and cognitive impairment and suggests a possible protective role for antioxidants such as uric acid.

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5

Markers for cognitive decline

5.1

Plasma Amyloid Beta Peptides and the Risk of Cognitive Decline

ABSTRACT

Background: The plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio has been suggested as a premorbid biomarker to identify people who are at increased risk of developing dementia. We investigated if the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio is not only associated with the risk of dementia, but also with cognitive decline at large.

Methods: We studied the relation between baseline plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels and the risk of dementia in 1756 participants of a prospective population-based cohort study and examined the influence of time-to-event on this association. Furthermore, we studied the relation between plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels at baseline and the decline in cognitive function in 1452 participants during an average follow-up of 8.0 years.

Results: Participants with a lower plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio were at an increased risk of dementia, but the risk increase diminished with time since blood-draw. There was no association between the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio level and the risk of cognitive decline in participants who did not develop dementia during follow-up.

Conclusion: The risk of dementia associated with a lower plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio is particularly increased within the first years after blood-draw. Assessment of the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio alone is not sensitive enough to identify people at risk for cognitive decline at large.

INTRODUCTION

Peripheral levels of amyloid β peptides have been associated with an increased risk of dementia.[1-6] Furthermore, plasma $A\beta_{1-42}$ levels selectively decline compared to the $A\beta_{1-40}$ levels in the presymptomatic period of the disease, possibly reflecting the deposition of $A\beta_{1-42}$ in the brain.[2,3]

Graff-Radford et al. suggested that the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio might be a useful premorbid biomarker to identify people who are at increased risk of developing dementia and cognitive decline.[5]

We considered that if the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio indeed reflects the selective deposition of $A\beta_{1-42}$ in the brain, it might be a relatively late marker in the presymptomatic stages of the disease. We hypothesized that although the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio can mark an increased risk of dementia,[4] it may not or less associate with earlier stages of presymptomatic disease.

Therefore, we examined whether the relation between the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio and the risk of dementia depends on the time between blood draw and onset of dementia. Additionally, we assessed whether the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio related to the risk of cognitive decline beyond the cognitive decline seen in people who develop clinical dementia.

METHODS

Population

The Rotterdam Study is a prospective population-based cohort study that was conducted among all inhabitants aged 55 years and over of Ommoord, a district of Rotterdam, The Netherlands.[7] The study was conducted according to the Declaration of Helsinki, and the appropriate Medical Ethics Committees approved the study protocol. Written informed consent was obtained from all participants. As described previously, we assessed plasma amyloid β levels in a random sample of 1756 participants of the Rotterdam Study who were free of dementia and had their blood drawn at baseline (1990-1993).[4] They were all followed for incident dementia until the end of 2004.

For 34 of the these 1756 people we did not have cognitive test scores at baseline and 270 persons did not have at least one follow-up assessment of cognitive function, leaving 1452 people available for the analyses on the risk of cognitive decline.

Plasma amyloid β levels

At baseline, non-fasting blood samples were obtained into vacutainers containing sodium citrate. These samples were put on ice immediately and centrifuged within 60 minutes and aliquots of plasma were stored at -80˚ C. Plasma levels of Aβ were determined by a double-

antibody sandwich enzyme-linked immunosorbent assay (ELISA) method (Pfizer, USA).[4] The detection limits were 5-100 pg/mL for $A\beta_{1-42}$ and 10-1000 pg/mL for $A\beta_{1-40}$.

Diagnosis of dementia

At baseline and during follow-up examinations, dementia was diagnosed similarly according to a three-step protocol which has been described elsewhere.[4] The diagnosis of dementia was made in accordance with internationally accepted criteria for dementia (DSM-III) by a panel consisting of a neurologist, a neuropsychologist, and a research physician.

Cognitive decline

Cognitive function was assessed at the research center. Global cognitive function was measured with the Mini-Mental State Examination (MMSE)[8] at baseline (1990-1993) and during three follow-up examinations in 1993-1994; 1997-1999; and 2002-2004. We calculated individual rates of decline in MMSE-score on basis of a minimum of two and a maximum of four MMSE-scores with a random-effects model (SAS 6.12, PROC MIXED; SAS Institute, Cary, NC). We used all baseline and follow-up MMSE-measurements as outcome variable, the time of measurement as independent variable with time at baseline examination as $t = 0$, and the intercept and time of MMSE measurements as random effects. The estimated fixed effect and the individual random effect were added to obtain the estimated slopes and intercepts of the individual MMSE scores.[9] Participants with the largest decline in cognitive function, defined as an individual rate of decline in MMSE-score during follow-up (mean (SD) 8.0 (3.8) years) in the top 25% of the distribution, were classified as cognitive decliners.

Additional measurements

Plasma creatinine concentrations were measured with an automated enzymatic procedure (Roche, Mannheim, Germany). Genotyping for APOE genotype was done on coded DNA specimens.

Statistical analyses

We studied whether the relation between the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio and the risk of dementia varied with time-to-event in the sample of 1756 participants, using Cox' proportional hazard models. Especially, we investigated the risk of dementia associated with the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio within the first 5 years after blood-draw; 5-10 years after blood-draw; and more than 10 years after blood-draw.

We investigated the association between plasma $A\beta_{1-42}$ and the risk of cognitive decline across tertiles of plasma $A\beta_{1-40}$ for the 1452 participants with at least one follow-up assessment of cognitive function with logistic regression. To examine whether associations were driven by persons who actually developed dementia, these analyses were repeated after we had excluded persons with incident dementia.

All analyses were adjusted for age, sex and additionally for plasma creatinine concentration and APOE genotype. Analyses were performed using SPSS (release 12.0.1; SPSS inc., Chicago, Illinois) and SAS (release 6.12 ; SAS Institute, Cary, NC).

RESULTS

Characteristics of the 1756 participants in the cohort at risk for dementia and of the 1452 participants in whom we could assess cognitive decline are shown in Table 1. Of the 1756 participants, 166 developed dementia during a mean follow-up of 9.3 years. By definition, of

Table 1. Baseline characteristics

	Total cohort at risk for dementia	Total cohort at risk for cognitive decline
No	1756	1452
Age (years)	68.6 ± 8.6	67.4 ± 8.0
Women (%)	61	60
Aβ1-42 (pg/mL)	17.8 (14.7-21.7)	17.5 (14.5-21.2)
Aβ1-40 (pg/mL)	192.0 (163.0-228.0)	189.0 (160.0-222.0)
Aβ1-42/Aβ1-40 ratio	0.09 (0.08-0.11)	0.09 (0.08-0.11)
Creatinine (µmol/L)	82.2 ± 17.2	81.9 ± 16.5
MMSE-score (points)	27.7 ± 1.8	27.9 ± 1.7

Data are presented as mean ± SD, percentage or median (IQR)
Abbreviations: MMSE, Mini-Mental State Examination; SD, standard deviation ; IQR, interquartile range

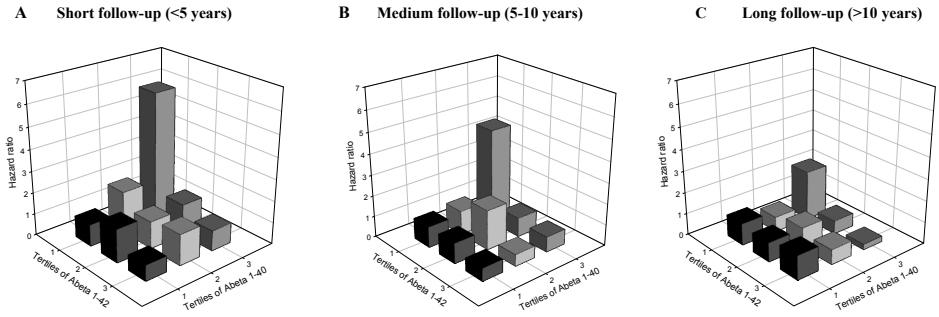

Figure 1. Hazard ratio's for dementia by levels of Aβ1-42 and Aβ1-40 peptides.
Cox' proportional hazard models were used, adjusted for age and sex. Tertile combination 1-1 was the reference tertile combination. The figure on the left (A) shows the relation between Aβ peptides and the risk of dementia in the first 5 years of follow-up for 262 participants. Mean follow-up was 2.6 years during which 66 cases of dementia developed. The figure in the middle (B) shows the relation between Aβ peptides and the risk of dementia after 5-10 years of follow-up for 296 participants. Mean follow-up was 7.7 years during which 64 cases of dementia developed. The figure on the right (C) shows the relation between Aβ peptides and the risk of dementia after more than 10 years of follow-up for 1198 participants. Mean follow-up was 11.2 years during which 36 cases of dementia developed.

the 1452 participants with repeated cognitive test data, 363 participants fulfilled the criteria for cognitive decline during a mean follow-up of 8.0 years.

Figure 1 shows that the participants who had a plasma $A\beta_{1-42}$ level in the lowest tertile of the distribution in combination with a plasma $A\beta_{1-40}$ level in the highest tertile of the distribution were at increased risk of developing dementia but that this increased risk dwindled with increasing duration of follow-up (compared to persons with both plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels in the lowest tertile of the distribution, age- and sex-adjusted hazard ratio for dementia with onset within 5 years after Aβ-measurement 5.97 (95% CI 1.87;19.03); for dementia with onset 5-10 years after Aβ-measurement 4.53 (95% CI 1.00;20.59); and for dementia with onset more than 10 years after Aβ-measurement 2.27 (95% CI 0.28;18.37).

Figure 2A shows that participants who had a plasma $A\beta_{1-42}$ level in the lowest tertile of the distribution in combination with a plasma $A\beta_{1-40}$ level in the highest tertile of the distribution, were more likely to show cognitive decline (age- and sex-adjusted odds ratio compared to persons with both plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels in the lowest tertile of the distribution 2.58 (95% CI 1.06;6.40). This appeared entirely due to people developing dementia, as after exclusion of persons who developed dementia during follow-up, the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio was no longer associated with the risk of cognitive decline (age- and sex-adjusted odds ratio for lowest $A\beta_{1-42}$ and highest $A\beta_{1-40}$ tertile versus the reference combination 0.67 (95% CI 0.14;3.15); figure 2B.

Additional adjustments for plasma creatinine concentrations and APOE genotype did not affect any of the estimates.

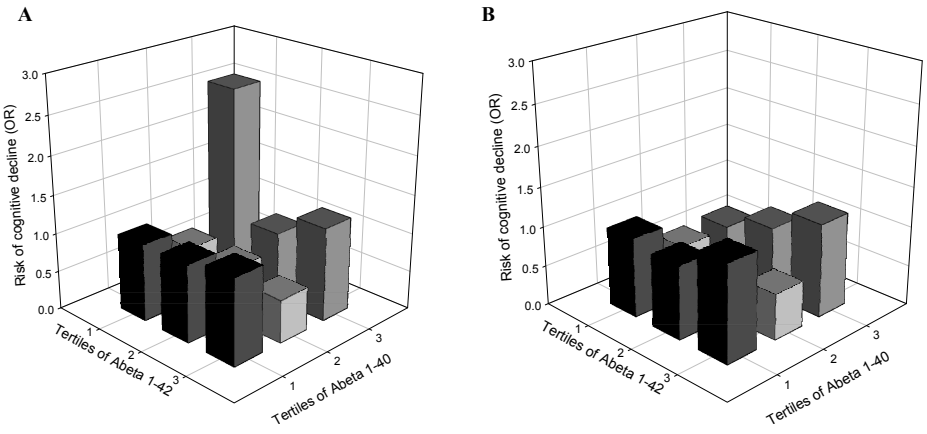

Figure 2. Risk of cognitive decline according to tertiles of baseline plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels. Logistic regression models were used, adjusted for age and sex. Cognitive decline was defined as an annual decline in cognitive function in the top 25% of the distribution. Tertile combination 1-1 was the reference tertile combination. On the left (A) the relation between tertile combination and risk of cognitive decline is shown for the total sample (n = 1452). On the right (B), the relation between tertile combination and the risk of cognitive decline is shown for the sample without incident dementia cases (n = 133) and therefore includes 1319 participants.

DISCUSSION

We showed that although a lower plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio is associated with a higher risk of incipient dementia, this risk diminishes with increasing time-to-event. Furthermore, the plasma $A\beta_{1-42/}A\beta_{1-40}$ ratio was not associated with the risk of cognitive decline in persons who did not develop dementia during follow-up. These findings suggest that although the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio may mark a relatively late stage in the preclinical development of dementia, it is not an adequate marker of the risk of dementia or cognitive decline in earlier stages of the preclinical disease process.

Some studies reported strong associations between the plasma $A\beta_{1-42/}A\beta_{1-40}$ ratio and the risk of dementia,[4,5] yet others found a relation between higher plasma $A\beta_{1-42}$ levels and an increased risk of dementia.[1,2] Together with our present findings, these discrepancies challenge the usefulness of the plasma $A\beta_{1-42/}A\beta_{1-40}$ ratio as a single premorbid biomarker for dementia. This fits the notion that biomarker profiles rather than single biomarkers are likely required to accurately identify persons at risk of dementia.[10] Our data support that biomarkers can be identified in plasma and may play an important role in dementia-risk profiling of presymptomatic individuals. Moreover, our findings illustrate that risk profiles may differ according to the development stage of the underlying disease process. Time-to-event may become an important factor to take into account when developing biomarker panels to screen for presymptomatic dementia.

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6

General discussion

The main objective of the research described in this thesis was to gain insight in the role of potentially modifiable determinants of cognitive decline and dementia in the elderly. I specifically focused on the role of glucose metabolism, vascular and inflammatory mechanisms and the influence of genetic factors on the pathogenesis of cognitive impairment and dementia, and additionally investigated preclinical biomarkers that have been suggested to be useful in the identification of people at risk for cognitive impairment and dementia.

In this chapter, I will first discuss some methodological considerations that apply to the studies described in this thesis. Second, the main findings of the research are addressed, and I will discuss how they relate to current knowledge on the development of cognitive impairment and dementia in the elderly. Finally, I will evaluate the clinical implication of these findings and suggest some directions for future research.

METHODOLOGICAL CONSIDERATIONS

How to measure cognitive function

In recent years, the amount of research on cognitive function and decline in the general population has rapidly increased. Interest in the pathophysiology of cognitive impairment has lead to a number of large population-based studies that examined the relation between potential risk factors and cognitive function and decline.[1-4] This increasing attention for studying cognitive function and decline is a positive development, although the great variety in neuropsychological tests that are used sometimes obstructs the comparability of results across studies: apart from the Mini-Mental State Examination,[5] there is little overlap in cognitive tests between studies. If consensus could be reached upon the use of some specific neuropsychological tests to assess distinct domains of cognitive function in these large, population-based studies, this would make it easier to compare results across studies. Fortunately, this issue was less of a problem in the studies discussed in this thesis (the Rotterdam Study, the Leiden 85-plus Study and PROSPER) as the neuropsychological test batteries in the different populations were quite comparable.

Selective attrition

Although research on determinants of cognitive decline and dementia in the very old is expanding, in most studies there is a clear underrepresentation of oldest old individuals. Extrapolating results from younger persons may be unwarranted in light of recent findings that suggest that risk factors that play a role at younger ages are not important anymore when people survive until higher ages.[6,7] Large numbers of very old participants are needed to study the potentially age-specific effects of possible risk factors for cognitive decline and dementia.

Furthermore, it is well recognized that epidemiologic studies of aging-related conditions can suffer from potentially large selection bias. Selective attrition in these studies of old people could affect the age-specific risk estimates, comparison between studies, and biological interpretation of the results.[8] This phenomenon may also have played a role in some of the studies presented in this thesis. For instance, in the investigation of the relation between blood pressure and cognitive function later in life, participants with a relatively low blood pressure and good cognitive function at baseline were more likely to be included in the study sample than participants with higher blood pressure and worse cognitive function at baseline. Selective attrition could have influenced our results, especially in the oldest age group of the Rotterdam Study where a large proportion of the participants who were present at baseline were not available at follow-up anymore. This was one of the reasons for combining our results on the relation between blood pressure and cognitive function from the Leiden 85-plus Study with those from the Rotterdam Study.

Cross-sectional vs. longitudinal study design

It is a generally accepted view that cross-sectional data are inferior to longitudinal data, as they would not allow for causal inference. In **chapter 2.1**, I presented the results of our investigation of the influence of selection by health and survival on estimates of age-associated cognitive function, and showed how this phenomenon influences both cross-sectional and longitudinal study designs. Although the causality of a relation may be difficult to infer from cross-sectional data, our findings indicated that the same selective mechanisms apply to longitudinal data. In a cross-sectional study participation may be selective as healthy people tend to have higher participation rates, yet longitudinal estimates will be based on a further selection of these healthy participants at baseline: those who complete follow-up. The choice for a longitudinal study design will therefore not necessarily provide a superior estimate of age-associated cognitive function over a cross-sectional design, as the influence of health related selection should be carefully taken in consideration in the assessment of cognitive function using either design.

MAIN RESEARCH FINDINGS

Measuring cognitive function

In **chapter 2.1**, I described our study on the measurement of cognitive function that showed that participants with complete follow-up were healthier and had better age-specific cognitive scores than those with incomplete follow-up. This health selection influenced estimates of cognitive function and decline in both cross-sectional and longitudinal study designs. Furthermore, the length of follow-up affected the estimation of cognitive decline, with a short follow-up resulting in a larger estimated rate of decline than a longer follow-up. Even

advanced statistical models such as linear mixed models could not properly account for the selective attrition that was present.

Many studies have addressed the problem of defining meaningful estimates of cognitive decline in the presence of selective attrition or "censoring by death", although it remains unclear which approach is most appropriate.[9] Principal stratification,[10] and "joint modeling" of both random effects in longitudinal covariates and the survival components,[11] are suggested tools to deal with the selection bias in longitudinal epidemiological studies, but their usefulness in this specific setting needs further clarification. In an accompanying commentary to our paper, Hernán et al.[8] showed how selection bias due to censoring by death can influence estimates in epidemiological studies. This further addresses the issue that selection on health and survival is often present in large epidemiological studies and has implications for comparison of estimates among studies as well as for biological interpretation of the results.[8] In general, the assessment of cognitive function in the elderly requires sufficient consideration of the possible influence of selection by health and survival.

Glucose metabolism and cognitive function

Although diabetes mellitus has been shown to associate with an increased risk of dementia and cognitive decline,[12-14] it is unclear if people in a "pre-diabetic" state, when impaired fasting glucose levels are present, already suffer from impaired cognitive function or an accelerated rate of cognitive decline.[15-18] In our analyses of data on fasting glucose levels and cognitive function and decline in a large sample of participants from PROSPER and the Rotterdam Study (**chapter 3.1**), we could not find a clear relation between elevated fasting glucose levels or increased insulin resistance and cognitive function and decline in people without a history of diabetes. These data suggest that cognitive decline accelerates strongly once diabetic but not with lesser degrees of dysglycaemia. As a result, preventing individuals at risk from developing diabetes through lifestyle changes, may also lead to large societal gains by preventing such individuals from undergoing accelerated cognitive decline.

In **chapter 3.2,** I described how an evolutionary conserved pathway that is related to glucose metabolism associates with cognitive function in 1015 participants from both cohorts of the Leiden 85-plus Study. In accordance with previous findings on old age survival, we found that genetically reduced insulin/IGF-1 signaling (IIS) was associated with better cognitive function in women, but not in men. These current findings are in accordance with the available data from model organisms that showed favourable effects of genetically induced lower IIS activity on both life span and health span.[19-21] Furthermore, the observed sex-specific involvement of IIS pathway activity in the development of cognitive impairment that we found, is supported by previous findings.[19,22] It is possible that improved learning and memory play an important role in the success of seeking food elsewhere, which is especially important for the female sex, as females must obtain sufficient energy to support the survival and development of

their offspring as well as themselves. Suggested mechanisms that could underlie the relation between reduced IIS pathway activity and better cognitive function in females include increased stress resistance, and reduced amyloid β-induced autophagosome accumulation,[22-24] although these results were mainly based on *C.elegans* data. To conclude, we showed that genetically reduced insulin/IGF-1 signaling seems to be beneficial for cognitive function in women, but not in men, which further indicates the involvement of the insulin/IGF-1 signaling pathway in the regulation of health span.

Vascular and inflammatory factors and cognitive function

In **chapter 4.1**, I presented the results of our investigation on the relation between blood pressure and cognitive function, and more specifically the influence of age on this relation. Several studies have shown that higher blood pressure levels at middle-age relate to cognitive impairment later in life,[1,25-27] possibly as a result of atherosclerotic changes in large and small cerebral vessels, ischaemic brain lesions, or disturbances in endothelial or brain cell permeability.[1,26,28] However, notwithstanding a vast amount of investigations, there is no general agreement on the relation between blood pressure and later cognitive function across different age groups.[28] Conflicting results in old aged participants have lead to the suggestion that age might influence the relation between blood pressure and cognitive function in the sense that blood pressure might not be a risk factor for cognitive impairment anymore in the oldest age categories. Moreover, three of four large trials of antihypertensive medication with dementia as a secondary endpoint that have been published did not find a reduction in incidence of dementia in the treatment group.[29-32]

We found in combined analyses of data from the Rotterdam Study and the Leiden 85-plus Study that indeed, higher blood pressure levels were associated with worse cognitive function later in life in participants aged up to 74 years, but that this relation reversed in older participants. In the oldest old, higher blood pressure was related to better cognitive function later in life. Although the possible influence of selective attrition on our results should not be ignored, there are other mechanisms that could underlie these findings. An alternative explanation is that in the oldest old a higher blood pressure is necessary to maintain adequate cerebral perfusion.[33-35] With increasing age, basal cerebral blood flow decreases, possibly caused by impaired cerebral autoregulation through atherosclerosis or endothelial dysfunction.[36,37] In the oldest old, a higher blood pressure may therefore be required to prevent cerebral hypoperfusion and preserve cognitive function. Results from the HYVET-trial, a randomised, placebo-controlled trial that included only participants of 80 years or over, did not show beneficial effects of antihypertensive treatment on incidence of dementia.[38]

In conclusion, we found that the relation between baseline blood pressure levels and cognitive function later in life differs across age groups. High blood pressure is a risk factor for

cognitive impairment up to age 75 years but may preserve cognitive function thereafter. Risks and benefits of blood pressure lowering therapy in the oldest old have to be determined.

Inflammation has repeatedly been suggested to play a role in the development of dementia and cognitive impairment by several lines of investigation. Animal models showed a relation between proinflammatory cytokines and neurodegeneration,[39] and inflammatory markers were found in the neighbourhood of senile plaques in the brain of demented persons.[40] Moreover, higher levels of circulating inflammatory markers were associated with an increased risk of dementia in population-based studies.[41,42] How these inflammatory markers relate to cognitive function and decline in the early stages of the development of dementia is not yet clear. In **chapter 4.2**, I described the results of our investigation on the association between inflammatory markers and cognitive function and decline in combined analyses of data from the Rotterdam Study and the Leiden 85-plus Study. We showed that systemic markers of inflammation were only moderately associated with cognitive function and decline.

Serum uric acid is known as an independent risk factor for myocardial infarction, stroke, and cardiovascular mortality,[43-45] that exerts its damage through mechanisms such as stimulation of vascular smooth muscle cell proliferation; its inflammatory properties; and a direct effect on endothelial function by impairing nitric oxide production.[43,44,46] Remarkably enough, as a major natural antioxidant, uric acid also accounts for a substantial part of the antioxidative capacity of the plasma.[47] This beneficial property might reduce oxidative stress and protect against the detrimental effect of free radicals. In the development of cognitive impairment and dementia, both vascular pathology and oxidative stress are involved.[48-50] Therefore, the different properties of uric acid might have contradictory effects on the risk of dementia and on cognitive function. In **chapter 4.3**, we studied the relation between serum uric acid levels and the risk of dementia as well as the relation between serum uric acid levels and several domains of cognitive function later in life, in the Rotterdam Study. We found in an overall analysis that serum uric acid levels were not associated with the risk of dementia or with cognitive function later in life. However, after adjustment for several cardiovascular risk factors, the protective effect of uric acid was unmasked and higher serum uric acid levels were associated with a decreased risk of dementia and better cognitive function later in life. Although these findings are not in line with a previous study that associated increased serum uric acid levels with worse cognitive function in a cross-sectional sample of community dwelling participants, we were to our knowledge the first to assess the relation between serum uric acid and cognitive function and dementia in a prospective, population-based design with a large study sample and a vast number of cardiovascular risk factors that were assessed.

In conclusion, we found that higher serum uric acid levels are related to a decreased risk of dementia and better cognitive function later in life, but only after adjustment for several

cardiovascular risk factors. This corroborates the notion that oxidative stress is involved in the pathogenesis of dementia and cognitive impairment.

Markers for cognitive decline

In **chapter 5.1**, I described the results of our investigation of the role of plasma amyloid β peptides as potential biomarkers for the risk of dementia and cognitive decline at large.

Senile plaques in the brain of Alzheimer's disease (AD) patients are a characteristic hallmark of the disease.[51] These plaques predominantly contain amyloid β (Aβ) peptides that are derived from the amyloid β precursor protein (APP) by proteolytic processing.[52] The majority of these Aβ peptides contain 40 amino acids ($Aβ_{1-40}$) but a small part consists of 42 amino acids ($Aβ_{1-42}$). This $Aβ_{1-42}$ peptide is the major constituent of the senile plaques and is thought to deposit first in the disease process, before $Aβ_{1-40}$.[53-55] Aβ peptides have been detected in cerebral spinal fluid (CSF), urine, skin and plasma.[56-58] Although the brain is considered as the origin of the deposited Aβ in the senile plaques, the source of Aβ peptides in the plasma remains unclear.[59] APP is produced by a variety of cells in and outside the brain, including platelets that could influence plasma Aβ levels.[60] It is unknown how the deposition of Aβ in the brain affects plasma Aβ levels, but a complex equilibrium between brain deposition and plasma levels of the Aβ peptides is suggested.[61-63] Plasma levels of Aβ peptides increase with age and increased plasma levels of Aβ peptides have been found in patients with early-onset, familial Alzheimer's disease and in patients with Down's syndrome, who are at higher risk of developing Alzheimer's disease.[64-66]

A previous study has shown that elevated plasma levels of Aβ peptides are associated with the development of dementia.[59] Furthermore, it was shown that plasma $Aβ_{1-42}$ levels selectively decline in the presymptomatic period of the disease, possibly reflecting the deposition of $Aβ_{1-42}$ in the brain.[59,67] In a recent study, Graff-Radford and colleagues postulated that a lower ratio of $Aβ_{1-42}/Aβ_{1-40}$ could indicate this selective decline of $Aβ_{1-42}$ in the presymptomatic period of the disease. They found an association between a lower plasma ratio of $Aβ_{1-42}/Aβ_{1-40}$ and an increased risk of dementia, and suggested the use of the plasma $Aβ_{1-42}/Aβ_{1-40}$ ratio as a premorbid biomarker to identify individuals at risk for developing Alzheimer's disease.[68] Our previous findings in a case-cohort study embedded in the Rotterdam Study showed that a lower plasma ratio of $Aβ_{1-42}/Aβ_{1-40}$ was associated with an increased risk of dementia, in accordance with the findings of Graff-Radford and colleagues.[69]

We considered that if the plasma $Aβ_{1-42}/Aβ_{1-40}$ ratio indeed reflects the selective deposition of $Aβ_{1-42}$ in the brain, it might be a relatively late marker in the presymptomatic stages of the disease. Furthermore, we hypothesized that although the plasma $Aβ_{1-42}/Aβ_{1-40}$ ratio can mark an increased risk of dementia, it may not or less associate with earlier stages of presymptomatic disease.

The results that I presented in **chapter 5.1** showed that although a lower plasma $Aβ_{1-42}/Aβ_{1-40}$ ratio was associated with a higher risk of incipient dementia, this risk diminished with

increasing time-to-event. Furthermore, the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio was not associated with the risk of cognitive decline in persons who did not develop dementia during follow-up. These findings suggest that although the plasma $A\beta_{1-42/}A\beta_{1-40}$ ratio may mark a relatively late stage in the preclinical development of dementia, it is not an adequate marker for the risk of dementia or cognitive decline in earlier stages of the preclinical disease process. Our data support the notion that biomarkers can be identified in plasma and may play an important role in dementia-risk profiling of presymptomatic individuals. Moreover, our findings illustrate that risk profiles may differ according to the development stage of the underlying disease process. Time-to-event may become an important factor to take into account when developing biomarker panels to screen for presymptomatic dementia.

CLINICAL IMPLICATIONS

One of the main messages with clinical relevance that arises from the research presented in this thesis is that the oldest old might differ more from younger individuals with respect to the mechanisms that underlie the development of cognitive impairment than is recognized at this moment. Some reluctance in the extrapolation of research results based on relatively young populations to the oldest old participants is warranted, as is shown in our study on blood pressure and cognitive function that indicates that current dogma of "lower is better" is not supported by our data on cognitive function in the elderly. These observational findings are supported by the results from the placebo-controlled clinical trials of antihypertensive medication with incidence of dementia or cognitive function as an outcome that all but one did not show a protective effect on cognitive function in the treatment group.[29-32] Recent findings from the HYVET-trial, that included only participants of 80 years or over, did not show beneficial effects of antihypertensive treatment on incidence of dementia.[38] Therefore, some reservation in the treatment of high blood pressure in the oldest old might be appropriate.

The results of our examination of fasting glucose levels in relation to cognitive function and decline showed that although diabetes mellitus is associated with impaired cognitive function, this is not the case for increased levels of fasting glucose. Suggestions to screen individuals for impaired fasting glucose levels based on their supposedly increased risk on cognitive impairment in this "pre-diabetic" state could not be supported by our data. However, preventing individuals at risk from developing diabetes through lifestyle changes, may lead to large societal gains by preventing such individuals from undergoing accelerated cognitive decline.

FUTURE RESEARCH

The work described in this thesis has provided additional insights in the relation between several potential risk factors for cognitive decline and dementia in the very old and I have tried to address and dispute several dogmas that were encountered.

The main message that can be derived from this work and which should be implemented in future research is that despite the well recognized potential gain in experienced health of the oldest old in our population from new insights in the development of cognitive decline and dementia, studies on this subject have not yet been placed in the limelight they deserve. Although there seems to be a general consensus that focus in research should lie on those biological mechanisms that are involved in the development of cognitive impairment in especially the oldest old, as the occurrence of cognitive impairment is predominantly present in the oldest people, the highest age groups remain to be underrepresented in most studies. Suggestions of age-specific relations of risk factors for cognitive decline and dementia, such as blood pressure, further emphasize the need for study samples in which the oldest old are sufficiently represented.

One of the methodological considerations that should be taken into account in future research is the vast amount of different neuropsychological tests that are being used in the investigation of determinants of cognitive impairment. The variability in tests designed to measure distinct domains of cognitive function may on the one hand increase the understanding of the relation between potential risk factors and cognitive function, but also makes the comparability of results across studies more difficult. Discussing the usefulness and appropriateness of the currently available tests in the prospect of reaching some sort of general agreement on neuropsychological tests may render more effect than the continuous development of additional tests.

Furthermore, our findings on the methodology of measuring cognitive function, and more specifically the influence of selection on health and survival in studies on age-associated cognitive function showed that there is still some ground to be covered in clarifying the methodological issues that can affect the estimation of cognitive function. There are indications that longer follow-up periods are needed in studies on cognitive decline, to minimize the potential learning effect between examinations. However, longer follow-up will most likely result in an increased number of participants who drop out before the end of follow-up, as our findings showed. The current inability to appropriately deal with the selective attrition that is present in studies on cognitive function and decline, through the handling of multiple and missing data with advanced statistical modeling, emphasizes the importance to investigate the influence of phenomena such as selection on health and survival on the estimation of cognitive function in these studies.

The shift in focus from the clinical endpoint of the dementia syndrome to studying changes in cognitive function in the developmental stages of the disease, that we made in the research described in this thesis, could be expanded even further. Increasing technological development in the imaging field has lead to the possibility to study subtle changes in brain structures that could underlie the first indication of cognitive decline that is not detected by neuropsychological testing. Investigating the relation between potential risk factors for dementia and cognitive decline and these structural brain changes, in combination with the effect of these changes on cognitive function in a later stage of the development of the disease could provide more insight in the biological mechanisms that underlie the eventually appearing cognitive impairment.

Finally, a better understanding of the impact of changes in cognitive abilities on the individually experienced quality of daily life of people may help in the choice for further research directions from a more clinical point of view. The research described in this thesis was mainly based on investigating the biological mechanisms that could underlie the development of cognitive impairment, although the personally experienced deprivation of cognitive abilities does not have to correspond fully with the decline in neuropsychological test scores. The identification of those cognitive domains that seem to have greatest impact on daily living might give us more insight in studying specific domains of cognition with respect to clinical relevance.

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7

Summary

7.1

Summary (in English)

The devastating impact of the loss of cognitive abilities that often accompanies the aging process is feared by many. Despite an increasing number of studies, the precise etiology of the disease is not known yet. There is accumulating evidence that a variety of causes, rather than a single factor, result in impaired cognitive function and the clinical symptoms of dementia. Glucose metabolism, vascular pathology and inflammation have all been suggested to play a role in the development of cognitive impairment and dementia.

The main objective of the work described in this thesis was the investigation of potentially modifiable risk factors, including factors involved in glucose metabolism, vascular pathology and inflammation in especially the early stages of the disease process. Furthermore, there have been studies that suggested that in general, possible risk factors that play a role at younger ages may not be important anymore when people survive until higher ages. Therefore, I additionally studied the age-specific effects of these risk factors in several large prospective studies including the Rotterdam Study, the Leiden 85-plus Study, and the PROSPER study, that were complementary with respect to the age of the participants and consequently allowed me to study the association between several potentially modifiable risk factors and cognitive function and decline over a wide age range in the general population. Finally, I investigated suggested preclinical biomarkers of dementia that might be used to identify people who are at increased risk of developing dementia.

I first explored the measurement of cognitive function, with respect to the effect of selection by health and survival on the estimation of cognitive function and decline in epidemiological studies. **Chapter 2** showed that selection for health and survival resulted in better age-specific cognitive test scores and less cognitive decline, and that this selection affects both cross-sectional and longitudinal studies. Subsequently, statistical methods handling multiple and missing data did not seem to fully correct for this bias. These results indicated that assessments of cognitive function in the elderly must take particular care in considering possible biases from health selection.

In **chapter 3**, the results from studies on the relation between glucose metabolism and cognitive function were presented. First, I investigated the influence of fasting glucose levels and insulin resistance on cognitive function in non-diabetic elderly from two independent prospective studies: the PROSPER study and the Rotterdam Study (**chapter 3.1**). Elevated fasting glucose levels and insulin resistance were not associated with worse cognitive function or a higher rate of cognitive decline in elderly subjects without a history of diabetes. These data suggest either a threshold for effects of dysglycaemia on cognitive function, or that factors other than hyperglycaemia-related pathways impair cognition in individuals with frank diabetes. In **chapter 3.2**, I described the relation between genetically reduced insulin/IGF-1 signaling (IIS) and cognitive function and decline, in participants from the Leiden 85-plus Study, aged 85 years and over. In addition to old age survival, genetically reduced IIS seemed to be beneficial for cognitive function in women.

Chapter 4 described the relation between vascular and inflammatory factors and cognitive function and decline. In **chapter 4.1**, I evaluated the age-specificity of the relation between blood pressure and cognitive function by examining the relationship between baseline blood pressure and cognitive function later in life across age groups. The data showed that high blood pressure increased the risk of cognitive impairment up to 75 years, but was associated with better cognitive function thereafter. This could mean that age-specific guidelines for blood pressure management are needed, as the current directive that 'lower is better' may not apply to blood pressure levels in the very old. In **chapter 4.2**, I investigated the relation between inflammatory markers and cognitive impairment in the early stages of the development of dementia, and found that systemic markers of inflammation were only moderately associated with cognitive function and decline. **Chapter 4.3** described the relation between serum uric acid, a cardiovascular risk factor as well as a major natural antioxidant, and the risk of dementia and cognitive impairment. The results showed that notwithstanding the associated increased risk of cardiovascular disease, higher levels of uric acid were associated with a decreased risk of dementia and better cognitive function later in life.

Chapter 5 showed the investigation of potential preclinical biomarkers of dementia. In **chapter 5.1**, I described the relation between plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels and the risk of dementia and evaluated the influence of time-to-event on this relation. Additionally, I studied the relation between plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels and the risk of cognitive decline in those who remained free of dementia. It seemed that the risk of dementia associated with a lower plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio was particularly increased within the first years after the biomarker assessment. There was no association between the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio and the risk of cognitive decline in those who did not develop dementia. This indicates that assessment of the plasma $A\beta_{1-42}/A\beta_{1-40}$ ratio alone is not sensitive enough to identify people at risk for cognitive decline at large.

In **chapter 6**, I evaluated the several methodological issues that were related to the work described in this thesis and discussed some suggestions for further research.

7.2

Samenvatting (Nederlands)

Het verwoestende effect van het verlies van het cognitieve vermogen, dat vaak samen gaat met het ouder worden, wordt door velen gevreesd. Ondanks een toenemend aantal studies is de exacte oorzaak van het achteruitgaan van de cognitie nog niet bekend. Het lijkt er steeds meer op dat het eerder een verzameling van oorzaken is en niet een enkele factor die leidt tot een verslechterende cognitie en de klinische symptomen van dementie. Glucose metabolisme, vasculaire mechanismen en inflammatie-processen zijn gesuggereerd als mogelijke factoren die hierbij een rol kunnen spelen.

De belangrijkste doelstelling van dit proefschrift is het onderzoeken van potentiële risicofactoren, zoals factoren die betrokken zijn bij het glucose metabolisme, vasculaire pathologie en inflammatie in met name de vroege stadia van het ziekteproces. Er zijn onderzoeken bekend die suggereren dat bepaalde risicofactoren die een rol spelen op jongere leeftijd, wellicht minder belangrijk zijn wanneer mensen op hogere leeftijd zijn. Ik heb daarom de mogelijke leeftijdsspecificiteit van deze risicofactoren onderzocht in verschillende, grote, prospectieve studie-cohorten, zoals de Rotterdam Studie, de Leiden 85-plus Studie en de PROSPER studie. Deze cohorten zijn aanvullend met betrekking tot de leeftijd van de deelnemers en boden daarmee de mogelijkheid om de relatie tussen potentiële risicofactoren en cognitieve functie en achteruitgang te bestuderen in verschillende leeftijdsgroepen in de algemene bevolking. Tenslotte heb ik enkele preklinische biomarkers voor dementie onderzocht die mogelijk gebruikt zouden kunnen worden om mensen te identificeren die een verhoogd risico lopen om dementie te ontwikkelen.

Ten eerste heb ik het meten van cognitieve functie onderzocht, met betrekking tot de invloed van selectie op gezondheid en overleven op de schatting van cognitieve functie en achteruitgang in epidemiologische onderzoeken. **Hoofdstuk 2** laat zien dat deze selectie op gezondheid en overleven resulteert in bettere leeftijdsspecifieke cognitieve test scores en minder cognitieve achteruitgang, en dat deze selectie een rol speelt in zowel cross-sectionele

als longitudinale onderzoeken. Vervolgens bleek ook dat statistische methodes die gebruikt worden om met meerdere metingen en missende data om te gaan, niet geheel corrigeren voor de verstoring door deze selectie. Deze resultaten suggereren dat er bij het bepalen van cognitive functie bij ouderen rekening moet worden gehouden met de mogelijke verstoring door selectie op gezondheid en overleving.

In **hoofdstuk 3** worden de resultaten van studies naar de relatie tussen glucose metabolisme en cognitieve functie gepresenteerd. In eerste instantie heb ik de invloed onderzocht van de glucose concentratie in het bloed en van insuline resistentie, op de cognitieve functie van ouderen die geen diabetes hadden. Dit is onderzocht in twee onafhankelijke, prospectieve cohorten: de PROSPER studie en de Rotterdam Studie (**hoofdstuk 3.1**). Verhoogde glucose concentraties en insuline resistentie waren niet geassocieerd met slechtere cognitieve functie of met een snellere cognitieve achteruitgang bij ouderen die geen diabetes hadden. Deze resultaten suggereren dat er oftewel een drempelwaarde bestaat voor effecten van dysglycaemie op cognitieve functie, of dat er andere factoren dan hyperglycaemie-gerelateerde mechanismen de cognitieve functie van mensen met langdurige diabetes beïnvloeden. In **hoofdstuk 3.2** heb ik de relatie beschreven tussen genetisch gereduceerde insuline/IGF-1 stimulatie (IIS) en cognitieve functie en achteruitgang, in deelnemers van de Leiden 85-plus Studie, die 85 jaar en ouder waren. Hieruit bleek dat een genetisch gereduceerde IIS naast een overlevingsvoordeel op hoge leeftijd ook gunstig lijkt te zijn voor cognitieve functie bij vrouwen.

Hoofdstuk 4 beschrijft de relatie tussen vasculaire en inflammatoire factoren, en cognitieve functie en achteruitgang. In **hoofdstuk 4.1**, heb ik de de leeftijdsspecificiteit onderzocht van de relatie tussen bloeddruk en cognitieve functie, door deze relatie te bekijken in verschillende leeftijdsgroepen van deelnemers. De data lieten zien dat hoge bloeddruk gerelateerd was aan slechtere cognitie tot aan een leeftijd van 75 jaar, maar dat op hogere leeftijden hoge bloeddruk gerelateerd was aan betere cognitieve functie. Dit kan betekenen dat er leeftijdsspecifieke voorschriften nodig zijn voor de behandeling van hoge bloeddruk, aangezien het huidige dogma "lager is beter" wellicht geen betrekking heeft op de bloeddruk van de oudste ouderen. In **hoofdstuk 4.2** staan de resultaten beschreven van het onderzoek naar de relatie tussen inflammatoire markers en cognitieve functie in het vroege stadium van de ontwikkeling van dementie. Dit onderzoek laat zien dat er slechts een bescheiden relatie tussen inflammatoire markers en cognitieve functie en achteruitgang is. In **hoofdstuk 4.3** heb ik de relatie onderzocht tussen ureumzuur (een cardiovasculaire risicofactor maar ook een belangrijke antioxidant) en het risico op dementie en een slechtere cognitie. Deze resultaten lieten zien dat ondanks het verhoogde risico op cardiovasculaire ziekte, hogere concentraties van ureumzuur geassocieerd waren met een lager risico op dementie en betere cognitieve functie.

Hoofdstuk 5 laat het onderzoek zien van enkele potentiële preklinische biomarkers voor dementie. In **hoofdstuk 5.1** beschrijf ik de associatie tussen plasma $A\beta_{1-42}$ en $A\beta_{1-40}$ con-

centraties en het risico op dementie en evalueer ik de invloed van de tijd tot de diagnose dementie op deze relatie. Daarnaast heb ik de relatie onderzocht tussen deze plasma $A\beta_{1-42}$ en $A\beta_{1-40}$ concentraties en het risico op een verstoorde cognitieve functie, in degenen die geen dementie ontwikkelden. Het bleek dat het risico op dementie dat geassocieerd was met een lagere $A\beta_{1-42}/A\beta_{1-40}$ ratio voornamelijk verhoogd was in de eerste jaren na het meten van de biomarkers. In degenen die geen dementie ontwikkelden was er geen relatie tussen de $A\beta_{1-42}/A\beta_{1-40}$ ratio en cognitieve functie. Dit lijkt erop te wijzen dat de bepaling van de $A\beta_{1-42}/A\beta_{1-40}$ ratio alleen niet gevoelig genoeg is om mensen te identificeren die een verhoogd risico op dementie lopen.

In **hoofdstuk 6.1** heb ik verschillende methodologische kwesties besproken die aan dit werk gerelateerd zijn en heb ik enkele voorstellen voor toekomstig onderzoek gedaan.

List of publications

Euser SM, Schram MT, Hofman A, Westendorp RGJ, Breteler MMB. Measuring cognitive function with age: the Influence of Selection by Health and Survival. *Epidemiology 2008;19:440-447*.

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About the author

Sjoerd Marijn Euser was born on December 30, 1979 in Herveld, the Netherlands. He graduated in 1998 at the "Hendrik Pierson College" in Zetten and started his Human Movement Sciences study at the "Vrije Universiteit" in Amsterdam in the same year.

After his graduation in September 2004, he worked at the Dutch Child Oncology Group (DCOG) in The Hague. In January 2005 he started the work described in this thesis in the Neuroepidemiology group of the Department of Epidemiology, Erasmus Medical Center in Rotterdam (Prof.dr. M.M.B. Breteler) in collaboration with the Department of Geriatrics and Gerontology, Leiden University Medical Center in Leiden (Prof.dr. R.G.J. Westendorp). In June 2007 he obtained a Master of Science in Clinical Epidemiology at the Netherlands Institute for Health Sciences in Rotterdam.