

Causes and Correlates of Brain Atrophy

A population-based MRI study

Tom den Heijer

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Causes and Correlates of Brain Atrophy
A population-based MRI study

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Een onderzoek in de algemene populatie

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Contents

1	Introduction	3
2	Vascular risk factors of brain atrophy on MRI	
2.1	Blood pressure levels and medial temporal lobe atrophy	7
2.2	Blood pressure levels and global brain atrophy	21
2.3	Plasma homocysteine levels	33
2.4	Alcohol intake	43
2.5	Apolipoprotein E genotype	57
3	Endocrine risk factors of brain atrophy on MRI	
3.1	Plasma estrogen levels	67
3.2	Estrogen receptor α polymorphisms	83
3.3	Type 2 diabetes	99
4	Clinical correlates of brain atrophy on MRI	
4.1	Memory performance	117
4.2	Dementia	131
4.3	Depression	143
5	General discussion	153
	Summary	171
	Samenvatting	175
	Dankwoord	178
	Publications	181
	About the author	183

Publications and manuscripts based on the studies described in this thesis

Chapter 2.1

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Chapter 2.2

den Heijer T, Skoog I, Oudkerk M, de Leeuw FE, de Groot JC, Hofman A, Breteler MMB. Association between blood pressure levels over time and brain atrophy in the elderly. *Neurobiology of Aging* 2003;24:307-313.

Chapter 2.3

den Heijer T, Vermeer SE, Clarke R, Oudkerk M, Koudstaal PJ, Hofman A, Breteler MMB. Homocysteine and brain atrophy on MRI of non-demented elderly. *Brain* 2003;126:170-175.

Chapter 2.4

den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, van Duijn CM, Hofman A, Breteler MMB. Alcohol intake in relation to brain MRI findings in non-demented elderly. Submitted.

Chapter 2.5

den Heijer T, Oudkerk M, Launer LJ, van Duijn CM, Hofman A, Breteler MMB. Hippocampal, amygdalar and global brain atrophy in different apolipoprotein E genotypes. *Neurology* 2002;59:746-748.

Chapter 3.1

den Heijer T, Geerlings MI, Hofman A, de Jong FH, Launer LJ, Pols HAP, Breteler MMB. Higher estrogen levels are not associated with larger hippocampi and better memory performance. *Archives of Neurology* 2003;60:213-220.

Chapter 3.2

den Heijer T, Schuit SCE, Pols HAP, van Meurs JB, Hofman A, Koudstaal PJ, van Duijn CM, Uitterlinden AG, Breteler MMB. Variations in estrogen receptor α gene and risk of dementia and brain atrophy on MRI. Submitted.

Chapter 3.3

den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, Breteler MMB. Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. *Diabetologia* 2003;46:1604-1610

Chapter 4.1

Hackert VH, den Heijer T, Oudkerk M, Koudstaal PJ, Hofman A, Breteler MMB. Hippocampal head size associated with verbal memory performance in nondemented elderly. *NeuroImage* 2002; 17:1365-1372.

Chapter 4.2

den Heijer T, Geerlings MI, Hoebeek FE, Hofman A, Koudstaal PJ, Breteler MMB. Hippocampal and amygdalar atrophy predict dementia in cognitively intact elderly. Submitted.

Chapter 4.3

den Heijer T, Tiemeier H, Koudstaal PJ, Hofman A, Breteler MMB. Hippocampal, amygdalar and frontal atrophy and the risk of incident depression in late life. Submitted.

Chapter 1

Introduction



In 1906, Alois Alzheimer described for the first time a form of dementia that later became known as Alzheimer's disease.¹ At necropsy, he had observed that the brain of a 51-year-old woman with progressive cognitive decline was filled with –at that time still anonymous– amyloid plaques and neurofibrillary tangles. Since then, numerous investigators saw in patients with dementia the same pathological findings that Alzheimer had seen. Clinically, Alzheimer's disease is recognized by a long period of progressive cognitive decline. Braak and Braak showed in the late eighties that the accumulation of plaques and tangles in the brain follows a predictable pattern over time that parallels this cognitive decline.² In their now widely accepted staging system, they identify a long phase where the medial temporal lobe is the first area to be afflicted whereas only in the later disease stages the pathology involves the isocortex. However, recent pathological studies show that brains of elderly patients, unlike the middle-aged patient that Alzheimer had observed, who in life receive a diagnosis of Alzheimer's disease have a rather mixed bag of brain pathology. Not only the traditionally recognized amyloid plaques and neurofibrillary tangles are observed but also cerebrovascular disease is found which could have contributed to the cognitive decline.³

The search for causes of Alzheimer's disease is hampered by its long preclinical period and the pathological diversity that contribute to clinical symptoms of Alzheimer's disease. Environmental or physiological factors that are present at time of a clinical diagnosis of Alzheimer's disease may not have effectuated the brain damage in the preclinical period. Furthermore, different key mechanisms could be involved in either the development of the specific Alzheimer pathology i.e. neurofibrillary tangles and amyloid plaques, or cerebrovascular disease such as lacunar infarcts and white matter disease. We took the approach of focusing on the preclinical stages of the disease by making magnetic resonance imaging (MRI) of the brain in non-demented elderly and assessing the extent of brain atrophy. The advantage of this approach is that brain atrophy on MRI, particularly atrophy of structures in the medial temporal lobe, is evident years before a clinical diagnosis of Alzheimer's disease and specifically reflects neuronal loss and neurofibrillary tangles.⁴ By identifying causes of atrophy on MRI, which we consider a preclinical sign of Alzheimer's disease, we hope to shed light on mechanisms by which Alzheimer's disease develops. The studies described in this thesis formed part of the Rotterdam Scan Study, a large population-based cohort study among elderly who were at time of MRI free of clinical dementia. Vascular risk factors are increasingly recognized to contribute to the clinical syndrome of Alzheimer's disease in late life,⁵ yet it is unclear whether this is only through induction of cerebrovascular disease that in itself causes cognitive decline or whether vascular factors also have more direct effects on brain atrophy. In chapter 2 of this thesis, the focus is on vascular risk factors in relation to brain atrophy on MRI. Chapter 3 is devoted to the endocrine factors plasma estradiol levels and genetic variation in the estrogen receptor α polymorphism. The potential of estrogen replacement therapy in preventing Alzheimer's

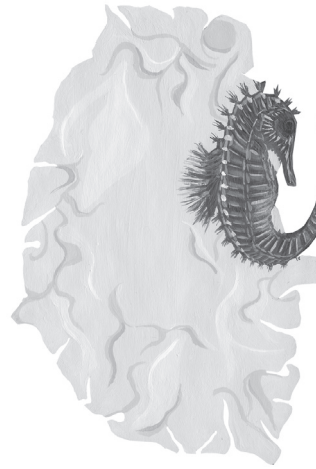
disease has recently received a lot of attention and there is considerable controversy on the role of estrogens in the etiology of Alzheimer's disease. Also in chapter 3, type 2 diabetes mellitus and insulin resistance are investigated as potential risk factors for brain atrophy on MRI. The functional significance and clinical correlates of atrophy on MRI are addressed in chapter 4. We examined whether atrophy on MRI was associated to memory performance, risk of dementia and depression. Finally, in chapter 5, the main findings in the context of current knowledge on the etiology of Alzheimer's disease, methodological aspects and suggestions for future research are discussed.

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Chapter 2

Vascular risk factors of brain atrophy on MRI



Chapter 2.1

Blood pressure and medial temporal lobe atrophy

Blood pressure may be associated to clinical Alzheimer's disease (AD) in several ways. High blood pressure levels can lead to cerebrovascular damage, which contributes to cognitive decline in AD. However, high blood pressure may also trigger the development of AD pathology, i.e. neurofibrillary tangles and amyloid plaques. In vivo, atrophy of the hippocampus and amygdala on MRI is a good and early indicator of AD pathology. We investigated the association of blood pressure levels with hippocampal and amygdalar atrophy on MRI. Additionally, we examined the association of markers of large vessel (carotid atherosclerosis) and small vessel disease (white matter lesions on MRI) with atrophy of the hippocampus and amygdala. In 1995-1996, 511 non-demented elderly (60-90 year) underwent brain MRI. Blood pressure levels were assessed concurrently with MRI and 5 years before the MRI. A high diastolic blood pressure 5 years before MRI predicted more hippocampal atrophy in persons untreated for hypertension. Conversely, in persons treated for hypertension a low diastolic blood pressure was related to atrophy. Small vessel disease, but not large vessel disease, coexisted with hippocampal and amygdalar atrophy. These results indicate that high blood pressure and markers of small vessel disease in the brain are associated with atrophy of structures affected by AD. A low blood pressure level may be either a cause or consequence of atrophy.

Introduction

Although Alzheimer's disease (AD) is originally considered to be a non-vascular disease, this view is challenged by observations that vascular factors contribute to the development of late-onset AD.¹⁻³ In this respect, the most frequently investigated vascular factor is blood pressure.^{4, 5} Long-term longitudinal studies have shown that a high blood pressure increases the risk of clinical AD.⁶⁻⁸ Yet cross-sectional studies^{9, 10} and studies with a shorter follow-up^{11, 12} reported a low blood pressure in patients with AD.

It is unclear whether and which structural brain changes could underlie the associations between blood pressure and clinical AD. High blood pressure levels can lead to cerebrovascular damage such as white matter lesions (WML) and small brain infarcts that contribute to cognitive decline in a patient with clinical AD.¹³⁻¹⁵ Alternatively, more direct links between blood pressure and AD are suggested by the observation that persons with hypertension have increased neurofibrillary tangles and brain atrophy at autopsy.^{16, 17} To explore the latter in vivo, we decided to study the relation between blood pressure and hippocampal and amygdalar atrophy on MRI in non-demented elderly. The hippocampus and amygdala are highly affected by amyloid plaques and neurofibrillary tangles even in the earliest stage of the development of Alzheimer's disease.¹⁸ Histopathological studies confirm that neuronal loss, neurofibrillary tangles and amyloid plaques at autopsy are highly correlated to atrophy visible on MRI.¹⁹⁻²¹ If the association between blood pressure and clinical AD is (partly) mediated through effects on the development of Alzheimer neuropathology in the medial temporal lobe, one would expect to find an association between blood pressure levels and atrophy of the hippocampus and amygdala on MRI. We additionally examined whether markers of small vessel disease in the brain (WML) or large vessel disease (carotid atherosclerosis) are associated to atrophy of the hippocampus and amygdala on MRI and modifies the association between blood pressure and atrophy on MRI.

Methods

Participants

The Rotterdam Study is a large population-based cohort study in the Netherlands designed to investigate prevalence, incidence and determinants of diseases in the elderly.²² Baseline examinations were done in 1990 to 1993. In 1995 to 1996, we randomly selected 965 living members (60-90 years of age) of the cohort in strata of sex and age (5 years) for participation in the Rotterdam Scan Study designed to study age-related brain changes on MRI.¹ As part of the eligibility criteria, we excluded from this selection people with dementia ($n=17$)²³ or MRI contraindications ($n=116$). Thus, 832 persons were eligible and invited. Among these, 563

participants gave their written informed consent to participate in the study, which included undergoing an MRI scan of the brain (response rate: 68%). Complete MRI data was available for 511 participants.²⁴ Participants were in general healthier than non-participants.²⁵ The study protocol was approved by the medical ethics committee of the Erasmus Medical Center.

MRI procedures

Standard T1, T2 and proton-density weighted axial MR images and a custom-made three-dimensional (3D) MRI sequence covering the whole brain were made using a 1.5 Tesla MR unit (VISION MR, Siemens, Erlangen, Germany). The MRI acquisition parameters have been described.^{24, 26}

MRI assessment of hippocampal and amygdalar volumes

We constructed a series of coronal brain slices (contiguous 1.5-mm slice thickness) from the 3D MRI, aligned to be perpendicular to the long axis of the hippocampus. We manually traced the boundaries of the hippocampus and amygdala on both sides on each slice with a mouse-driven cursor.²⁴ The summed surface was multiplied by slice thickness to yield estimates of the hippocampal and amygdalar volume (ml). The left and right-sided volumes were summed to yield the total hippocampal and amygdalar volume. As a proxy for head size, we measured on the middle sagittal MRI slice the intracranial cross-sectional area.²⁴ We corrected for head size differences across individuals by dividing the uncorrected volumes by the participant's calculated head size area and subsequently multiplying this ratio by the average head size area (men and women separately).²⁷

Assessment of blood pressure and vasculopathy

At baseline and time of MRI we assessed blood pressure with a random zero sphygmomanometer.²⁸ Participants were asked to bring all prescribed medications to the research center where a physician recorded the use. At baseline and time of MRI, participants underwent ultrasonography of the carotid arteries.²⁹ The presence of atherosclerotic plaques was determined at six locations: common carotid artery, carotid bifurcation, and internal carotid artery at the left and right side and summed (range 0-6). The intima-media thickness was measured by longitudinal two-dimensional ultrasound of the anterior and posterior wall of both common carotid arteries. We calculated the mean of these four locations. Cerebral WML on MRI were assessed on proton-density and T2 weighted axial MR images and were scored in the periventricular regions (range 0-9) and the subcortical regions (approximated volume).²⁶ We defined a group with severe WML on MRI similar to previous analyses³⁰ as having either a subcortical WML score or periventricular WML score in the upper quintile of the distribution. Brain infarcts were defined as focal hyperintensities on T2 weighted images, and, if present in

the white matter, with corresponding prominent hypointensity on T1 weighted images.³¹

Other measurements

Body mass index (BMI) was calculated as weight divided by the square of height. A physician assessed participants' smoking habits with a structured questionnaire and we categorized this into never, former or current smoking. Serum total cholesterol and high-density lipoprotein (HDL) were determined with an automated enzymatic procedure.

Data analysis

We assessed the relation between blood pressure continuously and in categories at baseline or at time of MRI and atrophy with multiple linear regression. As preliminary analysis and previous studies on AD and cognitive impairment,^{7 32-34} suggest differences in relations between persons with or without antihypertensive medication, we stratified for antihypertensive medication use. With multiple linear regression, we investigated the association between WML, carotid atherosclerosis and atrophy. Analyses were adjusted for age and sex and additionally for other cardiovascular factors. Finally, we repeated the analyses on blood pressure and atrophy in strata of severity of WML on MRI. Assumptions of the model were verified by residual diagnostics.

Results

Table 1 gives several characteristics of the study sample both at baseline and at time of MRI.

Table 1. Characteristics of the study sample at baseline (1990-1993) and time of MRI (1995-1996)

	Baseline (n=511)	Time of MRI (n=511)
Age, year	68.8 ± 8.0	73.4 ± 8.0
Sex, % women	49.1	49.1
Cholesterol / HDL ratio	5.4 ± 1.8	4.9 ± 1.5
Current smoking, %	19.6	16.3
BMI, kg/m ²	26.2 ± 3.4	26.3 ± 3.6
Diastolic blood pressure, mmHg	73.0 ± 10.8	76.5 ± 11.6
Systolic blood pressure, mmHg	136.5 ± 20.3	145.8 ± 20.3
Antihypertensive medication, %	30.3	38.9
Carotid plaques, score range 0-6	1.3 ± 1.5	1.6 ± 1.6
Intima-media thickness, mm	0.76 ± 0.14	0.87 ± 0.14
Periventricular WML on MRI, grade	-	2.7 ± 2.2
Subcortical WML on MRI, ml	-	1.80 ± 3.40
Infarcts on MRI, %	-	28.0

Values are unadjusted means ± SD or percentages

People using antihypertensive medication at both baseline and follow-up had on average smaller hippocampal (age and sex adjusted difference -0.15 , 95% CI -0.32 to 0.02 , $P=0.09$) and amygdalar volumes (-0.20 , 95% CI -0.34 to -0.06 , $P=0.005$) compared to people without antihypertensive medication. A higher diastolic blood pressure at baseline in persons untreated for hypertension was related to smaller hippocampal volumes (Figure 1). Per SD increase in diastolic blood pressure at baseline, we found in persons untreated a 0.10 ml smaller hippocampal volume (95% CI -0.19 to -0.02 , $P=0.02$). Diastolic blood pressure at time of MRI was not associated with hippocampal or amygdalar volume in persons without antihypertensive treatment (Figure 1). Conversely, in persons using antihypertensive medication, a lower diastolic blood pressure at time of MRI was related to smaller volumes on MRI, statistically

significant for the amygdala (Figure 1). Per SD increase in diastolic blood pressure at time of MRI, 0.10 ml (95% CI 0.00 to 0.20, $P=0.05$) larger amygdalar volumes were found. These associations did not change after adjusting for the cholesterol/HDL ratio, BMI or smoking.

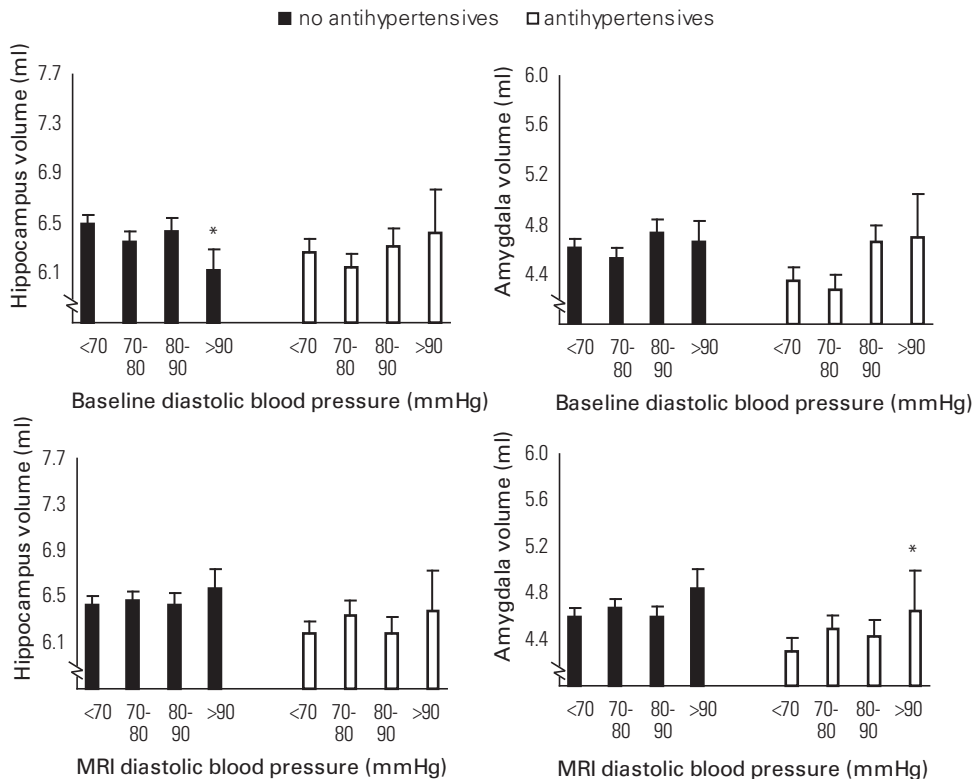


Figure 1. Association between diastolic blood pressure levels at baseline (upper panel) or at time of MRI (lower panel) and volumes of the hippocampus (left panel) and amygdala (right panel). Adjusted for age and sex and normalized to head size. * $P<0.05$ compared to diastolic blood pressure <70 mmHg

No associations were found with systolic blood pressure levels (Figure 2).

People with more carotid atherosclerosis at either baseline (data not shown) or time of MRI did not have smaller volumes on MRI (Table 2). People with more WML had smaller hippocampal or amygdalar volumes (Table 2). This relation did not disappear after adjusting for blood pressure levels, antihypertensive medication use, cholesterol/HDL ratio, BMI and smoking. People with infarcts on MRI did not have significantly smaller hippocampal or amygdalar vol-

umes (age and sex adjusted difference in hippocampal volume -0.10 ml (95% CI -0.27 to 0.07 , $P=0.24$) and in amygdalar volume -0.01 ml (95% CI -0.15 to 0.13 , $P=0.89$)).

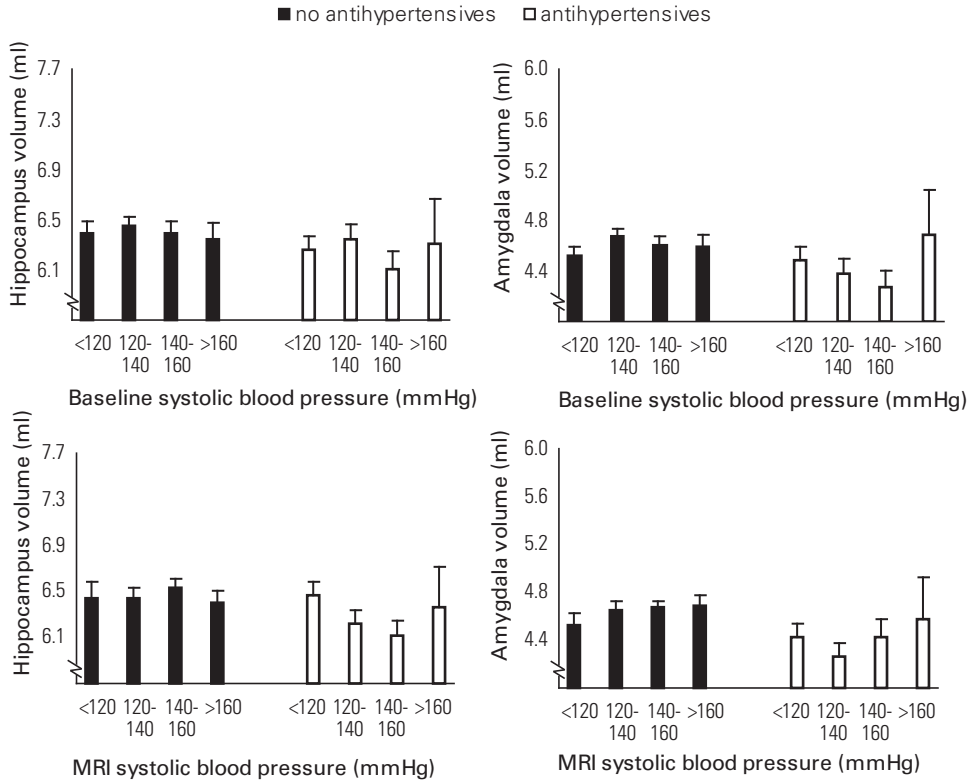


Figure 2. Association between systolic blood pressure levels at baseline (upper panel) or at time of MRI (lower panel) and volumes of the hippocampus (left panel) and amygdala (right panel). Adjusted for age and sex and normalized to head size

The association that we found in persons without antihypertensive treatment between a high diastolic blood pressure at baseline and hippocampal atrophy on MRI remained when excluding persons with severe WML on MRI (per SD increase in 0.13 ml smaller hippocampal volume (95% CI 0.03 to 0.23 , $P=0.02$). The association between concurrent low diastolic blood pressure level and more atrophy in persons using antihypertensive medications was however restricted to people with coexistent severe WML on MRI (Table 3).

Table 2. Cross-sectional associations between markers of vasculopathy and hippocampal and amygdalar volumes on MRI (n=511)

	Hippocampal volume (ml)		Amygdalar volume (ml)	
	Estimate (95% CI)	P	Estimate (95% CI)	P
Carotid atherosclerosis				
IMT	-0.01 (-0.09 ; 0.07)	0.84	-0.01 (-0.07 ; 0.06)	0.83
Carotid plaques	-0.00 (-0.09 ; 0.08)	0.92	-0.01 (-0.07 ; 0.06)	0.83
WML on MRI				
Periventricular	-0.11 (-0.19 ; -0.03)	0.01	-0.05 (-0.12 ; 0.02)	0.14
Subcortical	-0.09 (-0.17 ; -0.01)	0.02	-0.08 (-0.14;-0.02)	0.01

Values are adjusted regression coefficients (with 95% confidence interval (CI) and *P*-value) per SD increase in atherosclerosis or white matter lesions (WML) on MRI. Adjustments were made for age and sex

Table 3. Cross-sectional association between diastolic blood pressure level and volumes of hippocampus and amygdala on MRI according to WML severity on MRI

	Hippocampal volume (ml)		Amygdalar volume (ml)	
	Estimate (95% CI)	P	Estimate (95% CI)	P
No antihypertensive medication (n=313)				
Diastolic BP				
No or moderate WML* (n=245)	-0.01 (-0.12; 0.10)	0.83	0.06 (-0.02;0.15)	0.14
Severe WML* (n=68)	0.17 (-0.05;0.38)	0.12	0.06 (-0.11;0.22)	0.50
Antihypertensive medication (n=198)				
Diastolic BP				
No or moderate WML* (n=129)	-0.05 (-0.20;0.10)†	0.47	0.04 (-0.08;0.16)†	0.52
Severe WML* (n=69)	0.20 (0.01;0.39)	0.04	0.19 (0.02;0.36)	0.03

Values are adjusted regression coefficients (with 95% confidence interval (CI) and *P*-value) per SD increase in diastolic blood pressure level. * White matter lesions (WML) on MRI categorized according to upper quintile cut-off of distribution in either subcortical or periventricular WML.

†*P*-value of interaction term of diastolic blood pressure level and WML for hippocampus=0.03 amygdala=0.07

Discussion

We found in this community cohort of non-demented elderly that a high diastolic blood pressure in persons not treated for hypertension was associated with more hippocampal atrophy on MRI. Higher severity of WML coexisted with atrophy of the hippocampus and amygdala. Finally, in persons using antihypertensive medications a low diastolic blood pressure was related to more hippocampal and amygdalar atrophy.

The clinical distinction between vascular dementia and AD is sometimes difficult hampering studies investigating vascular risk factors in relation to clinically diagnosed AD.³⁵ In elderly people, dementia symptoms are mostly due to mixed disease i.e. both cerebrovascular damage and AD pathology contribute to the cognitive symptoms.³⁶ We had the opportunity to assess hippocampal and amygdalar atrophy on MRI that can be regarded as preclinical MRI markers of AD.^{19-21, 37} These assessments *in vivo* may help us clarifying whether vascular factors influence the AD pathology in the medial temporal lobe. However, we need to bear in mind that a small volume of the hippocampus or amygdala on MRI does not always reflect atrophy caused by incipient AD. For some persons a small regional brain volume will be innate or due to diseases other than AD. Future serial MRI studies can provide information to distinguish people with a high rate of brain volume loss due to AD pathology.³⁸

The results of studies on the association between blood pressure and AD are determined by the time period between blood pressure level assessment and AD diagnosis.^{5, 39} Longitudinal population studies with a long follow-up have shown high blood pressure levels in people who develop clinically overt AD several years later.⁶⁻⁸ Especially in persons not using antihypertensive medications, a higher blood pressure is a risk factor for clinical AD⁷ and cognitive impairment.^{32, 34} High blood pressure levels may lead to a spectrum of brain changes, which all could separately or in combination cause cognitive decline. Generalized brain atrophy,⁴⁰⁻⁴² WML,^{25, 41, 42} and infarcts on MRI³¹ are observed in persons with hypertension and are associated with cognitive decline and dementia.¹³ Another potential structural intermediate in the association between high blood pressure and clinical AD is damage to hippocampal neurones, as suggested by an autopsy study showing more neurofibrillary tangles and amyloid plaques in the hippocampus of persons with a high blood pressure.¹⁷ How exactly these pathological changes in the hippocampus develop due to high blood pressure is unclear. Long-standing hypertension and chronic brain hypoperfusion in rats may upregulate levels of nitric oxide in the hippocampus, lead to amyloid accumulation and memory loss.⁴³ The coexistence of WML, which reflects microangiopathy,⁴⁴ and atrophy of the hippocampus and amygdala on MRI has been reported before.⁴⁵ Its coexistence may be explained by both having a similar aetiology such as high blood pressure that in our sample was strongly related to WML.²⁵ Alternatively, as adjusting for blood pressure levels did not change the relation between WML and atrophy,

generalized microangiopathy may reduce cerebral blood flow to the hippocampus⁴⁶ and induce loss of hippocampal neurones.⁴⁷ Of interest, infarcts on MRI were not associated with the degree of atrophy of the hippocampus and amygdala on MRI. The majority of infarcts on MRI are lacunar infarcts³¹ due to microangiopathy. Unlike WML however, they are caused by acute occlusions of single small vessels possibly not affecting blood flow to the medial temporal lobe. Carotid atherosclerosis was not associated with atrophy of the hippocampus or amygdala suggesting that the association found between atherosclerosis and clinical AD⁴⁸ is not through effects on the hippocampus or amygdala but purely through WML or infarcts.

Studies in which blood pressure is assessed shortly before or at time of diagnosis of AD showed patients to have lower blood pressure levels compared to controls.^{9-12, 49, 50} Two hypotheses have been put forward to explain these associations with low blood pressure: (1) a low blood pressure is a secondary phenomenon of the dementia process or (2) a low blood pressure primarily contributes to development of dementia. Regarding the first hypothesis, the hippocampus and amygdala have a role in blood pressure regulation^{51, 52} and atrophy of these structures due to AD pathology could result in a decrease of blood pressure level. However, this being true, we would expect similar associations between atrophy and low blood pressure levels in all persons whereas in our study the association was strongest in those using antihypertensive medication. This is in line with the stronger association found between a low blood pressure and AD in persons on antihypertensive treatment.^{11, 12, 50} According to the second hypothesis, a too low blood pressure level can be detrimental to the brain.⁵³ Under normal conditions, cerebral autoregulatory mechanisms will keep up adequate cerebral blood flow despite a low systemic blood pressure level.⁵⁴ This is achieved by vasodilatation of the arterioles of the brain.⁵⁵ In persons with chronic hypertension and microangiopathy^{56, 57} the ability to vasodilate is reduced and a low systemic blood pressure might lead to hypoperfusion and ischemia of the brain particularly to the sensitive hippocampus and amygdala. Our finding that a low diastolic blood pressure level was particularly associated to atrophy in persons with antihypertensive medication (most likely with a history of chronic hypertension) and severe WML may fit this hypothesis.

To conclude, our findings support the idea that blood pressure has effects on atrophy of structures in the medial temporal lobe that have a central role in AD. However, as our study was cross-sectional we cannot infer causality of the associations. Prospective studies are needed to confirm or reject the associations we found.

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Chapter 2.2

Blood pressure and global brain atrophy

The relation between blood pressure level and degree of global brain atrophy is equivocal. We evaluated past and present blood pressure levels and change in blood pressure over 20 years in relation to the degree of cortical atrophy on magnetic resonance imaging (MRI). In 1995-1996, we measured blood pressure and performed MRI in 1,077 non-demented elderly (age 60-90 years). For 513 of these, we had information on a blood pressure level 20 years before. The degree of cortical atrophy was semi-quantitatively scored (range 0-15). In late life, a high (≥ 90 mmHg) and low (< 65 mmHg) diastolic blood pressure were associated with more cortical atrophy than a diastolic blood pressure level between 65-74 mmHg (adjusted difference 0.60 units (95% confidence interval (CI), 0.18-1.02) and 0.77 units (0.28-1.25), respectively). Persons whose diastolic blood pressure had declined more than 10 mmHg over 20 years had more cortical atrophy than those with stable blood pressure levels (adjusted difference 0.53 units, 0.05-1.02). Both high and declining diastolic blood pressure levels are associated with more global brain atrophy on MRI.

Introduction

Global brain atrophy is a common finding on magnetic resonance imaging (MRI) scans of elderly persons. Elderly with more pronounced brain atrophy perform worse on neuropsychological testing,¹ and may even be at increased risk to develop Alzheimer's disease.² Besides age, no definite risk factor for the development of severe brain atrophy has yet been identified. High blood pressure levels in mid or late life have been suggested to increase the degree of brain atrophy in later life,^{3,4} but other observational studies reported that a low blood pressure level or a decline in blood pressure are associated with severe brain atrophy.^{5,6} In a population-based sample of older people we investigated the relation between blood pressure levels and degree of brain atrophy on MRI. We investigated blood pressure levels 20 years before MRI, change in blood pressure over time, and concurrent blood pressure level in relation to the degree of brain atrophy on MRI.

Methods

Study sample

This study is based on data from the Rotterdam Scan Study, which was designed to investigate determinants and consequences of brain abnormalities on MRI in the elderly.⁷ In 1995-1996, 1,904 subjects aged 60-90 years were randomly selected in strata of age (5 years) and sex from two population cohort studies.^{8,9} We excluded 187 subjects who were demented, blind or had MRI contraindications at time of selection. Of the 1,717 eligible non-demented subjects, complete information including a cerebral MRI scan was obtained in 1,077 who gave written informed consent (participation rate 63%, mean age 72.2 years, 52% women). Subjects who consented to the MRI examination were on average younger and healthier than subjects who refused the MRI examination.¹⁰ In 513 subjects, originating from one of the cohorts (participation rate 59 %),⁸ we had information on a blood pressure level 20 years before MRI (measured from 1975-1978; age at that time between 40 and 70 years). The Medical Ethics Committee of Erasmus Medical Center, the Netherlands, approved the study.

Measurements

Measurements were done similarly at both examinations. Blood pressure was measured twice in a seated position with a random-zero sphygmomanometer. We used the average of these two measurements. In six subjects with MRI, we had no information on concurrent blood pressure level. Subjects showed prescribed medications to a physician who coded these according to the Anatomic-Therapeutic-Chemical index. Antihypertensive medications were medications falling into classification codes C02, C03 and C07. Diabetes mellitus was

considered present when subjects reported use of oral antidiabetic medication or insulin (classification code A10). A physician asked about smoking habits (current, never or former) and quantified this as pack-years of cigarettes smoked ($=\text{number of cigarettes per day} \times \text{year of smoking}/20$) (pack-years could only be quantified at time of MRI). Body mass index (BMI) was calculated as weight divided by height square.

MRI procedures

An axial T1, T2 and proton density weighted brain MRI scan was made with 1.5-Tesla scanners (Gyrosan, Philips NT, Best, The Netherlands or VISION MR, Siemens, Erlangen, Germany).¹⁰ Slice thickness was 5 or 6 mm with an interslice gap of 20 %. Images were optimized on the scanner screen and printed on laser hard copies. The extent of cortical atrophy was scored blinded to all clinical characteristics on a four point rating scale based on the size of the gyri and sulci from 0 (no cortical atrophy) to 3 (severe cortical atrophy) at five regions (frontal, parietal, temporal and occipital lobes and the insular region) with use of reference scans (Figure 1).

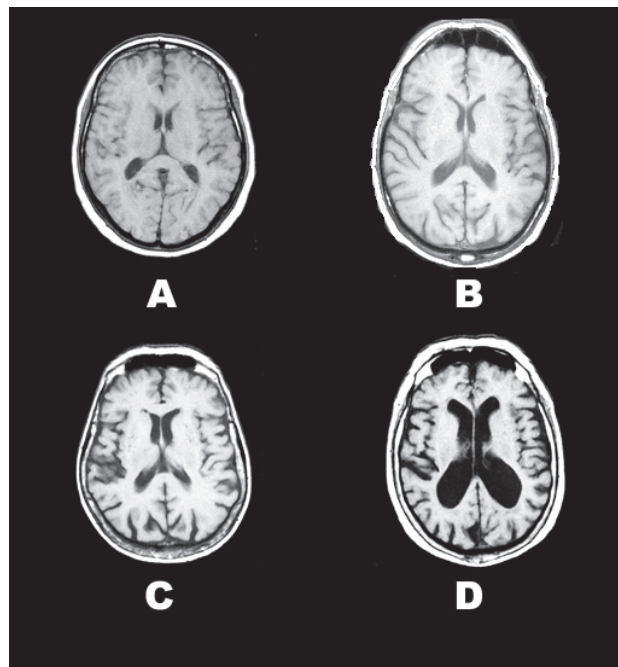


Figure 1. Axial brain slices at the level of the basal ganglia showing brains with sum score of cortical atrophy of A=0, B=5, C=10, D=15. Cortical atrophy was scored from 0-3 at the frontal, parietal, temporal and occipital lobes and the insular region

The sum of these five regions (0-15) was calculated. Two experienced raters scored all scans for cortical atrophy independent of each other. When the raters differed more than one point in any of the five regions a consensus reading was held, otherwise the mean score was calculated. Intra and interrater studies based on the first 200 scans showed good reproducibility for scoring cortical atrophy (intrarater weighted kappa 0.82 and interrater weighted kappa 0.81). White matter lesions were scored in periventricular (grade 0-9) and subcortical white matter regions (approximated volume) on the proton density scans.¹⁰ White matter lesions had to be hyperintense on the proton density and T2 weighted images, without prominent hypointensity on the T1 weighted images. We defined infarcts as focal hyperintensities on T2 weighted images.¹¹ Infarcts in the white matter also had to have corresponding hypointensities on T1 weighted images, in order to distinguish them from white matter lesions.

Data analysis

Age and sex adjusted linear regression was used to quantify the relation between several characteristics and atrophy. We used analysis of covariance (ANCOVA) to calculate adjusted means of cortical atrophy in blood pressure level categories. This was done for blood pressure levels 20 years before MRI, change in blood pressure level, and concurrent blood pressure level. When the ANCOVA suggested a linear association between the blood pressure variable and atrophy, we performed a multivariate linear model to calculate the increase in cortical atrophy units per 10 mmHg increase in blood pressure level. A possible U-shaped association between blood pressure measures and atrophy was evaluated by adding the quadratic term of the blood pressure variable to the linear model. We then report the *P*-value of the regression coefficient of the quadratic term. All analyses were adjusted for age, sex, and cigarette smoking (at examination in 1975-1978: smoking status; current, former, never; concurrent with MRI: pack-years of cigarette smoking). Additional adjustments were made for presence of diabetes, BMI, and white matter lesions. We evaluated whether relations differed according to antihypertensive medication use through stratified analyses. In addition, all analyses were repeated with the different regions of atrophy as dependent variables separately. Finally, we repeated all analyses excluding subjects with an infarct on MRI.

For missing data on categorical covariates, we used a missing indicator, whereas for missing data on continuous covariates we used the median value of the respective value as calculated from the total sample. Data were missing on cigarette smoking (*n*=24), diabetes (*n*=3), body mass index (*n*=3), and subcortical white matter lesions (*n*=4). All analyses were followed by residual analyses to confirm assumptions of the model.

Results

Table 1 shows characteristics of the total study sample at time of MRI and characteristics of the part with additional examinations 20 years before MRI.

Table 1. Characteristics of the total study sample in 1995-1996 (at time of MRI scan) and of subjects with additional examinations in 1975-1978

	Total study sample	Examinations in
	1995-1996 (n=1077)	1975-1978 (n=513)
Age, years	72 ± 7	51 ± 7
Sex, % women	52	53
Systolic blood pressure (mmHg)	147.3 ± 21.6	131.4 ± 17.0
Diastolic blood pressure (mmHg)	78.7 ± 11.7	81.3 ± 10.9
Antihypertensive medication, %	35	15
Smoking cigarettes, % current	16	37
Prevalent diabetes mellitus, %	6	1
Body mass index (kg/m ²)	26.7 ± 3.6	25.0 ± 3.0
Subcortical white matter lesions (ml)	1.4 ± 2.9	-
Periventricular white matter lesions, grade	2.4 ± 2.2	-
Infarcts on MRI, %	24	-
Cortical atrophy, range 0-15	5.56 ± 2.86	-

Values are unadjusted means ± standard deviation or percentages unless otherwise specified

The degree of cortical atrophy was significantly higher with increasing age (0.22 units per year (95% confidence interval (CI), 0.20 to 0.24) and more pronounced in men than in women (sex difference 0.90 units (95% CI, 0.56 to 1.24)). Users of antihypertensive medications in late life, but not in mid life, had more pronounced cortical atrophy (age- and sex-adjusted difference 0.52 units (95% CI, 0.23 to 0.82), and -0.26 units (95% CI, -0.75 to 0.22), respectively).

Figure 2 shows cortical atrophy according to concurrent blood pressure level. Subjects with a high diastolic blood pressure level (85-90 mmHg and above 90 mmHg) had adjusted 0.48 units (95% CI, 0.01 to 0.94) and 0.60 units (0.18 to 1.02) more cortical atrophy than subjects with a diastolic blood pressure level between 65 and 74 mmHg. In addition, subjects with a diastolic blood pressure level less than 65 mmHg had adjusted 0.77 units (95% CI, 0.28 to

1.25) more cortical atrophy than subjects with a diastolic blood pressure level between 65 and 74 mmHg. This U-shaped association between diastolic blood pressure level and degree of cortical atrophy was statistically significant (P of quadratic term = 0.02), and remained after additional adjustments for diabetes, BMI, and white matter lesions (P of quadratic term = 0.05). The association was similar for users and non-users of antihypertensive medications. Concurrent systolic blood pressure level was not related to the degree of cortical atrophy (Figure 2) (per 10 mmHg a decrease of -0.02 units (95% CI, -0.09 to 0.04)).

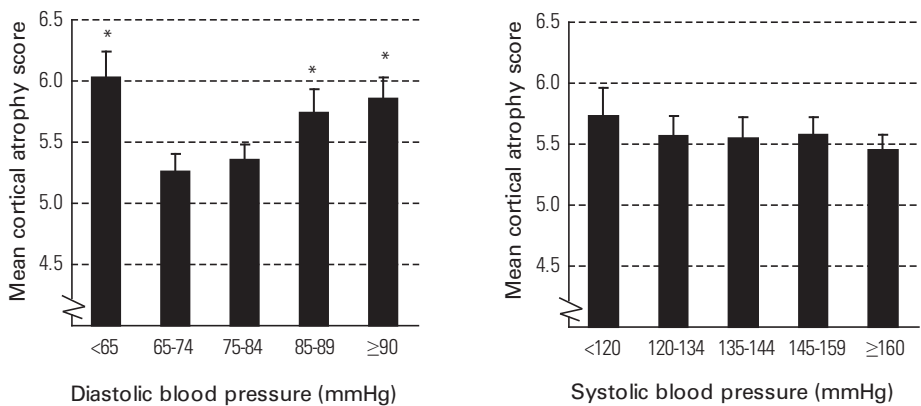


Figure 2. Cortical atrophy according to concurrent blood pressure levels. Values are adjusted means (+standard error) of cortical atrophy. Adjusted for age, sex, and pack-years of cigarette smoking. Numbers in the respective categories were for diastolic blood pressure 122, 271, 350, 140, 188 and for systolic blood pressure 99, 215, 186, 268, 303. *Adjusted mean in blood pressure level category differs from adjusted mean in diastolic blood pressure level category 65-74 mmHg $P<0.05$

Higher diastolic blood pressure levels 20 years before MRI predicted more cortical atrophy in later life in subjects without antihypertensive medication ($n=434$) (per 10 mmHg increase in diastolic blood pressure 0.17 units (95% CI, 0.00 to 0.34), but not in subjects with antihypertensive medication ($n=79$) -0.03 units (95% CI, -0.44 to 0.38). The association in subjects without antihypertensive medication weakened after adjusting for diabetes, BMI, and white matter lesions (per 10 mmHg increase in diastolic blood pressure 0.15 units (95% CI, -0.03 to 0.33). The number of subjects without antihypertensive medication allowed us to examine the association between previous diastolic blood pressure and atrophy in age categories of 40-50, 50-60 and 60-70. The association was strongest in the youngest (per 10 mmHg increase in diastolic blood pressure respectively 0.26 units (95% CI, 0.05 to 0.48); 0.12 (95% CI, -0.17 to 0.42); 0.13 (95% CI, -0.68 to 0.95). Systolic blood pressure levels 20 years before MRI were

not associated with the degree of cortical atrophy in later life (data not shown).

On average, subjects declined very little in diastolic blood pressure over 20 years (change -0.2 mmHg). A steeper decline in diastolic blood pressure was associated with significantly more cortical atrophy compared to a stable blood pressure level over time (Figure 3). Persons who had experienced a decline of more than 10 mmHg in diastolic blood pressure level had 0.53 units (95% CI, 0.05 to 1.02) more cortical atrophy than persons with a stable blood pressure level over time (change between -5 and $+5$ mmHg).

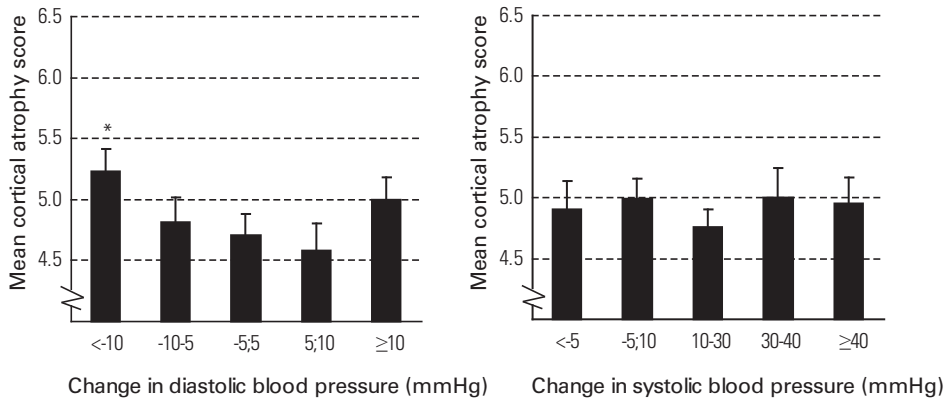


Figure 3. Cortical atrophy according to change in blood pressure level over 20 years time. Values are adjusted means (+standard error) of cortical atrophy. Adjusted for age, sex, and pack-years of cigarette smoking. Numbers in the respective categories were for diastolic blood pressure change 115, 81, 123, 76, 112 and for systolic blood pressure change 65, 120, 188, 60, 74. *Adjusted mean in blood pressure change category differs from adjusted mean in diastolic blood pressure level change from -5 to 5 mmHg $P < 0.05$

The decliners with ensuing late life diastolic blood pressure level less than 65 mmHg ($n=27$) had 1.27 units (95% CI, 0.45 to 2.13) more cortical atrophy than decliners with ensuing late life diastolic blood pressure level above 65 mmHg ($n=88$). Likewise, persons with a low diastolic blood pressure in late life who had experienced a decline of more than 10 mmHg ($n=27$) had 1.69 units (95% CI, -0.04 to 3.43) more cortical atrophy compared to persons with a low diastolic blood pressure without a preceding decline of more than 10 mmHg ($n=7$). The association between a decline of more than 10 mmHg in diastolic blood pressure and more cortical atrophy was present in persons without antihypertensive medications at anytime ($n=336$), (0.80 units (95% CI, 0.18 to 1.42)), whereas the association in chronic antihypertensive users was unclear due to small numbers ($n=56$). In persons who used antihypertensive medica-

tion only at late life ($n=98$), the decliners had 0.34 units (95% CI, -0.84 to 1.51) more cortical atrophy compared to subjects with a stable blood pressure level. There were only 23 subjects who used antihypertensive medication in earlier life without using antihypertensive medication in later life. An increase in diastolic blood pressure of more than 10 mmHg was also associated with more cortical atrophy (Figure 3), but the apparent U-shaped association between diastolic blood pressure change and atrophy was not statistically significant (P of quadratic term = 0.14). The associations did not change by additional adjustments for diabetes, BMI, and white matter lesions. Systolic blood pressure change over 20 years was not associated with the degree of cortical atrophy (Figure 3).

When analyzing the relation between blood pressure and the separate regions in which we measured cortical atrophy, no differential pattern emerged for any of the relations between blood pressure and atrophy. Finally, excluding subjects with an infarct on MRI did not materially change any of the results.

Discussion

The main finding of this study is that in a population of non-demented elderly a high diastolic blood pressure level 20 years before MRI predicted more pronounced cortical atrophy. Second, both concurrent high and low diastolic blood pressure levels were associated with more atrophy. Finally, subjects who had experienced a steep decline in diastolic blood over a 20-year period, and had subsequently a low diastolic blood pressure at late life, had more cortical atrophy than subjects with a stable blood pressure level over time. These associations were independent of other vascular risk factors, cerebral white matter lesions or infarcts on MRI. Some limitations of this study have to be considered. First, people had to be alive to be included in our study. Subjects with either very low or high blood pressure levels earlier in life may have preferentially died and hence could have been underrepresented in our sample. Most likely, this led us to underestimate the association between previous blood pressure levels or change in blood pressure level and brain atrophy. Some evidence that this played a role comes from our observation that higher previous blood pressure levels were associated with more atrophy especially in the youngest for whom higher blood pressure levels will not have a large impact on mortality rate. A second limitation is that our rating of brain atrophy is rather crude compared to that of others who used quantitative, yet time-consuming, techniques.² However, this crude rating should decrease the possibility of finding any association. Finally, we only had single measurements of blood pressure. Due to regression dilution this also would lead us to underestimate the effect of blood pressure on atrophy. Our finding that previous or later life high blood pressure levels are associated with more global brain atrophy in late life is in concordance with observations in clinical hypertensive pa-

tients¹²⁻¹⁴ and population studies.^{3, 4} As previously reported in our sample, high blood pressure levels were associated with severe white matter lesions and infarcts.^{11, 15} Given the fact that white matter lesions, infarcts and severe global brain atrophy commonly occur in the same brain,¹⁶ we considered that the relation between high blood pressure and atrophy could be due to the relation between blood pressure and other cerebrovascular pathology. However, adjusting for the severity of white matter lesions or excluding those with infarcts did not change the association between blood pressure and atrophy, suggesting that blood pressure is independently associated with atrophy. Biological support of an association between high blood pressure and atrophy comes from a pathological study that showed higher mid life blood pressure levels to be associated with more neurofibrillary tangles in the cortex and brain atrophy at death.¹⁷ However, this study was done in Honolulu comprising a different ethnical population under other environmental factors. Replication needs to be done in a Caucasian population such as ours. Another pathological study showed that elderly who died with hypertension had more senile plaques in the cortex than those without hypertension.¹⁸ Besides a high blood pressure level, we also found dropping blood pressure and lower concurrent blood pressure levels to be associated with more global brain atrophy, as was also reported by others.^{5, 6} From our sample with examinations 20 years before MRI, we observed that most of the subjects with low blood pressure levels in late life came from higher blood pressure levels earlier in life. Hence it may be that these previous higher blood pressure levels were responsible for more global brain atrophy at late life. However, it seems unlikely that this explains the whole association between a low or dropping blood pressure level and more global brain atrophy, given our observation that higher previous blood pressure levels were not very strongly associated with more global brain atrophy at late life. We can speculate on two interpretations of the association between low or dropping diastolic blood pressure levels and atrophy. First, a decline in blood pressure level over time may lead to mild ischemia of the brain.^{19, 20} In healthy subjects, a low systemic blood pressure level will not have a large impact on cerebral blood flow because the brain has the ability to keep cerebral blood flow constant by cerebral autoregulation.²¹ However, in subjects with impaired cerebral autoregulation, the lower limit of blood pressure at which cerebral autoregulation still functions is shifted towards a higher blood pressure level,²² and subsequently a low systemic blood pressure level may be inadequate for healthy brain perfusion.^{19, 23} As a second interpretation for the association between a drop in blood pressure and atrophy, one might speculate that atrophy of the brain, once it is severe enough, causes a decline in blood pressure levels. The central nervous system is involved in blood pressure regulation^{24, 25} and in the very elderly it was observed that those with more frontal or insular atrophy had on average lower blood pressure levels.⁵ Our results did however not indicate a differential association between a low blood pressure level and atrophy in specific regions. The association between low blood pressure and atrophy in

this non-demented population fit findings in patients with early Alzheimer's disease in which blood pressure levels drop, possibly due to an increase in atrophy.^{26, 27}

In summary, we found that in a non-demented population both a high and a declining blood pressure predicted more global brain atrophy on MRI. Prospective studies using several measurements of atrophy and blood pressure are required to unravel the chronology of these associations.

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Chapter 2.3

Homocysteine and brain atrophy

Patients with Alzheimer's disease have higher plasma homocysteine levels than controls but it is uncertain whether higher plasma homocysteine levels are involved in the early pathogenesis of the disease. Hippocampal, amygdalar and global brain atrophy on brain magnetic resonance imaging (MRI) have been proposed as early markers of Alzheimer's disease. In the Rotterdam Scan Study, a population-based study of age-related brain changes in 1,077 non-demented people aged 60 to 90 years, we investigated the association between plasma homocysteine levels and severity of hippocampal, amygdalar and global brain atrophy on MRI. We used axial T1 weighted MR images to visualise global cortical brain atrophy (measured semi-quantitatively; range 0-15) and a 3D HASTE (half-Fourier acquisition single-shot turbo spin echo) sequence in 511 participants to measure hippocampal and amygdalar volumes. We had non-fasting plasma homocysteine levels in 1,031 of the participants and in 505 of the participants with hippocampal and amygdalar volumes. Individuals with higher plasma homocysteine levels had on average more cortical atrophy [0.23 units (95% CI 0.07 to 0.38 units) per standard deviation increase in plasma homocysteine levels] and more hippocampal atrophy [difference in left hippocampal volume -0.05 ml (95% CI -0.09 to -0.01) and in right hippocampal volume -0.03 ml (95% CI -0.07 to 0.01) per standard deviation increase in plasma homocysteine levels]. No association was observed between plasma homocysteine levels and amygdalar atrophy. These results support the hypothesis that higher plasma homocysteine levels are associated with more atrophy of the hippocampus and cortical regions in elderly at risk of Alzheimer's disease.

Introduction

Patients with Alzheimer's disease or cognitive impairment have higher plasma homocysteine levels than cognitively unimpaired older subjects.¹⁻³ Recently, a high plasma homocysteine level was shown to be a strong, independent risk factor for the development of Alzheimer's disease.⁴ Elevated plasma total homocysteine level has emerged as a vascular risk factor⁵ and vascular factors may play an important role in the pathogenesis of Alzheimer's disease.⁶ Furthermore, homocysteine has direct neurotoxic effects on hippocampal and cortical neurones.⁷ ⁸ In Alzheimer patients, homocysteine was associated with atrophy of the medial temporal lobe and patients with higher homocysteine levels had a more rapid rate of atrophy over time.¹ Since Alzheimer's disease is characterised by a long prodromal period, its etiology may be better investigated in relation to early preclinical markers of the disease. Hippocampal, amygdalar and global brain atrophy on MRI may serve as such early markers.⁹⁻¹¹ The aim of this study was to investigate the association between plasma homocysteine levels and these brain MRI outcomes as putative early markers of Alzheimer's disease in a non-demented older population.

Methods

Study Sample

This study is based on data collected in the Rotterdam Scan Study, a population-based cohort study, designed to investigate the determinants and consequences of age-related brain changes in the elderly.⁶ In 1995-1996, we randomly selected 1,904 elderly participants (aged 60 to 90 years) stratified by gender and age (5 years) from two ongoing population-based cohort studies: the Rotterdam Study¹² and the Zoetermeer Study.¹³ The presence of dementia was assessed in a stepwise approach as used in the Rotterdam Study.¹⁴ First, participants were screened with the Mini-Mental State Examination (MMSE) and the Geriatric Mental State Schedule (GMS). Those scoring below 26 on the MMSE or more than 0 on the GMS were additionally assessed with the CAMDEX interview.¹⁵ Subjects thereafter suspected to be demented were examined by a neurologist. Finally, an expert panel reviewing all relevant information decided whether an individual was to be considered demented or not, based on criteria of the DSM-III-R. Additionally, persons who were blind or had contraindications for MRI were excluded, leaving 1,717 persons eligible. A total of 1,077 individuals participated (participation rate of 63 %) and gave written informed consent to a protocol, which was approved by the medical ethics committee of Erasmus Medical Center, Rotterdam, the Netherlands.

MRI acquisition

All 1,077 participants underwent an axial T1, T2 and proton-density weighted brain MRI scan in a 1.5-Tesla unit [Philips (n=514) and Siemens (n=563)].¹⁶ For the 563 subjects originating from the Rotterdam Study,¹² we added a 3D Half-Fourier Acquisition Single-Shot Turbo Spin Echo (HASTE) sequence to the protocol (inversion time 440 ms, repetition time 2800 ms, 128 contiguous sagittal slices of 1.2-mm, matrix 192x256, field of view 256x256). Two HASTE modules were sequentially acquired after the inversion pulse (effective echo time of 29 ms and 440 ms), of which the first was used for the volumetric assessments of the hippocampus and amygdala. Of the 563, fifty-two participants developed claustrophobia, leaving 511 participants with a HASTE sequence.

Hippocampal and amygdalar volumes

The HASTE sequence was used to reconstruct coronal slices (contiguous 1.5-mm slices) perpendicular to the long axis of the hippocampus (Figure 1).

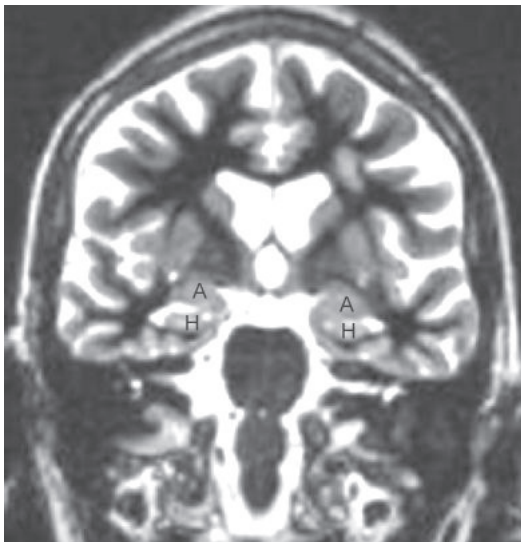


Figure 1. Coronal slice on which the hippocampus (H) and amygdala (A) are depicted

The left and right hippocampus and amygdala were manually traced using a mouse-driven cursor based on a reference atlas.¹⁷ Tracing proceeded from posterior to anterior, starting at the slice where the crux of the fornices was in full profile. The in-plane boundaries of the hippocampus were defined to include the subiculum, the CA1 through CA4 sectors of the hippocampus proper and the gyrus dentatus. Tracing of the amygdala included all of its nuclei.

As the anterior boundary of the amygdala is poorly defined in nature, we defined this to be the slice at the rostral extreme of the temporal stem. Volumes (ml) were calculated [sum of areas (mm²) X 1.5 mm/1000]. We measured the midsagittal area (cm²) by tracing the margin of the inner table of the skull in order to have a proxy for total intracranial volume.⁹ Two readers, who were blinded to clinical information, measured the 511 scans. Intra- and interreader studies based on 14 random scans showed good reproducibility. Intrarater intraclass correlation coefficients for the left and right hippocampus were $r=0.93$ and $r=0.90$, and interrater intraclass correlation coefficients were $r=0.87$ and $r=0.83$, respectively. For the left and right amygdala the intrarater intraclass correlation coefficients were $r=0.82$ and $r=0.78$, the interrater intraclass correlation coefficients were $r=0.80$ and $r=0.77$, respectively.

Global brain atrophy rating

The severity of global brain atrophy was scored on T1 weighted hard copies, blinded to clinical information, based on the widening of sulci and narrowing of gyri in comparison to reference scans. A score from 0 (no cortical atrophy) to 3 (severe cortical atrophy) at five different brain regions (frontal, parietal, temporal and occipital lobes and insular region) was given. The sum score of all five regions (range 0-15) was used for the analyses. The intrarater weighted kappa was 0.82, and the interrater weighted kappa was 0.81.

Plasma homocysteine measurements

Non-fasting blood samples were collected and processed at time of MRI as described previously.¹⁸ Blood samples were unavailable in 39 participants due to errors in the blood collection process. Plasma levels of total homocysteine were determined by fluorescence polarisation immunoassay on an IMx analyser (Abbott). Seven individuals with extreme values were excluded from the analyses since their plasma homocysteine levels fell outside the range 5-45 $\mu\text{mol/l}$ in order to minimize the effects of regression dilution bias. Finally, 1,031 participants were available for the analyses on global brain atrophy, and 505 participants for the analyses on hippocampal and amygdalar atrophy.

Covariates

We obtained information on the following covariates by interview and physical examination in 1995-1996: diabetes mellitus, hypertension (systolic blood pressure level ≥ 160 mmHg, or diastolic blood pressure level ≥ 95 mmHg, or use of blood pressure lowering medication), pack-years of cigarette smoking, vitamin supplements¹⁹ and serum creatinine levels (enzymatic assay). Presence of carotid artery plaques, and the intima-media thickness of the common carotid artery, were assessed as markers of atherosclerotic disease.²⁰ White matter lesions on MRI were scored in periventricular (grade 0-9) and subcortical regions (approximated vol-

ume).¹⁶ Infarcts on MRI were defined as focal hyperintensities on T2 weighted images, without prominent hypointensities on T1 weighted images.¹⁹

Data analysis

The relation between plasma homocysteine level and atrophy was evaluated using both homocysteine in quintiles and as continuous variable. Since homocysteine levels increase markedly with age, quintiles were defined in an age-specific manner for each of five-year age categories. We compared adjusted means of hippocampal and amygdalar volumes and global brain atrophy across the age-specific quintiles of plasma homocysteine by analysis of covariance (ANCOVA). The analyses were adjusted for age, sex, diabetes, hypertension, pack-years of cigarette smoking, serum creatinine, and, for the hippocampal and amygdalar analyses, midsagittal area. Because these analyses did not suggest a non-linear association between homocysteine and atrophy, we performed multivariate linear regression to calculate the change in atrophy per standard deviation (SD) increase in plasma homocysteine level. We investigated whether carotid atherosclerosis, white matter lesions or presence of infarcts on MRI mediated the association between homocysteine and atrophy by adding these covariates to the model. Assumptions of the models were confirmed by residual analyses.

Results

Selected characteristics of the total study sample and the subset with hippocampal and amygdalar volumes are shown in Table 1.

Table 1. Characteristics of the study sample in total and of the subset with hippocampal and amygdalar volumes

Characteristic	Total (n=1031)	Subset (n=505)
Age, years	72 ± 7	73 ± 8
Sex, % women	52	50
Total homocysteine (μmol/l)	11.5 ± 4.1	11.9 ± 4.3
Presence of diabetes, %	7	6
Presence of hypertension, %	52	53
Pack-years of cigarette smoking	19 ± 24	20 ± 25
Serum creatinine level (μmol/l)	88.9 ± 18.6	89.7 ± 19.9

Values are unadjusted means ± SD or percentages unless otherwise specified

Plasma homocysteine levels increased with increasing age (1.5 $\mu\text{mol/l}$ increase per 10 years) and were higher in men (sex difference 1.2 $\mu\text{mol/l}$). Sixty-one participants (5.9%) reported use of multivitamin supplements, and these individuals had lower plasma homocysteine levels than non-users (age and sex adjusted difference $-1.3 \mu\text{mol/l}$; 95% CI -2.3 to -0.3). Figure 2 shows the association between plasma homocysteine levels and hippocampal volumes. People with higher plasma homocysteine levels had smaller hippocampal volumes [difference in left hippocampal volume -0.05 (95% CI -0.09 to -0.01) and right hippocampal volume -0.03 (95% CI -0.07 to 0.01) per SD increase in plasma homocysteine level adjusted for age, sex, diabetes, hypertension, pack-years of cigarette smoking, creatinine levels and midsagittal area].

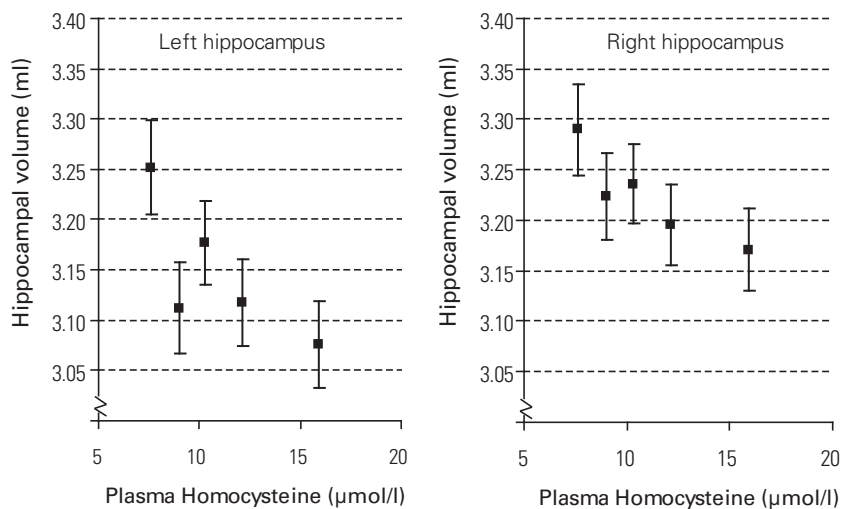


Figure 2. Hippocampal volumes according to age-specific quintiles of plasma homocysteine levels. The mean volumes (standard error) are plotted at the median of each quintile and are adjusted for age, sex, diabetes, hypertension, pack-years of cigarette smoking, creatinine levels and midsagittal area. Note that the left hippocampus is on average smaller than the right hippocampus

Further adjustment for carotid atherosclerosis, white matter lesions and infarcts did not change this association (data not shown). In contrast, there was a non-significant decrease in amygdalar volume with increasing plasma homocysteine levels (Figure 3). Per SD increase in plasma homocysteine level, the left amygdalar volume decreased -0.01 (95% CI -0.04 to 0.03) and the right amygdalar volume decreased -0.02 ml (95% CI -0.05 to 0.02).

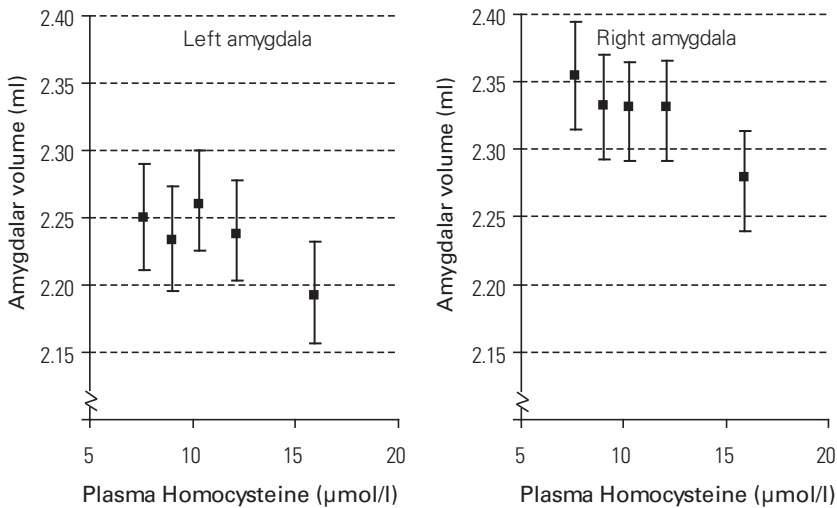


Figure 3. Amygdalar volumes according to age-specific quintiles of plasma homocysteine levels. The mean volumes (standard error) are plotted at the median of each quintile and are adjusted for age, sex, diabetes, hypertension, pack-years of cigarette smoking, creatinine levels and midsagittal area. Note that the left amygdala is on average smaller than the right amygdala

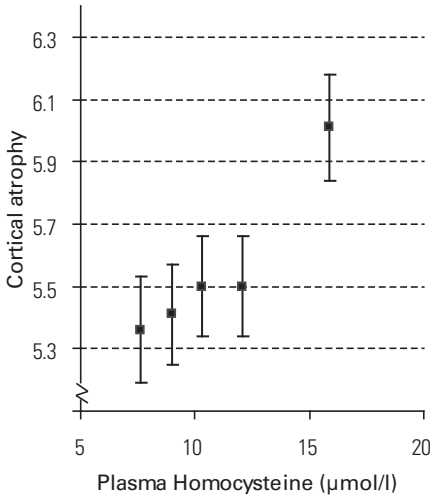


Figure 4. Global brain atrophy according to age-specific quintiles of plasma homocysteine levels. The mean atrophy score (standard error) is plotted at the median of each quintile and is adjusted for age, sex, diabetes, hypertension, pack-years of cigarette smoking and creatinine levels

Figure 4 shows the association between plasma homocysteine levels and severity of cortical atrophy. The degree of cortical atrophy increased with increasing plasma homocysteine levels [per SD 0.23 units more (95% CI 0.07 to 0.38)]. This association was unaltered after adjusting for carotid atherosclerosis [per SD 0.22 (0.06 to 0.37)] and slightly weakened after adjusting for white matter lesions and infarcts [per SD 0.19 (0.03 to 0.34)].

The results were not materially altered after exclusion of regular users of multivitamin supplements.

Discussion

This study found that increasing plasma homocysteine levels are associated with more hippocampal and cortical atrophy in an older non-demented population.

The chief strength of the present study is the population-based study design and the large number of volumetric assessments of the hippocampus and amygdala. However, a limitation was that the instruments used to assess global brain atrophy were somewhat imprecise.

Plasma homocysteine levels reflect vitamin status, renal function and genetic variations in the enzymes controlling homocysteine metabolism genes.⁵ Recently, it was shown that a low plasma folate level may predict more neocortical atrophy at death²¹ though homocysteine may have mediated this association. There is no available data on effects of genetic variations in homocysteine metabolism on brain atrophy. Two putative effects of homocysteine support a causal association between higher plasma homocysteine levels and brain atrophy. First, homocysteine damages the vascular walls²² from arteries.^{23, 24} People with more global brain atrophy have more frequently atherosclerosis in the carotid arteries and white matter lesions on MRI, which are assumed to be small vessel disease.^{25, 26} However, the association between plasma homocysteine levels and atrophy was unaltered by adjusting for carotid atherosclerosis and only partly reduced by adjusting for white matter lesions, suggesting that other pathways may be involved. Second, neurotoxic effects of homocysteine in cultures of cortical and hippocampal neurones could partly explain the associations.^{7, 8} These studies in rats showed that hippocampal neurones were even more sensitive to the effects of homocysteine⁷ than cortical neurones.⁸ Some individuals with hippocampal, and possibly global, brain atrophy are more likely to develop clinical Alzheimer's disease.^{10, 11} The findings of the present study suggest that higher homocysteine levels may be associated with early Alzheimer pathology. However, due to the cross-sectional design of the current study, it remains uncertain whether high homocysteine levels actually precede changes in pathology and cause the brain to shrink. Prospective studies using several atrophy and homocysteine measurements are necessary to unravel cause and consequence. The finding that high baseline homocysteine levels in patients with Alzheimer's disease predicted more rapid atrophy

of the medial temporal lobe over the following three years supports a causal association.¹ Our results confirm cross-sectional studies which show a high plasma homocysteine level to be associated with Alzheimer's disease and cognitive impairment.¹⁻³ Furthermore, they are in keeping with a prospective study which showed that a high plasma homocysteine level is an independent risk factor of Alzheimer's disease.⁴

Several large-scale randomised trials with folic acid-based vitamin supplements to lower homocysteine levels are currently being conducted and almost all of these trials include an assessment of cognitive function, some have sub-studies that also include MRI measurements. Further large-scale trials are required to assess whether lowering plasma homocysteine levels may prevent Alzheimer-related structural abnormalities or delay progression of clinical symptoms of Alzheimer's disease.

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Chapter 2.4

Alcohol intake and brain MRI findings

Background: *Light-to-moderate alcohol consumers have a lower risk of dementia and possibly Alzheimer's disease compared to abstainers. As vascular disease may contribute to symptoms of Alzheimer's disease, reduction of cerebrovascular disease in light alcohol consumers could account for these observations. However, a low concentration of alcohol may also have direct effects on the hippocampus, a brain structure highly affected by Alzheimer's disease.*

Objective: *We investigated alcohol intake in relation to brain magnetic resonance imaging (MRI) findings of presumed vascular origin (white matter lesions and infarcts) and findings more specifically found in early Alzheimer's disease (hippocampal and amygdalar atrophy).*

Design: *In a population-based sample of 1,074 non-demented elderly (age 60-90) we made brain MRI on which we rated white matter lesions and brain infarcts. In a subset of 509 people, hippocampal and amygdalar volumes on MRI were measured. Alcohol intake was assessed with a structured questionnaire. We categorized alcohol intake into lifetime abstaining, very light (<1 drink weekly), light (≥ 1 drink/week to <1 drink/day), moderate (≥ 1 drink/day to <4 drinks/day) and heavy (≥ 4 drinks/day).*

Results: *Light-to-moderate alcohol drinkers had less severe white matter lesions and brain infarcts on MRI compared to abstainers or heavy drinkers. Abstainers and very light drinkers had smaller hippocampal and amygdalar volumes on MRI than light-to-moderate drinkers, but only if carrying an apolipoprotein (APOE) $\epsilon 4$ allele.*

Conclusions: *Our data suggest that light-to-moderate alcohol intake is associated with a lower prevalence of vascular brain findings and -in APOE $\epsilon 4$ carriers- hippocampal and amygdalar atrophy on MRI.*

Introduction

Several prospective population-based studies have shown that light-to-moderate alcohol consumers have a lower risk of dementia compared to abstaining or heavy alcohol use.¹⁻⁴ Most studies find a relation with vascular dementia,^{2, 4, 5} consistent with the known beneficial effects of light-to-moderate alcohol consumption on vascular risk profile⁶ and risk of stroke.⁷ Vascular pathology likely contributes to the clinical syndrome in a large proportion of elderly Alzheimer patients.^{8, 9} A plausible explanation for the associations that are seen between alcohol consumption and overall dementia or Alzheimer's disease^{3, 4, 10} is therefore that this is through an effect on vascular pathology. Alternatively, alcohol in low amounts could have stimulatory effects on the release of acetylcholine in the hippocampus¹¹ and other non-vascular mechanisms might play a role.¹² A non-vascular relation between alcohol intake and Alzheimer's disease was indeed suggested by our observation in the Rotterdam Study that alcohol intake seemed only associated with Alzheimer's disease in carriers of the apolipoprotein (APOE) $\epsilon 4$ allele,² the risk allele for Alzheimer's disease.¹³

To further investigate what could underlie the relation between alcohol intake and risk of dementia, we decided to focus on the relation between alcohol intake and structural brain findings on MRI of non-demented elderly. We hypothesized that if the effect of alcohol on dementia risk were purely through an effect on vascular pathology, one would see a relation between alcohol intake and markers of cerebrovascular disease (brain infarcts, white matter lesions^{9, 14-16} but not between alcohol intake and putative presymptomatic MRI markers of Alzheimer's disease (hippocampal and amygdalar atrophy¹⁷⁻²²). We investigated this in the population-based Rotterdam Scan Study.

Methods

Study sample

This study was based on data collected in the Rotterdam Scan Study, a population-based cohort study designed to investigate determinants and consequences of age-related brain changes on MRI.²³ The study had its baseline examinations from 1995 to 1996. At that time, we made a random selection of 1,904 elderly aged 60 to 90 years originating from two population cohort studies in strata of age (5 years) and sex. After exclusion of persons with dementia,²⁴ or contraindications to undergo MRI (such as metal clips, pacemaker, or claustrophobia), 1,717 persons were eligible of whom 1,077 participated and gave written informed consent (participation rate 63%, mean age 72.2 years, 52% women). The medical ethics committee of Erasmus Medical Center approved the study protocol.

Alcohol intake

A physician questioned each participant at baseline examinations about alcohol intake with a structured questionnaire. Alcohol intake was coded into lifetime abstaining, former drinking and current drinking (defined as having consumed alcohol during the past 12 months). Current and former drinkers were asked about the amount of their alcohol consumption. The amount assessed with this questionnaire correlated well with the information obtained by a food frequency questionnaire administered five years before in part of the sample (Pearson $r=0.72$, $P<0.01$).²⁵ We categorized current alcohol drinkers into very light (<1 drink weekly), light (≥ 1 drink/week to <1 drink/day), moderate (≥ 1 drink/day to <4 drinks/day) and heavy (≥ 4 drinks/day) drinkers similar to our previous report on dementia.² For three participants information on alcohol intake was missing.

MRI acquisition

All participants underwent axial T1, T2 and proton-density weighted brain MRI scanning with a 1.5-Tesla unit (Philips or Siemens) at baseline.²⁶ Only the Siemens MRI unit, in which 563 participants had their MRI brain scan, allowed us to additionally include a custom-made three dimensional (3D) MRI sequence (Half-Fourier Acquisition Single-Shot Turbo Spin Echo²⁷ for volumetric assessment of the hippocampus and amygdala. Fifty-two of the 563 participants developed claustrophobia during the MRI acquisition and another two had no information on alcohol intake, leaving 509 participants with information on alcohol intake and hippocampal and amygdalar volumes.

Cerebrovascular disease on MRI

MRI measurements were performed blinded to clinical information of the participants.^{26, 28} White matter lesions were considered present if visible as hyperintense on proton-density and T2 weighted images, without prominent hypointensity on T1 weighted scans. Periventricular white matter lesions were scored semi-quantitatively from 0-9 and subcortical white matter lesions were counted in different size categories to approximate a total lesion volume (ml).²⁶ We defined infarcts as focal hyperintensities on T2 weighted images. Infarcts in the white matter also had to have corresponding hypointensities on T1 weighted images in order to distinguish them from white matter lesions. Infarcts on MRI were classified as silent or symptomatic.²⁸

Hippocampal and amygdalar volumes on MRI

We constructed a series of coronal brain slices (contiguous 1.5-mm slice thickness) from the 3D MRI, aligned to be perpendicular to the long axis of the hippocampus. We manually traced the boundaries of the hippocampus and amygdala on both sides on each slice with a

mouse-driven cursor.²⁷ The summed surface areas were multiplied by slice thickness to yield estimates of the hippocampal and amygdalar volume (ml). The left and right-sided volumes were summed to yield the total hippocampal and amygdalar volume. As a proxy for head size, we measured on the middle sagittal MRI slice the intracranial cross-sectional area.²⁷ We corrected for head size differences across individuals by dividing the uncorrected volumes by the subject's calculated head size area and subsequently multiplying this ratio by the average head size area (men and women separately).^{29, 30}

Covariates

The following covariates were assessed at baseline by interview and physical examination: pack-years of cigarette smoking, educational level, body mass index, diabetes mellitus and hypertension.²⁸ APOE genotype testing was performed³¹ and available for 969 participants in the total sample and 436 participants in the subset with hippocampal and amygdalar volume assessment; the remainder was mainly missing owing to no blood available. Participants were classified into carrier or non-carrier of an APOE $\epsilon 4$ allele; those with genotype APOE $\epsilon 2\epsilon 4$ ($n=22$ in the total sample and 9 in the subsample) were excluded in analyses considering APOE genotype.

Data analysis

We used multivariable linear regression to quantify the association between alcohol intake categories and white matter lesions and hippocampal and amygdalar volumes. With logistic regression, we calculated the adjusted odds ratio (95% confidence interval (CI)) of brain infarcts across alcohol intake categories using abstainers as the reference category. For tests of linear trend, we treated the categories of alcohol intake as continuous variable. For tests of quadratic trend, we squared the linear trend variable. As covariates we included age, sex and pack-years of cigarette smoking. Additionally, we adjusted for educational level, body mass index, diabetes and hypertension. We evaluated whether the effects of alcohol differed across sex and APOE genotype (carrier or non-carrier of the APOE $\epsilon 4$ allele) by performing stratified analyses and including interaction terms in the model.

Results

There were no differences in baseline characteristics between the total sample and the subsample with assessment of hippocampal and amygdalar volumes on MRI (Table 1). In Table 2 characteristics according to alcohol intake categories are given for the total sample. Former drinkers ($n=86$ in the total sample and $n=42$ in the subsample) more frequently had hypertension and diabetes mellitus compared to current drinkers supporting our presumption that

former drinkers could have stopped drinking due to illness. Therefore, former drinkers were excluded from analyses on alcohol intake and brain MRI findings.

Table 1. Characteristics of the total study sample and of the subsample with assessment of hippocampal and amygdalar volumes on 3D MRI

	Total sample (n=1074)	Subsample with 3D MRI (n=509)
Age, years	72 ± 7	73 ± 8
Women, %	52	49
Current alcohol intake, drinks/day	1.2 ± 1.3	1.2 ± 1.4
Former alcohol users, %	8	8
Pack-years of cigarette smoking	19 ± 24	20 ± 25
Primary education, %	35	31
Body mass index, kg/m ²	27 ± 4	26 ± 4
Presence of diabetes, %	7	6
Presence of hypertension, %	52	53
Presence of APOE ε4 allele, %*	28	27
Presence of symptomatic infarct on MRI, %	4	6

Values are means ± standard deviation or percentages.

*Available in 969 out of the 1074 and in 436 out of the 509.

Table 3 shows that light alcohol drinkers had the lowest degree of periventricular white matter lesions on MRI (adjusted difference compared to abstainers 0.69 (95% CI 0.25 to 1.13, $P = 0.002$); compared to heavy drinkers 0.79 (95% CI 0.17 to 1.41, $P = 0.01$)). They also had less subcortical white matter lesions but differences did not reach statistical significance (adjusted difference compared to abstainers 0.57 (95% CI -0.05 to 1.20, $P = 0.07$); compared to heavy drinkers 0.35 (95% CI -0.54 to 1.24, $P = 0.44$)). Brain infarcts on MRI were less frequently found in very light and light drinkers but none of the odds ratios reached statistical significance (Table 3). When we excluded persons with symptomatic infarcts on MRI ($n=42$), because they could have changed alcohol intake after the stroke, results remained similar. The associations did not change after additional adjustments for educational level, body mass index, diabetes and hypertension and were similar in men and women and in APOE ε4 carriers and non-ε4 carriers.

Table 2. Characteristics according to current alcohol intake in the total sample

	Alcohol intake				
	Abstainers (n=114)	Very light (<1/week) (n=195)	Light (≥1/week - <1/day) (n=247)	Moderate (1-3/day) (n=386)	Heavy (≥4/day) (n=46)
Former drinkers (n=86)					
Age, years	72 ± 8	73 ± 8	72 ± 8	72 ± 7	70 ± 7
Women, %	78	69	50	38	26
Pack-years of cigarette smoking	7 ± 16	14 ± 22	18 ± 22	23 ± 24	37 ± 33
Primary education, %	45	38	34	28	39
Body mass index, kg/m ²	27 ± 4	27 ± 4	27 ± 4	26 ± 3	26 ± 3
Presence of diabetes, %	10	7	5	5	4
Presence of hypertension, %	56	56	51	47	54
Presence of APOE ε4 allele, %	31	23	23	28	30
Presence of symptomatic infarct on MRI, %	6	3	2	5	4

Values are means ± standard deviation or percentages

Table 3. Vascular brain MRI findings according to alcohol intake in the total sample

	Alcohol intake				P value for Trend* Linear (Quadratic)
	Abstainers (n=114)	Very light (<1/week) (n=195)	Light (≥1/week - <1/day) (n=247)	Moderate (1-3/day) (n=386)	Heavy (≥4/day) (n=46)
White matter lesions					
Periventricular, mean grade ± SE†	2.73 ± 0.19	2.51 ± 0.14	2.04 ± 0.12	2.37 ± 0.10	2.83 ± 0.29 0.001 (0.001)
Subcortical mean volume (ml) ± SE†	1.58 ± 0.27	1.60 ± 0.20	1.01 ± 0.18	1.49 ± 0.14	1.36 ± 0.42 0.31 (0.36)
Brain infarcts					
Number (%)	34 (30)	43 (22)	52 (21)	94 (24)	9 (20)
Odds ratio (95% CI)‡	1.00 (ref)	0.63 (0.36-1.09)	0.65 (0.38-1.12)	0.86 (0.52-1.43)	0.80 (0.33-1.96) 0.14 (0.11)

* P-value for trend calculated from multiple linear regression models with alcohol intake in categories and its squared term.

† ANCOVA was used for calculating adjusted means in groups. Adjusted for age, sex, and pack-years of cigarette smoking.

‡ Logistic regression was used to calculate adjusted odds ratios. Adjusted for age, sex, and pack-years of cigarette smoking

Table 4 shows that overall there was no association between alcohol intake and hippocampal or amygdalar volumes on MRI. However, the effect of alcohol intake on volumes differed according to APOE genotype (P -value of interaction term = 0.02 for hippocampal volume and $P = 0.07$ for amygdalar volume). In carriers of the $\epsilon 4$ allele, alcohol intake was positively associated with hippocampal and amygdalar volumes, whereas in non- $\epsilon 4$ carriers there was no association (Figure 1). The associations between alcohol intake and hippocampal or amygdalar volumes did not change after additional adjustment for educational level, body mass index, diabetes and hypertension and were similar in men and women.

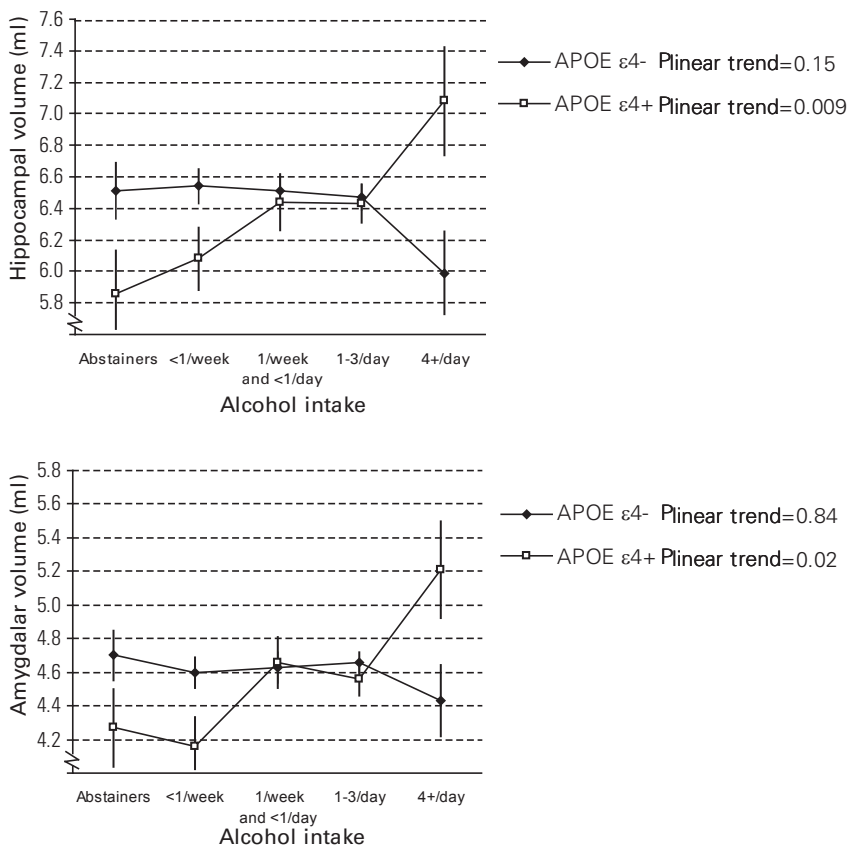


Figure 1. Hippocampal and amygdalar volume on MRI according to alcohol intake in strata of APOE genotype (non- $\epsilon 4$ carrier ($n=288$) and $\epsilon 4$ carrier ($n=101$)). Volumes were adjusted for age, sex and pack-years of cigarette smoking and normalized to average head size. Error bars indicate standard error of the mean

Discussion

In this population-based study in the elderly, we found that light-to-moderate alcohol drinkers had less cerebral white matter lesions and brain infarcts on MRI compared to abstainers and heavy drinkers. In APOE $\epsilon 4$ carriers, but not in non- $\epsilon 4$ carriers, we found a positive association between alcohol intake and hippocampal and amygdalar volumes on MRI.

The strength of our study is the large sample with brain MRI and volumetric assessments of the hippocampus and amygdala. Some methodological issues need to be discussed. First, we relied on self-reported alcohol intake, which may have led to underreporting or overreporting. Although we may misclassify absolute amounts of alcohol intake, the ranking of people according to their alcohol intake will be adequate.³² Second, we assessed average alcohol intake over a 1-year period before MRI examinations while it might be better to have information on the average alcohol intake over the lifetime. Finally, we had no detailed information on the type of alcoholic drink used. Previous studies showed that moderate beer, liquids or wine consumption are equally associated with a reduced risk of dementia,^{2, 4} though the Copenhagen City Heart Study found the association only for moderate wine consumption.¹⁰

Light-to-moderate alcohol drinking is associated with a reduced risk of cognitive impairment and dementia compared to lifetime abstention.^{1-4, 10, 33, 34} In line with the beneficial effects of alcohol on lipid profile, hemostatic factors,⁶ and atherosclerosis,³⁵ most investigators found a reduced risk of vascular dementia in light-to-moderate alcohol consumers.^{2, 4, 5} Consistent with these vascular effects of alcohol and with observations in the Cardiovascular Health Study (CHS),³⁶ we found that light-to-moderate alcohol consumers had less cerebral white matter lesions -in particular in the periventricular region- and infarcts on MRI. Different pathophysiological events may lead to either periventricular or subcortical white matter lesions.¹⁴ Especially the periventricular white matter is vulnerable to ischemia and severe periventricular white matter lesions are related to carotid atherosclerosis.³⁷ Light-to-moderate alcohol drinking has also been associated with a reduced risk of Alzheimer's disease in several,^{1, 3, 4, 10, 38} but not all studies.^{39, 40} Besides reducing cerebrovascular pathology which contributes to the development of symptoms of Alzheimer's disease^{8, 9, 24} alcohol in moderate amounts may also increase the release of acetylcholine of the hippocampus,¹¹ has antioxidative effects⁴¹ and induce the release of potentially beneficial prostaglandins.¹² The traditional Alzheimer pathology, i.e. amyloid plaques and neurofibrillary tangles accumulates in the brain with high predilection of the medial temporal lobe.⁴² This may lead to neuronal loss and atrophy in vivo detectable on MRI even in persons who have not yet developed clinical symptoms of dementia.¹⁷⁻²² APOE $\epsilon 4$ carriers are at increased risk to develop Alzheimer neuropathology,⁴³ atrophy of structures in the medial temporal lobe⁴⁴ and clinical Alzheimer's disease.¹³ Observations in the Rotterdam Study suggested that light-to-moderate alcohol consumption is associated with a reduced risk

of Alzheimer's disease only in APOE $\epsilon 4$ carriers.² Carmelli et al⁴⁵ also reported a stronger association of light drinking and better cognitive performance in APOE $\epsilon 4$ carriers. In our current study, we found that APOE $\epsilon 4$ carriers who were light-to-moderate alcohol drinkers had less hippocampal and amygdalar atrophy compared to abstainers. Although not all persons with hippocampal or amygdalar atrophy will develop Alzheimer's disease, they are at increased risk to develop clinical symptoms of Alzheimer's disease¹⁸ and, as such, our finding support the hypothesis that there could be a reduced risk of Alzheimer's disease for light-to-moderate alcohol drinkers with the APOE $\epsilon 4$ allele. Alcohol has antioxidative properties⁴¹ and this might suppress the highly sensitive peroxidation of the apolipoprotein $\epsilon 4$,⁴⁶ and thereby amyloid plaque formation in the medial temporal lobe^{47,48} or neurotoxicity of β -amyloid.⁴⁹ Surprisingly, the CHS found a lower risk of dementia in alcohol users to be more consistent among persons without the APOE $\epsilon 4$ allele.⁴ Possibly the younger population studied in the Rotterdam Study compared to the CHS led to these discrepancies because the APOE $\epsilon 4$ allele is a stronger risk factor for Alzheimer's disease at younger ages.¹³ The EVA study also found a reduced risk of cognitive deterioration with alcohol intake in non- $\epsilon 4$ carriers but in that study the APOE $\epsilon 4$ allele itself was not a risk factor for cognitive decline.⁵⁰

To conclude, our findings suggest that light-to-moderate alcohol intake is associated to brain MRI findings indicative of cerebrovascular disease and -in APOE $\epsilon 4$ carriers- to hippocampal and amygdalar atrophy on MRI. Given the possibility of confounding in observational studies on alcohol use, and the potentially dangerous effects of alcohol, recommendations to start drinking alcohol cannot be given. Our findings are however in line with observations that light-to-moderate alcohol consumption is associated with a reduced risk of dementia.

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Chapter 2.5

Apolipoproteine E genotype and brain atrophy

The $\epsilon 4$ allele of the APOE gene increases the risk for Alzheimer's disease (AD), whereas the $\epsilon 2$ allele may be protective. The authors assessed the impact of APOE genotype on hippocampal, amygdalar and global brain atrophy as putative markers of preclinical AD in a non-demented population. Carriers of $\epsilon 4$ had significantly more hippocampal and amygdalar atrophy than $\epsilon 3\epsilon 3$ subjects, but not more global brain atrophy. Carriers of $\epsilon 2$ did not have less brain atrophy than $\epsilon 3\epsilon 3$ subjects.

Introduction

The elevated risk of APOE allele $\epsilon 4$ for AD is well established, whereas the APOE allele $\epsilon 2$ may have a protective effect on AD. Hippocampal and amygdalar atrophy are putative early markers of AD. MRI studies investigating the impact of APOE genotype on the degree of hippocampal atrophy have yielded contradictory findings.¹⁻⁶ Some studies found demented persons carrying the $\epsilon 4$ allele to have more severe hippocampal atrophy^{1, 2} but other studies did not.^{3, 4} Suggestions that primarily the right hippocampus is affected in non-demented and demented $\epsilon 4$ carriers need further study.^{5, 6} Most studies, although not all,⁷ showed no effect of the $\epsilon 4$ allele on the degree of global brain atrophy, which occurs probably later in the course of AD.^{3, 6, 8} To our knowledge, no studies specifically evaluated the relation of the $\epsilon 2$ allele with the degree of hippocampal, amygdalar, and global brain atrophy. The aim of this study was to assess whether hippocampal, amygdalar, and global brain atrophy in non-demented elderly subjects differed according to APOE genotype. We hypothesized that $\epsilon 2$ carriers would have less and $\epsilon 4$ carriers more atrophy than those with the common APOE $\epsilon 3\epsilon 3$ genotype.

Methods

Subjects and procedures

In 1995 and 1996, we invited 1,904 elderly aged 60 to 90 years to participate as part of the Rotterdam Scan Study.⁹ We excluded persons who were demented, were blind or had MRI contraindications, leaving 1,717 persons eligible. Complete information, including a cerebral MRI scan, was obtained in 1,077 individuals, who gave written informed consent (participation rate 63%). The medical ethics committee of Erasmus University, Rotterdam, the Netherlands approved the study.

MRI examinations were done using a 1.5 Tesla MR unit and included an axial T1 weighted scan (repetition time [TR] 485 ms or 700 ms, echo time [TE] 14 ms, slice thickness 5 or 6 mm, interslice gap 20%). For volumetric measurements of the hippocampus and amygdala, a custom-made, inversion recovery - double contrast 3D half-Fourier acquisition single-shot turbo spin echo (HASTE) sequence was included (inversion time 440 ms, [TR] 2800 ms, 128 contiguous sagittal slices of 1.2-mm, matrix 192x256, field of view 256x256). Two HASTE modules were sequentially acquired after the inversion pulse (effective TE of 29 ms and 440 ms); the first was used for the volume measurements. This 3D sequence was done in a subsample of 563 subjects; 52 developed claustrophobia during the measurement, leaving 511 subjects. APOE genotyping was performed on coded DNA samples¹⁰ without knowledge of MRI measurements. APOE genotyping was present for 971 in the total sample and 437 in the subsample; the remainder was missing owing to no blood available. The allele frequencies

were $\epsilon_2=0.08$, $\epsilon_3=0.77$, and $\epsilon_4=0.15$, which was in Hardy-Weinberg equilibrium ($\chi^2=0.6$, $df=3$: $P>0.2$). Memory function was evaluated by a 15 word verbal word learning task that tests immediate and delayed recall (after 15 minutes).

MRI measurements

MRI measurements were done blinded to clinical characteristics. Global brain atrophy was scored on T1 weighted scans for cortical atrophy based on the size of gyri and sulci at five locations (0 [no cortical atrophy] to 3 [severe cortical atrophy]: total range 0 to 15), and for subcortical atrophy by the ventricle-to-brain ratio (average of assessments at three locations). Intra- and interreader studies showed moderate to good agreement. Hippocampal and amygdalar volumes were measured on coronal slices (1.5-mm, no interslice gap) reconstructed from the HASTE to be perpendicular to the long axis of the hippocampus. The left and right hippocampus and amygdala were manually traced with a mouse-driven cursor on a SUN work station featuring a Magic View 1000 program using boundaries similar to others.⁴ The anterior boundary of the amygdala was defined as the plane including the most anterior part of the temporal stem. Volumes were calculated by summing the areas multiplied by slice thickness. Intra- and interreader studies showed intraclass correlation coefficients exceeded $r=0.77$. We also measured the midsagittal area by tracing the inner skull in order to have a proxy for intracranial volume.

Data analyses

APOE genotypes were grouped in at least one APOE ϵ_2 allele (APOE ϵ_2+), APOE $\epsilon_3\epsilon_3$, or at least one APOE ϵ_4 allele (APOE ϵ_4+). Subjects with APOE $\epsilon_2\epsilon_4$ genotype were excluded ($n=22$ in the total study sample and $n=9$ in the subsample). The APOE group was entered as a categorical dummy variable (with APOE $\epsilon_3\epsilon_3$ as reference) into a multiple linear regression model (analysis of covariance). This model included age, sex and -in the hippocampal and amygdalar analyses- midsagittal area as covariates. We calculated adjusted means in cortical and subcortical atrophy and hippocampal and amygdalar volumes across APOE genotypes by entering the mean age, sex distribution, and -in the hippocampal and amygdalar analyses- mean midsagittal area in the linear regression model. Analyses were done for the left and right hippocampal and amygdalar volume separately and for the sum of left and right volumes. Finally, we repeated the analyses excluding persons with a memory score (immediate or delayed recall) <1.5 SD of the age- and education- adjusted means.

Results

Characteristics according to APOE genotype are shown in Table 1. The total study sample comprised 24 subjects homozygous for the $\epsilon 4$ allele, 10 of whom were in the subsample.

Table 1. Characteristics of study sample according to APOE genotype

Characteristic	APOE genotype*		
	APOE $\epsilon 2+$ (n=120)	APOE $\epsilon 3\epsilon 3$ (n=568)	APOE $\epsilon 4+$ (n=261)
Age, years	72 $\pm$ 7	73 $\pm$ 7	71 $\pm$ 7
Sex, % women	58	51	52
Mini-Mental State Examination	27.7 $\pm$ 2.0	27.5 $\pm$ 2.1	27.1 $\pm$ 2.5
Memory score < 1.5 SD, %†	7	9	15
Cortical atrophy, range 0-15	5.63 $\pm$ 2.71	5.56 $\pm$ 2.77	5.35 $\pm$ 2.82
Subcortical atrophy, ratio	0.321 $\pm$ 0.039	0.317 $\pm$ 0.036	0.314 $\pm$ 0.032
Hippocampus (ml)‡			
Left	3.12 $\pm$ 0.45	3.19 $\pm$ 0.46	3.12 $\pm$ 0.45
Right	3.21 $\pm$ 0.39	3.26 $\pm$ 0.45	3.19 $\pm$ 0.47
Amygdala (ml)‡			
Left	2.22 $\pm$ 0.30	2.25 $\pm$ 0.38	2.17 $\pm$ 0.41
Right	2.31 $\pm$ 0.36	2.35 $\pm$ 0.41	2.28 $\pm$ 0.40

Values are unadjusted means $\pm$ SD or percentages.

* Subjects with APOE $\epsilon 2\epsilon 4$ (n=22) were excluded.

† Percentage of subjects with memory score <1.5 SD below the age and education adjusted means.

‡ Hippocampal and amygdalar volumes were assessed in 428 subjects

Carriers of $\epsilon 4$ had more often a low memory score than those with APOE $\epsilon 3\epsilon 3$ ($P=0.01$). APOE genotype was not related to global brain atrophy (age- and sex- adjusted means [SEM] for cortical atrophy: $\epsilon 2+$ =5.63 [0.21], $\epsilon 3\epsilon 3$ =5.46 [0.09], $\epsilon 4+$ =5.57 [0.14], [$P=0.65$]; for subcortical atrophy $\epsilon 2+$ =0.321 [0.003], $\epsilon 3\epsilon 3$ =0.316 [0.001], $\epsilon 4+$ =0.316 [0.002], [$P=0.19$]). Carriers of $\epsilon 4$ had significantly smaller hippocampi and amygdalae than did subjects with APOE $\epsilon 3\epsilon 3$, both on the left and right side (Table 2). APOE $\epsilon 2$ carriers did not have larger hippocampal or amygdalar volumes compared to APOE $\epsilon 3\epsilon 3$ subjects. In contrast, their average volumes approximated those of $\epsilon 4$ carriers (difference in total hippocampal and total amygdalar volume for $\epsilon 2$ versus $\epsilon 4$ carriers 0.09 ml [-0.18 to 0.35] and 0.13 ml [-0.10 to 0.35]).

Hippocampal and amygdalar volumes decreased with increasing $\epsilon 4$ dose (difference with $\epsilon 3\epsilon 3$ subjects, total hippocampal volume: 1 allele -0.17 ml [95% CI -0.35 to 0.02], 2 alleles -0.71 ml [95% CI -1.22 to -0.20]; total amygdalar volume: 1 allele -0.17 ml [95% CI -0.33 to -0.02], 2 alleles -0.42 ml [95% CI -0.85 to 0.02]).

Table 2. Difference in hippocampal and amygdalar volume according to APOE genotype

		Difference in volume (95% CI) as compared to subjects with APOE $\epsilon 3\epsilon 3$ (n=259)*			
		Hippocampus		Amygdala	
	Number	Left	Right	Left	Right
APOE $\epsilon 2+$	52	-0.08 (-0.21 to 0.05)	-0.05 (-0.17 to 0.08)	-0.03 (-0.14 to 0.07)	-0.03 (-0.14 to 0.08)
APOE $\epsilon 4+$	117	-0.11 (-0.20 to -0.01)	-0.11 (-0.20 to -0.02)	-0.10 (-0.18 to -0.02)	-0.09 (-0.17 to -0.01)

*Values are differences (95% CI) in volume (ml), adjusted for age, sex and midsagittal area

Subjects with memory performance lower than 1.5 SD below the age- and education- adjusted means had smaller hippocampal (adjusted difference -0.25 ml [95% CI -0.50 to -0.01]) but not amygdalar volumes (-0.08 ml [95% CI -0.29 to 0.13]). When we excluded these subjects, we found a similar effect of $\epsilon 4$ on volumes (Table 3).

Table 3. Difference in hippocampal and amygdalar volume according to APOE genotype, in total sample and in sample after exclusion of subjects with low memory performance

		Difference in volume (95% CI) as compared to subjects with APOE $\epsilon 3\epsilon 3$ *			
		Hippocampus		Amygdala	
	Number	Total	Exclusion low memory performance†	Total	Exclusion low memory performance†
APOE $\epsilon 2+$	428	-0.13 (-0.37 to 0.12)	-0.20 (-0.45 to 0.05)	-0.06 (-0.27 to 0.14)	-0.13 (-0.35 to 0.08)
APOE $\epsilon 4+$	369	-0.21 (-0.39 to -0.03)	-0.21 (-0.40 to -0.02)	-0.19 (-0.34 to -0.04)	-0.17 (-0.33 to -0.01)

*Values are difference (95% CI) in volume (ml), adjusted for age, sex and midsagittal area.

†Subjects with memory score <1.5 SD below the age and education adjusted means were excluded

Discussion

We found that non-demented $\epsilon 4$ carriers had more hippocampal and amygdalar atrophy, but not more global brain atrophy, than those with the APOE $\epsilon 3\epsilon 3$ genotype. Suggestions from smaller studies in both nondemented ($n=54$) and demented ($n=42$) subjects that the $\epsilon 4$ allele especially affected the right hippocampus and amygdala could not be confirmed.^{5, 6} Contrary to expectations, $\epsilon 2$ carriers had more atrophy than those with the $\epsilon 3\epsilon 3$ genotype, but the difference was not statistically significant. Our finding that APOE genotype is not related to global brain atrophy is in keeping with previous studies in non-demented and demented elderly.^{3, 6, 8} However, the rating of global brain atrophy was less sensitive than our volumetric measurements and subtle differences in global brain atrophy across genotype may have been missed. We considered that the associations that we found in this non-demented population may reflect preclinical AD in $\epsilon 4$ carriers. Indeed, the $\epsilon 4$ carriers more often had low memory performance which was associated with smaller hippocampal volumes. However, excluding subjects with memory performance lower than 1.5 SD below the age- and education- adjusted means did not change results. Although we cannot exclude the possibility that $\epsilon 4$ carriers have smaller hippocampi and amygdalae throughout life, our data suggest that measurable hippocampal and amygdalar atrophy occurs before memory decline can be objectively determined.

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Chapter 3

Endocrine risk factors of brain atrophy on MRI



Chapter 3.1

Plasma estrogens, hippocampal atrophy and memory performance

Background: Estrogens may prevent cognitive decline and Alzheimer disease. Animal study findings have shown beneficial effects of estrogen on the brain, particularly on the hippocampus, a structure related to memory performance and early Alzheimer disease.

Objective: To investigate whether higher levels of endogenous estradiol in older women and men are associated with larger hippocampal volumes on magnetic resonance imaging and better memory performance.

Design and Setting: Cross-sectional analysis within the Rotterdam Scan Study, a population-based study in the Netherlands of elderly subjects who do not have dementia.

Participants: Two hundred ten women and 202 men, aged 60 to 90 years, with plasma levels of total estradiol and, in part, 162 women and 149 men also with levels of bioavailable and free estradiol.

Main Outcome Measure: Hippocampal volumes on magnetic resonance imaging and memory performance (delayed recall).

Results: Women with higher total estradiol levels had smaller hippocampal volumes and poorer memory performance -0.29 ml (95% confidence interval, -0.57 to -0.00) and -0.4 (95% confidence interval, -1.3 to 0.5) fewer words in delayed recall testing for the highest tertile compared with the lowest tertile. Similar inverse associations were found among bioavailable and free-estradiol levels, hippocampal volumes, and memory. In men, no association was observed between estradiol levels and hippocampal volume, but a trend was found for higher levels of total estradiol to be associated with poorer memory performance.

Conclusion: Our data do not support the hypothesis that higher levels of endogenous estradiol in older women and men are associated with larger hippocampal volumes and better memory performance.

Introduction

After menopause, women have low levels of circulating estrogens. Observational studies reported a reduced risk of cognitive decline and Alzheimer disease (AD) in postmenopausal women using estrogen replacement therapy.^{1, 2} In animals, estrogens protect hippocampal neurons exposed to amyloid- β ,³ improve synapse formation on dendritic spines in the hippocampus,⁴ and promote survival of hippocampal neurons, acting in concert with growth factors.⁵ Estrogen receptors are located throughout the brain, but especially in the hippocampus.⁶ Given the beneficial effects of estrogens on the hippocampus in animals, it has been hypothesized that estrogens may prevent hippocampal atrophy, a key feature of AD.⁷ The hippocampus plays a pivotal role in memory function and is one of the first regions affected in AD.⁸ The aim of this study was to investigate whether higher endogenous levels of estradiol were associated with larger hippocampal volumes on magnetic resonance imaging (MRI) and better memory performance. We examined this hypothesis in a population-based study of older women and men who did not have dementia.

Methods

Rotterdam Scan Study

This study is based on all participants in the Rotterdam Scan Study that originated from the Rotterdam Study. The Rotterdam Study is a large population-based cohort study in the Netherlands that started in 1990 and investigates the prevalence, incidence, and determinants of various chronic diseases among elderly participants.⁹ From 1995 to 1996, we randomly selected 965 living members (aged, 60-90 years) of this cohort in strata of sex and age (5 years) for participation in the Rotterdam Scan Study.¹⁰ After exclusion of individuals who had dementia ($n=16$),¹¹ or had contraindications to undergo MRI ($n=117$), 832 persons were eligible for our study. Among these, 563 participants gave their written informed consent to participate in the study (response rate, 68%), which included undergoing a MRI brain scan. The study was approved by the medical ethics committee of Erasmus MC, Rotterdam, the Netherlands.

MRI procedures

All subjects underwent T1-, T2-, and proton-density-weighted images in a 1.5-T magnetic resonance unit (VISION MR; Siemens, Erlangen, Germany).¹² For volumetric measurements of the hippocampus, a custom-made, inversion recovery—double contrast, 3-dimensional, half-Fourier acquisition single-shot turbo spin-echo (HASTE) sequence was included (inversion time, 440 milliseconds; repetition time, 2800 milliseconds; 128 contiguous sagittal slices of 1.2-mm; acquisition matrix, 192×256 pixels; and field of view, 256×256 cm). Two HASTE

modules were sequentially acquired after the inversion pulse (effective echo times of 29 milliseconds and 440 milliseconds). Each HASTE module combined nonselective radiofrequency excitations to provide a short interecho spacing of 3.9 milliseconds. The first HASTE module was used for the hippocampal volume measurement.

Hippocampal volumes

We reconstructed a series of coronal brain slices (contiguous 1.5-mm slices) based on the HASTE sequence, aligned to be perpendicular to the long axis of the hippocampus. All reconstructed slices were transferred to a workstation (Magic View 1000; Siemens) for volumetric assessment of the left and right hippocampi (Figure 1).

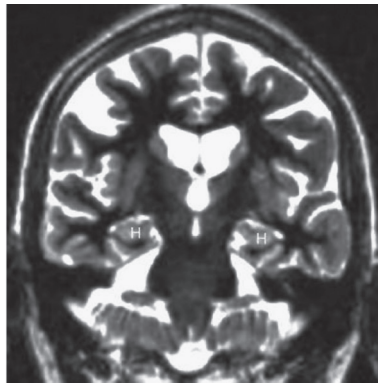


Figure 1. Coronal slice on which left and right hippocampus (H) are depicted

Referencing to an anatomical atlas,¹³ we manually traced the boundaries of both hippocampi on each slice using a mouse-driven pointer. We proceeded from posterior to anterior, starting on the slice where the crux of the fornices was in full profile. The alveus could often be used to delineate the boundary of the hippocampal head from the amygdala. Entering the outlined surface areas (expressed in millimeters squared), we multiplied the summed surface areas on each side with slice thickness to yield estimates of the left and right hippocampal volume (expressed in milliliters). In the current analyses the left and right hippocampal volumes were summed.

We also reconstructed a midsagittal slice (thickness 3.0 mm). The midsagittal area, which was used as a proxy for intracranial volume, was measured by tracing the inner table of the skull. Two raters (T.d.H. and a colleague) who were blinded to any clinical information related to the participants, assessed the scans. Studies performed on 14 random scans to evaluate intrarater and interrater correlation showed good overall agreement.

Memory performance

All participants underwent neuropsychological testing.¹² The Mini-Mental State Examination was administered to assess global cognitive function.¹⁴ Memory function was evaluated by a 15-word learning test based on the Rey Auditory Verbal Learning Test.¹⁵ This test consists of 3 learning trials in which 15 words have to be remembered. After 15 minutes, the subject is asked to recall as many words as possible (delayed recall). In the present study we used the delayed recall score because this score is most strongly associated with hippocampal volumes¹⁶ and with early AD.¹⁷

Blood measurements

Venapuncture was done in nonfasting subjects between 8:30 AM and 4 PM at baseline examination of the Rotterdam Scan Study (1990-1993). Blood samples were collected in 5-ml tubes containing a 0.5-ml sodium citrate solution. All tubes were stored on ice before and after blood sampling. Platelet-free plasma was obtained by 2-stage centrifugation (10 minutes at 1600g at 4°C and 30 minutes at 7000g at 4°C). Platelet-free samples were immediately frozen in liquid nitrogen and stored at -80°C. Assays were performed blinded to information on the subject. Plasma levels of estradiol and sex hormone-binding globulin were estimated with double antibody radioimmunoassays (ultrasensitive method for estradiol; Diagnostic Systems Laboratories, Webster, Tex). Because of the small volumes of plasma available, all estradiol levels are single sample estimations. Intra-assay coefficients of variation, determined on the basis of duplicate results of internal quality control pools with 3 different levels of each analyte, were below 4% for sex hormone-binding globulin and 18% for estradiol. Because inter-assay variations were 14% (sex hormone-binding globulin) and 21% (estradiol), results of all batches were normalized by multiplying all concentrations within a batch with a factor, which equalized results for the internal quality control pools. As measures of the levels of bioavailable and free estradiol, nonsex hormone-binding globulin-bound estradiol, and nonprotein-bound estradiol, respectively, were calculated on the basis of hormone and binding protein levels.^{18, 19} The median interval from blood sampling (1990-1993) to hippocampal volumes and memory testing (1995-1996) was 2.9 years (range, 1.6-6.6 years).

Covariates

Several variables may confound an association between estradiol levels and hippocampal volume or memory performance, such as age at the time of venapuncture, educational level, smoking habits, alcohol intake, body mass index (BMI) (calculated as weight in kilograms divided by the height in meters squared), depressive symptoms, apolipoprotein E (APOE) genotype, and for women, the variables included type of menopause, age at menopause, and use of hormone replacement therapy (HRT) (ie, those who ever used HRT or those who never

used HRT) for menopausal reports (Anatomical Therapeutic Chemical code g03). The level of education was dichotomized into primary education and lower vocational training and university education. We categorized smoking status into current, former, and never and made dummy variables for the analyses. Alcohol intake was recorded in grams per day.²⁰ Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D)²¹ at time of assessment of memory performance. Apolipoprotein E genotyping was done according to standard methods²² and was coded into non-APOE ϵ 4 carrier and APOE ϵ 4 carrier. Type of menopause was classified into natural or artificial (surgically or chemically induced). Age at natural menopause was defined as the age at which menses had not occurred for at least 1 year. Type and age of menopause were combined in 1 categorical variable with 4 groups: (1) artificial menopause, (2) natural menopause before the age of 48 years, (3) natural menopause between ages 48 and 52 years, or (4) natural menopause after the age of 52 years.

Study sample

To obtain the study samples for the analyses, we excluded 4 women who used HRT at the time of blood drawing, leaving 559 subjects (Figure 2). Because estradiol levels were measured as part of several blood measurements and small amounts of plasma were available, we randomly missed total estradiol levels for 147 subjects. Thus, 412 subjects for the analyses on total estradiol levels were included. Because of missing data on binding protein levels ($n=101$), we had bioavailable and free-estradiol levels in 311 subjects. These 311 subjects with bioavailable and free-estradiol levels were similar to the 248 subjects who were dropped from these analyses for age, hippocampal volume, and delayed recall score.

Data analysis

All analyses were performed in women and men separately. We first investigated whether there was a threshold in the relation between estradiol level and outcome by analysis of covariance (ANCOVA). Tertiles of total, bioavailable, and free estradiol were the group variable and we calculated adjusted mean hippocampal volumes and delayed recall scores in each tertile. Three consecutive models were used. The first adjusted for age and—in the hippocampal analyses—midsagittal area to account for intracranial volume.²³ After separately investigating several covariates, it appeared as if the BMI was the strongest confounder in the associations so the second model was adjusted, in addition to age and midsagittal area, for BMI. In the third model we additionally entered educational level, smoking, alcohol use, CES-D score, APOE genotype, and, for women, age at menopause, type of menopause, and use of ever taking HRT. Because the ANCOVA did not suggest a specific threshold in the relation between estradiol levels and outcome, we performed a multivariate linear regression analysis

to calculate the difference in hippocampal volume and delayed recall score per SD increase in estrogen levels. To investigate whether there was an indication of differential associations in age strata (<70 years and ≥70 years) or APOE genotype, we included in the multivariate linear model an interaction term and performed stratified analyses. Finally, we repeated the analyses excluding women with a history of HRT use.

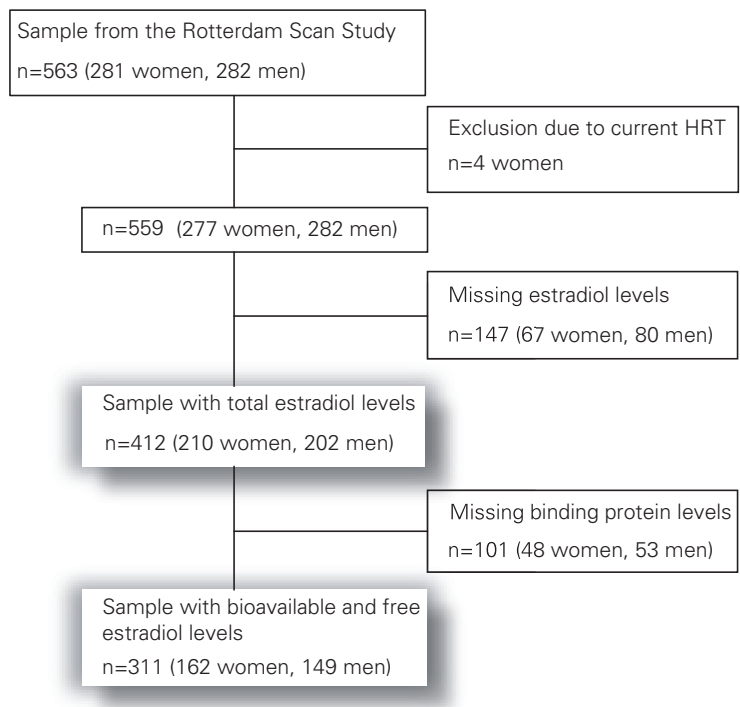


Figure 2. Description of study sample

Results

Of the 210 women and 202 men with total estradiol measurements, 24 women and 16 men had no hippocampal volume assessment (because they developed claustrophobia during the MRI) and 6 women and 8 men had no reliable delayed recall data (mostly owing to motivational problems). Characteristics of the study sample are given in Table 1. There were no differences in the characteristics between subjects with total estradiol levels and subjects without

total estradiol levels. Estradiol levels of women were lower than those of men, reflecting the fact that women were postmenopausal and were not taking HRT at time of venipuncture.

Table 1. Characteristics of study sample

Characteristic	Women (n=210)	Men (n=202)	Subjects without total estradiol levels (n=147)
Age, years	70 ± 8	69 ± 8	71 ± 8
BMI, kg/m ²	26.8 ± 3.9	26.1 ± 2.9	26.3 ± 3.2
Primary education, %	37	23	34
Current smoking, %	16	24	23
Alcohol, g/day	7.6 ± 11.5	17.4 ± 10.9	13.7 ± 17.0
CES-D score	7.0 ± 6.6	4.6 ± 5.3	5.0 ± 5.7
APOE genotype, % ε4 carrier	27	32	27
MMSE score	27.6 ± 2.3	27.8 ± 2.1	27.7 ± 2.0
Total estradiol median (range), pmol/l	13.4 (0.0-65)	45.3 (0.0-157)	-
Bioavailable estradiol median (range), pmol/l*	8.8 (0.0-49)	34 (0.0-120)	-
Free estradiol median (range), pmol/l*	0.32 (0.00-1.73)	1.22 (0.00-4.20)	-
Midsagittal area, cm ² †	143.1 ± 8.8	155.2 ± 9.9	149.3 ± 11.1
Hippocampus, ml†	6.17 ± 0.81	6.59 ± 0.89	6.36 ± 0.88
Delayed recall score‡	6.2 ± 2.8	5.5 ± 2.4	5.4 ± 2.6

Values are means ± standard deviation or percentages unless otherwise specified

*Values were present in 162 women and 149 men

†Values were present in 186 women and 186 men

‡Values were present in 204 women and 194 men. Higher scores reflect better performance

In women, estradiol levels were negatively correlated with age and positively correlated with BMI. In men, estradiol levels were negatively correlated with age and positively correlated with alcohol intake and CES-D score (Table 2). Hippocampal volumes and delayed recall scores decreased with increasing age (both in women and men, −0.03 ml/y and −0.1 words per year).

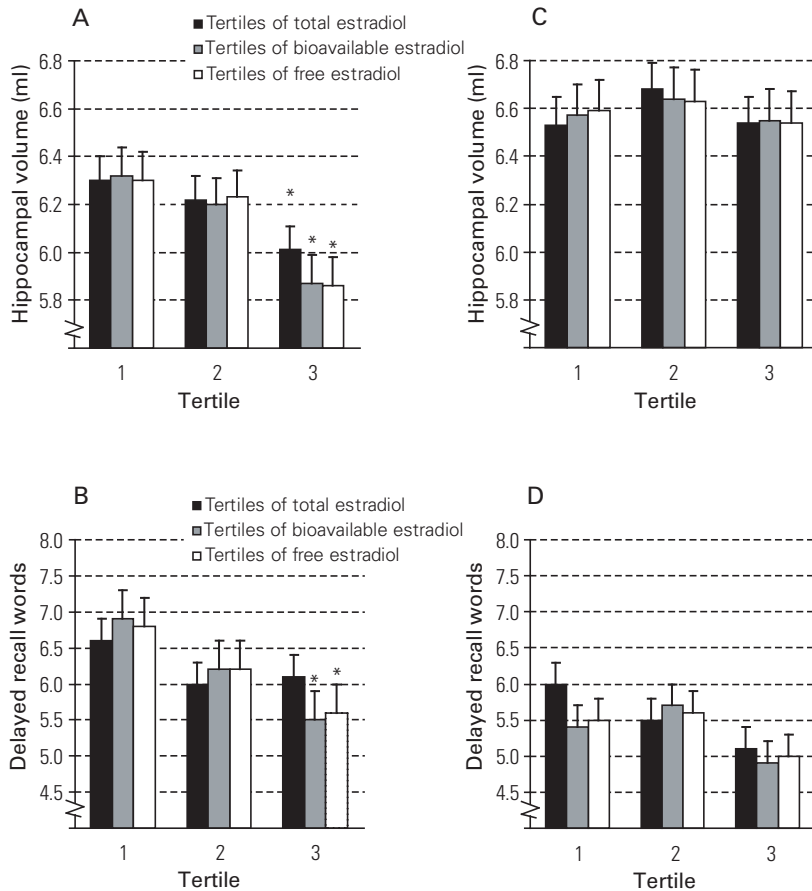


Figure 3. Adjusted hippocampal volumes (SE) (A and C) and delayed recall (SE) (B and D) across tertiles of total, bioavailable, and free-estradiol levels in women and men, respectively. A and B, Means are adjusted for age, educational level, BMI, alcohol, smoking, CES-D, type of menopause, age at menopause, ever use of HRT, APOE genotype, and the hippocampal volumes–midsagittal area. Tertile ranges (in picomoles per liter) were for total estradiol levels 0.0-7.3, 7.3-19, and 20-65; for bioavailable estradiol levels 0.0-5.2, 5.2-14, and 14-49; and for free-estradiol levels 0.00-0.19, 0.19-0.50, and 0.50-1.73. Asterisks indicate statistically significantly ($P < 0.05$) smaller than lowest tertile. C and D, Means are adjusted for age, educational level, BMI, alcohol, smoking, CES-D, APOE genotype, and the hippocampal volumes–midsagittal area. Tertile ranges (in picomoles per liter) were for total estradiol level 0.0-36, 36-52, and 52-157; for bioavailable estradiol level 0.0-27, 27-40, and 40-120; and for free-estradiol level 0.00-0.97, 0.97-1.49, and 1.49-4.20

Table 2. Correlations between several continuous variables in women (n=210) and in men (n=202)

	Total estradiol	Age	Education	BMI	Alcohol intake	CES-D
Age	-0.49*					
(per year)	<i>-0.62*</i>					
Education	-0.08	-1.02*				
(per grade)	<i>-0.87</i>	<i>-0.22</i>				
BMI	0.97*	0.24	-0.05*			
(per kg/m ²)	<i>1.04</i>	<i>0.04</i>	<i>-0.06</i>			
Alcohol intake	0.01	-0.13*	0.01	-0.03		
(per grams/day)	<i>0.33*</i>	<i>-0.04</i>	<i>-0.00</i>	<i>0.02</i>		
CES-D	-0.27	0.20*	-0.02	-0.04	0.02	
(per point)	<i>0.77*</i>	<i>-0.05</i>	<i>0.02</i>	<i>0.03</i>	<i>0.20</i>	
Midsagittal area	0.23	-0.22*	0.02*	0.01	-0.05	-0.10*
(per cm ²)	<i>0.14</i>	<i>-0.07</i>	<i>0.02</i>	<i>-0.01</i>	<i>-0.11</i>	<i>0.05</i>

Values are unadjusted regression coefficients in women and in men (*italic numbers*). The variables in the left column are the independent variables and the variables in the upper row the dependent variables.

* $P < 0.05$

Women

Estradiol level and hippocampal volume

In women, the age- and midsagittal-adjusted mean hippocampal volumes decreased across higher tertiles of total (SE) estradiol levels from 6.24 (0.10) ml to 6.22 (0.10) ml to 6.07 (0.10) ml, but there were no significant differences between tertiles. When the BMI was included as a covariate, the mean (SE) hippocampal volumes were 6.28 (0.10) ml; 6.21 (0.10) ml, and 6.04 (0.10) ml, respectively, but differences were again not statistically significant. Figure 3A shows that after additional adjustments for all other covariates the negative association between the total estradiol level and hippocampal volume became statistically significant (difference between the highest and lowest tertile of total estradiol -0.29 ml [95% confidence interval (CI), -0.57 to -0.00]). When we analyzed the total estradiol level as a continuous measure, we found a nonsignificant decrease in hippocampal volume per SD increase in total estradiol level (Table 3). There was a nonsignificant inverse relation between the bioavailable estradiol level and hippocampal volumes (the age- and midsagittal-adjusted means of hippocampal volume across higher bioavailable estradiol tertiles was 6.22 (0.12) ml, 6.17 (0.11) ml, and 6.00 (0.11)

ml. When the BMI was included in the model, a significant smaller hippocampal volume was found in women with bioavailable estradiol levels in the highest tertile of bioavailable estradiol compared with the lowest tertile (difference, -0.37 ml [95% CI, -0.71 to -0.03]). Additional adjustments did not change this inverse relation (Figure 3A). When we analyzed bioavailable estradiol levels as a continuous measure, we found a nonsignificant decrease of hippocampal volume of -0.09 ml (95% CI, -0.25 to 0.06) per SD (10-pmol/L) increase.

Table 3. Associations between total estradiol levels, hippocampal volumes and memory performance

Women	Per increase of 1 SD (14 pmol/l) in total estradiol level	
Adjusted for	Hippocampal volume (ml)	Delayed recall
Age, midsagittal area*	-0.01 (-0.12 to 0.10)	-0.1 (-0.4 to 0.3)
Age, midsagittal area*, BMI	-0.04 (-0.16 to 0.08)	-0.1 (-0.5 to 0.3)
Age, midsagittal area*, BMI, education, alcohol intake, smoking, CES-D, APOE genotype, age and type of menopause, ever use of HRT	-0.06 (-0.18 to 0.06)	-0.1 (-0.5 to 0.3)
Men	Per increase of 1 SD (23 pmol/l) in total estradiol level	
Adjusted for	Hippocampal volume (ml)	Delayed recall
Age, midsagittal area*	0.02 (-0.10 to 0.15)	-0.4 (-0.7 to -0.0)
Age, midsagittal area*, BMI	-0.01 (-0.05 to 0.03)	-0.4 (-0.7 to 0.0)
Age, midsagittal area*, BMI, education, alcohol intake, smoking, CES-D, APOE genotype	0.04 (-0.09 to 0.18)	-0.3 (-0.7 to 0.1)

Values are adjusted regression coefficients (95% CI) of the relation between total estradiol level and hippocampal volume/delayed recall.

*Adjusted for only in the hippocampal analyses

The relation between free estradiol levels and hippocampal volumes was similar to that of bioavailable estradiol levels (Figure 3A). The inverse associations between estradiol levels and hippocampal volumes were primarily seen in the oldest age group (in age < 70 years: per SD

increase in total estradiol level the fully adjusted difference in hippocampal volumes was 0.07 ml [95% CI, -0.09 to 0.23]; in age ≥ 70 years: -0.24 ml [95% CI, -0.44 to -0.04]; *P* of interaction term 0.04). The negative associations between estradiol level and hippocampal volumes were more pronounced in APOE $\epsilon 4$ carriers than in non-APOE $\epsilon 4$ carriers, although the interaction term was not statistically significant (Table 4).

Table 4. Associations between total estradiol levels and hippocampal volumes and memory performance in women according to APOE $\epsilon 4$ strata

Adjusted for	Hippocampal volumes		
	Non-APOE $\epsilon 4$ carrier	APOE $\epsilon 4$ carrier	<i>P</i> interaction term*
Age, midsagittal area, BMI, education, alcohol intake, smoking, CES-D, age and type of menopause, ever use of HRT	0.01 (-0.16 to 0.17)	-0.25 (-0.46 to -0.03)	0.07
Adjusted for	Delayed recall		
	Non-APOE $\epsilon 4$ carrier	APOE $\epsilon 4$ carrier	<i>P</i> interaction term*
Age, BMI, education, alcohol intake, smoking, CES-D, age and type of menopause, ever use of HRT	-0.10 (-0.61 to 0.41)	0.44 (-0.21 to 1.09)	0.29

Values are adjusted regression coefficients (95% CI) of the relation between total estradiol level (per 1 SD increase) and hippocampal volume/delayed recall

* *P*-value of interaction term (APOE strata x total estradiol level) in the multivariate linear regression analysis

Estradiol level and memory

The age-adjusted delayed recall score decreased nonsignificantly across total estradiol tertiles from 6.5 (0.3) to 6.1 (0.3) to 6.1 (0.3) words. Figure 3B shows that additional adjustments for other covariates did not substantially change these results. The fully adjusted difference in delayed recall words between the highest and lowest tertile of total estradiol levels was -0.4 (95% CI, -1.3 to 0.5). When we analyzed the total estradiol level as a continuous measure,

we found a nonsignificant decrease in delayed recall words (Table 3). There was a significant inverse relation between bioavailable estradiol tertiles and delayed recall (Figure 3B), although when we analyzed bioavailable estradiol levels continuously, we found a nonsignificant decrease in delayed recall words of -0.2 (95% CI, -0.7 to 0.2) per SD increase. The results were similar for the relation between the free estradiol level and delayed recall score (Figure 3B). Again, the inverse associations between the estradiol level and memory were stronger in the oldest age groups (data not shown). The associations were not different in APOE $\epsilon 4$ strata (Table 4). Exclusion of women who had used HRT in the past ($n=17$) did not change any of the above results.

Men

Estradiol level and hippocampal volume

In men, there was no association between estradiol levels and hippocampal volumes (age and midsagittal-adjusted hippocampal volumes in total estradiol tertiles: 6.55 [0.11] ml, 6.68 [0.11] ml, and 6.52 [0.11] ml). Additional adjustments for the other covariates did not change these estimates (Figure 3C and Table 3). There was also no association between bioavailable or free-estradiol levels and hippocampal volume (Figure 3C). There were no differential associations in age strata or in APOE $\epsilon 4$ strata (data not shown).

Estradiol level and memory

When we investigated the relation between estradiol levels and memory performance in men, a similar pattern as in women emerged. The age-adjusted delayed recall scores decreased across increasing total estradiol tertiles from 6.1 (0.3) to 5.4 (0.3) to 5.1 (0.3) words. When we analyzed the total estradiol level as continuous measure, we found a decrease in delayed recall with increasing levels (Table 3). Additional adjustments for covariates did not change this association (Figure 3D and Table 3). The association of delayed recall score with bioavailable and free estradiol levels showed a similar pattern as with the total estradiol level (Figure 3D). There were no differences in the associations in age strata or in APOE $\epsilon 4$ strata (data not shown).

Discussion

In this population-based study in elderly subjects with no dementia, we could not support the hypothesis that higher endogenous levels of estradiol are associated with larger hippocampal volumes on MRI and better memory performance. Women with higher estradiol levels had smaller hippocampal volumes and poorer memory performance than women with lower levels. In men, there was no relation between estradiol level and hippocampal volumes, but

memory performance was worse in those with higher levels of total estradiol compared with those with lower levels.

The strengths of this study are that it is population based and involves a large number of volumetric hippocampal assessments. Our study has several methodological limitations that need to be discussed. First, owing to the small amounts of plasma available, we had a large number of missing data on bioavailable and free-estradiol levels. Although these missing data were random, the smaller sample size will have resulted in less precise estimates of the associations. Second, blood was drawn several years before MRI and memory performance. It is difficult to determine if this influenced our results. It may have led to biased associations if subjects with estradiol levels on one side of the distribution selectively died between blood drawing and MRI measurement. This does not seem likely, however, because the interval between blood drawing and MRI was not long, and the relation between endogenous estradiol levels and mortality is not strong.²⁴ Third, HRT use in the Netherlands is infrequent compared with, for example, the United States. Our results were based on observations in non-HRT users and they may not be generalizable to HRT users.

The most consistent support for the hypothesis that estrogens prevent dementia comes from animal studies that showed that estrogens protect against cell loss in the hippocampus, increase neurite outgrowth, and have antioxidative properties.^{3, 25} In humans, observational studies on endogenous levels of estrogen in relation to cognitive function have been inconclusive. Some reported negative effects of higher endogenous estrogen levels on delayed visual reproduction²⁶ and attention tasks²⁷ in women and on the results of the MMSE, memory tasks,²⁸ and spatial performance²⁹ in men, while others showed in women positive effects of higher endogenous estrogen levels on verbal memory³⁰ or cognitive decline.³¹ Population-based studies on exogenous estrogens showed that women who used HRT had a reduced risk of AD^{1, 2, 32} but these studies may have been confounded by healthy-user effect. Also, other studies did not confirm this observation.³³⁻³⁵ Recent randomized trials with HRT in patients with AD showed no³⁶⁻³⁸ or a beneficial effect of exogenous estrogen on cognitive decline.³⁹ Together, the biological plausibility of the estrogen hypothesis in dementia is its strongest plea, whereas studies in humans are far from conclusive. Our results are contrary to what we expected in that we observed a small negative effect of higher estradiol levels on both hippocampal volumes in women and memory performance in women and men. As yet, we do not have a biological plausible explanation for these results. When interpreting these data, one has to keep in mind that the endogenous levels in our study sample of postmenopausal women are much lower than the levels obtained after supplemental use of exogenous estrogens. Therefore, our data do not reject the possibility that exogenous estrogens are related to larger hippocampal volumes and better memory performance.

Conclusion

This study does not support the hypothesis that higher endogenous estrogen levels in older women and men without dementia are associated with larger hippocampal volumes and better memory performance.

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Chapter 3.2

Estrogen receptor α polymorphisms, dementia and atrophy of the medial temporal lobe

The role of estrogens in Alzheimer's disease is controversial. We investigated the association between well-recognized, and potentially functional, polymorphisms in the estrogen receptor (ER) α gene and risk of Alzheimer's disease in a large prospective study of 6056 Caucasian elderly aged 55 years and over. Furthermore, we performed a meta-analysis based on published results of 5 other studies. In a subset of 468 non-demented elderly we assessed volumes of the hippocampus and amygdala, which have a high density of ER α , by means of brain magnetic resonance imaging (MRI). During a total of 35405 person-years of follow-up (mean per persons 5.8 years), 312 new cases of dementia were detected of whom 230 were diagnosed with Alzheimer's disease. Neither the PvuII nor the XbaI polymorphism or haplotypes thereof were associated with the risk of all-cause dementia or Alzheimer's disease, which was confirmed by the meta-analysis. In contrast, we found that non-demented women who carried the PvuII p allele or haplotype 'px' had smaller amygdalar volumes on MRI in an allele-dose dependent fashion. Further studies are required to investigate whether this smaller amygdalar volume has functional significance.

Introduction

In the last decade of the 20th century numerous cellular, molecular and epidemiological studies suggested a protective effect of estrogen on Alzheimer's disease. Estrogen protects against neuronal death,¹ improves survival of hippocampal neurones,² acts as an antioxidant,³ and stimulates cerebral blood flow.⁴ Observational studies reported that postmenopausal women who used estrogen replacement therapy have a lower risk of dementia.^{5, 6} Conversely, a large randomised trial on estrogen replacement therapy for preventing Alzheimer's disease was negative.⁷ This, in turn, is in line with findings from our group that higher endogenous estradiol levels, if anything, are associated with a higher risk of dementia.⁸

Estrogens exert most of their effects through intracellular activation of estrogen receptors. There are two known forms of estrogen receptors: the estrogen receptor α (ER α) and the estrogen receptor β , both expressed in the brain.^{3, 9} Several studies examined the PvuII and XbaI polymorphisms in the gene for ER α in association to Alzheimer's disease with different results.¹⁰⁻¹⁶ Except for one prospective study,¹⁰ most reports were based on case-control series with inherent limitations of selection.¹¹⁻¹⁶ The aim of the current study was to investigate polymorphisms in the ER α gene and risk of dementia in a large prospective population-based cohort study in the Netherlands. We included a meta-analysis of all published data. In a subset of the cohort, MRI of the brain was made to assess volumes of the hippocampus and amygdala. The hippocampus and amygdala are structures in the medial temporal lobe that express ER α at relatively high levels.⁹ If ER α polymorphisms are associated with macroscopic changes in brain volume, we would expect to find it for these two structures. An additional rationale for studying these structures is that hippocampal and amygdalar atrophy on MRI are seen early in Alzheimer's disease.^{17, 18} The putative risk on Alzheimer's disease associated with ER α polymorphisms could be mediated through effects on the hippocampus and amygdala. The ϵ 4 allele of the apolipoprotein E (APOE) is a well-known genetic risk factor for Alzheimer's disease,¹⁹ and estrogens influence regulation of APOE synthesis.²⁰ Previous reports suggested differential effects of the ER α genotype on risk of Alzheimer's disease within APOE ϵ 4 or non- ϵ 4 carriers,^{12, 14} therefore we performed stratified analyses according to APOE genotype.

Methods

Study population

This study is based on the Rotterdam Study, a large prospective population-based study in 7983 elderly aged 55 years or older that aims to assess determinants of diseases in later life.²¹ The Medical Ethics Committee of Erasmus Medical Center approved the study protocol and participants gave written informed consent. Baseline examinations took place from 1990 to

1993. A trained research assistant visited participants at home and obtained information on medication use, medical history and determinants of diseases. Participants were invited to visit the research center for clinical examinations by the research physicians. Re-examinations were performed in 1993 to 1994 and 1997 to 1999. Of the 7983 participants at baseline, 7528 (94.3%) were screened and examined for prevalent dementia. There were 482 participants diagnosed with prevalent dementia.²² Thus, 7046 participants comprised the cohort at risk for dementia of whom 6934 were of Caucasian origin.

Dementia diagnosis

Participants were examined for presence of dementia at the research center at follow-up visits. A three-step protocol was used as described in detail elsewhere.²³ Briefly, all participants were screened with the Mini-Mental State Examination (MMSE) and the Geriatric Mental State schedule (GMS), organic level. Screen positives (persons scoring below 26 on the MMSE or more than 0 on the GMS) underwent cognitive testing with the Cambridge examination for mental disorders in the elderly, which included an informant interview. Persons suspected of dementia were examined by a neurologist and underwent extensive neuropsychological testing. In addition to this in-person screening,²⁴ all participants were continuously monitored for development of dementia through linkage of the general practitioners' medical record system to the database of the Rotterdam Study. We also consulted the Regional Institute for Outpatient Mental Health Care (RIAGG) at a regular basis and checked all relevant reports. Surveillance of the population through the general practitioner and RIAGG reports continued up to December 31, 1999. A diagnosis of dementia and its subtypes were made according to international standard criteria by a panel consisting of a neurologist, neuropsychologist and study physician.^{25, 26}

Subset with MRI examinations

In 1995 to 1996, we made a random selection of 965 living members of the cohort in strata of age (5-years strata from 60 to 90) and sex for participation in the Rotterdam Scan Study, designed to study age-related brain changes on MRI.²⁷ As part of the eligibility criteria, we excluded persons who had developed dementia between baseline and time of selection ($n=17$) and persons who had contraindications to undergo MRI ($n=116$). This left 832 participants eligible. Complete MRI data, including a three-dimensional (3D) volumetric MRI sequence covering the whole brain, was obtained in 511 persons.

MRI procedures

We reformatted coronal brain slices (contiguous 1.5-mm slices) and a middle sagittal slice from the 3D MRI sequence. The coronal slices were orientated perpendicular to the long

axis of the hippocampus. The procedure of segmenting the hippocampus and amygdala on coronal slices has been described.²⁸ The left and the right hippocampus and amygdala were manually outlined on each slice with a mouse-driven cursor.²⁹ Absolute volumes (ml) were calculated by multiplying the areas on each slice by the slice thickness and left and right side were summed. As a proxy for head size, we measured on a reformatted middle sagittal MRI slice the intracranial cross-sectional area.²⁹ We corrected for head size differences across individuals by dividing the uncorrected volumes by the subject's calculated head size area and subsequently multiplying this ratio by the average head size area (men and women separately).^{18, 30}

ER α polymorphisms

Genomic DNA was isolated from peripheral leucocytes by standard procedures. Genotypes were determined using the Taqman allelic discrimination assay. Primer and probe sequences were optimized using the single nucleotide polymorphism assay-by-design service of Applied Biosystems. For details see <http://store.appliedbiosystems.com>. Reactions were performed on the Taqman Prism 7900HT 384 wells format. The coding for the ER α polymorphisms PvuII and XbaI follows that of previous studies on the ER α polymorphisms. Capital "P" and "X" stands for absence of a restriction site (indicating respectively nucleotide C and nucleotide G) and lower case letter "p" and "x" stands for presence of restriction site (nucleotide T and nucleotide A). Persons were classified as either non-carrier, heterozygous or homozygous for the allele. To increase genetic resolution we created also PvuII-XbaI haplotypes as reported previously.³¹ Haplotype alleles were coded as haplotype numbers 1 through 4 in order of decreasing frequency in the population (1=px, 2=PX, 3=Px, and 4=pX). We examined the polymorphisms PvuII and XbaI separately in relation to outcome to make our results comparable with earlier studies on dementia and examined haplotype 1 based on previous work from our group showing this to be the risk allele of osteoporosis.³¹

Analytical study samples

We missed genotyping of the ER α polymorphism in some persons because no blood was drawn or there were problems in the technical processing of the blood samples. Of the 6934 members of the cohort at risk for dementia, we determined the PvuII and XbaI genotype in 6056. The 878 persons without genotype were on average 4.8 years older ($P<0.001$); and more frequently women (67% versus 59%; $P<0.001$) compared to the 6056 participants with genotype. Of the 511 participants in the subset with MRI examinations, we determined the ER α genotype in 468 participants. The remaining 43 participants were similar in mean age ($P=0.31$) but more frequently women (63% versus 48%, $P=0.06$) compared to the 468 participants with genotype.

Other measurements

At baseline examinations, we ascertained by interview or physical examination level of education (primary versus lower vocational to university education), smoking status (current, former or never), body mass index (weight divided by the square of the height), age at menopause (either natural or surgically induced)³² and use of estrogen replacement therapy (ever versus never). APOE genotype was determined in 5281 participants of the total cohort and in 404 participants of the subset with MRI examination.³³ We classified participants into those with or without the $\epsilon 4$ allele.

Data analysis

Analyses were done separately in men and women. We compared baseline characteristics in ER α genotype using one-way analysis of variance or χ^2 analysis in the total study cohort. The hazard ratios of dementia and Alzheimer's disease were estimated using a Cox proportional hazard model in which time scale is the age of participants. Left truncation of the cohort was accounted for by using the "counting process" notation available in S-Plus.³⁴ In the subset with MRI examinations, we used analysis of covariance to compute age adjusted means of the brain volumes on MRI within genotype. Because preliminary analyses did not suggest a dominant or recessive effect of the polymorphisms, we analyzed the association using an allele-dose model. The genotype was entered as a linear term in the model to yield *P*-values of the allele-dose trend. Besides age, we additionally included the following confounders in the analyses that have previously been associated to dementia: level of education,²² body mass index,³⁵ smoking,³⁶ age at menopause,³⁷ and ever use of estrogen replacement therapy.³⁸

Meta-analysis

We identified studies on the ER α polymorphisms and Alzheimer's disease by searching Medline (1966 to May 2003) using "Alzheimer's disease" and "estrogen receptor" as key words. The reference list of each paper was checked for additional studies. Seven papers were identified¹⁰⁻¹⁶ of which five investigated Caucasian populations.^{10, 13-16} These five papers reported on six independent case-control series and one prospective study. Odds ratios (OR) of Alzheimer's disease with 95% confidence interval (CI) were computed for each study. In case a paper did not report results in men and women separately we contacted the authors to provide these data. From one author we could not retrieve the data and this study was excluded.¹⁶ The meta-analyses were based on the log odds ratios and their standard errors and we used random effects models to compute the combined OR of Alzheimer's disease across ER α genotype.

Results

Allele frequencies of the ER α polymorphisms were in Hardy-Weinberg equilibrium and similar to frequencies found in other Caucasian populations (Table 1).^{39, 40}

Table 1. ER α polymorphisms (PvuII and XbaI) in total study sample and in subset with MRI examinations

		Total sample (n=6056)		Subset with MRI (n=468)	
		Men (n=2483)	Women (n=3573)	Men (n=244)	Women (n=224)
PvuII*					
Allele	P	2326 (47)	3312 (46)	235 (48)	199 (44)
	p	2640 (53)	3834 (54)	253 (52)	249 (56)
Genotype	PP	539 (22)	780 (22)	58 (24)	39 (17)
	Pp	1248 (50)	1752 (49)	119 (49)	121 (54)
	pp	696 (28)	1041 (29)	67 (27)	64 (29)
Hardy-Weinberg†		0.64	0.40	0.72	0.16
XbaI*					
Allele	X	1720 (35)	2487 (35)	183 (38)	157 (35)
	x	3246 (65)	4759 (65)	305 (62)	291 (65)
Genotype	XX	290 (12)	445 (13)	37 (15)	23 (10)
	Xx	1140 (46)	1597 (45)	109 (45)	111 (50)
	xx	1053 (42)	1531 (43)	98 (40)	90 (40)
Hardy-Weinberg†		0.49	0.37	0.47	0.19

*Numbers (%).

†P-value for test of Hardy-Weinberg equilibrium

Baseline characteristics of the total study sample and of the subset with MRI examinations are given in Table 2. Characteristics across genotype were analyzed in the total study sample. There were no significant differences in age, educational level, smoking, body mass index or

ever use of hormone replacement therapy across genotype. Women with the pp genotype had a 0.5 years later age of menopause compared to women with the PP genotype ($P = 0.03$), which confirms our previous report in a smaller part of the cohort.³²

Table 2. Baseline characteristics of the total study sample and of the subset with MRI examinations

Characteristic	Total sample (n=6056)		Subset with MRI (n=468)	
	Men (n=2483)	Women (n=3573)	Men (n=244)	Women (n=224)
Age, yr	67.9 $\pm$ 7.9	69.8 $\pm$ 9.2	68.7 $\pm$ 7.8	68.7 $\pm$ 8.3
Primary education only, %	25	44	22	38
Current smoker, %	29	18	22	19
Body mass index, kg/m ²	25.8 $\pm$ 3.7	26.8 $\pm$ 4.1	26.0 $\pm$ 2.7	26.7 $\pm$ 3.9
Age at menopause, yr	-	48.8 $\pm$ 5.0	-	48.7 $\pm$ 5.1
Ever use of HRT, %	-	10	-	9
APOE, ϵ 4 carriers, %	28	28	31	28
MMSE, mean score	27.8 $\pm$ 1.7	27.5 $\pm$ 2.0	27.9 $\pm$ 1.8	27.7 $\pm$ 2.2

Values are means $\pm$ SD or percentages

The MMSE score at baseline was similar across genotype. After baseline, during 35405 persons-years of follow-up (mean per person 5.8 years), 312 participants developed dementia. A diagnosis of probable Alzheimer's disease was made in 230 participants. We found no association between ER α polymorphisms and risk of all-cause dementia or Alzheimer's disease, either in men or women (Table 3). This was corroborated by the meta-analysis (Figure 1). Additional adjustments for educational level, smoking, body mass index, age at menopause and use of estrogen replacement therapy did not change our findings. There was also no association between the ER α polymorphisms and dementia when we separately looked within APOE non- ϵ 4 carriers or ϵ 4 carriers (data not shown).

Table 3. The PvuII and XbaI ERα polymorphism and risk of incident dementia and Alzheimer’s disease in the total sample (n=6056)

All-cause dementia (n=312)				Alzheimer’s disease (n=230)			
Men (n=109)		Women (n=203)		Men (n=69)		Women (n=161)	
PvuII	n	RR (95% CI)	n	RR (95% CI)	n	RR (95% CI)	n
PP	28 (5)	1.0	41 (5)	1.0	14 (3)	1.0	34 (4)
Pp	56 (5)	0.90 (0.72-1.13)	96 (6)	1.02 (0.85-1.23)	39 (3)	1.06 (0.78-1.44)	74 (4)
pp	25 (4)	0.90 (0.78-1.05)	66 (6)	1.04 (0.94-1.15)	16 (2)	0.92 (0.76-1.12)	53 (5)
Trend		0.13		0.43		0.58	0.55
XbaI	n	RR (95% CI)	n	RR (95% CI)	n	RR (95% CI)	n
XX	15 (5)	1.0	20 (5)	1.0	9 (3)	1.0	16 (4)
Xx	47 (4)	0.87 (0.65-1.17)	98 (6)	1.25 (0.98-1.58)	31 (3)	0.92 (0.64-1.34)	76 (5)
xx	47 (5)	1.00 (0.87-1.14)	85 (6)	1.01 (0.91-1.12)	29 (3)	0.99 (0.83-1.17)	69 (5)
Trend		0.90		0.82		0.87	0.70

Values are number of cases in each genotype (% within genotype), relative risk (RR) (95% confidence interval (CI)) of dementia compared to reference genotype estimated with Cox proportional hazards models with age on the time scale. P-values for trend analyses with genotype as linear term in the model

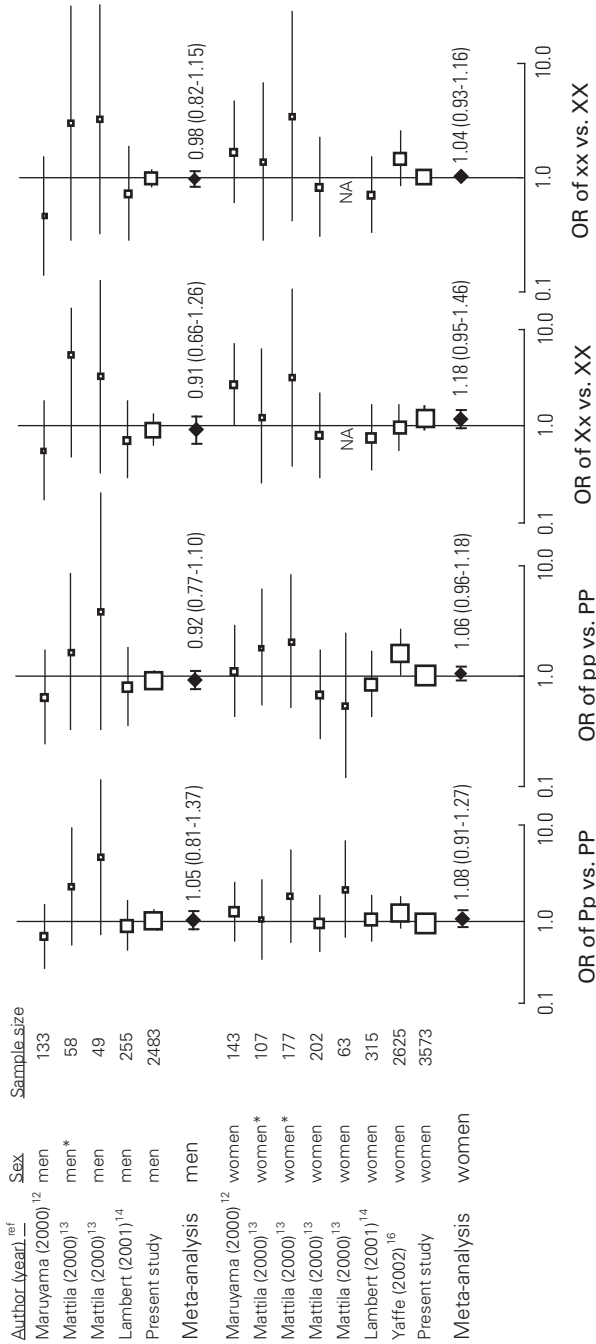


Figure 1. Odds ratio (OR) of Alzheimer's disease for the ER PvuII genotype pp or pP compared to PP genotype and the XbaI genotype xx or xX compared to XX genotype. Error bars denote 95% confidence interval. The areas of the boxes are proportional to the inverse variances of the estimated log odds ratios. * familial Alzheimer cases. NA not available due to no controls or cases with XX genotype

In the subset with MRI examinations ($n=468$), we found smaller hippocampal and amygdalar volumes on MRI in women with the pp or xx genotype compared to women with the PP or XX genotype (Table 4). There was an allele dose effect: per allele copy of the “p” allele the amygdala was -0.17 ml smaller (95% CI -0.30 to -0.05) and per allele copy of the “x” allele -0.17 ml (95% CI -0.30 to -0.04). The explained variance in amygdalar volume by the PvuII polymorphism above age was 3% (R square change 0.030, $F(1,221)=7.11$, $P=0.008$). There were 25 cases of incident dementia in the subset with MRI and excluding them did not change the results. There was no association between the ER α polymorphism and brain volumes in men. Additional adjustments for educational level, smoking, body mass index, age at menopause and use of estrogen replacement therapy did not change the results. There was no difference in the results when we stratified by APOE genotype.

Table 4. The PvuII and XbaI ER α polymorphism and brain volumes on MRI in the subset ($n=468$)

		Hippocampal volumes (ml)		Amygdalar volumes (ml)	
		Men ($n=244$)	Women ($n=224$)	Men ($n=244$)	Women ($n=224$)
PvuII					
	PP	6.56 ± 0.12	6.32 ± 0.13	4.73 ± 0.10	4.50 ± 0.10
	Pp	6.52 ± 0.08	6.22 ± 0.07	4.74 ± 0.07	4.45 ± 0.06
	pp	6.56 ± 0.11	6.06 ± 0.10	4.82 ± 0.09	4.18 ± 0.08
	Trend	0.99	0.10	0.49	0.008
XbaI					
	XX	6.38 ± 0.15	6.28 ± 0.16	4.72 ± 0.12	4.51 ± 0.13
	Xx	6.57 ± 0.09	6.28 ± 0.08	4.73 ± 0.07	4.47 ± 0.06
	xx	6.56 ± 0.09	6.06 ± 0.08	4.81 ± 0.07	4.24 ± 0.07
	Trend	0.41	0.08	0.41	0.01

Values are age adjusted mean volumes (standard error) and P values for trend analyses

The haplotype 1 (px) genotype followed exactly the PvuII genotype and therefore the analyses on haplotype 1 in association with outcome were similar as we report for the PvuII polymorphism.

Discussion

In this report based on a large population-based study and meta-analysis we show no effect of the PvuII and XbaI polymorphisms in the ER α gene on risk of dementia. However, we observed women with the p or x allele or haplotypes thereof to have smaller amygdalar volumes on MRI compared to women with the P or X allele. There was no differential effect according to APOE genotype.

Potential dangers of genetic association studies are population stratification or heterogeneity. This particularly holds for case-control studies in persons of mixed racial origin. In our study, this has played no role since all subjects were of Dutch Caucasian origin and can be considered ethnically homogeneous. Furthermore, allele frequencies were similar to those found in studies of other Caucasian subjects.⁴⁰

The disruption of ER α in ER-null mice causes memory impairment,⁴¹ a proinflammatory phenotype of microglia localized in the hippocampus and amygdala,⁴² and abolishment of the protective effect of estradiol on ischaemic brain injury.⁴³ Furthermore, brains of patients with Alzheimer's disease have less hippocampal neurons stained with ER α indicating a potential role of dysfunctional ER α in the pathogenesis of Alzheimer's disease.⁴⁴ The PvuII-XbaI polymorphisms in the ER α gene are attractive polymorphisms to investigate in association to Alzheimer's disease as they have been implicated in estrogen-related diseases such as osteoporosis,³¹ heart disease,⁴⁵ and breast cancer.⁴⁶ Also, we recently showed that women carrying the px haplotype were shorter. Given that the PvuII and XbaI polymorphisms are in strong linkage disequilibrium³¹ it is difficult to determine which of the two polymorphic sites is driving the associations. Both of them have an intronic localization and functional consequences on a biochemical level have still not been established. Interestingly though, Herrington et al.⁴⁷ recently showed that the T-allele of the PvuII polymorphism –corresponding to the “p” allele in our analyses– lacks a binding site for the transcription factor bMyb, which might result in lower ER α transcription. Nuclear ER α receptors bind to estrogen response elements in promotor regions of target genes which in turn stimulate expression of genes thought to be involved in neuroprotection.⁴⁸ Therefore, neurons expressing low ER α may be posed vulnerable to toxic events. Moreover, women with the p allele have a later age of menopause as shown in the current sample which extends our previous smaller sample³² and later age of menopause is associated with a higher risk of Alzheimer's disease.³⁷ Our findings however did not support the hypothesis that ER α PvuII-XbaI genotypes are associated to vulnerability to Alzheimer's disease. We did find the ER α PvuII-XbaI genotype to be associated to amygdalar volume in women. This could be a false positive finding. However, the population-based design, the size of the sample and the allele-dose effect of the relation supports a true association. Furthermore, genotype effects were similar, although weaker, for the hippocampus. This is in striking

similarity with the higher density of ER α in the amygdala compared to the hippocampus.^{9, 49} Although amygdalar atrophy is found in early Alzheimer's disease,¹⁸ exclusion of people who developed Alzheimer's disease during follow-up did not change the results. This limits the possibility that early Alzheimer's disease accounted for the findings on the amygdala. Does this smaller amygdalar volume then have any functional significance? The amygdala has several functions in emotional behavior and fear conditioning and amygdalar atrophy is reported in anxiety disorders.⁵⁰⁻⁵³ One may speculate that amygdalar atrophy could be the structural link between recently reported associations of polymorphisms in the ER α gene and anxiety.^{50, 51, 54} Future studies should include both amygdala volume and psychometric assessments of anxiety to test this hypothesis.

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Chapter 3.3

Diabetes mellitus and medial temporal lobe atrophy

Aim/hypothesis: Type 2 diabetes increases the risk not only of vascular dementia but also of Alzheimer's disease. The question remains whether diabetes increases the risk of Alzheimer's disease by diabetic vasculopathy or whether diabetes influences directly the development of Alzheimer neuropathology. In vivo, hippocampal and amygdalar atrophy on brain MRI are good, early markers of the degree of Alzheimer neuropathology. We investigated the association between diabetes mellitus, insulin resistance and the degree of hippocampal and amygdalar atrophy on magnetic resonance imaging (MRI) accounting for vascular pathology.

Methods: Data was obtained in a population-based study of elderly subjects without dementia between 60 to 90 years of age. The presence of diabetes mellitus and, in non-diabetic subjects, insulin resistance was assessed for 506 participants in whom hippocampal and amygdalar volumes on MRI were measured. We assessed the degree of vascular morbidity by rating carotid atherosclerosis, and brain white matter lesions and infarcts on MRI.

Results: Subjects with diabetes mellitus had more hippocampal and amygdalar atrophy on MRI compared to subjects without diabetes mellitus. Furthermore, increasing insulin resistance was associated with more amygdalar atrophy on MRI. The associations were not due to vascular morbidity being more pronounced in persons with diabetes mellitus.

Conclusions/interpretation: Type 2 diabetes is associated with hippocampal and amygdalar atrophy, regardless of vascular pathology. This suggests that type 2 diabetes directly influences the development of Alzheimer neuropathology.

Introduction

Type 2 diabetes increases the risk of stroke¹ and vascular dementia.² Recently, patients with type 2 diabetes were found to have an increased risk of the most common form of dementia, Alzheimer's disease.^{3, 4} The pathophysiological mechanism of the relation between diabetes mellitus and Alzheimer's disease is not clear. Diabetic vasculopathy can cause cerebrovascular brain damage, which is frequently found in patients with Alzheimer's disease.⁵ However, other, more direct effects of diabetes on the development of Alzheimer neuropathology can also be involved. Advanced glycation end products increase aggregation of proteins involved in Alzheimer's disease.⁶ Furthermore, dysfunction of insulin signalling in the brain has been implicated in the pathogenesis of Alzheimer's disease.⁷ The neuropathology of Alzheimer's disease occurs with greatest severity and in an early stage of the disease in the hippocampus and amygdala, brain structures in the medial temporal lobe.⁸ In vivo assessment of hippocampal volume on magnetic resonance imaging (MRI) of the brain provides a good estimate of the degree of Alzheimer neuropathology, even in elderly subjects without clinical symptoms of dementia.⁹ Several studies show that patients with mild Alzheimer's disease have smaller volumes of the hippocampus¹⁰⁻¹² and amygdala^{13, 14} on MRI compared to healthy control subjects. We examined the association between diabetes mellitus, insulin resistance and hippocampal and amygdalar atrophy on MRI using these as early MRI markers of Alzheimer's disease. We accounted for atherosclerosis and cerebrovascular disease to examine whether any association was caused by concomitant vascular disease.

Methods

Participants

The Rotterdam Study is a large population-based cohort study in the Netherlands that investigates the prevalence, incidence and determinants of chronic diseases in the elderly.¹⁵ Baseline examinations were done in 1990 to 1993. In 1995 to 1996, we randomly selected 965 living members (60-90 years of age) of the cohort in strata of sex and age (5 years) to participate in the Rotterdam Scan Study, designed to investigate age-related brain abnormalities on MRI.¹⁶ After excluding individuals who were demented ($n=17$)¹⁷ or had MRI contraindications ($n=116$), 832 people were eligible and invited. Among these, 563 participants gave their written informed consent and underwent MRI scanning of the brain (participation rate: 68 %). Participants were in general healthier than non-participants.¹⁸ The study protocol was approved by the medical ethics committee of the Erasmus Medical Center, Rotterdam, the Netherlands.

Assessment of diabetes mellitus and insulin resistance

Presence of diabetes mellitus was assessed at the baseline of the Rotterdam Study (1990-1993) and at time of MRI (1995-1996). Participants were considered to have diabetes mellitus if they reported use of oral anti-diabetic treatment or of insulin, or if they had a random serum glucose concentration greater than or equal to 11.1 mmol/l. In addition, if a post-load glucose concentration (2 h after a glucose drink of 75 g in 200 ml water) at baseline was greater than or equal to 11.1 mmol/l the participant was also considered to have diabetes mellitus. Glucose concentrations were measured by the glucose hexokinase method. Insulin resistance in non-diabetic subjects at baseline was assessed by the ratio of the post-load insulin concentration (Medgenix, Brussels, Belgium) over post-load glucose concentration.

MRI procedures

In 1995 to 1996, standard T1, T2 and proton-density weighted MR sequences of the brain were made using a 1.5 Tesla MR unit (VISION MR, Siemens, Erlangen, Germany).¹⁹ After these sequences were finished, an additional custom-made three-dimensional MRI sequence of the whole brain was acquired (named half-Fourier acquisition single-shot turbo spin echo²⁰). This three-dimensional MRI sequence was used for later volumetric assessments of the hippocampus and amygdala. A total of 52 participants developed claustrophobia during the MRI scanning period, leaving 511 participants with a completed three-dimensional MRI sequence.

Hippocampal and amygdalar volumes on MRI

For the 511 participants with a three-dimensional MRI sequence, we reformatted a series of coronal brain slices (contiguous 1.5-mm slices) aligned to be perpendicular to the long axis of the hippocampus and the middle sagittal slice. The procedure of segmenting the hippocampus and amygdala has been described in detail.²⁰ Briefly, we manually traced the boundaries of the hippocampi and amygdalae using a mouse-driven pointer (Figure 1), which yielded outlined areas (mm²). We proceeded from posterior to anterior, starting on the slice where the crux of the fornices of the hippocampus was in full profile. We multiplied the summed areas with slice thickness (1.5-mm) to calculate estimates of the left and right hippocampal and amygdalar volume (ml). Total hippocampal and amygdalar volumes were calculated by summing the left and right hippocampal volume and the left and right amygdalar volume. As a proxy for head size, we measured on the middle sagittal MRI slice the intracranial cross-sectional area.^{20, 21} We corrected for head size difference across the subjects as follows.^{13, 21} First, each subject's hippocampal or amygdalar volumes were divided by their measured head size area. Next, to obtain head size corrected, normalised volumes the ratios for each subject were multiplied by the average head size area (men and women separately).

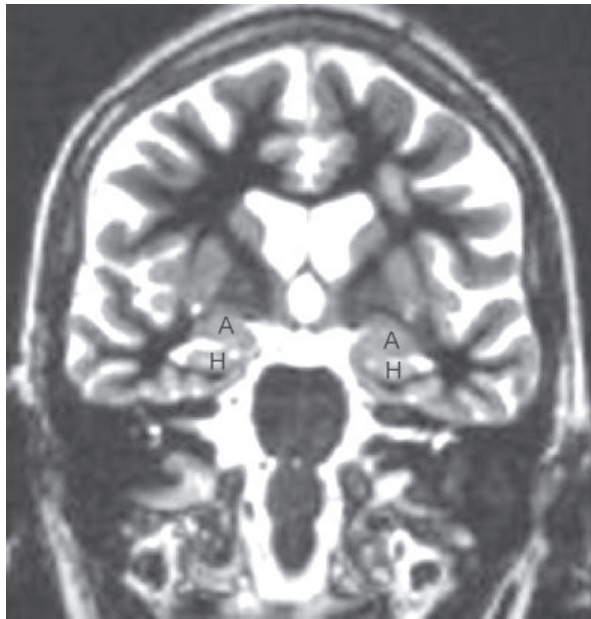


Figure 1. Coronal MRI slice on which the hippocampus (H) and amygdala (A) are depicted

Other measurements

We interviewed and gave physical examinations to the participants to obtain information on their: educational level (according to UNESCO²²), BMI (weight divided by the square of the height), pack-years of cigarette smoking, blood pressure and serum total cholesterol.²³ Memory function was evaluated with a 15-word learning test which consisted of three immediate learning trials and a delayed recall trial.¹⁹ For each participant, we calculated z-scores (individual test score minus mean test score divided by the standard deviation). We constructed a compound score for memory performance by averaging the z-score of the three immediate recall trials and the delayed recall trial.¹⁹ Apolipoprotein E (APOE) genotyping yielded the following alleles: $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$.²⁴ We classified participants into those with and without a $\epsilon 4$ allele because the $\epsilon 4$ allele is a strong risk factor of Alzheimer's disease.²⁵ Because the presence of the $\epsilon 2$ allele can reduce the risk of Alzheimer's disease,²⁵ we excluded persons with genotype $\epsilon 2\epsilon 4$ ($n=9$) in the analyses considering APOE genotype. To assess carotid atherosclerosis, participants underwent ultrasonography of the carotid arteries.²⁶ Presence of atherosclerotic plaques was determined at the common carotid artery, the carotid bifurcation, and the internal carotid artery at the left and right side and summed (range 0-6). The intima-media thickness was measured by longitudinal two-dimensional ultrasound of the anterior and posterior wall of both common carotid arteries. We calculated the mean of these four locations. Cerebral white

matter lesions were assessed on proton-density weighted axial MR images and were scored in the periventricular regions (range 0-9) and the subcortical regions (approximated volume).¹⁹ Brain infarcts were defined as focal hyperintensities on T2 weighted images, and, if present in the white matter, with corresponding prominent hypointensity on T1.²³

Data analysis

We missed information on the presence of diabetes mellitus in five participants, leaving a total of 506 participants for the analyses. We used multiple linear regression modelling to quantify the relation between diabetes, insulin resistance in non-diabetic subjects, and MRI volumes. Adjustments were made for age and sex. Additional adjustments included BMI, pack-years of cigarette smoking, blood pressure and serum cholesterol as co-variables. To investigate whether vascular disease was mediating any association between diabetes, insulin resistance and MRI volumes, we adjusted for carotid atherosclerosis, white matter lesions and brain infarcts on MRI. We repeated all analyses excluding subjects with infarcts on MRI. Finally, because the effect of diabetes on the risk of dementia might differ across APOE genotypes,⁴ we studied possible effect modification by APOE genotype through stratified analyses (non-carrier of the $\epsilon 4$ allele versus carrier of the $\epsilon 4$ allele). Assumptions of the model were verified by residual diagnostics. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

Selected characteristics of the study cohort according to the presence of diabetes mellitus are given in Table 1.

Table 1. Characteristics of participants with and without diabetes mellitus

Characteristic	No diabetes mellitus (n=465)	Diabetes mellitus (n=41)	Adjusted difference*
Age, years	73±8	77±8	4 (1; 6)
Women, %	50	34	-17 (-33; -0.6)
Education, primary only %	30	38	7 (-8; 20)
Memory performance, Z-score	0.03±0.92	-0.51±0.80	-0.33 (-0.60; -0.06)
Body mass index, kg/m ²	26.2±3.7	26.8±2.9	0.7 (-0.5; 1.8)
Pack-years of cigarette smoking	20±25	24±24	2 (-5; 10)
Diastolic blood pressure, mmHg	77±11	75±13	-1 (-5; 3)
Systolic blood pressure, mmHg	146±20	146±21	-1 (-8; 5)
Total cholesterol, mmol/l	5.8±1.0	5.9±1.3	0.2 (-0.2; 0.5)
Carotid plaques, total number	1.6±1.6	2.6±1.6	0.7 (0.2; 1.3)
Intima-media thickness, mm	0.86±0.14	0.93±0.13	0.04 (-0.01; 0.08)
Periventricular white matter lesions, grade	2.7±2.2	3.6 (2.2)	0.4 (-0.2; 1.1)
Subcortical white matter lesions, ml	1.7±3.4	2.3 (3.6)	0.01 (-1.0; 1.0)
Brain infarcts, %	27	44	11 (-3; 25)
APOE, ε4 carriers, %	27	31	5 (-11; 21)
Postload insulin, pmol/l†	422.0±312.6	-	-
Insulin resistance, pmol/mmolt†	65.1±40.1	-	-

Values are means ± standard deviation or percentages

*Age and sex adjusted difference (95% CI) in characteristic between participants with and without diabetes mellitus

†Present for 405 participants without diabetes

In total, 41 participants (8.1%) had diabetes mellitus. Twenty-six of them were treated with anti-diabetic medication at time of MRI. Their median age when they were diagnosed with diabetes mellitus was 64 years (range 43-84), suggesting that they all had type 2 diabetes. Of note, although all participants were clinically free from dementia, persons with diabetes mellitus performed worse on memory tests (Table 1). Persons with diabetes mellitus had more atherosclerotic plaques in the carotid arteries (Table 1). They also had more cerebral white matter lesions on MRI, but this was not statistically significant after accounting for age and sex differences. Brain infarcts were 1.7 times (95% CI 0.8 to 3.3) more frequent in subjects with diabetes mellitus compared to those without diabetes mellitus, after adjusting for age and sex.

Subjects with diabetes mellitus had smaller hippocampal and amygdalar volumes on MRI (Figure 2). Diabetes mellitus had a similar effect on the left and the right-sided brain volumes separately. Additional adjustments for BMI, pack-years of cigarette smoking, blood pressure and cholesterol, did not change the results.

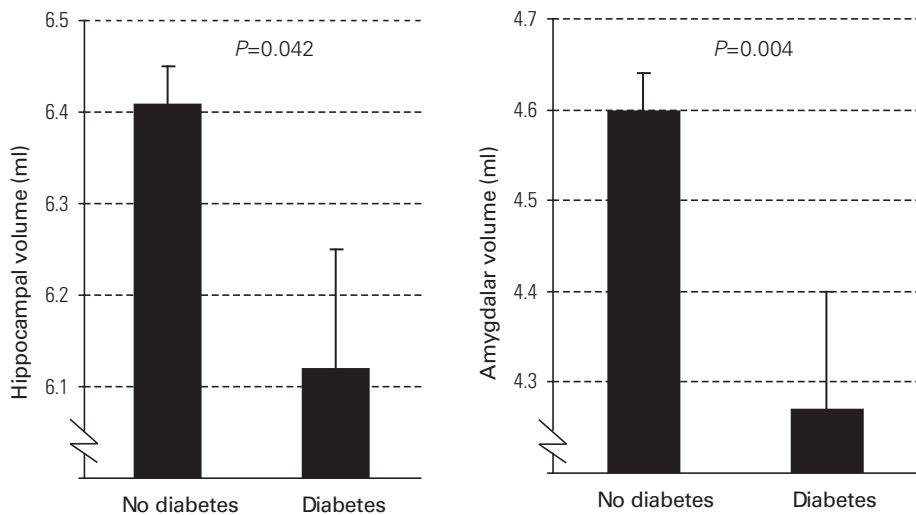


Figure 2. Hippocampal volumes and amygdalar volumes (+standard error) on brain MRI in participants with diabetes ($n=41$) and without diabetes ($n=465$). Volumes are adjusted for age and sex and normalised to average head size

Although subjects with diabetes mellitus had more vascular disease, accounting for markers of vascular disease did not change the association between diabetes and hippocampal or amygdalar volumes (Table 2). Exclusion of participants with infarcts ($n=142$) did not change the results either. There was no difference in association between diabetes and MRI volumes

according to APOE strata. In non-carriers of the $\epsilon 4$ allele, the age and sex adjusted difference in hippocampal volume between persons with and without diabetes mellitus was -0.30 (95% CI -0.66 to 0.06). In carriers of the $\epsilon 4$ allele the difference in hippocampal volume was -0.31 (95% CI -0.84 to 0.21). In non-carriers of the $\epsilon 4$ allele, the age and sex adjusted difference in amygdalar volume between persons with and without diabetes mellitus was -0.28 (95% CI -0.58 to 0.01). In carriers of the $\epsilon 4$ allele the difference in amygdalar volume was -0.41 (95% CI -0.86 to 0.04).

Table 2. Hippocampal and amygdalar volume on MRI in participants with and without diabetes mellitus accounting for markers of vascular disease

Difference adjusted for	Volume difference between participants with and without diabetes mellitus, ml (95% CI)			
	Hippocampus*	<i>P</i>	Amygdala*	<i>P</i>
Age and sex	-0.28 (-0.55 to -0.01)	0.042	-0.33 (-0.55 to -0.11)	0.004
Age, sex, and carotid atherosclerosis	-0.27 (-0.55 to 0.00)	0.053	-0.32 (-0.54 to -0.10)	0.005
Age, sex, white matter lesions and infarcts on MRI	-0.27 (-0.54 to 0.00)	0.053	-0.33 (-0.56 to -0.11)	0.003

Values are adjusted differences (95% CI and *P*-value) in MRI volumes (ml) between participants without diabetes mellitus ($n=465$) and participants with diabetes mellitus ($n=41$)

*Volumes are normalised to average head size

In non-diabetic participants ($n=465$), post-load insulin concentrations and insulin resistance were present for 405 participants. Persons with higher post-load insulin concentrations or insulin resistance had smaller amygdalar volumes, but not smaller hippocampal volumes on MRI (Table 3). Additional adjusting for BMI, pack-years of cigarette smoking, blood pressure, cholesterol, carotid atherosclerosis, white matter lesions and infarcts did not change the associations, nor did excluding participants with infarcts. The association between insulin resistance and amygdalar volumes on MRI was similar in APOE strata, although statistically significant only in non-carriers of the $\epsilon 4$ allele [non-carriers: adjusted difference in amygdalar volume per standard deviation increase in insulin resistance -0.11 (95% CI -0.19 to -0.04); carriers: -0.02 (95% CI -0.18 to 0.15)].

Table 3. Insulin resistance in participants without diabetes (n=405) in relation to hippocampal and amygdalar volumes on MRI

	Volume difference per SD increase in insulin concentrations and insulin resistance, ml (95% CI)			
	Hippocampus*	<i>P</i>	Amygdala*	<i>P</i>
Post-load insulin (per SD)				
Adjusted for				
Age and sex	-0.02 (-0.11 to 0.06)	0.57	-0.08 (-0.14 to -0.01)	0.020
Age, sex and carotid atherosclerosis	-0.03 (-0.11 to 0.05)	0.51	-0.08 (-0.14 to -0.01)	0.018
Age, sex, white matter lesions and infarcts on MRI	-0.02 (-0.11 to 0.06)	0.56	-0.08 (-0.15 to -0.02)	0.013
Insulin resistance (per SD)				
Adjusted for				
Age and sex	-0.00 (-0.09 to 0.08)	0.93	-0.08 (-0.15 to -0.02)	0.012
Age, sex and carotid atherosclerosis	-0.01 (-0.10 to 0.07)	0.75	-0.09 (-0.15 to -0.02)	0.008
Age, sex, white matter lesions and infarcts on MRI	-0.00 (-0.08 to 0.08)	0.95	-0.09 (-0.15 to -0.02)	0.008

Values are adjusted differences (95% CI and *P*-value) in MRI volumes (ml) per standard deviation (SD) increase in post-load insulin concentration and insulin resistance

*Volumes are normalised to average head size

Discussion

We observed that people with type 2 diabetes had more hippocampal and amygdalar atrophy on MRI than people without diabetes. Moreover, in persons without diabetes mellitus, insulin resistance was associated to amygdalar atrophy on MRI. The presence of atherosclerosis or cerebrovascular disease did not explain the associations.

The strengths of our study are its population-based design and the large sample with volumetric MRI. The prevalence of diabetes mellitus in our study was comparable to another Dutch population study,²⁷ leading to a moderate number of people with diabetes mellitus studied in the sample. However, the associations were robust and statistically significant sug-

gesting that we had sufficient power. A limitation of our study was the indirect assessment of insulin resistance through calculating the ratio of post-load insulin concentrations with glucose concentrations. This ratio however in non-diabetic subjects correlates well with the degree of insulin resistance assessed with precise clamping techniques.²⁸

Several studies have found an increased risk of Alzheimer's disease in people with diabetes mellitus.^{3, 4, 17 29-31} Other studies did not find this association or merely an association between diabetes mellitus and vascular dementia.³²⁻³⁵ Difficulties in diagnosing Alzheimer's disease in life and distinguishing it from vascular dementia could have resulted in different findings across studies. Alzheimer's disease is generally characterised by slow progression in clinical symptoms which is thought to reflect gradual development of the specific Alzheimer pathology over time.⁸ The pathological hallmarks of Alzheimer's disease, neurofibrillary tangles and amyloid plaques, occur in the most early stage of the disease in the hippocampus and amygdala⁹ causing neuronal loss and atrophy that can be visualised on MRI.³⁶ At this stage, dysfunction of the hippocampus could cause memory impairment, a well known early neuropsychological sign of Alzheimer's disease.³⁷ In our study, we used hippocampal and amygdalar atrophy on MRI in elderly subjects who were clinically free of dementia as markers of pre-clinical Alzheimer's disease. Several studies including our own²⁰ show that people with hippocampal atrophy on MRI have lower verbal memory performance.³⁸ Moreover, besides memory impairment, people with hippocampal atrophy on MRI frequently develop other symptoms of Alzheimer's disease later in life.^{11, 12} To our knowledge, no study has prospectively examined the specific role of amygdalar atrophy in Alzheimer's disease. However, patients with very mild Alzheimer's disease have equal volume losses in the hippocampus and amygdala on MRI compared to control subjects.^{13, 14} suggesting that atrophy of both hippocampal and amygdalar are early MRI markers of incipient Alzheimer's disease.

Three biological explanations support an association between diabetes mellitus and hippocampal and amygdalar atrophy on MRI. First, diabetes mellitus leads to vasculopathy and changes in lipid metabolism, which in turn could be associated to hippocampal and amygdalar atrophy. Although we found a clear association between diabetes mellitus and carotid atherosclerosis, the relation between diabetes and cerebrovascular disease, as noted by the degree of white matter lesions and brain infarcts on MRI, was not very strong. Moreover, when we accounted for vascular disease the relation between diabetes mellitus and atrophy on MRI remained the same. This is in line with a recent study in people free of cerebrovascular disease that showed impaired glucose tolerance to relate to hippocampal atrophy on MRI.³⁹ Thus, although diabetes mellitus is a vascular risk factor, other non-vascular pathways seem to play a role in the findings. A second biological explanation is that hyperglycaemia in diabetic patients is directly associated to hippocampal and amygdalar atrophy. A prospective study found that subjects with diabetes mellitus have increased amyloid plaques and neurofibrillary tangles

in the hippocampus at autopsy,⁴ though another post-mortem study did not.⁴⁰ In diabetic subjects, accelerated formation of advanced glycation endproducts can cross-link amyloid proteins leading to aggregation into the amyloid plaques.^{6, 41} In addition, glycation of the microtubule associated protein-tau could lead to formation of neurofibrillary tangles.⁴² Pointing to a third biological explanation was the finding that peripheral insulin resistance was associated to amygdalar atrophy on MRI. Insulin resistance is characterised by high plasma insulin concentrations and relatively normal glucose concentrations. Patients with Alzheimer's disease have higher plasma insulin concentrations compared with control subjects^{43, 44} but lower cerebrospinal-fluid insulin concentrations.⁴⁴ This suggests that insulin transport from plasma to the brain is diminished in Alzheimer patients.⁴⁴ Other investigations report that dysfunction of insulin signal transduction is involved in Alzheimer's disease.^{7, 45} Genetic variability in genes encoding for components of the insulin-signalling pathway is associated to Alzheimer's disease.⁴⁶ Insulin regulates metabolism of amyloid proteins, prevents tau phosphorylation⁷ and promotes neuronal survival,⁴⁷ all actions that in case of dysfunction of the insulin pathway can lead to Alzheimer's disease. Furthermore, the insulin-degrading enzyme is, in addition to its role in degrading insulin, important in cleaving amyloid protein in the brain.⁴⁸ Mice with hypofunction of this enzyme have increased accumulation of amyloid in the brain and increased plasma insulin concentrations, further supporting a connection between insulin and Alzheimer's disease.⁴⁹ It is postulated that patients with Alzheimer's disease try to compensate for impaired insulin signalling by increasing the amount of insulin receptors.⁵⁰ Interestingly, we found a restricted relation between insulin resistance and amygdalar atrophy on MRI. Higher plasma insulin concentrations or insulin resistance were not related to hippocampal atrophy on MRI, in agreement with others.³⁹ The hippocampus and amygdalar differ according to the amount of insulin receptors, the hippocampus having a higher density.⁵¹ A speculative explanation for the absence of a relation between insulin concentrations and hippocampal volumes is that the high density of insulin receptors in combination with high plasma insulin concentrations compensates for dysfunctions in the insulin-signalling pathway. However, the differential effects of insulin on the hippocampus and amygdala have yet to be confirmed.

In summary, in this community sample we found that people with type 2 diabetes have smaller hippocampal and amygdalar volumes on MRI, supporting the view that diabetes is a risk factor for Alzheimer's disease. Since atherosclerosis or cerebrovascular disease were not explaining the associations, it is likely that direct metabolic effects of diabetes mellitus are involved. Our finding that insulin resistance was associated to amygdalar atrophy on MRI is in line with suggestions that dysfunction of insulin signalling is involved in the pathogenesis of Alzheimer's disease.

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Chapter 4

Clinical correlates of brain atrophy on MRI



Chapter 4.1

Hippocampal atrophy and memory performance

The hippocampus plays a crucial role in the consolidation of memory. Anatomically, the hippocampal head, body, and tail are connected to separate regions of the entorhinal cortex, which conveys processed information from the association cortices to the hippocampus. Little is known, however, about the functional segregation along its longitudinal axis. In the present study, we investigated whether the hippocampal head, body, or tail is selectively involved in verbal memory performance. A total of 511 nondemented participants, aged 60-90 years, underwent a three-dimensional HASTE brain scan in a 1.5-T MRI unit. Hippocampal volumes were measured by manual tracing on coronal slices. Segmentation was performed in anterior-posterior direction on the basis of predefined cut-offs allocating 35, 45, and 20% of slices to the head, body, and tail, respectively. Memory performance was assessed by a 15-word learning test including tasks of immediate and delayed recall. To analyze the association between head, body, and tail volumes and memory performance, we used multiple linear regression, adjusting for age, sex, education, and midsagittal area as a proxy for intracranial volume. Participants with larger hippocampal heads scored significantly higher in the memory test, most notably in delayed recall [0.41 words per SD increase in left hippocampal head (95% CI 0.16, 0.67), 0.33 words per SD increase in right hippocampal head (95% CI 0.06, 0.59)]. Our data suggest selective involvement of the hippocampal head in verbal memory, and add to recent findings of functional segregation along the longitudinal axis of the hippocampus.

Introduction

The hippocampus is generally assumed to play a key role in the consolidation of memory, i.e. the integration of relevant new information and its transfer from short-term to long-term memory.¹ A host of magnetic resonance imaging (MRI) studies have found positive associations between total hippocampal volume and memory performance.²⁻⁵ Anatomically, the hippocampus can be divided into three distinct segments: (a) an anterior part, or head, (b) an intermediate part, or body, and (c) a posterior part, or tail.⁶ These subdivisions are reciprocally connected to separate regions of the entorhinal cortex.⁷ In addition, a number of studies suggest that the hippocampal head, body, and tail may have different functions. Presentation of novel information, for instance, was associated with activity in the left anterior hippocampus during functional MRI (fMRI),⁸ while a positron emission tomography (PET) study found memory impairment in amnesiacs and in Alzheimer patients to be associated with decreased glucose metabolism in the hippocampal head.⁹ A recent small volumetric MRI study demonstrated that impaired verbal memory in first-degree relatives of schizophrenic patients was associated with lower volumes in the anterior part of the hippocampus.¹⁰ The goal of our study was to investigate, in a large sample of nondemented elderly participants, whether MRI volumes of hippocampal head, body, and tail are differentially associated with verbal memory performance.

Methods

Participants

The Rotterdam Study is a large population-based cohort study in the Netherlands that started in 1990 and investigates the prevalence, incidence and determinants of various chronic diseases among elderly participants.¹¹ From 1995 to 1996, we randomly selected 965 living members (aged 60-90 years) of this cohort in strata of sex and age (5 years) for participation in the Rotterdam Scan Study.¹² After exclusion of individuals who were demented, blind, or had MRI contraindications, the number of eligible participants was 832. Subjects with dementia were excluded based on a stepwise approach as used in the Rotterdam Study.¹³ Subjects were screened using the Mini-Mental State Examination (MMSE)¹⁴ and the Geriatric Mental State Schedule (GMS). Those scoring below 26 on the MMSE or more than 0 on the GMS were additionally assessed by means of the CAMDEX interview.¹⁵ Subjects suspected of dementia based on the CAMDEX were examined by a neurologist. Finally, an expert panel reviewing all relevant information decided whether an individual was to be considered demented or not, based on the criteria of the DSM-III-R. Among the 832 eligible, 563 participants gave their written informed consent to participate in the present study (response rate: 68%), which

included undergoing a MRI brain scan. The study was approved by the medical ethics committee of the Erasmus Medical Center, Rotterdam, the Netherlands.

Magnetic resonance imaging acquisition

Brain scans were performed using a 1.5-Tesla MRI System (VISION MR, Siemens AG, Erlangen, Germany). The protocol included, in the following order, a) a sequence of proton-density images b) a sequence of T2-weighted images c) a sequence of T1-weighted images.¹⁶ Finally, for volumetric measurements of the hippocampus, a custom-made, inversion recovery - double contrast 3D half-Fourier acquisition single-shot turbo spin echo (HASTE) sequence was included (inversion time 440 ms, [TR] 2800 ms, 128 contiguous sagittal slices of 1.2-mm, matrix 192x256, field of view 256x256). Two HASTE modules were sequentially acquired after the inversion pulse (effective TEs of 29 ms and 440 ms), the first of that was used for the volume measurements. Fifty-two participants developed claustrophobia during MRI acquisition. Thus, complete MRI data were available from a total of 511 participants.

Hippocampal volume measurement

For each participant, we reconstructed a series of coronal brain slices (contiguous 1.5 mm slices) based on the 3D HASTE sequence, aligned to be perpendicular to the long axis of the hippocampus. All reconstructed slices were transferred to a Siemens Magic View 1000 workstation for volumetric assessment of the left and right hippocampus.

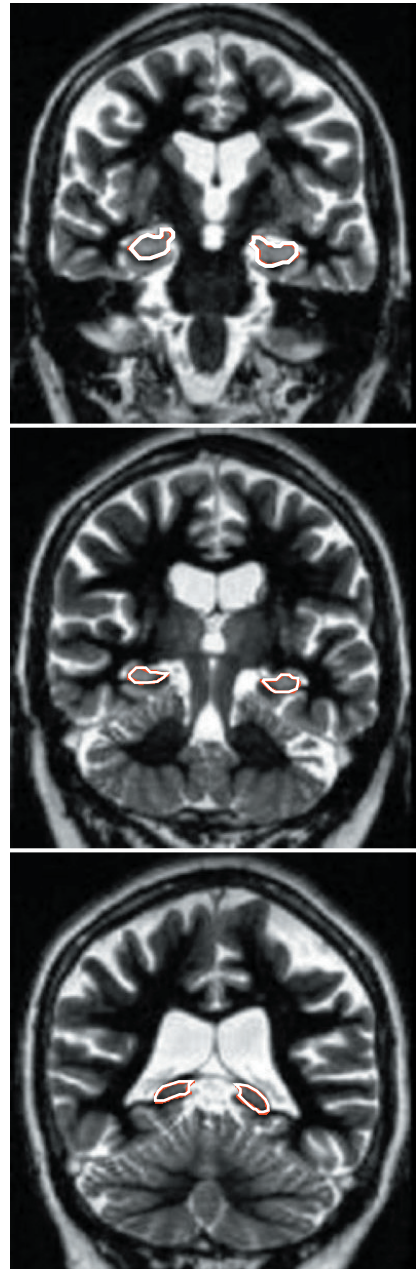


Figure 1. Hippocampal head (H), body (B) and tail (T) manually outlined on coronal images

Referencing to an anatomical atlas,⁶ we manually traced the boundaries of both hippocampi on each slice by means of a mouse-driven pointer (Figure 1). We proceeded from posterior to anterior, starting on the slice where the crux of the fornices was in full profile. Segmentation of the hippocampal head from the overlying amygdala was facilitated by identifying the alveus, which demarcated the undulating superior surface of the hippocampal head. The intraplane hippocampal boundaries were defined to include the CA1 through CA4 sectors of the hippocampus proper, the dentate gyrus, and the subiculum. The number of slices measured ranged from 16 to 31 (left hippocampus, median = 25) and from 18 to 31 (right hippocampus, median = 25) per individual. Entering the outlined surface areas (in mm²) into a spreadsheet program (Microsoft Excel 97), we multiplied the summed surface areas on each side with slice thickness to yield estimates of the left and right hippocampal volume (ml). We also reconstructed a midsagittal slice (thickness 3.0 mm). The midsagittal area, which we used as a proxy for intracranial volume, was measured by tracing the inner table of the skull. The 511 scans were randomly split between two raters who were blinded to any participant-related clinical information. Studies performed on 14 random scans to evaluate intra- and interrater correlation showed good overall agreement. Intrarater intraclass correlation coefficients were $r = 0.93$ for the left and $r = 0.90$ for the right hippocampus, whereas interrater intraclass correlation coefficients were $r = 0.87$ and $r = 0.83$, respectively. For the midsagittal area, intra- and interrater intraclass correlation coefficients were 0.995 and 0.96, respectively. To obtain separate volumes for the hippocampal head, body, and tail, we subdivided the hippocampus into three segments, such that the anterior 35% of coronal slices (or any rounded integer of slices closest to this cut-off) included the head with the hippocampal digitations, while the intermediate 45% represented the body of the hippocampus, and the remaining 20% the tail (Figure 2). These cut-offs were based on photographs of histological preparations.⁶ Grouping hippocampi by length (i.e. by number of slices), and plotting graphs of mean cross-sectional volumes over the anterior-posterior extent of the hippocampus, we confirmed that the 35% cut-off was a stable proxy for the boundary between head and body over the whole range of hippocampal lengths, as it invariably fell into, or near, the region of steepest slope corresponding to the anterior bend of the hippocampus. The cut-off between body and tail coincided well with the region where cross-sectional hippocampal volumes reached a minimum.

Measurement of memory function

Memory was assessed by means of a 15-word learning test, which is used to evaluate the ability to acquire and retain new verbal learning information based on Rey's auditive recall of words.¹⁷ To test immediate recall, participants were presented three times with a sequence of 15 words and subsequently asked to recall as many of these words as possible. Free delayed recall was tested 15 minutes later. Outcome variables were the mean of the number of

words recalled over the first three trials (as a summary score for immediate recall), and the number of words remembered after the 15-minute delay (as a score for free delayed recall). Immediate and delayed recall scores were normally distributed. The frequency of the highest and lowest scores (i.e., 0 and 15) was less than 3%. Therefore, no ceiling or floor effects were observed. As a measure of global cognitive function we used the MMSE.¹⁴

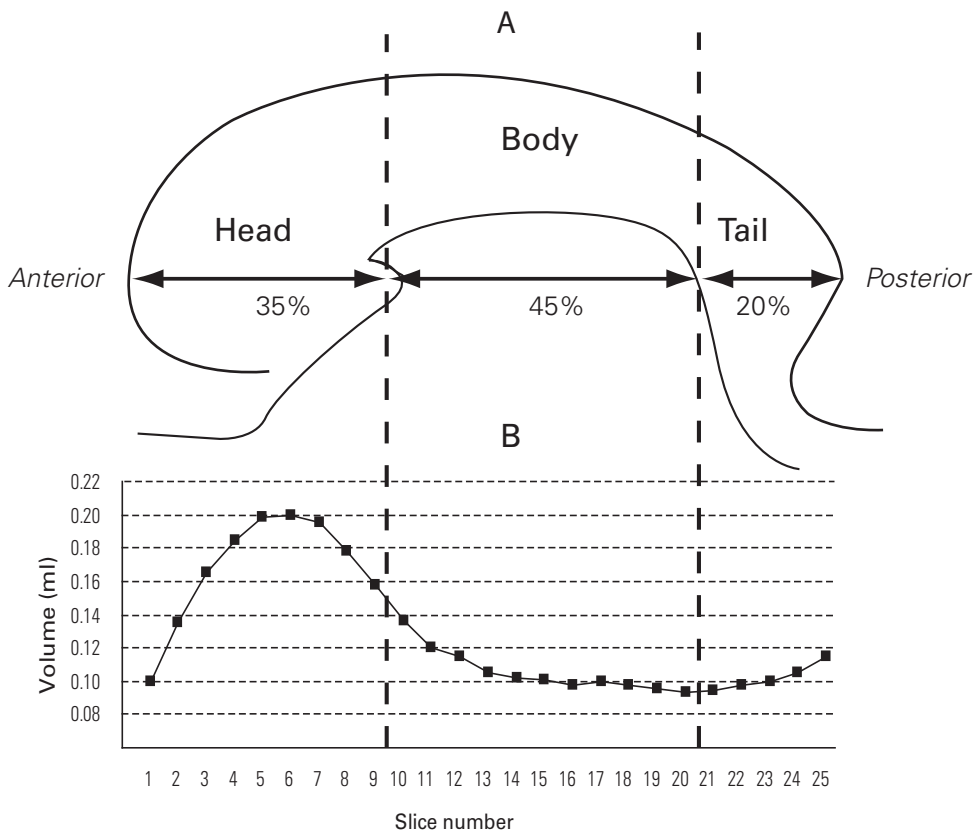


Figure 2. A. Sketch of right hippocampus as seen from above. Hippocampal head, body, and tail (dashed lines represent 35% and 20% cut-offs for the boundaries between head and body, and body and tail, respectively) are depicted.

B. Mean coronal volumes plotted over the anterior-posterior extent of the hippocampus (graph is based on hippocampi with a longitudinal extent of 25 slices).

Other measurements

Level of education according to UNESCO was obtained.¹⁸ The presence of depressive symptoms was determined by the Center of Epidemiologic Studies on Depression (CES-D) scale.¹⁹

Data analysis

To assess the association between hippocampal head, body and tail volumes and memory, we first constructed simple x,y scatter plots. As the scatter plots suggested approximately linear associations we decided to use multivariable linear regression for our analyses. We included all factors that were significantly correlated with hippocampal volumes (age, sex, education, midsagittal area) in our model. Thus, our model was represented by the following equation: memory performance = $B_0 + B_1 \times \text{age} + B_2 \times \text{sex} + B_3 \times \text{education} + B_4 \times \text{midsagittal area} + B_5 \times \text{hippocampal head volume (SD)} + B_6 \times \text{hippocampal body volume (SD)} + B_7 \times \text{hippocampal tail volume (SD)}$. The regression coefficients of the hippocampal volumes (B5-B7) reflect the average difference in number of words recalled between individuals for a given volume difference of 1 SD. Appropriate checks revealed no indication of nonlinearity. To confirm regional patterns of association and to check whether the association between the volumes of head, body and tail and performance in the 15-word learning test was independent of our chosen cut-off points, we performed additional slice-by-slice analyses. For this purpose, we transformed the number of slices to a standard of 20 for all hippocampi by means of linear interpolation. Thus, slice volumes were calculated for 5% intervals, given a relative length of 100% for all hippocampi. Each of the 20 slices was individually assessed for its potential association with memory performance, adjusting for age, sex, level of education, midsagittal area, and the volume of the remaining 19 slice locations combined.

Results

Relevant characteristics of all 511 participants are summarized in Table 1.

Table 1. Characteristics of the study sample (n=511)

Age, years	73 ± 8
Sex, % women	49
Highest education, %	
Primary	31
Intermediate/Higher	60
College/University	9
Depressive symptoms (CES-D), range 0-31	5.6 ± 6.1
15-Word Learning Test	
Immediate Recall, mean over three trials	6.57 ± 1.81
Delayed Recall	5.78 ± 2.63
MMSE score	27.7 ± 2.1
Midsagittal area, cm ²	149.1 ± 11.1
Total hippocampus, ml	
Left	3.15 ± 0.46
Right	3.22 ± 0.44
Hippocampal head, ml	
Left	1.46 ± 0.22
Right	1.52 ± 0.24
Hippocampal body, ml	
Left	1.18 ± 0.23
Right	1.19 ± 0.21
Hippocampal tail, ml	
Left	0.51 ± 0.12
Right	0.52 ± 0.11

Values are unadjusted means ± standard deviation or percentages

Volumes of the left and right hippocampus were highly correlated with each other ($r = 0.88$; $P < 0.001$). Volumes of hippocampal head, body and tail were positively and moderately correlated with each other (correlation between head and body $r = 0.45$ (left) and $r = 0.48$ (right), between head and tail $r = 0.30$ (left) and $r = 0.21$ (right), and between body and tail $r = 0.57$ (left) and $r = 0.63$ (right)). The number of immediate words recalled decreased 0.08 per year and of delayed recall words 0.11 per year.

Table 2 shows the association between hippocampal head, body and tail volumes and variables that may confound the association with memory performance. Age, sex, education and midsagittal area were significantly associated with subdivisational volumes. Slice-by-slice analysis revealed that the association between age and hippocampal volume was strongest in the region between 20% and 35%, corresponding to the posterior part of the hippocampal head.

Table 2. Association between several variables and hippocampal head, body and tail

	Head (ml)		Body (ml)		Tail (ml)	
	Left	Right	Left	Right	Left	Right
Age	-0.07	-0.08	-0.05	-0.06	-0.03	-0.03
(per 10 years)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)
Sex*	-0.07	-0.07	-0.00	0.00	0.03	0.03
	(0.002)	(0.001)	(0.85)	(0.90)	(0.007)	(0.002)
Education (range 0-7)	0.01	0.02	0.00	0.00	-0.00	0.00
	(0.02)	(0.008)	(0.44)	(0.10)	(0.37)	(0.34)
Depressive symptoms	-0.02	-0.03	0.01	0.00	0.00	-0.00
(per 10)†	(0.14)	(0.14)	(0.50)	(0.96)	(0.99)	(0.66)
Midsagittal area	0.06	0.08	0.04	0.04	0.01	0.01
(per 10 cm ²)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.15)	(0.03)

Values given are regression coefficients (P -values in parentheses)

*Regression coefficient adjusted for midsagittal area. Minus sign denotes that volume is smaller in women. Positive sign denotes that volume is smaller in men

†Score on the CES-D, range 0-31

Total hippocampus and memory

Table 3 summarizes the results of our multivariable linear regression analysis which demonstrated a strong positive and highly significant association between total hippocampal volumes and performance in the 15-word learning test, in both immediate recall and delayed recall.

Table 3. Association between total hippocampal volume and number of words recalled

	Immediate recall		Delayed recall	
	Difference per SD (95% CI)	<i>P</i>	Difference per SD (95% CI)	<i>P</i>
Left hippocampus	0.21 (0.05, 0.36)	0.008	0.41 (0.19, 0.64)	<0.001
Right hippocampus	0.22 (0.06, 0.38)	0.006	0.47 (0.23, 0.70)	<0.001

Values are adjusted difference in number of words recalled per standard deviation of hippocampal volume, with 95% confidence intervals (CI), and *P*-values. Adjusted for age, sex, education, and midsagittal area.

Table 4. Association between volume of hippocampal head (cut-off 35%), body (45%), and tail (20%) and number of words recalled

	Immediate recall		Delayed recall	
	Difference per SD (95% CI)	<i>P</i>	Difference per SD (95% CI)	<i>P</i>
Left Hippocampus				
Head	0.26 (0.09, 0.44)	0.003	0.41 (0.16, 0.67)	0.002
Body	-0.08 (-0.29, 0.14)	0.49	-0.03 (-0.35, 0.28)	0.83
Tail	0.11 (-0.08, 0.31)	0.26	0.19 (-0.09, 0.48)	0.19
	Difference per SD (95% CI)	<i>P</i>	Difference per SD (95% CI)	<i>P</i>
Right Hippocampus				
Head	0.19 (0.01, 0.37)	0.035	0.33 (0.06, 0.59)	0.015
Body	0.01 (-0.22, 0.20)	0.94	0.06 (-0.25, 0.36)	0.71
Tail	0.12 (-0.07, 0.31)	0.22	0.24 (-0.04, 0.51)	0.10

Values are adjusted estimates of the change in number of words recalled per increase in standard deviation of volume, with 95% confidence intervals (CI), and *P*-values. Adjusted for age, sex, education midsagittal area.

Hippocampal head, body, and tail and memory

Table 4 shows the association between volume of the hippocampal head, body, and tail and test performance. Individuals with larger hippocampal heads performed significantly better in the 15-word learning test, most notably in delayed recall. Estimates for the left hippocampal head tended to be slightly higher than estimates for the right hippocampal head. No significant associations were observed for the hippocampal body or hippocampal tail. The hippocampal head was the only region associated to the MMSE score [per SD 0.27 (95% CI 0.05-0.48) for the left hippocampal head; per SD 0.23 (95% CI 0.02-0.45) for the right hippocampal head]. Hippocampal body and tail were not associated to MMSE score. Slice-by-slice analyses of delayed recall performance revealed significant associations only for the most anterior part of the hippocampus (i.e., the first three slices of the left and right hippocampus), the region corresponding to the anterior half of the hippocampal head (Figure 3). No significant associations were observed at any other slice location.

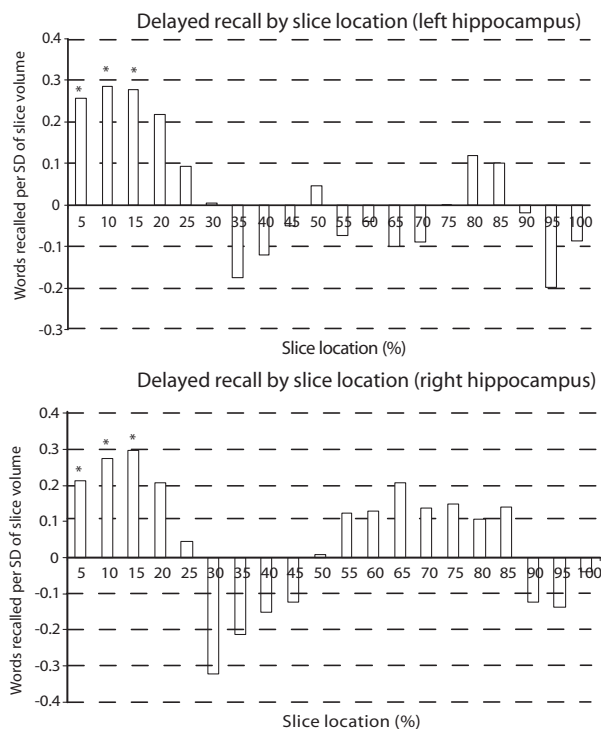


Figure 3. Association between left (top) and right (bottom) regional hippocampal volumes and number of words recalled in the delayed recall test (x-axis: location percentiles along the longitudinal axis of the hippocampus, y-axis: difference in the number of words recalled per standard deviation of hippocampal slice volume; * significant at the $P=0.05$ level). Adjusted for age, sex, educational level and midsagittal area

Discussion

We found that a larger volume of the hippocampal head, rather than the hippocampal body or tail, was significantly associated with better verbal memory performance, in tasks of both immediate and delayed recall.

Our large number of participants and the regional approach are obvious strengths of our study. Nevertheless, some methodological issues remain to be addressed. First, no precise definitions for the boundaries between hippocampal head, body and tail on MRI exist, and hence the cut-offs we chose are somewhat arbitrary. However, our slice-by-slice analysis suggested that the associations did not depend on the choice of cut-offs. Second, the method we used to define the hippocampal subdivisions was implicitly based on the assumption that all individual hippocampi, irrespective of their shape or length, share the same proportions regarding the longitudinal extent of the hippocampal head, body, and tail (i.e., 35%, 45%, and 20%, respectively). Although this assumption was mostly met, our subdivisions in volume were likely to be affected by some degree of random misclassification, leading to a weakening of the associations we found.

The observation that larger hippocampal volume is associated with better verbal memory is in keeping with numerous MRI studies,²⁻⁵ even though surprisingly some studies found an inverse association.^{20,21} More specifically, our study showed that hippocampal involvement in verbal memory performance may be limited to the head region. Few MRI studies investigated subdivisions of the hippocampus in relation to function. A study in taxi drivers showed the posterior hippocampus to be associated with spatial memory.²² Another study in psychopaths showed the posterior hippocampus to be associated with the degree of psychopathology.²³ A volumetric MRI study assessed the role of longitudinal subdivisions of the hippocampus in verbal memory in first-degree relatives of schizophrenic patients.¹⁰ This study found that amygdalar and anterior hippocampal volumes were significantly associated with delayed verbal recall, whereas no such association was shown for the posterior hippocampus. Most PET studies discussed in a review of medial temporal lobe (MTL) activation in fMRI and PET studies of episodic encoding and retrieval reported anterior medial temporal lobe activation, while the fMRI studies reviewed found activation mainly in the posterior part of the MTL.²⁴ A recent fMRI study observed activation of the left anterior hippocampus in participants presented with novel verbal stimuli, suggesting that the anterior hippocampus plays a crucial role in novelty encoding.⁸ No activation was observed in the posterior hippocampal region. A PET study measuring glucose metabolism in amnesic patients and in patients with Alzheimer's disease revealed that both groups had a significantly decreased glucose metabolism in the hippocampal head in comparison to controls.⁹ Again, no association was observed for the hippocampal tail or body.

Memory is acquired through the processing of incoming information by the association cortices which, by way of the parahippocampal and perirhinal cortex, send their output to the entorhinal cortex. Cells in the entorhinal cortex supply most of the fibers that enter the hippocampal formation through the perforant pathway, while most efferent connections from the hippocampus project back to the entorhinal cortex.²⁵ A recent study has shown that the perforant pathway is characterized by a topographical organization along the longitudinal axis of the hippocampal formation, such that a medial-to-lateral gradient in the entorhinal cortex corresponds to an anterior-posterior gradient in the hippocampal formation.⁷ This special architecture may be the underlying substrate for the functional segregation between hippocampal head, body, and tail that we and others have found.

The effect of hippocampal volume on number of words recalled may seem small and not clinically relevant. However, it is important to realize that these are not differences on an individual level, but average differences across groups of nondemented persons and adjusted for possible confounders. The effect size per SD increase in hippocampal volume in words recalled approximately equaled the effect of a 4-year age increase.

One explanation that would fit our data is that people with better memory have larger hippocampal heads. However, we also found a relation between hippocampal head volume and the MMSE score, which is a more global cognitive measure. This suggests that individuals who do not fulfill criteria of dementia but do have slight impairment of verbal memory or global cognitive function may have selective atrophy of the hippocampal head. Whether they are destined to develop clinically evident dementia is yet unclear. MRI in early Alzheimer's disease showed patients' hippocampi to be smaller than those of controls, while hippocampal head size in Alzheimer patients seems to be inversely related to disease severity.²⁶ Prospective studies suggested that smaller total hippocampal volume may predict dementia.^{5, 27 28} Possibly, subdividing the hippocampus into head, body or tail or even smaller subdivisions may show atrophy in regions specific for imminent Alzheimer's disease. Prospective follow-up investigations of the individuals participating in our study will enable us to confirm whether there is early - and possibly selective - involvement of the hippocampal head region in Alzheimer's disease. If this is the case, hippocampal head volume – in combination with other variables – may help to refine prediction of dementia and enhance the identification of at-risk individuals who might benefit from early intervention.

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Chapter 4.2

Hippocampal, amygdalar atrophy and incident dementia

Background: Patients with Alzheimer's disease have pronounced atrophy of the hippocampus and amygdala on magnetic resonance imaging (MRI). In persons with mild cognitive impairment, hippocampal atrophy predicts conversion to dementia. Whether atrophy is evident even before symptom onset is unclear. We studied the longitudinal relation between atrophy on MRI and incident dementia in a population-based cohort accounting for neuropsychological performance.

Methods: We obtained volumetric MRI assessments of the hippocampus and amygdala at baseline in a cohort of 511 elderly free of dementia (age 60-90 years). Each participant was asked about memory problems in daily life and cognitive performance was assessed. The cohort was followed for possible development of dementia by in-person screening and monitoring of medical records.

Results: During a total of 3043 person-year of follow-up (mean per person 6.0 years), we detected 35 incident dementia cases (26 with Alzheimer's disease). Those who developed Alzheimer's disease had on average 10% smaller volumes of the hippocampus and amygdala on MRI. The risk to develop dementia was higher in persons with hippocampal or amygdalar atrophy (age, sex and education adjusted relative risk per standard deviation decrease 2.99 (95% CI 1.96-4.57) for the hippocampus and 2.06 (95% CI 1.46-2.90) for the amygdala. In persons without memory complaints or cognitive impairment at baseline, the relation between atrophy on MRI and incident dementia was similar as that in the total population.

Conclusions: Atrophy of the hippocampus and amygdala on MRI predict dementia over a 6-year follow-up even in cognitively intact elderly.

Introduction

Alzheimer's disease is the most common cause of dementia in late life.¹ Strategies to prevent or delay the disease are under development.² Once available, it will be important to identify people at high risk to develop dementia who may benefit from such therapies. Magnetic resonance imaging (MRI) of the brain provides one potential tool for detecting the preclinical stages of the disease. Pathologically, Alzheimer's disease is characterized by dense accumulation of neurofibrillary tangles and amyloid plaques in the medial temporal lobe,³ leading to neuronal loss that is visible as atrophy on MRI.⁴ Several studies have shown in patients with Alzheimer's disease⁵⁻⁷ pronounced reductions in hippocampal and amygdalar volumes on MRI compared to healthy elderly. Patients with mild cognitive impairment (MCI), at high risk of developing Alzheimer's disease,⁸ have also smaller hippocampal volumes than healthy elderly.⁹⁻¹⁵ It is unclear whether in persons destined to develop dementia, atrophy on MRI is observed even before persons would be designated as having MCI. We focused on this question by studying the longitudinal relation between volumes of the hippocampus and amygdala on MRI and incident dementia in a population dwelling in the community. By taking into account memory complaints and neuropsychological performance at baseline we sought to investigate whether atrophy on MRI predicts dementia even in people without cognitive problems. We used data from the population-based Rotterdam Scan Study in whom 511 non-demented elderly had volumetric MRI assessments of the hippocampus and amygdala and whom we followed for an average period of 6 years.

Methods

Subjects

The Rotterdam Study is a large population-based cohort study in the Netherlands that started in 1990 and investigates the prevalence, incidence and determinants of chronic diseases among elderly participants.¹⁶ From 1995 to 1996, we randomly selected 965 living members (aged 60 to 90 years) of this cohort in strata of sex and age (5 years) for participation in the Rotterdam Scan Study, a study on age-related brain changes on MRI.¹⁷ As part of the eligibility criteria we excluded individuals who were demented,¹⁸ blind, or had MRI contraindications. This left 832 persons eligible for participation. Among these, 563 persons gave their written informed consent to participate in the present study (response rate: 68%). Complete MRI data, including a three-dimensional (3D) MRI sequence, was obtained in 511 persons.¹⁹ The study was approved by the medical ethics committee of the Erasmus Medical Center, Rotterdam, the Netherlands.

MRI assessments

At baseline examinations in 1995 to 1996, a three-dimensional (3D) MRI sequence covering the whole brain was made using a 1.5 Tesla MRI unit.¹⁹ We reformatted coronal slices (1.5-mm contiguous slices) from this 3D MRI sequence in such a way that they were perpendicular to the long axis of the hippocampus. The left and the right hippocampus and amygdala were manually outlined on each slice with a mouse-driven cursor.¹⁹ Absolute volumes (ml) were calculated by multiplying the areas on each slice by the slice thickness. We summed the left and right side to yield total volumes because the analyses did not suggest laterality of effects. As a proxy for head size, we measured on a reformatted middle sagittal MRI slice the intracranial cross-sectional area.¹⁹ We corrected for head size differences across individuals by dividing the uncorrected volumes by the subject's calculated head size and subsequently multiplying this ratio by the average head size area (men and women separately).^{7,20}

Ascertainment of incident dementia

All participants were free of dementia at baseline and we followed the cohort for incident dementia.¹⁸ Briefly, participants were screened at follow-up visits (1997-1999, 1999-2000, 2002-2003) with the Mini Mental State Examination (MMSE) and the Geriatric Mental Schedule and when screen positive assessed with the Cambridge Examination for Mental Disorders of the Elderly interview.²¹ Participants suspected of dementia were examined by a neurologist and underwent extensive neuropsychological testing. The MRI at baseline was not used in the diagnosis of dementia. In addition to these examinations in-person, we continuously monitored the medical records of all participants at the general practitioners' offices and the Regional Institute for Ambulatory Mental Health Care to obtain information on diagnosed dementia until January 1, 2003. A diagnosis of dementia and AD was made by a panel of a neurologist, neuropsychologist and research physician that used standard criteria.^{22, 23} The onset of dementia was defined as the date on which clinical symptoms allowed the diagnosis of dementia. Duration of follow-up for each participant was calculated from baseline examination until death, diagnosis of dementia or the end of follow-up whichever came first.

Other measurements

As memory impairment is the first detectable neuropsychological sign of incipient Alzheimer's disease,²⁴ we questioned persons on memory complaints (by a single question), and assessed memory performance (by a 15-word verbal learning task).²⁵ For each participant, we calculated z scores based on the results of the memory test ($z \text{ score} = \text{individual test score} - \text{mean test score} / \text{standard deviation}$). We constructed a compound score for memory performance by averaging the z scores of the total of the three immediate recall trials and the delayed recall trial of the 15-word verbal learning task.²⁵ In accordance with cur-

rent criteria, we defined MCI as having a memory score <1.5 standard deviation (SD) of age and education specific means and memory complaints.²⁶ To further reduce the possibility that the associations were only present in persons with low memory performance at baseline we also defined a group with a score of <1 SD below age and education specific means. Additionally we administered tests that assess psychomotor speed²⁵ and used a cut-off of <1 SD below age- and education adjusted means to indicate low performance of psychomotor tasks. We measured other characteristics on brain MRI that have previously been associated to dementia.¹⁸ White matter lesions were considered present if visible as hyperintense on proton-density and T2 weighted axial images, without prominent hypointensity on T1 weighted scans. Periventricular white matter lesions were scored semi-quantitatively from 0-9.²⁵ We defined infarcts as focal hyperintensities on T2 weighted images. Infarcts in the white matter also had to have corresponding hypointensities on T1 weighted images in order to distinguish them from white matter lesions. Subcortical brain atrophy was calculated by averaging the ventricle-to-brain ratio on T1 weighted images at the frontal horns, the occipital horns and the caudate nucleus.

Data analysis

With Cox proportional hazards models we calculated the adjusted relative risk of dementia per SD decrease in volumes on MRI. We made also age- and sex specific tertiles of volumes, which we denote as severe, moderate and no atrophy. Adjusted volume differences at baseline between those developing Alzheimer's disease and those remaining free of dementia were computed from these Cox models. We plotted this difference against the time after MRI that the diagnosis of Alzheimer's disease was made in three equally sized groups of time after MRI. In the basic model we adjusted for age, sex and level of education. Additional adjustments included periventricular white matter lesions, brain infarcts and subcortical atrophy on MRI.¹⁸ Analyses were repeated after excluding persons with memory complaints, with a MMSE <26 , with a low performance on psychomotor tasks (<1 SD below age and education adjusted means), with a low performance on memory tasks (<1 or <1.5 SD below age and education adjusted means) or MCI at baseline.

Results

Table 1 gives the baseline characteristics of the study sample. Sixteen persons fulfilled criteria of MCI at baseline (3%), a prevalence in line with other studies.²⁷

Table 1. Baseline characteristics of the study sample

Characteristic	n=511
Age, y	73.4 ± 8.0
Sex, % women	49
Primary education, %	31
MMSE, score	27.7 ± 2.1
Memory complaints, %	31
Speed performance	
	<1 SD*, % 15
Memory performance	
	<1 SD*, % 17
	<1.5 SD*, % 6
MCIT, %	3
Hippocampal volume, ml	6.38 ± 0.88
Amygdala volume, ml	4.57 ± 0.72

Values are mean (± SD) or percentages

*Defined as scoring less than 1 or 1.5 SD below the age and education adjusted means

†Presence of memory complaints and memory performance <1.5 SD below the age and education adjusted means in memory tests²⁶

During a total of 3043 person-years of follow-up (mean per person 6.0 years), there were 35 persons who developed dementia of whom 26 received a clinical diagnosis of Alzheimer's disease. Persons with severe atrophy of the hippocampus or amygdala had the highest risk to develop dementia, independent of other brain MRI measures (table 2 and 3). In people with no hippocampal atrophy, the incidence rate of dementia was 4.8 per 1000 person-years, whereas in people with severe hippocampal atrophy the incidence rate was 21.6 per 1000 person-years. For the amygdala we found in people with no atrophy an incidence rate of 2.9

per 1000 person-years and in people with severe atrophy 19.4 per 1000 person-years.

Table 2. Baseline hippocampal atrophy on MRI and risk of dementia

Atrophy severity†	Dementia	Relative risk (RR)			
		Adjusted for age, sex and education		Adjusted for age, sex, education and other MRI measures*	
		RR	95% CI	RR	95% CI
No atrophy (n=169)	5	1.00	Ref	1.00	Ref
Moderate atrophy (n=173)	9	1.81	0.61-5.39	1.87	0.63-5.60
Severe atrophy (n=169)	21	4.53	1.70-12.1	3.89	1.45-10.46
<i>P</i> trend		0.001		0.004	

Values are adjusted relative risks (RR) of dementia with 95% confidence interval (CI) compared to no atrophy and *P*-value of trend analysis

*The MRI measures adjusted for were severity of periventricular white matter lesions, brain infarcts, and severity of subcortical atrophy

†Based on age- and sex specific tertiles

Table 3. Baseline amygdalar atrophy on MRI and risk of dementia

Atrophy severity†	Dementia	Relative risk (RR)			
		Adjusted for age, sex and education		Adjusted for age, sex, education and other MRI measures*	
		RR	95% CI	RR	95% CI
No atrophy (n=170)	3	1.00	Ref	1.00	Ref
Moderate atrophy (n=171)	13	4.42	1.26-15.54	4.17	1.18-14.72
Severe atrophy (n=170)	19	7.33	2.15-24.96	6.01	1.74-20.77
<i>P</i> trend		0.001		0.003	

Values are adjusted relative risks (RR) of dementia with 95% confidence interval (CI) compared to no atrophy and *P*-value of trend analysis

*The MRI measures adjusted for were severity of periventricular white matter lesions, brain infarcts, and severity of subcortical atrophy

†Based on age- and sex specific tertiles

In the whole population, we found per SD decrease in hippocampal volume an age, sex and education adjusted relative risk of 2.99 (95%CI 1.96-4.57) and in amygdalar volume 2.06 (95% CI 1.46-2.90). This effect was similar after exclusion of persons with memory complaints, a low MMSE, low performance on psychomotor speed, low memory performance or MCI at baseline (Figure 1).

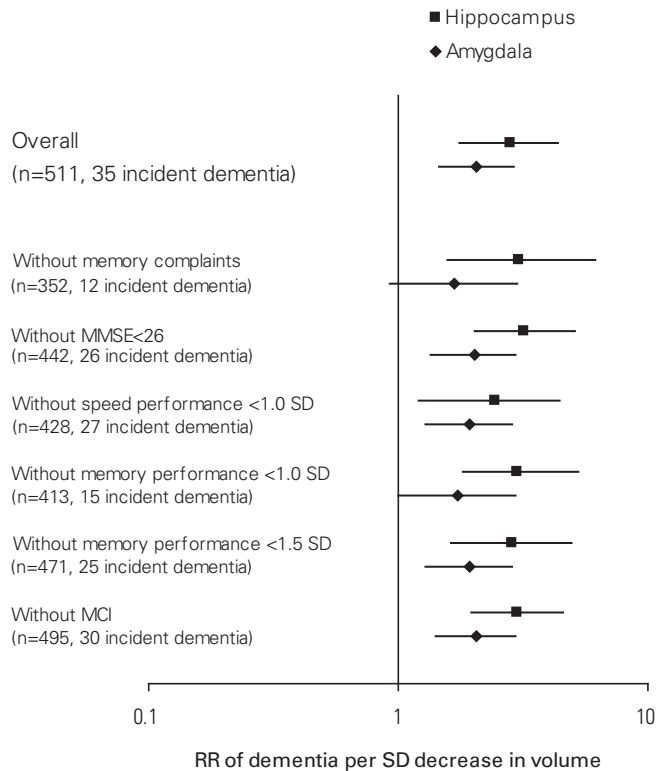


Figure 1. Relative risk of dementia with smaller hippocampal and amygdalar volumes on MRI (per SD decrease with 95% confidence interval). Analyses were done in the whole population and in persons without memory complaints, without a MMSE<26, without a low performance on psychomotor tasks (<1 SD below age and education adjusted means) without a low memory performance (<1 or 1.5 SD below age and education adjusted means) and without MCI at baseline. Adjustments were made for age, sex and education

Figure 2 shows that incident Alzheimer cases had significantly smaller hippocampal and amygdalar volumes at baseline than persons without incident dementia. Age, sex and education adjusted volumes were on average -10.7% (95% CI -19.3 to -2.1) smaller for the hippocampus and -10.3% (95% CI -19.9 to -0.8) smaller for the amygdala. Atrophy was also evident at baseline in people who received a diagnosis of Alzheimer's disease more than 5 years after the MRI but this was less pronounced.

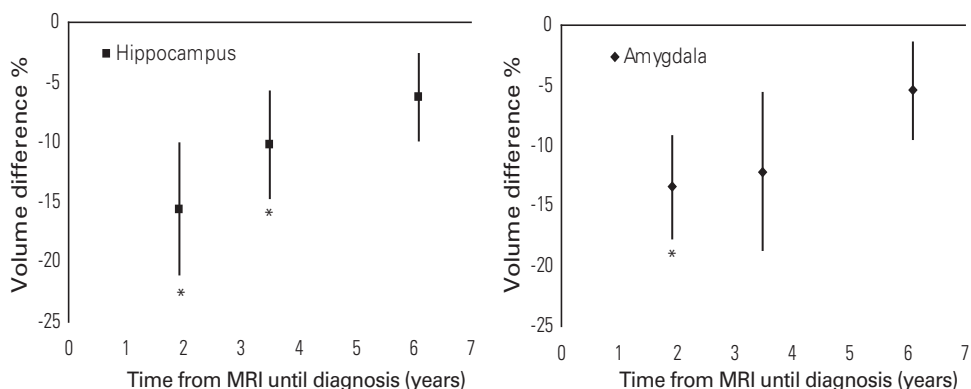


Figure 2. Volume difference at baseline MRI in hippocampus (left panel) and amygdala (right panel) of persons who developed AD ($n=26$) at different time points after baseline compared to the volume of people who were free of dementia and alive. We made tertiles according to time after baseline the diagnosis was made and plotted the differences (with standard error) at the median time point in these tertiles. Adjustments were made for age, sex and education. * $P<0.05$

Discussion

In this large cohort study in elderly people dwelling in the community, we found atrophy of the hippocampus and amygdala on MRI to predict dementia and Alzheimer's disease over a 6-year follow-up even in persons without memory complaints or low cognitive performance at baseline.

Despite our large cohort only few people developed clinical symptoms of dementia, as expected based on incident rates in the general population.²⁸ Vascular dementia could therefore not be analyzed as a separate entity. Clinical studies however suggest that patients with vascular dementia have a degree of hippocampal atrophy that lies between healthy elderly and

Alzheimer patients.²⁹ Another issue is that we only assessed the hippocampus and amygdala while recently some investigators propose that atrophy of the entorhinal cortex on MRI has a greater ability to predict Alzheimer's disease than the hippocampus.³⁰ However, difficulties in outlining the boundaries of the entorhinal cortex due to anatomic ambiguity make this assessment not easily applicable.¹⁴

As the neuropathology of Alzheimer's disease is found first, and remains most severe, in the hippocampal region, attention of imaging studies in Alzheimer's disease has been drawn to the hippocampus. Moreover, the in-vivo use of MRI of the hippocampus in Alzheimer's disease has been histologically correlated to neuronal loss and Alzheimer pathology.^{4, 31, 32} After early MRI studies showing atrophy of the hippocampus in patients with dementia of moderate severity,^{5, 6} later studies found atrophy also in patients with milder dementia.^{9, 15, 20} Atrophy on MRI is also observed before the diagnosis of dementia is made in high-risk populations such as MCI patients⁹⁻¹⁵ or persons at risk of autosomal dominant familial AD.^{33, 34} Within MCI patients, hippocampal atrophy severity predicts conversion to dementia independent of neuropsychological performance.^{26, 30, 35} We found that hippocampal and amygdalar atrophy on MRI predict dementia over a long follow-up period even when people have no memory complaints or cognitive impairment. This suggests that atrophy of structures in the medial temporal lobe is detectable several years before symptom onset. Although pathological studies suggested that the amygdala is affected slightly later in the Alzheimer process than the hippocampus,³ we found amygdalar atrophy to a similar extent as hippocampal atrophy in persons who develop dementia. Cross-sectional studies in mild AD patients also showed hippocampal and amygdalar atrophy to an equal degree.^{12, 20} Concerning the extent of atrophy, we found on average 10% smaller volumes in those destined to develop dementia. In persons with mild to moderate AD, volume reductions are between 25% and 40%³⁶ suggesting that atrophy accelerates in incipient AD.¹³ In a study in 5 middle-aged persons who developed familial AD within 3 years, a 16% volume difference in medial temporal lobe structures with healthy controls was observed.³⁴ In our elderly sample, the medial temporal lobe could be less affected by Alzheimer pathology as comorbid cerebrovascular disease may have contributed to the cognitive decline pushing people earlier over the threshold of dementia.^{18, 37-39} Our results were not changed when we took into account concurrent vascular brain changes on MRI suggesting that vascular brain pathology and atrophy on MRI reflect different underlying processes that both independently predict dementia in late life.

The emergence of potentially disease-modifying therapies for Alzheimer's disease urges the need to identify high-risk persons who may benefit from such therapies. Treatment or prevention might have the largest effect early in the disease process, in a stage where brain damage is not extensive and there are no or only few symptoms. A small hippocampus or amygdala on MRI in people without memory complaints or cognitive impairment may be indicative of

such a high-risk group early in the disease. However, we must bear in mind that the majority of people with atrophy do not develop dementia. Future studies are challenged to investigate whether other regions on MRI or other modalities can distinguish those with a small brain volume who are in the process of developing dementia and those who are not.

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Chapter 4.3

Brain atrophy and incident depression

Cross-sectional studies have suggested links between late-life depression and hippocampal, amygdalar and frontal atrophy on MRI. It is unclear whether atrophy in these structures could precede onset of depression. We followed a population-based sample of 459, non-demented, non-depressed elderly at baseline, over a three-year period during which 24 developed a depression. At baseline, MRI of the brain was made on which volumes of the hippocampus and amygdala and a score of frontal atrophy were assessed. Frontal atrophy, but not hippocampal or amygdalar atrophy, was associated with an increased risk of depression (per point increase odds ratio 2.0, (95% CI 1.1-3.8)).

Introduction

Several cross-sectional clinical studies found hippocampal, possibly amygdalar, and frontal lobe atrophy on magnetic resonance imaging (MRI) in patients with depression (reviewed by Sheline¹). It has been suggested that atrophy in these regions is a consequence of elevated, and neurotoxic, glucocorticoid levels accompanying the depression. However, atrophy could also be a cause of depression, as these structures have a role in mood regulation.¹ To investigate the latter hypothesis, we prospectively examined hippocampal, amygdalar and frontal brain atrophy on MRI in relation to risk of depression in the elderly.

Methods

Subjects

This study is based on the Rotterdam Scan Study, a population-based cohort study designed to investigate brain abnormalities on MRI.² The study protocol was approved by the Medical Ethics Committee of Erasmus Medical Center and participants gave written informed consent. The cohort consists of 1,077 non-demented elderly persons age 60 to 90 years (mean age 73, 49% women) who were examined at baseline in 1995-1996 and underwent brain MRI in a 1.5T scanner (VISION MR). During sample selection, we carefully excluded demented patients using a stepwise protocol.³ Axial T1 MRI sequences were made in all 1,077 participants. In 511 persons, we additionally obtained a three-dimensional (3D) MR sequence covering the whole brain. These 511 persons did not differ in several demographics from the original cohort of 1,077 persons.²

Incident depression

At baseline MRI, we excluded 43 participants with depressive symptoms based on the use of antidepressant medications or based on a score ≥ 16 on the Centre for Epidemiologic Studies Depression scale (CES-D), a cut-off widely used and highly sensitive for depression.⁴ This left 468 participants at risk of incident depression. Incident depression was detected in two ways. First, we invited all living members of the cohort for follow-up examinations in 1999-2000. From the total 432 living members, 334 (77%) agreed to participate and they were screened with the CES-D. Persons who refused ($n=98$) were on average 3.9 years older (T-test, $P<0.001$) but were similar with respect to sex distribution, CES-D score at baseline, and MRI assessments compared to participants. Persons with a CES-D score ≥ 16 at follow-up examinations were visited at home by a psychiatrist. The psychiatrist evaluated these participants with the Present State Examination, a semi-structured psychiatric interview,⁵ and made a diagnosis of major depression, minor depression, or dysthymia according to DSM-IV criteria.

As depression can occur between baseline and follow-up examinations and in persons who refused follow-up examinations, a second way to detect incident depression was through checking of medical records by two raters of all 468 participants at the general practitioners' offices for reports on treated depression until March 1st 2000. Five persons with no information from medical records and four persons with anxiety or other psychiatric disorders were excluded, leaving 459 participants for the analyses.

MRI acquisition and measurements

The MRI acquisition parameters are reported elsewhere.² Coronal MRI slices (contiguous 1.5-mm slices) were reformatted from the 3D MR sequence. The procedure of segmenting the hippocampus and amygdala has been described.² Briefly, we manually outlined on coronal slices the hippocampi and amygdalae on both sides with a mouse-driven cursor. Volumes were calculated by summing the areas multiplied by slice thickness. As a proxy for head size, we measured on the middle sagittal slice the intracranial area by tracing the inner skull. Volumes were divided by the intracranial area and the resulting ratio was standardized towards average head size (men and women separately). As one volumetric assessment was time-consuming (1-hour), the 459 scans were equally divided between two raters. Intra- and interrater studies based on 14 subjects done by both raters showed intraclass correlation coefficients to exceed $r=0.77$. We rated frontal cortical atrophy semi-quantitatively on axial T1 MRI slices. Two raters scored independently all the scans and gave a score of 0 (no atrophy) to 3 (severe atrophy) based on the average widening of the sulci of the frontal lobe and in comparison to reference scans. The central sulcus was the delimiter between the frontal and parietal lobe. In case of disagreement of more than 1 point between the raters a consensus reading was held, otherwise the mean score was calculated. Weighted kappa values of intra- and interrater agreement were 0.57 and 0.71 based on 200 scans. Other regions were similarly assessed for severity of atrophy (insular region, parietal lobe, occipital lobe and temporal lobe).

Data analysis

First, we used multiple linear regression modeling to compare hippocampal and amygdalar volumes and frontal atrophy score between persons with and without incident depression. Because the associations were similar for left or right-sided hippocampal or amygdalar volumes, we summed the left and right side to yield total volumes. Second, we calculated odds ratios (95% confidence interval CI) of incident depression by baseline MRI variables with logistic regression using the non-depressed as reference group. Adjustments were made for age and sex and additionally for baseline CES-D. Additionally, we excluded persons with a history of a depression before baseline (based on medical records), and persons who developed dementia in follow-up.³

Results

Hippocampal and amygdalar volumes on baseline MRI were similar in participants who developed a depression during follow-up ($n=24$ of whom 8 diagnosed by medical records) compared to those who did not ($n=435$) (Table 1). The corresponding odds ratios of incident depression were 0.9 (95% CI 0.6-1.4, $P=0.6$) per standard deviation (SD) decrease in hippocampal volume and 1.3 (95% CI 0.8-2.0, $P=0.2$) per SD decrease in amygdalar volume. In contrast, there was significantly more frontal atrophy in persons who developed depression during follow-up compared to persons who did not (Table 1); corresponding odds ratio per point increase 2.0 (95% CI 1.1-3.8, $P=0.031$). Adjusting for baseline CES-D did not change these results. Other regions for which we assessed cortical atrophy were not associated with depression. Persons with a history of depression at baseline ($n=14$) had not more frontal atrophy (adjusted difference compared to people with no history 0.10, 95% CI $-0.23;0.42$, $P=0.57$). Excluding them, of whom 4 developed a depression during follow-up, or persons with incident dementia ($n=15$ of whom 2 developed a depression) did not substantially change the association with frontal atrophy (odds ratio for frontal atrophy 2.2 (95% CI 1.1-4.4, $P=0.030$) and 1.8 (95% CI 0.9-3.5, $P=0.088$), respectively).

Table 1. Hippocampal, amygdalar volumes, and frontal brain atrophy at baseline MRI in relation to incident depression

MRI variable at baseline											
Hippocampal volume (ml)*						Amygdalar volume (ml)*					
Depression	Mean	Difference	95% CI	P		Mean	Difference	95% CI	P	Mean	Difference
No	6.38	-	-	-	4.59	-	-	-	-	1.22	-
Incident	6.47	0.09	-0.26;0.44	0.6	4.42	-0.17	-0.46;0.12	0.3	1.49	0.28	0.02;0.53
Frontal atrophy (grade, range 0-3)*											
Depression	Mean	Difference	95% CI	P		Mean	Difference	95% CI	P		
No											
Incident											

*Adjusted means in, and differences (with 95% confidence interval CI and P-value) between persons without depression (n=435) and with incident depression according to DSM-IV (n=24). Adjustments were made for age and sex differences between groups

Discussion

In this community-sample in the elderly, we observed no relation between hippocampal or amygdalar volumes on MRI and the risk of depression. Conversely, frontal brain atrophy was associated with an increased risk of depression.

A limitation of our study was the small number of incident depression cases, which explains the wide confidence intervals around the estimates. Furthermore, we recognize that currently more sophisticated, volumetric, assessments of the frontal lobe and specific regions in the frontal lobe are available^{6, 7} than at baseline in 1995. However, the fact that we found an association between our crude frontal atrophy assessment and incident depression suggests that we had enough power to detect associations between MRI variables and incident depression, strengthening the negative finding for the hippocampus and amygdala.

A reduced hippocampal or amygdalar volume on MRI in patients with late life depression has been observed though results are mixed.¹ Two explanations for this association have been proposed. First, a smaller hippocampal volume may be a consequence of depression due to associated hypercortisolemia. Elevated cortisol levels are neurotoxic to the hippocampus,⁸ but possibly this effect plays a role especially in early onset depression. A second explanation may be that volume reduction in the hippocampus and amygdala precedes onset of depression, given that these structures are involved in mood regulation.¹ In this report, we showed that neither hippocampal nor amygdalar atrophy on MRI increased the risk to develop depression. This observation strongly challenges the idea that atrophy of the hippocampus or amygdala precedes depression in later life. Consistent with this idea is a recent study that showed no hippocampal atrophy on MRI in persons with a first episode of depression, suggesting that reductions in hippocampal volume do not precede illness onset.⁹ Furthermore, persons with a longer disease duration have smaller hippocampal volume suggesting that volumes decrease after disease onset.^{9, 10} Longitudinal clinical studies should focus on whether hippocampal and amygdalar volume decrease in patients with depression, possibly due to lasting hypercortisolemia.

Most clinical studies in patients with late life major or minor depression found reductions in the frontal lobes on MRI.¹ Functional imaging studies have also implicated hypometabolism of the frontal lobes in depression.¹¹ The prefrontal cortex has high concentrations of glucocorticoid receptors potentially rendering it vulnerable to hypercortisolemic damage.¹ This suggests that damage to the frontal lobe is a result of depression. However, we observed that frontal atrophy on MRI is detectable before disease onset, and non-depressed persons with a history of depression had no increased frontal atrophy. This suggests that frontal atrophy indicates an increased vulnerability to develop depression in late life.

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Chapter 5

General discussion



This thesis presents studies on causes and correlates of brain atrophy on MRI in non-demented elderly. Studying causes of atrophy on MRI has potential advantages for unraveling the etiology of Alzheimer's disease compared to traditional studies associating risk factors to clinical Alzheimer's disease. First, as we focus on the preclinical period, we study factors that could initiate brain changes involved in the early pathogenesis of Alzheimer's disease. Second, the degree of brain atrophy on MRI is a continuous outcome in contrast to clinical Alzheimer's disease, and we have increased power to detect associations. Third, our study examined cognitively intact people and may therefore suffer less from selective attrition compared to studies based on patients with Alzheimer's disease.

Before we started to investigate potential causes of brain atrophy on MRI there was some indication that atrophy on MRI, in particular of the hippocampus and amygdala, could be observed in the preclinical period of Alzheimer's disease. However, we wanted to validate and quantify in our study sample the association between atrophy on MRI and future clinical symptoms of Alzheimer's disease. We further examined whether atrophy also predicted depression. In this chapter, I will first discuss a few methodological considerations. Then, I will discuss the main findings, the implications of our findings, and suggestions for future research.

Methodological considerations

The specific limitations of each study have been discussed in the previous chapters. In this part I will discuss two general issues, which pertain to all studies performed.

Study design

The studies described in this thesis were done as part of the Rotterdam Scan Study.¹ This is a population-based prospective study among 1,077 non-demented elderly between 60-90 years of age that started in 1995-1996. In all 1,077 persons, we had assessments of global brain atrophy on MRI. In 511 persons, we additionally had volumetric assessments of the hippocampus and amygdala. A specific feature of this design is that we invited people randomly from the general population. This is in contrast to clinical studies which use a selection of people who have memory problems and have taken the step to visit a physician. In etiological research we are interested in what triggers the first pathological signs, as the pathology starts before symptom onset. For this purpose, population studies are more suitable than clinical studies. Inherent to our approach is the fact that only a few people develop dementia, as expected in a low risk population. To investigate whether atrophy on MRI predicts dementia, we therefore needed a long follow-up duration and a large cohort in order to detect enough incident cases of dementia.

In 1995-1996, we assessed potential risk factors in persons at the same time that we made

the brain MRI on which we assessed atrophy. This cross-sectional design is a limitation that needs further discussion. In cross-sectional analyses, one cannot invariably draw the conclusion that the risk factor under study really is a cause of brain atrophy. Because we have no information on whether the risk factor preceded brain atrophy or vice versa, brain atrophy may have also resulted in physiological changes. When genetic determinants are concerned, this is not an issue as atrophy will not induce point mutations in DNA. However, in the analyses on alcohol intake and plasma homocysteine levels, which depend on folate and vitamin B12 intake, persons with brain atrophy and self-noticed cognitive decline could have changed food intake behavior. This in turn may induce associations between alcohol intake or homocysteine levels and brain atrophy. Although we tried to account for several confounding parameters, residual confounding is still possible. To resolve or limit the possibility that atrophy induced the change in a physiological marker, follow-up studies are needed where the baseline risk factor is associated with change in atrophy over time. These analyses are on the other hand limited by the fact that atrophy has developed over years due to lifetime cumulative exposure of determinants whereas the relation between determinant and change in atrophy over time may be less outspoken.

Brain atrophy as preclinical marker of Alzheimer's disease

Our studies were based on the major assumption that atrophy on MRI is a preclinical biomarker of Alzheimer's disease. To what extent does this assumption hold? Several studies have shown that patients with Alzheimer's disease have severe hippocampal, amygdalar and global brain atrophy on MRI compared to healthy elderly.² Preclinically, there are studies in at-risk patients such as those with genetic mutations³ or with mild cognitive impairment⁴ that show brain atrophy on MRI before a diagnosis of Alzheimer's disease can be made. Our own data (chapter 4.2) also indicated that even when there are no cognitive symptoms of incipient Alzheimer's disease, hippocampal and amygdalar atrophy on MRI predict a diagnosis of Alzheimer's disease several years later. These observations all favor the idea that atrophy on MRI can indeed be considered a relatively early preclinical marker of Alzheimer's disease. However, not every person with atrophy on MRI will later express clinical symptoms of Alzheimer's disease. Some will die before they have the chance to develop clinical symptoms of Alzheimer's disease. In others, a small brain volume on MRI may have been present since adulthood instead of being indicative of an underlying Alzheimer process. Finally, in several other diseases besides Alzheimer's disease, atrophy can occur such as in temporal lobe epilepsy (hippocampal/amygdalar atrophy)⁵ or anxiety (amygdalar atrophy).⁶ Therefore, when we identify a risk factor for brain atrophy on MRI, we have to be careful to interpret this as being a risk factor for the development of clinical Alzheimer's disease without further evidence. In chapter 3.2, we found that estrogen receptor α polymorphisms were associated with hippo-

campal and amygdalar atrophy on MRI yet not with dementia. Risk factors of atrophy should always be seen in light of evidence concerning these risk factors in Alzheimer's disease. Studies on risk factors for atrophy should not replace research of risk factors for clinical Alzheimer's disease but rather complement it in order to increase the understanding of what could elicit the preclinical brain changes.

Main findings

I will first discuss the main findings on putative causes of brain atrophy on MRI (chapter 2 and 3). Then, I discuss the clinical correlates of brain atrophy on MRI (chapter 4).

Causes of brain atrophy

Despite intensive research, the etiology of Alzheimer's disease remains largely unclear. Two aspects of the disease complicate etiological research. First, once symptoms of Alzheimer's disease are clearly evident, a long prodromal phase has been passed in which the brain pathology has accumulated. Physiological factors present at time of diagnosis of Alzheimer's disease, may not be the ones that effectuated the brain damage in the preclinical period. Second, late-onset Alzheimer's disease is pathologically a heterogeneous disease.⁷ The brain of a person who died with clinical symptoms of Alzheimer's disease is often destructed by various pathologies: besides neurofibrillary tangles and amyloid plaques in gray matter, lacunar infarcts and ischemia in the white matter are also frequently visible.⁸ Among individuals with clinical symptoms of Alzheimer's disease, the extent to which each pathology contributed to symptoms may differ, as well as the key mechanisms underlying these brain changes. We took the approach of investigating risk factors of brain atrophy on MRI *in vivo*, which is evident in the preclinical phase, and reflects a relatively distinct pathology. By doing so, we hoped to further increase insight into which risk factors contribute to the development of Alzheimer's disease. The risk factors we studied were not arbitrarily chosen but selected because of their putative role in Alzheimer's disease.

Vascular risk factors

A great deal of research over the last decade has added to the idea that vascular factors contribute to the development of clinical Alzheimer's disease in late life.⁹ Vascular factors can cause cerebrovascular damage, which enhances cognitive decline in a brain with existing Alzheimer pathology.¹⁰ However, cerebral ischemia can also upregulate amyloid- β in the hippocampi of rats¹¹ supporting a more direct link with Alzheimer's disease. We investigated several vascular risk factors in relation to brain atrophy on MRI.

A high blood pressure level is a well-recognized cardio- and cerebrovascular risk factor. The

relation between blood pressure and Alzheimer's disease is less clear.¹² Both mid-life high,^{13, 14} and late-life low¹⁵ blood pressure levels have been associated with the risk of clinical Alzheimer's disease. In line with these earlier observations, we found that both a high and a low or decreasing blood pressure level were associated with medial temporal lobe and global brain atrophy (chapter 2.1 and 2.2). Cerebrovascular damage as quantified by the extent of white matter lesions on MRI coexisted with severe hippocampal and amygdalar atrophy. One possibility is that cerebrovascular disease and brain atrophy have common shared risk factors such as high blood pressure levels. However, adjusting for blood pressure levels did not change this correlation. Possibly, microangiopathy of arterioles supplying the medial temporal lobe, visible as white matter lesions on MRI, may reduce cerebral blood flow leading to ischemia in the hippocampus and amygdala.¹⁶ The association between low blood pressure and atrophy on MRI as well as that between low blood pressure and Alzheimer's disease is difficult to interpret. Several regions of the brain, including the hippocampus and amygdala, are involved in blood pressure regulation and brain atrophy due to an Alzheimer process could have reduced blood pressure levels.^{17,18} Alternatively, a low blood pressure level in persons with a bad cerebral autoregulation could also cause cerebral ischemia, and thereby actually lead to the development of Alzheimer pathology.¹⁹

A high plasma homocysteine level has recently been suggested as a risk factor for Alzheimer's disease.²⁰ In the Rotterdam Scan Study, we previously found that a high homocysteine level may cause cerebrovascular damage such as silent brain infarcts and white matter lesions,²¹ pointing towards one potential explanation for the association between homocysteine levels and clinical Alzheimer's disease. In chapter 2.3 we report that a high homocysteine level also has effects on both hippocampal and global brain atrophy, on MRI, which were not reduced when accounting for cerebrovascular disease. This is in line with *in vitro* studies showing toxic effects of homocysteine on cortical and hippocampal neurons.^{22, 23}

A light-to-moderate alcohol intake may reduce cerebrovascular disease²⁴ and vascular dementia.²⁵ Concerning the risk of Alzheimer's disease, the effect of alcohol intake is less certain and a putative reduced risk may be confined to apolipoprotein (APOE) $\epsilon 4$ carriers who consume alcohol in light-to-moderate amounts.²⁵ Since we have assessments on MRI of both cerebrovascular disease (white matter lesions and infarcts) and findings more specific of Alzheimer pathology (hippocampal and amygdalar atrophy) in the Rotterdam Scan Study, we decided to study this issue further. Our data in chapter 2.4 showed, as expected, a reduced effect of alcohol on cerebrovascular disease in general. There was only a beneficial effect of light-to-moderate alcohol intake on hippocampal and amygdalar atrophy in APOE $\epsilon 4$ carriers further strengthening the idea that alcohol has an effect on the risk of Alzheimer's disease only in APOE $\epsilon 4$ carriers.

One of the strongest risk factor for Alzheimer's disease is carrying an APOE $\epsilon 4$ allele.²⁶ The

neurobiologic mechanism through which the $\epsilon 4$ allele confers an increased risk of Alzheimer's disease is not well understood. Some studies suggest that persons with the $\epsilon 4$ allele have increased cerebrovascular pathology²⁷ but the bulk of evidence suggests that APOE $\epsilon 4$ leads to clinical disease mainly via the specific Alzheimer pathology.²⁸ In vitro studies suggest that the $\epsilon 4$ protein isoform enhances aggregation of amyloid- β and formation of neurofibrillary tangles. APOE $\epsilon 4$ carriers have higher numbers of neurofibrillary tangles and amyloid plaques in the hippocampus compared to non- $\epsilon 4$ carriers.²⁹ Our finding that atrophy in the medial temporal lobe was more pronounced in $\epsilon 4$ carriers compared to persons with the APOE $\epsilon 3\epsilon 3$ genotype gave further support to our assumption that atrophy on MRI reflects Alzheimer pathology (chapter 2.5).

Taken together, we found several classical or more recently identified vascular risk factors to be associated to brain atrophy on MRI. This supports the idea that vascular factors could specifically trigger Alzheimer pathology in the preclinical phase.

Endocrine risk factors

Besides vascular risk factors, other risk factors have been suggested to be involved in the development of Alzheimer's disease.

A substantial body of evidence in animal studies suggest that estrogens have antioxidative properties³⁰ and beneficial effects on hippocampal neurons.³¹ Therefore in theory, a high estrogen level in the brain could reduce the risk of Alzheimer's disease. On the other hand, observational data in humans are far from conclusive on the role of estrogens in Alzheimer's disease. Before menopause, women have high endogenous estrogen levels and it could therefore be hypothesized that women with a long reproductive period, and hence a high lifetime cumulative exposure to estrogen, would have a lower risk of Alzheimer's disease. However, in the Rotterdam Study it was found that their risk to develop Alzheimer's disease was higher compared to those with shorter reproductive periods.³² After menopause, endogenous estrogen levels drop and in older women with a relatively high endogenous estradiol level the risk to develop dementia was highest.³³ In line with this finding, we showed in chapter 3.1 that higher endogenous levels of estrogens are not associated with a larger volume of the hippocampus on MRI or better memory performance. Endogenous levels after menopause are, however, far below levels obtained after estrogen replacement therapy and it could be that "supranatural" estrogen levels protect against Alzheimer's disease. Recent trials on estrogen replacement therapy in preventing or slowing of Alzheimer's disease do not support this idea.^{34, 35} In fact, elderly women who receive a combination of estrogen and progestin have a two times increased risk to develop Alzheimer's disease compared to women on placebo.³⁴ A similar kind of discrepancy between findings of basic sciences (estrogen is protective)³⁶ and randomized trials (estrogen is harmful)³⁷ has been observed in cardiovascular

research. In this field it has been suggested that genetic variations in the gene encoding for estrogen receptor α render some women more susceptible to the beneficial or harmful effects of estrogen.³⁸ Effects of estrogen replacement therapy on lipid profile are different across genotype³⁹ and polymorphisms in the estrogen receptor α are associated with risk of cardiovascular disease.⁴⁰ We found that polymorphisms in the estrogen receptor α gene are not associated with the risk of Alzheimer's disease (chapter 3.2). This makes it less plausible, though not impossible, that estrogen replacement therapy will have a beneficial effect on risk of dementia in women with a certain variation in the estrogen receptor α gene.

Other endocrine factors that we examined were diabetes mellitus and insulin resistance (chapter 3.3). Although these factors could also be categorized as vascular risk factors, evidence in Alzheimer research suggests that it is not the vascular risk component of diabetes mellitus and insulin resistance that causes the increased risk of Alzheimer's disease. Instead, a high glucose level or altered insulin signaling could have direct effects on the development of amyloid plaques and neurofibrillary tangles in the brain.^{41, 42} In line with this idea, we showed that persons with diabetes mellitus have increased atrophy of the hippocampus and amygdala on MRI. Of interest, the relation between diabetes mellitus and cerebrovascular disease was not strong and did not account for the associations with atrophy. The mechanisms that underlie the associations between diabetes mellitus and atrophy could be manifold. First, advanced glycation endproducts, of which persons with diabetes have increased levels, could trigger the aggregation of amyloid proteins into amyloid plaques.⁴¹ Second, glycation of the microtubule associated protein-tau could lead to formation of neurofibrillary tangles.⁴³ With respect to insulin, evidence from several lines of research has supported links with Alzheimer's disease. Insulin receptors are densely distributed in the brain⁴⁴ and once activated lead to a cascade of events that promote neuronal survival and prevent tau phosphorylation.⁴² Dysfunction of this pathway may therefore lead to Alzheimer's disease and genetic variations in the genes encoding for proteins in this insulin pathway have been associated to Alzheimer's disease.^{42, 44, 45} Plasma insulin levels may secondarily increase to compensate for this loss of receptor function. Furthermore, the insulin-degrading enzyme (IDE) is present in the brain and, in addition to insulin, also degrades amyloid proteins, thereby preventing plaque formation. Mice with hypofunction of this enzyme have more amyloid plaques and high insulin plasma levels⁴⁶ and variations in the gene encoding IDE have been found in patients with Alzheimer's disease.⁴⁷ In our study, interestingly, we found a specific effect of insulin resistance on amygdalar atrophy on MRI but not on hippocampal atrophy.

Specificity of measures of brain atrophy on MRI

We investigated putative causes of several different measures of brain atrophy on MRI (hippocampal, amygdalar, cortical and subcortical brain atrophy). Sometimes we found that a risk factor was specifically associated with one brain atrophy measure while not with the other atrophy measures. The APOE ϵ 4 allele for example was associated with hippocampal and amygdalar atrophy yet not with global brain atrophy. One possible source for different associations may be that global brain atrophy was less precisely measured (semi-quantitative) than the hippocampus and amygdala (volumetric). However, a more likely explanation is that anatomically different structures are under study or that the MRI measures have different pathological substrates.

Though frequently observed in Alzheimer patients,⁴⁸ there is no firmly defined pathological basis of global brain atrophy on MRI, which can be divided in cortical (sulcal widening) and subcortical (ventricular enlargement) atrophy. Although the name “cortical” atrophy would imply neuronal loss, there is only little neuronal loss with aging.⁴⁹ Sulcal widening may be the result of reduction of myelinated nerve fibers underneath the cortex in the white matter.⁴⁹ The nerve fibers that are lost presumably originate from the projecting pyramidal cells in the cortex. However, if there is little loss of cortical neurons, then the projecting myelinated axons must degenerate without the loss of the parental cell body. Subcortical atrophy indicates ventricular enlargement at the expense of white matter around the ventricles (hydrocephalus ex vacuo). Volumetric MRI studies have shown that brain white matter volume decreases with age⁵⁰ most likely due to loss of myelin with relative preservation of axons. Given that we do not have a pathological validation of global brain atrophy on MRI, it remains uncertain on which places (cortex or white matter) risk factors act. High blood pressure or homocysteine levels could for example induce neurofibrillary tangles and shrinkage of neurons in the cortex. However, effects such as ischemia in the white matter regions and loss of white matter volume could also be involved in the associations with global brain atrophy on MRI. Detailed segmentation of the brain in gray matter, white matter and cerebrospinal fluid volume and separately investigating the correlations with vascular factors could resolve this problem.

Compared to global brain atrophy on MRI, there is a stronger histological validation for hippocampal and amygdalar atrophy on MRI. Volumes of these structures on MRI are strongly positively correlated with neuronal count,^{51, 52} and negatively with the extent of the specific Alzheimer pathology, i.e. neurofibrillary tangles and amyloid plaques.⁵³ The risk factors that we found for hippocampal and amygdalar atrophy act therefore specifically on neurons of these brain structures or on the development of Alzheimer pathology for which these structures are highly susceptible. From the risk factors we studied blood pressure levels, homocysteine, alcohol intake, APOE gene, estrogen receptor α polymorphism and diabetes mellitus were more or less similarly associated with hippocampal and amygdalar atrophy on MRI. A notable

difference was insulin resistance, which was strongly associated with amygdalar atrophy but absolutely not with hippocampal atrophy on MRI (chapter 3.3). A recent study also found no association between insulin resistance and hippocampal volumes on MRI⁵⁴ but that study did not assess the amygdala. The biochemical properties of the Alzheimer pathology are, to my knowledge, similar in both structures and develop approximately at the same time in the process of Alzheimer's disease.⁵⁵ This suggests that differences between neurons of the hippocampus and amygdala result in differential effects of insulin resistance. The density of insulin receptors on hippocampal and amygdalar neurons are slightly different with the hippocampus having a higher density.⁵⁶ Possibly, the hippocampus is less sensitive for effects of insulin resistance than the amygdala due to its higher receptor density.

Clinical correlates of brain atrophy

The work we described on risk factors of brain atrophy on MRI started based on the belief that brain atrophy on MRI can be observed early in the process of Alzheimer's disease. To investigate the functional significance of brain atrophy on MRI in the preclinical period, we addressed the relation between brain atrophy and memory performance since memory impairment is the first preclinical neuropsychological sign of Alzheimer's disease.⁵⁷ Additionally, we quantified the association between brain atrophy on MRI and risk of dementia and risk of depression.

In chapter 4.1 we report that hippocampal atrophy on MRI of non-demented elderly correlates with worse memory performance. Persons with smaller volumes of the hippocampus on MRI, particularly of the head portion, performed worse on a verbal memory task. Since 1957, the medial temporal lobe has been identified to play an important role in memory function. Selective memory impairment was described after bilateral surgical removal of the medial temporal lobe in patient H.M.⁵⁸ However, the medial temporal lobe is a large region that includes the hippocampus, the amygdala and adjacent cortical areas. The precise contribution of each of these structures in memory function is just starting to be disentangled. Moreover, even within a structure, there may exist functional segregation. Response to novelty is, for example, mapped on the head portion of the hippocampus⁵⁹ whereas spatial memory is probably located in the posterior hippocampus.⁶⁰ The early observable verbal memory decline in incipient Alzheimer's disease is by most researchers attributed to the accumulation of Alzheimer pathology in the hippocampus.⁶¹ Damage to the hippocampus in this stage may go hand-in-hand with complaints of worse memory function and low memory performance. However, whether a person complains or not will also depend on personality traits or social factors. In chapter 4.2 we show that hippocampal atrophy on MRI predicts dementia even when a person did not report memory complaints or had a lower than average memory performance. This suggests that although the Alzheimer process already accumulates in the

hippocampus and can be detectable as atrophy on MRI, persons do not necessarily complain of bad memory or perform worse on memory tests. We found that amygdalar atrophy on MRI also predicts dementia. It is unclear whether in the preclinical phase of Alzheimer's disease amygdalar atrophy has functional correlates. Experiments in monkeys demonstrated that amygdalar damage does not impair memory.⁶² In line, we found no pronounced amygdala atrophy in persons with worse verbal memory (chapter 2.5). The amygdala consists of several nuclei which all could have separate functions but, in general, the amygdala functions in emotional information processing and fear conditioning.⁶³ In Japanese Alzheimer patients, worse memory of a traumatic event arising emotion (earthquake) was stronger correlated to amygdalar atrophy than to hippocampal atrophy on MRI.⁶⁴ Other studies showed increased anxiety in Alzheimer patients, which could possibly be contributed to damage of the amygdala by the Alzheimer process.⁶⁵

Clinical studies have shown atrophy of the hippocampus, the frontal lobe and sometimes amygdala in patients with depression compared to healthy controls.⁶⁶ Most consistently, this is observed in patients with a long duration of depression. The observation could be caused by hypercortisolemia which is neurotoxic and is present in patients with major depression. However, as the hippocampus, amygdala and frontal lobe, as part of the limbic system, are involved in emotion regulation it may also be that the presence of atrophy poses people at risk of mood disorders or a depression. We studied this latter hypothesis by investigating the longitudinal relation between atrophy and depression in persons who were non-depressed at baseline. While both hippocampal and amygdalar atrophy were not associated with incident depression, frontal atrophy was. These data make it unlikely that atrophy of the hippocampus and amygdala precedes a depression in late-life. In line, a recent clinical study showed no hippocampal atrophy in persons who entered the hospital with a first episode of depression but hippocampal atrophy was prominent in persons with multiple episodes of depression.⁶⁷ The frontal lobe, in particular the orbitofrontal cortex (gyrus rectus), has a connection to the limbic system and reductions in the gray matter of this area are observed in persons with depression.⁶⁸ What actually leads to this gray matter loss is unknown. Most researchers currently adhere to the "vascular depression hypothesis" of late life depression, stating that brain infarcts and white matter damage destruct brain pathways important for normal mood regulation. Hyperintensities in the basal ganglia coexist with a smaller orbitofrontal cortex volume suggesting a common, possibly vascular pathway of both pathologies.⁶⁸

Implications

Our findings were based on persons dwelling in the community who as a group are healthier than patients seen in clinical practice. However, I do think that our findings could have clinical implications. We showed that atrophy on MRI of the hippocampus and amygdala is a strong predictor of dementia independent of neuropsychological performance. In line, clinical studies in patients with mild cognitive impairment previously showed that atrophy of the hippocampus on MRI could predict progression to dementia.⁴ In case of finding a high degree of atrophy on MRI as of yet there is no therapeutical intervention available. However, these patients and caregivers can be advised and frequently followed. On the other hand, when finding no atrophy, patients may be reassured that their risk of progressing to dementia is low.⁴ Assessing MRI volumes by manual outlining of structures will be too labor intensive to implement in a normal diagnostic work-up. Visually rating of atrophy may be a good alternative⁶⁹ or automated segmentation methods⁷⁰ can be used.

We identified several risk factors for brain atrophy including vascular risk factors. Currently, it is not common knowledge that brain atrophy on MRI could have vascular determinants. Most frequently, brain atrophy on MRI is regarded as being normal with ageing and only when extensive be reported as indicative of neurodegeneration. Our findings suggest that persons with brain atrophy on MRI should at least be screened for potentially treatable risk factors such as high blood pressure level, diabetes mellitus and possibly high plasma homocysteine levels. It remains however to be demonstrated in clinical trials that treatment of these risk factors reduces the incidence of cognitive decline or dementia.

Suggestions for future research

The research described in this thesis was done in the framework of etiological research on Alzheimer's disease. The approach we took to investigate putative causes of a preclinical biomarker of Alzheimer's disease, in our case brain atrophy on MRI, has not been frequently pursued before. I think that this approach deserves further exploration. The following questions come to mind: which study sample to use? What biomarkers to use? What to do when we have identified a high-risk group?

Particular population-based samples could yield valuable information on the earliest preclinical changes. Clinical study samples are based on people with cognitive impairment and, as such, are further along the process of dementia than persons dwelling in the community. Because twenty-five percent of persons at age of 40 already have some neurofibrillary tangles in the brain,⁷¹ it is important to include persons in this young age range. A drawback of including middle-aged persons is that the incidence of dementia in this age group is very low.

Therefore, in a longitudinal setting, repeated measures of neuropsychological tests should be administered to detect subtle cognitive decline.

The biomarkers tested in the preclinical period can be manifold. Peripheral markers such as plasma amyloid- β levels⁷² or inflammatory markers⁷³ should be examined. Brain imaging could identify specific brain markers. We only addressed hippocampal, amygdalar and generalized brain atrophy on MRI whereas brain structures such as the entorhinal cortex may be affected by Alzheimer pathology even earlier.⁵⁵ Before jumping into assessing this structure by manual outlining, it will be more time-efficient to use automated protocols to label the entire brain.⁷⁴ In this way, we might also find other brain regions that previously have been unnoticed in the early development of Alzheimer's disease. For this purpose, high-resolution 3D MRI sequences should be administered on which, besides gray matter, also white matter lesions and brain infarcts can be quantified. Currently, it is beyond the resolution of MRI to visualize amyloid plaques or tangles and we merely see the consequences of these pathologies in terms of macroscopic volumes loss. Promising results have been made in postmortem tissue with high field strength MRI and binding molecules to detect amyloid plaques.⁷⁵ Another interesting imaging tool is functional MRI (fMRI) which visualizes functional changes in the brain. In 'at-risk' persons, differences in regional blood flow to brain regions during cognitive testing have been reported.⁷⁶ However, the development of paradigms used for fMRI is difficult and results will depend on how sensitive these paradigms are for specific changes in the preclinical phase.

To the extent that preclinical biomarkers predict Alzheimer's disease, they can be used as specific outcome measures in etiologic research. We found several environmental and genetic factors to be associated with brain atrophy on MRI. An interesting lead to further explore is the role of insulin metabolism in the etiology of Alzheimer's disease. In our study, insulin resistance was specifically associated with amygdalar atrophy on MRI but not with hippocampal atrophy or cerebrovascular disease. This suggests that Alzheimer patients with insulin resistance⁷⁷ might be a certain subgroup of patients with particularly severe amygdalar atrophy with less pronounced hippocampal atrophy or cerebrovascular disease. Genetic factors likely play a large role in the development of Alzheimer's disease. In interpreting genetic associations with brain volumes at late life, it must be kept in mind that identified genetic variations are present since conception. Some genetic factors may be involved in the development of the brain yet not in the etiology of Alzheimer's disease. The genetic variation in the estrogen receptor α polymorphism in association with amygdalar volumes on MRI may be examined in young populations, as this variation was not associated with Alzheimer's disease.

If we can reliably identify the preclinical period by a combination of biomarkers and neuropsychological tests, disease-modifying drugs, currently under development, could be tried in these people. Biomarkers could help in tracing the effects of treatment. Recently, a ran-

domized trial showed less pronounced hippocampal volume reduction on MRI over time in dementia patients using donepezil.⁷⁸ Before disease-modifying drugs are fully developed, clinical trials may study the effect of interventions in vascular risk profile in certain risk groups.

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Summary

Alzheimer's disease is a common disorder in the elderly that is clinically characterized by an insidious onset of memory decline followed by progressive decline in other cognitive domains. The cause of the disease is still largely unknown. From pathological studies it has become clear that damage to the brain occurs long before the onset of symptoms. Therefore, the emphasis of the studies described in this thesis was on brain changes during this preclinical period as visualized with magnetic resonance imaging (MRI) of the brain. The studies performed are part of the Rotterdam Scan Study, a population-based study among almost 1100 elderly who had no symptoms of dementia at time of MRI. On the MRI scans, we visualized the extent of brain atrophy, both generalized brain atrophy and of the hippocampus and amygdala, structures highly affected by Alzheimer pathology early in the disease. By establishing determinants of brain atrophy, we hoped to provide some clues on the pathogenesis of Alzheimer's disease. Additionally, we studied whether brain atrophy on MRI is clinically characterized by a decline in memory function, and whether it indicates an increased risk of developing clinically evident Alzheimer's disease and depression.

Chapter 2.1 and **2.2** illustrate the relationship between blood pressure and brain atrophy on MRI, both generalized atrophy and regional atrophy of the hippocampus and amygdala. Either a high or low diastolic blood pressure level was associated with increased brain atrophy. The biological mechanisms for these associations are still unknown, we provide some possible explanations. **Chapter 2.3** focuses on plasma homocysteine levels, as high levels of this amino acid have been associated with clinical Alzheimer's disease. Elderly with high plasma homocysteine levels were found to have more hippocampal and global brain atrophy on MRI. **Chapter 2.4** addresses the association between alcohol intake and both cerebrovascular disease and hippocampal and amygdalar atrophy on MRI. Light-to-moderate alcohol consumers have a lower risk of dementia compared to abstainers, particularly of vascular dementia. In accordance, we found a lower prevalence of cerebrovascular disease on MRI in light-to-moderate alcohol consumers compared to abstainers. Overall there was no effect of the degree of alcohol intake on hippocampal and amygdalar atrophy on MRI. However, within apolipoprotein E (APOE) $\epsilon 4$ carriers, light-to-moderate alcohol consumers had less atrophy compared to abstainers. This finding is in agreement with recent reports that APOE $\epsilon 4$ carriers who consume light-to-moderate amounts of alcohol have a reduced risk of Alzheimer's disease compared to $\epsilon 4$ carriers who abstain. In **chapter 2.5**, we studied the effect of the APOE genotype on degree of brain atrophy on MRI. Individuals with the $\epsilon 4$ allele, which is the risk allele in Alzheimer's disease, had more hippocampal and amygdalar atrophy but not more global brain atrophy compared to persons with the common $\epsilon 3\epsilon 3$ genotype.

In **chapter 3.1**, a study on plasma estradiol levels and hippocampal atrophy on MRI and memory performance is described. Based on animal studies showing beneficial effects of estrogen on hippocampal neurons, we formed the hypothesis that higher estradiol levels would be

associated with less hippocampal atrophy and better memory performance. This hypothesis was however not confirmed. The effect of polymorphisms in the estrogen receptor α gene on atrophy and risk of dementia is investigated in **chapter 3.2**. While the polymorphism was not associated with the risk of dementia, we found that women with certain genetic variations had smaller amygdalar volume on MRI. **Chapter 3.3** reports on the association between diabetes mellitus, insulin resistance and atrophy of the hippocampus and amygdala on MRI. Persons with diabetes mellitus had more atrophy on MRI compared to persons without diabetes mellitus. Furthermore, in non-diabetic persons, insulin resistance was associated with amygdalar atrophy on MRI.

Chapter 4.1 provides evidence that persons with more hippocampal atrophy on MRI perform worse on memory performance tasks. In particular, smaller hippocampal head volume was associated with decreased performance on a verbal learning task. In **chapter 4.2** we validated in our sample the assumption that atrophy on MRI could be observed in the preclinical period of Alzheimer's disease. Atrophy on MRI of either the hippocampus or amygdala predicted the development of clinical dementia within six years, even when persons were cognitively intact at time of MRI. In contrast, in **chapter 4.3** we show that hippocampal and amygdalar atrophy do not predict depression. However, frontal atrophy was associated with an increased risk of depression.

Finally, in **chapter 5**, methodological issues, the main findings and their implications, and suggestions for further research are discussed.

Samenvatting

De ziekte van Alzheimer is een veelvoorkomende ziekte in ouderen. Patiënten met de ziekte van Alzheimer merken een sluipende achteruitgang in geheugenfunctie waarna verslechtering van andere cognitieve functies volgt. De oorzaak van de ziekte is nog grotendeels onbekend. Uit pathologische studies is gebleken dat schade aan de hersenen reeds lang voor het begin van symptomen aanwezig is. We legden daarom in dit proefschrift de nadruk op veranderingen in de hersenen tijdens deze preklinische periode, zichtbaar gemaakt met behulp van “magnetic resonance imaging” (MRI) van de hersenen. De studies in dit proefschrift maken deel uit van de Rotterdam Scan Study, een studie onder bijna 1100 ouderen die ten tijde van de MRI geen klinische symptomen van dementie hadden. Op de MRI kwantificeerden we zowel gegeneraliseerde hersenatrofie, als atrofie van de hippocampus en amygdala, hersenstructuren die vroeg in het beloop van de ziekte van Alzheimer zijn aangedaan. Door het vinden van risicofactoren voor hersenatrofie, hoopten wij meer inzicht te krijgen in de ontstaanswijze van de ziekte van Alzheimer. Daarnaast bestudeerden we de vraag of hersenatrofie op MRI klinisch gekenmerkt wordt door geheugenachteruitgang, en of het een verhoogd risico op het krijgen van klinische symptomen van de ziekte van Alzheimer of een depressie aangeeft.

Hoofdstuk 2.1 en **2.2** illustreren het verband tussen bloeddruk en hersenatrofie, zowel gegeneraliseerde atrofie als specifieke atrofie van de hippocampus en amygdala. Een hoge- en een lage diastolische bloeddruk stonden beide in verband met meer hersenatrofie. De precieze mechanismen van deze associaties zijn nog onbekend, we geven enkele mogelijke verklaringen. **Hoofdstuk 2.3** bestudeert het plasma homocysteïne gehalte aangezien een hoge concentratie van dit aminozuur mogelijk een verhoogd risico van de ziekte van Alzheimer geeft. Ouderen met hogere plasma homocysteïne concentraties hebben meer hippocampus en gegeneraliseerde hersenatrofie op MRI. **Hoofdstuk 2.4** kijkt naar de associatie tussen alcohol consumptie en zowel cerebrovasculaire schade als hippocampus en amygdala atrofie. Ouderen die licht tot matig alcohol consumeren hebben een lager risico van dementie in vergelijking met geheel onthouders, in het bijzonder van vasculaire dementie. In dit kader vonden wij eveneens minder cerebrovasculaire schade op MRI in mensen die licht tot matig alcohol consumeren. Er was geen verband tussen alcohol consumptie en ernst van hippocampus of amygdala atrofie op MRI. Echter, in mensen die het $\epsilon 4$ allel van het apolipoproteïne E (APOE) gen hebben, is lichte tot matige alcohol consumptie geassocieerd met minder atrofie. In **hoofdstuk 2.5** bestuderen we het effect van het APOE genotype op de mate van hersenatrofie op MRI. Ouderen met het $\epsilon 4$ allel, het risico allel in de ziekte van Alzheimer, hebben meer hippocampus en amygdala atrofie, maar niet meer gegeneraliseerde atrofie, in vergelijking met ouderen met het $\epsilon 3\epsilon 3$ genotype.

In **hoofdstuk 3.1** beschrijven we een studie naar het verband tussen plasma oestrogenen concentratie, hippocampus atrofie en geheugen functie. Dierexperimenteel onderzoek heeft veelal positieve effecten van oestrogenen op neuronen in de hippocampus laten zien, dus

we formuleerden de hypothese dat ouderen met hogere plasma oestrogenen concentraties minder hippocampus atrofie zouden hebben, en beter zouden presteren op geheugen taken. Deze hypothese konden we echter niet bevestigen. Het effect van een genetische variatie in het gen van de oestrogeenreceptor α op atrofie en risico van dementie werd onderzocht in **hoofdstuk 3.2**. Alhoewel deze genetische variant niet met het risico van dementie in verband staat, hebben vrouwen met een bepaalde genetische variatie kleinere amygdala volumes. **Hoofdstuk 3.3** laat het verband zien tussen diabetes mellitus, insuline resistentie en hippocampus atrofie op MRI. Ouderen met diabetes mellitus hebben meer atrofie op MRI in vergelijking met ouderen zonder diabetes mellitus. In personen zonder diabetes mellitus, is insuline resistentie een risico factor voor amygdala atrofie.

Hoofdstuk 4.1 laat zien dat ouderen met meer hippocampus atrofie op MRI slechter presteren op geheugen taken. Specifiek atrofie van het “head” gedeelte van de hippocampus staat in verband met verminderde prestaties op een verbale geheugen taak. In **hoofdstuk 4.2** bestudeerden wij onze assumptie dat atrofie op MRI tijdens de preklinische periode van de ziekte van Alzheimer aanwezig is. Atofie van de hippocampus of amygdala op MRI voorspelt de ontwikkeling van dementie zes jaar later, zelfs wanneer ouderen ten tijde van de MRI cognitief goed functioneren. In **hoofdstuk 4.3** tonen wij aan dat hippocampus of amygdala atrofie geen voorspeller is van een depressie. Echter, de aanwezigheid van frontale atrofie op MRI geeft een verhoogd risico van depressie aan.

In **hoofdstuk 5** worden enkele methodologische aspecten, de belangrijkste bevindingen en hun implicaties, en suggesties voor verder onderzoek besproken.

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Tom den Heijer was born on February 9, 1976 in Rotterdam. He attended secondary school at the Blaise Pascal in Spijkenisse, which he passed in 1994. That same year, he started medical school at Erasmus University, Rotterdam. He obtained a Master's degree in Medicine in 1999 and started the work described in this thesis at the Department of Epidemiology & Biostatistics of Erasmus University, Rotterdam (Prof. dr. M.M.B. Breteler and Prof. dr. A. Hofman). As part of this project he visited the Institute of Clinical Neuroscience, University of Göteborg, Sweden (head Dr. I. Skoog). In 2001, he obtained a Master of Science degree in Clinical Epidemiology at the Netherlands Institute for Health Sciences for which he followed courses at Tufts University, Boston, USA. In February 2003, he became a medical doctor. March 1, 2004, he started his neurology training at Erasmus MC, Rotterdam (head: Prof. dr. P.A.E. Sillevius Smitt).