Hearing Function and Brain Health in the Elderly: Interrelations and risk factors

The Rotterdam Study

Pauline Helen Croll

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Interrelations and risk factors

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Gehoorfunctie en brein gezondheid in ouderen:
onderlinge relaties en risico factoren

De Rotterdam studie

Proefschrift

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The artwork on the cover is painted by the Dutch artist Willem Kalf entitled 'Stilleven met schelpen en koraal' (Still life with shells and coral) painted around 1690.[1] All the artwork (except for 'Three Musicians' by Rembrandt van Rijn) throughout this thesis are displayed at the Mauritshuis in The Hague, the Netherlands.

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1. Kalf W. Stilleven met schelpen en koraal. Den Haag, the Netherlands: Mauritshuis, 1690.

"Success isn't the result of arrogance – it's the result of belief"
Conor McGregor

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Chapter 2.2

Croll PH, Bos D, Vernooij MW, Arshi B, Lin FR, Baatenburg de Jong RJ, Ikram MA, Goedegebure A*, Kavousi M*. Carotid atherosclerosis is associated with poorer hearing in older adults. *Journal of American Medical Directors Association*. 2019; S1525-8610(19)30517-1.

Chapter 3.1

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Croll PH*, Vinke EJ*, Armstrong NM, Licher S, Vernooij MW, Lin FR, Baatenburg de Jong RJ, Goedegebure A, Ikram MA. Hearing loss and cognitive decline in the general Dutch population: a prospective cohort study. *Under review.*

* These authors contributed equally to the respective manuscript

SUPPLEMENTARY MATERIALS ACCOMPANYING THIS THESIS

All supplementary material can be found through the following QR-code:

Three Musicians (Allegory of Hearing); Rembrandt van Rijn[1]

1

General introduction

GENERAL INTRODUCTION

How would our mental life be like if we had no senses? What if you could not see, hear, touch, taste, smell, or sense the world around you in any other way? You would not be able to learn or to react, as both requires sensory input. Would we be able to think? What could we think about without any knowledge gained from our senses? To have a mental life, to perceive the world, we need sensation.[2]

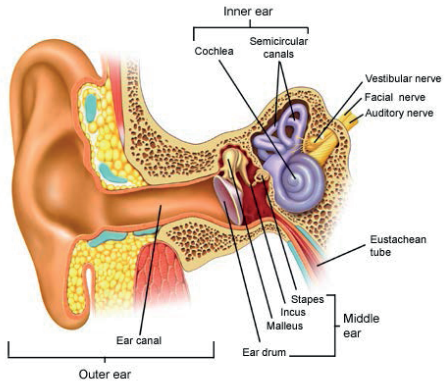

Figure 1. Anatomy of the ear. Source: University of Minnesota Duluth

Our ability to sense is the result of five complex sensory systems: touch, sight, hearing, smell and taste. The organs (skin, eyes, ears, nose, tongue respectively) associated with each sense, sends information to the brain to help us understand and perceive the world around us.[2] Unfortunately, as we grow older, the function of these organs decline. For example, due to degeneration of the olfactory receptor neurons, a high proportion of the elderly population lives with olfactory dysfunction.[3, 4] Another common disorder of the senses with ageing is visual impairment.[5] In addition to these two age-related conditions, hearing loss in the elderly is the most prevalent sensory dysfunction that affects quality of life and daily functioning.

Age-related hearing loss: pathophysiology and prevalence

Age-related hearing loss, also known as presbycusis, is characterized by reduced hearing sensitivity and speech understanding in noisy environments, slowed central processing of acoustic information, and impaired localisation of sound sources.[6] It has primarily been described as a condition caused by damage of the peripheral auditory system (**figure 1**).[6] More specifically, degeneration of the cochlear structures (**figure 1**), including the stria vascularis, the outer hair cells, and the cochlear neurons (**figure 2**), are prominent characteristics of this condition.[7] In terms of symptomatology, age-related hearing loss is characterized by a reduced ability to understand speech, followed by a reduced ability to detect, identify and localise sound sources.[8] Moreover, the abilities to understand speech in noise declines due to diminished central processing, which is also known as central hearing loss.[8] Overall, 10% of the global population has a hearing loss great enough to impair communication, with substantially increasing prevalence with higher age (40% in individuals older than 65 years and 80% in the population above the age of 80).[6, 9] Given the ageing of the population, the oc-

currence of hearing loss is rapidly increasing and the World Health Organization estimated that in 2018, 46 million people in the high income countries had a form of hearing loss, which is expected to increase to 58 million in 2030 and 72 million in 2050 (**figure 3**). With this increasing prevalence, it is inevitable that hearing loss will play an increasingly important role in adult health care. Although direct medical treatment is not possible, hearing aids and cochlear implants are available to partly compensate for the loss of auditory function and possibly delay further degeneration of the auditory system. Besides the use of hearing aids, delaying the onset or slow down the progression of hearing loss in itself may also prove beneficial.

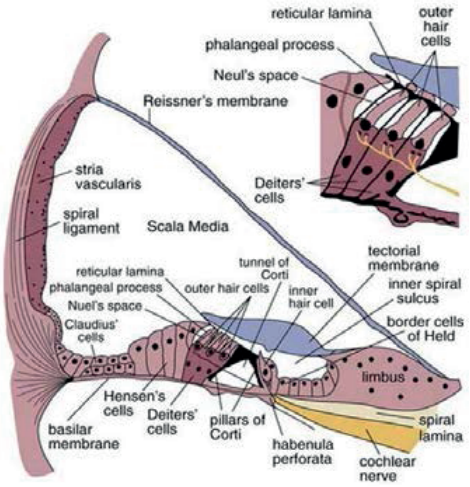

Figure 2. Cochlear anatomy. Source: Clinical Anatomy & Operative Surgery

Risk factors for hearing loss

To be able to delay the onset or slow down the progression of hearing loss, more in-depth knowledge is needed about its risk factors, specifically potentially modifiable ones. Several risk factors for hearing loss have been identified in the past, such as age, educational level, blood pressure, diabetes, smoking and exposure to excessive noise.[10, 11] As such, for example, maintaining a healthy blood pressure, lowering your risk of diabetes by conforming to a healthy lifestyle, and stopping smoking may prevent or delay the onset of hearing loss. Broadening this scientific and clinical knowledge with (longitudinal) population-based studies on risk factors may also support the prevention of potential adverse outcomes associated with hearing loss.

Adverse outcomes of hearing loss

As mentioned earlier, our senses are essential for mental development, so we might expect that a decline in hearing function, and thus a diminished input of auditory signals, will potentially have an impact on mental- and functional brain health. Previous studies have shown that hearing loss is associated with loneliness, social isolation, depression, and an increased risk of falls.[6, 12-14] In addition, recent epidemiological studies reported associations between hearing loss, accelerated cognitive decline and an increased risk of dementia.[15-20] However, the underlying pathway explaining this relationship remains unknown. Does one cause the other, or is a third factor the root cause? A clearer under-

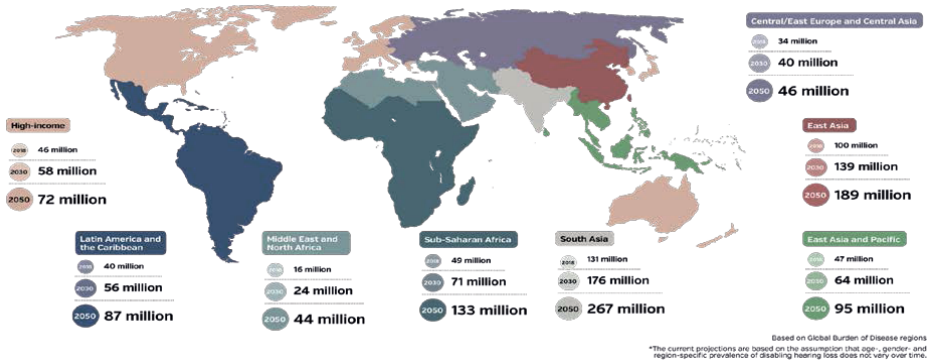

Figure 3. The projected number of people with hearing loss in different world regions until 2050. World Health Organization, Rapport on: WHO global estimates on prevalence of hearing loss. Source: http://who.int/en/news-room.

standing of the nature of the relationship between hearing loss and cognitive decline in the preclinical setting, before full-blown dementia is present, is critical if we are to minimize their impact, either in isolation or together, on quality of life, and to develop effective preventive and rehabilitation strategies. If hearing loss does contribute to accelerated neurodegeneration and cognitive decline, offering hearing aids or other rehabilitative treatments earlier in the course of auditory decline, may prove beneficial in preventing cognitive impairment and possibly even dementia.

Hearing loss and dementia: hypotheses on the potential underlying mechanism

Several hypotheses have been proposed to explain the association between hearing loss and cognitive decline (**figure 4**), of which two will be discussed in this thesis. First, there is the *'Common-Cause Hypothesis'* (**figure 4A**). This view proposes that a common mechanism may underlie both hearing loss and cognitive decline in the elderly and that hearing loss may present itself before the onset of cognitive decline and eventually cognitive impairment, rather than that the two are directly causally related to one another.[21] In regard to this hypothesis, in this thesis, I assess potential risk factors for both hearing function and brain health which are outlined further below. Even though there is support for the common-cause hypothesis, it has been demonstrated that age-related sensory degeneration is also at least in part independent of age-related

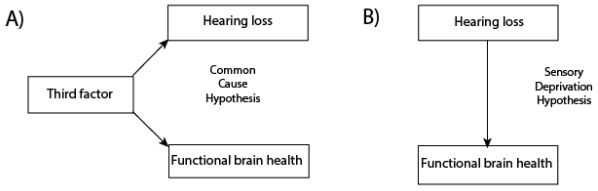

Figure 4. A summary of the hypotheses potentially explaining the relation between hearing loss and cognitive decline.

cognitive degeneration. Such independent contributions would not be observed if a single common-cause was underlying all decline.[22] Following this, there is the second and final hypothesis: the *'Sensory-Deprivation Hypothesis'* (**figure 4B**). This hypothesis poses that declines in perceptual function cause more permanent cognitive declines, possibly through neuroplastic changes that disadvantage general cognition in favour of processes supporting speech perception.[21] The current hypothesis emphasizes that such chronic reallocation of cognitive resources may produce permanent changes in cognitive performance over time. A potential mechanism underlying this hypothesis is thought to be deafferentation and atrophy in the auditory system as well as subsequent reorganization, due to long-term deprivation of sensory input.[21] To explore this hypothesis, I will investigate the interrelation between hearing function, brain health and cognitive function in several studies which are described in more detail below.

Aim of the current thesis

The aim of this thesis is three-fold. I firstly will explore potential risk factors for hearing loss, and secondly, I shall investigate potential risk factors for brain health. The risk factors in this thesis are selected based on current knowledge. To be more specific, cardiovascular disease, body composition, dietary patterns, and vitamin D levels are established risk factors for dementia.[23-26] Analysing these factors in relation to hearing function and brain health may thus add to the current knowledge in regard to the common-cause hypothesis. Thirdly, I will explore interrelations between hearing function and brain health, hopefully contributing to the current knowledge on the sensory-deprivation hypothesis. Important to note: as we do not have (enough) follow-up data yet, I will not be able to elucidate which hypothesis is the 'true' underlying pathway in the association between hearing loss and dementia. Nevertheless, this thesis will add considerably to the current knowledge. For the largest part, my research was embedded within the Rotterdam Study. The Rotterdam Study is an ongoing population-based study, which originated in 1990. It investigates determinants and consequences of ageing.[27] From 2005 onwards, magnetic resonance imaging (MRI) scanning of the brain on a 1.5 tesla MRI scanner was included in the study protocol.[28] Hearing assessment by means of pure-tone audiometry (as a measure of peripheral hearing loss), bone conduction, and a Digits in Noise test (as a measure of central hearing loss) was added to the core study protocol in 2011.[27]

In the following parts of this thesis I discuss various risk factors for hearing function and brain health and the interrelations of both. In **Chapter 2**, I focus on risk factors for hearing loss. **Chapter 2.1** describes the association between body composition (divided into body mass, fat mass, and fat-free mass), diet quality and hearing function. **Chapter 2.2** is dedicated to the association between carotid atherosclerosis, as a marker of generalized cardiovascular health, and hearing function. In **Chapter 3**, potential risk factors for brain health are highlighted. In **Chapter 3.1**, I focus on the relation between diet

quality and brain tissue volumes. Unique in this study is that I use a novel diet quality score. Next, **Chapter 3.2** describes the association between vitamin D status in the Rotterdam population and several markers of brain health, namely: brain tissue volume (total, grey matter, white matter, and the hippocampus), global white matter microstructure (fractional anisotropy and mean diffusivity), white matter hyperintensity volume and the presence and progression of lacunes and microbleeds. The final part of Chapter 3, **Chapter 3.3**, adresses the cross-sectional as well as the longitudinal association between body composition and comparable markers of brain health. In line with Chapter 2.1, I use measures of body mass, fat mass and fat-free mass. **Chapter 4** is dedicated to the interrelations between hearing function and brain health. As hearing loss has been found to be one of the biggest risk factors for tinnitus in the elderly,[29] **Chapter 4.1** focusses on the association between tinnitus and brain tissue volumes. **Chapter 4.2** is embedded within the Atherosclerosis Risk in Community Study, a population-based multisite study in the United States of America. Here, I discuss the association between hearing loss and microstructural integrity of the brain lobes (frontal, temporal, parietal, occipital), the medial temporal lobe structures (hippocampus and amygdala), and the white matter tracts in the brain. **Chapter 4.3** is dedicated to the longitudinal association between hearing loss and cognitive decline. Finally, in **Chapter 5**, I conclude with a review of my main findings in the context of the current knowledge and I will elaborate on future research in hearing function and brain health.

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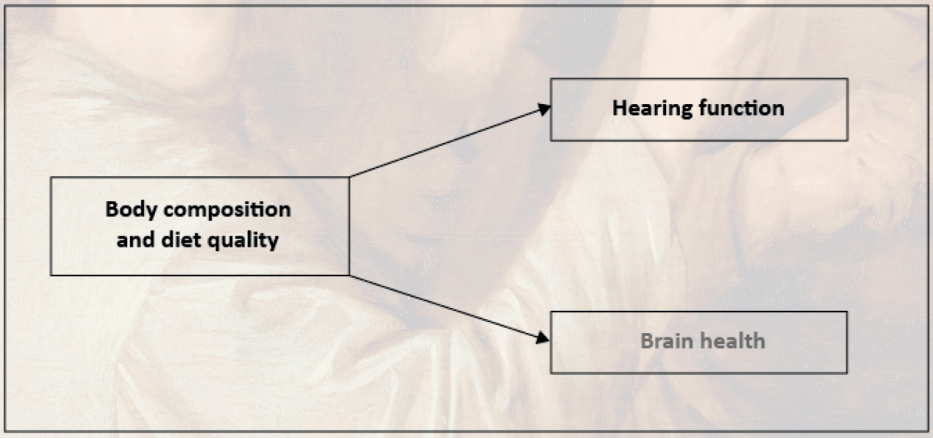
De vijf zintuigen: Het gehoor; Jan Miense Molenaer[1]

2

Risk factors for hearing function


Hearing function
Body composition and diet quality
Brain health

2.1

The association between obesity, diet quality and hearing loss in older adults

Pauline H. Croll, Trudy Voortman, Meike W. Vernooij, Robert J. Baatenburg de Jong, Frank R. Lin, Fernando Rivadeneira, M. Arfan Ikram, André Goedegebure

Aging, 2019

ABSTRACT

Background

With the ageing population, the prevalence of age-related hearing loss will increase substantially. Prevention requires more knowledge on modifiable risk factors. Obesity and diet quality have been suggested to play a role in the etiology of age-related hearing loss. We aimed to investigate independent associations of body composition and diet quality with age-related hearing loss.

Methods

We performed cross-sectional and longitudinal analyses (follow-up: 4.4 years) in the population-based Rotterdam Study. At baseline (2006-2014), 2,906 participants underwent assessment of body composition, diet, and hearing. Of these 2,906 participants, 636 had hearing assessment at follow-up (2014-2016). Association of body composition and of diet quality with hearing loss were examined using multivariable linear regression models.

Results

Cross-sectionally, higher body mass index and fat mass index were associated with increased hearing thresholds. These associations did not remain statistically significant at follow-up. We found no associations between overall diet quality and hearing thresholds.

Conclusions

This study shows that a higher body mass index, and in particular a higher fat mass index, is related to age-related hearing loss. However, whether maintaining a healthy body composition may actually reduce the effects of age-related hearing loss in the ageing population requires further longitudinal population based research.

INTRODUCTION

Age-related hearing loss is highly prevalent among older adults [2, 3], and is characterized by reduced hearing sensitivity and speech understanding, resulting from degeneration of the cochlea or the auditory nerves or both [4]. Hearing loss contributes to social isolation, depression, and possibly dementia [3, 5-8]. With a growing and ageing population, the number of people with hearing loss and its consequences will increase [2]. Therefore, prevention of hearing loss is key. But this requires more knowledge about modifiable risk factors.

One such risk factor may be obesity, which has been linked to increased hearing thresholds [5, 6, 9-11]. An important limitation is that previous studies mostly used body mass index (BMI) as a measure of body composition. Ageing is associated with a decrease in lean mass and an increase in fat mass, making BMI less suitable as an approximation of body composition in the elderly [12]. Moreover, cross-sectional studies are inconclusive about the true association between obesity and hearing and therefore more longitudinal research is needed.

It has been suggested that diet quality plays a role in the relation between body composition and age-related hearing loss. Studies observed a relation between food groups, such as fish and carbohydrates consumption, and hearing thresholds [13-15]. However, many complex interactions occur across different food components and nutrients [16] which triggered the interest to study effects of dietary patterns as a whole [16]. Two other studies examined the relation between diet quality and age-related hearing loss, and in both found an association between better diet quality and poorer hearing [14, 17]. But more research is needed to verify this association.

We aimed to investigate the association between detailed body composition (distinguishing between lean mass and fat mass) and age-related hearing loss and between overall diet quality and age-related hearing loss. We mutually adjusted for body composition and diet quality, therefore examining the independent relationship between body composition, diet quality and age-related hearing loss.

RESULTS

Table 1 shows the population characteristics. Mean age at baseline was 66.1 years (standard deviation (SD): 7.33), and 56.7% of the participants were women. Participants had a mean BMI of 27.3 kg/m^2 (SD: 4.1) and a median dietary adherence score of 7 (interquartile range: 6-8). Participants had a mean overall frequency hearing threshold of 24.1 decibel (dB) (SD: 12.1). Of the total group of 2,906 participants, 636 participants had a second hearing assessment at follow-up (median 4.4 years later, range 5.1) (Table 1).

Table 1. Population characteristics

Characteristics	Baseline	Follow-up
	N = 2,906	N = 636
Age, years	66.1 (7.3)	
Female, %	56.7	
Education level, %		
Primary	7.3	
Lower	36.6	
Middle	29.4	
High	25.9	
Hypertension, %	47.5	
Hypercholesterolemia, %	55.5	
Diabetes, %	8.2	
Smoking, %		
Never	32.1	
Former	52.4	
Current	15.0	
Physical activity, MET-hours per week	48.0 (IQR: 19.7-86.6)	
Hearing thresholds at baseline for all participants (N = 2,906)		
Overall frequency hearing loss, dB	24.1 (12.1)	
Low frequency hearing loss, dB	14.5 (9.3)	
High frequency hearing loss, dB	32.6 (17.4)	
Hearing thresholds for participants with 2 hearing assessments (N = 636)		
Overall frequency hearing loss, dB	30.0 (10.8)	32.5 (11.3)
Low frequency hearing loss, dB	17.6 (9.1)	18.1 (10.0)
High frequency hearing loss, dB	41.4 (15.7)	46.2 (15.9)
Body composition		
Length, cm	170.0 (9.3)	
Weight, kg	79.1 (14.4)	
Body mass index, kg/m^2	27.3 (4.1)	
Fat mass index, kg/m^2	9.9 (3.2)	
Fat-free mass index, kg/m^2	17.4 (2.1)	
Dietary characteristics		
Energy intake*	2,119 (IQR: 1,706-2,600)	
Dietary guideline adherence score*	7 (IQR: 6-8)	

Values are based on imputed data. Numbers of missings per variable were 360 for formerly smoking, 314 for never smoking and 207 for physical activity. Values are mean (standard deviation) for continuous variables or median (interquartile range) when indicated (*), percentages for dichotomous variables. MET: metabolic equivalent of task. dB: decibel. cm: centimetre. kg: kilogram. m: meter. IQR: interquartile range.

Cross-sectional results

After adjustment for relevant confounders (model 2), one SD higher BMI was associated with a 0.53 dB (CI: 0.04, 1.01) increase in hearing thresholds across all frequencies and with 0.42 dB (CI: 0.01, 0.82) increase in hearing thresholds in the lower frequencies (Table 2). Associations of BMI with hearing thresholds were mainly explained by fat mass index (FMI) rather than fat-free mass index (FFMI). One SD higher FMI was related to 0.58 dB (CI: 0.06, 1.09) increased hearing thresholds in all frequencies and with 0.43 dB (CI: -0.00, 0.86) increase in hearing thresholds among the lower frequencies (borderline non-significant: p = 0.05) (Table 2). When additionally adjusting for diet quality (model 3) effect estimates remained similar (Table 2). We did not find any associations between diet quality and hearing thresholds (Table 3, Supplementary tables 2, and 3). However, consumption of unsaturated fats and oils was associated with increased hearing thresholds and consumption of sugar containing beverages was associated with decreased hearing thresholds (Table 3). Effects estimates for most associations remained similar between model 1 and model 2 (Table 2, 3, Supplementary tables 2, and 3).

Table 2. The cross-sectional association between body composition and hearing thresholds

	All frequencies	Low frequencies	High frequencies
	Difference in dB (CI 95%)	Difference in dB (CI 95%)	Difference in dB (CI 95%)
Model 1			
Body mass index (SD)	**0.45 (0.09, 0.81)**	**0.35 (0.05, 0.65)**	**0.53 (0.01, 1.04)**
Fat mass index (SD)	**0.43 (0.05, 0.82)**	**0.37 (0.05, 0.70)**	0.47 (-0.08, 1.02)
Fat-free mass index (SD)	**0.50 (0.05, 0.94)**	0.30 (-0.08, 0.67)	0.64 (0.00, 1.28)
Model 2			
Body mass index (SD)	0.53 (0.04, 1.01)	0.42 (0.01, 0.82)	0.60 (-0.10, 1.30)
Fat mass index (SD)	0.58 (0.06, 1.09)	0.43 (-0.00, 0.86)	0.71 (-0.04, 1.46)
Fat-free mass index (SD)	0.39 (-0.18, 0.96)	0.36 (-0.12, 0.83)	0.35 (-0.48, 1.17)
Model 3			
Body mass index (SD)	0.52 (0.03, 1.00)	0.39 (-0.01, 0.80)	0.59 (-0.11, 1.29)
Fat mass index (SD)	0.56 (0.05, 1.08)	0.41 (-0.03, 0.84)	0.70 (-0.05, 1.45)
Fat-free mass index (SD)	0.39 (-0.18, 0.96)	0.35 (-0.12, 0.83)	0.34 (-0.48, 1.17)

All frequencies (0.25, 0.50, 1, 2, 4, and 8 kHz); low frequencies (0.25, 0.50, and 1 kHz); high frequencies (2, 4, and 8 kHz). Difference: represents the difference in dB per one SD higher body mass index, fat mass index, and fat-free mass index. CI: confidence interval. Model 1: adjusted for sex, age, age^2, and education. Model 2: additionally adjusted for energy intake, total brain volume, physical activity, smoking, alcohol, hypertension, hypercholesterolemia and type 2 diabetes. Model 3: additionally adjusted for diet quality score.

Longitudinal results

Body composition and diet quality were not related to change in hearing thresholds at follow-up (Supplementary tables 1, 4, 5, and 6). Some food groups did show a signifi-

Table 3. The cross-sectional association between diet quality, food groups and hearing thresholds – model 3

	All frequencies	Low frequencies	High frequencies
	Difference (CI 95%)	Difference (CI 95%)	Difference (CI 95%)
Diet quality	-0.09 (-0.34, 0.15)	-0.16 (-0.36, 0.05)	-0.05 (-0.40, 0.31)
Vegetables	-0.01 (-0.04, 0.01)	-0.02 (-0.04, 0.01)	-0.01 (-0.05, 0.03)
Fruit	-0.01 (-0.02, 0.01)	-0.00 (-0.02, 0.01)	-0.01 (-0.03, 0.01)
Whole grain products	-0.00 (-0.06, 0.06)	-0.03 (-0.08, 0.02)	0.03 (-0.06, 0.12)
Whole grains/total grains ratio	0.00 (-0.05, 0.06)	0.00 (-0.05, 0.05)	0.02 (-0.07, 0.10)
Legumes	6.93 (-2.72, 16.58)	8.04 (-0.00, 16.07)	4.51 (-9.46, 18.49)
Nuts	-0.01 (-0.31, 0.28)	-0.02 (-0.25, 0.22)	0.01 (-0.20, 0.22)
Dairy	0.00 (-0.01, 0.02)	-0.01 (-0.02, 0.01)	0.01 (-0.01, 0.04)
Fish	-0.06 (-0.26, 0.14)	-0.10 (-0.26, 0.07)	-0.06 (-0.35, 0.23)
Tea	-0.01 (-0.03, 0.01)	-0.01 (-0.02, 0.01)	-0.02 (-0.05, 0.01)
Unsaturated fats/total fats ratio	0.13 (-0.02, 0.29)	**0.16 (0.03, 0.29)**	0.13 (-0.09, 0.36)
Salt	0.00 (-0.00, 0.00)	-0.00 (-0.01, 0.00)	0.00 (-0.01, 0.01)
Alcohol	-0.07 (-0.36, 0.22)	-0.06 (-0.30, 0.19)	-0.09 (-0.52, 0.33)
Red and processed meat	0.04 (-0.06, 0.12)	0.05 (-0.02, 0.13)	0.01 (-0.13, 0.15)
Sugar containing beverages	-0.02 (-0.06, 0.01)	**-0.04 (-0.07, -0.01)**	-0.04 (-0.08, 0.00)

All frequencies (0.25, 0.50, 1, 2, 4, and 8 kHz); low frequencies (0.25, 0.50, and 1 kHz); high frequencies (2, 4, and 8 kHz). Difference represents difference in dB per 1 point increase in diet quality score on a scale ranging from 0 to 14 or a 10 gram increase for the individual food components. CI: confidence interval. Adjusted for sex, age, age^2, education, physical activity, smoking (former and current), alcohol intake, hypertension, hypercholesterolemia, prevalent diabetes mellitus, total brain volume, energy intake and BMI (model 3). We did not adjust for alcohol intake in grams in the assessment of alcohol with hearing thresholds. Significant effect estimates ($p<0.05$) are indicated in **bold**.

cant relationship with hearing thresholds over time. For all frequencies, we found that higher intake of nuts was associated with a 0.95 (CI: -1.52, -0.37) dB decrease of hearing thresholds, as well as in the higher frequencies where higher intake of nuts was associated with a 1.10 dB (-1.87, -0.33) decrease of hearing thresholds (Supplementary table 6). Moreover, for all frequencies we found that higher vegetable intake was associated with a 0.05 dB (CI: -0.09, -0.00) decrease in hearing thresholds (Supplementary table 6). Effects estimates for most associations remained similar between model 1 and model 2 (Supplementary tables 1, 4, 5, and 6). There were no significant interactions ($p<0.05$) between body composition and sex, diet quality and BMI, and between diet quality and sex. Effect sizes did not differ between men and women (data not shown).

DISCUSSION

In this large sample of community-dwelling individuals, we found that adiposity was associated with increased hearing threshold. Although not statistically significant, these effects estimates were similar at follow-up. We found no associations of overall diet quality with age-related hearing loss.

Strengths of our study included the population-based setting, the large sample size, and the standardized assessment of hearing thresholds with pure-tone audiograms and detailed measurement of body composition. Some limitations of this study should also be acknowledged. First, although we adjusted for possible confounders, there still might be residual confounding present. At last, the FFQ relies on an individual's capacity to recall their dietary behaviour over the past month. Recall bias in dietary behaviour could be a systematic bias.

We found that a higher BMI was associated with higher hearing thresholds in our cross-sectional analysis. BMI is an important marker for metabolic diseases,[18] and is a classic indicator for obesity. Other studies also confirmed this positive relationship between BMI and hearing thresholds.[6, 8-11, 19, 20] However, some studies found non-significant associations,[21-23] and therefore the true relationship between BMI and hearing thresholds in the elderly remains controversial. A new aspect of our study is that we differentiated between FMI and FFMI, which is thought to be a more accurate reflection of metabolic unhealthy people and metabolic healthy people.[24] As we found a significant effect for FMI and not for FFMI, it is possible that the absence of an association in other studies is explained by a more prominent contribution of FFMI to the BMI compared to FMI.

The fact that we find an association for FMI and not for FFMI is in line with the common idea that body composition influences hearing thresholds through vascular mechanisms. Hearing thresholds are associated with vasculopathies in metabolic diseases and therefore it is hypothesized that BMI is associated with the development of increased hearing thresholds.[9, 19, 24] The integrity of an individual's auditory hair cells is paramount to their ability to detect sound,[5] and a healthy blood flow and oxygen contribute to the health of these cells. As such, the underlying mechanism between age-related hearing loss and obesity may be due to the mechanical strain on the capillary walls caused by adipose tissue.[5, 24] An animal study found narrowed blood vessels in the stria vascularis in mice with obesity.[25] This is a heavily vascularized part of the cochlea, therefore highly sensitive to any cardiovascular changes.[3, 5, 26] A similar vascular mechanism might be active in human older adults.

With the growing prevalence of obesity,[5, 27] healthy diet may serve as a modifiable risk factor for both hearing loss and obesity. Two other studies examined the effects of dietary patterns on hearing thresholds. Contrary to what we have found, they both report significant associations between diet quality and hearing thresholds,[14, 17] al-

though the found association did not persist at follow-up.[17] Both studies did not adjust thoroughly for confounding, therefore residual confounding may be present in their results. Interestingly, the study of Spankovich et al.[14] compromises a broader age range, and found that diet quality was associated with hearing loss in their younger population. Similar to us, in their older population there was an association between body composition and hearing thresholds. As such, it might be that diet quality has a bigger effect in a younger population. More research has been conducted concerning food nutrients and age-related hearing loss, and those studies reported that sufficient intake of fish and whole grains, and moderate intake of alcohol are related to lower hearing thresholds,[13, 28-30] whereas we found a positive association between intake of unsaturated fats and oils and hearing thresholds and a negative association between intake of sugar containing beverages and hearing thresholds. Moreover, on follow-up we found that more consumption of vegetables and nuts associated with lower hearing thresholds, suggesting possible protective effects on hearing abilities. However, those results should be interpreted with caution and more (longitudinal) studies are needed to truly elucidate the association between specific food groups and hearing thresholds.

In our longitudinal analysis of body composition and age-related hearing loss, no significant associations were found, but effects estimates remained about the same as in the cross-sectional analysis. The absence of a significant effect might be explained by the fact that relative few people had a hearing assessment at follow-up but more likely, that the time interval might have been too short. To our knowledge, there are only two studies of a longitudinal origin,[8 22] in which the first does find a significant association and the latter does not. Clearly more evidence is needed to make any definite conclusions about body composition being a modifiable risk factor to prevent age-related hearing loss.

In conclusion, this study shows no association of diet quality with hearing loss and that a higher BMI is associated with hearing thresholds. This association with BMI is mainly driven by a higher FMI, suggesting involvement of metabolic and cardiovascular mechanisms, which may affect the cochlear function and suggesting that FMI is a better measure of body composition in age-related hearing loss. Whether a healthy body composition could serve as a preventive strategy for age-related hearing loss and thereby reducing the adverse consequences of hearing loss in older adults requires further longitudinal population-based research.

METHODS

Study design and subjects

This study was embedded in the Rotterdam Study, a population-based prospective cohort study in the Netherlands.[31] From 2011 onwards, hearing assessment was implemented in the study protocol, currently adding up to 6,494 audiograms. From this group, we excluded participants with no information on body composition (N = 1,155) and no information on diet quality (N = 868). Dietary assessment was performed between 2006 and 2012, and assessment of body composition was performed between 2009 and 2014. We finally excluded all hearing assessments performed later than 2014 (N = 1,565) resulting in a total sample of 2,906 participants for the cross-sectional analysis. Of this group, 636 participants had a second hearing assessment between 2014 and 2016. The Rotterdam Study has been approved by the medical ethics committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Body composition

Information on body weight and length was obtained by physical examination and BMI was calculated (kg/m^2).[31] A total body dual-energy X-ray absorptiometry (DXA) – scan was made from which bone mass, lean mass, and fat mass in kilograms was determined.[31] With the information obtained from the DXA-scan we calculated fat mass index (FMI, kg/m^2), and fat-free mass index (FFMI, kg/m^2). In this division, the sum of FMI and FFMI is BMI.

Diet quality

Diet quality was assessed with a validated self-administered semi-quantitative food-frequency questionnaire (FFQ) consisting of 389 items. The FFQ was found to be an appropriate measurement tool for ranking people according to their food intake.[32] As described in detail elsewhere,[32] we evaluated adherence (yes/no) to fourteen items (vegetables, fruit, (whole) grains, fats, nuts, legumes, dairy, fish, tea, red and processed meat, alcohol, sugar-containing beverages, and salt) of the Dutch dietary guidelines. An overall diet score ranging from 0-14 was calculated by adding up the scores for the fourteen food groups.[32] The Dutch Dietary Guidelines are based on internationally used dietary guidelines and on international literature about health effects of diet, as described in detail elsewhere.[32]

Hearing levels

Audiometric assessment was performed in a soundproof booth by one health care professional.[31] A computer-based audiometry system (Decos Technology Group, version 210.2.6 with AudioNigma interface) and TDH-39 headphones were used. Pure tone audiometry was performed to determine hearing thresholds in decibel (dB) hearing level, measured according to the ISO-standard 8253-1 (International Organization for Standardization [ISO], 2010). For both ears, air conduction (frequencies 0.25, 0.50, 1, 2, 4, and 8 kilohertz (kHz)) was tested. Masking was performed according to the method of Hood (Hood, 1960). The best hearing ear was determined by taking the average threshold over all frequencies. When hearing thresholds for both ears were equal, we alternately chose the left or right ear. The low-frequency hearing threshold is the average of 0.25, 0.50, and 1 kHz and the high-frequency hearing threshold is the average of 2, 4, and 8 kHz. We excluded participants with an air-bone gap of 15 dB or more in the better ear to eliminate conductive hearing loss.

Covariates

Information on smoking was collected through self-report and categorized into never, former and current.[31] Educational level was categorized as primary, lower, middle or higher.[31] Systolic and diastolic blood pressure was measured twice using a random zero-sphygmomanometer. Glucose was determined using the Hexokinase method. Using an automatic enzymatic procedure serum total cholesterol and high-density lipoprotein cholesterol were measured from fasting blood samples.[31] Hypertension was defined as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of blood pressure lowering medication.[31] Hypercholesterolemia was defined as total cholesterol concentration ≥ 6.2 mmol/L and/or the use of lipid-lowering medication.[31] Type 2 diabetes was defined as having fasting blood glucose concentration > 7.0 mmol/L and/or non-fasting blood glucose > 11.1 mmol/L and/or use of glucose-lowering medication. The LASA Physical Activity Questionnaire was used to assess physical activity. Physical activity data were recalculated into metabolic equivalent of task hours per week.[33]

Statistical analysis

The association of body composition (BMI, FMI, and FFMI) and of diet quality (overall score, and intake of the individual components per 10 grams) with hearing loss (all, low, and high frequencies) was examined using multivariable linear regression models. In the first model we adjusted for sex, age, age^2, and education. In the second model we additionally adjusted for total brain volume, education, physical activity, smoking (current and past), energy intake, alcohol intake, hypertension, hypercholesterolemia and type 2 diabetes. In the third model for body composition, we additionally adjusted for diet

quality score and in the second model for diet quality, we additionally adjusted for BMI. For hearing thresholds at follow-up the same regression models were used, additionally corrected for hearing levels at baseline and for time between hearing assessments. We explored whether associations differed by sex and if effects differed across BMI groups.

Missing data on covariates (<1%) were imputed using the multiple imputation algorithm (5 imputations) of SPSS. IBM SPSS statistics for Windows, version 24.0 (International Business Machines Corporation, Armonk, New York) was used.

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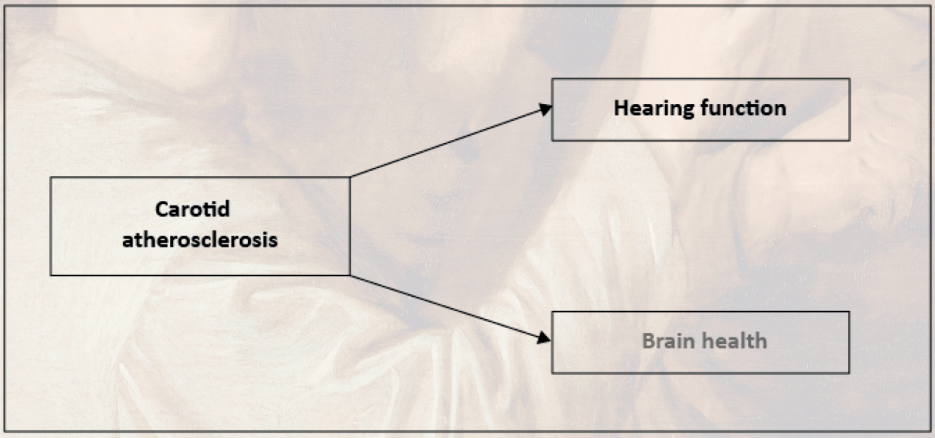
Hearing function
Carotid atherosclerosis
Brain health

2.2

Carotid atherosclerosis is associated with poorer hearing in older adults

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ABSTRACT

Objectives

Cardiovascular disease may be linked to hearing loss through narrowing of the nutrient arteries of the cochlea, but large-scale population-based evidence for this association remains scarce. We investigated the association of carotid atherosclerosis as a marker of generalized cardiovascular disease with hearing loss in a population-based cohort.

Design

Cross-sectional.

Setting

A population-based cohort study.

Participants

3,724 participants [mean age: 65.5 years, standard deviation (SD): 7.5, 55.4% female].

Methods

Ultrasound and pure-tone audiograms to assess carotid atherosclerosis and hearing loss.

Results

We investigated associations of carotid plaque burden and carotid intima media thickness (IMT) (overall and side-specific carotid atherosclerosis) with hearing loss (in the best hearing ear and side-specific hearing loss) using multivariable linear and ordinal regression models. We found that higher maximum IMT was related to poorer hearing in the best hearing ear [difference in dB hearing level per 1-mm increase IMT: 2.09 dB, 95% (CI): 0.08, 4.10]. Additionally, 3rd and 4th quartile plaque burden as compared to 1st quartile was related to poorer hearing in the best hearing ear (difference: 1.06 dB, 95% CI: 0.04, 2.08; and difference: 1.55 dB, 95% CI: 0.49, 2.60, respectively). Larger IMT (difference: 2.97 dB, 95% CI: 0.79, 5.14), 3rd quartile plaque burden compared to 1st quartile (difference: 1.24 dB, 95% CI: 0.14, 2.35), and 4th plaque quartile compared to 1st quartile (difference: 2.12 dB, 95% CI: 0.98, 3.26) in the right carotid were associated with poorer hearing in the right ear.

Conclusions and Implications

Carotid atherosclerosis is associated with poorer hearing in older adults, almost exclusively with poorer hearing in the right ear. Based on our results it seems that current therapies for the prevention of cardiovascular disease may also prove beneficial for hearing loss in older adults by promoting and maintaining inner ear health.

INTRODUCTION

Hearing loss among the older adult population is a growing public health problem causing reduced hearing sensitivity and impaired speech understanding.[1-6] Hearing loss contributes to depression, social isolation, reduced quality of life, and dementia,[7-18] and with an ageing population, the number of people with hearing loss and its consequences will increase.[19] At present, no treatment is available to cure hearing loss. Therefore, prevention of hearing loss might even be more effective but requires more in-depth knowledge on the etiology of hearing loss and possible modifiable risk factors.[6]

Hearing loss is the result of degeneration of the sensorineural structures of the cochlea and the stria vascularis. These parts of the inner ear are highly vascularized tissues, with the main blood supply coming from the labyrinth artery.[20] Given this vascularization, previous studies have focused on the association between cardiovascular risk factors,[11, 21-26] as well as more direct, generalized markers of atherosclerosis including carotid intima media thickness (IMT) and carotid plaque with hearing loss. These studies demonstrated associations between cardiovascular risk factors, markers of atherosclerosis and hearing loss.[22, 23, 25, 27-31] So far, few studies with small to moderate sample sizes have explored the association between atherosclerosis and hearing loss, and mainly assessed atherosclerosis or hearing loss by self-report. Moreover, hearing loss has solely been assessed in the best hearing ear whereas it is known that there are asymmetries between left and right auditory function, expressed in greater sensitivity for simple sounds and processing complex sounds such as speech in the right ear.[32-34] Thus, it could be hypothesized that the right ear may be more vulnerable to changes in cardiovascular health.

Therefore, we investigated the association between carotid atherosclerosis, as a marker of generalized atherosclerosis[27] measured by carotid IMT and plaque burden, and hearing loss within a large, well-characterized population-based cohort.

METHODS

Setting and study population

This study was embedded in the population-based Rotterdam study, the Netherlands, which originated in 1990, investigating determinants and consequences of ageing.[35] At study entry and subsequently every 3 to 4 years, all participants were invited for extensive examinations in the dedicated research centre. By 2008, 14,926 participants aged 45 years and older compromised this population-based study.[35]

Hearing assessment was added to the study protocol from 2011 onward. Between 2011 and 2014, 4,219 participants underwent pure-tone audiometry to assess hearing

abilities. Of those 4,219 participants, 4,190 participants also had data available on carotid IMT and plaque. We additionally excluded participants with present conductive hearing loss (n = 79) and those who did not have carotid ultrasound assessment and hearing assessment within the same year (n = 387), leaving 3,724 participants for the current analysis. Median time between atherosclerosis assessment and hearing assessment was 0.002 months.

All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Carotid atherosclerosis

Carotid plaque

By use of ultrasonography, the common carotid artery, carotid bifurcation, and internal carotid artery were visualized over a length as large as possible. Both left and right sides were analysed for the presence of plaques, which were defined as focal widenings relative to adjacent segments, with protrusion into the lumen, composed of calcified or non-calcified components. Researchers assessing the amount of plaques were blinded to all clinical information of the participants.[36] A weighted plaque score ranging from 0 to 6 was calculated by adding the number of sites at which a plaque was detected, divided by the total number of sites for which an ultra-sonographic image was available and multiplied by 6, which is the maximum number of sites.[36] Participants of whom at least 2 of the 6 sites did not have available information on the presence of plaques were excluded from the study. Additionally, we categorized plaque burden into quartiles.

Carotid intima-media thickness

The maximum IMT was determined as the maximum IMT of the near- or far-wall of the common carotid artery over a length of 1 cm. Subsequently, the average of left and right maximum common carotid IMT in millimetres was computed.[36]

Hearing

To determine decibel (dB) hearing levels, pure-tone audiometry was performed in a soundproof booth by 1 trained health care professional. A computer-based clinical audiometry system (Decos Technology Group, version 210.2.6 with AudioNigma interface) and TDH-39 headphones were used.[35] dB hearing levels were measured according to the ISO-standard 8253-1 [International Organization for Standardization (ISO), 2010]. Air conduction (frequencies 0.25, 0.50, 1, 2, 4, and 8 kHz) and bone conduction (0.50 and 4 kHz) were tested for both ears. According to the method of Hood, [37] masking was performed. dB hearing levels per ear were determined by taking the average thresholds over all frequencies. The better hearing ear was determined for every participant by comparing the average thresholds over all frequencies. If both ears were equal, we alternately chose

either the right or the left ear. Low-frequency dB hearing level was determined as the average of 0.25, 0.50, and 1 kHz and high-frequency dB hearing level was determined as the average of 2, 4, and 8 kHz. We categorized hearing loss into 3 categories: no hearing loss (0 – 20 dB), mild hearing loss (20 – 35 dB), and moderate/severe hearing loss (≥ 35 dB).[38] The last category contains 3 clinical categories (moderate, severe, and profound) that have been merged to maintain sufficient statistical power. Conductive hearing loss was present when participants had an air-bone gap of 15 dB or more.[35]

Covariables

Information on smoking was collected through questionnaire and was categorized into current, former, and never smoking. Educational level was assessed according to the standard classification of education, which allows comparison to international levels of education.[35, 39] In our analysis, we combined the 4 highest levels into 1 category, thus, obtaining 4 levels: (1) primary education; (2) lower-level vocational education; (3) medium-level secondary education; and (4) medium-level vocational training to university level. Systolic and diastolic blood pressure was measured twice using a random zero-sphygmomanometer and the average of the 2 measurements was used. Type 2 diabetes was defined as having fasting blood glucose concentration >7.0 mmol/L, a non-fasting blood glucose >11.1 mmol/L, use of glucose-lowering medication, or a combination of these. Using an automatic enzymatic procedure serum total cholesterol and high-density lipoprotein (HDL) cholesterol were measured from fasting blood samples. Information on body weight and length was obtained by physical examination and body mass index (BMI) was calculated. Information on use of blood pressure medication and lipid-lowering medication was collected. The prevalence of coronary heart disease (CHD) and stroke was determined at baseline interview, with verification from medical records.[35, 40]

Statistics

First, we assessed associations of degree of carotid plaque burden (second quartile, third quartile, and fourth quartile compared to first quartile) and IMT with decibel hearing levels in the best hearing ear (all, high, low frequencies) using multivariable linear regression models. In the first model, we adjusted for age and sex. In the second model, we additionally adjusted for age-squared (to account for nonlinear age effects), education, BMI, smoking, systolic and diastolic blood pressure, use of blood pressure-lowering medication, cholesterol levels (HDL and LDL), diabetes mellitus, and lipid-lowering medication use. Exploratory analyses were performed to account for possible confounding by prevalent coronary heart disease, stroke and antiplatelet medication use. Adding those variables into the models did not change the effect estimates and were therefore left out of the final analysis. Second, we performed a similar multivariable

linear regression analysis in which we studied the association of side-specific atherosclerosis (left and right carotid) and side-specific hearing loss (left and right ear). Third, we investigated the association of atherosclerosis with degree of hearing loss (no, mild, moderate/severe) using ordinal regression with similar multivariable adjusted models. The proportional-odds assumption was checked and it held for every association (P > .05). As prevalence of hearing loss increases substantially with age,[11] we further explored whether associations differed by midlife (50-70 years) vs late life (70-98 years). Additionally we checked whether associations differed by sex. All analyses were performed using IBM SPSS statistics for Windows, version 24.0 (International Business Machines Corporation, Armonk, New York). A *P* value ≤ 0.05 was considered statistically significant.

RESULTS

Population characteristics are described in Table 1. Mean age was 65.5 years (SD: 7.5), 55.5% were female. Mean maximum IMT was 1.0 mm (SD: 0.2). The first quartile included a plaque score of 0.00, second quartile included a plaque score of 0.10 to 1.49, the third quartile included a plaque score of 1.50 to 2.49, and the fourth quartile included a plaque score of 2.50 to 6.00. Mean hearing loss was 23.6 dB (SD: 12.1) across all hearing frequencies. Important to note for the interpretation of our results is that the amount of hearing loss is expressed in dB; that is, a higher dB value reflects greater hearing loss.

Table 1. Population characteristics (N = 3,724)

Characteristic	
Age, years	65.5 (7.5)
Age range, years	51.5 – 98.6
Female, %	55.5
Education level, %	
Primary	7.8
Lower-level vocational	36.9
Medium-level secondary	29.4
Medium-level vocational to university level	25.3
Systolic blood pressure, mmHg	139.6 (20.9)
Diastolic blood pressure, mmHg	83.1 (11.2)
Blood pressure medication use, %	40.9
Cholesterol, mmol/L	5.5 (1.1)
HDL-cholesterol, mmol/L	1.5 (0.4)
Lipid-lowering medication use, %	25.9

Table 1. Population characteristics (N = 3,724) (continued)

Characteristic	
Diabetes, %	9.1
Smoking, %	
Yes	17.1
No	82.9
Atherosclerosis	
Degree of total plaque burden, %	
1st quartile	19.4
2nd quartile	28.5
3rd quartile	21.6
4th quartile	27.5
Degree of left carotid plaque burden, %	
1st quartile	30.6
2nd quartile	24.5
3rd quartile	19.4
4th quartile	25.6
Degree of right carotid plaque burden, %	
1st quartile	30.3
2nd quartile	24.6
3rd quartile	20.4
4th quartile	24.7
Total maximum IMT, mm	1.0 (0.2)
Left maximum IMT, mm	1.0 (0.2)
Right maximum IMT, mm	1.0 (0.2)
Hearing loss	
All frequencies, dB	23.6 (12.1)
Low frequencies, dB	14.3 (9.2)
High frequencies, dB	32.0 (17.5)
Left ear, dB	27.0 (14.5)
Right ear, dB	26.2 (13.6)
Degree of hearing loss in the best hearing ear, %	
No	43.2
Mild	40.1
Moderate/severe	16.5

IMT: intima media thickness. dB: decibel. Values are mean (standard deviation) for continuous variables and percentages for dichotomous variables. The amount of hearing loss is expressed in dB; that is, a higher dB value reflects greater hearing loss. Degree of hearing loss: no 0–20 dB; mild = 20–35 dB; moderate/severe: ≥ 35 dB. Ranges for quartiles were 0, 0.50–1.49, 1.50–2.00, and 2.50–6.00 for overall plaque score, 0, 1.00–1.99, 2.00–2.99, and 3.00–6.00 for left carotid plaque score, and 0, 1.00–1.99, 2.00–2.99, and 3.00–6.00 for right carotid plaque score.

We found that higher maximum IMT and higher plaque burden were associated with poorer hearing in the best hearing ear for all, low, and high frequencies (Table 2; model 1). After additional adjustment for educational level and cardiovascular risk factors (model 2), we found that especially third quartile and fourth quartile plaque burden, as compared to first quartile plaque burden, related to poorer hearing across all frequencies [difference in dB hearing levels: 1.06 dB (95% CI: 0.04, 20.8), and difference: 1.55 dB (95% CI: 0.49, 2.60), respectively] (Table 2, model 2). Effect estimates in the low and high frequencies were comparable to all frequencies (Table 2, model 2). Additionally, larger maximum IMT related to poorer hearing in all hearing frequencies [difference in dB hearing levels per 1-mm increase in maximum IMT: 2.09 dB (95% CI: 0.08, 4.10)] (Table 2, model 2).

Table 2. Association between atherosclerosis and hearing loss in the better-hearing ear

Carotid atherosclerosis	**All hearing frequencies**	**Low hearing frequencies**	**High hearing frequencies**
	Difference in dB (95% CI)	Difference in dB (95% CI)	Difference in dB (95% CI)
Model 1			
Maximum IMT, mm	**2.76 (0.94, 4.59)**	**2.06 (0.53, 3.59)**	**3.35 (0.72, 5.99)**
Plaque burden			
1st quartile	*Reference*	*Reference*	*Reference*
2nd quartile	0.32 (-0.60, 1.23)	-0.01 (-0.78, 0.75)	0.57 (-0.75, 1.88)
3rd quartile	0.75 (-0.23, 1.73)	0.31 (-0.51, 1.13)	1.20 (-0.21, 2.61)
4th quartile	**1.52 (0.55, 2.49)**	**1.18 (0.36, 1.99)**	**1.95 (0.54, 3.35)**
Model 2			
Maximum IMT, mm	**2.09 (0.08, 4.10)**	1.36 (-0.32, 3.03)	2.82 (-0.09, 5.74)
Plaque burden			
1st quartile	*Reference*	*Reference*	*Reference*
2nd quartile	0.47 (-0.48, 1.41)	0.10 (-0.69, 0.88)	0.75 (-0.62, 2.12)
3rd quartile	**1.06 (0.04, 2.08)**	0.56 (-0.30, 1.41)	**1.59 (0.11, 3.08)**
4th quartile	**1.55 (0.49, 2.60)**	**1.08 (0.20, 1.96)**	**2.15 (0.62, 3.68)**

dB, decibel; CI, confidence interval; IMT, intima media thickness; mm, millimetre.
Difference represents the difference in dB hearing loss per 1-mm increase in maximum IMT or the difference in dB hearing loss in the better hearing ear per degree of plaque burden (2nd-, 3rd-, and 4th-quartile) compared to 1st-quartile plaque burden. The amount of hearing loss is expressed in dB; that is, a higher dB value reflects greater hearing loss. Model 1: adjusted for age and sex; model 2: additionally adjusted for age-squared, education, BMI, smoking, systolic blood pressure, diastolic blood pressure, use of blood pressure lowering medication, cholesterol (HDL and LDL), prevalent diabetes mellitus, and lipid-lowering medication use. Significant values ($P \leq 0.05$) are indicated in bold.

When investigating side-specific associations, we found that larger maximum IMT in the left carotid [difference: 2.03 dB (95% CI: 0.04, 4.03)] and in the right carotid [difference: 2.97 (95% CI: 0.79, 5.14)] was associated with poorer hearing exclusively in the right

ear (Table 3). Moreover, it appeared that fourth-quartile plaque burden, as compared to first-quartile plaque burden, in the left carotid is associated with poorer hearing in the right ear [difference: 1.68 dB (95% CI: 0.56, 2.81)] (Table 3). More interestingly, the third- and fourth-quartile plaque burden in the right carotid compared to first-quartile plaque burden [difference: 1.24 dB (95% CI: 0.14, 2.35), and difference: 2.12 dB (95% CI: 0.98, 3.26), respectively] was associated with poorer hearing in the right ear (Table 3). No associations were found for left carotid maximum IMT or plaque burden and left ear hearing loss (Table 3).

Table 3. Association between atherosclerosis and hearing loss per side

Carotid atherosclerosis	Left ear hearing loss,	Right ear hearing loss,
	Difference in dB (95% CI)	Difference in dB (95% CI)
Left carotid		
Maximum IMT, mm	-0.07 (-2.27, 2.14)	**2.03 (0.04, 4.03)**
Plaque burden		
First quartile	*Reference*	*Reference*
Second quartile	0.77 (-0.37, 1.91)	1.00 (-0.04, 2.04)
Third quartile	0.67 (-0.57, 1.90)	0.85 (-0.27, 1.98)
Fourth quartile	0.72 (-0.52, 1.95)	**1.68 (0.56, 2.81)**
Right carotid		
Maximum IMT, mm	2.15 (-0.26, 4.55)	**2.97 (0.79, 5.14)**
Plaque burden		
First quartile	*Reference*	*Reference*
Second quartile	0.07 (-1.06, 1.20)	0.02 (-1.02, 1.05)
Third quartile	0.99 (-0.22, 2.21)	**1.24 (0.14, 2.35)**
Fourth quartile	**1.65 (0.40, 2.90)**	**2.12 (0.98, 3.26)**

dB, decibel; CI, confidence interval; IMT, intima media thickness; mm, millimetre. *Difference* represents the difference in dB hearing loss per 1-mm increase in maximum IMT or the difference in dB hearing loss per degree of plaque burden (2^{nd}-, 3^{rd}-, and 4^{th}-quartile) compared to 1^{st}-quartile plaque burden. The amount of hearing loss is expressed in dB; that is, a higher dB value reflects greater hearing loss. Adjusted for age, age-squared, sex, education, BMI, smoking, systolic blood pressure, diastolic blood pressure, use of blood pressure lowering medication, cholesterol (HDL and LDL), prevalent diabetes mellitus, and lipid-lowering medication use. Significant values ($P \leq 0.05$) are indicated in bold.

Finally, we found that both side-specific, as well as overall higher carotid plaque burden and larger IMT was associated with poorer hearing in the better-hearing ear (Table 4). Interestingly, those associations seems to be explained by hearing status of the right ear. To be specific, larger overall maximum IMT and larger IMT in the right carotid were related with the odds of having a higher degree of hearing loss in the right ear [ordered log-odds: 0.50 (95% CI: 0.07, 0.92), ordered log-odds: 0.49 (95% CI: 0.10, 0.89), respectively] (Table 4). Moreover, fourth-quartile plaque burden, as compared to first-quartile

plaque burden, for the overall carotid, but also the left and right carotid, was related with the odds of having a higher degree of hearing loss in the right ear [ordered log-odds: 0.34 (95% CI: 0.12, 0.56), ordered log-odds: 0.26 (95% CI: 0.06, 0.46), and ordered log-odds: 0.32 (95% CI: 0.12, 0.52), respectively] (Table 4).

In general, the effect estimates of the association between atherosclerosis and hearing loss between midlife and late life (Supplementary Table 1) and between males and females (Supplementary Table 2) did not differ.

Table 4. Association between atherosclerosis and degree of hearing loss

Carotid atherosclerosis	Better-ear hearing loss	Left ear hearing loss	Right ear hearing loss
	Ordered log-odds (95% CI)	Ordered log-odds (95% CI)	Ordered log-odds (95% CI)
Overall			
Maximum IMT, mm	0.29 (-0.14, 0.71)	0.18 (-0.24, 0.59)	**0.50 (0.07, 0.92)**
Plaque burden			
1st quartile	*Reference*	*Reference*	*Reference*
2nd quartile	0.09 (-0.12, 0.30)	-0.01 (-0.21, 0.19)	0.11 (-0.09, 0.31)
3rd quartile	0.19 (-0.03, 0.42)	0.11 (-0.10, 0.33)	0.19 (-0.03, 0.41)
4th quartile	**0.31 (0.08, 0.53)**	0.15 (-0.06, 0.37)	**0.34 (0.11, 0.56)**
Left carotid			
Maximum IMT, mm	0.23 (-0.13, 0.58)	0.15 (-0.20, 0.50)	0.31 (-0.04, 0.67)
Plaque burden			
1st quartile	*Reference*	*Reference*	*Reference*
2nd quartile	**0.28 (0.09, 0.47)**	0.14 (-0.02, 0.30)	**0.22 (0.04, 0.41)**
3rd quartile	0.18 (-0.02, 0.39)	0.09 (-0.12, 0.29)	0.18 (-0.02, 0.38)
4th quartile	**0.28 (0.08, 0.48)**	0.16 (-0.16, 0.48)	**0.26 (0.06, 0.46)**
Right carotid			
Maximum IMT, mm	0.22 (-0.17, 0.61)	0.13 (-0.25, 0.52)	**0.49 (0.10, 0.89)**
Plaque burden			
1st quartile	*Reference*	*Reference*	*Reference*
2nd quartile	0.08 (-0.11, 0.27)	0.02 (-0.16, 0.20)	0.03 (-0.16, 0.21)
3rd quartile	0.13 (-0.08, 0.33)	0.06 (-0.14, 0.25)	0.12 (-0.08, 0.32)
4th quartile	**0.28 (0.07, 0.48)**	**0.25 (0.05, 0.44)**	**0.32 (0.12, 0.52)**

CI, confidence interval; IMT, intima media thickness; mm, millimetre; IMT, intima media thickness; mm, millimetre. Ordered log-odds represents the odds of having a higher degree of hearing loss (no hearing loss, mild hearing loss, or moderate/severe hearing loss) per 1-mm increase in maximum IMT or the odds of having a higher degree of hearing loss (no hearing loss, mild hearing loss, or moderate/severe hearing loss) per degree of plaque burden (2nd-, 3rd-, and 4th-quartile) as compared to 1st-quartile plaque burden. Adjusted for age, age-squared, sex, education, BMI, smoking, systolic blood pressure, diastolic blood pressure, use of blood pressure lowering medication, cholesterol (HDL and LDL), prevalent diabetes mellitus, and lipid-lowering medication use. Significant values ($P \leq 0.05$) are indicated in bold.

DISCUSSION

In this large sample of community-dwelling older individuals, we found that higher burden of carotid atherosclerosis as a measure of generalized atherosclerosis is associated with poorer hearing. Interestingly, this association was most prominent for hearing loss in the right ear.

Strengths of our study include the population-based setting, the large sample size, and the standardized assessment of hearing levels with pure-tone audiometry as well as atherosclerosis assessment. Some limitations of this study should also be acknowledged. First, this is a cross-sectional study, precluding inference on directionality. Second, the participants in this population-based cohort are mainly of European ancestry, which might limit the generalizability of our findings to other ethnicities. Third, data on labyrinthine artery atherosclerosis were unavailable, precluding inference on direct effects of atherosclerosis on hearing loss as blood supply to the inner ear directly comes from the labyrinthine artery.

Our results confirm previous reports regarding the association of the presence and burden of atherosclerosis with age-related hearing loss,[23, 29, 30, 41, 42] but were mainly of a cross-sectional nature, consisted of small sample sizes and assessed atherosclerosis and hearing loss by means of self-report. The only longitudinal study so far has reported an association of increased IMT and a larger number of plaques with a higher 5-year incidence of hearing impairment.[29] Although the reported study included a proper population-based study sample, the included age range (35-64 years) did not include the ages at which hearing loss is most pronounced. As hearing loss is highly prevalent at older ages, it is important to assess the association between atherosclerosis and hearing loss among older adults.

Interestingly, we found that generalized atherosclerosis is associated most prominently with right-ear hearing loss. The existence of asymmetries between right and left auditory function has been described earlier. It has been found that hearing levels in the right ear are poorer than hearing levels in the left ear in both young and older participants,[32-34, 43] for both central and peripheral auditory functions.[32] It has been hypothesized that this lateralization of central hearing abilities is associated with cerebral hemispheric topographic organization. Indeed, processing of phonetic information has been associated with higher activity in the left auditory cortex, resulting in a right ear advantage for complex auditory signals.[44] However, as detection of pure tones is dominated by peripheral function, it is more likely that our results are explained by peripheral hearing asymmetries. A study from 1983 found that with increasing age, the right ear was more vulnerable to peripheral auditory function changes than the left ear, [34] possibly because of a higher vulnerability than the left ear for risk factors such as atherosclerosis influencing inner ear health.[32] But, as the current study is of a cross-sectional design, we cannot determine

whether the right ear over time or with age is more vulnerable to peripheral damage. The above-mentioned hearing asymmetries might be further explained by differences in cochlear blood flow. Animal studies have consistently found poorer hearing in animals with lower cochlear blood flow.[45-47] Interestingly, a study in gerbils demonstrated that cochlear blood flow in the left ear was higher than cochlear blood flow in the right ear.[48] Therefore, we may speculate that abovementioned phenomenon is also applicable in humans. As such, our results might be explained by a lower right-cochlear blood flow and, possibly, the subsequent poorer right-ear hearing for older adults with atherosclerosis. Any change in cardiovascular health may affect the right ear more than the left ear. However, as it is not possible to measure cochlear blood flow in humans, this remains speculative and our results await further confirmation by other (longitudinal) population-based studies.

Nevertheless, as we found associations between both left and right carotid atherosclerosis and hearing loss, our results are more likely explained by a more generalized impact of atherosclerosis through the entire vascular system. We measured atherosclerosis in the carotid artery, but blood supply to the inner ear comes from the labyrinthine artery. The inner ear vessels may anastomose with the middle ear vessels, for which the external carotid artery is the most important supplier of blood.[20] Although not directly, the carotid externa might supply blood or, in light of our results, exert its atherosclerosis impact indirectly on the inner ear through this anastomose. Moreover, it is known that larger IMT in the carotid artery is correlated with atherosclerotic disease elsewhere in the arterial system, including the vertebrobasilar arteries, and with the risk of cardiovascular events.[27, 49, 50] As such, it has been suggested that carotid atherosclerosis can be used as an indicator of generalized atherosclerosis and cardiovascular health. Notably, a recent study reported an association between coronary atherosclerosis and hearing loss, supporting the generalized impact of atherosclerosis.[51] Thus, therapeutic and possibly other lifestyle interventions preventing or deferring progression of atherosclerosis through the entire vascular system [52] might be a promising strategy to prevent or delay the onset or progression of hearing loss in older adults as an added bonus in targeting cardiovascular disease.

CONCLUSION AND IMPLICATIONS

Carotid atherosclerosis is associated with poorer hearing in older adults. Interestingly, associations are predominantly found with poorer hearing in the right ear. The impact of atherosclerosis, therefore, seems to go beyond merely the risk of cardiovascular events. Early detection and prevention of atherosclerosis carries the promise to not only lower the risk of clinical cardiovascular events and mortality, but also prevent or delay the onset or progression of hearing loss in older adults by promoting and maintaining inner ear health.

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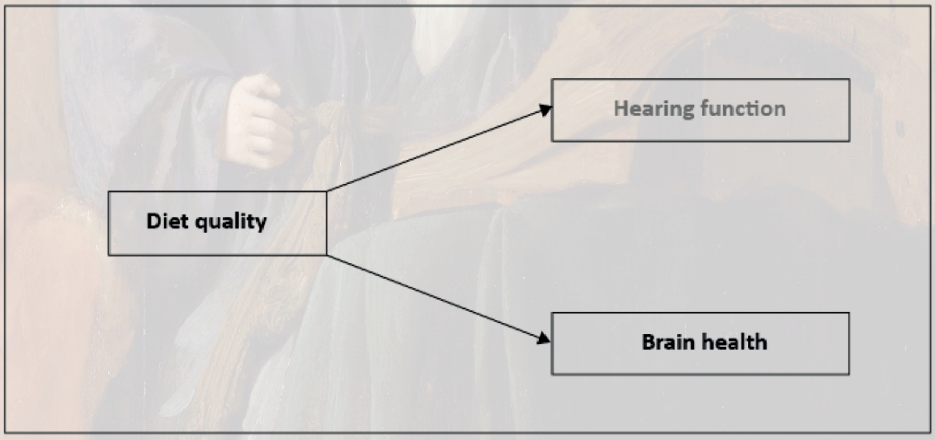
Minerva; Rembrandt van Rijn[1]

3

Risk factors for brain health


Hearing function
Diet quality
Brain health

3.1

Better diet quality relates to larger brain tissue volumes: the Rotterdam Study

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Neurology, 2018

ABSTRACT

Objective

To investigate the relation of diet quality with structural brain tissue volumes and focal vascular lesions in a dementia-free population.

Methods

From the population-based Rotterdam Study, 4,447 participants underwent dietary assessment and brain MRI scanning between 2005 and 2015. We excluded participants with an implausible energy intake, prevalent dementia or cortical infarcts, leaving 4,213 participants for the current analysis. A diet quality score (0-14) was calculated reflecting adherence to Dutch dietary guidelines. Brain MRI was performed to obtain information on brain tissue volumes, white matter lesion volume, lacunes and cerebral microbleeds. The associations of diet quality score and separate food groups with brain structures were assessed using multivariable linear and logistic regression.

Results

We found that better diet quality related to larger brain volume, grey matter volume, white matter volume, and hippocampal volume. Diet quality was not associated with white matter lesion volume, lacunes or microbleeds. High intake of vegetables, fruit, whole grains, nuts, dairy and fish and low intake of sugar-containing beverages were associated with larger brain volumes.

Conclusions

A better diet quality is associated with larger brain tissue volumes. These results suggest that the effect of nutrition on neurodegeneration may act via brain structure. More research, in particular longitudinal research, is needed to unravel direct versus indirect effects between diet quality and brain health.

INTRODUCTION

Diet is considered an important modifiable risk factor for dementia.[2-7] But the pathways underlying this association remain largely unknown. An important pathway may be through direct effects of diet on brain structures or focal vascular lesions,[8-15] as it is known that structural brain changes are an important risk factor for dementia.[8, 16] Moreover, a healthy diet is associated with better brain health and larger brain volumes.[9-15] However, studies performed on this research area were generally of limited sample size, considered only a limited age range, or used dietary adherence as a dichotomous variable rather than as a continuous variable.

Traditionally, epidemiological and animal studies on health effects of nutrition have focused on the effects of individual food nutrients and showed that specific nutrients such as B vitamins , vitamin E, and the *n*-3 fatty acid docosahexaenoic acid that can be found in for example vegetables, fruit and seafood, have neuroprotective effects.[10, 17] However, it is important to acknowledge that many complex interactions occur across different food components and nutrients, which has triggered the increasing interest to study effects of dietary patterns as a whole.[10] For example, adherence to a Mediterranean Diet showed protective effects against brain tissue loss,[13 18] including lower volumes of white matter hyperintensities.[19] Several other studies also linked other measures of diet quality to lower risk of dementia.[3, 6, 7]

Optimizing diet quality might be a suitable preventive strategy to maintain and augment cognition in healthy older adults.[2, 3] Hence, we investigated the association of dietary patterns and its components with structural brain volumes in a population-based sample of dementia-free middle aged and elderly individuals.

METHODS

Setting and study population

This cross-sectional study was embedded in the Rotterdam Study, a population-based community-dwelling cohort in the Netherlands since 1990 investigating determinants and consequences of ageing.[20] At study entry and subsequently every three to four years, all participants were invited to undergo extensive examinations in the dedicated research centre. By 2008, 14,926 individuals aged 45 years and older participated in the Rotterdam Study. For this study, 5,690 participants who visited the study centre between 2006-2012 for initial or re-examinations underwent extensive questionnaires on their dietary intake.[21] From 2005 onward, MRI scanning of the brain was included in the Rotterdam Study.[22] The MRI scans included in this study were performed between 2005 and 2015, and we excluded the participants without a brain MRI scan. This left

us with a total of 4,447 participants who had data on both dietary intake and a brain MRI scan. The median age interval between dietary assessment and MRI scanning was 0.13 months. From this group, we excluded participants with a reported daily energy intake of less <500 or >5,000 kcal/d (n = 162) and participants with prevalent dementia or cortical infarcts on MRI (n = 72), leaving a total of 4,213 participants for the current analyses.

The Rotterdam Study has been approved by the medical ethics committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG).

Dietary intake assessment

Dietary intake was assessed with a validated, self-administered, semi-quantitative food-frequency questionnaire (FFQ) consisting of 389 items. This FFQ was previously validated against the dietary history method and against nine-day food records in 2 other Dutch populations and was found to be an appropriate measurement tool for ranking people according to their food intake.[23, 24] For the different food items, questions about the number of servings per day and the frequency of consumption were included. Energy intake was calculated using the Dutch Food Composition Table (NEVO). Based on the information obtained from the FFQ, we evaluated adherence (yes/no) to 14 items of the Dutch dietary guidelines[25] (vegetables, fruit, whole grain products, legumes, nuts, dairy, fish, tea, whole grains of total grains, unsaturated fats and oils of total fats, red and processed meat, sugar-containing beverages, alcohol, and salt; Table 1). An overall diet score (0-14) reflecting adherence to the dietary guidelines was calculated by adding up the scores for the 14 above mentioned food groups, as described in more detail elsewhere.[21] For comparison with other studies, we additionally calculated a Mediterranean diet score based on sex and cohort specific median food intake of our study population, as previously described by Trichopoulou et al.[26]

Table 1. Population characteristics

Sample size	N =4,213
Women	56.8 %
Age, years	65.7 (10.8)
Age, years, range	45.5 – 97.5
Education, y	12.7 (3.9)
Lower	27.8 %
Middle	48.7 %

Table 1. Population characteristics (continued)

Sample size	N =4,213
Higher	23.5 %
Physical activity, MET-hours/week	59.9 (55.1)
Past or current smoking	14.5 %
Hypertension	22.2%
Hypercholesterolemia	52.0 %
Body mass index, kg/m^2	27.3 (4.1)
Type 2 diabetes	8.6 %
Dietary characteristics	
Energy intake, kcal/day	2,081 (1,684-2,542)[a]
Number of items adhered to (no.)	7 (6-8)[a]
Adherence to individual guideline components (%)	
Vegetables ≥200 g/day	44.2
Fruit 200 ≥ g/day	59.6
Whole grain products ≥ 90 g/day	63.6
Legumes ≥ 135 g/week	28.2
Nuts ≥ 15 g/day	21.8
Dairy ≥ 350 g/day	37.4
Fish ≥ 100 g/week	54.0
Tea ≥ 450 g/day	8.2
Whole grains ≥ 50% of total grains	79.3
Unsaturated fats and oils ≥ 50% of total fats	67.8
Red and processed meat ≤ 300 g/week	22.7
Sugar-containing beverages ≤ 150 g/day	80.6
Alcohol ≤ 10 g/day	57.7
Salt ≤ 6 g/day	63.5
Brain MRI tissue volumes	
Total brain volume, mL	932.01 (105.9)
Grey matter volume, mL	529.4 (62.4)
White matter volume, mL	402.7 (66.8)
Hippocampus volume, mL	7.7 (1.00)
Markers of cerebral small vessel disease	
Lacunes	4.6%
Microbleeds	20.6%
White matter lesion volume,[b] mL	8.2 (1.1)

Abbreviation: MET = metabolic equivalent of task. Values are based on imputed data. Numbers of missings per variable were 579 for amount of physical activity; 386 for total cholesterol; 316 for diastolic and systolic blood pressure; 302 for body mass index; 59 for years of education and 59 for highest obtained education level. [a] Values are mean (SD) for continuous variables or median (interquartile range) when indicated (*), percentages for dichotomous variables. [b] ln-transformed.

Magnetic resonance imaging

Brain MRI was performed on a 1.5T MRI scanner with a dedicated eight-channel head coil (software version 11x; General Electric Healthcare, Milwaukee, WI).[22] The scan protocol included a T1-weighted sequence, a proton-density weighted sequence and a fluid-inversion-recovery sequence.[22, 27] To quantify brain volume, grey matter volume, white matter volume, white matter lesion volume, hippocampal volume and intracranial volume, automated brain tissue classification was used. This quantification strategy was based on a k-nearest neighbour classifier algorithm, extended with an in-house developed white matter lesion segmentation.[22, 27] Furthermore, T1-weighted MR images were processed using FreeSurfer (version 5.1) to obtain the hippocampus volume.[28] Visual evaluation of all scans was performed to assess the presence and amount of lacunes, cortical infarcts, and cerebral microbleeds, using a strategy that has been previously described in detail.[22]

Other measurements in the Rotterdam Study

Information on cardiovascular risk factors, medication use, physical activity, and educational level was obtained by interview, physical examinations and blood sampling. Smoking data were collected through self-report and categorized into never, former, and current smoking. Educational level was categorized as lower, middle or higher education. Total years of education was calculated. Height and weight, blood pressure, glucose levels and cholesterol levels were measured and body mass index was calculated (kg/m^2). Systolic and diastolic blood pressure was measured twice using a random zero-sphygmomanometer. Glucose was determined by the hexokinase method. Using an automatic enzymatic procedure, serum total cholesterol and high-density lipoprotein cholesterol were measured from fasting blood samples.[29] Hypertension was defined as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of blood pressure lowering medication.[27] Hypercholesterolemia was defined as total cholesterol concentration ≥ 6.2 mmol/L and/or the use of lipid-lowering medication.[27] Type 2 diabetes was defined as having fasting blood glucose concentrations >7.0 mmol/L, non-fasting blood glucose >11.1 mmol/L, or use of glucose-lowering medication. The LASA (Longitudinal Ageing Study Amsterdam) Physical Activity Questionnaire was used to assess the amount of physical activity. This is a validated questionnaire,[30, 31] that consists of questions about walking, cycling, gardening, sports, and housekeeping.[21] For each participant, data were recalculated into MET (metabolic equivalent of task) hours per week.[32]

Statistical analysis

Given the skewed distribution of white matter lesion volume, we natural log-transformed these values and used these in the analyses. The association of the diet quality score

with total brain volume, grey matter volume, white matter volume, white matter lesion volume and hippocampal volume was examined using multivariable linear regression models. In the first model we adjusted for age, sex, educational level, total energy intake, and intracranial volume (as proxy for head size). In the second model, we additionally adjusted for smoking, body mass index, and physical activity. A third model was constructed in which we adjusted model 2 with the addition of diabetes, hypertension, and hypercholesterolemia.

The association of diet quality scores with the presence of lacunes and cerebral microbleeds was assessed using logistic regression models, and adjustments were similar to the above-mentioned models. To further explore whether associations of the overall diet quality score were explained by certain items of the dietary guidelines, we investigated associations of adherence to guidelines for specific food groups with the global and focal brain structures using the same models. We checked for effect modification by sex, and we checked for interaction by age by using interaction terms. To check whether associations were not driven by one specific food component we repeated our main analysis by excluding each of the 14 individual guidelines from the total dietary guideline score one at a time, and examining the effect on the estimates. Finally, to analyse the robustness of our dietary guideline score and the comparability of it toward other populations, we also conducted analyses to investigate the associations between the Mediterranean diet score and brain volumetric and between the Mediterranean diet score and focal vascular brain lesions using the above-mentioned models. Missing variables (<1%) were imputed using the multiple imputation algorithm (5 imputations) of SPSS. For the analyses, IBM SPSS statistics version 23 (IBM Corp, Armonk, NY) was used.

RESULTS

Table 1 shows the characteristics of the study population. Mean age at time of dietary assessment was 65.7 years (SD 10.8, range 45.5 – 97.5), and 56.7% of the participants were women. Participants had a median energy intake of 2,081 kcal/d (interquartile range 1,684-2,542) and had a median dietary guideline adherence score of 7 (interquartile range 6-8) on a theoretical range of 0 to 14. Participants had a total brain volume of 932.0 mL (SD 105.9).

We found that, after adjustment for age, sex, intracranial volume, education, energy intake, smoking, physical activity and body mass index (model 2), a higher diet quality score related to larger total brain volume, grey matter volume, white matter volume, and hippocampal volume (Table 2). Additional adjustment for other cardiovascular risk factors (model 3) did not change these results (Table 2). A higher diet score was neither associated with the presence of lacunar infarcts and microbleeds nor the volume of

Table 2. Diet quality and brain volume

	Total brain volume	Grey matter volume	White matter volume	Hippocampus volume
	Difference in mL (95% CI)	Difference in mL (95% CI)	Difference in mL (95% CI)	Difference in mL (95% CI)
Model 1	2.04 (1.24, 2.85)	0.85 (0.15, 1.55)	1.19 (0.42, 1.97)	0.02 (0.01, 0.03)
Model 2	2.03 (1.24, 2.83)	0.88 (0.18, 1.59)	1.15 (0.37, 1.93)	0.02 (0.00, 0.03)
Model 3	2.01 (1.21, 2.80)	0.89 (0.19, 1.60)	1.11 (0.33, 1.89)	0.02 (0.00, 0.03)

Difference in volume in mL per one point better adherence to the dietary guidelines. CI: confidence interval *Model 1:* adjusted for age, sex, intracranial volume, education, energy intake. *Model 2:* adjusted for age, sex, intracranial volume, education, energy intake, smoking, physical activity and body mass index. *Model 3:* adjusted for age, sex, intracranial volume, education, energy intake, smoking, physical activity, body mass index, diabetes, hypertension and hypercholesterolemia. Statistically significant effect estimates ($p < 0.05$) apply to all data.

Table 3. Diet quality and focal brain lesions

	White matter lesions*	Lacunes	Microbleeds
	Difference (95% CI)	OR (95% CI)	OR (95% CI)
Model 1	-0.01 (-0.02, 0.01)	1.01 (0.93, 1.10)	0.99 (0.95, 1.04)
Model 2	-0.01 (-0.02, 0.01)	1.01 (0.93, 1.10)	0.99 (0.95, 1.04)
Model 3	-0.01 (-0.02, 0.01)	1.01 (0.93, 1.10)	0.99 (0.95, 1.04)

Difference in volume per 1-point better adherence to the dietary guidelines. OR: Odds Ratio. CI: confidence interval. * log-transformed. *Model 1:* adjusted for age, sex, intracranial volume, education, energy intake. *Model 2:* adjusted for age, sex, intracranial volume, education, energy intake, smoking, physical activity and body mass index. *Model 3:* adjusted for age, sex, intracranial volume, education, energy intake, smoking, physical activity, body mass index, diabetes, hypertension and hypercholesterolemia. No statistically significant effect estimates ($p < 0.05$).

white matter lesions (Table 3). We found no prominent differences between men and women (Supplementary tables 1 to 4 [links.lww.com/WNL/A531]), and there was no interaction by age (p for interaction > 0.05).

Regarding specific food components, we observed that associations of diet quality with brain volumes were not driven by one single component. Guideline adherence for multiple components, such as vegetables, fruit, whole grains, nuts, dairy and fish was associated with larger total brain and white matter volumes (Table 4). Moreover, adhering to the guidelines for whole grains and dairy was associated with larger grey matter volumes, and adhering to the guidelines of sufficient fruit and low sugar-containing beverage intake was related to larger hippocampus volumes (Table 4). In line with this, excluding each of the food groups one by one from the score resulted in similar associations with brain volumes as observed for the total dietary guideline score (Supplementary table 5 [links.lww.com/WNL/A531]). As demonstrated in Supplementary tables 6 and 7, the effect estimates of the association between Mediterranean diet score and

Table 4. Adherence to dietary guidelines for specific food groups and brain volume

	Total brain volume	Grey matter volume	White matter volume	Hippocampus volume
	Difference in mL (95% CI)	Difference in mL (95% CI)	Difference in mL (95% CI)	Difference in mL (95% CI)
Vegetables	3.35 (0.31, 6.39)[a]	-0.47 (-3.16, 2.22)	3.82 (0.85, 6.79)[a]	0.03 (-0.02, 0.08)
Fruit	4.17 (1.10, 7.24)[a]	0.67 (-2.05, 3.39)	3.50 (0.50, 6.50)[a]	0.06 (0.01, 0.11)[a]
Whole grains	3.45 (0.32, 6.58)[a]	3.11 (0.34, 5.88)[a]	0.34 (-2.72, 3.40)	0.03 (-0.02, 0.08)
Legumes	0.08 (-3.13, 3.29)	0.98 (-1.79, 3.76)	-0.91 (-3.73, 1.92)	-0.00 (-0.06, 0.05)
Nuts	5.91 (2.26, 9.55)[a]	1.41 (-1.82, 4.63)	4.50 (0.94, 8.07)[a]	-0.01 (-0.07, 0.06)
Dairy	2.45 (-0.59, 5.49)	2.76 (0.10, 5.42)[a]	-0.31 (-3.28, 2.67)	0.02 (-0.03, 0.07)
Fish	2.44 (-0.47, 5.35)	-1.60 (-4.17, 0.97)	4.04 (1.20, 6.87)[a]	0.05 (-0.00, 0.10)
Tea	-0.20 (-5.43, 5.02)	2.94 (-1.68, 7.56)	-3.15 (-8.25, 1.96)	-0.01 (-0.09, 0.08)
Grains	5.39 (1.84, 8.94)[a]	2.43 (-0.71, 5.57)	2.95 (-0.52, 6.42)	0.04 (-0.02, 0.10)
Fats	2.10 (-0.97, 5.16)	-0.31 (-3.03, 2.41)	2.40 (-0.60, 5.41)	0.01 (-0.04, 0.06)
Red meat	2.29 (-1.25, 5.83)	3.01 (-0.12, 6.13)	-0.72 (-4.17, 2.74)	0.01 (-0.05, 0.07)
Sugar containing beverages	-1.57 (-5.25, 2.12)	-1.00 (-4.23, 2.22)	-0.56 (-4.17, 3.04)	0.09 (0.02, 0.15)[a]
Alcohol	2.43 (-0.57, 5.43)	1.50 (-1.16, 4.15)	0.94 (-2.00, 3.87)	-0.01 (-0.06, 0.04)
Salt	0.69 (-3.15, 4.54)	2.04 (-1.36, 5.44)	-1.35 (-5.10, 2.41)	-0.03 (-0.10, 0.03)

Difference in volume in millilitres (95% confidence interval) for adherence (yes/no) to the guideline for the specific food group. CI: confidence interval. Adjusted for age, sex, intracranial volume, education, energy intake, smoking, physical activity and body mass index. Cut off values for guidelines: vegetables ≥200 g/day, fruit ≥200 g/day, whole grain products ≥90 g/day, legumes ≥135 g/week, nuts ≥15 g/d, dairy ≥350 g/day, fish ≥100 g/week, tea ≥450 g/day, whole grains ≥ 50% of total grains, fats (unsaturated) ≥50% of total fats, meat (red and processed) ≤300 g/week, sugar-containing beverages ≤150 g/day, alcohol ≤10 g/d, salt ≤6 g/day (Table 1).

[a] Statistically significant effect estimates ($p < 0.05$).

brain volume and between the Mediterranean diet score and focal brain lesions were similar to the results found for the Dutch dietary guidelines, as can be seen in Tables 3 and 4.

DISCUSSION

In this large sample of community-dwelling individuals free of dementia, we found that better overall diet quality is related to larger total brain volume, grey matter, white matter, and hippocampal volumes. These associations were not driven by one specific food group, though several food groups contributed differentially to the effect on brain changes. In particular, sufficient intake of vegetables, fruit, nuts, whole grains, dairy, and fish and limited intake of sugar-containing beverages were related to larger brain tissue volumes and thus together promote brain health together. We found no effects of diet

quality on focal vascular brain lesions, such as white matter lesions, or the presence of lacunes or microbleeds.

Strengths of our study included the population-based setting and (quantitative) assessment of structural brain changes using imaging. In addition, we used a novel, validated, food-based diet score that can be used to rate overall diet quality of adults.[21] Contrary to other dietary guidelines, this guideline is completely based on food groups instead of individual nutrients,[21] which represents a more accurate reflection of eating patterns. However, some limitations of the current study should also be acknowledged. First, the FFQ relies on an individual's capacity to recall their dietary behaviour over the past month. Recall bias in dietary behaviour could be a systematic bias. For example, alcohol consumption is known to be underreported, and thus an underestimation of the actual alcohol intake in our population might be expected, leading to an underestimation of the true effect on the brain.[33] Second, the dietary guideline score is constructed using a dichotomous variable per component (i.e., adherent or non-adherent) which may have resulted in loss of information leading to an underestimation of the true effect. Third, this is a cross-sectional study, hampering the possibility to infer causality between determinant and outcome. Fourth, this dietary guideline score is developed for and validated in a Dutch population, which might restrict generalizability to other countries and its populations. However, our results indicate that the Mediterranean diet score developed by Trichopoulou at al.[26] showed the same associations between diet quality and brain volume and between diet quality and focal vascular lesions. This supports the generalizability of the Dutch dietary guidelines and suggests that overall diet quality is important for brain structure irrespective of the exact index used to define diet quality. Nevertheless, it is also important to acknowledge that it is still necessary to use population-specific dietary guidelines and corresponding diet scores to accurately estimate diet quality of populations. The widely used Mediterranean Diet, for example, has been found to predict mortality risk in Mediterranean populations, but it predicts mortality less so in non-Mediterranean populaitons.[34] Finally, although we tried to adjust for lifestyle factors and other factors that may relate to both diet quality and brain health, there still might be residual confounding from unmeasured confounders.

We found that better diet quality related to larger total brain volume, grey matter volume, white matter volume and hippocampal volume, supporting our hypothesis that direct structural changes in the brain are influenced by variations in diet quality. There are few other studies that examined the association between diet quality and brain health. Those that did examine the association between diet quality and brain health mostly incorporated a Mediterranean Diet. Similar results have been found in those studies, with better adherence to a Mediterranean Diet associated with lower rates of brain atrophy and larger grey and white matter volumes.[14,11] Regarding the potential pathways through which diet can influence the brain, there are several pos-

sibilities. First, nutritional factors could have a direct effect on neuronal heath. In a randomized controlled trial, the effects of a Mediterranean Diet on plasma brain-derived neurotrophic factor levels,[35] a nerve growth factor promoting survival and growth effects on neurons, was investigated.[36] The authors found higher plasma brain-derived neurotrophic factor levels in the experimental group compared to the control group.[35] In animal studies, comparable results have been found.[37] Dietary interventions in mice improved cerebrovascular health and enhanced neuroprotective mechanisms, leading to an increase of the synthesis of synaptic proteins and phospholipids and an improvement of functional connectivity in the brain.[37] These results highlight the potential of direct neuroprotective effects of diet quality on the brain, but other potential pathways should also be considered.

Another pathway could be the influence of diet quality on vascular risk and cerebrovascular disease. Changes in nutrition are thought to be a promising way to lower the risk of cerebrovascular disease.[38] However, in our study, we found that diet quality was not associated with focal vascular brain lesions (white matter lesions, lacunes, or cerebral microbleeds), not supporting this hypothesis. It is of interest that a cross-sectional study with 1,091 participants found that adhering to a Mediterranean Diet does relate to a lower volume of white matter lesions,[39] and also more generally, a healthy dietary pattern has been related to a lower cardiovascular risk.[40-42] A randomized trial which was conducted in Spain in 2013 reported a lower incidence of major cardiovascular events (relative risk reduction of approximately 30%) among high-risk persons whom received a Mediterranean Diet supplemented with extra-virgin olive oil or nuts compared to the control group.[43] The absence of an association in our study might be attributable to information loss as we used the presence (yes/no) of infarcts and microbleeds, possibly leading to an underestimation of the true effect. Another plausible explanation lies in the fact that most research in nutrition and cerebrovascular disease is performed in clinical studies. Participants are thus assigned to a diet, which might be healthier than what they normally eat, whereas the participants in our study report what they eat in general, which might be less healthy than the diets assigned to in clinical trials. Thirdly, residual confounding may underlie the association between diet and brain structure. Although we corrected for lifestyle factors such as education, energy intake, smoking, physical activity and body mass index, there still might be residual confounding. For example, socio-economic status (SES) might be a confounder in the relationship between brain health and diet quality. However, we do not have enough data pertaining to income and occupation, for example, to construct a proper SES variable, and therefore we used education as a proxy for SES.

Finally, we might be looking at an effect of neurodevelopment where variations in diet quality throughout life have different effects on brain structure and brain health. In a study conducted in Japan, researchers compared 2 types of breakfast in children aged 5

to 18 years.[44] The breakfast types, rice or bread, influenced different regions in the brain. The rice group had larger grey matter volumes in several regions, such as the left superior temporal gyrus, whereas the bread group had significantly larger grey matter volumes in several other regions, including the bilateral orbitofrontal gyri. This suggests that optimal nutrition is important for brain maturation.[44] Moreover, research in infants showed that breastfeeding is associated with improved developmental growth in late maturing white matter association regions, and that extended breastfeeding was associated with improved white matter structure and higher cortical thickness.[45-47] Again, this underlines the importance of nutrition on brain development and maturation and thus brain health.

Regarding the specific food groups in the diet quality score we used, most of the components contributed to the associations observed for overall diet quality with brain volumes. We found that sufficient intake of each - vegetables, fruit, nuts and whole grains - significantly related to larger total brain volume and larger white matter volumes. Multiple studies have addressed specific nutrient patterns and brain health. One study found "Alzheimer's disease-protective" nutrient patterns where vitamin B_{12}, vitamin D, and zinc were positively associated with AD brain biomarkers.[17] In addition, grey matter volume was negatively associated with intake of cholesterol, sodium, and saturated and trans-saturated fats. These nutrient patterns were linked to a higher intake of fruit, vegetables, whole grains, fish, low-fat dairy, and nuts and with a lower intake of sweets, fried potatoes, processed meat, high-fat dairy, and butter.[17] Other studies also found associations between higher intake of fish, whole grain, dairy (low-fat), and lower intake of meat, alcohol, and sugar-containing beverages and larger brain volumes such as grey matter volume and hippocampal volume.[11, 48, 49] However, intake of these components are correlated and therefore should be interpreted as a dietary pattern rather than as individual components. People consume a diet that consists of multiple nutrients that have interactive effects. Therefore, considering individual components might be inadequate to taking the additive and interactive effects of nutrients into account.[10] Moreover, we found similar effect estimates when excluding one food component at a time from the overall diet quality score, suggesting that the associations of overall diet quality were not driven by one specific food component and highlighting the importance of the overall diet quality.

This study suggests that a better overall diet quality is associated with larger brain tissue volumes, in which the additive and interactive effects of certain food groups, such as high consumption of fruit, vegetables, whole grains, nuts, dairy and fish and low consumption of sugar-containing beverages, support brain health. These results highlight the potential of nutrition influencing cognition and the risk of developing dementia through brain health. More research, in particular longitudinal population-based research, is needed to unravel direct vs indirect effects between diet quality and brain health.

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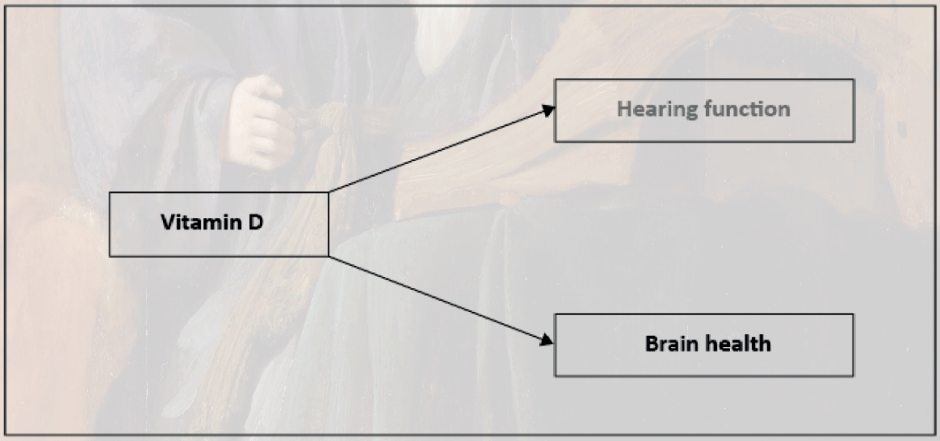
Hearing function
Vitamin D
Brain health

3.2

The association between vitamin D deficiency and MRI markers of brain health in a community sample

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Under review

ABSTRACT

Background and aim

Vitamin D deficiency has been linked to an increased risk of dementia. However, to strengthen current evidence and establish whether vitamin D can indeed play a role in early prevention of neurodegeneration, knowledge on underlying pathways is crucial. Therefore it was the aim of this study to investigate the association of vitamin D status with brain tissue volumes, hippocampus volume, white matter integrity, and markers of cerebral small vessel disease (CSVD) in a dementia-free population.

Methods

In this cross-sectional analysis, 2,716 participants free of dementia from the population-based Rotterdam Study underwent serum 25(OH)D concentration assessment and brain magnetic resonance imaging (MRI) scanning between 2006 and 2009. Outcomes of interest included brain tissue volume (total, white matter, grey matter and hippocampus volume), white matter integrity (fractional anisotropy (FA) and mean diffusivity (MD)), and markers of CSVD (white matter hyper intensity (WMH) volume, presence of lacunes and microbleeds). Associations between vitamin D status, both in categories and continuous, and these brain measurements were assessed using multivariable linear and logistic regression models, adjusting for lifestyle and other disease risk factors.

Results

We observed that vitamin D deficiency (25(OH)D <30 nmol/L) was independently associated with smaller brain tissue volume, smaller white matter volume and smaller hippocampus volume as compared to a sufficient vitamin D status (≥50 nmol/L). Vitamin D per 10 nmol/L increment and an insufficient (30-50 nmol/L) as compared to sufficient vitamin D status were not associated with the brain measures of interest. Moreover, vitamin D status was not associated with grey matter volume, white matter integrity or CSVD markers.

Conclusions

In this dementia-free population, vitamin D deficiency was associated with a smaller brain tissue volume and hippocampus volume. More research, in particular longitudinal, is needed to further elucidate the role of vitamin D in neurodegeneration.

INTRODUCTION

Vitamin D deficiency has been repeatedly linked to higher risk of dementia.[1-8] However, to strengthen current evidence and establish whether vitamin D can indeed play a role in early prevention of neurodegeneration, knowledge on underlying pathways is crucial. It has been hypothesized that the association of vitamin D with dementia might be explained by direct effects of vitamin D levels on brain health.

Indeed, previous studies found that higher vitamin D concentrations were associated with larger grey matter volume,[5] and that lower levels were associated with decreased white matter microstructural integrity (as reflected in lower fractional anisotropy (FA) and higher mean diffusivity (MD)),[9] and with markers of cerebral small vessel disease (CSVD) (white matter hyperintensity (WMH) volume and presence of lacunes and microbleeds).[10, 11] Contrary, another study reported an association of higher vitamin D concentrations with smaller white matter volume.[12] Interestingly, some studies reported that vitamin D deficiency was associated specifically with smaller hippocampus volumes,[13, 14] a part of the brain that plays a major role in learning and memory.[15] However, these previous studies had mostly small to moderate sample sizes, some included participants with dementia, and many studies did not extensively adjust for important confounding factors such as age and other lifestyle factors.[5, 10, 12]

Therefore, we studied the association of vitamin D status with several brain measures, including brain tissue volume (total, white matter, grey matter and hippocampus volume), white matter integrity (FA and MD), and markers of CSVD, in a large middle- and older-aged dementia-free population-based sample.

METHODS

Study design and study population

This cross-sectional study was embedded within the Rotterdam Study, an ongoing population-based prospective cohort study in the Netherlands investigating determinants and consequences of ageing since 1990.[16] At study entry and subsequently every three to four years participants were interviewed and underwent extensive examinations at the dedicated research centre in the district of Ommoord. By 2008, 14,926 individuals aged 45 years and over participated in the Rotterdam Study.

From 2005 onward, magnetic resonance imaging (MRI) scanning of the brain was included in the study protocol.[17] Between 2006 and 2009 blood samples were obtained from 3,425 participants. Of those 3,425 participants, 466 had no MRI data available and 125 had missing vitamin D data. From this group we further excluded participants with implausible MRI scan data (extremely low tissue volumes) (N = 22), participants with

prevalent dementia (N = 3), insufficient data to determine dementia status (N = 26) and those with a score < 24 on the Mini-Mental State Examination (MMSE) (N = 67).[18] None of these participants had cortical brain infarcts on MRI. The final population of analysis consisted of 2,716 participants, with a mean time interval between MRI scan and blood sample collection of 0.1 months (standard deviation (SD): 1.1).

Standard protocol approvals, registrations, and participant consents

The Rotterdam Study has been approved by the medical ethics committee of Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Vitamin D (25(OH)D) assessment

Vitamin D status was determined from 25-hydroxyvitamin D (25(OH)D) concentrations in blood. Fasting blood samples were collected at baseline. Serum 25(OH)D concentration was measured using an electrochemiluminescense-based assay (COBAS Roche Diagnostics GmbH, Germany). This assay has a functional sensitivity of 10 nmol/L (CV 18.5%), measuring a range of 7.5 nmol/L to 175 nmol/L, within-run precision of ≤6.5%, and intermediate precision of ≤ 11.5%.[19, 20] For the current analyses, vitamin D status was categorized into a 25(OH)D concentration of <30 nmol/L considered as deficient, a 25(OH)D concentration of 30-50 nmol/L was considered insufficient, and a 25(OH)D concentration ≥50 nmol/L was considered sufficient based on the vitamin D guidelines of the Institute of Medicine (USA).[8]

Assessment of brain volumetry, white matter microstructure and markers of CSVD

Brain MRI was performed using 1.5-T MRI system with a dedicated 8-channel head-coil (software version 11x; General Electric Healthcare, Milwaukee, WI).[17] The scan protocol included a T1 weighted sequence (voxel sixe 0.49 x 0.49 x 1.6 mm^3), a proton-density weighted sequence (voxel size 0.6 x 0.98 x 1.6 mm^3), and a fluid-attenuated inversion recovery sequence (voxel size 0.78 x 1.12 x 2.5 mm^3). To quantify intracranial volume, brain tissue volume, grey matter volume, white matter volume, and WMH volume, automated brain tissue classification was used.[17] This quantification strategy was based on a *k*-nearest neighbour classification algorithm.[21] Supratentorial intracranial volume was estimated by summing grey matter and white matter (the sum of normal-appearing white matter and WMH volume). To obtain hippocampus volume, T1-weighted MRI's were processed using FreeSurfer (version 5.1).[17, 22]

To obtain microstructural measures of the white matter, diffusion tensor imaging (DTI) (voxel size 3.3 x 2.2 x 3.5 mm^3) was used.[23] A single shot, diffusion weighted spin echo echo-planar imaging sequence was performed with maximum *b* value of 1,000 s/mm^2 in 25 noncollinear directions; 3 b_0 volumes were acquired without diffusion weighting. Using a standardized processing pipeline, diffusion data were pre-processed.[24] From this (in combination with the tissue segmentation), we derived global mean FA and MD in the normal-appearing white matter. FA is the degree of anisotropy in the normal-appearing white matter and is given as a ratio ranging from 0 (isotropic or non-directional) to 1 (unidirectional). MD is expressed in square millimetres per second. Visual ratings were performed for the presence of lacunes or microbleeds by trained raters.[17]

Covariate assessment

Trained interviewers conducted home interviews obtaining information on smoking, alcohol consumption and education. Smoking status was categorized as never, past or current smoker. Educational level was categorized as primary, lower, intermediate, and higher education. Physical activity data was collected using the LASA Physical Activity Questionnaire.[25] Metabolic equivalent of tasks (MET) scores were computed by summing the time spent in light, moderate and vigorous activity in MET-hours per week.[26] Depressive symptoms were assessed using the Centre for Epidemiology Depression Scale (CES-D) and were considered present with a score of ≥16.[27] Blood pressure was measured twice in sitting position with a random-zero sphygmomanometer and an average was computed. Total serum cholesterol, high-density lipoprotein (HDL) cholesterol, and serum glucose were measured in non-fasting participants using an automated enzymatic procedure. Hypertension was defined as a systolic blood pressure of ≥140 mmHg, and/or a diastolic blood pressure of ≥90, and/or antihypertensive medication use.[16] Diabetes was defined as fasting serum glucose of ≥7.0 mmol/L and/or use of antidiabetic medication.[16] Height and weight were measured at the research centre. Body mass index (BMI) was computed as weight divided by meters squared.[16] The MMSE is a validated screening tool and used to screen participants on their mental status.[28]

Statistical analyses

First, we present descriptive statistics for exposures, outcomes and covariates, for the whole study population and stratified by vitamin D categories. Subsequently, we used multivariable linear regression to examine associations of vitamin D status with brain tissue volumes (brain tissue volume, white matter volume, grey matter volume, and hippocampus volume), WMH volume (log-transformed), and global (standardized) measures of white matter integrity (FA and MD). Multivariable logistic regression was used to examine associations of vitamin D status with the presence of lacunes and microbleeds. In all analyses, vitamin D status was analysed both as a continuous variable and categorized

into deficient, insufficient, and sufficient vitamin D status, using sufficient as reference.[2] Assumptions of linearity and normality were checked using PP-plots and scatterplots of residuals, respectively. Assumption of no multicollinearity was checked using VIF values (<10) and homoscedasticity was checked by plotting standardized residuals against predicted residuals. We selected confounders based on theory or literature.[29] Model 1 was adjusted for age, age^2 (to account for non-linear age effects), sex, season of blood draw and intracranial volume (to account for inter-individual differences in head size). Model 2 was additionally adjusted for education, BMI, physical activity, smoking, alcohol consumption, prevalent diabetes mellitus, hypertension and hypercholesterolemia. We additionally adjusted for normal-appearing white matter volume in the analysis for vitamin D status with white matter microstructure. Multiple imputation (m = 10) with the expectation-maximization method was used for missing values of covariates (<3.16%), using data on exposure, outcome and other explanatory variables as predictors. As sensitivity analyses, we repeated our analyses excluding participants with a CES-D score ≥16 (N = 123), indicating presence of depressive symptoms.[30] Moreover, to exclude the possibility of reverse causation by cognitive status, we first repeated our analyses only in participants with a MMSE score of ≥ 29 (N = 1,373). Subsequently, we included participants with lower MMSE scores by two points at a time, down to a minimum score of 25. Level of statistical significance was set at 0.05 and two-tailed analyses were performed using IBM SPSS statistics version 24 (SPSS Inc., Armonk, NY, USA) and using RStudio; integrated development environment for R, version 3.5.1 (RStudio, Boston, MA).

RESULTS

Table 1 shows the baseline characteristics of the study participants. Mean age was 56.9 years (standard deviation (SD) 6.4), 55.4% was female. Mean 25(OH)D was 60.9 nmol/L (SD: 27.8) and blood drawing for vitamin D assessment was mainly performed in autumn (33.7 %). Of our study population, 12.4% was vitamin D deficient (25(OH)D<30 nmol/L), 25.1% had an insufficient vitamin D status (25(OH)D 30-50 nmol/L), and 62.4% of the population had a sufficient vitamin D status (25(OH)D ≥50 nmol/L). Generally, participants with lover levels of vitamin D had a higher BMI, were more often smokers, drank less alcohol, were less physically active and had a higher prevalence of hypertension (Table 1).

Table 2 shows the association of vitamin D with brain tissue volumes. Modelled per 10 nmol/L increment, we did not find associations between vitamin D concentration and brain tissue volumes, white matter integrity nor the presence of markers of CSVD. However, for categories of vitamin D status, we observed that participants with a deficient vitamin D status had smaller total brain tissue volume than those with a sufficient

vitamin D status (difference in mL brain volume as compared to sufficient vitamin D status: -5.02 [95% CI: -8.70, -1.35]). This association with total brain volume slightly attenuated after adjustment for confounders in model 2 (difference: -4.36 [95% CI: -8.07, -0.65]) (Table 2). Vitamin D deficiency as compared to a sufficient vitamin D status was associated with smaller white matter volumes (difference: -6.21 [95% CI: -10.21, -2.20] and difference: -5.67 [95% CI: -9.76, -1.59]; model 1 and 2 respectively), but not with grey matter volume (Table 2). We also observed that a vitamin D deficiency compared to a sufficient vitamin D status was associated with smaller hippocampus volume in both

Table 1. Population characteristics

Characteristics	Total sample (N = 2,716)	Vitamin D deficient (N = 338; 12.4%)	Vitamin D insufficient (N = 682; 25.1%)	Vitamin D sufficient (N = 1,696; 62.4%)
25(OH)D, nmol/L	60.9 ± 27.8	21.9 ± 4.9	39.3 ± 5.8	77.3 ± 21.3
Age, years	56.6 ± 6.4	56.3 ± 7.4	56.7 ± 6.7	56.6 ± 6.0
Age, range	45.5 – 87.8	45.6 – 87.8	45.5 – 87.1	45.8 – 84.1
Female, %	55.4	57.4	55.2	54.8
Education, %				
Primary	9.4	11.1	9.9	9.1
Lower	33.9	30.6	32.5	35.3
Intermediate	28.9	27.4	28.7	28.9
Higher	27.7	30.6	28.9	26.4
Body mass index, kg/m^2	27.5 ± 4.3	28.9 ± 5.2	28.2 ± 4.7	27.0 ± 3.9
Smoking, %				
Never	29.9	34.7	30.5	28.7
Ever	43.9	35.3	40.9	46.4
Current	26.3	30.0	28.6	24.9
Alcohol, grams/wk[a]	11.6 (2.6 – 23.4)	9.1 (1.6 – 21.8)	10.4 (1.8 – 22.6)	12.2 (3.4 – 24.2)
Physical activity, MET-h/wk[a]	50.6 (22.4 – 87.7)	43.0 (15.0 – 79.2)	46.0 (18.0 – 82.0)	54.3 (24.5 – 93.1)
Diabetes, %	9.0	14.6	12.3	6.6
Hypertension, %	47.2	51.3	50.5	44.9
Hypercholesterolemia, %	4.3	2.3	4.2	4.8
CES-D score[a]	3.0 (1.0 – 7.0)	4.0 (1.0 – 10.0)	3.0 (1.0 – 8.0)	3.0 (1.0 – 7.0)

Values are mean ± SD or median (interquartile range) when indicated ([a]) for continuous variables, and percentages for dichotomous variables. MET: metabolic equivalent of task. CES-D: Center for epidemiologic studies depression scale. CHD: coronary heart disease. 25(OH)D: 25-hydroxyvitamin D. nmol/L: nanomole/litre. IQR: interquartile range. Dates of season of blood draw: winter 21 December – 20 March; spring 21 March – 20 June; summer 21 June – 20 September; autumn 21 September – 20 December. Values are based on imputed data. Numbers missing per variable were 2 for BMI, 5 for smoking, 732 for alcohol in grams per week, 736 for MET/h per week, 8 for education, 26 for CHD, 22 for hypertension, 5 for total cholesterol, 7 for HDL cholesterol, 10 for systolic and for diastolic blood pressure and 261 for season of blood draw.

Table 2. The association between vitamin D status and brain volumetry

Vitamin D status		Brain tissue volume	Grey matter volume	White matter volume	Hippocampus volume
		Difference in mL (95% CI)	Difference in mL (95% CI)	Difference in mL (95% CI)	Difference in mL (95% CI)
25(OH)D per 10 nmol/L increment	Model 1	0.33 (-0.10, 0.75)	0.01 (-0.40, 0.42)	0.32 (-0.14, 0.79)	0.01 (-0.00, 0.02)
	Model 2	0.31 (-0.13, 0.75)	0.01 (-0.42, 0.43)	0.39 (-0.09, 0.88)	0.01 (-0.00, 0.02)
Vitamin D sufficient		*Reference*	*Reference*	*Reference*	*Reference*
Vitamin D insufficient	Model 1	-0.72 (-3.50, 2.06)	0.42 (-2.26, 3.09)	-1.14 (-4.17, 1.89)	-0.04 (-0.11, 0.03)
	Model 2	-0.26 (-3.06, 2.53)	0.53 (-2.19, 3.24)	-0.79 (-3.86, 2.29)	-0.02 (-0.09, 0.05)
Vitamin D deficient	Model 1	**-5.02 (-8.70, -1.35)**	1.18 (-2.35, 4.72)	**-6.21(-10.21, -2.20)**	**-0.12 (-0.21, -0.03)**
	Model 2	**-4.36 (-8.07, -0.65)**	1.31 (-2.29, 4.91)	**-5.67 (-9.76, -1.59)**	**-0.10 (-0.19, -0.01)**

Difference in mL represents the difference in brain volume per 10 nmol/L increase in vitamin D concentration or the difference in brain volume for vitamin D insufficient or deficient status as compared to sufficient vitamin D status. mL: milliliter. CI: confidence interval. 25(OH)D: 25-hydroxyvitamin D. nmol/L: nanomole/liter. Vitamin D sufficient: 25(OH)D ≥ 50nmol/L. Vitamin D insufficient: 25(OH)D 30-50 nmol/L. Vitamin D deficient: (25(OH)D < 30 nmol/L. Model 1 is adjusted for age, age^2, sex, season of blood draw and intracranial volume. Model 2 is additionally adjusted for education, BMI, smoking, alcohol consumption, physical activity, prevalent diabetes, hypertension, and hypercholesterolemia. Statistically significant values (p<0.05) are indicated in **bold.**

models (difference model 1: -0.12 [95% CI: -0.21, -0.03]; difference model 2: -0.10 [95% CI: -0.19, -0.01]) (Table 2). No differences were observed for insufficient as compared to sufficient vitamin D status. Also, 25(OH)D concentration or status was not associated with focal MRI-markers such as WMH volume, lacunes and microbleeds (Table 3) or with global measures of white matter integrity (FA and MD) (Table 4).

No significant interaction effects were found for gender, depressive symptoms or season (p >0.05). In the sensitivity analysis excluding all participants who scored ≥16 on the CES-D (depressive symptoms present) we observed similar results as reported in the whole study population (Supplementary table 1). There was no indication of reverse causality by cognitive status as results between vitamin D status and brain tissue volumes on MMSE scores were comparable to results in the entire population (Supplementary tables 2-4).

Table 3. The association between vitamin D status and markers of CSVD

Vitamin D status		White matter lesion volume*	Lacunes	Microbleeds
		Difference (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
25(OH)D per 10 nmol/L increment	Model 1	-0.01 (-0.02, 0.01)	0.96 (0.89, 1.04)	0.98 (0.94, 1.03)
	Model 2	0.00 (-0.01, 0.01)	0.98 (0.90, 1.06)	0.98 (0.94, 1.03)
Vitamin D sufficient *(25(OH)D ≥ 50nmol/L)*		*Reference*	*Reference*	*Reference*
Vitamin D insufficient *(25(OH)D 30-50 nmol/L)*	Model 1	0.06 (-0.01, 0.12)	0.99 (0.59, 1.66)	1.04 (0.77, 1.41)
	Model 2	0.03 (-0.03, 0.10)	0.93 (0.55, 1.57)	1.04 (0.76, 1.41)
Vitamin D deficient *(25(OH)D < 30 nmol/L)*	Model 1	0.05 (-0.04, 0.13)	1.29 (0.69, 2.41)	1.13 (0.77, 1.68)
	Model 2	0.02 (-0.07, 0.10)	1.15 (0.60, 2.20)	1.13 (0.76, 1.68)

* log-transformed. Difference represents the difference in the log of white matter lesion volume per 10 nmol/L increase in vitamin D concentration or the difference in the log of white matter lesion volume for vitamin D insufficient or deficient status as compared to sufficient vitamin D status. Odds ratio represents the odds of having lacunes or microbleeds per 10 nmol/L increase in vitamin D concentration or the odds of having lacunes or microbleeds for vitamin D insufficient and deficient status as compared to sufficient vitamin D status. CSVD: cerebral small vessel disease. CI: confidence interval. 25(OH)D: 25-hydroxyvitamin D. nmol/L: nanomole/liter. Model 1 is adjusted for age, age^2, sex, season of blood draw and intracranial volume. Model 2 is additionally adjusted for education, BMI, smoking, alcohol consumption, physical activity, prevalent diabetes, hypertension and hypercholesterolemia. Statistically significant values ($p<0.05$) are indicated in **bold.**

Table 4. The association between vitamin D status and white matter microstructure

Vitamin D status		Fractional anisotropy	Mean diffusivity
		Difference in SD (95% CI)	Difference in SD (95% CI)
25(OH)D per 10 nmol/L increment	Model 1	0.00 (-0.01, 0.02)	-0.00 (-0.02, 0.01)
	Model 2	0.00 (-0.01, 0.02)	-0.00 (-0.02, 0.01)
Vitamin D sufficient *(25(OH)D ≥ 50nmol/L)*		*Reference*	*Reference*
Vitamin D insufficient *(25(OH)D 30-50 nmol/L)*	Model 1	-0.07 (-0.16, 0.02)	0.03 (-0.05, 0.11)
	Model 2	-0.07 (-0.15, 0.02)	0.02 (-0.06, 0.10)
Vitamin D deficient *(25(OH)D < 30 nmol/L)*	Model 1	-0.03 (-0.14, 0.09)	0.01 (-0.09, 0.11)
	Model 2	-0.02 (-0.14, 0.10)	-0.01 (-0.12, 0.09)

Difference in SD represents the difference in standard deviation fractional anisotropy or mean diffusivity per 10 nmol/L increase or the difference in standard deviation fractional anisotorpy or mean difusitivy for vitamin D insufficient or deficient status as compared to sufficient vitamin D status. SD: standard deviation. CI: confidence interval. 25(OH)D: 25-hydroxyvitamin D. nmol/L: nanomole/liter. Model 1 is adjusted for age, age^2, sex, season of blood draw, intracranial volume and normal-appearing white matter volume. Model 2 is additionally adjusted for education, BMI, smoking, alcohol consumption, physical activity, prevalent diabetes, hypertension, and hypercholesterolemia. Statistically significant values ($p<0.05$) are indicated in **bold.**

DISCUSSION

In this large population-based study of Dutch middle-aged and older adults free from dementia we observed smaller brain tissue volume, smaller white matter volume and smaller hippocampus volume in persons with vitamin D deficiency as compared to persons with a sufficient vitamin D status. Vitamin D status was not associated with grey matter volume, white matter integrity, or with markers of CSVD.

Strengths of our study include the population-based setting, the large sample size and the standardized quantitative assessment of brain tissue volume, white matter integrity and markers of CSVD. However, some limitations should also be acknowledged. First, due to the cross-sectional design of this study, no causation or temporal direction of the association can be established. Second, although we adjusted for a wide range of covariates, residual confounding might be present from unmeasured or incompletely measured confounders, such as an overall healthier lifestyle resulting in a higher vitamin D status. Third, the Rotterdam Study population incorporated an almost exclusively Caucasian population, limiting generalizability towards other populations.

In our study we found an association of vitamin D status with total brain tissue and white matter volume but not with grey matter volume. These results are not only statistically significant, but based on the observed effect sizes may also be clinically relevant. In another population-based sample it was found that one year of ageing was equivalent to a 5.40 mL smaller brain tissue volume and a 2.30 mL smaller white matter volume.[31] Thus, the averagely 4.36 mL smaller brain tissue volume and 5.67 mL smaller white matter volume that we observed in those with vitamin D deficiency as compared to those with sufficient levels may indicate significant accelerated neurodegeneration on top of normal age-related changes. Previous studies on brain health in relation to vitamin D were inconsistent and have shown associations of vitamin D status with larger or even smaller total brain tissue, grey matter, and/or white matter volume.[5, 12, 32, 33] However, most of these studies did not exclude participants with cognitive impairment or dementia, thereby introducing the possibility to measure a reverse effect of cognitive decline on neurodegeneration rather than the effects of vitamin D status. We performed additional sensitivity analyses in our sample to rule out the reverse causality caused by preclinical cognitive impairment as an explanation for our results. In line with our results, another large prospective community-based study, the Framingham Heart Study, consisting of 1,139 participants (mean age 59.3 years), also found an association of vitamin D deficiency with lower total brain tissue volume.[33] However, further replication is needed, preferable from studies that have repeated measurements of both vitamin D status and brain measurements to infer on temporality.

Interestingly, we also observed an association of vitamin D deficiency with smaller volumes of the hippocampus compared to those with sufficient vitamin D levels. This

was also observed in three previous population-based studies.[14, 32, 33] Unfortunately only one of these three previous studies took cognitive status into account.[14, 32, 33] The hippocampus plays a critical role in cognition, especially in declarative memory and is one of the first parts in the brain to be affected in the case of Alzheimer's disease.[34, 35] Although total evidence remains sparse, the results from our study and these previous studies combined suggest a promising potential role of vitamin D for brain health.[5, 32, 33] And in light of above findings one could hypothesize that a sufficient vitamin D status promotes brain health directly through larger brain tissue volume and possibly even through larger tissue volume of the hippocampus. When interpreting these results, it is of importance to take into account that a higher BMI is related to vitamin D deficiency and that a high BMI has been reported to increase the risk of dementia. In our population we saw that participants with vitamin D deficiency have a higher BMI than participants with sufficient or insufficient vitamin D levels. We adjusted for BMI in our models, but because of the cross-sectional nature of our study could not further explore the role that BMI and changes in BMI may have in these associations. Longitudinal studies are warranted to study effects of overweight and obesity in the association between vitamin D and brain health.

A potential direct effect of vitamin D and brain health may go through neuronal health. Indeed, it has been found that higher levels of vitamin D support neuronal growth, maintenance and survival by the up-regulation of neurothrophins such as neurotrophin-3 (NT-3), glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF).[1, 36-38] Lower concentrations of BDNF have been found to be associated with reduced hippocampus volume.[39] Vitamin D can also modulate neurogenesis in the hippocampus and is neuroprotective as it activates the downregulation of L-type-voltage sensitive calcium channel (LVCC), which causes exitotoxic cell death in the hippocampus and upregulating vitamin D receptors.[1, 39, 40] Moreover, vitamin D deficiency may result in a lack of protective effects related to enhancement of amyloid-beta peptide clearance across the blood brain barrier.[40] Unfortunately, we do not have these kind of measures in the Rotterdam Study and are therefore unable to replicate those potential associations.

Contrary to the few other studies on these outcomes, we did not find an association between vitamin D status and white matter integrity or markers of CSVD.[10, 11, 14] One study among participants with mild cognitive impairment (N = 54) found an association between vitamin D deficiency and disruptions of neural white matter integrity, primarily in the frontal regions of the brain.[14] Regrettably, this study did not adjust profoundly for possible confounding factors. As such, significant results may still be (partly) confounded, increasing the risk of an overestimation of the true effect. In our study, we only used global measures of white matter integrity, which might limit sensitivity for possible associations between vitamin D and white matter integrity in specific regions

of the brain. For example, in light of our results it could be hypothesized that vitamin D deficiency is associated with degeneration in white matter fibre bundles connecting parts of the brain which are important for cognition. Moreover, other studies found an inverse association between vitamin D concentration and WMH volume,[14] as well as an inverse association between vitamin D concentration and the number of lacunes and microbleeds.[10, 11] But those latter two studies included participants with acute ischemic stroke or transient ischemic attack, therefore possibly introducing selection bias as those participants may already be further along the neurodegenerative-pathway due to these cerebrovascular events then participants without a history of cerebrovascular events.[10]

In conclusion, our study shows that vitamin D deficiency is associated with smaller brain tissue volume, smaller white matter volume and smaller hippocampus volume. These results suggest that an adequate vitamin D status is important for structural brain health, and probably most importantly, for hippocampus volume. This association with structural brain health may partly explain previously reported associations between vitamin D, cognitive decline and the risk of dementia. Although more research is needed to replicate these findings, our results support the importance of achieving a sufficient vitamin D status, for example by increasing vitamin D intake, supplementation, or through sufficient sun exposure.

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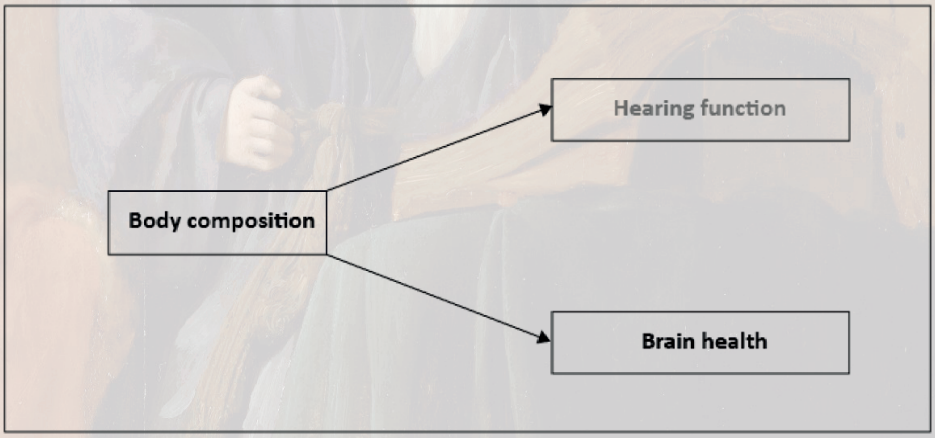
Hearing function
Body composition
Brain health

3.3

Body composition is not related to structural or vascular brain changes

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ABSTRACT

Background

It is known that obesity [measured with body mass index (BMI)] relates to brain structure and markers of cerebral small vessel disease (CSVD). However, BMI may not adequately represent body composition. Furthermore, whether those cross-sectional associations hold longitudinally remains uncertain.

Methods

Three thousand six hundred and forty-eight participants underwent baseline (2006-2014) dual-energy X-ray absorptiometry (DXA)-scan to obtain detailed measures of body composition and a magnetic resonance imaging (MRI) scan to assess brain structure. One thousand eight hundred and forty-four participants underwent a second MRI-scan at follow-up (2010-2017; median follow-up: 5.5 years). To assess cross-sectional and longitudinal associations (measures of change have been calculated) between body composition [BMI, fat mass index (FMI), fat-free mass index (FFMI)], and brain tissue volume (grey matter, white matter, hippocampus), white matter microstructure [fractional anisotropy (FA), mean diffusivity (MD)], and CSVD markers (white matter hyperintensity volume, lacunes, microbleeds) we used multivariable linear and logistic regression models.

Results

A higher BMI and FMI were cross-sectionally associated with smaller white matter volumes, (difference in Z-score per SD higher BMI: -0.064 [95% CI: -0.094, -0.035]) and FMI: -0.067 [95% CI: -0.099, -0.034], higher FA and MD. A higher FFMI was associated larger grey matter volume (difference: 0.060 [95% CI: 0.018, 0.101]). There was no statistically significant or clinically relevant association between body composition and brain changes.

Conclusions

Body composition, distinguishing between fat mass and fat-free mass, does not directly influence changes in brain tissue volume, white matter integrity and markers of CSVD. Cross-sectional associations between body composition and brain tissue volume likely reflect cumulative risk or shared etiology.

INTRODUCTION

The increasing prevalence of obesity is accompanied by numerous adverse health effects, including cognitive decline and dementia.[1, 2] Moreover, obesity has been linked with smaller brain tissue volumes, and decreased white matter integrity.[1, 3-5] On top of that, it has been found that obesity is associated with focal brain pathology in the form of cerebral small vessel disease (CSVD).[6]

An important limitation of previous studies is that obesity is generally assessed by body mass index (BMI).[1] However, ageing is associated with a decrease in lean mass and an increase in fat mass, making BMI less suitable as an approximation of obesity in the elderly.[1, 7] In a study looking at the association between body composition and metabolic syndrome the same "BMI problem" was encountered. Researchers found that fat mass index (FMI) was independently and positively associated with the presence of metabolic syndrome regardless of BMI and concluded that FMI is a better approximation of body composition.[8] A second limitation is that previous studies mainly assessed cross-sectional relations, precluding inference on directionality.

We studied the association of body composition, divided into body mass, fat mass and fat-free mass, with brain tissue volume, white matter integrity and markers of CSVD (white matter lesion hyperintensities (WMH), lacunes, and microbleeds) to evaluate whether fat mass and fat-free mass is a better approximation of body composition. Moreover, we aimed to assess the longitudinal association between body composition and brain health.

MATERIALS AND METHODS

Setting and study population

This study was embedded within the population-based Rotterdam Study. At study entry and subsequently every 3-4 years, all participants were invited to undergo extensive examinations. For the present study, 4,104 participants (2006-2014) underwent body composition assessment with a dual-energy X-ray absorptiometry (DXA) scan and a brain magnetic resonance imaging (MRI) scan[9]. From this group we excluded participants with cortical infarcts on MRI (*N = 164*), cancer (*N = 263*) and dementia (*N = 29*), leaving 3,648 participants for the cross-sectional analyses. Between 2010 and 2017 in a subsequent examination-round, 1,844 participants had a follow-up MRI-scan available for the longitudinal analysis. Reasons of dropout at follow-up or unavailability of follow up MRI information have been included in Supplemental figure 1.

The Rotterdam Study has received medical ethical approval according to the Population Screening Act: Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants provided written informed consent.

Body composition

Body weight and length were measured and BMI was calculated (kg/m^2).[9] A DXA – scan (iDXA, GE Lunar Healthcare, USA) was performed to determine fat-free mass and fat mass in kilograms.[9] From this, we calculated fat mass index (FMI) (kg/m^2), and fat-free mass index (FFMI) (kg/m^2).

Brain tissue volume, white matter integrity, and markers of CSVD

Brain-MRI was performed on a 1.5-tesla MRI scanner with a 8-channel head coil, using a standardized scan protocol which has been described in detail before.[9, 10] To quantify brain tissue volume, grey matter volume, white matter volume, WMH volume and intracranial volume, automated brain tissue classification was used. This quantification strategy was based on a *k*-nearest neighbour classifier algorithm, extended with an in-house-developed WMH segmentation.[10] Total brain volume did not include the cerebellum (used MRI segmentation only segments supratentorial brain tissue and not all MRI scans incorporated the entire cerebellum in the field of view). T1-weighted MR images were processed using FreeSurfer (version 5.1) to obtain the hippocampus volume.[9] White matter microstructure [fractional anisotropy (FA), mean diffusivity (MD)] was assessed with diffusion weighted imageing.[9, 11] Visual ratings were performed for presence of lacunes or microbleeds.[9]

Covariables

Information on energy intake, smoking, alcohol, education and physical activity was obtained from questionnaires and interviews as described in detail in the supplement.[9]

Statistics

We investigated associations of body composition with brain tissue volumes, WMH volume (log-transformed), and FA and MD as a ratio of intracranial volume (to adjust for absolute head size differences) using linear regression models. We calculated changes by subtracting baseline measurement from follow-up measurement, which were eventually standardized and used to assess the longitudinal association with linear regression models. The association of body composition with presence of lacunes and microbleeds at baseline and the progression of lacunes or microbleeds at follow-up for the longitudinal analysis was assessed with logistic regression models. Adjustments were made for age, age^2, sex, and education (model 1). We additionally adjusted for energy intake, smoking, physical activity, and alcohol (model 2). Adding height, hyper-

Table 1. Population characteristics

Characteristics	Population for cross-sectional analysis	Population for longitudinal analysis
	Baseline N = 3,648	Baseline N = 1,844
Age, years	65.9 (11.1)	60.9 (9.9)
Age range, years	45.7 – 97.8	44.1 – 90.2
Female, %	57.3	55.9
Education level, %		
Primary	8.1	7.1
Lower	38.5	35.4
Middle	30.1	29.3
Higher	22.4	27.9
Hypertension, %	44.1	34.3
Hypercholesterolemia, %	50.1	46.9
Diabetes, %	8.4	7.4
Smoking, %		
Never	33.0	33.3
Former	52.1	49.7
Current	14.4	16.4
Physical activity, MET-hours per week	44.7 (18.0-83.8)[a]	46.3 (19.6 – 83.8)[a]
Energy intake, kcal/day	2,090.5 (1,689.6 - 2,571.6)[a]	2,2567.0 (1,777.8 – 2,647.2)[a]
Alcohol intake, g/day	7.3 (1.1 - 17.6)[a]	8.5 (1.7 – 20.0)[a]
Body composition		
Length, cm	168.4 (9.4)	169.5 (9.4)
Weight, kg	77.4 (13.6)	77.9 (13.6)
Body mass index, kg/m^2	27.2 (3.9)	27.0 (3.8)
Fat mass index, kg/m^2	9.6 (3.3)	9.3 (3.3)
Fat-free mass index, kg/m^2	17.6 (2.1)	17.4 (2.2)
Brain MRI volumetry		
Brain tissue volume, mL	930.2 (103.3)	949.4 (101.8)
Grey matter volume, mL	528.3 (60.8)	536.2 (61.9)
White matter volume, mL	401.8 (64.9)	413.2 (62.9)
Fractional anisotropy	0.3 (0.0)	0.3 (0.0)
Mean diffusivity	0.7 (0.0)	0.7 (0.0)
Markers of cerebral small vessel disease		
Lacunes, presence %	7.2	4.7
Microbleeds, presence %	19.7	16.1
White hyperintensity volume	8.2 (1.1)*	7.9 (1.0)*

MET: metabolic equivalent of task. kcal: kilocalories. g: gram. cm: centimetre. kg: kilogram. m: meter. mL: millilitre. Values are mean (standard deviation) for continuous variables or median (interquartile range) when indicated ([a]), percentages for dichotomous variables. *; ln-transformed.

Table 2. The associations between body composition and brain tissue volume and CSVD markers – model 1

	Brain tissue volume	Grey matter volume	White matter volume	Hippocampus volume	Fractional anisotropy	Mean diffusivity	White matter hyperintensity volume*	Lacunes	Microbleeds
	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Odds ratio (95% CI) *p-value*	Odds ratio (95% CI) *p-value*
Cross-sectional									
Body mass index (kg/m^2)	-0.025 (-0.050, 0.001) *0.056*	0.042 (0.010, 0.073) *0.009*	-0.064 (-0.094, -0.035) *0.000*	-0.022 (-0.050, 0.006) *0.128*	0.061 (0.031, 0.091) *0.000*	0.045 (0.018, 0.072) *0.001*	0.008 (-0.018, 0.034) *0.565*	1.075 (0.944, 1.223) *0.276*	0.931 (0.854, 1.015) *0.103*
Fat mass index (kg/m^2)	-0.033 (-0.061, -0.005) *0.020*	0.034 (-0.001, 0.068) *0.055*	-0.067 (-0.099, -0.034) *0.000*	-0.019 (-0.015, 0.012) *0.224*	0.098 (0.065, 0.130) *0.000*	0.075 (0.045, 0.104) *0.000*	0.000 (-0.028, 0.029) *0.982*	1.080 (0.933, 1.250) *0.301*	0.945 (0.859, 1.040) *0.250*
Fat-free mass index (kg/m^2)	-0.007 (-0.040, 0.027) *0.697*	0.060 (0.018, 0.101) *0.005*	-0.059 (-0.098, -0.021) *0.003*	-0.028 (-0.065, 0.0094) *0.137*	-0.022 (-0.062, 0.017) *0.268*	-0.021 (-0.056, 0.014) *0.246*	0.024 (-0.010, 0.058) *0.171*	1.070 (0.905, 1.264) *0.430*	0.899 (0.804, 1.005) *0.061*
Longitudinal									
Body mass index (kg/m^2)	0.004 (-0.029, 0.036) *0.810*	-0.006 (-0.048, 0.036) *0.789*	0.011 (-0.031, 0.054) *0.604*	-0.050 (-0.097, -0.004) *0.034*	0.029 (-0.031, 0.089) *0.339*	0.052 (-0.007, 0.111) *0.084*	-0.004 (-0.046, 0.038) *0.861*	1.003 (0.816, 1.232) *0.979*	0.978 (0.852, 1.123) *0.754*
Fat mass index (kg/m^2)	0.012 (-0.022, 0.047) *0.485*	0.004 (-0.040, 0.049) *0.846*	0.011 (-0.035, 0.056) *0.645*	-0.033 (-0.082, 0.017) *0.192*	0.048 (-0.014, 0.110) *0.130*	0.061 (0.000, 0.122) *0.050*	-0.009 (-0.054, 0.036) *0.686*	0.906 (0.732, 1.120) *0.362*	0.921 (0.800, 1.061) *0.257*
Fat-free mass index (kg/m^2)	-0.017 (-0.061, 0.027) *0.448*	-0.031 (-0.087, 0.026) *0.291*	0.012 (-0.046, 0.069) *0.684*	-0.089 (-0.151, -0.026) *0.006*	-0.026 (-0.108, 0.055) *0.527*	0.017 (-0.063, 0.098) *0.672*	0.010 (-0.047, 0.067) *0.726*	1.149 (0.946, 1.397) *0.162*	1.080 (0.945, 1.233) *0.259*

* In-transformed. CI: confidence interval. Cross-sectional differences represent the difference in standard deviation brain tissue volume (total brain tissue, grey matter, white matter, hippocampus volume, fractional anisotropy, mean diffusivity and white matter hyperintensity volume) as a ratio of intracranial volume per standard deviation higher body mass index, fat mass index, or fat-free mass index. Cross-sectional odds ratios represent the odds of the presence of lacunes and microbleeds per standard deviation higher body mass index, fat mass index, or fat-free mass index. Longitudinal differences represent the difference in standard deviation change in brain tissue volume (total brain tissue, grey matter, white matter, hippocampus volume, fractional anisotropy, mean diffusivity and white matter hyperintensity volume) as a ratio of intracranial volume per standard deviation higher body mass index, fat mass index, or fat-free mass index. Longitudinal odds ratios represent the odds of progressing from no presence of lacunes and microbleeds to presence or the odds of progressing to more lacunes or microbleeds at follow-up per standard deviation higher body mass index, fat mass index, or fat-free mass index. Effect estimates were considered statistically significant with a p-value < 0.05. Cross sectional models are adjusted for age, age^2, sex, and education. Longitudinal models were additionally adjusted for time between MRI scans.

Table 3. The associations between body composition and brain tissue volume and CSVD markers – model 2

	Brain tissue volume	Grey matter volume	White matter volume	Hippocampus volume	Fractional anisotropy	Mean diffusivity	White matter hyperintensity volume*	Lacunes	Microbleeds
	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Difference in Z-score (95% CI) *p-value*	Odds ratio (95% CI) *p-value*	Odds ratio (95% CI) *p-value*
Cross-sectional									
Body mass index (kg/m^2)	-0.028 (-0.060, 0.003) *0.075*	0.039 (0.001, 0.076) *0.043*	-0.066 (-0.101, -0.030) *0.000*	-0.016 (-0.049, 0.018) *0.357*	0.078 (0.043, 0.112) *0.000*	0.060 (0.029, 0.091) *0.000*	0.013 (-0.018, 0.043) *0.409*	1.134 (0.980, 1.131) *0.091*	0.918 (0.830, 1.015) *0.095*
Fat mass index (kg/m^2)	-0.035 (-0.070, -0.001) *0.045*	0.033 (-0.008, 0.075) *0.113*	-0.069 (-0.108, -0.030) *0.001*	-0.008 (-0.044, 0.029) *0.685*	0.114 (0.076, 0.152) *0.000*	0.092 (0.058, 0.127) *0.000*	0.006 (-0.028, 0.039) *0.728*	1.137 (0.962, 1.344) *0.131*	0.927 (0.829, 1.038) *0.188*
Fat-free mass index (kg/m^2)	-0.014 (-0.056, 0.027) *0.498*	0.051 (0.002, 0.101) *0.041*	-0.061 (-0.107, -0.014) *0.010*	-0.034 (-0.077, 0.010) *0.130*	-0.002 (-0.047, 0.044) *0.944*	-0.010 (-0.051, 0.032) *0.646*	0.028 (-0.012, 0.068) *0.165*	1.149 (0.948, 1.392) *0.156*	0.895 (0.786, 1.018) *0.092*
Longitudinal									
Body mass index (kg/m^2)	0.029 (-0.012, 0.069) *0.165*	0.008 (-0.041, 0.057) *0.756*	0.028 (-0.021, 0.077) *0.270*	-0.041 (-0.098, 0.016) *0.163*	0.004 (-0.070, 0.078) *0.918*	0.025 (-0.043, 0.092) *0.475*	0.005 (-0.045, 0.055) *0.840*	0.943 (0.725, 1.227) *0.662*	0.979 (0.831, 1.154) *0.804*
Fat mass index (kg/m^2)	0.039 (-0.005, 0.083) *0.080*	0.005 (-0.011, 0.021) *0.567*	0.032 (-0.021, 0.085) *0.233*	-0.019 (-0.080, 0.042) *0.546*	0.014 (-0.063, 0.091) *0.728*	0.021 (-0.049, 0.091) *0.557*	-0.004 (-0.057, 0.0105) *0.897*	0.835 (0.632, 1.104) *0.206*	0.948 (0.801, 1.122) *0.534*
Fat-free mass index (kg/m^2)	0.003 (-0.052, 0.058) *0.920*	-0.011 (-0.078, 0.055) *0.741*	0.016 (-0.051, 0.083) *0.643*	-0.093 (-0.171, -0.015) *0.019*	-0.023 (-0.126, 0.079) *0.655*	0.030 (-0.063, 0.124) *0.521*	0.027 (-0.041, 0.095) *0.444*	1.160 (0.896, 1.501) *0.260*	1.041 (0.885, 1.225) *0.629*

* ln-transformed. CI: confidence interval. kg: kilogram. m: meter. Cross-sectional differences represent the difference in standard deviation brain tissue volume (total brain tissue, grey matter, white matter, and hippocampus volume) and the difference in standard deviation fractional anisotropy, mean diffusivity and white matter hyperintensity volume as a ratio of intracranial volume per standard deviation higher body mass index, fat mass index, or fat-free mass index. Cross-sectional odds ratios represent the odds of the presence of lacunes and microbleeds per standard deviation higher body mass index, fat mass index, or fat-free mass index. Longitudinal differences represent the difference in standard deviation change in brain tissue volume (total brain tissue, grey matter, white matter, and hippocampus volume) and the difference in standard deviation fractional anisotropy, mean diffusivity and white matter hyperintensity volume as a ratio of intracranial volume per standard deviation higher body mass index, fat mass index, or fat-free mass index. Longitudinal odds ratios represent the odds of progressing from no presence of lacunes and microbleeds to presence or the odds of progressing to more lacunes or microbleeds at follow-up per standard deviation higher body mass index, fat mass index, or fat-free mass index. Effect estimates were considered statistically significant with a p-value < 0.05. Cross sectional models are adjusted for age, age^2, sex, education, energy intake, smoking, physical activity, and alcohol consumption. Longitudinal models were additionally adjusted for time between MRI scans.

tension, hypercholesterolemia, and diabetes to the regression models did not change the effect estimates and were therefore left out of the final analysis. For the longitudinal analysis we additionally adjusted for time between MRI-scans. We ran the same analyses, with brain tissue volumes, WMH volume, and FA and MD unstandardized (while adjusting for intracranial volume in the regression model) to evaluate clinical relevance of the associations. We compared our results with previously reported brain tissue changes (independent from inter-individual differences in head size) ranging from 3.6 millilitres to 5.4 millilitres with one year of ageing.[12, 13] We checked for interaction between body composition and sex and between body composition and age (< 60 versus ≥ 60 years) as effects of BMI might differ between mid- and late-life.[7] We were powered (alpha level: 0.05; power level: 0.80) to detect minimal differences of 2.028 millilitre brain tissue volume. A p-value < 0.05 was considered statistically significant. IBM SPSS statistics version 24.0 (IBM Corp, Armonk, NY, USA) was used for analyses.

RESULTS

Baseline characteristics are described in Table 1. Mean age was 65.9 years (standard deviation (SD): 9.8), 57.3% was female. Mean BMI was 27.2 kg/m^2 (SD: 3.9). Participants with a follow-up MRI scan compared to those with only baseline MRI were younger, higher educated, and healthier (Supplementary table 1).

We found that cross-sectionally a higher BMI and FMI were related with smaller white matter volumes (difference in Z-score per SD higher BMI: -0.064 [95% CI: -0.094, -0.035]; FMI: -0.067 [95% CI: -0.099, -0.034]), higher FA (BMI difference: 0.061 [95% CI: 0.031, 0.091]; FMI difference: 0.098 [95% CI: 0.065, 0.130]) and higher MD (BMI difference: 0.045 [95% CI: 0.018, 0.072]; FMI difference: 0.075 [95% CI: 0.045, 0.104]), and a higher FFMI was related with larger grey matter volumes (difference: 0.060 [95% CI: 0.018, 0.101]) (Table 2, model 1). Effect estimates did not change between the different models (Table 3, model 2), nor were there differences in effects of body composition indices between the various outcomes of interest. Though statistically significant, cross-sectional results might not be clinically relevant as effect estimates of change in brain tissue volumes, as well as their corresponding confidence intervals, reported in millilitre difference per one point increase in BMI, FMI, or FFMI are small and do not exceed clinically relevant amounts of tissue changes (Supplementary tables 2 and 3).

Only the longitudinal association between FFMI and change in hippocampus volume was statistically significant (difference: -0.089 [95% CI: -0.151, -0.026]) (Table 2; model 1), and did not change across models. However, it is questionable whether the direction of this association is biological plausible, and when reporting this association in millilitre (Supplementary table 3; model 2), it is borderline statistically significant (difference in

millilitre per one point increase in FFMI: -0.027 [95% CI: -0.055, 0.000]). We did not find any statistically significant or clinically relevant longitudinal associations between body composition, brain tissue volumes, white matter integrity, or markers of CSVD (Tables 2 and 3; Supplementary tables 2 and 3). Both cross-sectional and longitudinal results did not differ between midlife and late life (data not shown), or between males and females (Supplementary table 4).

DISCUSSION

We found that detailed measures of body composition (higher BMI and higher FMI) related to smaller white matter volume and decreased white matter microstructure and that higher FFMI related to higher grey matter volumes. However, expressing those cross-sectional associations in millilitre difference, raises the question whether those are also clinically relevant. No statistically significant or clinically relevant associations were found longitudinally (median follow-up: 5.5 years).

Strengths of this study are the large sample size, the distinction of body composition, the wide range of brain imaging markers and the longitudinal design. However, some limitations should also be acknowledged. First, due to the observational design we cannot exclude the possibility of residual confounding. Second, we did not adjust for possible changes in body composition over time in the longitudinal analysis.

Previous studies showed that body composition relates to brain structure,[1, 2] which is replicated in some of our cross-sectional results. Currently there are no established thresholds for rates of brain atrophy. However, in two other population-based studies, change in brain tissue volume with one year of ageing were found to range from 3.6 millilitres to 5.4 milliliters.[3, 4] Against this background, it is questionable whether a (cross-sectional) 0.496 millilitre smaller brain volume per one-point increase in BMI (independent of age) and other (longitudinal) effect estimates, are also clinically relevant. The absence of a longitudinal association did not result from insufficient power, as we were able to minimally detect a change of 2.028 millilitre brain tissue volume, which is smaller than a clinically relevant change of 5 millilitre brain volume.[3] Though, the absence of a longitudinal association might result in part from selection bias. Indeed, it appeared that participants with MRI at follow-up compared to those without follow-up were younger, higher educated and healthier. Another explanation might be that the effects of body composition in midlife and late life may be reversed.[5, 6] But, we could not confirm effect modification by age in our study. Other longitudinal studies are inconclusive in their results, both reporting non-significant associations, and significant associations between body composition and various brain measurements,[6-10] probably due to different methodologies. In a study investigating whether the effect of obesity

on the brain increased with age they found no significant results and thus suggested that obesity may act as a modifier of brain atrophy rather than it being a direct cause.[11] Cardiovascular risk factors (hypertension, glucose, cholesterol, plasma triglyceride) are known to co-occur frequently with obesity and have independent negative effects on brain structure.[12-15] Therefore, although we did not find significant associations between body composition and markers of CSVD, it might be that the effects of vascular risk factors may be less pronounced in their effects on brain structure. And it might also be that solely body composition does not influence brain health, but it is rather the interplay of cardiovascular risk factors altogether that may impact brain health negatively.[16, 17]

Since BMI may not be a good approximation for body composition,[18] we used FMI and FFMI. Surprisingly, both cross-sectionally and longitudinally, regression estimates between the different body composition measurement (BMI, FMI and FFMI) and brain MRI data did not differ.

Based on our findings it seems that cross-sectional associations between body composition and brain health likely reflect cumulative risk or shared etiology. Moreover, it seems that body composition, decomposed into fat mass and fat-free mass, does not influence brain changes or the presence of CSVD. Therefore, we cannot conclude that FMI and FFMI is a better approximation of adiposity than BMI to study brain health. Other longitudinal studies are needed to replicate our findings.

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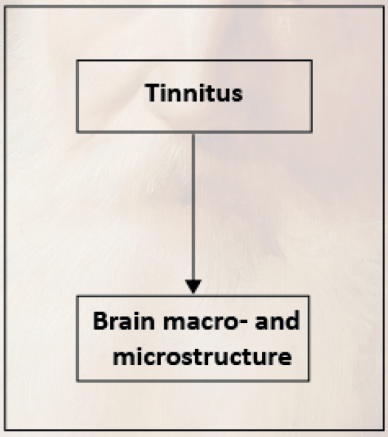
Tronie van een oude man; Jan Lievens[1]

4

Interrelations between hearing function and brain health


Tinnitus
Brain macro- and microstructure

4.1

Tinnitus and its central correlates: a neuro-imaging study in a large ageing population

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Under review

ABSTRACT

Objective

To elucidate the association between tinnitus and brain tissue volumes and white matter microstructural integrity.

Methods

2,616 participants (mean age 65.7 years [standard deviation: 7.5]; 53.9% female) of the population-based Rotterdam Study underwent tinnitus assessment (2011-2014) and magnetic resonance imaging (MRI) of the brain (2011-2014). Associations between tinnitus (present vs absent) and total, grey, and white matter volume and global white matter microstructure were assessed using multivariable linear regression models adjusting for demographic factors, cardiovascular risk factors, depressive symptoms, MMSE score and hearing loss. Finally, we assessed potential regional grey matter density and white matter microstructural differences on a voxel-based level again using multivariable linear regression.

Results

Participants with tinnitus (21.8%) had significantly larger brain tissue volumes (difference in standard deviation: 0.09 [95% CI: 0.06, 0.13]), driven by larger white matter volumes (difference: 0.12 [95% CI: 0.04, 0.21]) independent of hearing loss. There was no association between tinnitus and grey matter volumes nor with global white matter microstructure. On a lobar level, tinnitus was associated with larger white matter volumes in each lobe, not with grey matter volume. Voxel-based results did not show regional specificity.

Conclusion

We found that tinnitus in older adults is associated with larger brain tissue volumes, driven by larger white matter volumes, independent of age and hearing loss. Based on these results, it may be hypothesized that tinnitus has more of a neurodevelopmental origin potentially increasing the risk of developing future tinnitus in people with relatively larger brain tissue volumes from a young age onwards.

BACKGROUND

Tinnitus is a poorly understood and common disorder, often debilitating in the daily life of people with tinnitus.[2] The disorder can be characterized by the perception of a sound while there is no objective corresponding external sound source.[3, 4]
Hearing loss is suggested to be one of the most important risk factors for tinnitus: 90% of the people with chronic tinnitus have some form of hearing loss and the acoustic characteristics of the tinnitus sound correspond to the region of hearing loss.[2, 3, 5, 6] However, several observations indicate that tinnitus also has a central component to its pathogenesis, regardless of the peripheral damage that might trigger it.[7] Moreover, about 10% of individuals with tinnitus have normal hearing abilities.[2, 3] Recently, interest in the association between brain volume and brain function and tinnitus has increased. However, observed findings are often contradictory, some reporting regional cortical thickness reductions and functional alterations in individuals with tinnitus whereas other studies do not find significant associations.[4-13] These inconsistencies might be explained by high heterogeneity of individuals with tinnitus, differences in sample selection, imaging methodology and data analysis, and relatively small sample sizes. Moreover, previous studies mostly focused on auditory regions in the brain or the limbic system, disregarding potential whole brain associations.[3]

Therefore, the aim of this study was to assess the association between tinnitus and brain tissue volumes and white matter microstructure in a large population-based sample. Furthermore, we explored the association between tinnitus and the brain independent of hearing loss, to possibly disentangle peripheral versus central components contributing to prevalent tinnitus.

METHODS

Study setting and population

This cross-sectional study is embedded in the Rotterdam Study, a prospective, population-based study initiated in 1989 that investigates determinants and consequences of ageing.[14] The entire study population consists of 14,926 individuals aged ≥45 years from the Ommoord area, a suburb of Rotterdam, the Netherlands, who undergo extensive examinations at the research centre at study entry and subsequent visits every 3 to 4 years.

For this study, 4,773 participants who visited the study centre between 2011 and 2014 for initial or re-examinations underwent home interview on the presence or absence of tinnitus. Of the 4,151 participants with available tinnitus data, 2,661 participants also had MRI scanning of the brain (2011 – 2014). The median time interval between tinnitus

assessment and MRI scanning was 4.0 months (SD: 3.5). We excluded participants with cortical brain infarcts on MRI (N = 45), leaving a total of 2,616 participants for the current analysis.

The Rotterdam Study has been approved by the medical ethics committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). All participants provided written informed consent to participate in the study.

Tinnitus assessment

Tinnitus was assessed during a home interview. Participants were asked if they experience or recently have experienced sounds in the head or in the ears, without an objective external sound source being present. Possible answers were: no, never; yes, less than once a week; yes, more than once a week but not daily; yes, daily.[14] For the current study, tinnitus was investigated as a binary variable; not present (no, never; yes, less than once a week) or present (yes, more than once a week but not daily; yes, daily). Because of the heterogeneity of the origin, and often temporary character of tinnitus present less than once a week, this was not recorded as prevalent tinnitus.

Magnetic resonance imaging

Brain MRI was performed on a 1.5-tesla MRI scanner with a dedicated 8-channel head coil (software version 11x; General Electric Healthcare, Milwaukee, WI).[15] The entire scan protocol and sequence details have been described elsewhere.[15]

Brain tissue volumes

For brain tissue volumes, T1-weighted, proton density-weighted, and the fluid-attenuated inversion recovery scans were used for automated segmentation of supratentorial grey matter, white matter, cerebrospinal fluid (CSF), and white matter hyperintensities.[16] Total brain tissue volume was the sum of grey matter, normal-appearing white matter, and white matter hyperintensity volume. Supratentorial intracranial volume was estimated by summing grey matter and white matter (normal-appearing white matter and white matter hyperintensity volume) and CSF volumes.[15] A multi-atlas approach was used to obtain lobar brain volumes (frontal, parietal, temporal, occipital) from all participants.[17]

White matter microstructural integrity

To obtain microstructural measures, diffusion tensor imaging (DTI) was used. A single shot, diffusion weighted spin echo echo-planar imaging sequence was performed with maximum *b* value of 1,000 s/mm^2 in 25 noncollinear directions; 3 b_0 volumes were acquired without diffusion weighting. Using a standardized processing pipeline, diffusion data were preprocessed.[18] From this (in combination with the tissue segmentation),

we derived global mean fractional anisotropy (FA) and mean diffusivity (MD) in the normal-appearing white matter. FA is the degree of anisotropy in the normal-appearing white matter and is given as a ratio ranging from 0 (isotropic or non-directional) to 1 (unidirectional). MD is expressed in square millimetres per second.

Voxel based morphometry of white matter tracts

We performed a voxel-based analysis of diffusion tensor MRI data using FSL software for preprocessing.[19] FA and MD maps were nonlinearly registered to the standard FA template from the FSL package with a 1 x 1 x 1 mm^3 voxel resolution. In addition, a Rotterdam Study specific tract-atlas was created.[19] White matter tract segmentation masks of every participant were registered to Montreal Neurological Institute (MNI) template in the same way as FA and MD maps and then merged to one tract probability atlas image.[19] To map voxels from voxel-based analysis, a 10% probability cut-off was used to define tract boundaries microstructure.

Voxel based morphometry of grey matter density

Using an optimized protocol with FSL software, voxel-based analysis of the grey matter was performed.[19] Grey matter density maps derived from T1-weighted images were nonlinearly registered to the MNI template. A spatial modulation procedure was applied to preserver local grey matter volume, i.e. voxel densities were multiplied by the Jacobian determinants of transformation field. Subsequently, images were smoothed using an isotropic Gaussian kernel of 3 mm (full width half maximum 8 mm). The location of the voxels were defined based on Hammer atlas segmentation.[20]

Covariates

Educational level was categorized as lower, middle, or higher education. Height (meter) and weight (kilograms) were measured and body mass index (kg/m^2) was calculated. Systolic and diastolic blood pressure were measured twice using a random sphygmomanometer. Hypertension was defined as systolic blood pressure ≥ 160 mm Hg, diastolic blood pressure ≥ 90 mm Hg, and/or the use of blood pressure-lowering medication.[14] Using an automatic enzymatic procedure, serum total cholesterol and high-density lipoprotein cholesterol were measured from fasting blood samples. Hypercholesterolemia was defined as total cholesterol concentration ≥ 6.2 mmol/L and/or the use of lipid-lowering medication.[14] Self-reported smoking data were categorized into never, former, and current smoking. Alcohol consumption, in grams per day, was assessed through self-report by means of the Food-Frequency Questionnaire. The LASA Physical Activity Questionnaire was used to assess the amount of physical activity, recalculated into metabolic equivalent of task hours per week.[14] The MMSE was administered during home interview to assess global cognitive functioning.[14] To assess depressive symptoms,

the Center for Epidemiological Studies Depression scale was used. To determine hearing levels in decibel (dB), pure tone audiometry was used according to the ISO-standard 8253-1,[14] measured on different air conduction frequencies (0.25-8 kilohertz).

Statistical analysis

First, we investigated whether characteristics differed between participants with and without tinnitus, using T-tests, χ^2-tests and Mann-Whitney U-Tests when appropriate. Second, we explored the association of tinnitus with brain tissue volume (total, white matter, grey matter) and global white matter microstructural integrity (FA and MD) using multivariable linear regression models. In the first model we adjusted for age, sex, education, hearing loss, and intracranial volume (to adjust for intra-individual differences in head sizes). The second model was additionally adjusted for smoking, alcohol, physical activity, body mass index, hypertension, hypercholesterolemia, depressive symptoms and MMSE-score. Third, we performed a similar multivariable linear regression analysis investigating the association of tinnitus and lobar grey and white matter volume (frontal, temporal, parietal, occipital lobe) for the left and right hemisphere separately. Fourth, we performed the same multivariable linear models for the association between tinnitus and every voxel of the brain measures in the VBM analysis. It is important to note that, even though we expect that potential brain differences occur before tinnitus onset, we present our analyses and results with tinnitus as the determinant and brain measures as the outcome. Since the design of the current study is cross-sectional, results can be interpreted both ways, and we believe presenting results in the current order facilitates interpretation.

In sensitivity analyses, we explored whether results between tinnitus and brain volumes differed by degree of hearing loss (normal hearing: 0 – 20 dB; mild hearing loss: 20 – 40 dB; moderate/severe hearing loss: >40 dB). Next, to disentangle potential peripheral involvement, we used similar multivariable models in a sub-group of participants (N = 355) whom did not have a hearing threshold level above 20 dB on any of the measured hearing frequencies. Finally, we stratified by sex.

IBM SPSS statistics version 24.0 (IBM Corp, Armonk, NY, USA) was used for data handling and R statistical software version 3.5.1 was used for analyses. A p-value < 0.05 was considered statistically significant in the analyses between tinnitus, brain tissue volumes and white matter microstructure. For VBM, as the voxels throughout the brain are correlated, the actual number of independent tests was calculated using 10,000 permutations. The significant p-value threshold for $\alpha = 0.05$ was estimated separately for FA, MD and grey matter: 5.91×10^{-8} , 6.49×10^{-8} and 2.99×10^{-7} respectively.

RESULTS

Baseline characteristics are described in Table 1. Mean age was 65.7 years (standard deviation (SD): 7.5), 53.9% was female. Tinnitus was present in 21.8% of the study population (men: 51.8%; women: 48.2%, p-value: 0.002). Participants with tinnitus had a higher hearing threshold than those without tinnitus (28.8 dB (SD: 17.1); 22.5 dB (SD: 14.5) respectively, p-value: <0.001).

Table 1. Population characteristics

	Total sample (N = 2,616)	Participants with tinnitus (N = 570; 21.8%)	Participants without tinnitus (N = 2,046; 78.2%)	p-value
Age, years	65.7 (7.5)	65.7 (7.3)	65.8 (7.6)	0.789
Age, range	51.8-97.8	51.9-91.7	51.8-97.8	
Female, %	53.9	48.2	55.5	0.002
Hearing loss, dB	27.0 (15.3)	28.8	25.7	<0.001
Degree of hearing loss, %				<0.001
Normal: <20 dB	39.4	26.3	43.1	
Mild: 20-40 dB	46.3	51.1	45.0	
Moderate/severe: >40 dB	14.3	22.6	11.9	
Body mass index, kg/m^2	27.3 (4.0)	27.4 (4.0)	27.2 (4.0)	0.396
Education level, %				0.770
Primary	6.8	7.7	6.6	
Lower	35.4	35.6	35.6	
Middle	30.6	30.9	30.5	
Higher	26.5	26.5	26.5	
Smoking, %				0.003
Never	32.6	26.8	34.3	
Past	50.8	55.2	49.6	
Current	16.2	17.9	15.8	
Physical activity, MET[a]	46.5 (18.8, 85.3)	43.8 (18.9, 82.0)	46.9 (18.8, 85.8)	0.374
Alcohol, g/day[a]	8.0 (1.4, 19.0)	6.6 (1.1, 18.5)	8.3 (1.4, 19.1)	0.355
Hypertension, %	65.6	65.6	65.4	0.940
Hypercholesterolemia, %	51.9	54.4	51.3	0.127
MMSE <24, %	1.5	1.8	1.4	0.073
Depressive symptoms, %	8.6	10.5	8.1	0.487

Values are mean (standard deviation (SD)) for normally distributed continuous variables, median[a] (interquartile range) for non-normally distributed continuous variables. Values are percentages for dichotomous variables. dB: decibel. kg: kilogram. m: meter. MET: metabolic equivalent of task. g: gram. MMSE: Mini-Mental State Examination. Tinnitus was defined as a binary variable; either not present (no, never; yes, less than once a week) or present (yes, more than once a week but not daily; yes, daily). T-test were used for normally distributed variables, χ^2-test for dichotomous variables, and Mann-Whitney U-Test for non-normally distributed variables to see whether characteristics were significantly different ($p<0.05$) between participants with and without tinnitus.

Global brain tissue volumes and white matter microstructural integrity

We found that participants with tinnitus had statistically significantly larger brain tissue volumes (difference in SD brain tissue volume in participants with tinnitus as compared to participants without tinnitus: 0.07 [95% CI: 0.03, 0.10]) (model 1, Table 2), which was driven by larger white matter volume (difference: 0.12 [95% CI: 0.05, 0.19]) (model 1, Table 2). Additionally adjusting for other relevant confounders (model 2) did not change the effect estimates (difference total brain tissue volume: 0.09 [95% CI: 0.06, 0.13]; difference white matter volume: 0.12 [95% CI: 0.04, 0.21]) (Table 2). We did not find statistically significant associations between tinnitus and grey matter volume and white matter microstructural integrity (Table 2).

Table 2. The association between tinnitus and brain tissue volume and white matter microstructural integrity

		Total brain volume	Grey matter volume	White matter volume	Fractional anisotropy	Mean diffusivity
		Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)
Tinnitus; present versus absent	Model 1	**0.07 (0.03, 0.10)**	-0.02 (-0.08, 0.04)	**0.12 (0.05, 0.19)**	**0.00 (0.00, 0.00)**	0.00 (0.00, 0.00)
	Model 2	**0.09 (0.06, 0.13)**	0.02 (-0.05, 0.09)	**0.12 (0.04, 0.21)**	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)

Difference represents the difference in SD brain tissue volume (total, grey matter, white matter) or the difference in SD white matter microstructural integrity (fractional anisotropy, mean diffusivity) in participants with tinnitus as compared to participants without tinnitus. SD: standard deviation. CI: confidence interval. Model 1: adjusted for age, sex, education, hearing loss and intracranial volume. Model 2: additionally adjusted for smoking, alcohol intake, physical activity, body mass index, hypertension, hypercholesterolemia, depressive symptoms, and MMSE-score. Significant effect estimates ($p<0.05$) are indicated in **bold**.

Lobar brain tissue volumes

Associations for participants with tinnitus as compared to participants without tinnitus remained statistically significant on a lobar level (both left and right hemisphere) solely for larger white matter tissue volume across all the different lobes (frontal, temporal, parietal, and occipital) (Table 3; model 1 and 2). No statistically significant associations were found for grey matter volume on a lobar level (Table 3).

Voxel-based morphometry

We conducted exploratory voxel-based analysis to identify if tinnitus was associated with regional white matter integrity and grey matter density on a voxel level. The analyses showed that tinnitus was associated with higher FA as compared to participants without tinnitus in several white matter fibre bundles (Figure 1). However, these associations did not show regional specificity and were not statistically significant (Figure 1, Supplementary table 1). No statistically significant associations were found between tinnitus and voxel based white matter MD and grey matter density (Figure 1, Supplementary tables 2 and 3).

Table 3. The association between tinnitus and lobe specific tissue volumes

		Left frontal lobe	Right frontal lobe	Left temporal lobe	Right temporal lobe	Left parietal lobe	Right parietal lobe	Left occipital lobe	Right occipital lobe
		Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)
	Grey matter								
Tinnitus present versus absent	Model 1	-0.03 (-0.09, 0.04)	-0.03 (-0.10, 0.03)	0.01 (-0.04, 0.07)	0.01 (-0.05, 0.07)	-0.01 (-0.07, 0.06)	-0.02 (-0.08, 0.05)	0.01 (-0.06, 0.08)	-0.01 (-0.08, 0.05)
	Model 2	0.01 (-0.06, 0.08)	0.00 (-0.07, 0.07)	0.06 (-0.01, 0.13)	0.05 (-0.02, 0.12)	0.06 (-0.02, 0.14)	0.04 (-0.05, 0.12)	0.05 (-0.04, 0.13)	0.03 (-0.05, 0.11)
	White matter								
Tinnitus present versus absent	Model 1	**0.13 (0.06, 0.20)**	**0.13 (0.06, 0.20)**	**0.10 (0.03, 0.17)**	**0.12 (0.05, 0.19)**	**0.12 (0.04, 0.19)**	**0.13 (0.06, 0.20)**	**0.12 (0.04, 0.20)**	**0.11 (0.03, 0.19)**
	Model 2	**0.12 (0.03, 0.21)**	**0.13 (0.04, 0.21)**	**0.11 (0.02, 0.19)**	**0.13 (0.04, 0.22)**	**0.13 (0.04, 0.22)**	**0.14 (0.06, 0.23)**	**0.13 (0.03, 0.23)**	**0.12 (0.03, 0.22)**

Difference represents the difference in SD brain tissue volume in participants with tinnitus as compared to participants without tinnitus. SD: standard deviation. CI: confidence interval. Model 1: adjusted for age, sex, education, hearing loss and intracranial volume. Model 2: additionally adjusted for smoking, alcohol intake, physical activity, body mass index, hypertension, hypercholesterolemia, depressive symptoms, and MMSE-score. Significant effect estimates ($p<0.05$) are indicated in **bold**.

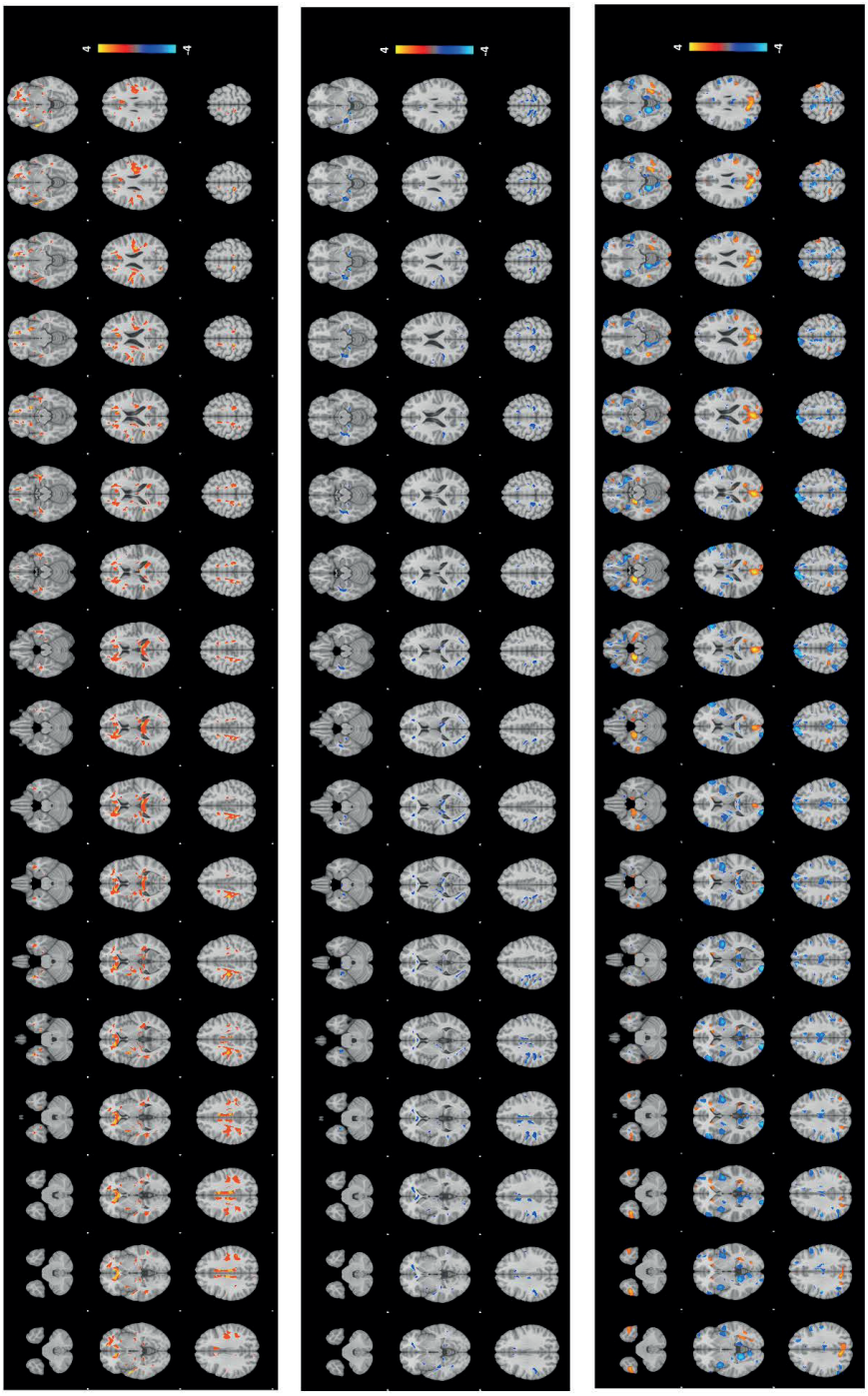

Figure 1.

Figure 1. A: Axial projection of white matter voxels: fractional anisotropy associated with tinnitus. Colours reflect the tendency of the association: blue for a negative direction (decrease of white matter fractional anisotropy in tinnitus), red for a positive direction (increase of white matter fractional anisotropy). **B:** Axial projection of white matter voxels: mean diffusivity associated with tinnitus. Colours reflect the tendency of the association: blue for a negative direction (decrease of white matter mean diffusivity), red for a positive direction (increase of white matter mean diffusivity). Higher white matter mean diffusivity indicates decreased white matter microstructural integrity. **C:** Axial projection of voxels: grey matter density associated with tinnitus. Colours reflect the tendency of the association: blue for a negative direction (decrease of grey matter), red for a positive direction (increase of grey matter). No statistically significant associations were found (see supplementary tables for exact outcomes per area).

Table 4. The association between tinnitus and brain tissue volume and white matter microstructural integrity – stratified by degree of hearing loss

		Total brain volume	Grey matter volume	White matter volume	Fractional anisotropy	Mean diffusivity
		Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)	Difference in SD (95% CI)
				Normal hearing (0 – 20 dB)		
Tinnitus present versus absent	Model 1	**0.07 (0.01, 0.12)**	-0.07 (-0.17, 0.02)	**0.17 (0.07, 0.28)**	0.00 (0.00, 0.00)	0.00 (-0.01, 0.00)
	Model 2	**0.10 (0.03, 0.17)**	-0.02 (-0.15, 0.11)	**0.18 (0.03, 0.32)**	0.00 (0.00, 0.00)	0.00 (-0.01, 0.00)
				Mild hearing loss (20 – 40 dB)		
Tinnitus present versus absent	Model 1	**0.08 (0.02, 0.14)**	-0.01 (-0.10, 0.08)	**0.14 (0.03, 0.25)**	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
	Model 2	**0.10 (0.05, 0.16)**	0.02 (-0.08, 0.13)	**0.14 (0.00, 0.27)**	0.00 (0.00, 0.00)	0.00 (-0.01, 0.00)
				Moderate/severe hearing loss (> 40 dB)		
Tinnitus present versus absent	Model 1	0.04 (-0.04, 0.12)	0.04 (-0.09, 0.17)	0.02 (-0.13, 0.18)	0.00 (0.00, 0.01)	0.00 (-0.01, 0.01)
	Model 2	0.05 (-0.04, 0.14)	0.07 (-0.07, 0.22)	0.00 (-0.18, 0.18)	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)

Difference represents the difference in SD brain tissue volume (total, grey matter, white matter) or the difference in SD for white matter microstructural integrity (fractional anisotropy, mean diffusivity) in participants with tinnitus as compared to participants without tinnitus. SD: standard deviation. CI: confidence interval. dB: decibel. Model 1: adjusted for age, sex, education, hearing loss and intracranial volume. Model 2: additionally adjusted for smoking, alcohol intake, physical activity, body mass index, hypertension, hypercholesterolemia, depressive symptoms, and MMSE-score. Significant effect estimates ($p<0.05$) are indicated in **bold**.

When stratifying by degree of hearing loss, similar associations between tinnitus and brain tissue volume were found (Table 4). In a subgroup of participants (N = 355; of whom 37 reported tinnitus) with no threshold above 20 dB on any of the measured frequencies, similar results were found as in the group with normal hearing; tinnitus was associated with larger brain tissue volumes, fully driven by larger white matter volumes (Supplementary table 4). Associations did not differ between males and females (Supplementary table 5).

DISCUSSION

In a large population-based sample of older adults we found that participants with tinnitus, independent of degree of hearing loss and age, had significantly larger brain tissue volumes as compared to participants without tinnitus. This association was entirely driven by larger white matter volumes. Tinnitus was not associated with grey matter volume or global white matter microstructural integrity. Regional analyses on a lobar or voxel-based level did not show regional specificity for these findings.

There is a known strong relation between hearing loss and tinnitus.[1] As hearing loss has previously been associated with smaller brain tissue volumes and decreased white matter microstructure,[2, 3] we had expected similar results: an association between tinnitus and smaller brain volumes and compromised white matter microstructure. Conversely, we found that individuals with tinnitus had larger white matter volumes, which was also independent of hearing loss. These results suggest that tinnitus is not related with ageing processes such as neurodegeneration. Indeed, another study reported no associations between tinnitus and white matter volume changes. They suggested that decreased white matter volume may be explained by comorbid hearing loss, which is again largely determined by age.[4] In line with this, several other studies proposed that grey matter changes, which is also known to decrease with age,[5] are attributable to the age-related hearing loss rather than the tinnitus per se.[4, 6] In light of our results, it may be hypothesized that tinnitus is associated with neurodevelopmental aspects in earlier life. To put it differently, people with larger brain tissue volume from an early age onwards may be more at risk for tinnitus at later ages then people with smaller brain tissue volumes. To truly state whether tinnitus indeed has a neurodevelopmental origin, longitudinal research in children, adolescents and young adults with and without tinnitus is needed. One study in a middle-aged population with tinnitus (mean age: 59 years [SD: 8.3]) reported larger grey matter volumes of the left auditory cortex, thus indicating that larger brain volumes in individuals with tinnitus may already be present in middle-aged adulthood.[7] However, to our knowledge, no study has explored these associations in a younger population yet.

A meta-analysis on tinnitus and functional-MRI detected regions of aberrant neural activity mainly in the non-auditory brain regions, including the parahippocampus, insula, cerebellum, cuneus, and thalamus.[8] Interestingly, we found that tinnitus was associated with larger white matter volumes in every lobe, whereas it could be expected that especially the temporal lobe would have been associated with tinnitus as it encompasses the auditory cortex. Thus, our results, in accordance with above mentioned meta-analysis, might point towards a more generalized effect of tinnitus on the brain, or vice versa. Longitudinal data is needed on both brain measurements and tinnitus, including data on tinnitus duration and onset, to truly determine whether people with larger white matter volumes are more sensitive for tinnitus, or the other way around, that tinnitus leads to cortical reorganization and aberrant neural activity.

Moreover, though not statistically significant, we found that tinnitus tended to relate to increased white matter microstructure of the white matter tracts based on a VBM analysis. Previous VBM studies mostly found associations between the prevalence of tinnitus and reduced cortical thickness in the bilateral temporal and frontal lobes,[9] reduced white matter volumes[10] and decreased white matter integrity.[9] Yet, we could not replicate these findings. Results between studies remain conflicting, probably due to methodological differences such as participant selection (clinical populations versus the general population), small sample sizes and focusing on specific regions of interest of the brain instead of whole brain analyses. Furthermore, most previous studies failed to appropriately adjust for effects of ageing, which may have led to residual confounding by age and its associated neurodegeneration.[5]

Another key feature of our analysis is that we explored associations between tinnitus and the brain taking into account the amount of hearing loss to disentangle possible central versus peripheral components contributing to tinnitus. Our results indicated that the association between tinnitus and brain tissue volumes is independent of hearing loss. This association attenuated in a sub-sample of participants with no hearing threshold above 20 dB on any of the measured hearing frequencies, again supporting a strong central component of tinnitus. It has been hypothesized that a peripheral trigger is associated with the onset of tinnitus.[11] However, based on our results it may be argued that central processes play a large role in maintaining tinnitus or being at risk for developing tinnitus. Still, we cannot infer on what causes tinnitus; whether there is one sole pathophysiological mechanism or multiple. Longitudinal studies are needed to unravel whether the incidence of tinnitus is associated with either peripheral or central processes or with both.

Strengths of our study included the large population-based sample, the (quantitative) assessment of brain structure and microstructure using imaging and the availability of extensive information on potential confounding factors. A limitation of the current study is its cross-sectional nature, hampering the possibility to infer causality between

determinant and outcome. Moreover, due to incomplete data we could not investigate the severity of the tinnitus complaints. Nor did we have information on the time since tinnitus onset and which ear was affected. On top of this, even though we extensively adjusted for potential confounders, residual confounding may still be present. Moreover, even though a 1.5 tesla MRI scanner is widely used, both in research and in clinical settings, a higher field strength would have the advantage to more sensitively image relatively small structures.

To conclude, we found that tinnitus is associated with larger brain tissue volumes, driven by larger white matter volumes, independent of hearing loss and age. Thus, it may be hypothesized that tinnitus has more of a neurodevelopmental origin potentially increasing the risk of developing future tinnitus in people with larger brain tissue volumes from a young age onwards. Future (longitudinal) population-based studies are warranted to elucidate the role of peripheral damage and central processes in the pathophysiology of tinnitus.

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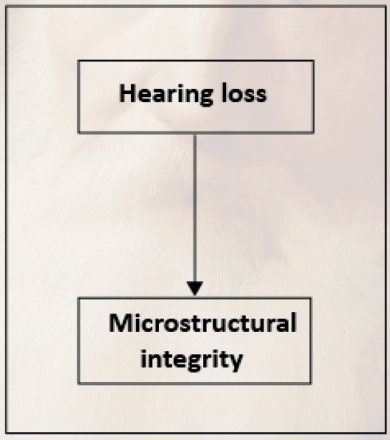
Hearing loss
Microstructural integrity

4.2

Hearing loss and microstructural integrity of the brain in a dementia-free population

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Under review

ABSTRACT

Background

Hearing loss has been associated with smaller macro structural brain volumes, which is believed to at least partly explain the association between hearing loss and neurodegenerative disease. However, microstructural changes precede macro structural changes. Therefore, we aimed to assess the association between hearing loss and microstructural integrity of the brain.

Methods

1,086 dementia-free participants (mean age = 75.2 [SD: 4.9], 61.4% female) of the population-based Atherosclerosis Risk in Communities (ARIC) study underwent hearing assessment (2016-2017) and magnetic resonance imaging (MRI) of the brain (2011-2013). Hearing was assessed with pure-tone audiometry. Microstructural integrity (fractional anisotropy (FA) and mean diffusivity (MD)) of multiple brain regions was determined with diffusion tensor imaging (DTI). Multivariable linear regression models were used to investigate the association between hearing loss and microstructural integrity of the lobes, temporal lobe gyri, medial temporal lobe structures and white matter (WM) tracts, adjusting for relevant confounders.

Results

Hearing loss was associated with lower WM microstructural integrity in the temporal lobe (mean difference in standardized FA for moderate/severe hearing loss as compared to normal hearing: -0.19 [95% CI: -0.35, -0.03]; and mean difference in standardized MD for moderate/severe hearing loss as compared to normal hearing: 0.15 [95% CI: 0.01, 0.28]). Hearing loss was associated with lower grey matter (GM) microstructural integrity of the hippocampus, but not the amygdala. Moreover, hearing loss was associated with lower WM microstructural integrity of the limbic tracts and the uncinate fasciculus.

Conclusion

Our results demonstrate that hearing loss is associated with lower WM microstructural integrity in the temporal lobe, the limbic tracts and the uncinate fasciculus and lower GM integrity in the hippocampus, brain regions that are important for different cognitive processes. However, the results should be interpreted with caution as this study is of a cross-sectional design.

BACKGROUND

Recently hearing loss has been identified as a promising modifiable risk factor for dementia.[1] Several hypotheses explaining a potential underlying pathway have been proposed, including the sensory-deprivation hypothesis, describing a direct causal relationship in which hearing loss leads to neuroplastic changes in the brain.[2]

Indeed, hearing loss has been associated with smaller brain tissue volumes.[3,4] However, recent studies in the underlying microstructure of brain tissue showed that with aging, microstructural changes precede gross morphological changes.[5] As such, it has been suggested that microstructural degeneration might be a more sensitive and/or earlier marker of neurodegeneration than macro structural atrophy.[5] Therefore, interest in a potential association between hearing loss and microstructural integrity has increased.

Some studies (mostly cross-sectional and consisting of small sample sizes) have reported associations between hearing loss and lower microstructural integrity in the auditory cortex and white matter (WM) tracts.[6-10] So far, only one population-based study reported associations between lower global and tract-specific WM microstructural integrity and poorer hearing.[11] However, they did not assess microstructural integrity of the temporal lobe, where important areas for auditory processing as well as brain structures most relevant in neurodegenerative disease are located.[12] Moreover, diffusion tensor imaging (DTI) was performed on a 1.5 tesla magnetic resonance imaging (MRI) scanner whereas high(er) field strengths are preferred for relatively small structures,[13] such as the limbic tracts. Importantly, these WM tracts might be of interest in regard to hearing loss as they connect parts of the temporal lobe with other regions of the brain that are involved in cognitive processes.[14] Traditionally, DTI has mainly been used to assess WM microstructure. However, interest in the microstructure of the grey matter (GM) has increased. Specifically, it has been reported that high GM mean diffusivity (MD) in the hippocampus was associated with lower memory performance.[15] To our knowledge, no study has assessed a potential association between hearing loss and GM microstructure.

Against this background, we aimed to elucidate the association between hearing loss and WM microstructural integrity of the lobes (frontal, temporal, parietal, occipital), WM microstructural integrity of the gyri of the temporal lobe (superior, middle, inferior), GM MD of medial temporal lobe structures (hippocampus and amygdala), and the microstructural integrity of WM tracts residing in four groups of WM fibre bundles (association, limbic, commissural, sensorimotor) in a population-based sample of older adults.

METHODS

Setting and study population

The Atherosclerosis Risk in Communities (ARIC) Study is an ongoing population-based prospective cohort study of 15,792 participants aged 45-64 years at recruitment in 1987-1989 from four US communities (Washington County, Maryland; Forsyth County, North Carolina; Jackson, Mississippi; and Minneapolis, Minnesota).[16, 17]

In visit 6 (2016 – 2017) assessment of hearing was included in the study protocol and 3,655 participants underwent hearing assessment. Of those 3,655 participants, 1,204 participants underwent magnetic resonance imaging (MRI) of the brain at visit 5 (2011 – 2013). Invitation criteria for a brain MRI-scan are described in detail elsewhere.[17] From this sample we excluded participants with prevalent dementia (N = 10), participants with implausible MRI data (N = 4), and participants with probable cognitive impairment (N = 101) (MMSE score ≤ 23 if participants had an educational level of high-school degree or less, or a MMSE score ≤ 25 when participants had an educational level of some college or more). Additionally, due to low numbers we excluded participants if race was other than black or white, or if non-white from Minneapolis and Washington County (N = 3), resulting in an analytical sample of 1,086 participants. The institutional review boards of all participating institutions approved this study and participants provided written informed consent.

Hearing assessment

Pure tone air conduction audiometry was conducted in a sound-treated booth. Air conduction thresholds were obtained at standard frequencies from 0.5 kHz to 8 kHz by trained technicians using an Interacoustics AD629 audiometer (Interacoustics A/S, Assens, Denmark). For participants with a home visit or who were in a long-term care facility, pure tone audiometry was conducted with a portable audiometer and supra aural headphones (Shoebox Audiometer, Ottawa, Canada), after ensuring that the ambient levels of noise in the room were acceptable for valid testing. All thresholds were measured in decibels (dB) hearing level. For all participants, the threshold levels for the pure-tone speech frequencies of 0.5, 1, 2, and 4 kHz in the better hearing ear were averaged to obtain a pure tone average in accordance with the World Health Organization.[18] We categorized pure tone average according to clinically relevant cut points for hearing impairment: normal hearing: ≤ 25 dB; mild: 26-40 dB; moderate/severe > 40 dB. As few participants had severe hearing loss (N = 35; 3.2%), moderate and severe hearing impairment were combined in one category to maintain sufficient statistical power.

Brain imaging and image processing

At ARIC-NCS identical protocols for 3-T brain magnetic resonance imaging (MRI) were used at each study site. The entire scan protocol is described in detail elsewhere.[17]

The protocol included a 3D T1 weighted scan and an axial fluid-attenuated inversion recovery (FLAIR) sequence for atlas region registration and tissue segmentation. For microstructural imaging of the brain, DTI was used. The DTI scans used the Siemens product echo planar imaging (EPI) pulse sequence and diffusion gradient set, namely a single b = 0 volume followed by 64 b = 1,000 s/mm^2 diffusion directions uniformly spread over the whole sphere. An isotropic 2.7 mm voxel resolution was used, and the echo time (TE) was 87 ms for all scanners.

In post-processing the DTI images were simultaneously corrected for eddy current distortion and volume-to-volume head motion by affinely registering each of the diffusion weighted volumes to the b = 0 volume. EPI sequences are also affected by distortions at changes in magnetic susceptibility, such as for example air/tissue/bone interfaces around the sinuses and ear canals. To correct for these distortions, the diffusion weighted data were nonlinearly registered to the T1-weighted anatomical reference scan using the BrainSuite program,[19] which also produced fractional anisotropy (FA) and mean diffusivity (MD) images in each subject's T1-weighted space after fitting diffusion tensors to the data by way of a weighted least-squares scheme. All FLAIR-to-T1 and DTI-to-T1 registrations were manually examined to catch and correct or remove gross registration failures.[17, 20]

DTI measures of FA and MD were used to assess microstructural integrity of lobar and deep WM regions. MD was used to assess microstructural integrity of the temporal lobe GM areas. There is no preferred direction of water diffusion in the GM, therefore GM FA is near 0 and thus it is to be expected that the true GM FA is below the noise limit of DTI scans. FA is a unit less measure of the directional constraint placed on water molecules by cell membranes. MD is the directionally averaged diffusivity of the water molecules, reported here in mm^2/s. Lower WM microstructural integrity is reflected by lower levels of FA and higher levels of MD. Lower GM microstructural integrity is reflected by a higher MD. An in-house atlas derived from the STAND400 template[21] was used to delineate lobar and deep WM regions. For every participant, tissue segmentations from T1-weighted and FLAIR images were intersected with each WM region.[17] Voxels with a greater than 50% probability of being WM, including WM hyperintensities, were used to calculate global FA and MD. To exclude edge voxels that were primarily cerebrospinal fluid, an upper cut-off of MD < 0.002 mm^2/s was applied.[20] We grouped the atlas regions into four regions of interest: frontal, temporal (combined left and right), parietal and occipital lobes. The combination was done by averaging within each group, weighted by the number of voxels in each WM region, to create WM FA and MD measures per structure. Important to note: the diffusion tensor model assumes a single, homogeneous tissue in each measurement voxel, and as such cannot disambiguate changes in individual tissue components to changes in mixing fraction for voxels containing more than one tissue or fibre bundle. However, when corrected for the macro structural effects that

capture most of the compositional differences, differences in MD do essentially reflect changes in the integrity of the tissue for both WM and GM. Next, the JHU "Eve" atlas was nonlinearly registered to the FA image and used to select 18 different regions - tracts and GM structures - in subject native space.[22] Regions were categorized into GM, sensorimotor fibres, association fibres, limbic fibres and commissural fibres. Region-specific measurements of microstructural integrity were obtained by taking median measures inside each region, with subsequent combination of left and right measures. DTI values, both global and region-specific, were measured using fully automated methods (no readers involved).

Covariates

All time-varying covariates are based on data collected at ARIC visit 5. Demographic covariates (collected at visit 1) included date of birth, sex, race (black – Mississippi, black – North Carolina, white – North Carolina, white – Maryland, white – Minnesota), and education.[16] Intracranial volume, normal-appearing WM volume, GM volume and WM hyperintensity volume were quantified via in-house algorithms.[17, 23] All scans included a sagittal T1-weighted 3D volumetric Magnetization Prepared Gradient Echo pulse sequence and a FLAIR sequence. T1-weighted sequences were used for the quantification of brain volumes, FLAIR sequences were primarily used to detect WM hyperintensities.[23] Self-reported information on current and past cigarette smoking was collected and coded as ever or never smoking. Information on alcohol intake was obtained through self-report and defined as alcohol use yes/no. Body mass index (BMI) was defined as weight divided by height squared (kg/m^2). Blood pressure levels were assessed using a random-zero sphygmomanometer after resting for five minutes. The average of the second and third of three consecutive measurements was used to calculate systolic and diastolic blood pressure levels.[24] Hypertension was considered present if diastolic blood pressure was ≥ 90 mm Hg, systolic blood pressure was ≥ 140 mm Hg, and/or the use of antihypertensive treatment.[16] High density lipoprotein (HDL) – cholesterol was measured enzymatically after precipitation of the apolipoprotein B containing lipoproteins. Low density lipoprotein (LDL) – cholesterol was calculated using the Friedewald formula.[24] Hypercholesterolemia was considered present if total cholesterol was ≥ 6.2 mmol/L and/or the use of lipid-lowering medication. Diabetes was defined as a fasting serum glucose level ≥ 126 mg/dL, a non-fasting serum glucose level ≥ 200 mg/dL, and/or a history of diabetes, insulin therapy or oral hypoglycaemic medication use.[16]

Statistical methods

First, we assessed associations of hearing loss continuously and by degrees of hearing loss (mild and moderate/severe hearing loss as compared to normal hearing)) and lobar WM microstructural integrity (frontal, temporal, parietal, and occipital lobe) using

multivariable linear regression models. In the first model we adjusted for age, sex, race per centre, level of education, intracranial volume, normal-appearing WM volume, and WM hyperintensity volume. In the second model we additionally adjusted for smoking, alcohol intake, BMI, hypertension, hypercholesterolemia and diabetes mellitus. Adding age^2 (to account for potential non-linear effects of age), time between visit 5 and 6, the interaction between hearing loss and age and the interaction between hearing loss and sex did not add significantly to the models and were therefore left out of the final analysis. Second, we performed a similar multivariable linear regression analysis in which we studied the association of hearing loss and WM microstructural integrity of the temporal lobe gyri (superior, medial, inferior). Third, we explored associations between hearing loss and GM microstructural integrity of medial temporal lobe structures (hippocampus and amygdala) with the same multivariable linear regression models, though we adjusted for GM volume instead of WM volume. Fourth, we assessed the association of hearing loss and microstructural integrity of specific WM fibre bundles using the same multivariable linear regression models. Effect estimates did not change between models and we therefore report only the full models. To allow comparison between brain regions, we standardized FA and MD values. IBM SPSS statistics version 25.0 (IBM Corp, Armonk, NY, USA) and RStudio; integrated development environment for R, version 3.5.1 (RStudio, Boston, MA, USA) were used for data handling and statistical analyses. Results were considered statistically significant with a p-value ≤ 0.05 (indicated by *) or a p-value ≤ 0.01 (indicated by **). Tests between hearing loss and WM fibre bundles were additionally adjusted for multiple comparisons of the number of tracts studied using a Sidak correction. The number of independent tests were estimated which resulted in a significance threshold at p ≤ 0.0022 (indicated by ***).

RESULTS

Population characteristics are described in Table 1. Mean age was 75.2 years (standard deviation [SD]: 4.9) with a range of 67.0 – 90.0 years. 667 participants (61.4%) were female. Average hearing threshold was 32.4 dB (SD: 13.4) and 338 participants (31.1%) had normal hearing function (hearing threshold ≤ 25 dB).

Hearing loss was associated with lower WM microstructural integrity in the temporal lobe, but not in the frontal, parietal or occipital lobe (Table 2). Specifically, we found that moderate/severe hearing loss as compared to normal hearing was associated with lower FA in the temporal WM (mean difference in standardized FA: -0.19 [95% CI: -0.35, -0.03]). Moreover, hearing loss per 10 dB increase was associated with an on average 0.04 higher standardized MD (95% CI: 0.00, 0.08) and participants with moderate/severe hearing loss had on average a 0.15 higher standardized MD (95% CI: 0.01, 0.28) in the temporal

Table 1. Population characteristics

	N = 1,086
Age, years (SD)	75.2 (4.9)
Age, range in years	67.0 – 90.0
Female, N (%)	667 (61.4)
Education, N (%)	
Basic	106 (9.8)
Intermediate	415 (38.2)
Advanced	563 (51.8)
Center, N (%)	
Forsyth County, North Carolina	277 (25.5)
Jackson, Mississippi	250 (23.0)
Minneapolis, Minnesota	271 (25.0)
Washington County, Maryland	285 (26.2)
Body mass index, kg/m^2 (SD)	28.8 (5.7)
Smoking, yes N (%)	536 (49.4)
Alcohol use, yes N (%)	559 (51.5)
Hypertension, yes N (%)	786 (72.4)
Hypercholesterolemia, yes N (%)	623 (57.4)
Diabetes, yes N (%)	341 (31.4)
Hearing abilities	
Hearing threshold better hearing ear, dB (SD)	32.4 (13.4)
Degree of hearing loss better hearing ear, N (%)	
Normal hearing (≤ 25 dB)	338 (31.1)
Mild (26-40 dB)	454 (41.8)
Moderate/severe (≥ 40 dB)	294 (27.1)
White matter fractional anisotropy, by region	
Frontal lobe (SD)	0.28 (0.02)
Temporal lobe (SD)	0.29 (0.02)
Parietal lobe (SD)	0.30 (0.02)
Occipital lobe (SD)	0.22 (0.02)
White matter mean diffusivity, by region, 10^{-4} mm^2/s	
Frontal lobe (SD)	8.50 (0.50)
Temporal lobe (SD)	8.80 (0.55)
Parietal lobe (SD)	8.70 (0.56)
Occipital lobe (SD)	8.60 (0.59)

dB: decibel. Values are mean (standard deviation) for continuous variables and number (percentages) for dichotomous variables.

Table 2. The association between hearing loss in the better hearing ear and lobe specific white matter microstructure

	Frontal lobe	Temporal lobe	Parietal lobe	Occipital lobe
	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)
			Fractional anisotropy	
Hearing loss, per 10 dB	0.01 (-0.03, 0.05)	-0.04 (-0.08, 0.01)	0.01 (-0.04, 0.05)	0.04 (-0.01, 0.08)
Degree of hearing loss				
None	*Reference*	*Reference*	*Reference*	*Reference*
Mild	-0.00 (-0.12, 0.12)	-0.13 (-0.26, 0.01)	-0.04 (-0.16, 0.09)	-0.02 (-0.16, 0.11)
Moderate/severe	0.03 (-0.11, 0.17)	-0.19 (-0.35, -0.03)**	-0.00 (-0.15, 0.14)	0.08 (-0.08, 0.23)
			Mean diffusivity	
Hearing loss, per 10 dB	-0.02 (-0.06, 0.02)	0.04 (0.00, 0.08)*	0.01 (-0.03, 0.05)	-0.02 (-0.07, 0.02)
Degree of hearing loss				
None	*Reference*	*Reference*	*Reference*	*Reference*
Mild	-0.01 (-0.13, 0.11)	0.07 (-0.05, 0.18)	0.06 (-0.06, 0.18)	0.00 (-0.12, 0.13)
Moderate/severe	-0.02 (-0.16, 0.12)	0.15 (0.01, 0.28)*	0.07 (-0.07, 0.21)	-0.06 (-0.21, 0.09)

Difference represents the mean difference in standardized fractional anisotropy or mean diffusivity per 10 dB increase in hearing thresholds or the mean difference in standardized fractional anisotropy or mean diffusivity per degree of hearing loss (mild, moderate/severe) as compared to normal hearing. Normal hearing ≤ 25 dB. Mild hearing loss: 26 – 40 dB. Moderate/severe hearing loss: ≥ 40 dB. Adjusted for age, sex, race per centre, level of education, intracranial volume, normal-appearing white matter volume, white matter hyperintensity volume, body mass index, hypertension, hypercholesterolemia, smoking (yes/no), alcohol use (yes/no), and prevalent diabetes mellitus. dB: decibel. CI: confidence interval. Lower white matter microstructural integrity is indicated by lower fractional anisotropy and higher mean diffusivity. * $p \leq 0.05$. ** $p \leq 0.01$.

WM compared to participants with normal hearing (Table 2). Those associations were not driven by a specific gyrus in the temporal lobe as effect estimates of the associations between hearing loss and WM microstructural integrity in the superior, medial and inferior temporal gyrus were all comparable (Figure 1; Supplementary table 1).

Hearing loss appeared to be associated with lower GM microstructural integrity in the hippocampus as reflected in higher levels of GM MD, not in the amygdala (Table 3). Associations between hearing loss and the hippocampus were most prominent for moderate/severe hearing loss as compared to normal hearing (mean difference in standardized GM MD: 0.25 [95% CI: 0.11, 0.39]).

Most prominent associations between hearing loss and WM microstructural integrity of the WM fibre bundles were found for the limbic fibres (Figure 2). Hearing loss per 10 dB increase was associated with lower WM FA in the cingulate gyrus part of cingulum, parahippocampal part of the cingulum, fornix (body and column), and the fornix cres stria terminalis as well as with higher WM MD in the parahippocampal part of the cingulum (Figure 2; Supplementary table 2). Mild and moderate/severe hearing loss as

Table 3. Hearing loss in the better hearing ear and grey matter microstructure of medial temporal lobe structures

	Hippocampus mean diffusivity	Amygdala mean diffusivity
	Difference (95% CI)	Difference (95% CI)
Hearing loss, per 10 dB	0.08 (0.04, 0.12)***	-0.01 (-0.05, 0.04)
Degree of hearing loss		
None	*Reference*	*Reference*
Mild	0.13 (0.01, 0.25)*	-0.02 (-0.16, 0.11)
Moderate/severe	0.25 (0.11, 0.39)***	0.03 (-0.13, 0.19)

Difference represents the mean difference in standardized MD per 10 dB increase in hearing thresholds or the mean difference in standardized mean diffusivity per degree of hearing loss (mild, moderate, severe) as compared to normal hearing. Normal hearing ≤ 25 dB. Mild hearing loss: 26 – 40 dB. Moderate/severe hearing loss: ≥ 40 dB. Adjusted for age, sex, race per centre, level of education, intracranial volume, grey matter volume, body mass index, hypertension, hypercholesterolemia, smoking (yes/no), alcohol use (yes/no), and prevalent diabetes mellitus. Lower grey matter microstructural integrity is indicated by higher mean diffusivity. * $p \leq 0.05$. ** $p \leq 0.01$. *** $p \leq 0.001$.

compared to normal hearing were both associated with lower WM FA in all limbic fibre bundles (except for mild hearing loss and the cingulate gyrus). Moreover, mild hearing loss was associated with higher WM MD in the parahippocampal part of the cingulum and moderate/severe hearing loss was associated with higher WM MD in the cingulate gyrus and parahippocampal part of the cingulum (Figure 2; Supplementary table 2). No statistically significant associations were found between hearing loss and WM FA in the association fibres. However, hearing loss (per 10 dB increase and moderate/severe hearing loss as compared to normal hearing) was associated with higher WM MD in the uncinate fasciculus (Figure 3; Supplementary table 3). No clinically relevant associations were found between hearing loss and WM microstructural integrity of the sensorimotor fibres and the commissural fibres (Supplementary tables 4 and 5).

DISCUSSION

We found in a dementia-free population-based sample, that hearing loss was associated with lower WM microstructural integrity in the temporal lobe, independent of macro structural measures, but not in the frontal, parietal or the occipital lobe. The association with temporal microstructure was not driven by a specific temporal lobe gyrus. Interestingly, hearing loss was also associated with higher GM MD in the hippocampus. Moreover, we found that hearing loss was associated with lower WM microstructural integrity in the limbic tracts and in the uncinate fasciculus (part of the association tracts).

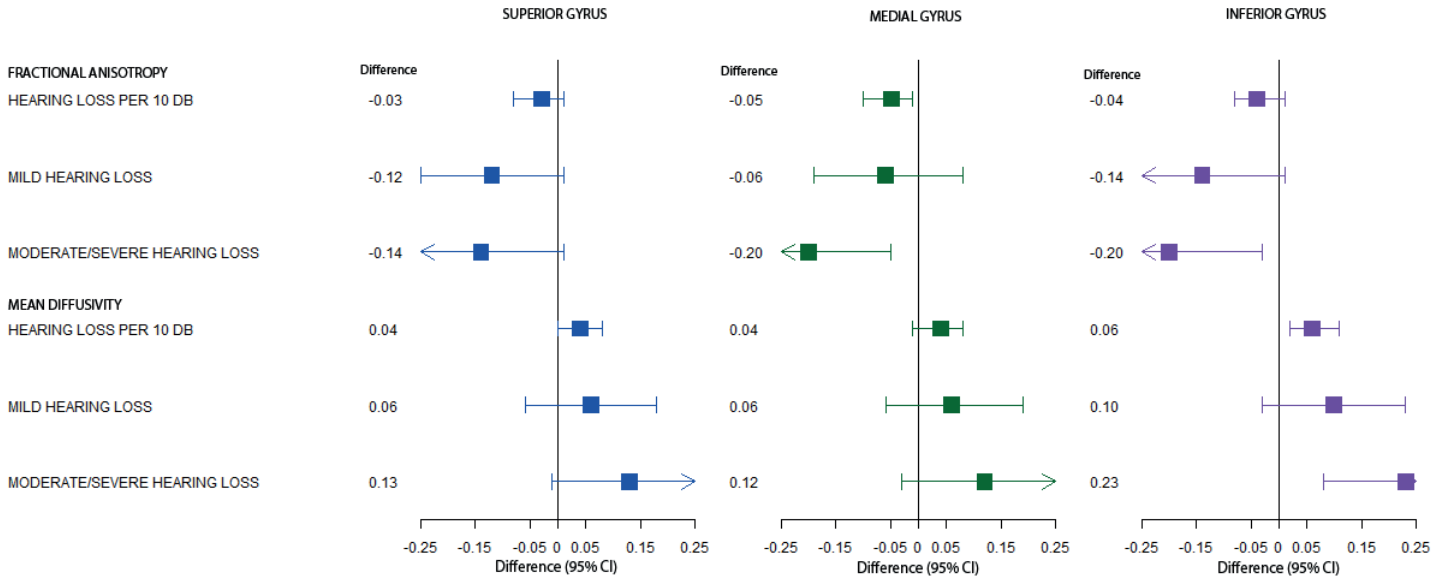

Figure 1. Forest plot of the association between hearing loss in the better hearing ear (per 10 dB increase or mild or moderate/severe hearing loss as compared to normal hearing) and white matter microstructural integrity of the temporal lobe gyri. Difference represents the mean difference (with corresponding 95% CI) of standardized fractional anisotropy or standardized mean diffusivity per 10 dB increase or for mild or moderate/severe hearing loss as compared to normal hearing. Values are adjusted for age, sex, race per centre, level of education, intracranial volume, normal-appearing white matter volume, white matter hyperintensity volume, hypertension, hypercholesterolemia, body mass index, smoking (yes/no), alcohol use (yes/no), and prevalent diabetes mellitus. HL: hearing loss. dB: decibel. SD: standard deviation. CI: confidence interval. Lower white matter microstructural integrity is indicated by lower fractional anisotropy and higher mean diffusivity. For effect estimates with exact 95% CI and statistical significance values, see Supplemental table 1.

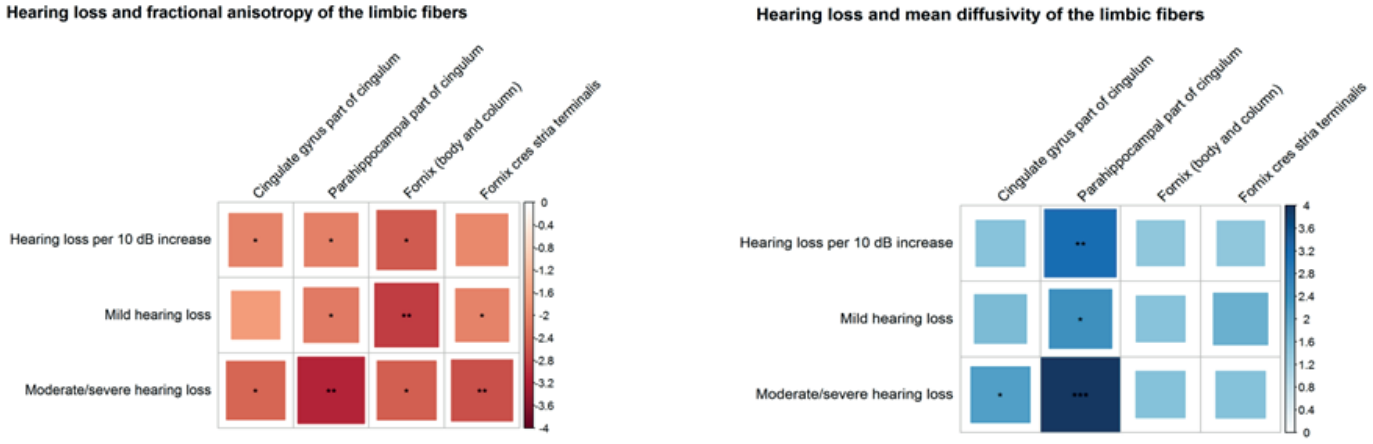

Figure 2. Heat map of the association between hearing loss in the better hearing ear (per 10 dB increase or mild or moderate/severe hearing loss as compared to normal hearing) with white matter microstructural integrity of the limbic tracts reflected by the standardized fractional anisotropy (red; left) and standardized mean diffusivity (blue; right) of the white matter microstructure. Colours and sizes of the blocks correspond to t-values (for effect estimates, see supplemental table 2), with blue and red indicating positive and negative associations, respectively. Values are adjusted for age, sex, race per centre, level of education, intracranial volume, normal-appearing white matter volume, white matter hyperintensity volume, hypertension, hypercholesterolemia, body mass index, smoking (yes/no), alcohol use (yes/no), and prevalent diabetes mellitus. Lower white matter microstructural integrity is indicated by lower fractional anisotropy and higher mean diffusivity. Larger blocks indicate stronger associations, and significance levels as indicated by asterisks: *p ≤ 0·05; **p ≤ 0·01; ***p ≤ 0·0022.

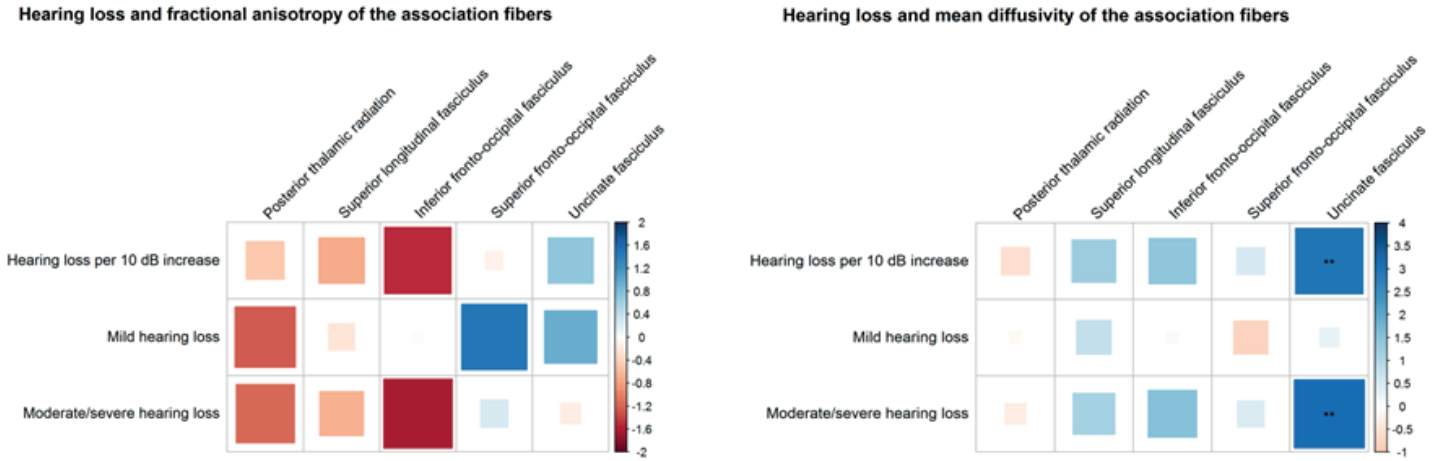

Figure 3. Heat map of the association between hearing loss in the better hearing ear (per 10 dB increase or mild or moderate/severe hearing loss as compared to normal hearing) with white matter microstructural integrity of the association tracts reflected by the standardized fractional anisotropy (red; left) and standardized mean diffusivity (blue; right) of the white matter microstructure. Colours and sizes of the blocks correspond to t-values (for effect estimates, see supplemental table 3), with blue and red indicating positive and negative associations, respectively. Values are adjusted for age, sex, race per centre, level of education, intracranial volume, normal-appearing white matter volume, white matter hyperintensity volume, hypertension, hypercholesterolemia, body mass index, smoking (yes/no), alcohol use (yes/no), and prevalent diabetes mellitus. Lower white matter microstructural integrity is indicated by lower fractional anisotropy and higher mean diffusivity. Larger blocks indicate stronger associations, and significance levels as indicated by asterisks: *p ≤ 0·05; **p ≤ 0·01; ***p ≤ 0·0022.

Strengths of this study include the population-based setting and (quantitative) assessment of microstructural integrity of the brain using diffusion imaging. Moreover, our sample includes a racially diverse population. We also had extensive data on other measurements, enabling us to adjust for potential confounding. In particular, we adjusted analyses for macro structural volumes of relevant brain tissues, so the microstructural differences we found have added value over previously found macro structural changes. Some limitations of the current study should also be acknowledged. First, this is a cross-sectional study, hampering the possibility to infer causality between determinant and outcome. Second, DTI resolution in ARIC is limited in the temporal lobes. EPI distortion is stronger around the inferior surface of the temporal lobe, hampering temporal lobe results. However, if this would have influenced our results, it would have been in the negative direction. Third, as hearing has been measured prior to MRI scanning of the brain, a possible interpretation of our findings is that compromised microstructural integrity leads to hearing loss (i.e., reverse causation). However, there is little (biological) evidence that neurodegeneration precedes hearing loss.[1]

To our knowledge there is only one other population-based study assessing the association between WM microstructural integrity and hearing loss.[3] They reported a whole brain association between lower WM microstructural integrity and higher levels of hearing loss. In contrast, we only found significant associations between increased hearing levels and lower microstructural integrity of the temporal lobe. This contrast may be explained by the fact that the authors of the first study did not consider lobe-specific associations.[3] It is known that the auditory cortex is located in the temporal lobe.[4] Therefore, a whole brain association in the former study may have been driven by associations between hearing loss and the microstructural integrity of the temporal lobe.[3] Indeed, other studies report significant associations between increased levels of hearing loss and microstructural degeneration of the temporal lobe.[5, 6] More specifically, it is known that the auditory regions are located in the superior part of the temporal lobe.[7] Surprisingly, we did not find that associations within the temporal lobe were driven by the superior temporal gyrus. This may point towards a more generalized effect of hearing loss on neurodegeneration of the temporal lobe. However, it may also be an effect of aging in general. The temporal lobe is one of the first areas in the brain to be affected by neurodegeneration with increasing age.[8] Moreover, the current study is of a cross-sectional design, and the mean age is high (75.2 years). Due to this design and the relatively high age of our population we might be unable to adjust for potential residual confounding by age. As such, there is a possibility that our results are partially explained by age-related neurodegeneration, not by hearing loss per se. We tried to circumvent this by adjusting for age^2 in our models as well, but to truly exclude residual effects of aging, longitudinal data are needed.

Interestingly, we found that hearing loss was associated with higher GM MD in the hippocampus. To our knowledge, we are the first study to report this association in humans. A recent study in mice found that hearing loss affected the hippocampus, reflected in altered pre- and post-synaptic markers.[9] Even though our results are promising, care must be taken when interpreting them. The field of diffusion imaging has been growing substantially in the previous years, but mainly in the field of WM microstructural integrity.[10, 11] GM microstructural integrity is a relatively new field in the area of diffusion imaging and up to now it remains unclear what actual accelerated degeneration of the GM microstructure is, apart from 'normal' age-related changes. Interestingly though, it has been found that high GM diffusivity in the hippocampus, before any hippocampal atrophy is visible, is associated with an increased risk for cognitive impairment and dementia.[12] Moreover, another study reported that in dementia-free individuals, higher GM MD was associated with worse performance on memory tasks.[13] However, more longitudinal research is warranted, to establish more in-depth knowledge on GM microstructural degeneration with aging.

Our results did not show an association between hearing loss and GM microstructural integrity of the amygdala. Hearing loss has been associated with depression,[14] for which the amygdala is an important area in the brain.[15] A study from South-Korea found smaller amygdala volumes in patients with hearing loss compared to controls. An absence of an association between hearing loss and the amygdala in our study may be explained by selection bias as people with depressive symptoms may either be less inclined to participate at all in the study or have dropped out of the study at an earlier stage.

In tract-specific WM analyses, significant associations were found between higher levels of hearing loss and lower microstructural integrity of the different tracts located in the limbic fibre bundles and the uncinate fasciculus (part of the association tracts). These results might shed more light on the association between hearing loss and cognitive decline, as the limbic system is a network of cortical and subcortical centres and WM tracts that modulate memory, emotions, and behavior.[16] Moreover, the uncinate fasciculus connects limbic regions in the temporal lobe with areas in the frontal lobe which are involved in behavior.[17] Our results with the uncinate fasciculus are comparable to those of the Rotterdam Study, also reporting associations between lower WM integrity in the uncinate fasciculus and higher levels of hearing loss.[3] However, they did not find associations with the limbic fibre bundles, which is probably explained by different MRI field strengths between studies (1.5 tesla in Rotterdam vs. 3 tesla in the current study) and imaging resolutions, and consequently the limited ability in the Rotterdam Study to track the smaller limbic tracts. Other studies also reported reduced FA values in several WM pathways leading into and out of the auditory cortex. However, those studies consisted of small sample sizes and mostly involved young to middle-aged adults.[6]

Our results may support the sensory-deprivation hypothesis of the effect of hearing on cognition and dementia, as it could be argued that hearing loss has a direct effect on microstructural neurodegeneration of different areas in the brain involved in cognition. However, we should also consider the so-called common-cause hypothesis.[1] This hypothesis states that there is a third factor both causing hearing loss and dementia through central nervous system-wide functional decline, rather than that those two are causally related to one another. As such, greater sensitivity in one domain could identify impairments in that domain prior to the other, leading to the appearance of a false causal relationship.[1] From this view, our results may be explained by a third upstream common cause, rather than that hearing loss is actually related to lower microstructural organization of the brain. To explore whether hearing loss is really related to accelerated neurodegeneration of microstructural organization independent of normal aging effects, longitudinal data are warranted.

To conclude, this study provides new promising evidence pointing towards a direct effect of hearing loss on neurodegeneration as hearing loss appeared to be associated with lower microstructural integrity in the temporal lobe, the hippocampus and the WM tracts of the brain. However, as this study is of a cross-sectional origin we should be cautious with interpreting these results as residual effects of aging on neurodegeneration may still be present. Longitudinal studies in other population-based samples are highly warranted to further elucidate longitudinal associations between hearing loss and potential accelerated degeneration of microstructural integrity of the brain independent of age-effects.

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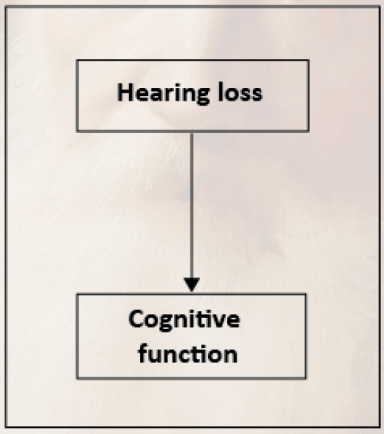
Hearing loss
Cognitive function

4.3

Hearing loss and cognitive decline in the general Dutch population: a prospective cohort study

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* These authors contributed equally to the respective manuscript

Under review

ABSTRACT

Background

Hearing loss has recently been suggested to be a promising modifiable risk factor for cognitive decline and dementia. Since both hearing and cognitive abilities steeply deteriorate with advancing age, it is uncertain whether effects of hearing loss on cognitive decline extend beyond this age-related decline. Therefore, it was our aim to elucidate whether hearing loss accelerates cognitive decline over time, independent of ageing effects.

Methods

Of a total of 3,739 participants from the population-based Rotterdam Study**,** 3,590 non-demented participants (mean age: 64.4 years [SD: 6.9], 59.3% women) were eligible for analysis at baseline, and a maximum of 837 participants were eligible for the longitudinal analysis. Hearing loss was defined by elevated hearing thresholds or reduced speech-understanding at baseline. Cognitive function was measured at baseline and at follow-up (4.4 years [SD: 0.2]) with different cognitive tests. Multivariable linear regression analysis was used for the cross-sectional analysis. Linear mixed models were used to assess the longitudinal association between varying degrees of hearing loss at baseline and cognitive decline over time while adjusting for age, level of education, cardiovascular risk factors and the interaction of age and follow-up time to take into account a faster decline in cognitive function in older participants compared to younger participants.

Results

Hearing loss was associated with lower overall cognitive function at baseline. Moreover, hearing loss was associated with accelerated cognitive decline over time on the 15-WLT while adjusting for age, education and cardiovascular risk factors. After additionally adjusting for the interaction between age and follow-up time, we found that hearing loss did not accelerate cognitive decline anymore.

Conclusions

Hearing loss appeared to be associated with lower cognitive function at baseline and accelerated cognitive decline on the 15-WLT. The association between hearing loss and accelerated cognitive decline attenuated and was non-significant after additional adjustment for non-linear age effects. More evidence is needed to ensure the role of hearing loss as a modifiable risk factor for cognitive decline, whilst paying attention to potential strong effects of age.

INTRODUCTION

Recently, hearing loss has been put forward as a promising modifiable risk factor for cognitive decline and dementia.[1-5] Both the prevalence of hearing loss and dementia will increase substantially due to the ageing of the worldwide population.[1, 6, 7] With the increasing prevalence of both conditions, it is of great importance to determine if hearing loss is independently associated with cognitive decline. As such, more can be said on whether hearing rehabilitative treatments may potentially delay the progression of cognitive decline.

Several longitudinal studies reported associations between hearing loss and poorer cognitive function,[8] and with an increased risk of dementia.[3, 4, 6, 9-12] Despite these promising results, several methodological issues should be considered. First, both hearing loss and cognitive impairment are heavily dependent on age, reflected in a steep increase of the prevalence of both with increasing age.[7, 13] Therefore, it is of great importance to thoroughly adjust for both linear and non-linear age effects in the association between hearing loss and cognition. To our knowledge, only one other study incorporated age non-linearly in their models, but they did not adjust for the fact that older people may decline faster over time on cognitive abilities compared to their younger counterparts, as can be accomplished by adding an interaction between age and follow-up time into statistical models.[8] Second, some studies rely on self-reported measures of cognitive impairment,[14, 15] or a limited battery of neuropsychological tests for cognitive assessment.[5, 9, 16-26] This potentially increases the likelihood of misclassification of cognitive impairment,[27] especially in those with higher levels of hearing impairment. Lower scores on cognitive tests may be falsely attributed to cognitive impairment, as individuals might not be able to hear the instructions properly.[28, 29] Third, hearing loss does not necessarily accurately reflect an inability to follow speech in noisy environments.[30] To our knowledge, only one other study incorporated a measure of speech understanding in their analyses.[26]

Against this background, we aimed to elucidate whether hearing loss accelerates cognitive decline over time in a large population-based study. We measured hearing loss, including speech understanding, and repeatedly assessed cognitive functioning with an extensive set of neuropsychological tests. We examined whether trajectories of cognitive decline differed across degrees of hearing impairment while adjusting for both linear and non-linear effects of age.

METHODS

Study Setting and Population

This study is embedded in the Rotterdam Study, a prospective, population-based cohort study. The Rotterdam Study was initiated in 1989 and investigates determinants and consequences of ageing. Details of the study have been described previously.[31] The entire study population consists of 14,926 individuals aged ≥45 years from the Ommoord area, a suburb of Rotterdam, the Netherlands, who undergo extensive examinations at the research centre at study entry and subsequent visits every 3 to 4 years. In 2011, hearing assessment was introduced into the study protocol. For the present study, we sampled two study populations, described in detail below.

Hearing loss and cognitive function: cross-sectional study population

In total, 3,739 participants underwent baseline hearing assessment (2011-2014). We excluded participants with probable conductive hearing loss (air-bone gap ≥ 15 dB; N = 83), participants with a history of dementia or those who were insufficiently screened for dementia at baseline (N = 51), and participants who developed dementia during follow-up (N = 15), leaving 3,590 participants with baseline hearing assessment. From those 3,590 participants, data was available on different cognitive tests, namely the MMSE (N = 3,584), the Stroop test (N = 3,500), the Word Fluency test (WFT) (N = 3,536), the Letter Digit Substitution test (LDST) (N = 3,507), the Word Learning test (WLT) (N = 3,239), and the Purdue Pegboard test (PPT) (N = 3,264). There were 3,498 participants with both data on hearing thresholds and speech understanding in noise.

Hearing loss and cognitive decline: longitudinal study population

Data on the different cognitive tests from participants who were re-invited for follow-up measurements and with available cognitive data at baseline, were available at follow-up (2015-2016) for the longitudinal analysis. At follow-up, 837 participants had data available for the MMSE, 764 participants for the Stroop test, 519 participants for the WFT, 780 participants for the LDST, 755 participants for the WLT, and 714 participants for the PPT. The mean time interval between cognitive baseline assessment and re-examination was 4.4 years (SD: 0.2). See supplementary methods for an explanation regarding the attrition rate.

Participant consent

The Rotterdam Study has been approved by the medical ethics committee of the Erasmus MC (registration number MEC 02.1015) and the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071271-159521-PG). All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Hearing assessment

Hearing thresholds measured with pure-tone audiometry

To determine hearing loss expressed by hearing thresholds in decibel (dB), pure-tone audiometry was performed in a soundproof booth.[31] A computer-based audiometry system (Decos Technology Group, version 210.2.6, AudioNigma interface) and TDH-39 headphones were used. dB hearing levels were measured according to the ISO-standard 8253-1 (International Organization for Standardization, 2010). Air conduction (frequencies 0.25-8 kilohertz [kHz]) and bone conduction (0.5 and 4 kHz) were tested for both ears while masking according to the method of Hood.[32] The best hearing ear was determined by taking the average hearing thresholds over all frequencies and identified by the lowest hearing threshold of one of both ears. Of the best hearing ear, we determined the average speech frequencies threshold (average of 0.5, 1, 2, and 4 kHz) levels. Finally, we determined degrees of hearing loss: normal hearing (0 – 20 dB), mild hearing loss (20 – 35 dB), moderate hearing loss (35 – 50 dB), and severe hearing loss (≥ 50 dB).[31, 33]

Speech understanding in noise measured with the digits-in-noise test

To measure speech understanding in noise, we derived a signal-to-noise ratio (SNR; in dB) from the digits-in-noise (DIN) test, a 3-minute test of speech understanding in noise.[34] Both speech and noise signal were presented in the participant's better hearing ear. Pre-recorded male-spoken speech-signal consisted of 24 digit triplets. Initially, the triplet was presented at 0 dB SNR. In case of an incorrect response, the next triplet was presented more intensely. After the first correct response, the speech level was decreased and a new stimulus was presented. For a correct response, the intensity was decreased again, while an incorrect response lead to an increase of the response. This was repeated until 24 triplets were repeated. SNR was the average of the last 20 triplets. We defined hearing categories based on optimal SNR cut points defined by clinically relevant degree of hearing loss using Youden's Index (Supplementary figure 1).[35]

Cognitive assessment

Cognitive function was assessed in detail with an extensive neuropsychological test battery comprising the MMSE, the Stroop test (adjusted interference score; inverted as higher scores indicate worse performance), the WFT (amount of animals named within 60 seconds), the LDST (number of correct digits within 60 seconds), the 15-WLT (total number of words remembered at least 10 minutes after immediate recall), and the PPT (sum score of three trials). Results of the WLT are not negatively influenced by hearing status, as the 15 different words are visually presented to the participants. Any practice effects are limited due to the average interval between baseline assessment and re-examination.

Covariates

During home interviews, educational level was assessed and categorized as primary education, lower education, intermediate vocational education and higher education. Smoking habits were assessed during the same interview and subsequently classified into never, former and current smoking.[31] Alcohol consumption was assessed through self-report with the food-frequency questionnaire,[36] and we subsequently calculated daily alcohol consumption in grams.[36] Systolic and diastolic blood pressures were measured twice on the right arm with a random-zero sphygmomanometer; the mean of these readings was used for the analyses. Use of antihypertensive medication was assessed by interview.[31] Participants were screened for dementia at baseline and follow-up examinations using a protocol described in detail elsewhere.[37]

Statistical analysis

We investigated whether baseline characteristics differed between participants with just a baseline assessment and participants with both a baseline and a follow-up assessment using T-tests, χ^2-tests, and Mann-Whitney U-Tests when appropriate. Subsequently, we present three sequential analyses to examine the association between hearing loss and cognitive function and cognitive decline.

First, we assessed the cross-sectional association between hearing loss (all frequencies, speech frequencies, degrees of hearing loss and SNR) and cognitive functioning at baseline using multivariable linear regression models. We adjusted for age, age^2 sex, education, alcohol consumption, smoking behaviour, systolic- and diastolic blood pressure, and use of blood pressure lowering medication. All SNR analyses were additionally adjusted by PTA hearing levels for all frequencies.

Second, we used linear mixed models with random intercepts and slopes to elucidate the longitudinal association between degrees of hearing loss (mild, moderate or severe compared to normal hearing defined by either PTA or SNR) and cognitive trajectories per test. Linear mixed models were used as this approach is able to account for different follow-up times between participants and does not assume independence of the repeated measures. In each model, we entered follow-up time in years after baseline measurement to use as time variable. For adjustment, we used the same models as described above. In a second model, a two-way interaction between age and follow-up time was added to account for possible slope differences for cognition over time, depending on the baseline age. All SNR analyses were additionally adjusted by PTA hearing thresholds. Next to the linear effects of hearing loss on cognition, an interaction of hearing loss and follow-up time was incorporated in all models, to allow slope differences in the relationship between cognitive functioning and time explained by degree of hearing loss. The linear hearing loss term (intercept difference) and the interaction term between hearing loss and follow-up time (slope difference) are the main terms of

interest in this longitudinal analysis. For SNR analysis, random slopes were not included as the models failed to converge.

Third, we performed similar linear mixed models to study the longitudinal association between hearing levels (all frequencies, speech frequencies, and SNR) and cognitive trajectories per test.

In sensitivity analyses, we explored whether longitudinal associations between hearing levels and cognitive trajectories differed between men and women and between mid-life (51 – 70 years) compared to late life (70 – 99 years). Moreover, to explore whether models in the longitudinal analysis were potentially over-parameterized, we re-ran analyses using repeated measures ANOVA. Following this statistical approach, results were comparable. As such, we chose to report the results as found with the linear mixed models.

IBM SPSS Statistics version 25 (International Business Machines Corporation, Armonk, New York) and RStudio; integrated development environment for R, version 3.5.1. (RStudio, Boston, Massachusetts) were used for statistical analyses. Analyses with linear mixed models were done using the "*lme*" function of the R-package "*nlme*".[38]

RESULTS

Table 1 shows the baseline characteristics of the study population. Mean age was 64.4 years (SD: 6.9). 59.3% of our population were female. Participants had a mean all frequency hearing threshold of 20.8 dB (SD: 9.7). 50.7% of the population had normal hearing threshold levels. For speech understanding in noise, mean SNR was -4.06 dB (SD: 4.2). Participants with a follow-up assessment compared to participants with only a baseline assessment were significantly older, had a lower alcohol intake and were unhealthier (Supplementary table 1).

Cross-sectional results

Table 2 shows the cross-sectional association between hearing loss and cognitive function. Elevated hearing thresholds and diminished speech in noise understanding were associated with lower scores on all cognitive tests, and appeared to be most pronounced for participants with severe hearing loss as compared to normal hearing on the Stroop test, WFT, LDST and the PPT (Table 2).

Longitudinal results

In the first model, mild hearing loss showed statistically significant intercept differences, compared to normal hearing thresholds on the WFT, LDST, and the PPT (Table 3). In line with this, mild and moderate degrees of hearing loss, showed intercept differences for

Table 1. Baseline characteristics

Baseline characteristics	
Age, years	64.4 (6.9)
Age, range	51.7 - 98.6
Female, %	59.3
Education level, %	
Primary	7.2
Lower	36.8
Intermediate vocational	28.4
Higher	27.1
Alcohol consumption, gram	8.0 (IQR: 1.4-19.1)
Smoking, %	
Never	33.5
Past	49.4
Current	16.7
Systolic blood pressure, mmHg	138.4 (20.5)
Diastolic blood pressure, mmHg	82.9 (11.2)
Use of blood pressure lowering medication, %	39.0
Hearing thresholds: pure-tone audiometry	
All-frequency hearing loss, dB	20.8 (9.7)
Speech frequency hearing loss, dB	18.1 (9.2)
Degree of hearing loss, %	
Normal (0 – 20 dB)	50.7
Mild (20 -35 dB)	40.1
Moderate (35 – 50 dB)	7.9
Severe (≥ 50 dB)	0.9
Speech understanding in noise: Digits-in-noise test	
Signal-to-noise ratio*, dB	-4.06 (4.2)
Degree of hearing loss, %	
Normal (0 – 20 dB)	46.3
Mild (20 – 35 dB)	23.3
Moderate/severe (35 – 50 dB)	30.4
Cognitive abilities	
Mini-Mental State Examination score[a]	29.0 (27.0 – 29.0)
Stroop Test interference score[a]	44.5 (37.9 – 54.1)
Word Fluency Test score[a]	23.0 (19.0 – 27.0)
Letter Digit Substitution Test score[a]	30.0 (26.0 – 35.0)
Word Learning Test delayed recall score[a]	8.0 (6.0 – 10.0)
Purdue Pegboard Test sum score[a]	36.0 (33.0 – 39.0)

Values are mean (standard deviation) for continuous variables or [a] median (interquartile range) for non-normally distributed continuous variables and percentages for categorical variables. *Available in 3,498 participants. The amount of hearing loss is expressed in dB, i.e. a higher dB value reflects more hearing loss.

Table 2. Effect estimates of hearing loss and cognitive function based on the cross-sectional analysis

Hearing loss	Mini-Mental State Examination score	Stroop Test interference score	Word Fluency Test score	Letter Digit Substitution Test score	Word Learning Test delayed recall	Purdue Pegboard Test sum score
	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)
Hearing loss measured with pure-tone audiometry						
Hearing thresholds per 10 dB increase						
All frequencies	-0.04 (-0.14, 0.06)	-0.63 (-1.31, 0.04)	**-0.42 (-0.65, -0.20)**	**-0.38 (-0.62, -0.14)**	**-0.11 (-0.23, -0.00)**	**-0.33 (-0.52, -0.14)**
Speech frequencies	0.01 (-0.09, 0.11)	-0.49 (-1.15, 0.18)	**-0.37 (-0.59, -0.15)**	**-0.27 (-0.51, -0.03)**	-0.10 (-0.21, 0.01)	**-0.28 (-0.47, -0.09)**
Degree of hearing loss						
Normal (0-20 dB)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Mild (20-35 dB)	-0.07 (-0.25, 0.12)	-0.75 (-2.18, 0.67)	**-1.02 (-1.50, -0.55)**	-0.42 (-0.93, 0.09)	-0.20 (-0.44, 0.03)	**-0.52 (-0.92, -0.13)**
Moderate (35-50 dB)	-0.10 (-0.44, 0.23)	-1.84 (-4.05, 0.37)	**-0.77 (-1.50, -0.03)**	-0.66 (-1.45, 0.12)	-0.31 (-0.68, 0.05)	**-0.83 (-1.45, -0.21)**
Severe (≥ 50 dB)	**-0.98 (-1.94, -0.02)**	0.02 (-4.58, 4.61)	**-1.88 (-3.40, -0.37)**	**-1.91 (-3.54, -0.28)**	-0.59 (-1.35, 0.16)	**-1.38 (-2.67, -0.09)**
Hearing loss measured with the digits-in-noise test						
Speech understanding in noise per 1 dB increase						
Speech reception threshold	**-0.07 (-0.10, -0.04)**	**-0.59 (-0.96, -0.23)**	-0.03 (-0.12, 0.05)	**-0.19 (-0.30, -0.09)**	**-0.07 (-0.12, -0.02)**	-0.03 (-0.06, 0.00)
Degree of hearing loss*						
Normal (≤-5.55 dB)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Mild (-5.55 - -3.80 dB)	-0.14 (-0.37, 0.09)	-2,08 (-4.75, 0.59)	**-0.80 (-1.45, -0.14)**	**-0.85 (-1.59, -0.11)**	**-0.41 (-0.77, -0.05)**	0.01 (-0.19, 0.22)
Moderate/severe (> -3.80 dB)	**-0.36 (-0.64, -0.08)**	**-6.19 (-9.38, -2.99)**	**-0.92 (-1.71, -0.14)**	**-1.56 (-2.45, -0.66)**	**-0.53 (-0.96, -0.09)**	**-0.29 (-0.54, -0.04)**

Difference: represents the difference in cognitive score per 10 dB increase in hearing acuity or the difference in cognitive score per 1 dB increase in speech understanding in noise or the difference in degree of hearing loss (both hearing acuity (PTA) and speech understanding (DIN)) as compared to normal hearing. All frequencies: 0.25, 0.50, 1, 2, 4, and 8 kHz. Speech frequencies: 0.5, 1, 2, and 4 kHz. The amount of hearing loss is expressed in dB, i.e. a higher dB value reflects more hearing loss. CI: confidence interval. dB: decibel. *Defined by digits-in-noise score cut-offs. Adjusted for age, age^2, sex, education, alcohol consumption, smoking, diastolic and systolic blood pressure, and use of blood pressure lowering medication. Analyses using speech understanding were further adjuster for hearing acuity. Statistically significant effect estimates ($p < 0.05$) are indicated in **bold.**

Table 3. Effect estimates of the degree of hearing loss and cognitive function based on the longitudinal analysis (intercept differences)

Degree of hearing loss		Mini-Mental State Examination score	Stroop Test interference score	Word Fluency Test score	Letter Digit Substitution Test score	Word Learning Test delayed recall	Purdue Pegboard Test sum score
		Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)
Degrees of hearing loss as measured with pure-tone audiometry							
Normal (0-20 dB)	Model 1	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Mild (20-35 dB)	Model 1	-0.08 (-0.38, 0.23)	0.11 (-3.45, 3.67)	**-1.11 (-2.19, -0.04)**	-0.64 (-1.79, 0.51)	-0.18 (-0.71, 0.36)	**-1.01 (-1.84, -0.17)**
Moderate (35-50 dB)	Model 1	-0.14 (-0.50, 0.21)	-1.76 (-5.91, 2.39)	-1.09 (-2.34, 0.16)	-1.07 (-2.41, 0.27)	-0.37 (-0.99, 0.26)	-0.80 (-1.77, 0.17)
Severe (≥50 dB)	Model 1	-0.33 (-0.98, 0.32)	-1.70 (-9.39, 6.00)	-2.00 (-4.33, 0.33)	-2.42 (-4.92, 0.07)	-0.26 (-1.41, 0.86)	-0.89 (-2.79, 1.00)
Normal (0-20 dB)	Model 2	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Mild (20-35 dB)	Model 2	-0.09 (-0.39, 0.21)	0.12 (-3.43, 3.68)	**-1.17 (-2.24, -0.09)**	-0.69 (-1.84, 0.46)	-0.22 (-0.75, 0.32)	**-1.03 (-1.87, -0.20)**
Moderate (35-50 dB)	Model 2	-0.17 (-0.53, 0.18)	-1.73 (-5.88, 2.42)	-1.23 (-2.48, 0.03)	-1.19 (-2.54, 0.15)	-0.47 (-1.10, 0.15)	-0.87 (-1.84, 0.11)
Severe (≥50 dB)	Model 2	-0.39 (-1.04, 0.27)	-1.72 (-9.41, 5.96)	-2.24 (-4.57, 0.10)	**-2.65 (-5.16, -0.14)**	-0.46 (-1.61, 0.69)	-1.04 (-2.95, 0.86)
Degrees of hearing loss as measured with digits-in-noise test							
Normal (≤-5.55 dB)	Model 1	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Mild (-5.55- -3.80 dB)	Model 1	-0.04 (-0.32, 0.25)	-1.27 (-4.53, 1.98)	-0.88 (-1.69, -0.07)	-0.92 (-1.83, -0.01)	-0.59 (-1.03, -0.16)	0.05 (-0.20, 0.30)
Moderate/severe (>-3.80 dB)	Model 1	-0.27 (-0.60, 0.07)	-5.30 (-9.18, -1.42)	-1.07 (-2.04, -0.11)	-1.50 (-2.60, -0.40)	-0.73 (-1.25, -0.21)	-0.31 (-0.60, -0.02)
Normal (≤-5.55 dB)	Model 2	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Mild (-5.55- -3.80 dB)	Model 2	-0.05 (-0.34, 0.23)	-1.63 (-4.87, 1.62)	-0.92 (-1.73, -0.11)	-0.95 (-1.86, -0.03)	-0.62 (-1.05, -0.18)	0.04 (-0.21, 0.29)
Moderate/severe (>-3.80 dB)	Model 2	-0.03 (-0.64, 0.04)	-5.96 (-9.83, -2.08)	-1.15 (-2.12, -0.19)	-1.55 (-2.65, -0.45)	-0.77 (-1.29, -0.25)	-0.33 (-0.62, -0.03)

Difference: represents the intercept difference in cognitive score per degree hearing loss (both hearing threshold as measured with pure-tone audiometry and speech understanding in noise as measured with the digits-in-noise test) as compared to normal hearing. CI: confidence interval. dB: decibel. Model 1: adjusted for age, sex, education, alcohol consumption, smoking, diastolic and systolic blood pressure, and use of blood pressure lowering medication. Model 2: additionally adjusted for the interaction between age and follow-up time. Analyses using speech understanding were further adjuster for hearing thresholds as measured with pure-tone audiometry. Statistically significant effect estimates ($p < 0.05$) are indicated in **bold**.

Table 4. The additional change in cognitive score per year attributed to different degrees of hearing loss based on the longitudinal analysis (slope differences)

Degree of hearing loss		Mini-Mental State Examination score	Stroop Test interference score	Word Fluency Test score	Letter Digit Substitution Test score	Word Learning Test delayed recall	Purdue Pegboard Test sum score
		Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)	Difference (95% CI)
Degrees of hearing loss as measured with pure-tone audiometry							
Normal (0-20 dB)	Model 1	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Mild (20-35 dB)	Model 1	-0.01 (-0.10, 0.07)	-0.13 (-1.29, 0.43)	-0.00 (-0.24, 0.23)	0.01 (-0.18, 0.21)	-0.09 (-0.20, 0.03)	0.02 (-0.16, 0.19)
Moderate (35-50 dB)	Model 1	-0.04 (-0.14, 0.06)	-0.47 (-1.47, 0.52)	-0.05 (-0.35, 0.24)	0.00 (-0.22, 0.23)	**-0.17 (-0.30, -0.03)**	-0.01 (-0.22, 0.19)
Severe (≥50 dB)	Model 1	-0.05 (-0.23, 0.13)	-1.39 (-3.20, 0.43)	-0.19 (-0.96, 0.58)	0.30 (-0.14, 0.73)	-0.03 (-0.27, 0.22)	-0.15 (-0.54, 0.24)
Normal (0-20 dB)	Model 2	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Mild (20-35 dB)	Model 2	-0.00 (-0.09, 0.08)	-0.23 (-1.09, 0.63)	0.04 (-0.20, 0.27)	0.04 (-0.16, 0.24)	-0.06 (-0.18, 0.06)	0.03 (-0.14, 0.21)
Moderate (35-50 dB)	Model 2	-0.02 (-0.12, 0.08)	-0.03 (-1.05, 0.98)	0.03 (-0.28, 0.33)	0.06 (-0.17, 0.30)	-0.11 (-0.25, 0.03)	0.03 (-0.18, 0.24)
Severe (≥50 dB)	Model 2	-0.01 (-0.19, 0.18)	-0.47 (-2.35, 1.41)	-0.02 (-0.80, 0.75)	0.42 (-0.03, 0.88)	0.09 (-0.17, 0.35)	-0.05 (-0.46, 0.36)
Degrees of hearing loss as measured with the digits-in-noise test							
Normal (≤-5.55 dB)	Model 1	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Mild (-5.55- -3.80 dB)	Model 1	-0.03 (-0.12, 0.05)	-0.15 (-1.01, 0.71)	0.06 (-0.14, 0.25)	0.04 (-0.15, 0.24)	0.07 (-0.04, 0.18)	-0.04 (-0.11, 0.03)
Moderate/severe (>-3.80 dB)	Model 1	-0.07 (-0.15, 0.00)	-0.45 (-1.20, 0.30)	0.04 (-0.14, 0.21)	-0.04 (-0.21, 0.13)	0.04 (-0.06, 0.13)	-0.02 (-0.09, 0.04)
Normal (≤-5.55 dB)	Model 2	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Mild (-5.55- -3.80 dB)	Model 2	-0.03 (-0.11, 0.06)	0.06 (-0.79, 0.91)	0.08 (-0.12, 0.27)	0.06 (-0.14, 0.25)	0.08 (-0.03, 0.19)	-0.03 (-0.11, 0.04)
Moderate/severe (>-3.80 dB)	Model 2	-0.04 (-0.12, 0.03)	0.10 (-0.66, 0.87)	0.10 (-0.08, 0.28)	0.00 (-0.17, 0.18)	0.07 (-0.03, 0.17)	-0.01 (-0.08, 0.05)

Difference: represents the additional change in cognitive score per year increase in follow-up time per degree hearing loss (both hearing threshold as measured with pure-tone audiometry and speech understanding in noise as measured with the digits-in-noise test) as compared to normal hearing. CI: confidence interval. dB: decibel. Model 1: adjusted for age, sex, education, alcohol consumption, smoking, diastolic and systolic blood pressure, and use of blood pressure lowering medication. Model 2: additionally adjusted for the interaction between age and follow-up time. Analyses using speech understanding were further adjuster for hearing thresholds as measured with pure-tone audiometry. Statistically significant effect estimates ($p < 0.05$) are indicated in **bold.**

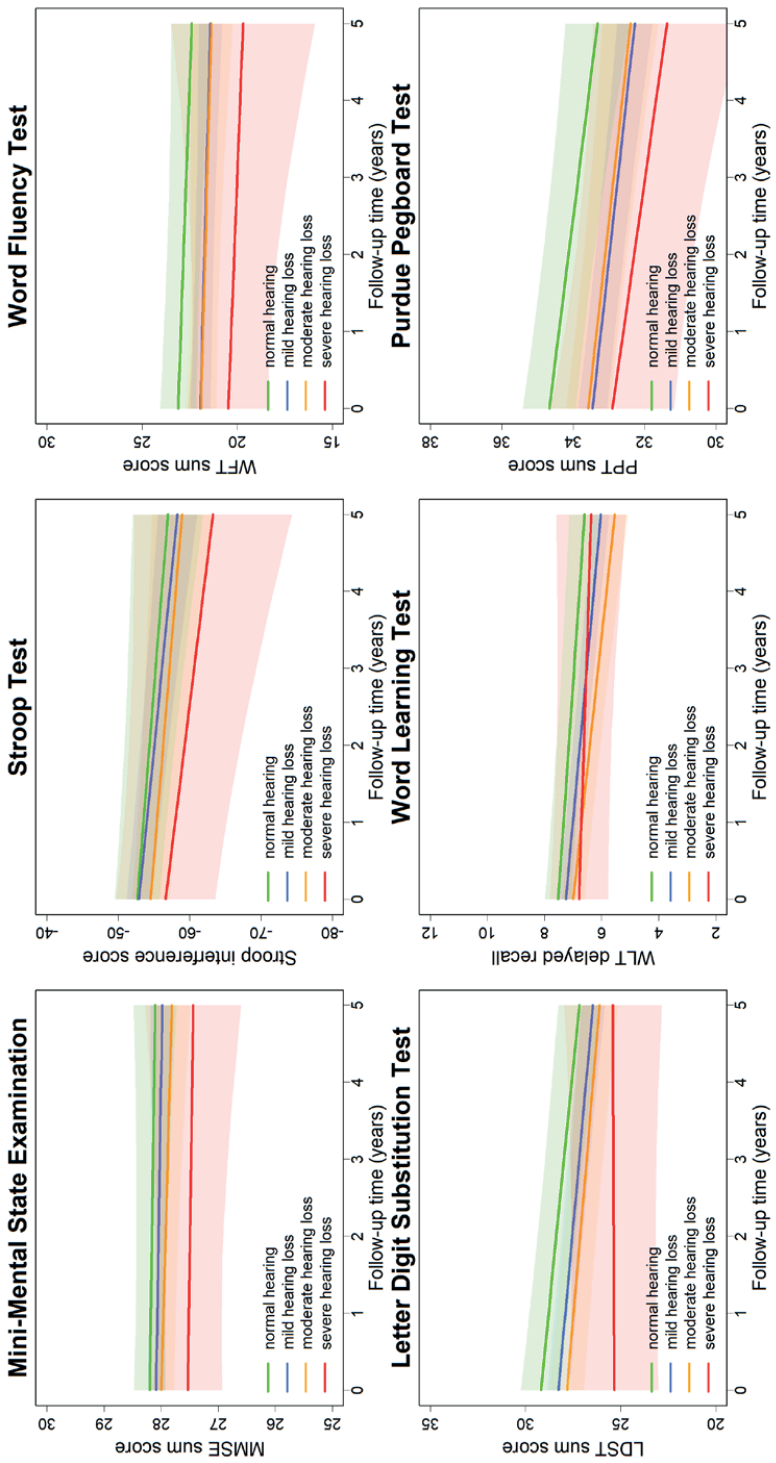

Figure 1. Estimated cognitive function trajectories over time for different degrees of hearing loss as measured with pure-tone audiometry, with corresponding 95% confidence intervals: adjusted for age and sex.

all cognitive tests, though not statistically significant (Table 3; model 1). Longitudinally, any hearing loss, compared to normal hearing thresholds, modified the slope of cognitive decline for all tests over time, though only statistically significant for the 15-WLT in participants with moderate levels of hearing loss (Table 4; model 1). Comparable slope differences, albeit not statistically significant, were found for any hearing loss, as compared to normal speech understanding in noise (Table 4, model 1). Interestingly, the significant slope difference of the 15-WLT becomes statistically non-significant, and slope differences of other cognitive tests becomes small or close to zero (Table 3; model 2; figure 1) after additional adjustment for the interaction between age and follow-up time. Comparable results are found for degrees of hearing loss as measured with the DIN test (Table 4; model 2).

Moreover, assessing hearing levels continuously showed that the additional change in cognitive functioning attributable to hearing loss were small and non-significant for both hearing thresholds and speech understanding in noise (Supplementary table 2). Results did not differ between males and females or between midlife and late-life (Supplementary tables 3 and 4).

DISCUSSION

In this large population-based study in non-demented older adults, we found that hearing loss was associated with poorer cognitive functioning, expressed by lower scores on the MMSE, Stroop test, WFT, LDST, 15-WLT and the PPT. After adjustment for the possible non-linear effects of age on cognitive change during follow-up, we did not find that hearing loss for either hearing thresholds or speech understanding in noise accelerates cognitive decline over time.

Strengths of this study are its prospective and longitudinal population-based design, the large sample size and the standardized assessment of hearing thresholds with pure-tone audiometry and a speech-in-noise test as well as cognitive functioning with an elaborate neuropsychological assessment. However, the following limitations of this study must be considered. First, although we extensively adjusted for age and other important confounders, residual confounding might still be present. Second, as hearing assessment has been added to the study protocol in 2011, dementia incidence of participants with a baseline hearing assessment is small (N = 15), precluding the possibility to analyse whether hearing loss is associated with an increased risk of dementia.

Our cross-sectional results were comparable with other studies, reflected in lower scores on cognitive tests with higher levels of hearing loss.[1-3] In our longitudinal analysis we found an accelerated decline in memory function (as measured with the 15-WLT) with moderate hearing loss, which is comparable to the results and effect estimates of

other population-based studies.[4, 5] Importantly, with further adjustment for confounding by age, this association became weaker and statistically non-significant. The prevalence of both hearing- and cognitive impairment increases substantially with age.[6, 7] Moreover, it is also important to consider, especially in longitudinal studies with a wider age range, that older individuals may decline faster on cognitive test performance between baseline and follow-up measurement than their younger counterparts.[8] Therefore, we added the interaction between baseline age and follow-up time into our statistical models, which seemed to explain most of the effects of hearing loss on memory function as the slope difference becomes statistically non-significant in the second model. Moreover, (non-significant) slope differences of the other cognitive tests also became small or close to zero in the second model as compared to the first model. To our knowledge, only one other study incorporated non-linear effects of age in their statistical model.[4] Therefore, verification in future studies is needed to explore whether effects of hearing loss on cognitive decline extend beyond 'normal' age-related decline of cognitive function.

Besides elevated hearing thresholds, speech understanding in noise could contribute towards accelerated cognitive decline. The ability to understand speech in noise is a complex process in which elements of peripheral processing interact with more centrally located elements of auditory processing.[9] As such, it may be hypothesized that a potential association with cognitive functioning may even be stronger when specifically speech understanding is reduced. Interestingly, we found the same (non-significant) results between speech understanding in noise and cognitive decline. This may be explained by the fact that there is a high correlation between hearing loss based on audiometry and speech in noise results in our population.[9]

It is also worthwhile considering whether found associations in our and previous studies might be driven by confounding and/or bias. The absence of an effect of hearing loss on cognitive decline in the current study is not explained by selection bias, as the sample with both a baseline- and a follow-up measurement were significantly older than the participants with just a baseline measurement. Moreover, significant associations in other studies may be explained by the possibility that poorer hearing influences certain neuropsychological tests which rely heavily on auditory function rather than cognition per se,[8, 10] which is replicated in our study with significant cross-sectional as well as intercept differences on cognitive test scores among degrees of hearing loss. Also, hearing loss in older adults may lead to more medical attention, resulting in over-diagnosis of cognitive impairment.[10] Moreover, it has been proposed that upstream common causes, i.e., inflammation, vascular pathology, and other systemic neurodegenerative processes, may lead to both hearing loss and cognitive decline through central nervous system-wide functional decline, rather than that those two are related to one another.[8] As such, greater sensitivity in one domain could identify impairments in that domain prior to the other, leading to the appearance of a false direct association.[8, 10]

We should also acknowledge that our follow-up time (mean = 4.4 years) may have been too short to capture a possible small, but significant effect of hearing loss on cognition. Cognitive decline with age is gradual;[8] therefore, studies with sufficient follow-up time are needed to truly capture trajectories of cognitive function. Epidemiological evidence has shown that elevated blood pressure in mid-life, an established modifiable risk factor of dementia, increases the risk of cognitive impairment 20-30 years later.[11-14] In contrast, another study with a follow-up of 8 years did not find an association between hypertension and cognitive functioning.[15] The differences in these results suggest that the follow-up time would need to be longer to show a potential association of hearing loss with cognitive decline.

In conclusion, hearing loss was significantly associated with accelerated decline on the 15-WLT measuring memory function. Notably, this association seemed to be driven by non-linear effects of age. Future, population-based studies are needed to confirm the role of hearing loss as a potential modifiable risk factor for cognitive decline, whilst paying attention to a probable strong effects of age on cognition.

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Democritus, de lachende filosoof; Johannes Moreelse[1]

5

General discussion

GENERAL DISCUSSION

In recent years, age-related hearing loss in epidemiological research has been growing from a new kid on the block, into an established and relevant outcome assessed in population-based studies. Once regarded as an inevitable part of ageing, increased interest in the etiology and potential adverse outcomes of age-related hearing loss revealed to the scientific and medical world that hearing loss is a problem in the elderly population that should be acknowledged and treated properly. Several population-based studies reported that hearing loss is associated with social isolation, loneliness, and depression, but also with an increased risk of dementia.[2, 3] Especially the latter association has put hearing loss on the map as a condition seriously affecting quality of life, general health and psychosocial well-being in the elderly. Moreover, it has inspired interest into hearing loss as a potentially modifiable factor in neurological diseases.[4] However, the underlying pathway explaining this association remains unknown. Large, population-based studies provide the unique opportunity to further elucidate if and how hearing loss in the elderly is associated with an increased risk of dementia. As such, more can be said on whether hearing rehabilitative treatments may potentially alter or delay the progression of cognitive decline and dementia onset.

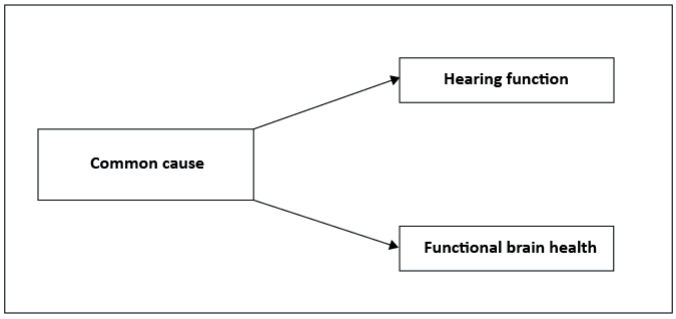

Figure 1. Common-cause hypothesis

The objective of this thesis was to gain new insights into the '*common-cause hypothesis*' (**figure 1**) and the '*sensory deprivation hypothesis*' (**figure 2**), two hypotheses proposing potential underlying mechanisms in the recently discovered association between hearing loss and dementia.[4] Specifically, I explored potential risk factors for hearing loss on the one hand, and risk factors for brain health on the other hand. Identifying common risk factors for both hearing function and brain health may shed more light on the *'common-cause hypothesis'*. Subsequently, I have addressed potential direct interrelations between hearing loss and brain health, to further explore the

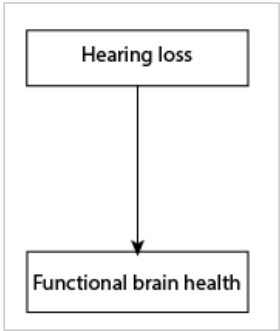

Figure 2. Sensory-deprivation hypothesis

'sensory-deprivation hypothesis'. Most studies described in this thesis were embedded within the prospective, population-based Rotterdam Study.[5] One study in this thesis was embedded within the Atherosclerosis Risk in Communities (ARIC) study from the United States of America.

In this chapter I will first review and discuss the main findings described in this thesis. Next, I will discuss methodological issues which should be considered when interpreting the findings. Finally, I will conclude with potential implications of my research with regard to clinical practice and future research.

MAIN FINDINGS

Risk factors for hearing function

Currently, several demographic- and lifestyle factors are known to contribute to elevated hearing thresholds in the elderly. One of the biggest demographic risk factors is age, reflected by exponentially increasing hearing thresholds with higher age.[6] Even though this is essential information to lay the foundation for further research into hearing loss by defining the prevalence and potential societal impact of hearing loss within an ageing population, it is obviously a non-modifiable risk factor. Therefore, other studies mostly focused on risk factors that are potentially modifiable. One of those well-established risk factors is smoking, reflected in a higher incidence of hearing impairment in smokers.[7-9] On top of this, smoking cessation virtually eliminates an increased risk of developing hearing impairment.[7] Following these promising results, I was interested in potential preventive effects of other lifestyle- and cardiovascular risk factors on hearing function.

The risk factors explored in the current thesis, for both hearing function and brain health, were selected based on existing knowledge. To be more specific, I was especially interested in factors known to be (also) associated with dementia. As such we might be able to draw conclusions about preventive factors for hearing function and brain health and eventually about the common-cause hypothesis.

Adherence to a healthy dietary pattern has been reported to lower the risk of dementia.[10] Thus, it may be hypothesized that diet is a potential common cause in the association between hearing loss and dementia. In the relatively few studies examining nutritional factors and hearing loss, it was seen that sufficient consumption of fish, meat, vitamin C, vitamin B_{12} and moderate intake of fat and alcohol was related with lower hearing thresholds.[11-15] The downside of assessing individual food components is that it does not acknowledge the complex interactions that occur across different food items and nutrients.[16] Surprisingly, overall dietary pattern as a risk factor for hearing loss is relatively unexplored. Two other population-based studies found cross-sectional associations between better diet quality and lower hearing thresholds, however they did

not replicate this association at follow-up.[17, 18] In **chapter 2.1**, it appeared that dietary composition did not affect hearing function, both at baseline and over time. Translating my results into clinically relevant measures of ageing within hearing function (+1 dB per year),[19] counterintuitively it appeared that adhering to a healthier dietary pattern was equivalent to 0.2 years of ageing. However, it is questionable whether such a small difference should be regarded as a clinically relevant effect on hearing abilities. Even though other studies confirmed protective effects of certain individual food components, it is questionable whether overall dietary pattern directly affects hearing function. Regardless of the non-significant association between diet quality and hearing levels, it is well-known that an unhealthy diet is the biggest contributor to obesity, which is currently one of the larger public health issues.[20] On top of this, obesity has been identified as a risk factor for dementia[20, 21] and it has been argued that obesity has a detrimental effect on hearing function,[22] making it a potential common cause. Most studies exploring the association between obesity and hearing loss used BMI as a measure of body composition.[22] However, BMI does not differentiate between metabolic healthy and unhealthy body mass. Therefore, dividing BMI further into fat mass index (FMI) and fat-free mass index (FFMI) may be a more accurate reflection of body composition. Especially since the cochlea is a heavily vascularized organ and consequently is prone to any change in cardiovascular health, associations between BMI and hearing loss may be largely explained by the effects of FMI. Indeed, in the Rotterdam Study I found that higher BMI, and especially a higher FMI, were related to higher hearing thresholds (**chapter 2.1**). However, a higher BMI and/or a higher FMI did not result in a statistically significant faster decline in hearing function over time. This may be partly due to a relatively short follow-up period (average of 4.4 years). Nonetheless, the results in this study can be considered relevant as the effect of obesity on hearing function is comparable to one year of ageing in hearing function.[19] Besides these nutritional- and cardiovascular risk factors, previous studies have focused on more direct, generalized markers of cardiovascular disease such as atherosclerosis. And indeed, during the last years, it has become clear that higher atherosclerotic burden leads to worse hearing function.[23] In light of the common-cause hypothesis, it is known from previous studies in the Rotterdam Study population that higher atherosclerotic burden leads to a higher risk of dementia.[24] On top of this, it was reported that carotid atherosclerosis is related with cognitive decline apart from normal age-related declines.[24, 25] In this thesis I found that higher plaque burden and increased intima media thickness of the carotid artery resulted in higher hearing thresholds (**chapter 2.2**). On top of this, atherosclerosis specifically seemed to exert its influence on hearing loss in the right ear, not in the left ear. Clinically, the effect of atherosclerosis is even more striking. Overall, higher atherosclerotic burden is related to 2 – 3.5 years of ageing in hearing function. From a clinical perspective, the impact of atherosclerosis seems to go beyond merely the risk of cardiovascular events.[26] Thus,

early detection and prevention of atherosclerosis by therapeutic- or lifestyle interventions carries the promise to not only lower the risk of clinical cardiovascular events and mortality, but also to delay the onset or slow down the progression of hearing loss by promoting and maintaining inner ear health.

Promoting hearing function

Unfortunately we are unable to cure hearing loss. Therefore it is of great importance to focus on the prevention of accelerated decline, beyond 'normal' age-related degeneration of hearing function by identifying modifiable risk factors. From the above studies it seems that promoting cardiovascular health through maintaining an optimal body composition and as such prevent cardiovascular disease may prove beneficial in promoting hearing function and maintaining inner ear health in the elderly population. Even though it is extremely important to adhere to a healthy dietary pattern for multiple reasons, based on our results it does not seem likely that diet quality is related to hearing function. Yet, we should keep in mind that an unhealthy diet is one of the largest contributors to obesity. Whereas diet quality might not have a direct effect on hearing function, it may exert its effects indirectly through body composition.

Risk factors for brain health

With the ageing of the population, not only the prevalence of hearing loss is increasing, also the number of dementia cases is showing a steep upward trend.[4] For this reason, research has focused on the prevention of cognitive impairment and dementia in the preclinical phases and identified some promising modifiable risk factors. For example, epidemiological evidence has shown that elevated blood pressure in mid-life increases the risk of cognitive impairment 20-30 years later.[27, 28] Thus, treating hypertension by medical- or lifestyle interventions may prevent cognitive decline and lower the risk or delay the onset of dementia. As such, preventing accelerated neurodegeneration, apart from 'normal' declines in brain volume and function with age, may prove as an effective strategy in lowering the risk of dementia. Yet, more in-depth knowledge on modifiable risk factors for brain health is needed.

Aside from the non-significant results between diet quality and hearing loss in **chapter 2.1**, the evidence in regard to dietary factors, brain health and dementia have been more conclusive. Specifically, we know that B vitamins, vitamin E, and the *n*-3 fatty acid docosahexaenoic acid that can be found in vegetables, fruit and seafood, have neuroprotective effects.[16, 29] However, people do not only consume fish, vegetables or vitamin E. Therefore, overall dietary pattern is a more realistic and accurate reflection of daily life. Evidence has shown that better adherence to the Mediterranean Diet in other European populations supports brain health, reflected in larger grey- and white matter volumes.[30, 31] Using the Rotterdam Study dietary guidelines in relation to brain

measures,[32] participants with healthier dietary patterns had larger brain tissue volumes. Specifically, adhering to a healthier diet was related to larger white matter volumes. Putting it differently, eating unhealthier appeared to be equivalent to 0.5 years of ageing in brain health.[33] Interestingly, diet quality did not seem to influence the volume of white matter hyperintensities, nor the presence of lacunes or microbleeds (**chapter 3.1**). Zooming in on specific dietary vitamins, it is known that vitamin D deficiency is associated with an increased risk of dementia and has previously been linked to worse hearing function, making vitamin D status a potential common cause.[34, 35] Unexpectedly, vitamin D in relation to brain health in dementia-free individuals remains relatively unexplored. In **chapter 3.2** I found that participants with vitamin D deficiency had on average smaller white matter volumes, which was equivalent to 1.6 years of ageing. Moreover, deficient vitamin D levels seemed to result in smaller hippocampus volumes, an area in the brain important for memory. Dietary pattern and vitamin D levels may be directly related to brain health through neuroprotective effects of both. Additionally, an indirect link may also be present. For example, it is plausible that the people with a healthy dietary pattern and high vitamin D levels may be higher educated and as such are more aware of the beneficial health effects of adhering to a healthy lifestyle. Concluding from my results, overall diet quality and vitamin D status may be a promising modifiable risk factor in the prevention of dementia by supporting brain health in the preclinical phases. As such, it is of importance to raise awareness of the beneficial effects of eating healthy and maintaining optimal vitamin D levels (possibly through vitamin D supplementation) in the general population and amongst general practitioners. Additionally, from an economic standpoint, promoting a healthy dietary pattern may be supported by lowering prices of healthy products and/or increase the prices of unhealthy products and possibly target (grocery store) advertisements at healthy foods and/or ban advertisements that recommend purchases of unhealthy products. Applying such strategies may also have additional beneficial effects in the battle against the so-called obesity epidemic.[20] As stated earlier, obesity may be a common-cause in the association between hearing loss and dementia.[21, 36] Previous studies mostly used BMI as a measure of obesity in relation to brain health.[36-38] Though, this does not take into account that in the elderly fat mass tends to decrease and lean mass tends to increase, making BMI a less suitable strategy to infer on obesity in the elderly.[21, 39] Additionally, it may be hypothesized that especially unhealthy fat mass may have negative effects on brain health through system wide cardiovascular disease.[25] Unexpectedly, differentiating between FMI and FFMI did not show an association (both statistically significant and clinically relevant) between higher FMI and changes in brain volume, white matter microstructure, nor the presence of markers of cerebrovascular disease such as white matter hyperintensity volume, lacunes and microbleeds (**chapter 3.3**). This may be partly explained by selection bias in the study population as the sample with a follow-up MRI scan were younger and healthier

than the participants with just a baseline MRI scan. Moreover, equal to **chapter 3.1**, we might be dealing with an insufficient follow-up time to capture a potential small, but significant effect of obesity on brain health.

Promoting brain health

With increasing age, our brain will undergo so-called normal age-related changes, such as decreasing cell function and volume, increasing cerebrospinal fluid volume, the formation of white matter hyperintensities, decreased microstructural organization, and potential formation of lacunes and microbleeds (**figure 3** shows an example of A; a microbleed, and B; white matter hyperintensities).[40] Nowadays, no treatment exists to prevent this age-related neurodegeneration. However, great promise may lay in preventing accelerated neurodegeneration when no cognitive decline or cognitive impairment is present yet. From our results, in a dementia-free population, it can be concluded that adhering to a healthy lifestyle by consuming an overall healthy dietary pattern and maintaining sufficient vitamin D levels might directly support brain health. Although we could not confirm an effect of obesity on neurodegeneration, maintaining a healthy body composition may indirectly support brain health as it is plausible that all lifestyle factors are highly intertwined with one another.[21]

Age related hearing loss and brain health: the common-cause hypothesis

Identifying potential common-causes in the association between hearing loss and dementia is important to truly establish whether hearing loss is directly related to brain health, cognitive function, and eventually dementia. Based on my results it is doubtful

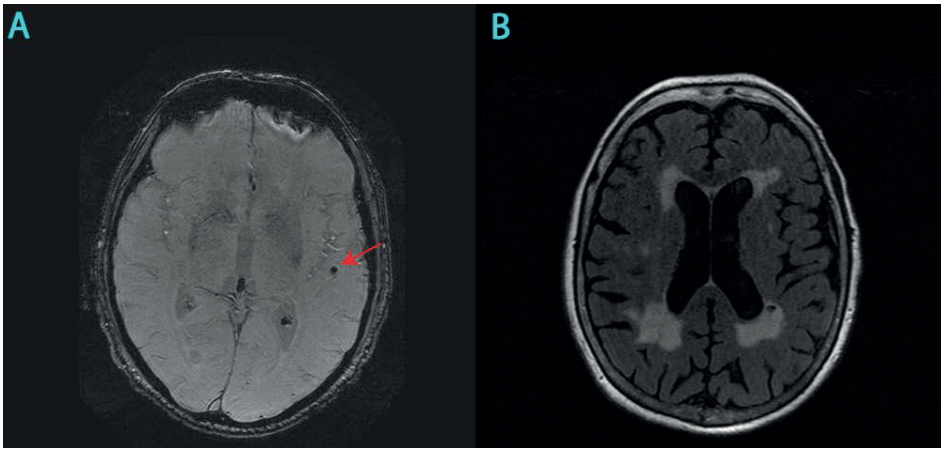

Figure 3. A) Axial slice of a susceptibility weighted image (SWI). The red arrow indicates a microbleed. B) Axial slice of a T2-weighted fluid attenuated inversion recovery (FLAIR) image on which white matter hyperintensities are visible. Images were obtained by means of a 1.5 tesla MRI scanner.

that dietary pattern and obesity on their own are a third factor causing both hearing loss and cognitive impairment. Yet, cardiovascular disease and vitamin D levels may actually be a common-cause as they both relate to worse hearing function and diminished brain health. As such, they might also be important confounders in previously found associations between hearing loss and dementia. Even though I did not find a direct relation of obesity and diet quality on either brain health or hearing function, we should not disregard them entirely as a potential common-cause. It is plausible that the accumulation of several lifestyle factors and cardiovascular risk factors all together exerts its effects on neurodegeneration and/or hearing loss. In future studies exploring hearing loss as a modifiable risk factor for dementia, it might be worthwhile to adjust for cardiovascular disease, vitamin D status and other lifestyle factors. If positive associations continue to exist after this adjustment, then there is more ground to make statements about a potential direct association between hearing function and brain health. Moreover, it might be of interest to explore whether hearing loss could act as a mediator in the association between cardiovascular risk factors, cardiovascular disease and dementia.

Interrelations between hearing function and brain health

Even though there is support for the common-cause hypothesis, it has been demonstrated that age-related sensory degeneration is also at least in part independent of cognitive degeneration. Such independent effects would not be observed if there is a single common-cause underlying all decline.[41] Hence, in recent years, interest has also increased in a potential direct link between hearing function and brain health. It has been argued that impoverished auditory input results in permanent cognitive changes, possibly through neuroplastic changes that disadvantage general cognition in favour of processes supporting speech perception.[41] Such chronic reallocation of cognitive resources may produce permanent changes in structural and functional brain health over time. Even though research on the association between hearing loss, cognition and dementia has increased exponentially, it is necessary to replicate previously found findings in various population-based samples.

Tinnitus and brain health

Besides hearing impairment, tinnitus is a common auditory disorder in the adult population.[42] It is characterized by the perception of a sound, without an objective corresponding sound source being present. Research has shown that hearing loss is one of the biggest risk factors for tinnitus: 90% of the people with tinnitus also have hearing loss.[42] Besides peripheral involvement, it has been argued that there is a central process contributing to the pathogenesis of tinnitus.[43] A cross-sectional analysis within the Rotterdam Study reported associations between higher levels of hearing loss and smaller brain tissue volumes.[44] As hearing loss is highly related to tinnitus, it might be

argued that the same central processes occur in individuals with tinnitus as in those with hearing loss, i.e. having tinnitus is associated with smaller brain volumes. Interestingly, I found the opposite. In **chapter 4.1** I have described how in the Rotterdam Study population, having tinnitus is associated with larger instead of smaller brain volumes. These associations were independent of age and amount of hearing loss. Another study reported no significant associations between tinnitus and brain volumes.[45] These authors suggested that smaller brain volumes may be explained by comorbid hearing loss, which is largely determined by age. Other studies also proposed that grey matter changes, which is known to degenerate with age,[40] are attributable to age-related hearing loss rather than the tinnitus per se.[45, 46] This might suggest that tinnitus has more of a neurodevelopmental origin potentially increasing the risk of developing future tinnitus in people with larger brain tissue volumes from a young age onwards. My results add to the knowledge on the pathophysiology of tinnitus. Nonetheless, it is plausible that tinnitus does not play a role in the association between hearing loss and dementia.

Hearing loss and brain health

Typically, research in ageing of the brain has mostly focused on macro structural neurodegeneration, e.g. cell loss and the formation of white matter hyperintensities visible on MRI-scans. Besides degeneration of the macrostructure, changes in the underlying microstructure occur which are invisible to the human eye. These changes even take place before macro structural cell loss or the formation of white matter hyperintensities sets in.[47] Therefore, it has been suggested that degeneration of the underlying microstructure is an earlier, more sensitive marker of neurodegeneration.[47] Following this, it was seen in the Rotterdam Study that higher levels of hearing loss were related to diminished white matter microstructural integrity, independent of macro structural brain measures.[48] Until recently, this was the only large population-based study exploring the association between hearing loss and microstructural integrity of the brain. In an American population-based sample, I identified that hearing loss had a negative effect on white matter microstructure in the temporal lobe and in several white matter limbic fibres (**chapter 4.2**). Moreover, I found that hearing loss was associated with lower grey matter microstructure of the hippocampus. Research on diffusion imaging so far mostly focused on the white matter microstructural degeneration of the brain. Fewer studies researched grey matter microstructure, even though there is evidence that grey matter microstructure degenerates with increasing age.[49] Therefore, it is unclear whether found associations with lower grey matter microstructural integrity in the hippocampus are also clinically relevant. Nonetheless, my results support the sensory-deprivation hypothesis, describing a direct causal relationship in which diminished auditory input leads to neuroplastic changes in the brain.

Hearing loss and cognitive decline

Moving further downstream from brain health towards cognitive function, it has been repeatedly reported that hearing loss is associated with accelerated cognitive decline.[4] Whilst this is an important and promising finding in the search towards preventive factors for dementia, it is important to realize that both hearing- and cognitive function are heavily dependent on age.[50, 51] Therefore, it remains unclear whether the effects of hearing loss on cognitive function are independent of concurrent ageing effects. Whilst adjusting for baseline age takes account of differences in hearing- or cognitive function due to age at baseline, it fails to take into account that older people will decline faster in cognitive function over time compared to their younger counterparts. Adding an interaction of age and follow-up time into statistical models will filter out these ageing effects over time. This is supported by our findings in **chapter 4.3**. Even though we saw that any hearing loss accelerated decline of memory functioning, this association disappeared after adding above mentioned interaction into our models. Yet is important to acknowledge that our relatively short follow-up time (4.4 years) and limited amount of repeated measurements (2 cognitive assessments) may have resulted in these non-significant results. Cognitive decline with age is gradual,[52] thus studies with sufficient follow-up time are needed to identify potential small, but significant effects. Indeed, a study with 8-year of follow-up time did not find a significant association between hypertension, a well-known risk factor for dementia,[4] and risk of cognitive impairment.[53] On the other hand, in a study with 20-30 years of follow-up researchers reported an increased risk for cognitive impairment related to hypertension during mid-life.[27, 28] This underlines the need for sufficient follow-up time to capture an effect of hearing loss on cognitive decline.

Age related hearing loss and brain health: the sensory-deprivation hypothesis

In this thesis I have found some promising evidence pointing towards a direct link between hearing loss and brain health, namely an independent association between higher levels of hearing loss and diminished brain health as reflected in lower microstructural integrity. On top of this, hearing loss had a negative impact on cognitive function. Yet, age-related hearing loss did not accelerate cognitive decline over time. This finding underlines the need for two things to truly establish whether hearing loss is independently related to brain health, cognitive decline and dementia. First, longitudinal data with repeated measurements with sufficient follow-up time is warranted. Second, in statistical models it is essential to take the strong effects of ageing and other confounders into account. As I will describe in more detail below, it is difficult to truly filter out the confounding effects of ageing. Due to these strong effects, it should be considered that age might act as a third factor, a common-cause, in the association between hearing loss and brain health.

METHODOLOGICAL CONSIDERATIONS

Study design

All studies described in this thesis are embedded within population-based cohort studies. Most of them (**chapter 2.1 & 2.2; chapter 3.1 – 3.3; chapter 4.1 & 4.3**) were part of the Rotterdam Study; an ongoing prospective population-based cohort study in the area of Ommoord, Rotterdam, the Netherlands, initiated in 1989.[5] **Chapter 4.2** was embedded within the Atherosclerosis Risk in Communities (ARIC) study, an ongoing cohort study from four US communities (Washington County, Maryland; Forsyth County, North Carolina; Jackson, Mississippi; and Minneapolis, Minnesota) from 1987 onwards.[3, 54] Population-based cohort studies provide the unique opportunity to accurately study the incidence, but also the etiology of a large variety of diseases. Besides this, another specific advantage of population-based studies is that the findings may be generalized to a large portion of the population.[55] Despite these advantages, there are also some limitations that should be acknowledged.

Population-based studies, like all other studies, may be subject to specific types of bias. To be more specific, selection bias, information bias and confounding may play a role in associations between a determinant and an outcome and increase the risk of potential false negative results or false positive results. Despite the best efforts to minimize these biases through procedures such as random sampling from the general population, blinded measurements, maintaining high response rates, and adjustment for potential confounders in statistical analyses, the results in any study may still suffer from some residual confounding and/or bias.

A number of studies in this thesis are of a cross-sectional design. Even though the Rotterdam Study and the ARIC study have a considerable history of data collection, hearing assessment has only been added into the core study protocol in both studies in 2011 and in 2016, respectively. Moreover, magnetic resonance imaging (MRI) of the brain has been included into the core study protocol of the Rotterdam Study in 2005 and in ARIC in 2011 (see **figure 4** for an overview of the Rotterdam Study). As such, few follow-up data is available for particularly hearing function, limiting some of our studies towards a cross-sectional design (**chapters 2.2; 3.1; 3.2; 4.1; 4.2**). A well-known limitation of cross-sectional studies is the lack of the ability to establish a temporal effect, i.e. determine whether the determinant actually precedes the outcome. In above mentioned chapters, it can only be speculated that the determinants and outcomes are related to one another in that specific order. However, biologically it is very unlikely that for example in **chapter 2.2** higher levels of hearing loss will lead to increased plaque burden and higher intima media thickness in the carotid artery, or that in **chapter 4.2** lower microstructural integrity will cause more hearing loss.[41]

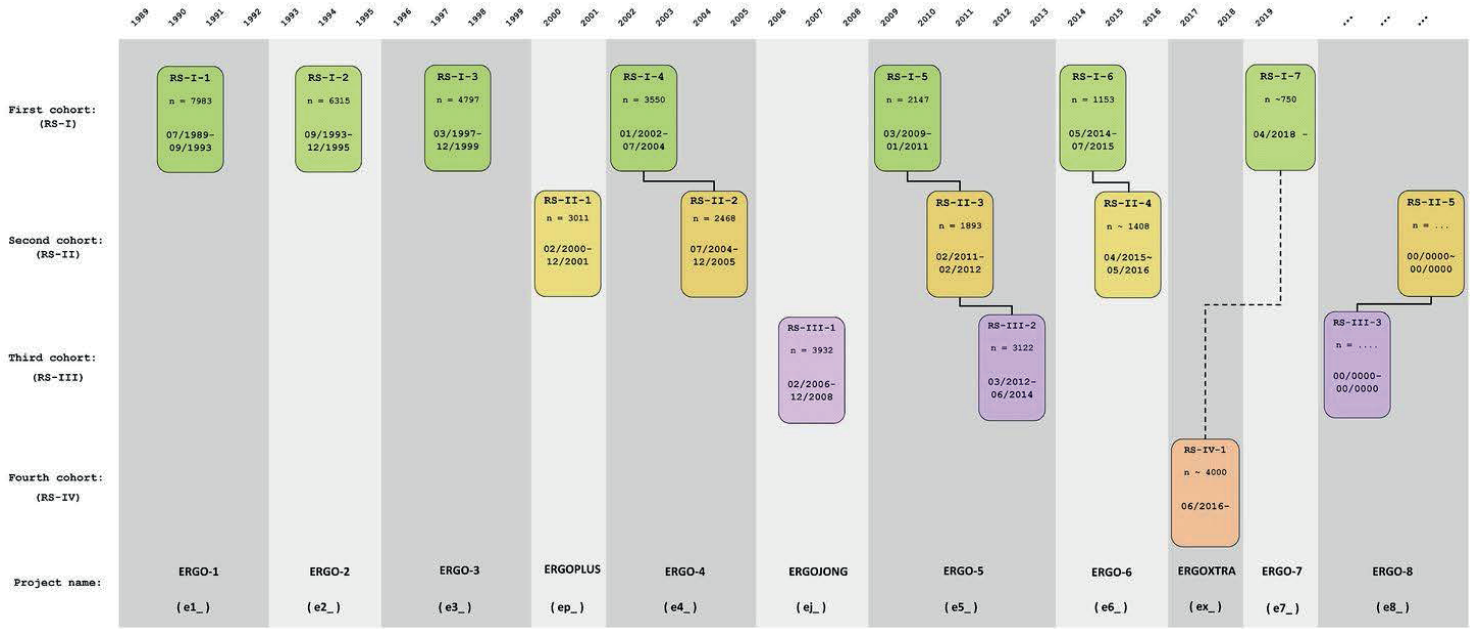

Figure 4. Overview of the Rotterdam Study cohorts and follow up scheme

Effects of age in cross-sectional studies

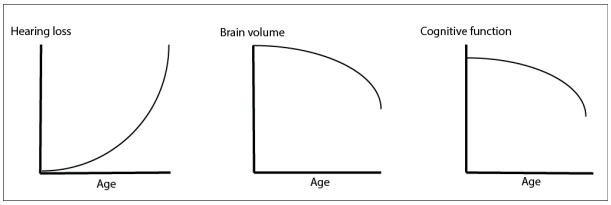

Figure 5. Hypothetical trend of hearing function, brain volume and cognitive function with ageing

Besides these limitations in the cross-sectional studies concerned, I might be dealing with residual confounding by age effects. Especially when I am exploring potential direct associations between hearing loss, brain health and cognitive function. The amount of hearing loss increases, and brain volume and cognitive function decreases in a non-linear fashion with older age (see for a hypothetical example: **figure 5**).[40, 50, 51, 56, 57] Adjusting for the linear term of age or the non-linear term of age (age^2 or the spline of age) in statistical models will sufficiently adjust for baseline age differences, i.e. it will take into account that due to age, older people will have smaller brain volumes or a lower cognitive function at baseline. However, residual confounding due to strong, far-reaching effects of age might still be present. Not only hearing loss, brain health and cognitive function are heavily dependent on age, the confounders in our statistical models are highly related to age as well. In **Table 1**, I have listed every determinant, outcome or confounder used in any of my studies ordered by age group. Measurements of these variables have been obtained during research visit 5 (e5; **figure 3**) of the Rotterdam Study, the same study visit as when hearing assessment was added to the study protocol. In this table we see that the levels, amount or presence of almost every factor increases substantially with age. So for example, we see in the eldest group of the Rotterdam Study population (80 – 99 years), lower levels of physical activity (median MET h/week of 20.0), higher plaque burden (median plaque score of 2.5), higher levels of hearing loss (mean hearing threshold of 45.8 dB), and a lower score on the 15-word learning test (mean score of 5.9) as compared to the youngest participants of the Rotterdam Study (51 – 65 years; MET h/week: 42.0; plaque score: 1.0; hearing threshold: 18.0 dB; 15-word learning test score: 9.0, respectively). So it is extremely complex in cross-sectional studies to truly adjust for effects of ageing as it is highly intertwined with every variable in the different studies throughout this thesis. Following this reasoning, we might be looking at effects of ageing instead of a direct effect of vitamin D deficiency (**chapter 3.2**) on brain health as the lowest levels of vitamin D are measured in the eldest age group. This might also be the case in **chapter 4.2** in which I report associations between higher levels of hearing loss and lower microstructural integrity. Both hearing function and brain health decline steeply with age (presence of severe hearing impairment in youngest participants: 0.4%; vs in oldest participants: 11%; total brain volume in youngest participants: 959.8 mL; vs in

Table 1. Overview of characteristics of participants (cohort I, II, and III) of the Rotterdam Study during visit 5 (2009-2015) by age groups.

	Entire sample	Participants 51 - 65 years	Participants 65 - 80 years	Participants 80 – 99 years
	N = 6,279 (100%)	N = 1,996 (31.8%)	N = 3,379 (53.8%)	N = 904 (14.4%)
Demographic				
Age, years	69.6 (9.2)	59.3 (3.6)	71.8 (4.4)	84.5 (3.5)
Female, N (%)	3,593 (57.2)	1,140 (57.1)	1,898 (56.2)	555 (61.4)
Educational level, N (%)				
Primary	538 (8.6)	141 (7.1)	252 (7.5)	145 (16.0)
Lower/intermediate	2,475 (39.4)	627 (31.4)	1,459 (43.2)	389 (43.0)
Intermediate vocational	1,830 (29.6)	612 (30.7)	975 (28.9)	273 (30.2)
Higher vocational	1,341 (21.4)	609 (30.5)	644 (19.1)	88 (9.7)
Lifestyle factors				
Physical activity, MET h/week*	40.1 (15.7 – 79.4)	42.0 (18.0 – 76.7)	44.4 (17.5 – 85.5)	20.0 (8.3 – 49.3)
Smoking yes, N (%)	3,270 (52.1)	942 (47.2)	1,820 (53.9)	508 (56.2)
Alcohol consumption, grams p/day*	6.8 (0.9 – 17.4)	8.0 (1.3 – 19.2)	7.3 (0.9 – 17.5)	2.7 (0.0 – 11.6)
Dietary adherence score*	7.0 (6.0 – 8.0)	7.0 (6.0 – 8.0)	7.0 (5.0 – 8.0)	7.0 (5.0 – 8.0)
Vitamin D status, nmol/L	60.9 (27.7)	59.4 (27.2)	64.5 (28.5)	54.2 (31.1)
Cardiovascular risk factors				
Body mass index, kg/m^2	27.5 (4.3)	27.3 (4.5)	27.7 (4.2)	27.0 (4.0)
Fat mass index, kg/m^2	10.0 (3.3)	9.8 (3.4)	10.1 (3.2)	9.9 (3.1)
Fat-free mass index, kg/m^2	17.5 (2.1)	17.5 (2.2)	17.5 (2.0)	17.1 (1.9)
Systolic blood pressure, mmHg	143.9 (22.2)	132.4 (18.2)	147.8 (21.0)	155.9 (23.5)
Diastolic blood pressure, mmHg	83.5 (11.2)	82.1 (10.8)	84.4 (11.1)	83.3 (11.9)
Anti-hypertensive medication use, N (%)	2,962 (47.2)	638 (32.0)	1,741 (51.5)	583 (64.5)
Total cholesterol, mmol/L	5.4 (1.1)	5.6 (1.1)	5.4 (1.1)	5.2 (1.1)
HDL-Cholesterol, mmol/L	1.5 (0.4)	1.5 (0.5)	1.5 (0.4)	1.5 (0.4)
Statin use, N (%)	1,756 (28.0)	384 (19.2)	1,094 (32.4)	278 (30.8)
Diabetes mellitus, N (%)	557 (8.9)	141 (7.1)	325 (9.6)	91 (10.1)
Lipid lowering medication use, N (%)	1,926 (30.7)	482 (24.1)	1,159 (34.3)	285 (31.5)
Cardiovascular disease				
Maximum intima media thickness*	1.0 (0.9 – 1.1)	0.9 (0.8 – 1.0)	1.0 (0.9 – 1.1)	1.1 (1.0 – 1.3)
Plaque score*	1.5 (0.5 – 2.5)	1.0 (0.0 – 2.0)	1.5 (0.5 – 3.0)	2.5 (1.0 – 4.0)
Hearing function				
All frequency hearing loss, dB	25.7 (13.5)	18.0 (9.1)	29.3 (12.1)	45.8 (13.9)
Low frequency hearing loss, dB	15.6 (10.6)	11.1 (7.0)	17.4 (9.9)	29.9 (15.6)
Speech frequency hearing loss, dB	22.8 (13.3)	16.0 (8.9)	25.9 (12.3)	42.0 (15.2)
High frequency hearing loss, dB	34.7 (19.0)	23.8 (13.8)	40.3 (17.2)	60.5 (15.2)
Degree of hearing loss, N (%)				
Normal hearing (0 – 20 dB)	1,631 (26.0)	1,145 (57.4)	482 (14.3)	4 (0.4)
Mild hearing loss (20 – 35 dB)	1,607 (25.6)	550 (27.6)	1,004 (29.7)	53 (5.9)
Moderate hearing loss (35 – 50 dB)	702 (11.2)	68 (3.4)	500 (14.8)	134 (14.8)
Severe hearing loss (>50 dB)	249 (4.0)	14 (0.7)	134 (4.0)	99 (11.0)

Table 1. Overview of characteristics of participants (cohort I, II, and III) of the Rotterdam Study during visit 5 (2009-2015) by age groups. (continued)

	Entire sample	Participants 51 - 65 years	Participants 65 - 80 years	Participants 80 – 99 years
	N = 6,279 (100%)	N = 1,996 (31.8%)	N = 3,379 (53.8%)	N = 904 (14.4%)
Brain tissue volume				
Total brain tissue volume, mL	920.5 (62.9)	959.8 (97.4)	912.1 (101.8)	853.9 (92.7)
White matter volume, mL	393.9 (65.9)	414.8 (63.0)	390.4 (63.3)	354.6 (63.3)
Grey matter volume, mL	526.6 (62.9)	545.1 (63.3)	521.7 (60.0)	499.4 (59.8)
Cognitive function				
Mini-mental state examination score*	28.0 (27.0 – 29.0)	29.0 (28.0 – 30.0)	28.0 (27.0 – 29.0)	27.0 (25.0 – 29.0)
Word Learning Test – delayed recall	7.6 (3.0)	9.0 (2.7)	7.2 (2.8)	5.9 (2.8)

Values are mean (standard deviation) for normally distributed continuous variables or median (interquartile range) when indicated (*), percentages for dichotomous variables. MET: metabolic equivalent of task. nmol: nanomole. L: litre. kg: kilogram. m: meter. mmHg: millimetres of mercury. mmol: millimole. dB: decibel. mL: millilitre.

oldest participants: 853.9 mL; **Table 1**). Therefore, to elucidate whether determinant and outcomes of interest are truly directly related to one another, independent of age and other confounding factors, longitudinal studies are preferred above cross-sectional designs as they have the potential to filter out effects of ageing more thoroughly for which I will present an example below.

Effects of age in longitudinal studies

Multiple studies have shown that higher levels of hearing loss are related to accelerated cognitive decline and an increased risk of dementia.[2-4, 58, 59] These studies have only taken baseline age (whether it being the linear or non-linear term of age) into account as a confounding factor in their analyses. However, it is plausible that older people will decline faster over time on cognitive function compared to their younger counterparts. For example, hypothetical participant Y (80 years) will decline faster on cognitive functioning between T0, T1 and T2 then hypothetical participant X (65 years old) as a consequence of his or her older age (**figure 6**). Not taking into account this faster decline in cognition in older participants, may result in an overestimation of the true effect of hearing loss on cognitive decline. This faster decline due to age can be accounted for by adding the interaction between age and follow-

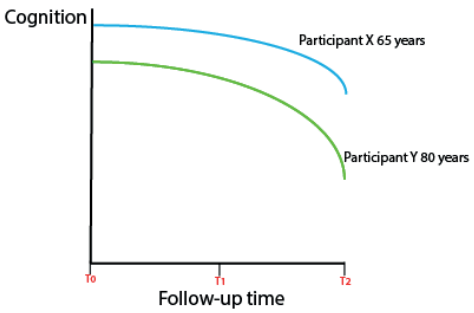

Figure 6. Hypothetical difference in cognitive trajectories over time in two participants.

up time into statistical models. In **chapter 4.3** I explored the longitudinal association between hearing function and cognitive decline in two steps. First, I applied a model similar to statistical models in previous studies and found comparable results, namely an accelerated decline on memory function due to moderate hearing loss as compared to normal hearing abilities. However, when adding the interaction between age and follow-up time in a second model, hearing loss was no longer significantly associated with an accelerated decline on memory function. This likely indicates that the association in my study was driven by (residual) effects of age on cognitive function. As such, I believe it is important, when investigating factors influencing brain health, cognitive decline or risk of dementia, to thoroughly adjust for strong effects of ageing.

Measuring hearing function

Throughout this thesis, I have used a somewhat crude measure of hearing loss, namely the average over all-, low-, speech-, or high frequencies. As such, I have disregarded potential subtypes of hearing loss, which have been described previously.[57] These subtypes encompass the metabolic-, the sensory-, and a mixed phenotype of age-related hearing loss.[60] The metabolic type is thought to result from the deterioration of the stria vascularis in the cochlear lateral wall (**figure 6**), which normally produces endolymph to maintain the endocochlear potential. Elderly with a metabolic type of hearing loss typically show audiograms that exhibit mild, flat hearing loss at the lower frequencies (10-40 dB) and gradually sloping hearing loss at higher frequencies (30-60 dB).[60] The sensory phenotype is thought to be related to damage to sensory cells in the inner ear (**figure 7**) and loss of the cochlear amplifier due to environmental exposures, such as excessive noise or ototoxic drugs, resulting in steeply sloping 50-70 dB thresholds shifts that predominantly affect the higher frequencies. The mixed phenotype is thought to reflect combined metabolic declines and sensory damage. This leads to audiograms marked by mild, flat hearing loss at the lower hearing frequencies and steeply sloping hearing loss at the higher hearing frequencies. Interestingly, it is thought that audiometric phenotypes are stable over time, although hearing thresholds do increase with older age.[60] Yet, if a transition is seen between phenotypes, it is usually observed in the sensory phenotype. Re-

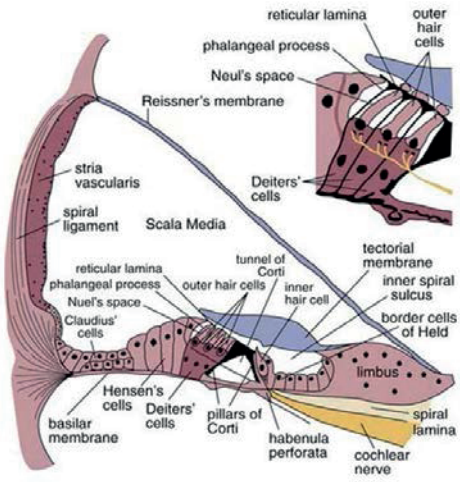

Figure 7. Cochlear anatomy. Source: Clinical Anatomy & Operative Surgery

searchers have proposed that sensory hearing loss might be relatively unaffected by ageing.[60] Nevertheless, from 70 years of age onwards, individuals with a sensory form of hearing loss also start to exhibit hearing loss typical for the metabolic phenotype, thus transitioning from the sensory type, to the mixed phenotype. Moreover, the metabolic and the mixed phenotype is most often seen in the older ages and are therefore regarded as the phenotypes which are typical for age-related hearing loss.[60] Indeed, the Rotterdam Study participants do have hearing thresholds typical for age-related hearing loss. As can be seen in **Table 1**, the increase in hearing thresholds with age is largest in the high frequencies (from 23.8 dB towards 40.3 dB and 60.5 dB per increasing age group) whereas thresholds in the low frequencies increase in a slower fashion (from 11.1 dB towards 17.4 dB and 29.9 dB per increasing age group). Unfortunately, there is no data available on noise exposure within the Rotterdam Study leading to the inability to differentiate between a metabolic and sensory phenotype. Nevertheless, exploring differences in results between the low- and high hearing frequencies may serve as a proxy to discern between these phenotypes. If certain factors would specifically harm hearing thresholds within age-related hearing loss, it can be expected that the largest effect is seen for the high hearing frequencies as compared to the low hearing frequencies. In **chapter 2.1** and **chapter 2.2** I examined differences between low- and high frequencies in regard to the effect of body composition and cardiovascular disease on hearing function. Regardless of the fact that the Rotterdam Study population does show hearing thresholds typical for age-related hearing loss, no differences were seen in the effect on high- versus low hearing frequencies for both body composition and cardiovascular disease. If certain factors would be more detrimental for a specific phenotype of hearing loss, I did not find evidence in this thesis pointing towards such a phenomenon.

Measuring brain health

Magnetic resonance imaging

Throughout this thesis I have mostly used MRI data from the Rotterdam Study. In the dedicated research centre, MRI scans are made with a 1.5 tesla MRI scanner.[61] Even though a 1.5 tesla MRI scanner is widely used, both in research and in clinical settings, a higher field strength would have the advantage to more sensitively image relatively small structures or markers of cerebral small vessel disease such as microbleeds.[62-64] The potential lower detection of the scanner used could explain the absence of any association between the determinants of interest and the risk of having microbleeds (**chapter 3.1, 3.2 and 3.3**). Moreover, in **chapter 4.2,** in which I had data available on brain microstructure imaged with a 3 tesla MRI scanner from the ARIC study, I saw an effect of hearing loss in the white matter microstructure of the limbic tracts, a relatively small white matter area. The study of Rigters et al.,[48] did not find such an association, which may (partly) be explained by the fact that this study used diffusion data obtained

by a 1.5 tesla MRI scanner. Thus, I might have missed particular associations in the Rotterdam Study between risk factors, hearing loss and measures of brain health for which scanning at a higher field strength would be preferred.

Unfortunately I have not (yet) used data on functional changes of the brain as a measure of brain health. Even though functional changes in brain health are a known marker of neurodegeneration. In normal ageing, neurodegeneration is characterized by grey- and white matter atrophy and the formation of white matter hyperintensities. Additionally, it is thought that these brain changes are preceded by changes in the functional organization of the brain.[65] The functional dynamics of the brain can be investigated by means of functional MRI (fMRI). fMRI indirectly reflects neural activity by measuring MRI signal fluctuations caused by variations in blood oxygenation and flow resulting from changes in neural metabolic demand.[66] Indeed, with increasing age the functional organization of the brain appears to decrease.[66] Whether hearing loss is potentially related to reduced functional connectivity of the brain may be an interesting research question in the search towards the underlying pathway in the association between hearing function and dementia. Some small studies did explore this relation and reported that higher levels of hearing loss were accompanied by decreased functionality of the auditory cortex,[67] and disrupted spontaneous activity in different brain regions amongst which the superior temporal gyrus and the parahippocampal gyrus.[68] Large population-based studies on hearing loss and functional connectivity do not exist yet, underlining the potential and the need to study this possible relation.

In none of the studies in the current thesis I specifically focused on the primary auditory cortex, an area in the brain involved in sound processing, which is located in the superior temporal gyrus. Possibilities were explored to obtain information for participants on auditory cortex volume. Unfortunately, the precise location of the primary auditory cortex remains controversial, due to highly differentiating sizes and shapes of the auditory cortex between individuals.[69] Especially in large population-based studies, identifying the auditory cortex for every participant would be highly time consuming and prone to erroneous classification. Instead, information on the superior temporal gyrus can be used as a proxy, as it is certain that the primary auditory cortex, in whatever size or shape, is located there.[69] Unexpectedly, I did not find that associations in the temporal lobe were predominantly driven by effects in the superior temporal gyrus in **chapter 4.2**. Rather, the effects were found for the entire temporal lobe. This may point towards effects of ageing in general rather than direct effects of hearing loss on neurodegeneration as it is known that the temporal lobe is one of the first structures to degenerate with older age.[40]

Cognitive function and dementia

As hearing assessment has been added into the Rotterdam Study protocol in 2011, just one follow-up measurement on cognitive function was available for the study I conducted, with a mean follow-up time of 4.4 years. The fact that I did not find a significant association between hearing loss and cognitive decline, may thus be explained by this relatively short follow-up time. Moreover, in this thesis I did not explore whether hearing loss was associated with an increased risk of dementia. So far, studies identifying hearing loss as a risk factor for dementia were mostly conducted in the United States and in a some other European countries, but not in the Netherlands so far. For purposes of generalizability and confirmation of previous results, it would add considerably to the current knowledge to assess this association in the Rotterdam Study. Unfortunately, incidence of dementia in participants with a baseline hearing assessment is limited (N = 15), precluding the possibility to infer on risk of developing dementia in participants with higher baseline levels of hearing loss.

CLINICAL IMPLICATIONS AND FUTURE RESEARCH

In the past decade, epidemiological studies have aided considerably to the current knowledge on hearing loss as a risk factor for dementia. Yet, we still have a long way ahead of us to truly elucidate how hearing loss is related to dementia and how we may potentially prevent accelerated degeneration of both hearing function and brain health. Worldwide, the number of persons developing hearing loss and dementia is still increasing, and will likely continue to do so, given the ageing of the population.[4, 57] Findings described in this thesis may first contribute to identifying potential modifiable risk factors for both hearing function and brain health. Second, our findings add to the current knowledge on a potential mechanism underlying the association between hearing loss and dementia. Yet, several aspects with regard to understanding the underlying mechanism between hearing function and dementia still remains unclear. In this final part of my thesis, I concentrate on the clinical implications of these findings and potential future directions.

Risk factors for hearing function and brain health

Taking together all the results in the current thesis it can be argued that adhering to a healthy lifestyle is key in healthy ageing. Preventing overweight, atherosclerosis and vitamin D deficiency may prove beneficial for both hearing function and brain health. This can be accomplished, as indicated in my results, by adhering to an overall healthy dietary pattern which may have either direct effects on hearing function and brain health, or indirect effects by promoting general cardiovascular health. Moreover, it will

likely maintain sufficient vitamin D levels, prevent overweight and obesity and through this lower the risk of cardiovascular disease. Other risk factors that are well-known to have a negative impact on brain health, such as sedentary behaviour, smoking, and consuming (too much) alcohol, are relatively unexplored in regard to hearing function. As such, it may be of interest to explore these lifestyle factors in relation to hearing function in future population-based studies. This could also provide information on which subgroups are more susceptible for lifestyle factors influencing hearing function and brain health. Such specific information could then be used to develop targeted interventions. On top of this, promoting adherence to a healthy lifestyle not only caries the promise to support hearing function and brain health, it will also have a beneficial effect on other (chronic) diseases associated with an unfavourable lifestyle. To put it differently, it will improve public health in general. For future studies, in the Rotterdam Study or in any other population-based sample, it is essential to have longitudinal data with multiple repeated measurements of both the determinant and outcome of interest. Only then it can be derived whether for example an unhealthy diet accelerates neurodegeneration besides normal age-related changes or that atherosclerosis increases the incidence of hearing impairment. Moreover, as lifestyle factors and cardiovascular risk factors are highly intertwined it could be of interest to explore both lifestyle factors and/or cardiovascular risk factors together in regard to hearing function and brain health instead of assessing them separately. This might shed more light on the interaction between these factors and the overall effect on hearing abilities and neurodegeneration.

On a completely different note, due to the globally changing climate, interest in vegetarian and plant-based diets has increased across the general population. Not consuming any meat and fish or eating no animal products at all is proven to protect the environment, but preliminary evidence also has shown health benefits on an individual level. For example, in the early 1990s it was reported that participants whom consumed large amounts of meat as compared to participants who had not eaten meat in 30 years, were twice as likely to develop dementia.[70] Unfortunately, of the 5,690 participants in the Rotterdam Study who underwent dietary assessment, only few adhere to a vegetarian or plant-based diet (N = 133 [2.3%], N = 79 [1.4%], respectively), limiting power and thus possibilities to research effects of these diets on general health, cognitive function and dementia risk. Nevertheless, results in the Rotterdam Study imply a beneficial effect of adherence to a diet higher in plant-based foods and lower in animal-based foods on the development of diabetes type 2 and obesity.[71, 72] Thus, eating vegetarian and plant-based might be a promising strategy in healthy ageing.

Hearing loss as a risk factor for dementia in population-based studies

Whilst considerable effort has been made to investigate the direct association between hearing loss, brain health and cognitive function, more evidence is still needed to en-

sure that hearing loss is indeed a risk factor for dementia. This can be accomplished by collecting data on hearing- and cognitive function and incident dementia over sufficient follow-up time with multiple repeated measurements of both hearing thresholds and cognitive function in varying populations with wide age ranges. Moreover, researchers should take careful notice of concurring ageing effects, possibly confounding previously found results. Additionally, other potential common-causes such as lifestyle- and cardiovascular risk factors should also be taken into account in statistical models to avoid potential false positive results. If hearing loss is indeed directly and independently related to accelerated structural and functional neurodegeneration, it is of great importance to collect detailed information on hearing aid use, and study whether this can slow down or modify the cognitive decline associated with hearing loss. Only then we might be able to say more about the clinical relevance of hearing loss as a (modifiable) risk factor for dementia. Whenever this data on hearing aids is available, we could conduct a so-called target trial emulation in population-based samples.[73] Unfortunately, data on when participants first started using a hearing aid is missing in the Rotterdam Study. Moreover, using data from a Dutch population likely introduces selection bias, as hearing aids are refunded at hearing thresholds from 35 dB or greater. On top of this, even though the benefits of hearing aid use among older adults with hearing loss have been well documented, actual hearing aid use is poor.[74] For example, in a survey conducted in 2011 in Germany, France and the United Kingdom showed that between 4.7% and 12.4% of people never used the hearing aid that they own.[75] Furthermore, actual hearing aid purchase and/or use appears to be highly dependent on several audiological and non-audiological determinants such as degree of hearing loss (the higher the degree of hearing loss, the better the compliance to hearing aid use), perception of hearing handicap, sex (females use it more than males), education (higher education associated with better compliance) and income (those with higher incomes used hearing aids more regularly).[75] Thus, we should keep in mind that we might be dealing with a selected population and results from studies might not be generalizable towards the entire population that has a hearing impairment great enough to be eligible for a hearing device. Nevertheless, it might also be of interest to investigate the effect of hearing aid use in groups of populations with all degrees of hearing loss (and not restrict research to for example individuals with moderate hearing loss), individuals with mild cognitive impairment (MCI) or even in individuals with full-blown cognitive impairment/dementia. To do so, we would need clinical trials offering hearing aids at different degrees of hearing loss and/or cognitive status.

Hearing loss as a risk factor for dementia in clinical trials

A recent study showed that in individuals with hearing loss and without any form of a hearing device, cortical brain reorganization takes place. This is reflected by increased

cortical activation of the auditory cortex next to activity in the somatosensory regions of the brain when presented with a somatosensory stimuli.[76] This reorganization can be explained by increased reliance on other sensory modalities to speech perception in those with hearing loss.[76] Interestingly, some preliminary, but promising evidence is already showing the benefits of hearing aid use or cochlear implantation on brain health both in humans and in animals.[77, 78] In congenital deaf cats it was recorded that the primary auditory cortex develops significantly different as compared to their normal hearing counterparts. After the congenital deaf cats received cochlear implantation, functional organization of the auditory cortex was significantly changed by the new hearing experience. The largest difference that was recorded in these cats was a substantial larger activated cortical area.[78] Moreover, in a case report of a 9-year old girl with unilateral sudden-sensorineural hearing loss, who was eligible for cochlear implantation, researchers examined potential cortical changes before and after cochlear implantation.[79] Pre-cochlear implantation, auditory stimulation of the patient's normal hearing ear resulted in temporal and frontal activation. However, this frontal activation appeared to be absent after the cochlear implantation, suggesting a decrease in listening effort with the hearing device.[79] Even though above mentioned results should be interpreted with caution as it concerns just a single case and animal research which may not apply to humans, results might point towards a reversible effect of cortical reorganization when hearing devices are implemented. Nevertheless, we are mostly interested in the effects of such devices in the elderly. To achieve benefits of cochlear implants in older adults, some cortical plasticity is needed to restore a level of speech understanding. Therefore it is encouraging that cochlear implants seem to catch on well in elderly patients with the highest degrees of hearing loss.[80] In a French study with 9 participants, aged 48-66 years with single-sided deafness, three measurements were obtained before cochlear implantation, and 6 and 12 months post cochlear implantation. Even though it is a small sample size, cortical modification occurred rapidly at 6 and 12 months post implantation in response to cortical auditory evoked potentials. On top of this, in a small randomized trial with elderly veterans participants (aged 66 – 80 years) who received a hearing aid reported improved social and emotional functioning and better cognitive functioning as compared to those who did not receive a hearing aid after 4 months of hearing aid use.[77] So, even in elderly groups of participants, preliminary evidence is available pointing towards favourable cortical reorganization and improvements in social- and cognitive functioning after the implementation of hearing devices.[80]

To further elucidate this effect, (larger) randomized clinical trials, applying both in-depth auditory function testing, extensive cognitive testing and detailed non-invasive brain imaging studies in an elderly population are needed. In the United States of America researchers have initiated the Ageing and Cognitive Health Evaluation in Elders (ACHIEVE) study and the Hearing Equity through Accessible Research and Solutions

(HEARS) study, two randomized controlled trials aiming to determine efficacy of hearing treatment in reducing cognitive decline in older adults.[81] As both studies are still ongoing, results are not available yet. Whenever these studies show that hearing devices do delay or slow down cognitive decline in at-risk older adults, this could have a very promising clinical, social, and public health impact as use of hearing devices is a relatively easy and inexpensive intervention. Moreover, exploring potential beneficial effects may not only be of interest in participants without cognitive decline and mild degrees of hearing loss, it could also prove useful to offer hearing aids at higher degrees of hearing loss or when individuals are already diagnosed with MCI or dementia. Implementing hearing aids at a MCI stage might show whether such an intervention would lead to a slower cognitive decline and/or a potential later onset of cognitive impairment. Applying hearing aids in the dementia phase may feel counterintuitive, yet it might reduce caregiver burden due to improved communication between the patient and the caregiver. The same goes for individuals with severe degrees of hearing loss. Restored/better communication as a result of hearing devices could lead to improved general health, as individuals better understand instructions given by for example doctors about medical prescriptions and/or treatments. Which again possibly results in lower rates of rehospitalisation and reduced health care costs.

In conclusion, hearing loss and neurodegeneration impacts the public health enormously on the level of physical- and psychological well-being but also by increasing the risk of adverse (neurological) outcomes. Given the ageing of the population and the accompanying rise of age-related diseases such as hearing impairment and dementia, huge amounts of work are still needed to promote and support healthier ageing. This thesis has highlighted some interesting avenues to explore further in this regard.

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Stilleven met boeken en een viool; Jan Davidsz de Heem[1]

6

Summary / Samenvatting

SUMMARY

Chapter 1, the general introduction, provides the rationale and aim of this thesis. Recently, hearing loss has been identified as a promising modifiable risk factor for cognitive decline and dementia. However, the mechanism underlying this association remains unknown. Several hypotheses have been proposed, amongst which are the common-cause hypothesis and the sensory-deprivation hypothesis. The common-cause hypothesis states that there is a third, common factor, both causing hearing loss and impoverished functional brain health. The sensory-deprivation hypothesis proposes that hearing loss has a direct, permanent negative effect on brain health. Therefore, it was the aim of this thesis to firstly, identify potential risk factors for both hearing function and brain health which may potentially be a third, common factor in the association between hearing loss and dementia. Established risk factors for dementia were selected to investigate as a potential common-cause. Secondly, I explored potential direct interrelations between hearing function and structural and functional brain health. Throughout this thesis, I have used data of the population-based Rotterdam Study and the Atherosclerosis Risk in Communities Study.

Risk factors for hearing function

Due to the ageing population, the prevalence of hearing loss will increase substantially in the coming years. At present, there is no treatment to cure hearing loss. Therefore, more in-depth knowledge is needed on potential modifiable risk factors to slow down the progression of declining hearing function. In **Chapter 2**, the focus is on potential risk factors for hearing loss.

The inner ear is a heavily vascularized organ and as such prone to any change in cardiovascular health. Therefore, research has focused on cardiovascular risk factors in regard to hearing function. Interestingly, I found that higher body mass and higher fat mass were associated with higher levels of hearing loss (**Chapter 2.1**). Though, this association disappeared at follow-up. Yet, estimates may indicate a clinically relevant effect of obesity on hearing thresholds. In **Chapter 2.1** I did not find an association between overall dietary pattern and hearing loss, both at baseline and at follow-up. Even though a healthy diet is extremely important, such a diet will likely not support hearing function with ageing. Besides cardiovascular risk factors, I was also interested in a potential association between cardiovascular disease, an established risk factor for cognitive decline and dementia, and hearing loss. In **Chapter 2.2** I discovered that carotid atherosclerosis as reflected by higher intima media thickness and higher plaque burden, was associated with higher levels of hearing loss, Interestingly, this association was predominantly found for hearing loss in the right ear, not in the left ear. As such, interventions targeted

at treating and/or preventing cardiovascular disease may also benefit hearing function as an added bonus.

Risk factors for brain health

The ageing brain undergoes so-called 'normal, age-related changes' reflected in macro structural atrophy of the grey- and white matter, formation of white matter hyperintensities and white matter microstructural degeneration. A the moment, we are unable to prevent this normal age-related neurodegeneration. Though, we might be able to prevent accelerated neurodegeneration beyond these age-related changes by identifying modifiable risk factors for brain health. **Chapter 3** is dedicated to identifying potential risk factors for brain health in dementia-free participants.

In **Chapter 3.1** I report that a healthier overall dietary pattern is associated with larger brain volumes, driven by larger white matter volumes. Dietary pattern was not associated with markers of cerebral small vessel disease such as white matter hyperintensity volume and the presence of lacunes and microbleeds. In line with this, in **Chapter 3.2**, I identified that vitamin D deficiency was linked to smaller brain volumes, especially smaller white matter volume and a smaller hippocampus volume. Again, markers of cerebral small vessel disease were not affected by vitamin D status. Both studies point towards the importance of adhering to a healthy dietary pattern and maintaining a sufficient vitamin D level, which may either directly or indirectly support brain health. From previous studies it is known that obesity inflates the risk of dementia. However, studies have mostly taken BMI as the only measure of body composition, thereby disregarding potential differences between healthy- and unhealthy body mass. **Chapter 3.3** describes that a higher body mass index (BMI) and a higher fat mass index (FMI) were associated with diminished brain health at baseline. However, I could not find that body composition had a significant influence on brain changes over time. Although it is important to maintain a healthy body weight for several health outcomes, based on my results, it is questionable that body composition in itself is a modifiable risk factor for brain health.

The common-cause hypothesis

Concluding from the 2nd and 3rd chapter, it seems unlikely that body composition and dietary pattern individually are a common-cause in the association between hearing loss and dementia. Yet, we should keep in mind that these factors are probably highly intertwined with other lifestyle- and cardiovascular risk factors. All these factors together might have a detrimental effect on brain health and hearing function. Interestingly, it appeared that sufficient vitamin D status is protective for brain health and that cardiovascular disease was linked to elevated hearing thresholds. Moreover, we know from other research that vitamin D also has a protective effect on hearing levels and that cardiovascular disease is linked to smaller brain volumes. Therefore, these two factors

could very well be a common-cause. Adjusting for these factors and other lifestyle- and cardiovascular risk factors in future studies investigating a direct link between hearing loss, cognitive decline and dementia could be of interest to further disentangle the potential direct link between hearing function and risk of dementia.

Interrelations between hearing function and brain health

Most population-based studies have focused on the association between hearing loss, cognitive decline and dementia. However, it is also of great importance to determine whether hearing loss is related to brain health before dementia or cognitive impairment is present. As such, we might be able to identify promising windows of opportunity in the intervention of hearing loss and its potential beneficial effects on brain health. Therefore, the final chapter, **Chapter 4**, is dedicated to the potential direct link between hearing function and brain health.

In **Chapter 4.1** I explored the association between tinnitus and brain health. Interestingly, it appeared that having tinnitus was associated with larger brain tissue volumes, fully driven by larger white matter volumes. This association was independent of hearing function and age. Thus, tinnitus possibly has more of a neurodevelopmental origin than a neurodegenerative. All in all, it is questionable whether tinnitus, even though highly prevalent in the elderly, is involved in the association between hearing loss and dementia. In **Chapter 4.2**, I identified an association between hearing loss and differences in white- and grey matter microstructure. To be more specific, higher hearing thresholds were associated with lower microstructural organization of the temporal lobe, the limbic fibre tracts and the hippocampus. These results encompass promising new evidence pointing towards a direct link between hearing loss and neurodegeneration in dementia-free individuals. However, longitudinal data is warranted. **Chapter 4.3** is dedicated to the association between hearing loss and cognitive decline. At baseline, I found that hearing loss is associated with lower cognitive functioning across several cognitive domains. Longitudinally it was seen that hearing loss accelerated declining memory functioning over time. Though, this association attenuated when adjusting for the fact that older people decline faster on cognitive functioning over time as compared to their younger counterparts.

In **Chapter 5**, the general discussion, the main findings are discussed in light of current scientific knowledge. Methodological considerations, clinical implications of the findings, and directions for future research are also discussed in this chapter.

SAMENVATTING

In **Hoofdstuk 1**, de algemene introductie, beschrijf ik de achtergrond van dit proefschrift en het doel van mijn onderzoek. In de afgelopen jaren is gebleken uit verschillende populatie studies dat gehoorverlies bij ouderen een mogelijke modificeerbare risico factor is voor cognitieve achteruitgang en dementie. Echter is nog onbekend wat het mechanisme achter dit verband is. In de literatuur zijn er verschillende hypothesen geopperd. Twee daarvan zijn de *'gemeenschappelijke oorzaak hypothese'* en de '*sensorische-deprivatie hypothese'.* De eerste schetst dat er een derde, gemeenschappelijke factor is die zowel gehoorverlies als veranderingen in het brein veroorzaakt. De tweede omschrijft dat gehoorverlies een direct, permanent negatief effect heeft op de gezondheid van het brein. Zodoende was het doel van dit proefschrift tweeledig. Ten eerste, wilde ik potentiele risico factoren voor gehoorfunctie en breingezondheid identificeren die eventueel een derde factor zijn in de relatie tussen gehoorverlies en dementie. De onderzochte risicofactoren in dit proefschrift zijn gebaseerd op bekende risicofactoren voor dementie. Ten tweede was ik geïnteresseerd in de directe relatie tussen gehoorfunctie en structurele en functionele brein gezondheid. In dit proefschrift heb ik data van twee populatie studies gebruikt, namelijk de Rotterdam Studie en de Atherosclerosis Risk in Communities Studie.

Risico factoren voor gehoorverlies

Met de toenemende vergrijzing zal de prevalentie van gehoorverlies substantieel toenemen. Heden ten dage is er geen behandeling die bestaand gehoorverlies kan genezen. Daarom is het van belang om meer kennis te vergaren van potentiele modificeerbare risicofactoren die de progressie van gehoorverlies wellicht kunnen vertragen. In **Hoofdstuk 2** heb ik me gericht op mogelijke risicofactoren voor gehoorverlies.

Het binnenoor is een sterk gevasculariseerd weefsel en is daarom erg gevoelig voor (kleine) veranderingen in cardiovasculaire gezondheid. In een cross-sectionele studie heb ik aangetoond dat een hoger BMI (body mass index) en een hoger FMI (fat-mass index) geassocieerd was met meer gehoorverlies (**Hoofdstuk 2.1**). Echter verdween deze relatie bij de tweede meting. In andere woorden, een hoger BMI en FMI resulteerde niet in een versnelde achteruitgang van de gehoorfunctie over tijd. Echter, ondanks dat er geen statistisch significante associatie was, was er wel voldoende grond om aan te nemen dat er een klinisch relevante associatie is tussen obesitas en een afnemende gehoorfunctie. In hetzelfde hoofdstuk kon ik geen significante en/of relevante relatie aantonen tussen dieet patroon en verhoogde gehoordrempels, zowel op het eerste meetmoment als over tijd. Ondanks dat het erg belangrijk is om gezond te eten, zal het waarschijnlijk geen substantieel effect hebben op het voorkomen van snellere achteruitgang van de gehoorfunctie. Behalve levensstijl- en cardiovasculaire risicofactoren,

was ik ook geïnteresseerd in cardiovasculaire ziekte, een bekende risicofactor voor cognitieve achteruitgang en dementie. In **Hoofdstuk 2.2** vond ik dat slagaderverkalking in de halsslagaders gelinkt is aan hogere gehoordrempels, specifiek in het rechteroor. Zodoende is het goed mogelijk dat interventies die specifiek gericht zijn op het voorkomen van slagaderverkalking, ook indirect een positieve werking hebben op het gehoor.

Risicofactoren voor breingezondheid

Het verouderende brein ondergaat zogenaamde normale, leeftijd gerelateerde veranderingen, gekenmerkt door macro structurele atrofie van de grijze- en witte hersenstof, vorming van witte stof laesies en micro structurele achteruitgang van de witte en grijze hersenstof. Ondanks dat het onwaarschijnlijk is dat we deze normale neurodegeneratie helemaal tegen kunnen gaan, is het van groot belang om versnelde neurodegeneratie zoveel als mogelijk te voorkomen. Dit kunnen we doen door risicofactoren te identificeren die de gezondheid van het brein beïnvloeden voordat er sprake is van dementie. **Hoofdstuk 3** is toegewijd aan het identificeren van mogelijke modificeerbare risicofactoren voor brein gezondheid in deelnemers vrij van dementie.

Door eerdere studies is aangetoond dat ongezonde voeding gelinkt is aan versnelde cognitieve achteruitgang en een verhoogd risico op dementie. In **Hoofdstuk 3.1** rapporteer ik dat een gezonder voedingspatroon geassocieerd is met grotere brein volumes, specifiek met grotere witte stof volumes. De kwaliteit van het voedingspatroon van deelnemers in de Rotterdam Studie was echter niet geassocieerd met indicatoren van cerebrale vaatziekte zoals de hoeveelheid witte stof laesies en de aanwezigheid van lacunes en microbloedingen. In **Hoofdstuk 3.2** vond ik dat vitamine D deficiëntie gerelateerd was aan kleinere brein volumes, ook weer voornamelijk te zien in kleinere witte stof volumes. Daarnaast lieten mijn resultaten zien dat vitamine D deficiëntie geassocieerd was met lagere hippocampus volumes, een belangrijk gebied in de hersenen voor geheugen. Ook hier zag ik dat vitamine D status geen invloed had op indicatoren van cerebrale vaatziekte. Beide studies onderstrepen het belang van gezonde voeding en het behouden van een optimale vitamine D status, wat dan wel direct dan wel indirect de gezondheid van het brein bevordert. Eerdere studies hebben laten zien dat overgewicht en obesitas een negatieve invloed heeft op het risico van dementie. Echter hebben deze studies voornamelijk BMI genomen als maat voor overgewicht en het is belangrijk om er rekening mee te houden dat BMI niet differentieert tussen gezonde en ongezonde lichaamsmassa. **Hoofdstuk 3.3** omschrijft dat een hoger BMI en een hoger FMI geassocieerd is met verminderde gezondheid van het brein. Echter hebben mijn resultaten niet kunnen aantonen dat lichaamssamenstelling de gezondheid van het brein ook over tijd beïnvloedt. Ondanks dat het zeer belangrijk is om overgewicht te voorkomen omwille van verschillende gezondheid risico's, zien we dit effect op basis van bovenstaande resultaten niet voor de gezondheid van het brein.

De gemeenschappelijke oorzaak hypothese

Wanneer we de resultaten in hoofdstuk 2 en 3 bij elkaar in overweging nemen, kunnen we concluderen dat het onwaarschijnlijk is dat lichaamssamenstelling en voedingspatroon op zichzelf een gemeenschappelijke oorzaak zijn van zowel gehoorverlies als cognitieve achteruitgang en dementie. Echter is het goed om ons te realiseren dat deze twee factoren zeer waarschijnlijk sterk samenhangen met andere levelsstijl- en cardiovasculaire risicofactoren. Ondanks dat we dus geen directe relatie zien tussen voedingspatroon en lichaamssamenstelling op het brein en het gehoor, is het wel aannemelijk dat verschillende levensstijl- en cardiovasculaire factoren samen van invloed zijn op de gezondheid van het brein en het gehoor. Een interessante bevinding was dat vitamine D status wellicht een beschermende werking heeft op de gezondheid van het brein en dat cardiovasculaire ziekte gerelateerd was aan verhoogde gehoordrempels. Bovendien weten we van andere studies dat toereikende vitamine D gehaltes een beschermend effect heeft op gehoorverlies en dat cardiovasculaire ziekte gerelateerd is aan kleinere brein volumes. Zodoende zouden deze twee factoren zeer goed een derde factor kunnen zijn in de relatie tussen gehoorverlies en dementie. Corrigeren voor deze factoren en voor andere levensstijl- en cardiovasculaire factoren in toekomstige studies kunnen ons dichterbij een antwoord brengen hoe gehoorverlies, cognitieve achteruitgang en dementie exact aan elkaar gerelateerd zijn.

Onderlinge relaties tussen gehoorfunctie en brein gezondheid

Overwegend veel populatie-studies hebben zich geconcentreerd op de directe relatie tussen gehoorverlies, cognitieve achteruitgang en dementie. Echter is het van essentieel belang om te onderzoeken of gehoorverlies gerelateerd is aan structurele en functionele gezondheid van het brein voordat er sprake is van dementie. Zodoende zouden we mogelijk veelbelovende preklinische stadia kunnen ontdekken waar behandeling van gehoorverlies positieve effecten heeft op de gezondheid van het brein en mogelijk het risico op dementie verlaagd. In **Hoofdstuk 4** van dit proefschrift bespreek ik de resultaten die ik heb gevonden op het gebied van een mogelijk directe link tussen gehoorfunctie en de gezondheid van het brein in ouderen vrij van dementie.

Hoofdstuk 4.1 rapporteert resultaten van een studie waarin ik de relatie tussen tinnitus en breingezondheid heb onderzocht. Hier bleek dat het hebben van tinnitus geassocieerd was met grotere brein volumes, specifiek met grotere witte stof volumes. Deze relatie was onafhankelijk van gehoorfunctie en leeftijd. Zodoende is het aannemelijk dat tinnitus meer te maken heeft met de ontwikkeling van het brein in plaats van met de veroudering van het brein. Zodoende is het de vraag of tinnitus, ondanks dat het veel voorkomt bij ouderen, betrokken is in de relatie tussen gehoorverlies en dementie. In **Hoofdstuk 4.2** heb ik een directe relatie aangetoond tussen mate van gehoorverlies en verschillen in micro structurele integriteit van het brein. Anders gezegd, het bleek dat

verhoogde gehoordrempels geassocieerd zijn met verminderde microstructuur van de temporale kwab, de limbische witte stof banen en de hippocampus. Dit zijn veelbelovende resultaten en ondersteunen de hypothese dat er wellicht een directe link is tussen gehoorverlies en breinveranderingen. Echter is het van groot belang om deze resultaten te repliceren in longitudinaal onderzoek. **Hoofdstuk 4.3** is toegewijd aan de relatie tussen gehoorverlies en cognitieve achteruitgang. Op het eerste meetmoment zag ik dat gehoorverlies gerelateerd was aan slechtere cognitieve functie in verschillende domeinen zoals globale cognitie, geheugen en executief functioneren. Longitudinaal rapporteerde ik dat meer gehoorverlies geassocieerd was met versnelde achteruitgang van de geheugen functie. Echter verdween deze associatie wanneer ik rekening hield met het feit dat oudere mensen over tijd sneller achteruitgaan in cognitieve functies in vergelijking met jongere mensen.

Tot slot bediscussieer ik in **Hoofdstuk 5** de belangrijkste bevindingen, methodologische overwegingen, de klinische implicaties van mijn onderzoek en presenteer ik een aantal suggesties voor toekomstig onderzoek.

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Meisje met de parel; Johannes Vermeer[1]

7

Dankwoord
PhD Portfolio
List of publications
About the author

DANKWOORD

Daar is hij dan! Mijn boekje! Toen ik begon aan mijn promotie is mij de vraag gesteld: "*Maar promoveren, dan zit je toch in je eentje in een kamer onderzoek te doen en ben je heel eenzaam?*" Maar dat is zeker niet het geval op de 28e. Een aantal mensen wil ik in het bijzonder noemen.

Meike en Arfan, oneindig veel dank voor het vertrouwen dat jullie me in 2016 hebben gegeven (ondanks dat ik geen antwoord wist op de vraag: "*Wat is Epidemiologie?*").

Meike, een moeder en een professor. Je bent een voorbeeld voor velen! Woorden schieten tekort om te omschrijven hoe bevoorrecht ik me voel met jou als één van mijn mentoren.

Arfan, mede dankzij jou heb ik mijn promotie als een geweldige tijd ervaren. Jouw enthousiasme voor het onderzoek, je scherpe en kritische blik en jouw enorme enthousiasme zijn erg inspirerend en hebben mij onwijs veel geleerd de afgelopen jaren.

Als iemand mij 10 jaar geleden had verteld dat ik zou gaan promoveren bij de KNO, dan had ik diegene niet geloofd. Rob en André, veel dank dat jullie mij hebben geïntroduceerd in dit mooie vak. Ik vond het een uitdaging om me wegwijs te maken in dit voor mij nieuwe en onbekende specialisme. En daarvoor had ik me geen betere mentoren kunnen wensen. En er valt nog zoveel moois te doen! Ik kijk zeer uit naar wat er komen gaat in onze toekomstige samenwerking.

Lieve Sanneke en Gerda, het voelt alweer als een eeuwigheid geleden, maar jullie hebben mij voor het eerst in aanraking gebracht met het vak wetenschap. Door jullie immens aanstekelijke ambitie en bevlogenheid voor onderzoek, was mijn passie voor dit vak dan ook snel ten volste aangewakkerd. Ik kijk altijd met veel plezier terug op onze samenwerking!

Dear Frank and Jennifer, my stay in Baltimore was a first for me: on my own in a foreign country! I couldn't have wished for more kind and hospitable hospital hosts, who made me feel at home from the start. Thank you so much for having me and I sincerely hope we will see each other again in the future.

Mijn lieve paranimfen Eline en Eline, what's in the name?

Elien/Eline, tegelijkertijd begonnen wij in het Erasmus. In het begin wat onwennig, maar al gauw is er een zeer dierbare vriendschap ontstaan. Jouw eindeloze geduld wanneer ik mijn computer uit het raam wilde gooien door jouw grote passie R, onze

heerlijke gesprekken en dagelijkse grappen en grollen. En wat een enorme rijkdom dat ik jou als paranimf achter me heb staan!

Meinardi, Rat, Tiet, E, Terror en alle andere namen die hier niet gepast zijn. Geen woorden zijn er bij ons nodig. In Leiden al mijn maatje en gelukkig is afstand bij ons voorlopig geen probleem. Laten we afspreken dit zo te houden en als we oud, bejaard, grijs en uitgezakt zijn samen een bejaardenhuis op z'n kop te zetten.

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Lieve Men, mijn alles. Dank dat je bent wie je bent en met je eeuwige rust de chaos in mijn kop tot orde weet te roepen. Let's grow old together (en met Har).

PHD PORTFOLIO

Name PhD Student:	Pauline H. Croll
Erasmus MC Department:	Otorhinolaryngology, Head and Neck Surgery, Radiology and Nuclear Medicine and Epidemiology
Research School:	NIHES
PhD Period:	August 2016 – December 2019
Supervisors:	Prof. R.J. Baatenburg de Jong, prof. M.W. Vernooij, prof. M.A. Ikram, dr. A. Goedegebure

1. PhD training	Year	ECTS
Research skills		
Master of Science in Clinical epidemiology, NIHES	2016-2017	70
Integrity in Science, Erasmus MC	2018	0.3
Course on R, MolMed	2016	1.8
International conferences		
Alzheimer's Association International Conference, London, the UK: poster presentation	2017	1.6
Hearing Across the Lifespan, Como, Italy: oral presentation	2018	1.0
European Atherosclerosis Society Congress, Maastricht, the Netherlands: oral presentation	2019	1.3
Alzheimer's Association International Conference, LA, the US: oral and poster presentation	2019	1.3
In depth courses, seminars, workshops and research visits		
Weekly research seminars, department of epidemiology, Erasmus MC	2016-2019	4.0
Praktische Neuroanatomie en Neuroradiologie	2018	0.4
Memorabel, ZonMw	2018	0.3
Visiting researcher Johns Hopkins University, Baltimore, the United States of America	2019	2.0

2. Teaching activities		
Teaching assistant		
Principles of Research in Medicine and Epidemiology, NIHES, Rotterdam, the Netherlands	2018	2.0
Practice of epidemiologic analysis, NIHES, Rotterdam, The Netherlands	2018	2.0
Supervisor		
Master thesis of M. Boelens: Association of vitamin D status with brain volumetry	2018	4.0
Junior MedSchool student: Tinnitus and the brain	2018	1.5
Master thesis of M. Adank: Grey matter differences in hearing loss using deep learning	2019	4.0

3. Other		
Reviewer		
Reviewing activities for various journals	2017- present	1.0

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Croll PH*, Boelens M*, Vernooij MW, van de Rest O, Zillikens MC, Ikram MA, Voortman T. The association between vitamin D deficiency and MRI markers of brain healthy in a community sample. *Under review.*

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ABOUT THE AUTHOR

Pauline Croll was born on December 15, 1990 in Veldhoven (the Netherlands), and grew up in Eindhoven. After graduating in 2009 from the 'Lorentz Casimir Lyceum' in Eindhoven, she started her Bachelor's degree in Psychology at Leiden University, which she obtained in April 2013. Subsequently, she started her Master's degree in Clinical Neuropsychology in August 2013 at Leiden University. She undertook her clinical training at the Bronovo hospital at the department of Geriatrics under the supervision of Prof. G.J. Blauw, dr. C. Jurgens and dr. M.N. Witjes-Ané. Her Master thesis was on cognitive function in patients with Hereditary Cerebral Haemorrhage With Amyloidosis – Dutch type conducted at the department of Radiology in the Leiden University Medical Center under supervision of dr. S. van Rooden and dr. J. van der Grond. Pauline obtained her master degree in August 2015 after which she stayed as a researcher at the department of Radiology at Leiden University Medical Centre.

From 2016 onwards, she conducted her PhD-project as described in this thesis at the departments of Otorhinolaryngology, Head and Neck Surgery (under supervision of Prof. R.J. Baatenburg de Jong [head of the department] and dr. A. Goedegebure), Radiology and Nuclear Medicine (under supervision of Prof. M.W. Vernooij) and Epidemiology (under supervision of Prof. M.A. Ikram [head of the department]).

In 2017 Pauline obtained her Master's degree in Health Sciences (specialisation: Clinical Epidemiology) at the Netherlands Institute for Health Sciences (NIHES). In 2019 she won the 2nd price for her poster presentation at the Annual Research on Aging Showcase at Johns Hopkins University, Baltimore (MA), the United States of America. Pauline will continue to work as a researcher at the departments of Epidemiology, Otorhinolaryngology, Head and Neck Surgery, and Radiology and Nuclear Medicine.

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