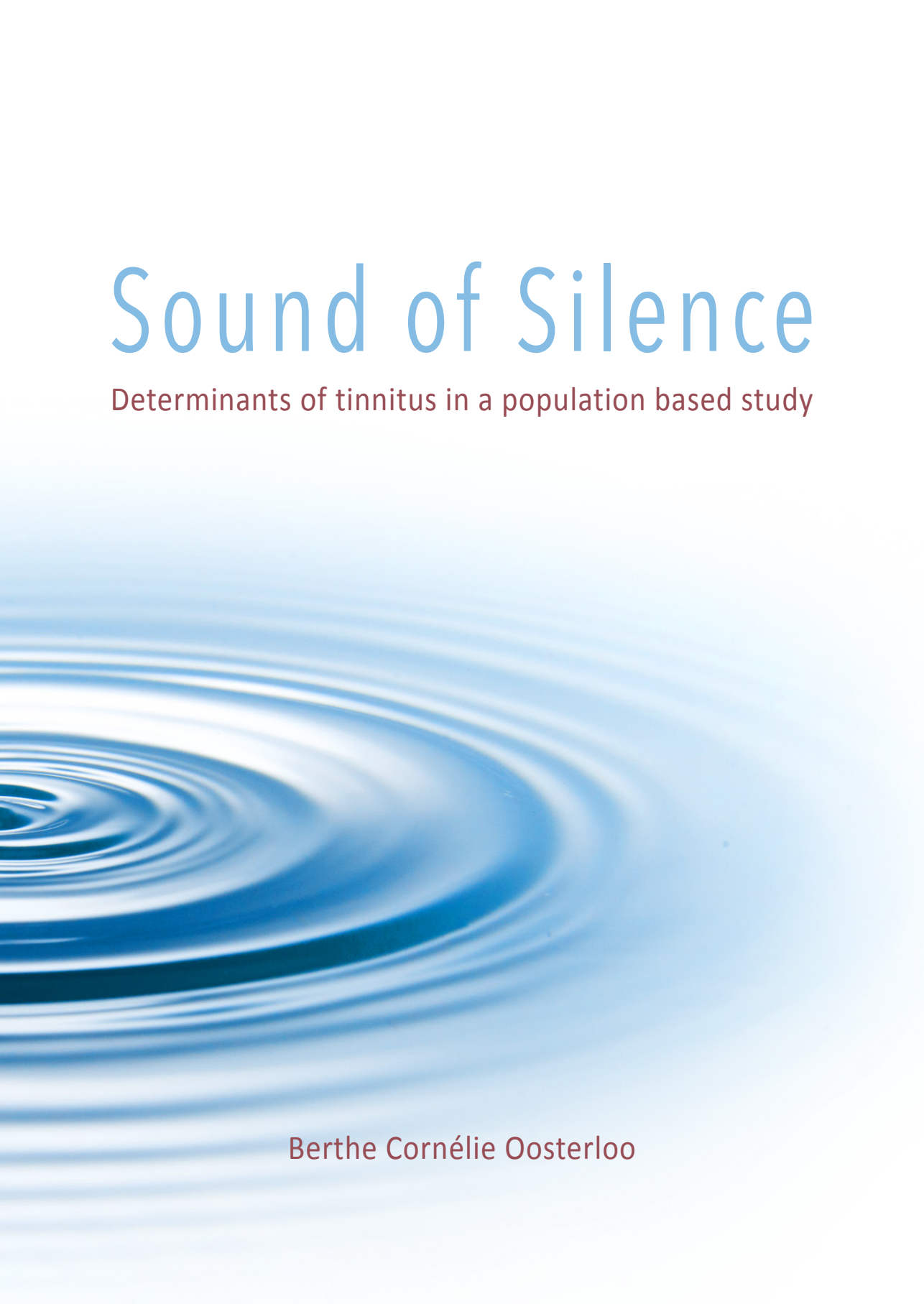
Sound of Silence

Determinants of tinnitus in a population based study



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The Sound of Silence

Determinants of tinnitus within the rotterdam study

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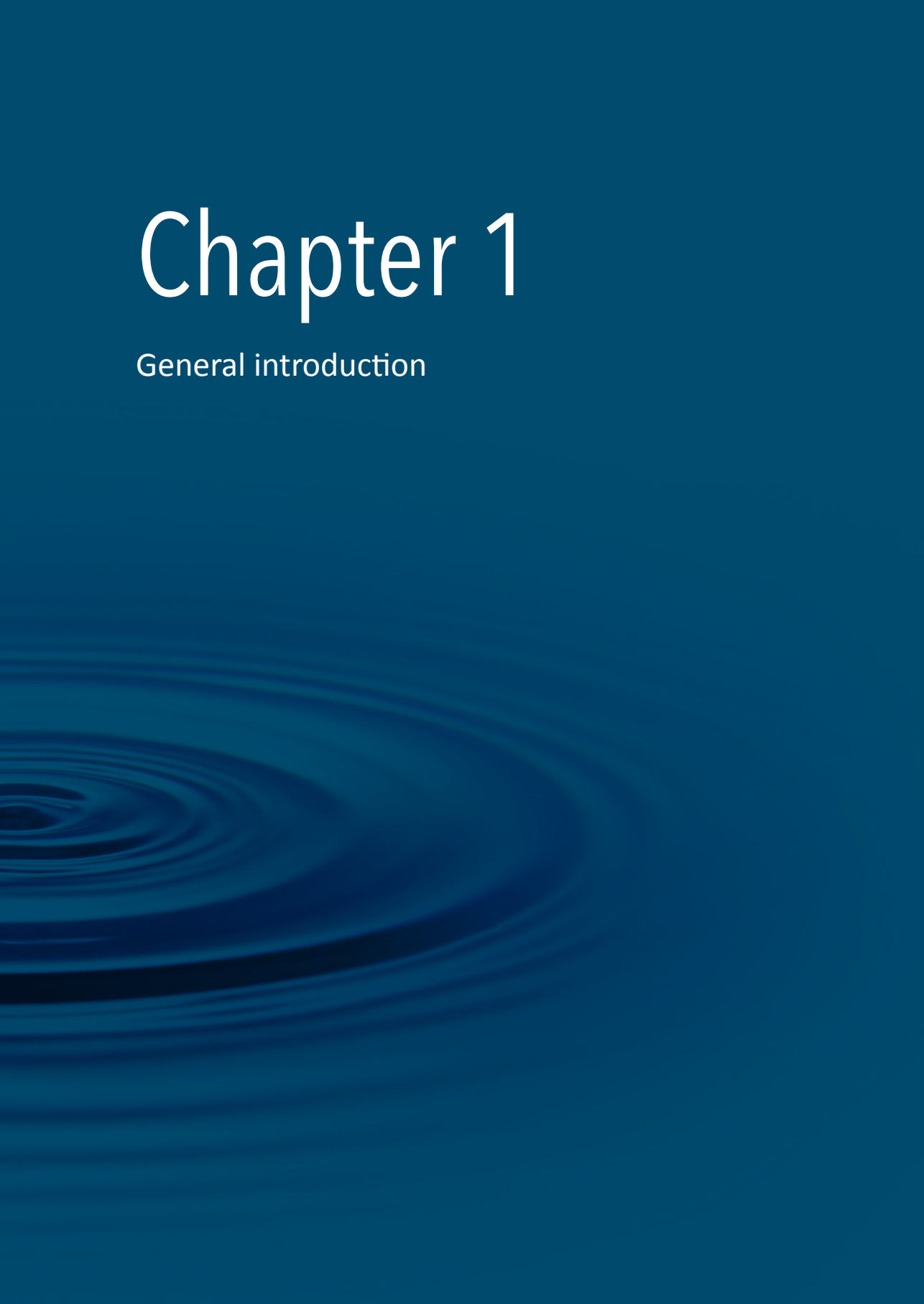
Chapter 1

General introduction



Imagine hearing a beep or buzz all day, every day, that nobody else hears but that interferes with almost everything you do, even sleeping. It sounds like a nightmare that could drive anyone crazy. Nearly everyone has experienced a beep or buzz in their ears at some point in their life. The name for this disease is tinnitus, after the Latin word for ringing: *'tinnere'*. For most, it will disappear. Unfortunately, it is reality for a growing number of people that this beep or buzz remains present, which can have an enormous impact on the quality of life.

In the Netherlands, the incidence of tinnitus in the first line of care is approximately 1.3 per 1000 patients per year in the period 2000-2002[1]. A recent meta-analysis showed that tinnitus prevalence largely differs between studies, ranging from 5.1-42.7% in the general population[2]. Tinnitus can already be present in children[3], becomes more common in young adults[4] and is most prevalent in middle-aged and older adults, where prevalence becomes stable around 70 years of age[2]. In young adults an important risk factor for chronic tinnitus is cumulative noise exposure[5], whereas in middle-aged and older adults hearing loss is the main risk factor[6].

In tinnitus research the burden of disease can differ between different study populations. The main group are those with 'any' tinnitus, so any person that hears a beep/buzz/sound in their head or ears. A distinct subgroup are the people with 'clinical tinnitus', those who have entered the health care system for their tinnitus complaints. It appears that many people from the general population report to have tinnitus, but for most the burden of disease is low or non-existent[7]. At the other end of the spectrum are the patients that enter the health care system searching for relieve or even lose the lust to live[8]. Unfortunately, there is no causal treatment for tinnitus available. A 2019 European multidisciplinary guideline for tinnitus diagnostics, assessment and treatment concluded that there are countless investigated treatment options for tinnitus[9]. Nevertheless, at the moment, cognitive behavioural therapy has been proven effective in reducing tinnitus complaints in all patients and the use of hearing aids can be effective in people with additional hearing loss[9].

Historical perspective on tinnitus

It appears that tinnitus has been around for as long as we know, and people were searching for the cause and a treatment. In 1984 professor Dafydd Stephens published a paper in which he investigated historical texts on tinnitus and its treatment, which will be summarized in the next paragraphs [10]. In one of the earliest medical texts, written by the Egyptians on papyrus rolls in the sixteenth century B.C., tinnitus is

referred to as 'a bewitched ear' and even a treatment is mentioned. A later papyrus from Crocodilopolis (second century B.C.) refers to treatments for 'humming in the ears'. Mesopotamian clay tablets described treatments for tinnitus and subdivided three types, 'singing of the ears', 'whispering of the ears' and 'speaking of the ears'. Interestingly, in these texts a first mention was made towards a possible etiology: 'If when the hand of a ghost seizes a man, his ears sing…'.

For a long time, the theory by the Greek philosopher Empedocles (504-433 B.C.) on the disbalance of the humours as cause for all diseases was the leading theory on the origin of tinnitus and therefore also the basis of treatment. The compendium 'De Medicina' by Celsus (around 30 A.D.) has a large section devoted to ear disease and three paragraphs specifically to tinnitus treatment, specified by origin. Celsus divided tinnitus origin into 'stemming from a cold', or 'from disease in the head' or 'when it is due to other causes it is probably sinister'.

In the Middle Ages, the theory of the humours was still important in the causation of disease, although it had matured over time and place. Tinnitus was seen as 'wind trapped in the brain', but there were still many theories on how this could happen. Paul of Aegina (625-690 A.D.) divided tinnitus into three categories; that 'due to fevers', 'chronic noises produced by thick and viscid humours' and 'chronic hissing sounds'. A little later Avicenna (980-1036 A.D.) wrote his *Canon*, which became one of the leading medical textbooks in the middle ages. He described several types of tinnitus: '… sometimes comes because of certain medicines which cause a retention of the humours and winds in certain parts of the brain' (the first description of ototoxic tinnitus), 'tinnitus caused by viscous humours stopping up the ear', 'tinnitus caused by fevers', 'tinnitus caused by excitement of the senses' and 'tinnitus caused by cold viscous humours'. An Englishman from the same period, Gilbertus Anglicus (1180-1250), was the first to describe tinnitus due to 'feebleness of the ears', most probably referring to tinnitus due to hearing loss.

In the Renaissance, knowledge of the anatomy and physiology of the ear increased rapidly. Nonetheless, tinnitus remained a disease about which little was known. Duverney (1648-1730) was the first to report tinnitus not being an 'excess of wind in the ears' but rather a distortion of perception within the auditory mechanical system[11]. He was also the first to make the distinction between 'true tinnitus' and 'false tinnitus'. Regarding true tinnitus as 'the perception of a sound that is internal' and false tinnitus as 'a sound that is not internal'. The next noteworthy tinnitus classification was by Gaspar Itard (1775-1838) who is seen as the founder of audiological medicine. He subdivided tinnitus into true, false and fantastic tinnitus. Where 'true tinnitus' reflects

accentuation of physiological noises, 'false tinnitus' is not related to normal physiological noises and 'fantastic tinnitus' reflects auditory hallucinations. This sub-classification proposed by Itard was the backbone for many new treatment strategies for tinnitus in years to follow. Throughout the nineteenth century, after Itard's 'Traite des maladies de l'oreille (1821)', no revolutionary new ideas on tinnitus classification or aetiology were posed. Equally during the twentieth century, Itard's classification of tinnitus remained the backbone of tinnitus research. During this period, tinnitus research was primarily focused on finding a treatment for the disease[10]. At the end of the twentieth century Jastreboff proposed a more integrative model of tinnitus generation and manifestation[12]. He was the first to argue that not only the auditory pathways are involved in tinnitus generation, but that the cortices, limbic system and prefrontal cortex are involved in tinnitus perception[12]. This resulted in the acceptation that emotional well-being is associated with tinnitus.

Current perspective on tinnitus

Only more recently, the classification of true and false tinnitus has changed into 'objective' and 'subjective' tinnitus [13]. Objective tinnitus is tinnitus that can be heard by both physician and patient, whereas subjective tinnitus is only heard by the patient him or herself[13].

Objective tinnitus has a physical origin. Patients may regard their tinnitus as pulsatile, either synchronous with the heartbeat (a vascular origin of the tinnitus) or asynchronous (usually having a myoclonal origin in the middle-ear or from the palatal muscle)[14]. Objective tinnitus is far less common than subjective tinnitus, and is not part of the scope of this thesis.

The exact pathophysiology of tinnitus remains unknown. Following the Jastreboff model, it is accepted that tinnitus reflects a complex interplay between peripheral and central components in the auditory pathway[15,16]. In addition, psychosocial wellbeing is recognized as an important factor in tinnitus perception. Tinnitus has been frequently associated with a range of psychological conditions, such as depression, anxiety, irritability, sleep disturbances, subjective distress and intense worrying[17]. It is unknown how reduced psychosocial wellbeing and tinnitus are related, it appears that either can cause or attenuate the other[18].

Three possible independent tinnitus subtypes are proposed; 1. cochlear tinnitus, 2. peripheral-dependent central tinnitus and 3. peripheral-independent central tinnitus[19]. Cochlear tinnitus refers to an origin in damage of the outer hair cells, which

may cause spontaneous firing and is transferred through the cochlear nerve to the central auditory pathway and cortex[19]. Peripheral-dependent central tinnitus is thought to be due to cochlear damage resulting in a lack of input in which any part of the central auditory pathway may respond in overcompensating[20]. In the case of peripheral-independent central tinnitus, any part of the central auditory pathway may induce tinnitus in absence of cochlear damage[21]. These studies on tinnitus pathophysiology provide a framework which is important for a better understanding of tinnitus and subsequent for effective therapeutic strategy development.

In summary, tinnitus is a symptom of a phenomenon in which the sub-types cannot be easily distinguished in at an individual level. It is thought that at least two or more triggers are needed to induce tinnitus[22]. These triggers can be hearing loss, noise damage, emotional distress and somatosensory factors. Despite the increased insight into pathophysiological mechanisms, it remains unclear why one person develops tinnitus and another does not, even though they have a seemingly similar profile. A strategy to determine clinically relevant subtypes, is to use epidemiological studies to find risk factors for tinnitus. Insight in those risk factors will hopefully help in developing a profile of a tinnitus patient that can be used for subtyping and subsequent therapeutic strategies.

Epidemiological evidence from population-based studies has shown that older age, male sex and lower education level are demographic risk factors for tinnitus[2]. Hearing loss, both conductive and sensorineural, is reported to be the most important risk factor for tinnitus[9]. However, there are many people that suffer from tinnitus but do not show any hearing loss on conventional audiometry[23-25]. Depression, anxiety and sleeping issues are also frequently reported as risk factors for tinnitus in both clinical[26-28] and population-based studies[6,29-34]. Additionally, several metabolic risk factors appear to be associated with tinnitus, including hyperthyroidism, diabetes, hypertension, hypercholesterolemia and anaemia[6,35-37]. Another important risk factor for tinnitus is the use of ototoxic medication, as tinnitus is often reported as a side effect of many known drugs[38].

Aim of this thesis

The main goal for this thesis is to further specify and identify determinants for tinnitus. To achieve this we investigated the age-dependency of tinnitus and its relation with hearing loss and hearing function, brain morphology and psychosocial well-being in people with tinnitus, and several modifiable risk factors of tinnitus.

All studies included in this thesis are embedded within the Rotterdam Study, which is one of the longest running population-based studies in the world, since 1989. It was setup to investigate the determinants and consequences of ageing[39]. Hearing function and tinnitus assessment were added to the study protocol in 2011. Tinnitus is assessed during a home interview with the question 'Do you experience sounds in the head or in (one of) the ears (such as whizzing, peeping or humming) without an objective external sound source?'. Tinnitus severity was assessed with the screening version of the tinnitus handicap inventory (THI-s)[40,41]. Pure-tone audiometry is used to assess peripheral hearing function, whereas the Digits In Noise test assesses central hearing as well.

In **chapter 2** we will explore the prevalence of tinnitus and its relationship with hearing loss. First, in **chapter 2.1** we study the occurrence of tinnitus in a general population of middle-aged and elderly participants and its relationship with prevalent hearing loss. In **chapter 2.2** we determined how tinnitus interferes with speech in noise understanding, a measure for hearing in difficult circumstances.

In **chapter** 3 we will investigate the association of modifiable risk factors with tinnitus. Cardiovascular health is a risk factor for many diseases, including hearing loss [35]. We therefore were interested whether cardiovascular health would also be associated with tinnitus, which we explore in **chapter 3.1**. Next, in **chapter 3.2 and chapter 3.3** we investigate the association between ototoxic medication and hearing loss and tinnitus. Ototoxicity is commonly reported as a side effect of many often prescribed drugs[38].

In **chapter 4** we will explore how prevalent tinnitus affects the brain and vice versa. This is firstly done in **chapter 4.1** in which we compared brain tissue volumes from magnetic resonance imaging (MRI) data between participants with and without tinnitus. **Chapter 4.2** describes the association of tinnitus with mental health status, both cross-sectionally as well as longitudinally.

Finally, in **chapter 5**, we will conclude with a general discussion of the studies embedded in this thesis in relation to literature and current clinical practice. Additionally we will propose some future directions for tinnitus research.

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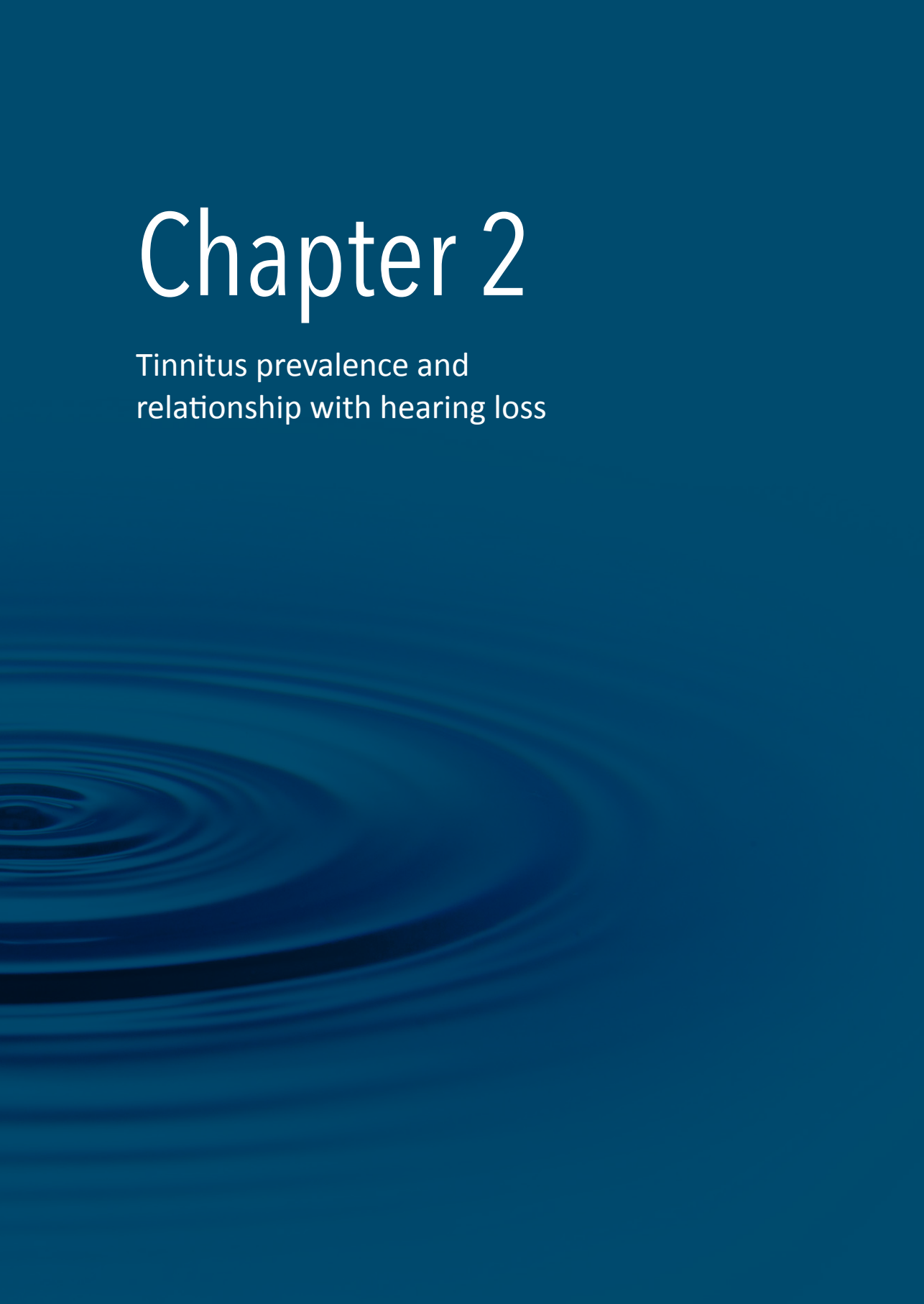
Chapter 2

Tinnitus prevalence and relationship with hearing loss



Chapter 2.1

Prevalence of tinnitus in an aging population and its relation to age and hearing loss

Otolaryngoly, Head&Neck surgery, 2020

Berthe C. Oosterloo; Pauline H. Croll; Robert J. Baatenburg de Jong;
M. Kamran Ikram; André Goedegebure

Abstract

Objectives

Tinnitus is a common hearing-related disorder, which may have a large impact on daily life. With aging populations worldwide, it is important to gain insight in the occurrence of tinnitus at older ages and to understand its relationship with age-related hearing loss. We investigated the prevalence of tinnitus among a general aging population, across age-strata and hearing status.

Study design

Cross sectional.

Setting

The population-based Rotterdam Study.

Subjects and methods

6,098 participants underwent tinnitus assessment and 4,805 had additional hearing assessment. We determined tinnitus prevalence per 5-year age-groups. Hearing impairment was defined as ≥25 dB HL worse ear pure tone average (0.5, 1, 2, 4 kHz). We investigated with multivariable logistic regression the association between hearing impairment and tinnitus. Tinnitus handicap was assessed in 663 participants with daily tinnitus with the Tinnitus Handicap Inventory, screening version (THI-s).

Results

Tinnitus was prevalent in 21.4% (N=1,304). Prevalent tinnitus was evenly distributed over 5-year age-groups. Participants with hearing impairment were more likely to have tinnitus (OR: 2.27 (95%CI: 1.92, 2.69)) compared to those without hearing impairment. The median THI-s score is 4 (IQR 0-10), i.e. slight handicap. 14.6% of the participants reported a moderate or severe handicap (THI-s ≥16).

Conclusions

In a general elderly population, 1 in 5 persons has tinnitus. Of those with tinnitus, for 1 per 10 persons the presence of tinnitus interfered with daily life. Participants with hearing impairment were twice as likely to have tinnitus. Despite the age dependent occurrence of hearing impairment, no such age-dependency was found for tinnitus.

Introduction

Tinnitus is a common disorder in the adult population[1]. Tinnitus is defined as a sound that is heard in the absence of an objective external sound-source. For some, tinnitus is not bothersome at all, whereas others might experience it as very disturbing and warrants health care[2,3]. In spite of the clear definition of subjective tinnitus as phenomenon in literature, there is no consensus about when tinnitus becomes pathological. The lack of gold standard for pathological tinnitus leads to a wide variety in reported prevalence. In several population based studies of 18 years and older, tinnitus prevalence ranges from 9% up to 35%[1].

Various risk factors for tinnitus, such as otological, audiological, personal, socio-economic and disease related factors have been reported[4]. It is generally accepted that hearing impairment is one of the leading risk factors associated with tinnitus[5,6]. As the worldwide population is aging[7], the prevalence of age-related hearing impairment is increasing accordingly[8,9]. As such, it can be expected that tinnitus prevalence will increase as well[10]. However, limited data are available on the age-dependency of tinnitus in a population of older adults. Most studies that investigated tinnitus and age-dependency did so in middle aged populations[1]. The studies that investigated older population, reported prevalence numbers of 8.2% up to 30.3%, in which age-dependency was reported by several studies[11-15], others did not[16-22]. There is still a lack of understanding about the prevalence and age-dependency of tinnitus in the general, aging population and its association with age-related hearing loss.

Therefore, in this study, we aimed to determine (a) the prevalence of tinnitus in an aging population-based sample, (b) its age distribution and association with sex and highest achieved education, taking in account the potential underlying association with hearing impairment, and (c) the handicap associated with prevalent tinnitus.

Methods

setting and study population

This cross-sectional study was embedded in the Rotterdam Study, a prospective, population-based cohort study. The Rotterdam Study was initiated in 1989 and investigates determinants and consequences of aging. Details of the study have been described elsewhere[23]. The entire study population consists of 14,926 individuals aged ≥ 45 years living in the well-defined Ommoord district in the city of Rotterdam, the Netherlands[23]. All participants were invited to undergo extensive examinations

in the dedicated research center at study entry and subsequently every 3 to 4 years. In total, almost 80% of the inhabitants aged 50 years and older who were invited to participate in the study between February 2011 and December 2016 were tested, including audiometry. Participation rates did not significantly vary among age groups.

Tinnitus and hearing assessment were introduced into the core study protocol in 2011. Of the 6,168 eligible participants, 6,098 complete cases were included who underwent home interview regarding the presence or absence of tinnitus and 663 participants filled out the Tinnitus Handicap Inventory screening version (THI-s) between 2011 and 2016 in the current study. Of the participants with information on tinnitus status, 4,805 underwent hearing assessment in the dedicated study center between 2011 and 2016.

Tinnitus assessment

Tinnitus assessment was performed through a home interview. Participants were asked if they experienced sounds in the head or in (one of) the ears (such as whizzing, peeping, or humming) without an objective external sound source being present. Possible answers to this question were: 'no, never'; 'yes, less than once a week'; 'yes, more than once a week but not daily' and 'yes, daily'.

For the current study, tinnitus was investigated 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'). Because of the heterogeneity of the origin and the often temporary character of tinnitus, presence of less than once a week was not recorded as prevalent tinnitus. All participants who answered that they experienced tinnitus were asked whether it interferes with daily life ('yes' or 'no').

Only participants suffering from tinnitus on a daily basis were asked to fill out the simplified Tinnitus Handicap Inventory (THI-s)[24]. This inventory consists of 10 items, with a possible score of 0, 2 or 4 per item, which includes questions on the interference of tinnitus in daily life. A score of ≥16 represented a moderate/severe handicap[24,25].

Hearing assessment

Audiometric assessment was performed by one trained health care professional in a soundproof booth. For the audiometric assessment, a computer-based audiometry system (Decos Technology Group, version 210.2.6 with AudioNigma interface) and TDH-39 headphones were used[23]. To determine hearing levels in decibel hearing level (dB HL), pure tone audiometry was used according to the ISO-standard 8253-1[26]. Air conduction thresholds for both ears were measured on different frequencies (0.25,

0.5, 1, 2, 4, and 8 kilohertz (kHz)). Masking was performed according to the method of Hood[27]. Conductive hearing losses (air-bone gap >15 dB HL) were not excluded as the origin of the hearing loss does not seem to matter in tinnitus induction[28].The worse hearing ear was determined by taking the average dB HL over all measured frequencies. The worse hearing ear is chosen as this is the most probable ear for tinnitus to occur in[29]. Pure tone average hearing thresholds, averaged over 0.5, 1, 2, 4 kHz, were determined based on the worse hearing ear[29]. Hearing impairment was determined as an average threshold ≥25 dB HL[30].

Covariables

Sex, age (years) and highest achieved educational level were investigated as covariables. Educational level was categorized as lower, middle, or higher education according to the UNESCO International Standard Classification of Education[31].

Statistical analysis

We investigated the prevalence of tinnitus in several steps. First, we compared the differences in demographic characteristics (sex, age and highest achieved education) between participants with and without tinnitus. We used a t-test, one-way ANOVA, Mann Whitney U-test and X^2-test when appropriate. Second, we performed a multivariable logistic regression analysis for the association between hearing impairment and tinnitus adjusted for sex and age. We repeated this analysis whilst stratifying in 5-year age groups. Next, we described the severity of the tinnitus complaints, as reported with the THI-s. The THI-s score was described as median (interquartile range (IQR)) and % with a score of ≥16, i.e. reporting a relevant tinnitus associated handicap. We compared demographics between participants with a relevant handicap vs. a low handicap. Finally we performed a sensitivity analysis with an altered definition of tinnitus (only daily) and no tinnitus (never tinnitus) in the demographics of the population and between participants with and without tinnitus according to this definition.

Results

Tinnitus prevalence and demographic characteristics

Out of 6,098 participants, we found that 21.4% reported tinnitus (Table 1). The prevalence of tinnitus did not vary significantly between the different age groups: it ranged between 23.2% in the 65-70-year-old group and 19.9% in the 80-85-year-old group, X^2-test p=0.585 (Table 1, Figure 1). Participants with prevalent tinnitus were more often male than participants without tinnitus (46.5% vs. 41.0%, p<0.001).

A similar difference in the proportion of males was found in the 60-75 years age groups, not in the other age groups. There was no difference in highest achieved education between the participants with and without tinnitus, neither in the entire population, nor in either age group (Table 1).

Tinnitus and hearing impairment

The average hearing threshold in the study population was 30.5 dB HL (SD: 17.3) (Table 1). In all age groups, except that of >85 years of age, participants with tinnitus had a significantly higher average hearing threshold (Table 2).

Table 1. Participant characteristics, comparing tinnitus with no tinnitus

	Total N	Total population	Tinnitus	No tinnitus	P value
N (%)	6,098	6,098	1,304 (21.4)	4,794 (78.6)	
Male (%)	6,098	2,570 (42.1)	606 (46.5)	1,964 (41.0)	<0.001
Age	6,098	69.4 (10.1)	69.3 (9.8)	69.5 (10.2)	0.644
Age group	6,098				0.585
50-55		382 (6.3)	79 (6.1)	303 (6.3)	
55-60		829 (13.6)	172 (13.2)	657 (13.7)	
60-65		953 (15.6)	191 (14.6)	762 (15.9)	
65-70		1,382 (22.7)	321 (24.6)	1,061 (22.1)	
70-75		780 (12.8)	174 (13.3)	606 (12.6)	
75-80		622 (10.2)	135 (10.4)	487 (10.2)	
80-85		677 (11.1)	135 (10.4)	542 (11.3)	
≥85		473 (7.8)	97 (7.4)	376 (7.8)	
Education (%)	6,098				0.149
Lower		2,953 (48.4)	661 (50.7)	2,292 (47.8)	
Middle		1,749 (29.4)	374 (28.7)	1,420 (29.6)	
Higher		1,351 (22.2)	269 (20.6)	1,082 (22.6)	
Hearing threshold, dB HL	4,805	30.5 (17.3)	35.4 (19.2)	29.1 (16.5)	<0.001
Hearing impairment ≥25 dB HL (%)	4,805	1,547 (32.2)	449 (43.2)	1,098 (29.2)	<0.001
Tinnitus impairment daily life (%)	6,098	160 (2.6)	160 (12.3)	-	
THI-s score‡	663	4 (0, 10)	4 (0, 10)	-	
THI-s ≥16 (%)	663	97 (1.6)	97 (14.6)	-	

Values are mean (standard deviation (SD)) for normally distributed continuous variables. ‡Median (interquartile range (IQR)) for non-normally distributed continuous variables. Categorical variables are given as N, (%). dB HL: decibel. Hearing impairment was averaged over the 0.5, 1, 2, 4 kHz frequencies in the worst ear. THI-s: Tinnitus Handicap Inventory – screening version. THI-s ≥ 16; the participants with a THI-s score ≥ 16 represent participants suffering from a moderate/severe handicap from tinnitus.

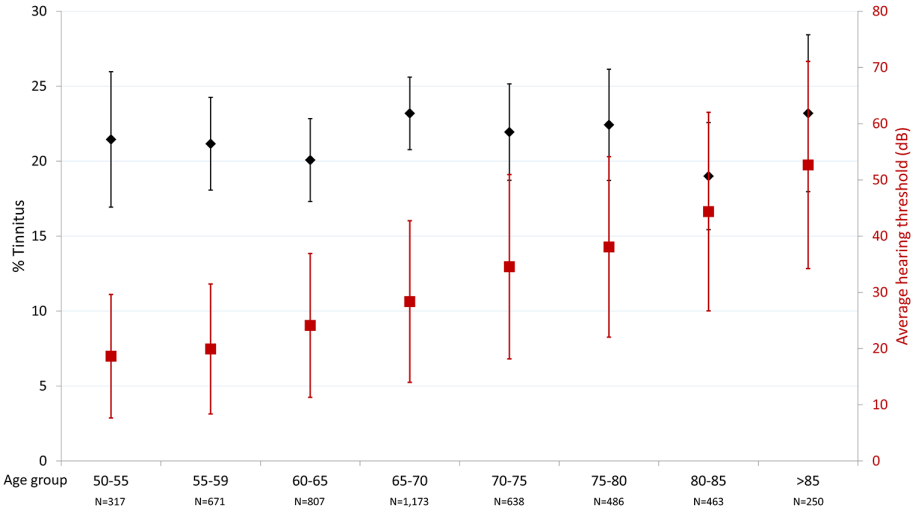


Figure 1. In participants with hearing assessment (N = 4,805), tinnitus prevalence (95%CI) and average hearing threshold (+/- 1SD) per 5-year age groups. Hearing threshold: 0.5, 1, 2, 4 kHz in the worse hearing ear.

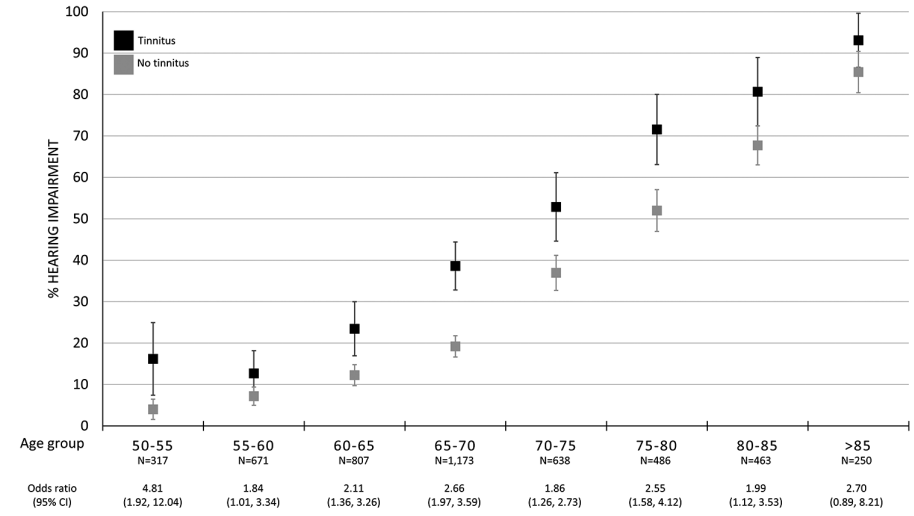


Figure 2. The prevalence of hearing impairment in participants with and without tinnitus (N=4,805), per age category. Odds ratio's (OR) are adjusted for age and sex. Hearing impairment: ≥25dB HL over 0.5- 4 kHz in worse hearing ear.

Table 2. Participant characteristics, comparing tinnitus with no tinnitus, per 5-year age-category

		Total N	Total population	Tinnitus	No tinnitus	P value
50-55 years	N (%)	382	382	79 (20.7)	303 (79.3)	
	Male (%)	382	159 (41.6)	36 (45.6)	123 (40.6)	0.424
	Education (%)	382				0.457
	Lower		125 (32.7)	28 (35.4)	97 (32.0)	
	Middle		144 (37.7)	25 (31.6)	119 (39.3)	
	Higher		113 (29.6)	26 (32.9)	87 (28.7)	
	Hearing threshold, dB HL	317	18.7 (11.0)	22.1 (10.8)	17.7 (10.9)	0.004
	Hearing impairment ≥25 dBHL (%)	317	21 (6.6)	11 (16.2)	10 (4.0)	<0.001
	Tinnitus impairment daily life (%)	382	8 (2.1)	8 (10.1)	-	
	THI-s score±	39	4 (0, 8)	4 (0, 8)	-	
	THI-s ≥16 (%)	39	6 (1.6)	6 (15.4)	-	
55-60 years	N (%)	829	829	172 (20.7)	657 (79.3)	
	Male (%)	829	360 (43.4)	80 (46.5)	280 (42.6)	0.406
	Education (%)	829				0.711
	Lower		289 (34.9)	63 (36.6)	226 (34.4)	
	Middle		263 (31.7)	56 (32.6)	207 (31.5)	
	Higher		277 (33.4)	53 (30.8)	224 (34.1)	
	Hearing threshold, dB HL	671	19.9 (11.6)	23.4 (14.8)	19.0 (10.3)	0.001
	Hearing impairment ≥25 dBHL (%)	671	56 (8.3)	18 (12.7)	38 (7.2)	0.036
	Tinnitus impairment daily life (%)	829	27 (3.3)	27 (15.9)	-	
	THI-s score±	87	4 (0, 12)	4 (0, 12)	-	
	THI-s ≥16 (%)	87	14 (1.7)	14 (16.1)	-	
60-65 years	N (%)	953	953	191 (20.0)	762 (80.0)	
	Male (%)	953	406 (42.6)	98 (51.3)	308 (40.4)	0.007
	Education (%)	953				0.596
	Lower		439 (46.1)	93 (48.7)	346 (45.4)	
	Middle		253 (26.5)	51 (26.7)	202 (26.5)	
	Higher		261 (27.4)	47 (24.6)	214 (28.1)	
	Hearing threshold, dB HL	807	24.1 (12.8)	28.6 (13.2)	23.0 (12.4)	<0.001
	Hearing impairment ≥25 dBHL (%)	807	117 (14.5)	38 (23.5)	79 (12.2)	<0.001
	Tinnitus impairment daily life (%)	953	27 (2.8)	27 (14.1)	-	
	THI-s score±	90	6 (0, 14)	6 (0, 14)	-	
	THI-s ≥16 (%)	90	22 (2.3)	22 (24.4)	-	

Table 2. Continued

		Total N	Total population	Tinnitus	No tinnitus	P value
65-70 years	N (%)	1,382	1,382	321 (23.2)	1,061 (76.8)	
	Male (%)	1,382	624 (45.2)	167 (52.0)	457 (43.1)	0.005
	Education (%)	1,382				0.192
	Lower		691 (50.0)	154 (48.0)	537 (50.6)	
	Middle		356 (25.8)	95 (29.6)	261 (24.6)	
	Higher		335 (24.2)	72 (22.4)	263 (24.8)	
	Hearing threshold, dB HL	1,173	28.4 (14.4)	34.0 (17.3)	26.7 (12.9)	<0.001
	Hearing impairment ≥25 dBHL (%)	1,173	278 (23.7)	105 (38.6)	173 (19.2)	<0.001
	Tinnitus impairment daily life (%)	1,382	37 (2.7)	37 (11.5)	-	
	THI-s score±	165	4 (0, 10)	4 (0, 10)	-	
	THI-s ≥16 (%)	165	21 (1.5)	21 (12.7)	-	
70-75 years	N (%)	780	780	174 (22.3)	606 (77.7)	
	Male (%)	780	340 (43.6)	92 (52.9)	248 (40.9)	0.005
	Education (%)	780				0.384
	Lower		419 (53.7)	100 (57.5)	319 (52.6)	
	Middle		229 (29.4)	50 (28.7)	179 (29.5)	
	Higher		132 (16.9)	24 (13.8)	108 (17.8)	
	Hearing threshold, dB HL	638	34.6 (16.4)	40.5 (20.3)	32.9 (14.7)	<0.001
	Hearing impairment ≥25 dBHL (%)	638	258 (40.4)	74 (52.9)	184 (36.9)	0.001
	Tinnitus impairment daily life (%)	780	20 (2.6)	20 (11.5)	-	
	THI-s score±	75	2 (0, 8)	2 (0, 8)	-	
	THI-s ≥16 (%)	75	10 (1.3)	10 (13.3)	-	
75-80 years	N (%)	622	622	135 (21.7)	487 (78.3)	
	Male (%)	622	260 (41.8)	57 (42.2)	203 (41.7)	0.911
	Education (%)	622				0.030
	Lower		337 (54.2)	84 (62.2)	253 (52.0)	
	Middle		191 (30.7)	29 (21.5)	162 (33.3)	
	Higher		94 (15.1)	22 (16.3)	72 (14.8)	
	Hearing threshold, dB HL	486	38.1 (16.0)	44.5 (17.2)	36.2 (15.2)	<0.001
	Hearing impairment ≥25 dBHL (%)	486	274 (56.4)	78 (71.6)	196 (52.0)	<0.001
	Tinnitus impairment daily life (%)	622	17 (2.7)	17 (12.5)	-	
	THI-s score±	79	4 (0,10)	4 (0,10)	-	
	THI-s ≥16 (%)	79	11 (1.7)	11 (13.9)	-	

Table 2. Continued

		Total N	Total population	Tinnitus	No tinnitus	P value
80-85 years	N (%)	677	677	135 (19.9)	542 (80.1)	
	Male (%)	677	276 (40.8)	48 (35.6)	228 (42.1)	0.168
	Education (%)	677				0.366
	Lower		351 (51.8)	77 (57.0)	274 (50.6)	
	Middle		228 (33.7)	42 (31.1)	186 (34.3)	
	Higher		98 (14.5)	16 (11.9)	82 (15.1)	
	Hearing threshold, dB HL	463	44.4 (17.7)	49.5 (19.1)	43.2 (17.1)	0.003
	Hearing impairment ≥25 dBHL (%)	463	325 (70.2)	71 (80.7)	254 (67.7)	0.017
	Tinnitus impairment daily life (%)	677	16 (2.4)	16 (11.9)	-	
	THI-s score‡	82	4 (0,8)	4 (0,8)	-	
	THI-s ≥16 (%)	82	9 (1.3)	9 (11.0)	-	
≥ 85 years	N (%)	473	473	97 (20.5)	376 (79.5)	
	Male (%)	473	145 (30.7)	28 (28.9)	117 (31.1)	0.668
	Education (%)	473				0.964
	Lower		302 (63.8)	62 (63.9)	240 (63.8)	
	Middle		130 (27.5)	26 (26.8)	104 (27.7)	
	Higher		41 (8.7)	9 (9.3)	32 (8.5)	
	Hearing threshold, dB HL	250	52.7 (18.4)	55.3 (19.2)	51.9 (18.2)	0.221
	Hearing impairment ≥25 dBHL (%)	250	218 (87.2)	54 (93.1)	164 (85.4)	0.125
	Tinnitus impairment daily life (%)	473	8 (1.7)	8 (8.2)	-	
	THI-s score‡	46	2 (0,4)	2 (0,4)	-	
	THI-s ≥16 (%)	46	4 (0.8)	4 (8.7)	-	

Values are mean (standard deviation (SD)) for normally distributed continuous variables. ‡Median (interquartile range (IQR)) for non-normally distributed continuous variables. Categorical variables are given as N, (%). dB HL: decibel. Hearing impairment was averaged over the 0.5, 1, 2, 4 kHz frequencies in the worst ear. THI-s: Tinnitus Handicap Inventory – screening version. THI-s ≥ 16; the participants with a THI-s score ≥ 16 represent participants suffering from a moderate/severe handicap from tinnitus.

The prevalence of hearing impairment was 25.4% in the entire population and significantly higher in the participants with tinnitus compared to those without tinnitus (43.2% vs. 29.2%, respectively, p<0.0001) (Table 1). Participants with prevalent tinnitus in the youngest age group in our population (50-55 years of age) had more often hearing impairment (16.2%) than participants without tinnitus (4.0%, p<0.001, Table 2, Figure 2). The increase of the prevalence of hearing impairment is similar in participants with and without tinnitus (Table 2, Figure 2). Participants with hearing

Table 3. Sensitivity analysis comparing daily tinnitus to never tinnitus.

	Total N	Total population	Tinnitus	No tinnitus	P value
N (%)	4,920	4,920	827 (16.8)	4,093 (83.2)	
Male (%)	4,920	2,100 (42.7)	399 (48.2)	1,701 (41.6)	<0.001
Age	4,920	69.6 (10.1)	69.5 (9.6)	69.7 (10.2)	0.690
Age group	4,920				0.170
50-55		290 (5.8)	45 (5.4)	245 (6.0)	
55-60		670 (13.6)	104 (12.5)	566 (13.8)	
60-65		751 (15.3)	115 (13.9)	636 (15.5)	
65-70		1,105 (22.5)	210 (25.4)	895 (21.9)	
70-75		646 (13.1)	119 (14.4)	527 (12.9)	
75-80		506 (10.3)	89 (10.8)	417 (10.2)	
80-85		572 (11.6)	93 (11.2)	479 (11.7)	
≥85		380 (7.7)	52 (6.3)	328 (8.0)	
Education (%)	4,920				0.478
Lower		2,357 (47.9)	409 (49.5)	1,948 (47.6)	
Middle		1,467 (29.8)	246 (29.7)	1,221 (29.8)	
Higher		1,096 (22.3)	172 (20.8)	924 (22.6)	
Hearing threshold, dB HL	3,896	30.4 (17.1)	37.0 (19.2)	29.0 (16.3)	<0.001
Hearing impairment ≥25 dB HL (%)	3,896	1,264 (25.7)	320 (47.5)	944 (29.3)	<0.001
Tinnitus impairment daily life (%)	4,920	110 (2.2)	110 (13.3)	-	
THI-s score‡	663	4 (0, 10)	4 (0, 10)	-	
THI-s ≥16 (%)	663	97 (2.0)	97 (11.7)	-	

Values are mean (standard deviation (SD)) for normally distributed continuous variables. ‡Median (interquartile range (IQR)) for non-normally distributed continuous variables. Categorical variables are given as N, (%). dB HL: decibel. Hearing impairment was averaged over the 0.5, 1, 2, 4 kHz frequencies in the worst ear. THI-s: Tinnitus Handicap Inventory – screening version. THI-s ≥ 16; the participants with a THI-s score ≥ 16 represent participants suffering from a moderate/severe handicap from tinnitus.

impairment were twice as likely to have tinnitus compared to participants without hearing impairment, OR: 2.27 (95% CI: 1.92, 2.69), a result that was found across all age groups (Figure 2).

Tinnitus handicap

Of all participants with tinnitus (N=1,304), 160 participants (12.3%) reported that their tinnitus interfered with daily life. This reflected 2.6% of the entire population.

The THI-s was available for 76% of the participants with daily tinnitus (Table 1). The median THI-s score was 4 (IQR 0 - 10), i.e. representing no or a slight handicap. A relevant tinnitus handicap (score ≥ 16) was found in 14.6% (N = 97) of the participants that filled out the THI-s. The median THI-s score was 4 for almost all age categories. The prevalence of a relevant handicap hardly showed differences between age categories, except for a slightly higher percentage in the group 60-65 years old (Table 2.) Neither did we find a significant difference between the sexes (male 12.2%, female 16.8%, p=0.092), nor by hearing threshold (no hearing impairment 14.0%, hearing impairment 16.1%, p=0.481).

Sensitivity analysis

Finally we ran a sensitivity analysis, in which 'tinnitus' was defined as only 'daily tinnitus' and 'no tinnitus' as 'never tinnitus'. Here, we found that an altered definition of tinnitus did not lead to significant differences in the results (Table 3 as compared to Table 1).

Discussion

In this study, we found that the prevalence of tinnitus was 21.4% in a general Dutch population-based sample of older adults (50 years and older), using a definition of tinnitus being present more than once a week regardless of the tinnitus burden. For 1 in 10 persons with tinnitus, the presence of tinnitus interfered with their daily life. Furthermore, participants with hearing impairment were twice as likely to have tinnitus. Despite the age dependent occurrence of hearing impairment, no such age-dependency was found for tinnitus.

In this study we found a similar prevalence of tinnitus over the age groups, whereas the proportion of participants with hearing impairment was as expected much higher in the older age groups. Interestingly, we found a similar increase of % hearing impairment above the age of 55 years both for tinnitus and no-tinnitus participants. This suggests that tinnitus in itself is, unlike hearing impairment, probably not associated with aging processes. We propose several possible mechanisms for this. First, aging in itself does not put individuals at a greater risk of developing tinnitus. This implies that age-related change/decline of the brain does not lead to an increased vulnerability for developing tinnitus. Second, although hearing loss in general is an important risk factor for tinnitus, age-related aspects of hearing impairment are not likely to induce tinnitus. One of the explanations may be the gradual development of age-related hearing impairment. It is suggested that a sudden lack of input to the

brain from the cochlea can result in tinnitus[32-35]. In contrast to this hypothesis stands age-related hearing impairment, which is a slowly progressing disease of the auditory system and therefore the brain has time to adjust to the increasing lack of input[35]. Another possible explanation is that the pathophysiology of age-related hearing impairment is principally different from other types of hearing loss, like noise-induced hearing loss, that are more likely to induce tinnitus than others[33,36,37]. We therefore hypothesize that tinnitus and hearing impairment in the elderly co-occur, but the age-related aspect of hearing impairment does not seem to contribute to the found association between hearing impairment and tinnitus.

The reported prevalence of tinnitus in our cohort of 21.5%, is in the middle of the range reported by the McCormack review (5.1%-42.7%) consisting of both larger and smaller populations[1]. More specifically, tinnitus prevalence from other large population-based studies ranges from 9.6% up to 30.3%[14,15,22,38-40]. To our knowledge, no other study has yet reported the prevalence of tinnitus in 5-year age intervals, in which we, unexpectedly, found no differences. This is in contrast to what was reported in the review by McCormack , where the authors stated: "*The prevalence figures generally show an increase in tinnitus prevalence as age increases*."[1]. It should be noted though, that this statement is based on studies reporting tinnitus prevalence in populations of 20 years of age and older and not solely in an elderly population, like our study. Only a few studies describe prevalence trends in 10 year intervals in populations consisting of older participants (>45 years). These studies report ambiguous conclusions about tinnitus prevalence in these older participants, both an increased prevalence[11,14] and a similar prevalence[12,13] with increasing age. Interestingly, these four studies are comparable in their assessment of tinnitus and consist of larger populations (N>1320), similar to our current study. Comparing tinnitus prevalence between studies is complicated as there is no gold standard for the assessment. The frequency of tinnitus being present is one of the main differences in definition between studies. This frequency ranges between 'daily >5 minutes' or 'ever'[1]. For example, when we alter the definition of tinnitus in our study the reported prevalence will change as well. The prevalence would increase from 21.4% to 32.9% when applying a broader definition including any form of tinnitus. Which might result in effect dilution as it increases the chance of misclassifying temporary tinnitus related to specific conditions, such as noise exposure, as chronic tinnitus. Conversely, if we would classify participants with daily tinnitus as prevalent tinnitus, the prevalence in our population decreased towards 13.6%. This number decreased further towards 2.1% in our population when tinnitus was defined as experiencing tinnitus on a daily basis and when tinnitus interferes with daily life.

Population-based studies have shown that the handicap associated with tinnitus is generally mild, yet for some it interferes with life on a daily basis[4,41,42]. This is similar to what we found in the current study: bothersome tinnitus was reported by 1 out of 10 participants with prevalent tinnitus. Of these participants, most answered to be bothered by tinnitus on a daily basis. Of the participants with daily tinnitus, 11.7% had a score higher than 16 points on the THI-s, reflecting moderate or worse handicap associated with tinnitus. One should be careful to extrapolate these results to clinical tinnitus populations. The clinical tinnitus population is a highly selected group with a large burden of disease which is probably only a subgroup of our participants that report tinnitus to interfere with daily life[10].

Even though hearing impairment is regarded as the main risk factor for tinnitus, there are other potential risk factors for tinnitus that may affect the prevalence, like depression, anxiety, cardiovascular risks or genetics[4,15,43-45]. Deteriorated mental health is often reported in mainly clinical tinnitus populations and to be associated with a high tinnitus burden[44-46], As the Rotterdam Study consists of relatively healthy older individuals with a low tinnitus burden, we do not expect this to affect the overall tinnitus prevalence reported in the present study.

The current study is one of the larger population-based studies investigating tinnitus prevalence and its relation to hearing impairment measured with pure-tone audiometry. The large sample size and pure-tone audiometry allowed for proper investigation of the association of hearing impairment in an elderly population. Some limitations in the current study should also be acknowledged. First, it remains unknown in which ear the tinnitus is present, which would have allowed for closer investigation of the association with hearing impairment. Second, no information was available on tinnitus onset and duration available. Third, this study was of a cross-sectional origin, limiting the ability to infer on causality.To conclude, tinnitus is present in 1 out of 5 older adults, and every 1 out of 10 with tinnitus experience severe tinnitus that is interfering with daily life. Participants with hearing impairment were twice as likely to have tinnitus compared to participants without hearing impairment. In spite of the strong age-related character of hearing impairment, no such age-dependency was found for the prevalence of tinnitus.

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Chapter 2.2

The role of hearing loss in speech in noise comprehension in individuals with tinnitus

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Abstract

Background

Tinnitus is a prevalent auditory disorder that frequently co-occurs with hearing loss. It is suggested that tinnitus might have negative impact on speech perception. However, studies thus far have not been able to disentangle tinnitus, hearing loss and speech in noise intelligibility**.** We therefore investigated whether there is an association between tinnitus and speech understanding in noise, independent of hearing loss.

Methods

Of 4,211 participants from the population-based Rotterdam Study (mean age 67.8 (SD 8.9), 57.3% female) data was available on tinnitus, pure-tone audiometry and digits in noise test. We compared the speech reception threshold in noise (SRT_n) in participants with and without tinnitus for the whole population as well as for subgroups stratified for average hearing threshold in 10-dB strata. Additionally we regressed tinnitus on SRT_n with a multivariable regression model, adjusting for sex, age, highest achieved education and cognitive function.

Results

Participants with tinnitus (20.8%) had a higher SRT_n (-3.6 dB (SD 3.7) vs. -4.6 dB (SD 3.1)). This difference remained only in the subgroups of participants with hearing loss, between 0.6 to 0.8 dB difference in the SRT_n for the different subgroups. In the fully adjusted model tinnitus was associated with 0.2 dB (95%CI 0.00, 0.39) SRT_n increase.

Conclusion

We have shown that tinnitus is associated to speech intelligibility in noise, but it is a small effect, only found in people with co-occurring hearing loss.

Introduction

Tinnitus - a sound heard in absence of an objective external acoustical source for this sound - is found in anywhere between 5% and 40% of the adult population[1]. Even though tinnitus is a prevalent problem, there is an incomplete understanding of its pathophysiology. Only in a very small amount of cases the tinnitus is explained by sound generation in or near the ear, so-called objective tinnitus, while in the majority of cases it concerns subjective tinnitus based on self-report[2]. A recent review on the pathophysiology of tinnitus concludes that both peripheral and central components of the auditory system are involved, combined with certain central non-auditory functions[3]. The peripheral component mainly consists of cochlear damage, which triggers the central auditory system and generates tinnitus. The cochlear problems can be of diverse origin, such as noise-induced hearing impairment, Meniere's disease and age-related hearing impairment, but most are a reflection of hair cell damage or cochlear synaptopathy[4]. Cochlear hearing impairment can therefore be considered to be one of the most important risk factors for tinnitus initiation[5,6]. The central component, or central auditory system, is suggested to be more important in tinnitus generation and maintenance than the peripheral auditory system[3,7,8]. Central tinnitus generators are found in and around the primary auditory cortex as well as in many non-auditory higher order processing centers, such as attentional, memory and emotional systems[3,7].

The interaction between the peripheral and central components of tinnitus is implied to influence the processing of sound, especially in complex acoustical conditions[9]. A systematic review on speech understanding in noise in tinnitus patients hypothesized that tinnitus patients have difficulty with speech understanding in noise due to a central mechanism[10]. In this review, it was indicated that tinnitus patients have a poorer speech comprehension in noise. Additionally it is suggest that hearing impairment contributes to even worse hearing in noise intelligibility[10]. However, the summarized studies in this review have relatively small numbers of participants, all less than 100. Only a few of the previous studies compared participants with and without hearing impairment but none assessed the role of the hearing threshold [11-16]. It is important to take the effect of hearing loss itself in account , as the amount of hearing loss is strongly associated with speech in noise understanding[17-20]. The aim of our study is to investigate whether there is an association between tinnitus and speech understanding in noise, independent of hearing loss, in a large population-based study.

Methods

Setting and study population

This cross-sectional study was embedded in the Rotterdam Study, a population-based cohort study including older individuals living in the well-defined Ommoord district in the city of Rotterdam, the Netherlands. The Rotterdam Study investigates determinants and consequences of aging[21]. All participants were invited to undergo extensive examinations in the dedicated research center at study entry and subsequently every 3 to 4 years. By 2008, 14,926 individuals aged 45 years and older had participated in the Rotterdam Study.

For this study, participants were included who visited the study center between 2011 and 2016 for initial or re-examination. Baseline tinnitus interview data was available from 6,157 participants. 4,808 of the participants underwent audiometry and the Digits in Noise test (DIN).

Tinnitus assessment

General subjective hearing abilities and tinnitus were assessed during a home interview. For tinnitus complaints in particular, participants were asked if they have experienced sounds in the head or in (one of) the ears, such as whizzing, peeping, or humming, without an objective external sound source being present. Possible answers to this question were: 'no, never'; 'yes, less than once a week'; 'yes, more than once a week but not daily'; 'yes, daily'.

For the present study, tinnitus was investigated 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'). Because of the heterogeneity of the origin and the often temporary character of tinnitus present less than once a week, this was not recorded as prevalent tinnitus.

Only participants suffering from tinnitus on a daily basis were asked to fill out the simplified Tinnitus Handicap Inventory (THI-s)[22]. This inventory consists of 10 items, with a possible score of 0, 2 or 4 per item, which included questions on the interference of tinnitus in daily life. A score of ≥16 represented a moderate/severe handicap[22,23].

Digits in noise test (DIN)

Speech in noise performance was assessed with the DIN-test. Prior to the DIN, a pure-tone audiogram was measured. Afterwards, the DIN was measured in the best ear (average of 0.5 and 4 kHz in the pure tone audiogram) using TDH-39P headphones

with MX-41/AR cushions. When the average loss in both ears was equal, we measured alternately the left or right ear. The DIN test consists of lists with 24 digit triplets. The digits are presented against a background of 70 dB SPL speech shaped noise, which is unchanged throughout the test. First, the starting level of the speech is determined by repeatedly presenting the first triplet, increased by 4 dB, until it is first heard correctly. The measurement then follows an adaptive up-down procedure, using 2-dB steps. For instance: when the participant gives an incorrect response, the subsequent triplet is presented at a level 2 dB higher than the triplet before. A stable speech reception threshold in noise (SRT_n) is only reached after a number of presentations, therefore the first responses are skipped for the calculation. The overall SRT_n was calculated by taking the average SRT_n of the separate digit triplets 5-24. The last triplet is not actually presented, but its level is calculated from the response to triplet 23[24]. To assess the quality of SRT_n measurements, the measurement error was determined by taking the mean of all the individual within-test SRT_n deviations. Subjects with an average SRT_n deviation of more than +2 standard deviations (4dB) above mean were considered outliers as this represents an unreliable test and excluded from further analysis (n=210).

Pure-tone audiometry

All audiometric examinations took place in a sound-treated booth with a clinical audiometer (Decos audiology workstation, version 210.2.6, with AudioNigma interface; Decos Audiology, Inc., Peachtree City, GA) [21]. To determine hearing levels in decibel (dB), pure-tone audiometry was used according to the ISO-standard 8253-1[25]. Air conduction thresholds for both ears were measured on several frequencies (0.25, 0.5, 1, 2, 4, and 8 kilohertz (kHz)). Masking was performed according to the method of Hood[26]. A pure tone average over 0.5, 1, 2, 4 kHz ($PTA_{0.5-4}$) was calculated in the ear in which the digits in noise test (DIN) was performed. Participants with an air-bone gap of >15dB in that ear were excluded (N=210). Additional analyses were done with a high frequency average (2, 4, 8khz (PTA_{2-8})). A subgroup including only participants without any sign of hearing impairment, i.e. no frequency threshold >20dB, was investigated separately. Participants with higher hearing thresholds (severe hearing impairment, $PTA_{0.5-4}$ >50dB) were excluded as the hearing threshold is too close to the static noise level of 70dB which is part of the DIN test (N=177).

Other measurements in the Rotterdam Study

Amongst many other parameters, highest achieved educational level was also noted, using the UNESCO classification[27]. To calculate a general cognitive factor (g-factor) we performed a principal component analysis incorporating the color-word interference subtask of the Stroop test, LDST, verbal fluency test, delayed recall score of the 15-

WLT, DOT and Purdue pegboard test. For tests with multiple subtasks we chose only one subtask in order to prevent highly correlated tasks distorting the factor loadings. Principal component analysis was performed on complete case data of 3,009 persons. The g-factor was identified as the first unrotated component of the principal component analysis and explained 49.8 % of all variance in the cognitive tests. This is a typical amount of variance accounted for by the g-factor[28].

Statistical analysis

Analyses were done in a stepwise manner. First, descriptive analyses were used to assess and compare the differences in participant characteristics in participants with and without tinnitus. Continuous data were described as mean (standard deviation (SD)) when normally distributed, categorical variables were described as number (%). A t-test and chi-squared test were used respectively.

Second, we wanted to assess both the average hearing threshold and hearing threshold per frequency, to find whether this differed between participants with and without tinnitus. Ultimately the goal was to eliminate differences in hearing acuity as much as possible so differences in the DIN-test could be attributed to tinnitus. To achieve this, the average threshold per frequency was assessed for participants with and without tinnitus in the whole population. Subsequently an average audiogram is plotted for participants with and without tinnitus with an average $PTA_{0.5\text{-}4}$ >20dB, i.e. participants considered to have hearing impairment, stratified for $PTA_{0.5\text{-}4}$ 20-30 dB, $PTA_{0.5\text{-}4}$ 30-40dB and $PTA_{0.5\text{-}4}$ 40-50dB.

Third, we compared the SRT_n in participants with and without tinnitus stratified for their average hearing threshold, all frequencies <20dB, $PTA_{0.5\text{-}4}$ <20 dB, $PTA_{0.5\text{-}4}$ 20-30 dB, $PTA_{0.5\text{-}4}$ 30-40dB and $PTA_{0.5\text{-}4}$ 40-50dB, with a t-test. Finally, we defined a multivariable linear regression model for the SRT_n when having tinnitus, with model 1 adjusted for $PTA_{0.5\text{-}4}$, sex and age and model 2 additionally adjusted for g-factor and highest achieved education. This step was repeated for $PTA_{2\text{-}8}$ as this average covers the frequencies mostly associated to tinnitus and in an attempt to rule out difference in hearing loss in the group with and without hearing loss even more. These analyses were repeated in a subgroup with a THI-s score, comparing participants with a score ≥16 to those with a lower score, investigating whether tinnitus severity changes the SRT_n.

Results

Table 1 describes the investigated population (N=4,211). The mean age was 67.8 (SD 8.9) ranging between 51.5 and 98.6. Tinnitus was prevalent in 20.8% of the population. The mean $PTA_{0.5\text{-}4}$ was 19.9 dB (SD 8.8), as participants with a $PTA_{0.5\text{-}4}$ >50dB were excluded. All frequency thresholds were < 20 dB in 11.6% of the population. The $PTA_{0.5\text{-}4}$ over the frequencies was between 20 and 50dB for 47.9% of the population. The mean SRT_n was -4.41 (SD 3.25) with a mean intra-test standard deviation (STD) of 2.17 (SD 0.53). The tinnitus group (n=877) differed from the no tinnitus group (n=3,334) in being less often female and in having a higher SRT_n and $PTA_{0.5\text{-}4}$.

Figure 1 shows the average pure-tone audiogram thresholds for the two subpopulations with and without tinnitus. The group with tinnitus has higher average thresholds over all measured frequencies, sloping steeper in the high frequencies (Figure 1, Supplementary table 1). In participants with hearing impairment, the average thresholds were very similar for participants with and without tinnitus, except for $PTA_{0.5\text{-}4}$ 20-30dB at 0.5, 4, 8 kHz (Supplementary table 1).

Figure 2 and table 2 show the SRT_n for participants with and without tinnitus, stratified for hearing threshold. Stratification for $PTA_{0.5\text{-}4}$ results in a higher (less favorable) SRT_n for participants with tinnitus compared to participants without tinnitus in the groups $PTA_{0.5\text{-}4}$ 20-30 dB (-3.79 dB (SD 2.41) vs. -4.40 dB (SD 2.08), $p<0.001$, respectively) and $PTA_{0.5\text{-}4}$ 30-40 dB (-1.07 dB (SD 3.44) vs. -1.71 (SD 3.59), $p=0.05$, respectively). When stratified for high-frequency hearing impairment ($PTA_{2\text{-}8}$), a higher SRT_n was found for participants with tinnitus compared to participants without tinnitus in the group $PTA_{2\text{-}8}$ 30-40 dB (-4.50 dB (SD 2.40) vs. -4.92 (SD 1.91), $p=0.042$, respectively). The fully adjusted linear regression model showed an association of tinnitus with a SRT_n increase of 0.20 dB (95%CI 0.00, 0.39) (Table 3).

A THI-s score was available for 484 participants. Severe tinnitus, a THI-s score ≥16, was present in 14.7% (N=71). Participants with a high THI-s score did not have a higher SRT_n compared to participants with a low THI-s score (-2.68 dB (SD 4.72) vs.-3.44 dB (SD 3.56), p=0.113 respectively). In the group $PTA_{0.5\text{-}4}$ <20 the unadjusted SRT_n was significantly higher for participants with a high THI-s score (-5.22 (SD 1.57) vs. -6.00 (SD 1.48), p=0.011, respectively) (Table 2). In the adjusted models a high THI-s score was not associated with a different SRT_n (Table 3).

Table 1. Description of the investigated population

	Investigated population (N=4,211)	**No tinnitus (N=3,334)**	**Tinnitus (N=877)**	**p-value**
Age, years	67.8 (8.9)	67.9 (9.0)	67.5 (8.7)	0.273
Age, range	51.5-98.6	51.5-98.6	51.9-94.7	
Female, %	57.3	58.4	53.5	0.009
SRT_n, dB	-4.41 (3.25)	-4.62 (3.11)	-3.62 (3.66)	<0.001
STD, dB	2.17 (0.53)	2.15 (0.52)	2.24 (0.56)	<0.001
Tinnitus, %				
Never	79.2			
Yes	20.8			
PTA, dB	21.6 (10.6)	20.9 (10.4)	24.3 (10.7)	<0.001
PTA, %				<0.001
<20 dB	52.1	55.1	41.0	
20-30 dB	27.4	26.3	31.5	
30-40 dB	14.2	13.1	18.6	
40-50	6.3	5.6	8.9	
All frequencies <20dB, %	11.6	13.0	6.0	<0.001
Education level, %				0.822
Primary	7.1	7.1	7.0	
Lower	38.6	38.2	40.0	
Middle	29.4	29.5	29.1	
Higher	24.2	24.4	23.4	
G-factor	0.0 (1.0)	0.0 (1.0)	-0.0 (0.9)	0.921

Table 1. Characteristics of the investigated population. Participants with and without tinnitus are compared with descriptive statistics. SRT_n: speech reception threshold – in noise based on the Digits in noise (DIN) test. STD: intra participant DIN standard deviation. PTA: Pure tone average over 0.5, 1, 2, 4 kHz. The G-factor is a principal component analysis incorporating the color-word interference subtask of the Stroop test, LDST, verbal fluency test, delayed recall score of the 15-WLT, DOT and Purdue pegboard test

Table 2. Signal to noise ratio difference between participants with and without tinnitus, stratified on hearing loss.

			All frequencies <20dB	PTA <20 dB	PTA 20-30 dB	PTA 30-40 dB	PTA 40-50 dB
PTA (0.5-4)	No tinnitus	Mean	-6.53	-6.01	**-4.40**	**-1.71**	**1.22**
		SD	1.19	1.54	**2.08**	**3.59**	**5.08**
	Tinnitus	Mean	-6.66	-5.85	**-3.79**	**-1.07**	**1.96**
		SD	1.13	1.49	**2.41**	**3.44**	**5.22**
	THI-s < 16	Mean	-6.44	**-6.00**	-3.65	-1.34	1.40
		SD	1.27	**1.48**	2.33	3.42	4.61
	THI-s ≥ 16	Mean	6.73	**-5.22**	-3.86	1.47	5.48
		SD	0.98	**1.57**	2.35	3.83	6.47
PTA (2-8)	No tinnitus	Mean	-6.53	-6.38	-5.71	**-4.92**	-3.85
		SD	1.19	1.34	1.65	**1.91**	2.59
	Tinnitus	Mean	-6.66	-6.43	-5.54	**-4.50**	-3.40
		SD	1.1	1.25	1.65	**2.40**	2.56
	THI-s < 16	Mean	-6.44	-6.37	-5.75	-4.47	-3.30
		SD	1.27	1.35	1.64	2.52	2.69
	THI-s ≥ 16	Mean	6.73	-6.11	-5.05	-4.11	-2.53
		SD	0.98	1.25	1.27	2.19	3.94

Table 2. Mean Speech Reception Threshold – in noise (SRT_n) per stratum based on pure tone averages (PTA) over 0.5-4 kHz and 2-8 kHz. **Bold** indicates a significant difference between the thresholds of participants with and without tinnitus or a high or low THI-s score. ($p<0.05$).

Discussion

In this study we found that only among participants with an average hearing threshold >20dB, participants with tinnitus have a higher SRT_n than participants without tinnitus. When adjusting for the difference in hearing impairment between the groups with and without tinnitus, tinnitus remains associated with a higher SRT_n. More severe tinnitus marginally increased the SRT_n.

The results of our present study seem to corroborate the findings of other studies in which people with tinnitus generally have a higher SRT_n[10]. However, this previous finding may be mediated by hearing impairment as participants with tinnitus generally have worse hearing than participants without tinnitus. In our study we were able to do a separate analysis for the group of participants without hearing loss ($PTA_{0.5-4}$

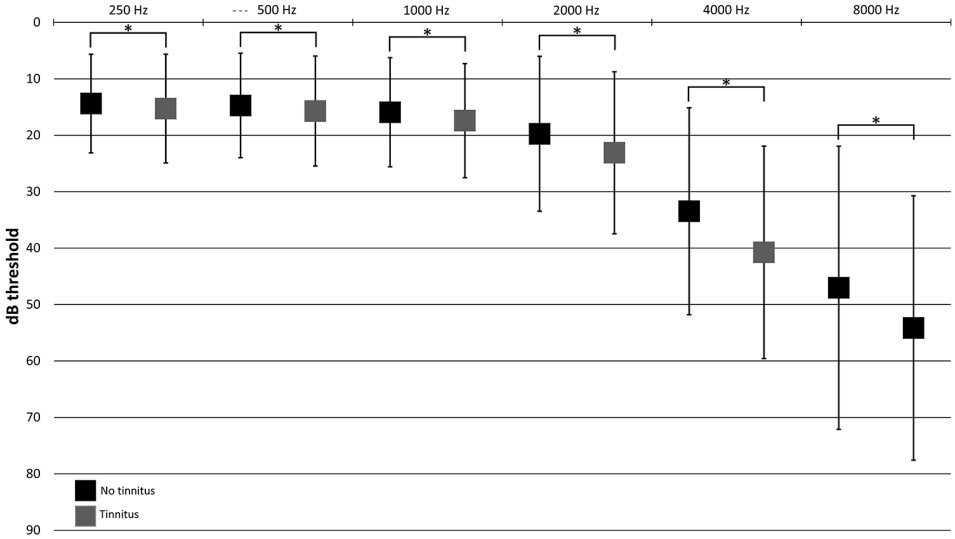

Figure 1. Average audiogram for all participants
Figure 1. Audiogram for the $PTA_{0.5-4}$, N=4,211. * reflects a significant difference (t-test, $p<0.05$) in average at the specific frequency between participants with and without tinnitus.

<20dB). For this group, we did not find a difference in SRT_n between tinnitus and non-tinnitus participants, contrary to what has been reported before[15,16,29,30]. This suggests that hearing loss may indeed be a mediating factor in the association between tinnitus and speech understanding. There are several major differences between the present study and previously published studies. Firstly, our present study is embedded in a population-based study, which is in strong contrast to the selected clinical populations investigated before. Secondly, the number of investigated participants in our study is much higher than the 15, 19, 28 and 29 participants described by Mertens et al., Hennig et al., Tai et al. and Ryu et al. respectively, resulting in a more reliable effect estimate. A third difference is the average age of the investigated populations, as the Rotterdam Study participants are older adults. This may have contributed to the difference in results among normal hearing participants. People with more severe complaints (clinical population) and younger age may be more susceptible to an interaction between tinnitus and speech in noise. Even though we did not find an adjusted difference in SRT_n for the total group of participants with more severe tinnitus based on the THI, a difference in SRT_n was found for the subgroup with normal hearing.

In the group with hearing loss, we did find that participants with tinnitus and mild hearing impairment ($PTA_{0.5-4}$ <40dB) have higher SRT_n s than participants without

Table 3. Adjusted signal to noise ratio difference between participants with and without tinnitus

		$PTA_{0.5-4}$	PTA_{2-8}
		dB SRT_n change (95%CI)	**dB SRT_n change (95%CI)**
Tinnitus vs. no tinnitus	Model 1	**0.19**	0.17
		-0.00, 0.39	-0.00, 0.35
Tinnitus vs. no tinnitus	Model 2	**0.20**	0.16
		0.00, 0.39	-0.00, 0.34
THI-s ≥16 vs. THI-s < 16	Model 1	0.23 -0.52, 0.98	0.35 -0.50, 1.19
	Model 2	0.15 -0.59, 0.90	0.24 -0.59, 1.08

Table 3. Linear regression analyses for speech reception threshold – in noise (SRT_n). dB SRT_n change reflects the decibels SRT_n threshold change by having tinnitus vs. not having tinnitus. PTA: pure tone average over 0.5-4 and 2-8 kHz. THI-s: tinnitus handicap inventory-screening version, a score ≥16 represents severe tinnitus. dB: Decibel. CI: Confidence interval. Model 1 is adjusted for age, sex, average hearing threshold, model 2 additionally for highest achieved education and the g-factor. Significant estimates (p<0.05) are indicated in **bold**.

tinnitus and mild hearing impairment. Once the $PTA_{0.5-4}$ 40dB hearing threshold has been passed, we found no statistically significant difference between participants with and without tinnitus, probably due to a lack of statistical power. This might also explain the large variability as expressed by the SD. Additionally, the SD decreased in the larger better-hearing groups resolving some of the variability due to differences in hearing acuity. As expected, the SRT_n increased with hearing loss, but the difference within a stratum remained similar around 0.6dB SRT_n. The poorer SRT_n found for people with tinnitus and hearing loss corroborates previously published results in which participants are generally matched on the presence or absence of hearing impairment[10]. However, no study matched on subgroups of hearing loss within the study.

Speech comprehension in noise is subject to many factors influencing ones capacity to perform well on such a task. The most important factor is ones hearing acuity. The SRT is highly correlated with the pure tone hearing threshold[17,20]. Smits compared the SRT_n of the DIN with sentence SRT_n and found a correlation coefficient of 0.96[31]. The DIN is therefore very sensitive to hearing impairment. In this study we used several strategies to adjust for the mediating role of hearing impairment in the SRT_n threshold and tinnitus presence. The sample size of this study allowed for stratification on hearing threshold for both a mid-frequency average ($PTA_{0.5-4}$) and high-frequency average (PTA_{2-8}). The $PTA_{0.5-4}$ comprises the speech reception frequencies[32], whereas

the PTA_{2-8} comprises the higher frequencies, which are more likely to be affected by tinnitus. Participants with tinnitus had on average steeper slopes in their audiograms than the participants without tinnitus. This suggests that there might be different underlying pathophysiological mechanisms for tinnitus-associated hearing impairment, resulting in higher SRT_n s for participants with tinnitus.

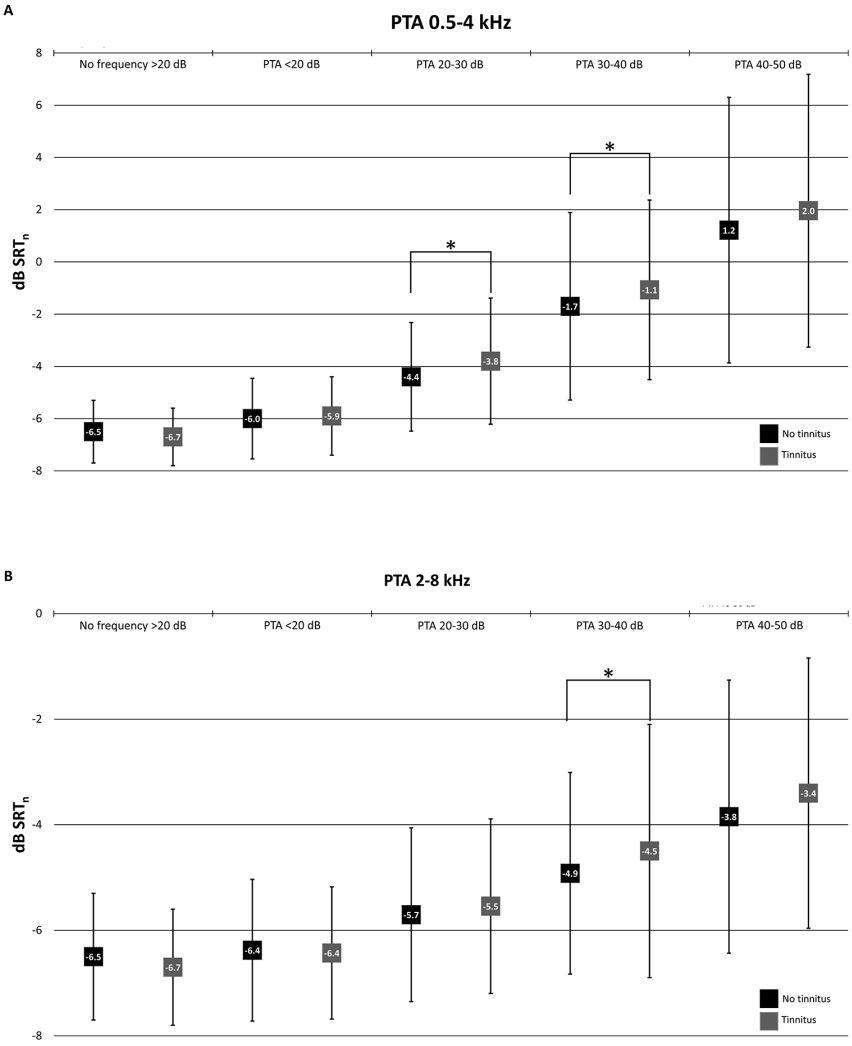

Figure 2 (A, B). Mean SRT_n per average hearing stratum
A. For $PTA_{0.5-4}$; B For PTA_{2-8}. Mean SRT_n per stratum on average hearing threshold.
* reflects a significant difference (t-test, $p<0.05$) in SRT_n between participants with and without tinnitus.

In addition to hearing impairment, cognitive capacities are reported to also be of major influence on performance in a speech in noise task[33]. This might comprise another mechanism underlying the higher SRT_n s in participants with tinnitus, as tinnitus reduces cognitive capacity[34]. It can be hypothesized that when someone has both hearing impairment and tinnitus, this combination is a large cognitive burden. Cognitive and linguistic skills contribute to the top-down processes that are involved in the understanding of sentences and words in noise. The DIN was developed to primarily measure the auditory speech recognition abilities in noise and to depend minimally on this top-down processing[20]. We additionally adjusted for the cognitive functioning in the multivariable regression analysis, but did not find this to alter our results.

The clinical relevance of the differences in SRT_n found in the multivariable regression can be debated. In an unadjusted model, we found a 1dB increase in SRT_n for participants with tinnitus compared to participants without. In the fully adjusted model, we found an increase of only 0.2dB in the SRT_n. This small increase is not of clinical significance/relevance, as it will not be noticed in daily life by an individual listener. However, from a more theoretical perspective it is an interesting finding that tinnitus is associated with poorer speech understanding, which might contribute to hypothesis generation as to which structures contribute to tinnitus.

Strengths of this study are that we have described a large population-based sample, with participants of whom several measures are available. This allows us to account for factors such as the hearing threshold and cognitive functioning. The large number of participants contributes strongly to the interpretability of the results. A limitation to this study is that we do not know in which ear the tinnitus is present or for how long the tinnitus has existed. The results would have been sounder if the audiogram and SRT_n had been investigated in the tinnitus ear. However, a consensus appears to have been reached in literature that central processes are involved in tinnitus maintenance[3,35]. These central processes can be localized in the central auditory system, so the effect of tinnitus on hearing in noise may be argued to occur not only in the tinnitus-ear. Additionally it would have been interesting to have information on the perceived loudness of the tinnitus to further differentiate in the mechanism of the involvement of tinnitus in speech in noise understanding.

This study is to our knowledge the first large scale population-based study to investigate the role of tinnitus in speech in noise understanding. We have shown that tinnitus is associated with poorer speech intelligibility in noise, but it is a small effect that is found only in people with co-occurring hearing loss.

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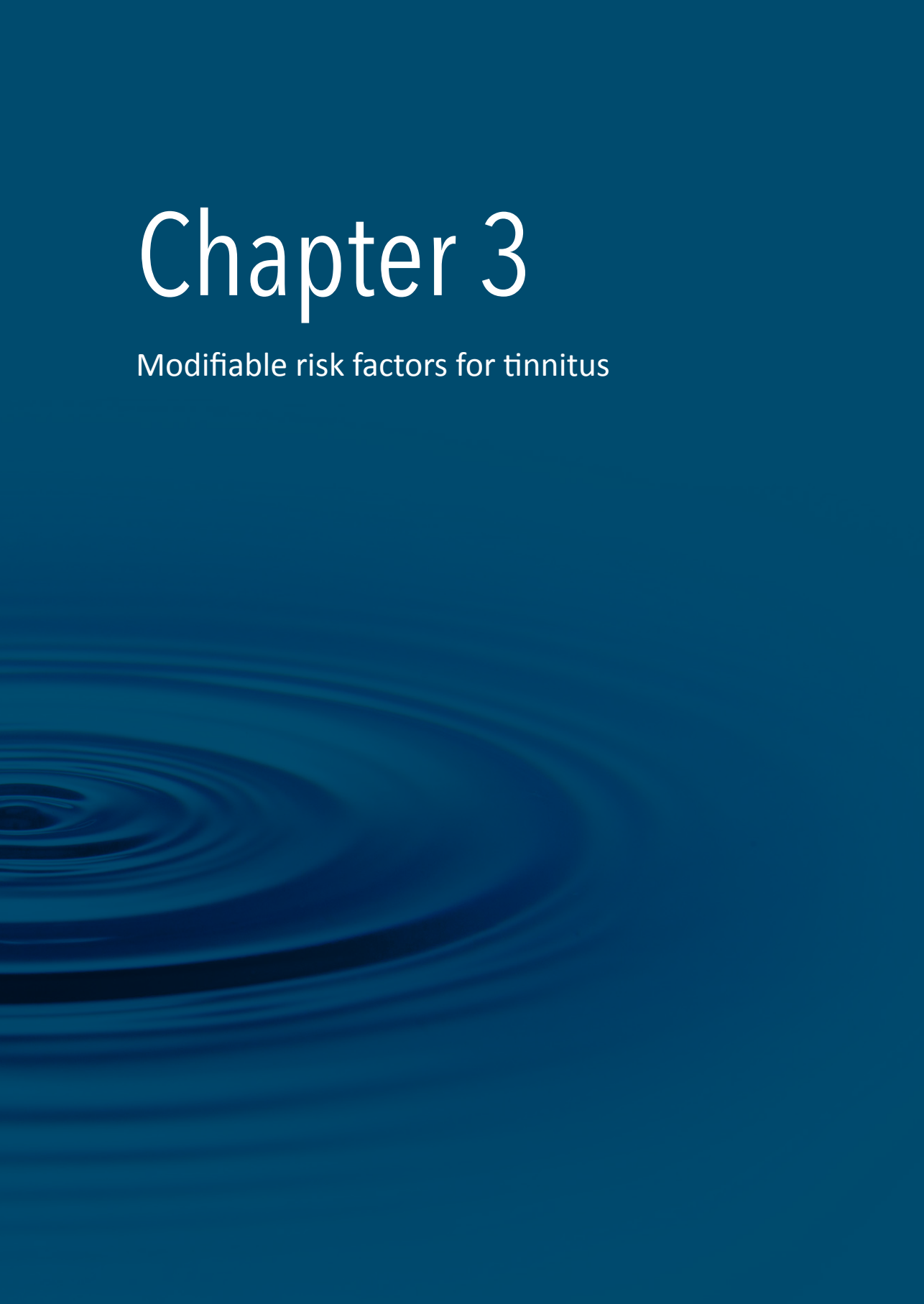
Chapter 3

Modifiable risk factors for tinnitus



Chapter 3.1

Poor cardiovascular health increases risk for tinnitus; results from the Rotterdam study

Submitted

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Abstract

Background

Tinnitus is a prevalent auditory disorder with a poorly understood pathophysiology. Individual cardiovascular risk factors have been associated with a higher risk for tinnitus, but it is unknown whether the risk increases when multiple risk factors are combined. We have investigated the association between an overall cardiovascular health (CVH) scoring tool and tinnitus.

Methods

Within the population-based Rotterdam Study, data was available for 5,435 participants (mean age: 68.6 (SD 9.5) years, 43.4% male) on tinnitus, CVH and pure-tone audiometry. We defined CVH according to the American Heart Association's definition from the smoking, BMI, physical activity, healthy diet score, total cholesterol, blood pressure and fasting glucose variables. Each of these 7 variables were categorized as poor, intermediate or ideal. Using logistic regression analyses, we explored the association between tinnitus and a non-optimal CVH, adjusted for age, sex, education and hearing threshold. Analyses were repeated stratified on hearing loss (≥ 25 dBHL).

Results

Non-optimal CVH was significantly associated with tinnitus (OR: 1.50 [95%CI 1.03, 2.18]). We found a borderline-significant association between tinnitus and non-optimal CVH in absence of hearing loss (OR: 1.59 [95%CI 0.98, 2.57]) and no significant association among those with hearing loss (OR: 1.25 [95%CI 0.65, 2.42]).

Conclusion

In this sample of older individuals from the general population we found that non-optimal cardiovascular health was associated with a higher likelihood for tinnitus. This suggests that poor cardiovascular health may be a relevant factor in initiating or maintaining chronic tinnitus.

Introduction

Tinnitus, a sound heard in absence of an objective external sound source, is a highly prevalent disorder and can have a major impact on individuals' daily life. Even though between 5% and 40% of the general adult population report experiencing tinnitus[1], the aetiology of the disorder is still poorly understood.

Both brain (central hearing and other central processes) and cochlea (peripheral hearing) are described to have a role in tinnitus pathophysiology[2]. Several groups of risk factors for tinnitus are known, of which hearing loss, mental health problems, genetics and cardiovascular risk factors are mostly reported[3-7]. Major individual cardiovascular risk factors such as smoking, hypertension, diabetes, hyperlipidaemia and high body mass index (BMI), have been associated individually with an increased risk for tinnitus[7-10]. However, these individual factors can be interrelated and may have an additional effect when more than only one is sub-optimal. Therefore it is interesting to investigate these as a composite outcome. In 2010, the American Heart Association (AHA) introduced a composite score for cardiovascular health, comprising BMI, smoking, diet quality, physical activity, blood pressure, cholesterol, and fasting glucose[11]. This score was introduced primarily as a prevention strategy for cardiovascular disease. Improved cardiovascular health will not only reduce cardiovascular disease[12], but might as well improve many other diseases that might be caused by similar mechanisms.

Reduced cardiovascular health might deteriorate both brain health and cochlear health[13,14], resulting in a higher risk for hearing loss[15] and possibly tinnitus. Hearing loss is regarded as the most important risk factor for tinnitus[2,16]. This raises the question whether reported associations between individual cardiovascular health factors and tinnitus may be mediated by hearing loss. Moreover, It is not yet known if the risk for tinnitus increases across the spectrum of cardiovascular health and when multiple individual metrics are sub-optimal.

The aim of this study is therefore to assess the association between tinnitus and cardiovascular health, as defined by the AHA cardiovascular health score, and subsequently the individual metrics in this composite outcome, in a population based study. Additionally, we explore this association independently from hearing loss, to disentangle the potential mediating role of hearing loss in the associations.

Methods

Setting and study population

This cross-sectional study was embedded in the Rotterdam Study, a prospective, population-based cohort study. The Rotterdam Study was initiated in 1989 and investigates determinants and consequences of aging. Details of the study have been described elsewhere[17]. The study population consists of 14,926 individuals aged ≥ 45 years living in the well-defined Ommoord district in the city of Rotterdam, the Netherlands[17]. All participants were invited to undergo extensive examinations in the dedicated research center, at study entry and subsequently every 3 to 4 years.

Tinnitus assessment was introduced in the study protocol in 2011. From then on, all participants contributing to the Rotterdam Study were eligible for tinnitus assessment, using the baseline tinnitus measurement. This has to date resulted in 6,168 eligible participants for this study. Of these, 6,157 underwent a home interview on the presence or absence of tinnitus and 668 participants filled out the Tinnitus Handicap Inventory screening version (THI-s) between 2011 and 2016. Of the participants with information on tinnitus status, 5,435 had information available on at least one cardiovascular factor. Of this group, 4,712 participants also underwent hearing assessment.

Tinnitus assessment

Tinnitus assessment was performed through a home interview. Participants were asked if they experienced sounds in the head or in (one of) the ears (such as whizzing, peeping, or humming) without an objective external sound source being present. Possible answers to this question were: 'no, never'; 'yes, less than once a week'; 'yes, more than once a week but not daily' and 'yes, daily'.

For the current study, tinnitus was investigated as a binary variable; either absent ('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 the often temporary character of tinnitus, presence of less than once a week was not recorded as prevalent tinnitus. Only participants suffering from tinnitus on a daily basis were asked to fill out the simplified Tinnitus Handicap Inventory (THI-s)[18]. This inventory consists of 10 items, with a possible score of 0, 2 or 4 per item. The outcome was dichotomized at a score of ≥16, this represents a moderate/severe tinnitus handicap[18,19].

Cardiovascular health

We used a measure of overall cardiovascular health in line with the one proposed by the AHA[11]. This measure includes seven metrics of cardiovascular health (CVH): three health factors (total cholesterol, fasting glucose and BP) and four health behaviors (BMI, diet, smoking and physical activity)[11]. We used the AHA definitions of poor, intermediate and ideal health categories.

The thresholds for these categories were based on data available from existing guidelines and from reviews of the literature[11]. For each metric, a participant received 0 points if that metric fell into the poor category, 1 point for the intermediate category, or 2 points for the ideal category. A maximum score of 14 could be reached. Participants with prevalent cardiovascular disease (including coronary heart disease, stroke, and heart failure) were not excluded from the analysis; instead each of their metric scores were subtracted by 1, resulting in a maximum total CVH-score of 7 for these subjects[11]. For the health factor metrics (total cholesterol, blood pressure, and fasting plasma glucose), current treatment for hypercholesterolemia, hypertension, or diabetes were accounted for by subtracting 1 from the corresponding metric score[11]. For analysis, the total CVH-score was dichotomized into two groups: Optimal CVH (a score of 11–14) versus non-optimal CVH (a score of 0–10)[20].

Individual covariates

Information on educational level was obtained through interviewing and categorized as primary, lower, middle, or higher education. To assess hearing function, air conduction thresholds were obtained at octave frequencies 0.25 – 8 kilohertz (kHz) according to the ISO-standard 8253-1[17]. A pure tone average (PTA) was calculated over 0.5-4 kHz for the best hearing ear. Hearing loss was defined as a PTA ≥25dB[21]. Height (meters) and weight (kilograms) were measured at the research center and body mass index (BMI, kg/m2) was calculated. Self-reported smoking data were obtained from the interview and were categorized into never, former, and current smoking. Dietary intake was measured with a 389-item Food-Frequency Questionnaire (FFQ)[22]. Diet quality was defined as adherence to the Dutch dietary guidelines. For all participants we examined adherence (yes/no) to fourteen items of the guidelines: vegetables, fruit, whole-grains, legumes, nuts, dairy, fish, tea, wholegrains, fats and oils, red and processed meat, sugar-containing beverages, alcohol and salt. Total adherence was calculated as sum-score of the adherence to the individual items (0-14). For the analyses we divided the dietary quality score into tertiles (low [0-6], medium [6-8] and high adherence [8-14]). Alcohol consumption was assessed in grams per day through the FFQ[22]. The LASA Physical Activity Questionnaire was used to assess the amount of physical activity, the number of minutes moderate or vigorous

activity per week were calculated[23]. Systolic and diastolic blood pressure (BP) were measured twice, using a random sphygmomanometer. Hypertension was defined as systolic BP ≥ 160 millimeter mercury (mmHg), diastolic BP ≥ 90 mmHg, and/or the use of blood pressure-lowering medication[17]. Using an automatic enzymatic procedure, serum total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides were measured from fasting blood samples. Hypercholesterolaemia was defined was a total cholesterol concentration of ≥6.2 mmol/L or the use of lipid-lowering drugs[17]. Prevalent diabetes was defined on the basis of WHO criteria for fasting glucose, ≥7.0 mmol/L or use of antidiabetic therapy[24]. The use of blood pressure-lowering medication, antidiabetic therapy and lipid-lowering drugs was identified from pharmacy records.

Statistical analysis

First, we described characteristics for the entire investigated population. Second, using logistic regression analyses, we explored the association between tinnitus and a non-optimal CVH compared to an optimal CVH. Subsequently we separately explored the association for each individual metric of the AHA CVH-score for an intermediate or poor health as opposed to ideal health, with tinnitus. Confounder adjustment was achieved in several models. Model 1 was adjusted for age, sex and highest achieved education. Model 2 was additionally adjusted for the average pure-tone hearing thresholds (PTA). These models were repeated using a stricter definition of tinnitus as outcome, i.e. participants with a relevant tinnitus handicap (a THI-s score≥16, representing a moderate or worse handicap) compared to participants without tinnitus. Additionally, we performed a sensitivity analysis in which we excluded participants with prevalent cardiovascular disease or who were using medication. The regression analyses for tinnitus presence were repeated in this group on the metrics of CVH-score. Finally, we repeated the analyses for prevalent tinnitus stratified for the presence of hearing loss and subsequently stratified by age (midlife (<67.5 years old) vs. late-life (>67.5 years old)).

To reduce selection bias due to missing data, multiple imputations of covariates were performed and pooled results of 5 imputed datasets were presented[25]. IBM SPSS statistics version 24.0 (IBM Corp, Armonk, NY, USA) was used for data handling and analyses. A p-value < 0.05 was considered statistically significant, however principle emphasis was given to findings with a p-value <0.001. All p-values were two sided.

Results

A population of 5,435 participants were included for the analyses, of which the characteristics are shown in Table 1 (description of the population) and Table 2 (cardiovascular health (CVH) related). Chronic tinnitus was prevalent in 21.7% of participants. The mean age was 68.6 years (standard deviation (SD): 9.5) and 43.4% was male. The average hearing threshold was 23.9 dB and hearing loss was present in 39.9% of participants. Optimal CVH was present in 5.3% of the population.

Regression analyses showed that non-optimal CVH was significantly associated with tinnitus (OR: 1.50 [95%CI 1.03, 2.18]), but not significantly with a relevant tinnitus handicap (OR: 4.57 [95%CI 0.63, 33.03]) (model 2, Table 3). Regarding the specific health behaviour metrics (BMI, smoking, diet quality and physical activity), only current smoking compared with never smoking was significantly associated with higher odds for tinnitus and none were associated with a relevant tinnitus handicap (Table 3). Looking at the specific health factors (blood pressure, total cholesterol, fasting glucose), only poorer total cholesterol levels were significantly associated with tinnitus (both general and with a relevant handicap) as compared to ideal cholesterol levels (Table 3). Sensitivity analyses in which participants were excluded with either medication usage or prevalent CVD, revealed that all significant associations found between a non-optimal CVH and the individual metrics, remained with similar effect estimates (Table 4).

In analyses stratified on hearing loss, we found a borderline-significant association between tinnitus and non-optimal CVH in absence of hearing loss (OR: 1.59 [95%CI 0.98, 2.57]) and no significant association among those with hearing loss(OR: 1.25 [95%CI 0.65, 2.42]), Supplement Table 1. When the dataset is stratified on age, a significant association was found for non-optimal CVH with tinnitus in midlife (OR: 1.56 [95%CI 1.04, 2.35]) and not in late-life (OR: 1.24 [95%CI 0.54, 2.86]), Supplement Table 2.

Table 1. Population characteristics

	Total sample (N=5,435)
Age, years	68.6 (9.5)
Male	2,360 (43.4)
Education	
Primary	417 (7.7)
Lower	2,110 (39.2)
Intermediate	1,602 (29.8)
Higher	1,255 (23.3)
PTA, dB	23.9 (13.8)
Hearing loss ≥25 dB, yes	1,881 (39.9)
Tinnitus	1,180 (21.7)
Tinnitus handicap[a]	4 (0, 8)
Relevant tinnitus handicap, yes	92 (13.8)
Prevalent CV disease, yes	726 (13.5)
Body mass index, kg/m^2	27.5 (4.4)
Smoking	
Never	822 (15.3)
Former	2,837 (52.7)
Current	1,726 (32.1)
Diet quality, total score[a]	7 (6, 8)
Physical activity, min moderate activity/wk [a]	390 (195, 720)
Hypertension, yes	3,820 (70.3)
BP lowering medication, yes	2,498 (46.1)
Systolic BP, mmHg	140.9 (21.0)
Diastolic BP, mmHg	81.5 (11.6)
Hypercholesterolaemia, yes	3,003 (55.3)
Lipid-lowering medication, yes	1,751 (32.3)
Total cholesterol, mmol/L	5.5 (1.1)
Diabetes Mellitus, yes	678 (12.9)
Antidiabetic therapy, yes	517 (9.25)
Fasting glucose, mmol/L	5.8 (1.3)

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). Tinnitus handicap was assessed with the Tinnitus Handicap Inventory – screening version, a relevant handicap was a score ≥16. Prevalent cardiovascular disease: coronary heart disease, stroke or heart failure. Diet quality was regarded as adherence to Dutch dietary guidelines. PTA: pure tone average (best hearing ear over 0.5-4 kilo Hertz). dB: decibel. CV: cardiovascular. kg: kilogram. m: meter. wk: week. BP: blood pressure. mmHg: millimeters mercury. mmol: millimol. L: liter. Values are mean (standard deviation (SD)) for normally distributed continuous variables, median[a] (interquartile range) for non-normally distributed continuous variables. Values are N (%) for categorical variables.

Table 2. Cardiovascular health categories based on the AHA score, description of the population

	Total sample (N=5,435)
Optimal CV health, yes	202 (5.3)
Health behaviors	
BMI	
Ideal (<25 kg/m^2)	1,436 (26.4)
Intermediate (25-30 kg/m^2)	2,378 (43.8)
Poor (>30 kg/m^2)	1,620 (29.8)
Smoking	
Ideal (never)	822 (15.3)
Intermediate (former)	2,837 (52.7)
Poor (current)	1,726 (32.1)
Diet quality	
Ideal (>8 points)	737 (17.1)
Intermediate (6-8 points)	1,555 (36.1)
Poor (<6 points)	2,015 (46.8)
Physical activity	
Ideal (>150 min moderate activity/wk)	3,458 (72.9)
Intermediate (1 - 150 min moderate activity/wk)	1,162 (21.4)
Poor (<1 min moderate activity/wk)	123 (2.3)
Health factors	
Blood pressure	
Ideal (SBP <120 mmHg and DBP <80 mmHG)	456 (8.5)
Intermediate (SBP 120-140 mmHg or DBP 80-90 mmHg, or treated)	1,238 (23.0)
Poor (SBP>140 mmHg or DBP >90 mmHg, or treated)	3,694 (68.6)
Total cholesterol	
Ideal (<5.2 mmol/L)	821 (15.7)
Intermediate (5.2-6.2 mmol/L)	2,110 (40.4)
Poor (>6.2 mmol/L)	2,288 (42.1)
Fasting glucose	
Ideal (<7.8 mmol/L)	2,454 (47.0)
Intermediate (7.8-11.1 mmol/L, or treated)	1,891 (36.2)
Poor (>11.1 mmol/L, or treated)	874 (16.7)

Optimal cardiovascular health (CVH): total score of ≥11 based on the American Heart Association (AHA) score for CVH. Each of the 7 components can contribute 0 (Poor), 1 (Intermediate) or 2 (Ideal) points to the total score. Prevalent cardiovascular disease (coronary heart disease, stroke or heart failure) subtracted 1 point from each category (maximum score of 7). The use of lipid-lowering drugs, blood pressure lowering drugs or antidiabetic therapy subtracted 1 point of the score in that category. Diet quality was regarded as adherence to Dutch dietary guidelines. kg: kilogram. m: meter. wk: week. mmHg: millimeters mercury. mmol: millimol. L: liter. All values are N (%).

Table 3. The association between cardiovascular health and tinnitus

	Tinnitus vs. no tinnitus		Relevant tinnitus handicap vs. no tinnitus	
	Model 1	**Model 2**	**Model 1**	**Model 2**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Non-optimal CVH (vs. optimal)	**1.50 (1.04, 2.15)**	**1.50 (1.03, 2.18)**	4.69 (0.65, 33.86)	4.57 (0.63, 33.03)
Health behaviours				
BMI Ideal (<25 kg/m^2)	ref	ref	ref	ref
Intermediate (25-30 kg/m^2)	1.07 (0.91, 1.26)	1.06 (0.90, 1.25)	1.49 (0.84, 2.64)	1.47 (0.82, 2.61)
Poor (>30 kg/m^2)	1.16 (0.97, 1.38)	1.13 (0.94, 1.35)	1.80 (0.99, 3.26)	1.71 (0.94, 3.12)
Smoking Ideal (never)	ref	ref	ref	ref
Intermediate (former)	1.04 (0.68, 1.59)	0.99 (0.64, 1.52)	1.30 (0.38, 4.41)	1.04 (0.28, 3.86)
Poor (current)	**1.22 (1.04, 1.42)**	**1.20 (1.03, 1.40)**	1.11 (0.70, 1.78)	1.07 (0.67, 1.73)
Diet quality Ideal (>8 points)	ref	ref	ref	ref
Intermediate (6-8 points)	0.96 (0.77, 1.19)	0.98 (0.79, 1.23)	0.83 (0.46, 1.51)	0.87 (0.47, 1.59)
Poor (<6 points)	0.99 (0.68, 1.45)	0.97 (0.66, 1.43)	0.64 (0.31, 1.30)	0.62 (0.30, 1.26)
Physical Activity Ideal (>150 min moderate activity/wk)	ref	ref	ref	ref
Intermediate (1 - 150 min moderate activity/wk)	**1.21 (1.03, 1.42)**	**1.18 (1.00, 1.40)**	1.42 (0.88, 2.31)	1.38 (0.84, 2.28)
Poor (<1 min moderate activity/wk)	1.37 (0.91, 2.07)	1.36 (0.90, 2.06)	0.62 (0.08, 4.61)	0.59 (0.08, 4.37)
Health factors				
Bloodpressure Ideal (SBP <120 mmHg, DBP <80 mmHG)	ref	ref	ref	ref
Intermediate (SBP 120-140 mmHg or DBP 80-90 mmHg, or treated)	0.94 (0.72, 1.23)	0.93 (0.71, 1.22)	1.82 (0.68, 4.82)	1.80 (0.68, 4.79)
Poor (SBP>140 mmHg or DBP >90 mmHg, or treated)	1.06 (0.82, 1.36)	1.04 (0.81, 1.33)	1.73 (0.68, 4.41)	1.69 (0.66, 4.34)
Total cholesterol Ideal (<5.2 mmol/L)	ref	ref	ref	ref

Table 3. Continued

	Tinnitus vs. no tinnitus		Relevant tinnitus handicap vs. no tinnitus	
	Model 1	**Model 2**	**Model 1**	**Model 2**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Intermediate (5.2-6.2 mmol/L)	**1.53 (1.23, 1.89)**	**1.51 (1.21, 1.87)**	**2.50 (1.09, 5.73)**	**2.45 (1.07, 5.62)**
Poor (>6.2 mmol/L)	**1.43 (1.16, 1.77)**	**1.44 (1.16, 1.79)**	**2.52 (1.10, 5.82)**	**2.53 (1.10, 5.62)**
Fasting glucose Ideal (<7.8 mmol/L)	ref	ref	ref	ref
Intermediate (7.8-11.1 mmol/L, or treated)	0.98 (0.85, 1.14)	0.96 (0.83, 1.11)	0.93 (0.59, 1.46)	0.91 (0.58, 1.45)
Poor (>11.1 mmol/L, or treated)	1.03 (0.86, 1.25)	1.00 (0.83, 1.21)	0.59 (0.29, 1.19)	0.56 (0.27, 1.14)

Odds ratios (OR) and 95% confidence interval (CI) for the association of non-optimal CVH (and all 7 metrics) with tinnitus or tinnitus with a relevant handicap as compared to no tinnitus. Model 1 is adjusted for age, sex and highest achieved education. Model 2 is additionally adjusted for the average hearing threshold over 0.5-4kHz. Optimal cardiovascular health (CVH): total score of ≥11 based on the American Heart Association (AHA) score for CVH. Each of the 7 components can contribute 0 (Poor), 1 (Intermediate) or 2 (Ideal) points to the total score. Prevalent CV disease subtracted 1 point from each category (maximum score of 7). The use of lipid-lowering drugs, blood pressure lowering drugs or antidiabetic therapy subtracted 1 point of the score in that category. Diet quality was regarded as adherence to Dutch dietary guidelines. dB: decibel. CV: cardiovascular. kg: kilogram. m: meter. Cm: centimeter. Min: minutes. Wk: week. g: gram. BP: blood pressure. mmHg: millimeters mercury. Mmol: millimol. L: liter. **Bold** values represent significant associations.

DISCUSSION

In a large population-based sample of middle-aged and older individuals, we found that a non-optimal cardiovascular health was associated with a higher likelihood for tinnitus. This association was mainly present in participants without hearing loss and in younger participants.

This is the first study to find that a poorer cardiovascular health (CVH) as a composite outcome is associated with a higher likelihood for tinnitus. There is only one previous study, performed among participants of the Jackson Heart Study (JHS), that investigated the association between tinnitus and the AHA CVH score, but did not find an association[15]. One other study within the JHS neither reported an association for individual cardiometabolic risk factors and prevalent tinnitus or tinnitus handicap[26].

Table 4. Sensitivity analysis, unadjusted for CVD or medication usage.

	Tinnitus vs. no tinnitus	
	Model 1	**Model 2**
	OR (95% CI)	**OR (95% CI)**
Non-optimal CV health (vs. optimal)	1.47 (1.02, 2.11)	1.46 (1.01, 2.14)
Health behaviours		
BMI Ideal (<25 kg/m^2)	ref	ref
Intermediate (25-30 kg/m^2)	1.07 (0.88, 1.31)	1.07 (0.90, 1.26)
Poor (>30 kg/m^2)	1.07 (0.88, 1.31)	1.05 (0.86, 1.28)
Smoking Ideal (never)	ref	ref
Poor (current)	1.19 (1.02, 1.40)	1.18 (1.00, 1.38)
Diet quality Ideal (>8 points)	ref	ref
Intermediate (6-8 points)	0.95 (0.76, 1.18)	0.97 (0.77, 1.22)
Poor (<6 points)	0.95 (0.63, 1.44)	0.93 (0.62, 1.41)
Physical Activity Ideal (>150 min moderate activity/wk)	ref	ref
Intermediate (1 - 150 min moderate activity/wk)	1.24 (1.01, 1.51)	1.21 (0.98, 1.49)
Poor (<1 min moderate activity/wk)	-	-
Health factors		
Bloodpressure Ideal (SBP <120 mmHg, DBP <80 mmHG)	ref	ref
Intermediate (SBP 120-140 mmHg or DBP 80-90 mmHg, or treated)	0.92 (0.70, 1.21)	0.92 (0.69, 1.21)
Poor (SBP>140 mmHg or DBP >90 mmHg, or treated)	0.91 (0.69, 1.20)	0.87 (0.66, 1.16)
Total cholesterol Ideal (<5.2 mmol/L)	ref	ref
Intermediate (5.2-6.2 mmol/L)	1.44 (1.15, 1.80)	1.44 (1.15, 1.80)
Poor (>6.2 mmol/L)	1.32 (1.05, 1.67)	1.35 (1.06, 1.70)

Table 4. Continued

	Tinnitus vs. no tinnitus	
	Model 1	**Model 2**
	OR (95% CI)	**OR (95% CI)**
Fasting glucose Ideal (<7.8 mmol/L)	ref	ref
Intermediate (7.8-11.1 mmol/L, or treated)	0.99 (0.65, 1.15)	0.97 (0.83, 1.14)
Poor (>11.1 mmol/L, or treated)	0.97 (0.65, 1.45)	0.92 (0.62, 1.37)

Odds ratios (OR) and 95% confidence interval (CI) for the association of non-optimal CVH (and all 7 metrics) with tinnitus as compared to no tinnitus. Model 1 is adjusted for age, sex and highest achieved education. Model 2 is additionally adjusted for the average hearing threshold over 0.5-4kHz. Participants with prevalent CV disease or those using bloodpressure lowering drugs, lipid lowering drugs or antidiabetic drugs were excluded from these analyses. Optimal cardiovascular health (CVH): total score of ≥11 based on the American Heart Association (AHA) score for CVH. Each of the 7 components can contribute 0 (Poor), 1 (Intermediate) or 2 (Ideal) points to the total score. Diet quality was regarded as adherence to Dutch dietary guidelines. dB: decibel. CV: cardiovascular. kg: kilogram. m: meter. Cm: centimeter. Min: minutes. Wk: week. g: gram. BP: blood pressure. mmHg: millimeters mercury. Mmol: millimol. L: liter. **Bold** values represent significant associations.

Whereas we found augmented effect estimates in participants with a relevant tinnitus handicap, or severe tinnitus. A few key differences between the JHS and the Rotterdam Study should be noted that may explain these different outcomes. The first is the ethnicity of the population, primarily African-American in JHS vs. primarily Caucasian in the current analysis, with each its own cardiovascular risk profile and prevalence of the risk factors[27,28]. Next should be noted that our sample size is much larger (N=5,435 vs. N=1,314 respectively), but prevalence of tinnitus is lower (21.7% vs. 30.0% respectively). Finally the prevalence of hearing loss is higher in our study (39.9% vs. 30%, respectively) which might have resulted in extra power for our stratified analyses on hearing loss. Combined, these differences possibly (partly) explain the different outcomes.

There are many individual factors of CVH that are of interest for the association of CVH with tinnitus. In this study we have chosen the definition of CVH as proposed by the American Heart Association, an often used composite score, containing modifiable risk factors[11]. This score contains metrics that independently are associated with tinnitus[7-10]. However, we were unable to reproduce most of these associations for the individual risk factors. We think this can be attributed to the fact that most studies

were done in younger populations, and did not adjust as extensively for hearing loss. Nevertheless, we did find an association between current smoking and poorer cholesterol health and tinnitus. The effect size we found for current smoking is similar to the one reported in a meta-analysis of 20 studies (this study OR: 1.20 [95%CI 1.03, 1.40]; meta-analysis OR: 1.21 [95%CI 1.09, 1.35])[10]. Smoking is known to cause both large and small vessel disease resulting in both damage of the brain[29], but also of the cochlea[30], which both can contribute to an increased risk for tinnitus. Next, we found throughout all analyses that poorer cholesterol health (i.e. elevated cholesterol concentrations or treatment for hypercholesterolemia) was associated with an increased likelihood for tinnitus. This is in line with what is reported from the KNHANES study[7]. One smaller study reported a decreased risk for tinnitus when hyperlipidaemia is treated[31]. Notably, one should be careful interpreting the association found in our study, as the intermediate and poor health groups are partly containing participants who are using lipid lowering drugs. One type of lipid lowering drugs, atorvastatin, is reported to be tinnitus inducing[32]. Still, we found a similar association between elevated cholesterol concentrations and tinnitus in participants free of any lipid lowering drug or cardiovascular disease. Chronic hypercholesterolemia is a known risk factor for poor vascular health and subsequently brain damage[33]. It is additionally reported to structurally alter the cochlea through lipid accumulation in both the stria vascularis and Corti organ, resulting in a higher sensitivity of the cochlea for hearing loss or potentially tinnitus[34,35]. These mechanisms can contribute to tinnitus development. We therefore consider higher cholesterol concentrations to be a risk factor for tinnitus.

Hearing loss is considered to be the most important risk factor for tinnitus[6,7]. Therefore, it is important to investigate the role of hearing loss in the observed associations between cardiovascular health and tinnitus. We found an association between a non-optimal CVH and tinnitus in participants without hearing loss, which is mitigated among participants with hearing loss. Hearing loss is mainly a problem of the elderly[36] and CVH decreases while ageing[37]. We therefore stratified on age to further investigate whether the association between CVH and tinnitus is a direct effect or whether it is an ageing effect. Participants under 67.5 years of age, most of whom do not have hearing loss, had an increased likelihood for tinnitus with a non-optimal CVH. This association was absent in the older individuals of our population. It could be hypothesized that in the elderly other factors contribute to tinnitus risk than poor CVH or hearing loss, as both are so highly prevalent in the elderly. However, the association found in the younger individuals of our population could suggest a direction towards a pathophysiological pathway of tinnitus based on microangiopathy. The general idea at the moment is that tinnitus is partly a result of (in some cases

transient) peripheral, cochlear damage, but that central brain changes are essential for the tinnitus to remain[2,16]. Different aspects of poorer CVH might contribute to microangiopathy of the cochlea or the brain, which might not in all cases result in tinnitus, but do appear to contribute to an increased risk for tinnitus. We hypothesize that the mechanism of the association between poor CVH and tinnitus can be found in brain damage mostly, as the main associations were found within the group without significant hearing loss. Still, more research is warranted to elucidate on the possible causal role of CVH as risk factor for tinnitus.

A major strength of this study is the population-based nature of the study and the large sample size, which allows for thorough adjustment for hearing loss and age. This allowed us to show that there is an ageing effect in the investigated associations. Furthermore, this is the first study to investigate the association between tinnitus and CVH with an often used and published on composite outcome, the AHA-score[11]. Some side notes can be placed to our current study. We have chosen for a slightly altered diet score compared to the one proposed by the AHA. The 14 food-groups diet score we used, as opposed to the 5 food-groups diet score originally proposed by Lloyd-Jones et al., is more applicable for the Dutch population and in line with the Dutch dietary guidelines[22]. Furthermore, one needs to be aware that the study was done in a Caucasian population, thus limiting the ability to extrapolate the results to other ethnicities as each ethnicity has its own cardiovascular risk profile. Lastly, the cross-sectional design of the study limits the ability to infer on causality.

In conclusion, in this sample of older individuals from the general population we found that non-optimal cardiovascular health was associated with a higher likelihood for tinnitus. This association was mainly present in the younger participants, with a low prevalence of hearing loss. This suggests that poor cardiovascular health may be a relevant factor in initiating or maintaining chronic tinnitus. Future studies are warranted to elucidate further on the causality and pathophysiological mechanisms explaining this association.

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Supplemental material

Supplement table 1. The association between cardiovascular health and tinnitus– stratified on hearing loss

	Tinnitus vs. no tinnitus			
	No hearing loss		**Hearing loss (>25dB)**	
	Model 1	**Model 2**	**Model 1**	**Model 2**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Non-optimal CV health (vs. optimal)	**1.61 (1.01, 2.58)**	1.59 (0.98, 2.57)	1.23 (0.65, 2.34)	1.25 (0.65, 2.42)
Health behaviours				
BMI Ideal (<25 kg/m^2)	ref	ref	ref	ref
Intermediate (25-30 kg/m^2)	1.12 (0.89, 1.41)	1.09 (0.86, 1.39)	1.01 (0.79, 1.29)	1.00 (0.78, 1.28)
Poor (>30 kg/m^2)	1.26 (0.97, 1.64)	1.21 (0.94, 1.58)	1.02 (0.79, 1.32)	1.01 (0.79, 1.31)
Smoking Ideal (never)	ref	ref	ref	ref
Intermediate (former)	0.45 (0.16, 1.30)	0.48 (0.17, 1.37)	1.24 (0.75, 2.05)	1.19 (0.72, 1.99)
Poor (current)	**1.33 (1.07, 1.65)**	**1.31 (1.06, 1.63)**	1.06 (0.84, 1.34)	1.07 (0.85, 1.36)
Diet quality Ideal (>8 points)	ref	ref	ref	ref
Intermediate (6-8 points)	0.96 (0.74, 1.25)	0.97 (0.74, 1.27)	0.96 (0.66, 1.58)	0.98 (0.67, 1.42)
Poor (<6 points)	0.94 (0.62, 1.43)	0.92 (0.61, 1.40)	1.01 (0.65, 1.58)	1.01 (0.65, 1.59)
Physical Activity Ideal (>150 min moderate activity/wk)	ref	ref	ref	ref
Intermediate (1 - 150 min moderate activity/wk)	1.19 (0.93, 1.51)	1.18 (0.92, 1.51)	1.20 (0.95, 1.49)	1.19 (0.95, 1.48)
Poor (<1 min moderate activity/wk)	1.03 (0.49, 2.14)	0.99 (0.47, 2.09)	1.66 (0.99, 2.78)	1.64 (0.97, 2.76)
Health factors				
Bloodpressure Ideal (SBP <120 mmHg, DBP <80 mmHG)	ref	ref	ref	ref
Intermediate (SBP 120-140 mmHg or DBP 80-90 mmHg, or treated)	1.03 (0.74, 1.44)	1.03 (0.74, 1.44)	0.75 (0.45, 1.23)	0.74 (0.45, 1.23)

Supplement table 1. Continued

	Tinnitus vs. no tinnitus			
	No hearing loss		Hearing loss (>25dB)	
	Model 1	Model 2	Model 1	Model 2
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Poor (SBP>140 mmHg or DBP >90 mmHg, or treated)	1.07 (0.78, 1.47)	1.06 (0.77, 1.45)	0.88 (0.56, 1.41)	0.88 (0.55, 1.40)
Total cholesterol Ideal (<5.2 mmol/L)	ref	ref	ref	ref
Intermediate (5.2-6.2 mmol/L)	**1.77 (1.31, 2.40)**	**1.78 (1.32, 2.40)**	1.27 (0.93, 1.72)	1.26 (0.93, 1.71)
Poor (>6.2 mmol/L)	**1.68 (1.25, 2.26)**	**1.70 (1.26, 2.29)**	1.20 (0.88, 1.62)	1.20 (0.89, 1.63)
Fasting glucose Ideal (<7.8 mmol/L)	ref	ref	ref	ref
Intermediate (7.8-11.1 mmol/L, or treated)	0.88 (0.71, 1.09)	0.87 (0.70, 1.08)	1.08 (0.86, 1.35)	1.08 (0.86, 1.34)
Poor (>11.1 mmol/L, or treated)	1.06 (0.80, 1.40)	1.03 (0.78, 1.36)	0.98 (0.76, 1.27)	0.98 (0.75, 1.27)

Odds ratios (OR) and 95% confidence interval (CI) for the association of non-optimal CVH (and all 7 metrics) stratified on the presence of hearing loss (≥25dB). Model 1 is adjusted for age, sex and highest achieved education. Model 2 is additionally adjusted for the average hearing threshold over 0.5-4kHz. Optimal cardiovascular health (CVH): total score of ≥11. Each of the 7 components can contribute 0 (Poor), 1 (Intermediate) or 2 (Ideal) points to the total score. Prevalent CV disease subtracted 1 point from each category (maximum score of 7). The use of lipid-lowering drugs, blood pressure lowering drugs or antidiabetic therapy subtracted 1 point of the score in that category. Diet quality was regarded as adherence to Dutch dietary guidelines. dB: decibel. CV: cardiovascular. kg: kilogram. m: meter. Cm: centimeter. Min: minutes. Wk: week. g: gram. BP: blood pressure. mmHg: millimeters mercury. Mmol: millimol. L: liter. **Bold** values represent significant associations.

Supplement table 2. The association between cardiovascular health and tinnitus– stratified on midlife vs. late life.

	Tinnitus vs. no tinnitus			
	Midlife (<67.5 years)		**Late life (>67.5 years)**	
	Model 1	**Model 2**	**Model 1**	**Model 2**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Non-optimal CV health (vs. optimal)	**1.57 (1.06, 2.35)**	**1.56 (1.04, 2.35)**	1.25 (0.56, 2.79)	1.24 (0.54, 2.86)
Health behaviours				
BMI Ideal (<25 kg/m^2)	ref	ref	ref	ref
Intermediate (25-30 kg/m^2)	1.17 (0.93, 1.46)	1.12 (0.89, 1.42)	0.97 (0.76, 1.23)	0.97 (0.76, 1.23)
Poor (>30 kg/m^2)	1.26 (0.98, 1.62)	1.20 (0.93, 1.54)	1.04 (0.81, 1.33)	1.02 (0.79, 1.32)
Smoking Ideal (never)	ref	ref	ref	ref
Intermediate (former)	0.77 (0.31, 1.90)	0.78 (0.31, 1.97)	1.13 (0.69, 1.83)	1.05 (0.64, 1.73)
Poor (current)	**1.27 (1.03, 1.56)**	**1.25 (1.02, 1.54)**	1.18 (0.84, 1.48)	1.15 (0.92, 1.44)
Diet quality Ideal (>8 points)	ref	ref	ref	ref
Intermediate (6-8 points)	1.00 (0.77, 1.30)	1.05 (0.80, 1.38)	0.90 (0.64, 1.26)	0.89 (0.63, 1.26)
Poor (<6 points)	1.00 (0.67, 1.48)	0.98 (0.66, 1.46)	0.98 (0.61, 1.58)	0.96 (0.59, 1.55)
Physical Activity Ideal (>150 min moderate activity/wk)	ref	ref	ref	ref
Intermediate (1 - 150 min moderate activity/wk)	1.25 (1.00, 1.57)	1.23 (0.97, 1.55)	1.18 (0.95, 1.47)	1.16 (0.92, 1.46)
Poor (<1 min moderate activity/wk)	0.90 (0.41, 1.97)	0.85 (0.38, 1.91)	**1.65 (1.00, 2.71)**	**1.67 (1.01, 2.77)**
Health factors				
Bloodpressure Ideal (SBP <120 mmHg, DBP <80 mmHG)	ref	ref	ref	ref
Intermediate (SBP 120-140 mmHg or DBP 80-90 mmHg, or treated)	0.97 (0.72, 1.31)	0.97 (0.71, 1.32)	0.80 (0.43, 1.48)	0.76 (0.40, 1.42)
Poor (SBP>140 mmHg or DBP >90 mmHg, or treated)	1.07 (0.81, 1.42)	1.04 (0.78, 1.38)	0.90 (0.51, 1.60)	0.86 (0.48, 1.53)

Supplement table 2. Continued

	Tinnitus vs. no tinnitus			
	Midlife (<67.5 years)		Late life (>67.5 years)	
	Model 1	Model 2	Model 1	Model 2
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Total cholesterol Ideal (<5.2 mmol/L)	ref	ref	ref	ref
Intermediate (5.2-6.2 mmol/L)	**1.58 (1.18, 2.10)**	**1.60 (1.19, 2.14)**	**1.46 (1.08, 1.98)**	**1.40 (1.03, 1.91)**
Poor (>6.2 mmol/L)	**1.59 (1.20, 2.12)**	**1.63 (1.22, 2.18)**	1.26 (0.92, 1.72)	1.24 (0.90, 1.71)
Fasting glucose Ideal (<7.8 mmol/L)	ref	ref	ref	ref
Intermediate (7.8-11.1 mmol/L, or treated)	0.94 (0.77, 1.16)	0.93 (0.76, 1.15)	1.03 (0.83, 1.27)	1.00 (0.80, 1.24)
Poor (>11.1 mmol/L, or treated)	1.02 (0.77, 1.35)	0.97 (0.73, 1.29)	1.02 (0.80, 1.31)	1.00 (0.78, 1.30)

Odds ratios (OR) and 95% confidence interval (CI) for the association of non-optimal CVH (and all 7 metrics) stratified on age (<67.5 years and >67.5 years). Model 1 is adjusted for age, sex and highest achieved education. Model 2 is additionally adjusted for the average hearing threshold over 0.5-4kHz. Optimal cardiovascular health (CVH): total score of ≥11. Each of the 7 components can contribute 0 (Poor), 1 (Intermediate) or 2 (Ideal) points to the total score. Prevalent CV disease subtracted 1 point from each category (maximum score of 7). The use of lipid-lowering drugs, blood pressure lowering drugs or antidiabetic therapy subtracted 1 point of the score in that category. Diet quality was regarded as adherence to Dutch dietary guidelines. dB: decibel. CV: cardiovascular. kg: kilogram. m: meter. Cm: centimeter. Min: minutes. Wk: week. g: gram. BP: blood pressure. mmHg: millimeters mercury. Mmol: millimol. L: liter. **Bold** values represent significant associations.

Chapter 3.2

Macrolide-associated ototoxicity: a cross-sectional and longitudinal study to assess the association of macrolide use with tinnitus and hearing loss

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ABSTRACT

Introduction

Macrolides are widely prescribed antibiotics for many different indications. However, there are concerns about adverse effects such as ototoxicity.

Aim

To investigate whether macrolide use is associated with tinnitus and hearing loss in the general population.

Methods

Cross-sectional (n=4,286) and longitudinal (n=636) analyses were performed within the population-based Rotterdam Study. We investigated with multivariable logistic regression models the association between macrolides and tinnitus, and with multivariable linear regression models the association between macrolides and two different hearing thresholds (both ears, averaged over 0.25, 0.5, 1, 2, 4 and 8 kHz and 2, 4 and 8 kHz). Both regression models were adjusted for age, sex, systolic blood pressure, alcohol, smoking, BMI, diabetes, education level, estimated glomerular filtration rate, and other ototoxic or tinnitus-generating drugs. Cumulative exposure to macrolides was categorized according to the number of dispensed Defined Daily Doses (DDDs) and duration of action.

Results

In the fully adjusted model, ever use of macrolides was associated with a 25% higher likelihood of prevalent tinnitus (OR=1.25; 95% CI 1.07-1.46). This association was more prominent in participants with a cumulative dose of more than 14 DDDs and among users of intermediate- or long-acting macrolides. Macrolide use in between both assessments was associated with more than a twofold increased risk on incident tinnitus. No general association was found between macrolides and hearing loss. Only a trend for a higher hearing threshold in very recent users (≤3 weeks) was observed.

Conclusions

Macrolide use was significantly associated with both prevalent and incident tinnitus. Macrolide-associated tinnitus was likely cumulative dose-dependent.

INTRODUCTION

Macrolides are among the most frequently prescribed classes of antibiotics worldwide[1], and are indicated for atypical respiratory tract infections[2], sexually transmitted diseases[3], and gastro-intestinal infections with *Helicobacter pylori*[4], or *Campylobacter spp*[5]. Besides the antibiotic effect, macrolides have significant immunomodulatory and antiviral effects[6]. For Europe, the outpatient use of macrolides increased over time from 1997 to 2009[7]. In the Netherlands, use of intermediate-acting macrolides (mainly clarithromycin) decreased by more than 10%, whereas use of azithromycin increased by more than 20%[7]. However, widespread use of macrolides expose people to the risk of adverse effects such as gastro-intestinal adverse effects, bacterial resistance, QTc prolongation, and ototoxicity[8].

Several previous studies investigated the association between macrolide use and ototoxicity. Ototoxicity comprises both sensorineural hearing loss (SNHL) and tinnitus. A Cochrane review based on four randomised controlled trials found that hearing loss is more often reported in participants using macrolides[8]. Another systematic review concluded that SNHL is associated with either oral or intravenous macrolide usage, even when administered at standard oral doses[9]. Some studies reported that SNHL is dose-dependent and reversible[10,11], whereas other studies found that it is irreversible[12,13]. Other larger-scale studies, a retrospective cohort and a case-control study, found no increased risk for SNHL of macrolides in comparison to other antibiotics[14,15]. Overall, no association was found between macrolide antibiotics and SNHL in a recent meta-analysis (OR 1.20 ; 95% CI 0.96-1.49)[16]. Tinnitus has been associated with macrolides in case reports[17,18]. One COPD patient withdrew from a trial because of newly developed tinnitus in the erythromycin treatment arm[19].

Previous studies on macrolide usage and hearing loss had limitations and gave contradictory results. Most studies consisted of limited populations. Larger studies were based on health claims data[14,15]. Additionally, limited studies reported on the association between macrolide usage and tinnitus. Therefore, we investigated in a large, population-based sample of older adults both the cross-sectional and longitudinal association of any dispensed oral macrolide prescription with hearing loss and tinnitus. Additionally, we investigated whether there was a cumulative effect and whether time since discontinuation influenced any association.

MATERIALS AND METHODS

Study setting

This study was embedded in the Rotterdam Study; a prospective, population-based cohort study. The Rotterdam Study (RS) was initiated in 1989 and investigates determinants and consequences of aging. Details of the study have been described elsewhere[20]. The entire study population consists of 14,926 individuals aged ≥ 45 years living in the well-defined Ommoord district in the city of Rotterdam, the Netherlands[20]. Participants were invited to undergo extensive examinations in the dedicated research centre at study entry and subsequently every 3 to 4 years. All participants were registered in one of the seven community pharmacies participating in the Rotterdam Study. Information was available on all drug dispensing data from study entry including drug names, international non-proprietary names, Anatomical Therapeutic Chemical (ATC) codes, dosage forms, dates of delivery, number of prescriptions, prescribed daily dosages, and duration of the prescription. In addition, home interviews are performed. Moreover, participants are continuously monitored for major morbidity and mortality through linkage of records from general practitioners, specialist letters, hospitalization registries, and municipality to the study database[20].

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). The Rotterdam Study has been entered into the Netherlands National Trial Register (www.trialregister.nl) and into the World Health Organization International Clinical Trials Registry Platform (who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians[20].

Study design

First, the association of macrolides with tinnitus and hearing loss was studied in a cross-sectional analysis, embedded in the Rotterdam Study. Tinnitus and hearing assessment were implemented in the study protocol in 2011, and the first group of participants was invited for their second hearing assessment in 2015[21].
Participants from cohorts RS-I-6, RS-II-3 and RS-III-2 (February 2011-July 2015) who underwent pure-tone audiometry and whose pharmacy data was available, were included in the cross-sectional study (Figure S1). Participants with conductive hearing loss, defined as an air-bone gap of 15 decibel (dB) or more in the best hearing ear, were excluded.

Second, participants without tinnitus at baseline were followed-up longitudinally to test whether macrolides were associated with incident tinnitus. For these analyses, data from cohort RS-II-3 and RS-II-4 were analysed (Figure S1).

Tinnitus assessment

Tinnitus assessment was performed through a home interview. Participants were asked if they experienced sounds in the head or in (one of) the ears (such as whizzing, peeping, or humming) without an objective external sound source being present. All possible answers to this question were classified into a binary variable in which tinnitus was either absent ('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 the often temporary character of ringing in the ears, presence of less than once a week was not recorded as prevalent tinnitus[22]. Incident tinnitus was defined as tinnitus in participants with no tinnitus present at the first interview in 2011-2012 but who reported tinnitus symptoms at the follow-up in 2015-2016.

Hearing assessment

Audiometric assessment was performed by one trained health care professional in a soundproof booth. For the audiometric assessment, a computer-based audiometry system (Decos Technology Group, version 210.2.6 with AudioNigma interface) and TDH-39 headphones were used[20]. To determine hearing levels in dB, pure tone audiometry was used according to the ISO-standard 8253-1[23]. Masking was performed according to the method of Hood[24]. Air conduction thresholds for both ears were measured on different frequencies (0.25, 0.5, 1, 2, 4, and 8 kilohertz (kHz)). Bone conduction thresholds were measured at 0.5 and 4 kHz to exclude possible conductive hearing losses. Two pure tone average hearing thresholds were calculated for mean of both ears, averaged over 0.25, 0.5, 1, 2, 4 and 8 kHz ($PTA_{0.25\text{-}8}$) and 2, 4 and 8 kHz ($PTA_{2\text{-}8}$). Since we expect ototoxicity to be detectable at high frequencies first, these results are discussed in the main text, the other results are discussed in the supplement. Hearing loss was defined as a $PTA_{0.25\text{-}8} \geq 35$ dB based on the cut-off for moderate hearing loss according to the Global Burden of Disease classification[25], with the inclusion of 0.25 and 8 kHz. The decline of hearing loss was calculated as the difference between the hearing thresholds at follow-up and the hearing thresholds at the first audiometric assessment.

Assessment of macrolide use

Complete information on all filled prescriptions on a day-to-day basis are obtained in automated format from all community pharmacies in the Ommoord region[20]. Information was retrieved using the ATC codes for oral macrolides and combinations

with oral macrolides for eradication of *H. pylori* (Table S1) in the number of dispensed Defined Daily Doses (DDDs). Antibiotics are only available on prescription in the Netherlands.

Ever use of macrolides was defined as any dispensed oral macrolide prescription between January 1st 1995 and the date of the first hearing test for the cross-sectional analyses, and between the first hearing test and the second hearing test for the longitudinal analyses. The cumulative macrolide dose on the day of the first hearing test was calculated and divided into two groups: 1-14 DDDs or >14 DDDs. Because of the potential reversibility, use of macrolides was categorized into very recent use (≤3 weeks), recent use (3 weeks – 3 months) or past use (>3 months) before the day of the hearing test. The types of macrolides were categorized as short- (J01FA01, J01FA02), intermediate- (J01FA06, J01FA09, A02BD04) and long-acting (J01FA10) according to their mean plasma elimination half-life[7].

Covariables assessment

Highest achieved educational level was noted, using the United Nations Educational, Scientific and Cultural Organization (UNESCO) classification[26]. Smoking data was collected through self-report and categorized into never, former or current smoker. Alcohol consumption, in grams per day, was assessed through self-report by means of the Food-Frequency Questionnaire[27]. Prevalent diabetes was defined on the basis of WHO criteria for fasting glucose, ≥7.0 mmol/L or use of glucose lowering drugs[28]. Height (meters) and weight (kilograms) were measured at the research centre and body mass index (BMI, kg/m2) was calculated. Waist circumference (centimeters) was measured at the research centre. Systolic blood pressure (SBP) was measured twice using a random sphygmomanometer. Serum creatinine was measured with an enzymatic assay at ergo-5. The estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration formula assuming that the Rotterdam Study has no all-black participants. Age, sex, smoking status, alcohol consumption, and educational level were interviewed by a research assistant at the participant's home. The dispensed prescriptions of other ototoxic or tinnitus-generating drugs, according to Altissimi et al. and Lanvers-Kaminsky et al.[29,30], were retrieved using the ATC codes. Ever use of irreversible ototoxic drugs was defined as any dispensed prescription between January 1st 1995 and the date of the first hearing test. Participants were considered current users of reversible ototoxic drugs if the hearing measurement occurred within a prescription episode.

Statistical analyses

Descriptive analyses were performed to assess and compare the differences in participant demographic characteristics. Continuous data were described as mean

(standard deviation (SD)) and categorical variables are described as number (N (%)). An independent samples T-test, and X^2-test or Fisher's Exact Test were used respectively to test differences in characteristics between never and ever macrolide users.

For the cross-sectional analyses, we evaluated the association between use of macrolide antibiotics and tinnitus with a multivariable logistic regression model. Second, we evaluated the effect of macrolide antibiotics on $PTA_{0.25\text{-}8}$ and $PTA_{2\text{-}8}$ hearing thresholds with a multivariable linear regression model. Third, we evaluated the effect of macrolide antibiotics on hearing loss ($PTA_{0.25\text{-}8} \geq 35$ dB) with a multivariable logistic regression model. A sensitivity analysis was performed to test the robustness of the used tinnitus definition. Both the logistic and linear regression models were adjusted for age and sex in a first model, and additionally adjusted for SBP, alcohol, smoking, education level, BMI, diabetes, eGFR, and other tinnitus-generating or ototoxic drugs in a second model. Missing data on alcohol consumption was dealt with using the last observation carried forward method because of the high percentage of missing values for this covariable (12%). Missing data on other variables were not imputed (<3.2%). To study whether the ototoxicity was dependent on cumulative dosing, we expressed the use of macrolides in DDDs/patient between January 1st 1995 and the first hearing test, and between the first and the second hearing test. To study if the ototoxicity is irreversible or reversible, and in the latter case, how long this effect is measurable after macrolide use is discontinued, we included time between last use and the hearing assessment in our model. Finally, we longitudinally assessed the association between macrolide use and incident tinnitus with this method in subjects without tinnitus at baseline, and with hearing thresholds at follow-up adjusting for the hearing threshold at baseline. Data analyses were performed using IBM SPSS Statistics® version 25, a p-value of <0.05 was considered significant.

RESULTS

Study population

In total, 4,309 participants from cohorts RS-I-6, RS-II-3 and RS-III-2 without conductive hearing loss at baseline had pure-tone audiometry available of both ears of whom all had pharmacy data. Of these participants, 23 who gave no informed consent for follow-up were excluded. The study population for the cross-sectional analyses on hearing loss and on tinnitus comprised 4,286 and 4,276 participants, respectively (Figure 1A). A subset of 636 participants was available for the longitudinal analysis. The median follow-up time was 4 years (min: 2 years; max: 5 years). After exclusion

of the participants with missing data and baseline tinnitus, 625 participants were included in the longitudinal analysis on hearing loss and 499 in the longitudinal logistic regression analysis on tinnitus (Figure 1B).

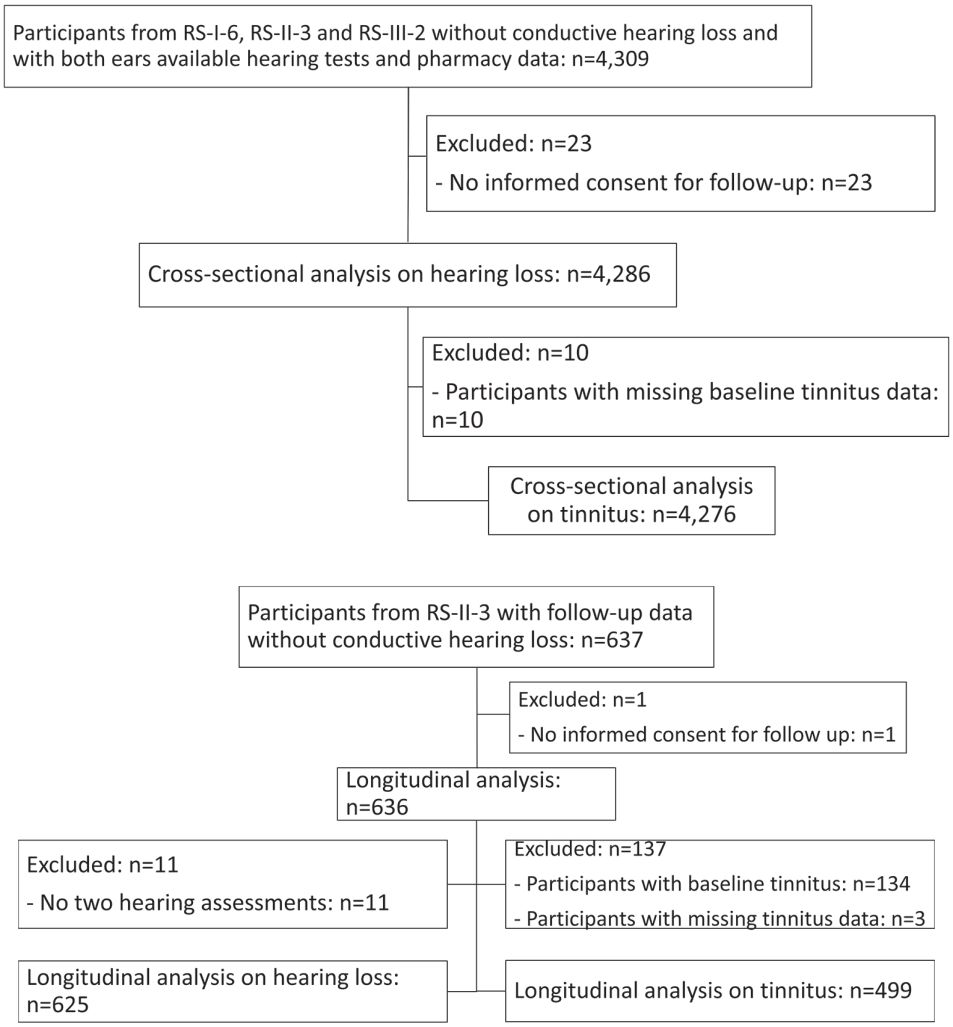

Figure 1. Flow diagram of study population
A The cross-sectional analysis.
B The longitudinal analysis. Abbreviations are as follows: RS, Rotterdam Study

Baseline characteristics

Table 1 describes the baseline characteristics of the study population. The mean age at baseline was 68 ± 10 years, and the majority of participants included in this study were female (56%). Baseline characteristics of never and ever macrolide users are presented in Table 1. Compared to never users, macrolide users were significantly more often female and had a higher BMI, a lower alcohol consumption, used more often other tinnitus-generating drugs, and ototoxic drugs. The lower alcohol consumption among macrolide users was driven by a higher proportion of females (generally drinking less) among ever macrolide users.

Macrolide use

At baseline, a total of 1,871/4,286 (44%) participants had ever received one or more macrolide prescription(s) (Table 1). The most frequently dispensed macrolides were clarithromycin and azithromycin. Clarithromycin in combination with pantoprazole and amoxicillin (A02BD04) was the only combination preparation dispensed for eradication of *H. pylori*. The median cumulative dose among the users was 12 DDDs, with the highest cumulative dose for clarithromycin users (Table S2). A total of 71/636 (11%) participants had received one or more macrolide prescription(s) between both hearing assessments. Azithromycin was more often dispensed than clarithromycin. Spiramycin and roxithromycin were not dispensed during this period (Table S2).

The association of macrolide use with tinnitus

In total, 898 (21%) participants reported tinnitus at baseline. Of them, 35% (n=315) experienced ringing in (one of) the ears more than once a week, and 65% (n=583) daily. The proportion of patients reporting tinnitus was 20% among never users and 23% among ever macrolide users (p=0.010, Chi^2). As shown in Table 2, ever use of macrolides was significantly associated with a 25% higher likelihood of tinnitus (aOR 1.25; 95% CI 1.08-1.45) in model 1. This association remained statistically significant after adjusting for a range of potential confounders (aOR 1.25; 95% CI 1.07-1.46). The association was more prominent in participants with a cumulative dose of more than 14 DDDs, and among users of intermediate- or long-acting macrolides (Table 2). A stronger association was found if tinnitus was defined as daily present (aOR 1.32; 95% CI 1.09-1.58), and slightly weaker when having tinnitus less often than once a week was included (aOR 1.23; 95% CI 1.07-1.40). Figure 2 represents the results of the multinomial regression analysis.

The association of macrolide use with incident tinnitus

The mean follow-up time was not different for participants with incident tinnitus (1,605 days) and those without tinnitus (1,603 days) (p=0.901). The 4-year cumulative incidence of tinnitus in the total study population was 11%. The incidence of tinnitus

Table 1. Baseline characteristics of the study population for cross-sectional analyses

	Total (n=4,286)	Never ML users (n=2,415)	Ever ML users (n=1,871)	p-value
RS-I, n (%)	727 (17)	407 (17)	320 (17)	
RS-II, n (%)	1,103 (26)	601 (25)	502 (27)	0.290
RS-III, n (%)	2,456 (57)	1,407 (58)	1,049 (56)	
Age in years, mean (SD)	68 (10)	68 (10)	69 (10)	0.238
Female, n (%)	2,404 (56)	1,244 (52)	1,160 (62)	<0.001
BMI in kg/m², mean (SD)	27 (4)	27 (4)	28 (4)	<0.001
Diabetes, n (%)	529 (13)	295 (13)	234 (13)	0.784
Never smoker, n (%)	1,371 (32)	798 (33)	573 (31)	
Former smoker, n (%)	2,211 (52)	1,215 (51)	996 (54)	0.152
Current smoker, n (%)	669 (16)	381 (16)	288 (16)	
Primary education, n (%)	336 (8)	184 (8)	152 (8)	0.107
Lower/intermediate general education or lower vocational education, n (%)	1,613 (38)	876 (37)	737 (40)	
Intermediate vocational education or higher general education, n (%)	1,278 (30)	725 (30)	553 (30)	
Higher vocational education or university, n (%)	1,018 (24)	601 (25)	417 (22)	
Alcohol consumption (g/day), mean (SD)	8.5 (8.4)	8.7 (8.7)	8.1 (8.1)	0.019
Alcohol consumption LOCF (g/day), mean (SD)	7.8 (8.5)	8.1 (8.7)	7.3 (8.1)	0.005
Systolic blood pressure in mmHg, mean (SD)	140 (21)	140 (21)	141 (21)	0.124
eGFR in ml/min/1.73m², mean (SD)	77 (15)	76 (15)	77 (15)	0.365
Current use other tinnitus-generating drugs, n (%)	1,360 (32)	714 (30)	646 (35)	0.001
Ever use of other irreversible ototoxic drugs, n (%)	8 (0.2)	0 (0.0)	8 (0.4)	0.002
Current use of other reversible ototoxic drugs, n (%)	180 (4.2)	96 (4.0)	84 (4.5)	
Tinnitus, n (%)	898 (21)	472 (20)	426 (23)	0.010
$PTA_{2\text{-}8,}$ mean (SD)	40 (20)	40 (20)	40 (19)	0.456
$PTA_{0.25\text{-}8,}$ mean (SD)	29 (14)	29 (14)	29 (14)	0.746
Hearing loss ($PTA_{0.25\text{-}8}$≥35 dB), n (%)	1,318 (31)	755 (31)	563 (30)	0.410

Abbreviations are as follows: ML, Macrolide; RS, Rotterdam Study; SD: Standard Deviation; BMI, Body Mass Index; LOCF, Last Observation Carried Forward; eGFR, estimated Glomerular Filtration Rate; PTA Pure-Tone Average; dB decibel. The numbers of the missing values are not shown in this table, but are as follows: BMI 12; diabetes 124; smoking 35; education 41; alcohol consumption 497; alcohol consumption LOCF 3; systolic blood pressure 44; eGFR 135 and tinnitus 10.

was 19% in the participants who used macrolides in between both tinnitus assessments, while this was 10% in the non-users (p=0.034, Chi²). Macrolide use between both tinnitus assessments was associated with more than a twofold increased risk on incident tinnitus (Table 3).

Table 2. Logistic regression analysis on the association between macrolide therapy and tinnitus

	Tinnitus cases/ total n (%)	Model 1 aOR [95% CI], p-value	Model 2 aOR [95% CI], p-value
Users		n=4,276	n=4,072
Never users	472/2,409 (20)	Ref.	Ref.
Ever users	426/1,867 (23)	1.25 [1.08; 1.45], p=0.004	1.25 [1.07; 1.46], p=0.006
Cumulative dose		n=4,276	n=4,072
Never users	472/2,409 (20)	Ref.	Ref.
1-14 DDDs	251/1,148 (22)	1.18 [0.99; 1.40], p=0.063	1.19 [0.99; 1.43], p=0.058
>14 DDDs	175/719 (24)	1.36 [1.12; 1.66], p=0.002	1.34 [1.08; 1.65], p=0.007
Macrolide type*		n=3,686	n=3,513
Never users	472/2,409 (20)	Ref.	Ref.
Short-acting	15/110 (14)	0.70 [0.40; 1.24], p=0.224	0.70 [0.38; 1.26], p=0.231
Intermediate-acting	145/625 (23)	1.33 [1.02; 1.74], p=0.034	1.31 [1.00; 1.73], p=0.051
Long-acting	121/542 (22)	1.25 [0.98; 1.58], p=0.071	1.29 [1.01; 1.66], p=0.044

Model 1 was adjusted for age and sex. Model 2 was additionally adjusted for SBP, alcohol (LOCF), smoking, education level, BMI, diabetes, eGFR, current use of tinnitus-generating drugs and other ototoxic drugs, and $PTA_{0.25\text{-}8}$. Significant estimates (p<0.05) are indicated in **bold**. *adjusted for cumulative dose. Macrolides were categorized as short- (J01FA01, J01FA02), intermediate- (J01FA06, J01FA09, A02BD04), and long-acting (J01FA10) according to their mean plasma elimination half-life.[7] Abbreviations are as follows: DDDs, Defined Daily Doses; SBP, Systolic Blood Pressure; LOCF, Last Observation Carried Forward; BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate; PTA, Pure-Tone Average.

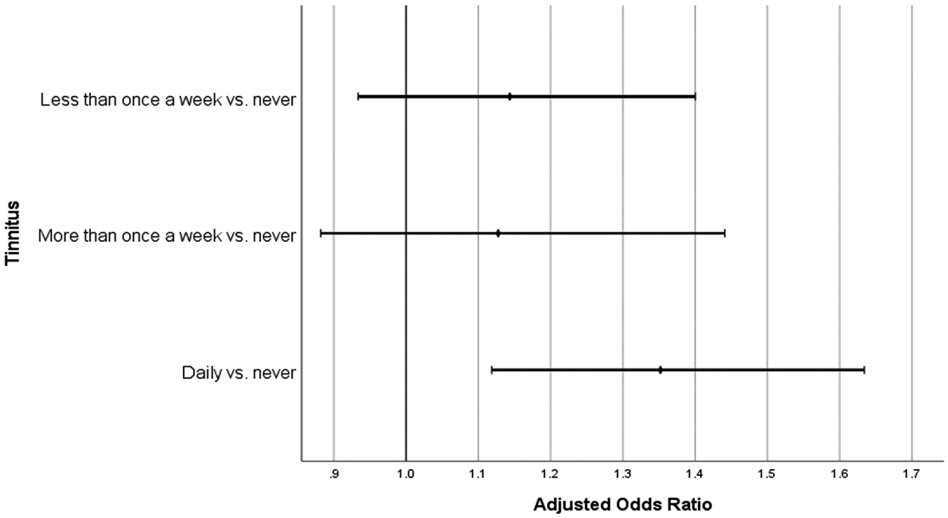

Figure 2. Forrest plot representing adjusted odds ratios and 95% confidence intervals of multinomial logistic regression analysis for the association between ever macrolide use and tinnitus.
Adjusted for age, sex, SBP, alcohol (LOCF), smoking, education level, BMI, diabetes, eGFR, current use of tinnitus-generating drugs, and other ototoxic drugs and PTA0.25-8. Abbreviations are as follows: SBP, Systolic Blood Pressure; LOCF, Last Observation Carried Forward; BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate; PTA, Pure-Tone Average

Hearing threshold

In total, mean PTA_{2-8} was 39.8 (SD 19.6) dB and mean $PTA_{0.25-8}$ was 29.1 (SD 14.2) dB at baseline. Almost one third had hearing loss ($PTA_{0.25-8}$ ≥35 dB) (Table 1). No significant difference between never and ever macrolide users was observed. The results of the linear analysis with PTA_{2-8}, $PTA_{0.25-8}$ as outcome and the logistic regression analysis with hearing loss ($PTA_{0.25-8}$ ≥35 dB) can be found in Table 4, S3 and S4, respectively. As presented in these tables, no significant association between macrolide use and hearing threshold or hearing loss was observed. Only very recent use (≤3 weeks) tended to be associated with higher hearing thresholds (difference=4.34 dB; 95% CI -6.28; 14.96).

The association of macrolide use with hearing threshold

The average decline of hearing threshold was 4.17 dB/4.42 years in the participants who used macrolides between the 3rd and 4th visit, and 5.04 dB/4.38 years in the non-users. No significant difference in PTA_{2-8} at follow-up between users and non-users was observed (Table 5).

Table 3. Logistic regression analysis on the association between macrolide therapy and incident tinnitus

	Tinnitus cases/ total n (%)	Model 1 aOR [95% CI], p-value	Model 2 aOR [95% CI], p-value
Use between both tinnitus assessments		n=499	n=476
No macrolide use	44/442 (10)	Ref.	Ref.
Macrolide use	11/57 (19)	**2.25 [1.08; 4.68], p=0.030**	2.21 [0.96; 5.06], p=0.062

Model 1: adjusted for age and sex. Model 2: adjusted all variables in model 1 + SBP, alcohol (LOCF), smoking, education level, BMI, diabetes, eGFR, current use of tinnitus-generating drugs, and other ototoxic drugs and $PTA_{0.25\text{-}8}$

Abbreviations are as follows: SBP, Systolic Blood Pressure; LOCF, Last Observation Carried Forward; BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate; PTA, Pure-Tone Average

Table 4. Linear regression analysis on the association between macrolide therapy and $PTA^{2\text{-}8}$

	Number	Model 1 Difference [95% CI], p-value	Model 2 Difference [95% CI], p-value
Users		n=4,286	n=4,072
Never users	2,415	Ref.	Ref.
Ever users	1,871	-0.19 [-1.06; 0.68], p=0.671	-0.40 [-1.29; 0.49], p=0.383
Recent use		n=4,285	n=4,071
Never users	2,415	Ref.	Ref.
Very recent use	9	9.23 [-0.12; 18.58], p=0.053	4.34 [-6.28; 14.96], p=0.423
Recent use	38	-0.27 [-4.85; 4.31], p=0.907	-0.64 [-5.22; 3.95], p=0.786
Past use	1,823	-0.22 [-1.09; 0.65], p=0.620	-0.40 [-1.30; 0.50], p=0.386
Macrolide type		n=3,696	n=3,513
Never users	2,415	Ref.	Ref.
Short-acting	110	-1.86 [-4.58; 0.87], p=0.183	-2.08 [-4.82; 0.66], p=0.137
Intermediate-acting	628	0.56 [-0.69; 1.82], p=0.379	0.53 [-0.76; 1.81], p=0.422
Long-acting	543	-1.01 [-2.35; 0.32], p=0.136	-1.23 [-2.60; 0.15], p=0.080

Estimates represent the decibel (dB) change in hearing threshold for a both ear pure tone average (PTA) over both 2, 4 and 8 kilohertz (kHz) ($PTA_{2\text{-}8}$) for macrolide usage. Model 1 was adjusted for age and sex. Model 2 was additionally adjusted for SBP, alcohol (LOCF), smoking, education level, BMI, diabetes, eGFR, and other ototoxic drugs. Significant estimates ($p<0.05$) are indicated in **bold**. Macrolides were categorized as short- (J01FA01, J01FA02), intermediate- (J01FA06, J01FA09, A02BD04), and long-acting (J01FA10) according to their mean plasma elimination half-life.[7] Abbreviations are as follows: SBP, Systolic Blood Pressure; LOCF, Last Observation Carried Forward; BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate.

Table 5. Linear regression analysis on the association between macrolide therapy and PTA_{2-8} at follow-up

	Number	Model 1 Difference [95% CI], p-value	Model 2 Difference [95% CI], p-value
Use between both hearing assessments		n=625	n=605
No macrolide use	557	Ref.	Ref.
Macrolide use	68	-0.72 [-2.44; 0.99], p=0.407	-0.76 [-2.08; 0.56], p=0.260

Model 1 was adjusted for PTA_{2-8} at baseline, age and sex. Model 2 was additionally adjusted for SBP, alcohol (LOCF), smoking, education level, BMI, diabetes, eGFR, and other ototoxic drugs. Abbreviations are as follows: SBP, Systolic Blood Pressure; LOCF, Last Observation Carried Forward; BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate

DISCUSSION

This is the first large population-based study investigating the association between macrolide use and tinnitus. We observed that macrolide use was associated with both prevalent and incident tinnitus. We did not observe a general association between macrolide use and hearing loss. Only a trend of a higher hearing threshold in very recent macrolide users (≤3 weeks) was found.

Ever use of macrolides was associated with a 25% higher likelihood for prevalent tinnitus. The association with tinnitus was already present after short-term use (1-14 DDDs), but reached statistical significance from cumulative doses of 14 DDDs onwards. This finding suggests a dose-response relation. Although cases of tinnitus in users of short-acting erythromycin have been described[18,19], we observed the strongest effect in intermediate- and long-acting macrolides.

Tinnitus can be triggered anywhere along the auditory pathway; from the ear to the central auditory pathways[31]. Patients may have tinnitus due to SNHL. Several mechanisms of macrolide-induced ototoxicity have been described. An experimental study showed that azithromycin and clarithromycin (but not erythromycin) have a reversible ototoxic effect on the inner ear in guinea pigs, namely a reversible reduction of the transient evoked otoacoustic emissions[32]. Two cases showed absence of evoked auditory brainstem potential in waves I to III during treatment with erythromycin and normalization after treatment[33]. A histologic case report found oedema of the stria vascularis, but this could be confounded by the administration of furosemide[34]. However, since we observed a consistent association with tinnitus but only a trend to a higher hearing thresholds in very recent users, this might suggest that patients can recover from macrolide-associated temporary hearing loss, but develop tinnitus. It can be hypothesized that the transient hearing loss due to macrolide usage might

induce tinnitus, but that other central pathways are necessary for the tinnitus to persist[35].

Another possible explanation is macrolide-associated 'central' tinnitus, which is tinnitus generated in auditory brain centres by deviant neural activity[31]. The impairment of central nervous system function through erythromycin is considered because some patients reported central complications[30]. However, clarithromycin has been more closely linked to psychiatric side effects than other macrolides and this can possibly be attributed to GABA-A antagonism[36]. The finding that adjusting for $PTA_{0.25\text{-}8}$ strengthens the association between macrolide use and tinnitus, contributes to this 'central tinnitus hypothesis'[35]. However, more research is needed to further investigate these hypotheses.

We could not find an association between the use of macrolides and hearing loss, which is consistent with a recent meta-analysis[16]. The association found in previous larger-scale studies was likely due to confounding by indication[15]. The authors attributed this to the underlying infectious or inflammatory process. Very recent use (≤3 weeks) tended to increase the hearing threshold, though the group size was small. Still, it seems to be associated with a higher risk on hearing loss ($PTA_{0.25\text{-}8} \geq 35$ dB) which was absent when macrolide use occurred longer than 3 weeks before hearing measurement. This finding is in accordance with prior research. According to a systematic review, SNHL was reversible with macrolide cessation alone in 70/78 cases and in the reversible cases, improvement occurred within hours to days[9]. According to another review, ototoxic SNHL resolves indeed within 1–3 weeks after cessation of treatment in most cases[30].

The major strength of our study is the population-based and prospective design. Most studies are patient-based and to the best of our knowledge, this is the first population-based study to show the effect of macrolide use on tinnitus. Another strength of our study is that pure-tone thresholds were measured as an objective measurement of hearing loss instead of a definition based on ICD-9 codes in other larger-scale studies and thus minimizing information bias. In this way, we can objectively measure all hearing losses, including the minimal ones when patients do not seek medical help. Furthermore, a strength is that in addition to the cross-sectional analysis (n=4,286), we also performed a longitudinal analysis in a subset (n=636). However, our study had a few potential limitations including the unavailability of hospital pharmacy data. Missing data on the use of intravenous macrolides and oral macrolides during hospitalization may lead to underestimation of exposure. However, the research question was not the risk of high-dose intravenous administrations, but rather the risk of long-term use. Therefore, it was not possible to adjust for other ototoxic or tinnitus-generating drugs dispensed by the hospital pharmacy such as intravenous aminoglycoside exposure. Another limitation is the lack of information on noise

exposure. However, we used education level as a proxy for noise exposure since occupations associated with noise exposure are strongly associated with lower education level[37]. To minimize indication bias, we excluded patients with conductive hearing loss, defined as an air-bone gap of 15 dB or more in the best hearing ear. In addition, otitis media is rare in adults and does not often cause hearing loss[38]. Antibiotics are not indicated in otitis media, but if oral treatment is initiated, amoxicillin is preferred. Macrolides are only preferred in case of penicillin allergy[39], therefore indication bias is unlikely in this study. It should be noted that there is no gold standard tinnitus definition, causing a widespread inconsistency across studies[40]. However, the tinnitus assessment and definition in our study was similar in comparison with other studies[40]. Our results are further strengthened by the sensitivity analyses showing a stronger association when we defined tinnitus as daily ringing in (one of) the ears instead of more than one day per week.

In conclusion, we found that macrolide use is consistently associated with tinnitus. This is the first large population-based study to show this association. More in depth studies are needed to confirm this association and investigate the pathophysiological mechanism. Furthermore, clinicians should be aware of this additional adverse effect especially when macrolide antibiotics are prescribed long term.

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SUPPLEMENT

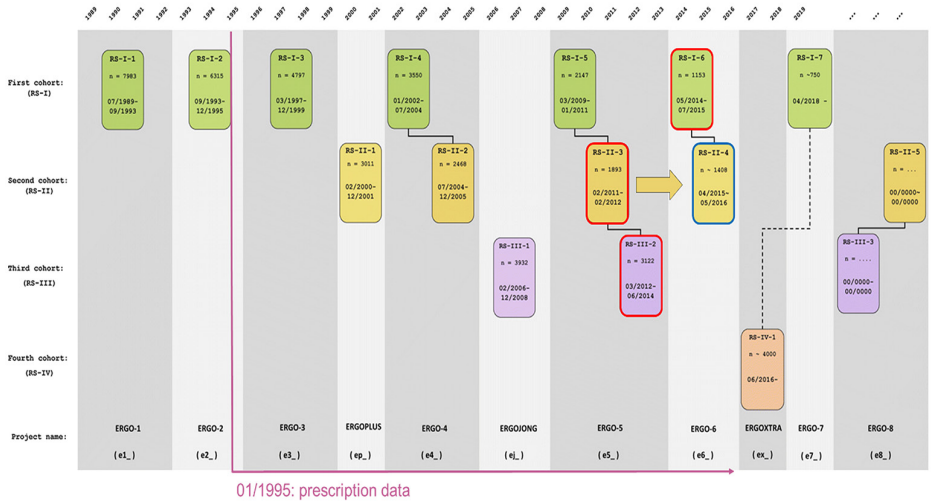

Figure S1. Adapted diagram of examination cycles of the Rotterdam Study (RS) [20]. Red circles show baseline examinations considered for the cross-sectional analysis. The blue circle shows the re-examination of cohort II used for the longitudinal analysis.

Table S1. Codes used to identify oral macrolides

3rd level, pharmacological subgroup (ATC code)	4th level, chemical subgroup (ATC code)	5the level, chemical substance (ATC code)
Macrolides, lincosamides and streptogramins (J01F)	Macrolides (J01FA)	Erythromycin (J01FA01) Spiramycin (J01FA02) Roxithromycin (J01FA06) Clarithromycin (J01FA09) Azithromycin (J01FA10)
Combinations of antibacterials (J01R)	Combinations of antibacterials (J01RA)	Azithromycin, fluconazole, secnidazole (J01RA07)
Drugs for peptic ulcer and gastro-oesophageal reflux disease (A02B)	Combinations for eradication of Helicobacter pylori (A02BD)	Pantoprazole, amoxicillin and clarithromycin (A02BD04) Omeprazole, amoxicillin and clarithromycin (A02BD05) Esomeprazole, amoxicillin and clarithromycin (A02BD06) Lansoprazole, amoxicillin and clarithromycin (A02BD07) Lansoprazole, clarithromycin and tinidazole (A02BD09) Pantoprazole, amoxicillin, clarithromycin and metronidazole (A02BD11) Rabeprazole, amoxicillin and clarithromycin (A02BD12)

Abbreviations are as follows: ATC, Anatomical Therapeutic Chemical

Table S2. Use of macrolides

	Cross-sectional (n=4,286)		Longitudinal (n=636)	
	Use, n (%)	Cumulative dose among the users in DDDs, median (Q1-Q3)	Use, n (%)	Cumulative dose among the users in DDDs, median (Q1-Q3)
All macrolides	1,871 (43.7)	12 (7-21)	71 (11.2)	7 (5-14)
Erythromycin	344 (8.0)	8 (6-14)	5 (0.8)	7 (5-7)
Spiramycin	4 (0.1)	6 (5-9)	0 (0.0)	N.A.
Roxithromycin	67 (1.6)	7 (7-10)	0 (0.0)	N.A.
Clarithromycin	1,078 (25.2)	14 (7-21)	22 (3.5)	12 (7-14)
Azithromycin	1,033 (24.1)	5 (5-10)	46 (7.2)	5 (5-10)
Clarithromycin, pantoprazole & amoxicillin	97 (2.3)	7 (7-7)	3 (0.5)	7 (7-7)

Abbreviations are as follows: DDDs, Defined Daily Doses

Table S3. Linear regression analysis on the association between macrolide therapy and $PTA_{0.25-8}$

	Number	Model 1 β [95% CI], p-value	Model 2 β [95% CI], p-value
Users		n=4,286	n=4,072
Never users	2,415	Ref.	Ref.
Ever users	1,871	-0.22 [-0.86; 0.43], p=0.512	-0.42 [-1.08; 0.24], p=0.213
Recent use		n=4,285	n=4,071
Never users	2,415	Ref.	Ref.
Very recent use	9	**7.41 [0.46; 14.36], p=0.037**	4.59 [-3.26; 12.44], p=0.252
Recent use	38	0.34 [-3.07; 3.74], p=0.846	0.03 [-3.36; 3.42], p=0.985
Past use	1,823	-0.26 [-0.91; 0.39], p=0.438	-0.44 [-1.10; 0.23], p=0.196
Macrolide type		n=3,696	n=3,513
Never users	2,415	Ref.	Ref.
Short-acting	110	-1.50 [-3.53; 0.53], p=0.149	-1.65 [-3.68; 0.38], p=0.112
Intermediate-acting	628	0.05 [-0.88; 0.98], p=0.917	0.01 [-0.94; 0.96], p=0.978
Long-acting	543	-0.53 [-1.52; 0.47], p=0.299	-0.73 [-1.74; 0.28], p=0.158

Estimates represent the decibel (dB) change in hearing threshold for a both ear pure tone average (PTA) 0.25, 0.5, 1, 2, 4, 8 kHz ($PTA_{0.25-8}$) for macrolide usage.
Model 1 was adjusted for age and sex. Model 2 was additionally adjusted for SBP, alcohol (LOCF), smoking, education level, BMI, diabetes, eGFR, and other ototoxic drugs. Significant estimates ($p<0.05$) are indicated in **bold**.
Macrolides were categorized as short- (J01FA01, J01FA02), intermediate- (J01FA06, J01FA09, A02BD04), and long-acting (J01FA10) according to their mean plasma elimination half-life.[7]
Abbreviations are as follows: SBP, Systolic Blood Pressure; LOCF, Last Observation Carried Forward; BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate

Table S4. Logistic regression analysis on the association between macrolide therapy and hearing loss (PTA0.25-8≥35 dB)

	$PTA_{0.25\text{-}8}$≥35 dB cases/total (%)	Model 1 aOR [95% CI], p-value	Model 2 aOR [95% CI], p-value
Users		n=4,286	n=4,072
Never users	755/2,415 (31)	Ref.	Ref.
Ever users	563/1,871 (30)	0.91 [0.78; 1.08], p=0.279	0.88 [0.74; 1.04], p=0.145
Recent use		n=4,285	n=4,071
Never users	755/2,415 (31)	Ref.	Ref.
Very recent use	4/9 (44)	3.61 [0.82; 16.00], p=0.091	2.62 [0.50; 13.80], p=0.256
Recent use	13/38 (34)	1.30 [0.58; 2.92], p=0.531	1.22 [0.53; 2.82], p=0.637
Past use	546/1,823 (30)	0.90 [0.76; 1.06], p=0.210	0.87 [0.73; 1.03], p=0.115
Macrolide type		n=3,696	n=3,513
Never users	755/2,415 (31)	Ref.	Ref.
Short-acting	33/110 (30)	0.77 [0.47; 1.29], p=0.324	0.75 [0.44; 1.25], p=0.267
Intermediate-acting	212/628 (34)	0.91 [0.72; 1.15], p=0.429	0.89 [0.70; 1.13], p=0.338
Long-acting	137/543 (25)	0.79 [0.60; 1.03], p=0.076	0.76 [0.58; 1.01], p=0.055

Model 1 was adjusted for age and sex. Model 2 was additionally adjusted for SBP, alcohol (LOCF), smoking, education level, BMI, diabetes, eGFR, and use of other ototoxic drugs. Significant estimates ($p<0.05$) are indicated in **bold**.

Macrolides were categorized as short- (J01FA01, J01FA02), intermediate- (J01FA06, J01FA09, A02BD04), and long-acting (J01FA10) according to their mean plasma elimination half-life.[7]

Abbreviations are as follows: SBP, Systolic Blood Pressure; LOCF, Last Observation Carried Forward; BMI, Body Mass Index; eGFR, estimated Glomerular Filtration Rate; PTA, Pure-Tone Average

Chapter 3.3

Ototoxicity of antibiotic eardrops in the population-based Rotterdam Study

Submitted

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ABSTRACT

Background

Controversy remains regarding the ototoxic effect of antibiotic eardrops, despite their frequent usage in the treatment of infections of the external and middle ear. No population-based studies have investigated the possible ototoxic effect of ototopical antibiotic treatments.

Methods

We collected data for 3,676 participants within the Rotterdam study (mean age: 69.3 (SD 9.6) years, 44.2% male) on ototopical antibiotic prescriptions (neomycin/ polymyxin B/ corticosteroid; framycetin/ gramicidin/ dexamethasone; oxytetracyclin/ polymyxin B/ hydrocortisone; bacitracin/ colistin/ hydrocortisone), pure-tone audiometry and self-reported tinnitus. Hearing thresholds were averaged over both ears, for frequencies 2 to 8 kHz (PTA_{2-8}) and for all measured audiometric frequencies ($PTA_{0.25-8}$). We excluded participants with an air-bone gap >5dB. We regressed a history of one or more ototopical antibiotic prescriptions on hearing threshold averages and self-reported tinnitus.

Results

A history of one or more ototopical prescriptions was associated with increased hearing thresholds (PTA_{2-8} 2.77dB (95%CI 1.56-3.99), $PTA_{0.25-8}$ 2.39dB (95%CI 1.49 – 3.29)). This effect was found throughout all different ototopical antibiotics, and reached statistical significance in neomycin/ polymyxin B/ corticosteroid; oxytetracyclin/ polymyxin B/ hydrocortisone; bacitracin/ colistin/ hydrocortisone prescriptions. No significant association was found for tinnitus.

Conclusion

In this population-based study we found that ototopical antibiotic prescriptions are associated with poorer sensorineural hearing. Although the effect size was limited, it is important for prescribers to be aware of this possible ototoxic effect.

INTRODUCTION

Ototoxicity is the adverse effect of a drug to cause damage to the cochlea, resulting in sensorineural hearing loss and tinnitus[1]. Several drug groups are known for their ototoxic action, including platinum-based chemotherapeutics, quinines, salicylates, loop diuretics, and macrolide and aminoglycoside based antibiotics[2]. Due to their efficacy against gram-negative bacteria, aminoglycosides are a common component of ototopical antibiotic preparations. Antibiotic eardrops usually contain a combination of corticosteroids, aminoglycosides and polymyxin B. They are frequently prescribed, both by general practitioners and otorhinolaryngologists, to treat infectious diseases of the ear, including otitis externa, acute otitis media, post-tympanostomy tube otorrhea, and chronic (suppurative) otitis media or cholesteatoma[3].

In case of an intact tympanic membrane, the application of aminoglycoside eardrops is considered safe. There are concerns of eardrop related ototoxicity especially in the presence of a non-intact tympanic membrane, due to either a perforation or a tympanostomy tube. In animal studies, it is indeed well established that aminoglycoside eardrops administered directly to the middle ear cause ototoxicity[4]. Substances can enter the inner ear via the permeability of the round window, but systemic absorption of locally applied eardrops has also been reported[5]. Literature shows conflicting evidence on ototoxicity of ear drops. Some studies and guidelines argue against a large effect, claiming that the inflamed middle ear mucosa prevents antibiotics from entering the cochlea via the round window. They do however warn to stop using the eardrops after cessation of the ottorhea. In contrast, several other studies report ototoxicity, although their sample size is usually limited to several cases[6-8]. Apparently, this does not withhold otorhinolaryngologists from prescribing these eardrops, even in the presence of a non-intact tympanic membrane[9]. This may be due to a lack of alternatives or the clinical experience that ototoxic complications are rare.

We investigated the association between the prescription of ototopical antibiotic drops and hearing loss and tinnitus in a large population. This study was conducted in a large sample of older adults, which enabled us to investigate cumulative ototoxic effects, adjusting for age and other known contributors to hearing loss.

METHODS

Setting

This study was embedded in the Rotterdam Study; a prospective, population-based cohort study. The Rotterdam Study was initiated in 1989 and investigates determinants and consequences of aging. Details of the study have been described elsewhere[10]. The entire study population consists of individuals aged ≥ 45 years living in the well-defined Ommoord district in the city of Rotterdam, the Netherlands[10]. Participants were invited to undergo extensive examinations in the dedicated research center at study entry and subsequently every 3 to 4 years. All participants were registered in one of the seven community pharmacies participating in the Rotterdam Study. Information on all drug dispensing data was available from study entry including drug names, international non-proprietary names, Anatomical Therapeutic Chemical (ATC) codes, dosage forms, dates of delivery, number of prescriptions, prescribed daily dosages, and duration of the prescription.

Hearing assessment was introduced into the core study protocol in 2011. For the current study, we included all participants with at least one hearing assessment between 2011 and 2016, resulting in a total number of 4,712 participants. Participants were excluded from analyses if they had received a prescription ≤90 days (maximum prescription length in the Netherlands) before the hearing test (N=40), in order to minimize the risk of confounding by indication. Additionally, participants were excluded when one or more covariables were missing (N= 649), or with suspected conductive hearing loss in either ear, defined as an air-bone gap >5 dB (N= 373). This resulted in a complete-case population of 3,676 participants.

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). The Rotterdam Study has been entered into the Netherlands National Trial Register (www.trialregister.nl) and into the World Health Organization International Clinical Trials Registry Platform (who.int/ictrp/network/primary/en/) under shared catalog number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Hearing assessment

Audiometric assessment was performed as described previously[11]. In short, for both ears air conduction thresholds were measured at frequencies 0.25, 0.5, 1, 2, 4, and 8 kilohertz (kHz), and bone conduction thresholds were measured at 0.5 and 4 kHz. Ototoxicity involves sensorineural hearing loss, so only participants without conductive

hearing loss (air-bone gap averaged over 0.5 and 4 kHz below or equal to 5dB in both ears) were included.

The outcome was defined based on two different pure tone average (PTA) hearing thresholds: a high frequency average over 2, 4 and 8 kHz ($PTA_{2\text{-}8}$), and an average over all measured frequencies ($PTA_{0.25\text{-}8}$). The PTA was first calculated per ear and subsequently averaged over both ears. We also investigated asymmetry between the ears in an individual, by calculating these values separately per ear and taking the (absolute) difference between them.

Tinnitus assessment

Tinnitus assessment was performed through a home interview. Participants were asked if they experienced sounds in the head or in (one of) the ears (such as whizzing, peeping, or humming) without an objective external sound source being present. Possible answers to this question were: 'no, never'; 'yes, less than once a week'; 'yes, more than once a week but not daily' and 'yes, daily'. Tinnitus was investigated as a binary variable; either absent ('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 the often temporary character of tinnitus, presence of less than once a week was not regarded as prevalent tinnitus.

Antibiotic exposure

Auricular antibiotic prescriptions in this study population were retrieved from the pharmacy records. Based on ATC codes (S02CA03, S02CA05, S02CA06, S02CA07), we identified the following auricular antibiotic prescriptions, between brackets the abbreviation that will be used in the text: neomycin/ polymyxin B/(any) corticosteroid (neomycin); framycetin/ gramicidin/ dexamethasone (framycetin); oxytetracyclin/ polymyxin B/ hydrocortisone (oxytetracyclin); bacitracin/ colistin/ hydrocortisone (bacitracin). Several participants had received multiple types of drugs. We studied the number of drug prescription as a dichotomous outcome (0 versus ≥1 prescription) and as a dose effect (1, 2 or ≥3 prescriptions).

Covariables

Highest achieved educational level was noted, using the UNESCO classification[12]. Smoking data was collected through self-report and categorized into 'never' or 'ever'. Alcohol consumption, in grams per day, was assessed through self-report by means of the Food-Frequency Questionnaire[13]. Prevalent diabetes was defined on the basis of WHO criteria for fasting glucose, ≥7.0 mmol/L or use of glucose lowering drugs. Systolic blood pressure was measured twice using a random sphygmomanometer. Exposure to other orally administered ototoxic drugs (minocyclin, erythromycin,

spiramycin, roxithromycin, azithromycin, clarithromycin, hydroquinone, furosemide, bumetanide and acetylsalicylic acid[14]) was collected from pharmacy records, categorized as 'never' or '≥1 prescriptions'.

Statistical analyses

Descriptive analyses were used to assess and compare the differences in demographic characteristics of participants. Continuous data were described as mean (standard deviation (SD)) when normally distributed and as median (inter quartile range (IQR)) when not normally distributed. Categorical variables are described as number (N (%)). An independent samples t-test, Mann-Whitney-U-test and X^2-test were used for description of the investigated population, respectively.

Several regression models were defined. The models were constructed with forward step wise selection of candidate covariables with a p-value <0.10. The tested covariables were: sex, age, age^2, highest level of education achieved, smoking status, BMI, systolic blood pressure, diabetes mellitus, alcohol usage, other ototoxic drugs, and the use of any of the other investigated antibiotics. Covariables were selected for the final model based on statistical significance and contribution to the model based on the Akaike Information Criteria, resulting in adjustment for age, age^2, sex, highest achieved education level, use of any of the other investigated antibiotics and smoking status.

We regressed a history of 1 or more prescriptions of any auricular antibiotic prescription (neomycin, framycetin, oxytetracyclin and bacitracin) linearly on both $PTA_{2\text{-}8}$, $PTA_{0.25\text{-}8}$ and logistically on tinnitus. Subsequently, we investigated the association of the number of prescriptions (1, 2, ≥3) on $PTA_{2\text{-}8}$, $PTA_{0.25\text{-}8}$ and tinnitus. IBM SPSS statistics version 24.0 (IBM Corp, Armonk, NY, USA) was used for data handling and for analyses. A p-value < 0.05 was considered statistically significant.

RESULTS

The total investigated population consisted of 3,676 participants, mean age was 69.3 years [SD 9.6] and 44.2% were males (Table 1). Of this population, 560 (15.2%) individuals had received one or more auricular antibiotic prescriptions in the past (specified in Table 2). These participants were slightly older (71.0 years [SD 9.8] vs. 69.0 years [SD 9.5], p<0.001) and had more often received other ototoxic drugs (55.9% vs. 42.6%, p<0.001) compared to participants who had not received any auricular antibiotics. They also had on average higher hearing thresholds at every measured frequency (Figure 1), which is reflected in the pure-tone averages we studied: $PTA_{2\text{-}8}$ 44.7 dB [SD 19.3] vs. 38.9 dB [SD 18.9], p<0.001) and $PTA_{0.25\text{-}8}$ 32.5 dB [SD 14.6] vs.

28.0 dB [SD 13.2], p<0.001. The air-bone gap was not significantly different between both groups (Table 1). Tinnitus was more often reported by those who had received an antibiotic ototopical prescription as compared to no antibiotic prescription, 24.8% vs. 20.5% respectively (p=0.020).

Table 1. Description of the investigated sample, comparing participants with and without a history of ototopical antibiotic prescriptions.

	Investigated population (N =3,676)	Any antibiotic ≥1 prescription (N=560)	No antibiotic prescription (N=3,116)	p-value
Age, years	69.3 (9.6)	71.0 (9.8)	69.0 (9.5)	<0.001
Age, range	51.5-100.7	51.9-96.7	51.5-100.7	
Male	1,625 (44.2)	256 (45.7)	1,369 (43.9)	0.435
Education level				0.097
Lower	249 (6.8)	45 (8.0)	204 (6.5)	
Middle	1,431 (38.9)	233 (41.6)	1,198 (38.4)	
Higher	1,996 (54.3)	282 (50.4)	1,714 (55.0)	
Other ototoxic drug prescriptions*	1,639 (44.9)	313 (55.9)	1,326 (42.6)	<0.001
$PTA_{2\text{-}8}$, dB	39.8 (19.1)	44.7 (19.3)	38.9 (18.9)	<0.001
$PTA_{0.25\text{-}8}$, dB	28.7 (13.5)	32.5 (14.6)	28.0 (13.2)	<0.001
Tinnitus	777 (21.1)	139 (24.8)	638 (20.5)	0.020
Left-right asymmetry $PTA_{2\text{-}8}$, dB‡	5.0 (3.3, 10.0)	5.0 (3.3, 11.7)	5.0 (3.3, 10.0)	0.285
Left-right asymmetry $PTA_{0.25\text{-}8}$, dB‡	3.3 (1.7, 6.7)	4.2 (1.7, 7.5)	3.3 (1.7, 6.7)	0.012
Air-bone gap left ear, dB	0.9 (1.4)	0.9 (1.4)	0.9 (1.4)	0.939
Air-bone gap right ear, dB	0.9 (1.4)	0.9 (1.4)	1.0 (1.4)	0.148
Smoking, ever	2,509 (68.3)	414 (73.9)	2,095 (67.2)	0.002
BMI, kg/m^2	27.4 (4.2)	28.0 (4.3)	27.3 (4.2)	<0.001
Systolic blood pressure, mmHg	138.4 (19.8)	138.5 (19.8)	138.4 (19.8)	0.907
Diabetes mellitus	431 (11.7)	75 (13.4)	356 (11.4)	0.183
Alcohol, g/day‡	6.4 (1.6, 8.6)	6.4 (0.5, 8.6)	6.4 (1.6, 8.6)	0.178

Values are mean (standard deviation (SD)) for normally distributed continuous variables. ‡Median (interquartile range (IQR)) for non-normally distributed continuous variables. Dichotomous variables are given as N, (%). Corticosteroid is either dexamethasone, fluocinolone or hydrocortisone. $PTA_{2\text{-}8}$: both ear pure tone average over 2, 4 and 8 kilohertz (kHz). $PTA_{0.25\text{-}8}$: both ear pure tone average over 0.25, 0.5, 1, 2, 4, 8 kHz. dB: decibel. *shows N, (%) for 1 or more prescriptions.

Table 2. Distribution of the different auricular antibiotic prescriptions

	Total number of prescriptions			
	0	1	2	≥3
All ototopical antibiotic	3,116 (84.8)	300 (8.2)	112 (3.0)	148 (4.0)
Neomycin/Polymyxin B/Corticosteroid	3,235 (88.0)	258 (7.0)	90 (2.4)	93 (2.5)
Framycetin/Gramicidin/Dexamethason	3,565 (97.0)	68 (1.8)	20 (0.5)	23 (0.6)
Oxytetracyclin/Polymyxin B/Hydrocortison	3,587 (97.6)	48 (1.3)	18 (0.5)	23 (0.6)
Bacitracin/Colistin/Hydrocortison	3,593 (97.7)	51 (1.4)	15 (0.4)	17 (0.5)

Number of participants (N, %) with the total number of prescriptions per drug, ABG ≤5dB.

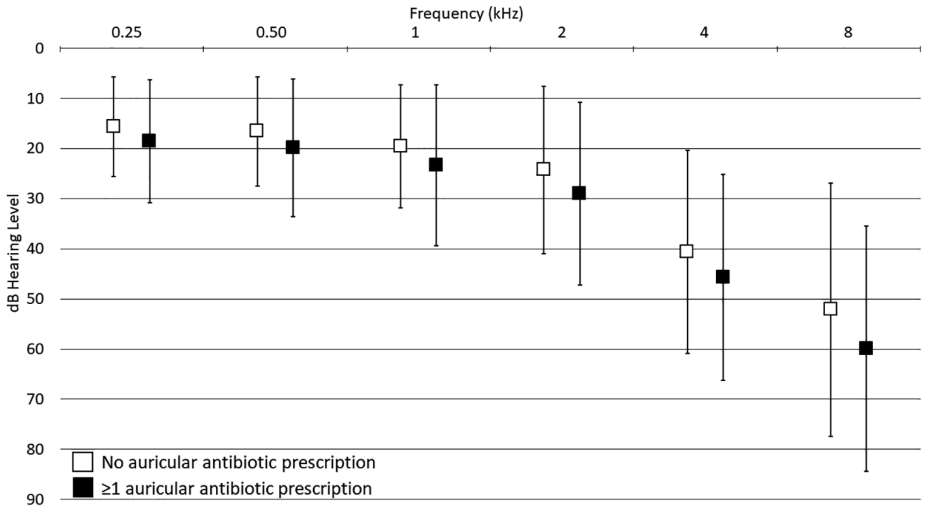

Figure 1. Average audiogram

Hearing threshold per frequency, averaged over both ears, comparing participants with and without a history of antibiotic ototopical prescriptions. For each frequency, the thresholds were significantly different ($p<0.001$).

Regression analyses showed that participants who received ≥1 prescription of any auricular antibiotic had higher hearing thresholds ($PTA_{2\text{-}8}$ 2.77 dB [95%CI 1.56, 3.99], $PTA_{0.25\text{-}8}$ 2.39 dB [95%CI 1.49, 3.29]), as listed in Table 3. A positive association with increased hearing thresholds was found in all antibiotic prescriptions. One or more prescriptions of three specific antibiotic eardrops, neomycin, oxytetracyclin and bacitracin, were significantly associated with higher values of both $PTA_{2\text{-}8}$ (neomycin: 1.97 dB [95%CI 0.54, 3.39]; oxytetracyclin: 4.24 dB [95%CI 1.30, 7.18]; bacitracin: 2.59

Table 3. Effect estimates of one or more ototopical antibiotic prescriptions compared to no ototopical prescriptions.

		≥1 prescription	1 prescription	2 prescriptions	≥3 prescriptions
		Effect estimate (95% CI)	**Effect estimate (95% CI)**	**Effect estimate (95% CI)**	**Effect estimate (95% CI)**
Any antibiotic	PTA_{2-8}	**2.77 (1.56, 3.99)**	**1.96 (0.53, 3.39)**	1.53 (-0.02, 3.07)	**3.89 (1.98, 5.80)**
	$PTA_{0.25-8}$	**2.39 (1.49, 3.29)**	**1.38 (0.37, 2.39)**	1.02 (-0.07, 2.12)	**3.36 (1.99, 4.73)**
	Tinnitus	1.10 (0.88, 1.37)	0.96 (0.71, 1.29)	1.23 (0.79, 1.92)	1.30 (0.89, 1.90)
Neomycin/ Polymyxin B/ Corticosteroid	PTA_{2-8}	**1.97 (0.54, 3.39)**	1.29 (-0.46, 3.03)	2.15 (-0.75, 5.05)	**3.71 (0.62, 6.80)**
	$PTA_{0.25-8}$	**1.67 (0.64, 2.71)**	1.25 (-0.00, 2.50)	1.87 (-0.21, 3.94)	**3.33 (1.11, 5.56)**
	Tinnitus	1.11 (0.86, 1.43)	1.05 (0.77, 1.44)	0.99 (0.59, 1.67)	1.49 (0.90, 2.45)
Framycetin/ Gramicidin/ Dexamethasone	PTA_{2-8}	2.19 (-0.47, 4.84)	3.08 (-0.23, 6.40)	1.90 (-4.15, 7.96)	-0.19 (-5.84, 5.47)
	$PTA_{0.25-8}$	1.55 (-0.37, 3.47)	2.14 (-0.25, 4.54)	1.77 (-2.60, 6.13)	-0.17 (-4.26, 3.92)
	Tinnitus	1.02 (0.64, 1.62)	1.00 (0.56, 1.80)	0.98 (0.34, 2.82)	1.10 (0.40, 2.98)
Oxytetracyclin/Polymyxin B/Hydrocortisone	PTA_{2-8}	**4.24 (1.30, 7.18)**	**6.19 (2.28, 10.10)**	1.42 (-4.93, 7.76)	2.45 (-3.22, 8.12)
	$PTA_{0.25-8}$	**4.43 (2.30, 6.56)**	**6.13 (3.30, 8.96)**	3.44 (-1.13, 8.02)	2.07 (-2.02, 6.16)
	Tinnitus	0.93 (0.55, 1.56)	1.08 (0.55, 2.11)	2.47 (0.92, 6.62)	**0.11 (0.01, 0.81)**
Bacitracin/ Colistin/ Hydrocortisone	PTA_{2-8}	**2.59 (1.30, 3.89)**	1.23 (-2.60, 5.05)	**7.88 (0.91, 14.85**)	**8.91 (2.34, 15.48)**
	$PTA_{0.25-8}$	**3.23 (1.00, 5.46)**	0.80 (-1.96, 3.56)	4.61 (-0.41, 9.64)	**10.05 (5.30, 14.81)**
	Tinnitus	1.09 (0.65, 1.85)	1.19 (0.62, 2.29)	0.60 (0.16, 2.26)	1.33 (0.46, 3.88)

Effect estimates for ≥1 prescription of auricular antibiotics as compared to none represent the decibel (dB) difference in hearing threshold for a pure tone average (PTA) over 2, 4 and 8 kilohertz (kHz) (PTA_{2-8}) and 0.25, 0.5, 1, 2, 4, 8 kHz ($PTA_{0.25-8}$), averaged over both ears, or the likelihood (odds ratio) for tinnitus. Additionally, a trend analysis for cumulative exposure was done. All models are adjusted for usage of the other investigated oral antibiotics and for age, age^2, highest level of education achieved and smoking status. Significant estimates ($p<0.05$) are indicated in **bold**.

dB [95%CI 1.30, 3.89]) and $PTA_{0.25-8}$ (neomycin: 1.67 dB [95%CI 0.64, 2.71]; oxytetracyclin: 4.43 dB [95%CI 2.30, 6.56]; bacitracin: 3.23 dB [95%CI 1.00, 5.46]), Table 3. For framycetin we only found a trend towards higher hearing thresholds (PTA_{2-8}: 2.19 dB [95%CI -0.47, 4.84], $PTA_{0.25-8}$: 1.55 dB [95%CI -0.37, 3.47]). For all antibiotics combined, and specifically neomycin and bacitracin, we found an increase in average hearing threshold for both PTA_{2-8} and $PTA_{0.25-8}$ per additional prescription (Table 3). No association with tinnitus was found for all antibiotics as a group nor for any of the individual antibiotics separately, Table 3.

To exclude any possible effect of confounding by indication, we repeated the analysis in all participants with an even stricter criterion by excluding any conductive hearing loss (ABG = 0 dB or negative in both ears). This greatly reduced the sample size (Supplementary table 1), but there was still an effect noticeable on hearing threshold averages in participants with a history of ototopical antibiotic prescription (Supplementary table 2).

DISCUSSION

To our knowledge, this is the first population-based study investigating the ototoxicity of antibiotic eardrops in older adults. We found that a history of one or more ototopical antibiotic prescriptions was associated with increased hearing thresholds but not significantly with tinnitus. This effect was specifically found in prescriptions of Neomycin/Polymyxin B/corticosteroid (Neomycin), Oxytetracyclin/Polymyxin B/Hydrocortisone (Oxytetracyclin) and Bacitracin/Colistin/Hydrocortisone (Bacitracin), and less clearly in prescriptions of Framycetin/Gramicidin/Dexamethasone (Framycetin).

The ototoxic effect of aminoglycosides is well known, they may cause hearing loss due to apoptosis of outer hair cells from base to apex[1]. This effect was found for all aminoglycoside containing eardrops, but not for Framycetin (also called Neomycin B[15]), which may be due to a lack of statistical power. Interestingly, we found the strongest positive association between hearing loss and the polymyxin containing drugs, Neomycin (Polymyxin B), Oxytetracyclin (Polymyxin B) and Bacitracin (colistin is another name for Polymyxin E). It is well documented that systemic administration of polymyxins is dose-dependently nephrotoxic and possibly reversibly neurotoxic, but no records of ototoxicity are mentioned[16].

The effect size of the ototopical prescriptions on hearing thresholds was relatively small from a clinical perspective (2 to 4 dB). However, it can still be considered as a substantial contribution to the multifactorial origin of hearing loss, so the effect should not be neglected, especially in populations at risk for hearing loss. The effect size might even increase when the ototopical medication is used in limited ear disease or

when it is administered too long after cessation of the disease it was initially prescribed for. Probably due to small sample size, we could not demonstrate a dose-effect relationship, but we certainly cannot exclude it either.

An important discussion point is whether the increased hearing thresholds are a result of ototoxicity of the ototopical antibiotics or a result of the initial disease they were prescribed for. First, by selecting only participants with an air-bone gap of 5 dB or less, we already excluded many possible otological diagnoses with a conductive or mixed hearing loss. The increased hearing thresholds in the group with a history of antibiotic eardrops thus have a sensorineural origin. This statement is supported by the sensitivity analysis of participants with no air-bone gap, which yielded results in the same direction. Next, the general risk of sensorineural hearing loss due to an ear infection is low. Acute otitis media, an infection of the middle ear, is reported to induce sensorineural hearing loss in approximately 9.3% of adults[17]. According to a recent study of the Dutch General Practitioner's data, the incidence of acute otitis media in adults aged 64 years and older is low, i.e. 2.7/1000 person years[18]. Therefore, it seems unlikely that the increased hearing thresholds we found are due to sensorineural hearing loss triggered by acute otitis media. Although we cannot firmly state that the increased hearing thresholds are a result of ototoxicity of the eardrops, we do think the evidence points in that direction. The mechanisms of action still need to be elucidated, but there are several possibilities. Direct diffusion to the cochlea via the round window in a perforated ear drum is probably the most important one. Systemic absorption[5] or even permeation through an intact tympanic membrane, which has been demonstrated in animal studies[19], are alternative options. The precise pathophysiological pathways should be topic of future studies.

One of the major strengths of this study is the large sample size combined with post treatment audiometry, including bone conduction thresholds. In addition, we were able to adjust for several confounders for hearing loss, although residual confounding cannot be fully excluded. There certainly are some limitations as well, as pretreatment audiometry was not available, nor information on diagnosis, otoscopy or length of treatment. Although the included ATC codes were specific for otological disease, we cannot exclude other applications, for example ophtalmological use. We do however know that the ear drops were retrieved at the pharmacy by the participants.

In conclusion, this is the first population-based study to investigate the association between ototopical drops and ototoxicity. We found that prescriptions of Neomycin/Polymyxin B/corticosteroid, Framycetin/Gramicidin/Dexamethasone, Oxytetracyclin/Polymyxin B/Hydrocortisone and Bacitracin/Colistin/Hydrocortisone are associated with poorer sensorineural hearing. A direct causal relationship is difficult to establish, but we still think physicians should be aware of this possible ototoxic effect especially in patients with pre-existent hearing loss.

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SUPPLEMENTAL

Supplementary table 1. Distribution of the different auricular antibiotic prescriptions in participants with no conductive hearing loss.

	Total number of prescriptions			
	0	**1**	**2**	**≥3**
Any antibiotic	1,423 (85.2)	133 (8.0)	46 (2.8)	68 (4.1)
Neomycin/Polymyxin B/Corticosteroid	1,471 (88.1)	115 (6.9)	43 (2.6)	41 (2.5)
Framycetin/Gramicidin/Dexamethason	1,630 (97.6)	20 (1.2)	10 (0.6)	10 (0.6)
Oxytetracyclin/Polymyxin B/Hydrocortison	1,626 (97.4)	23 (1.4)	8 (0.5)	33 (0.8)
Bacitracin/Colistin/Hydrocortison	1,632 (97.7)	24 (1.4)	5 (0.3)	9 (0.5)

Number of participants (N, %) with the total number of prescriptions per drug, ABG 0dB.

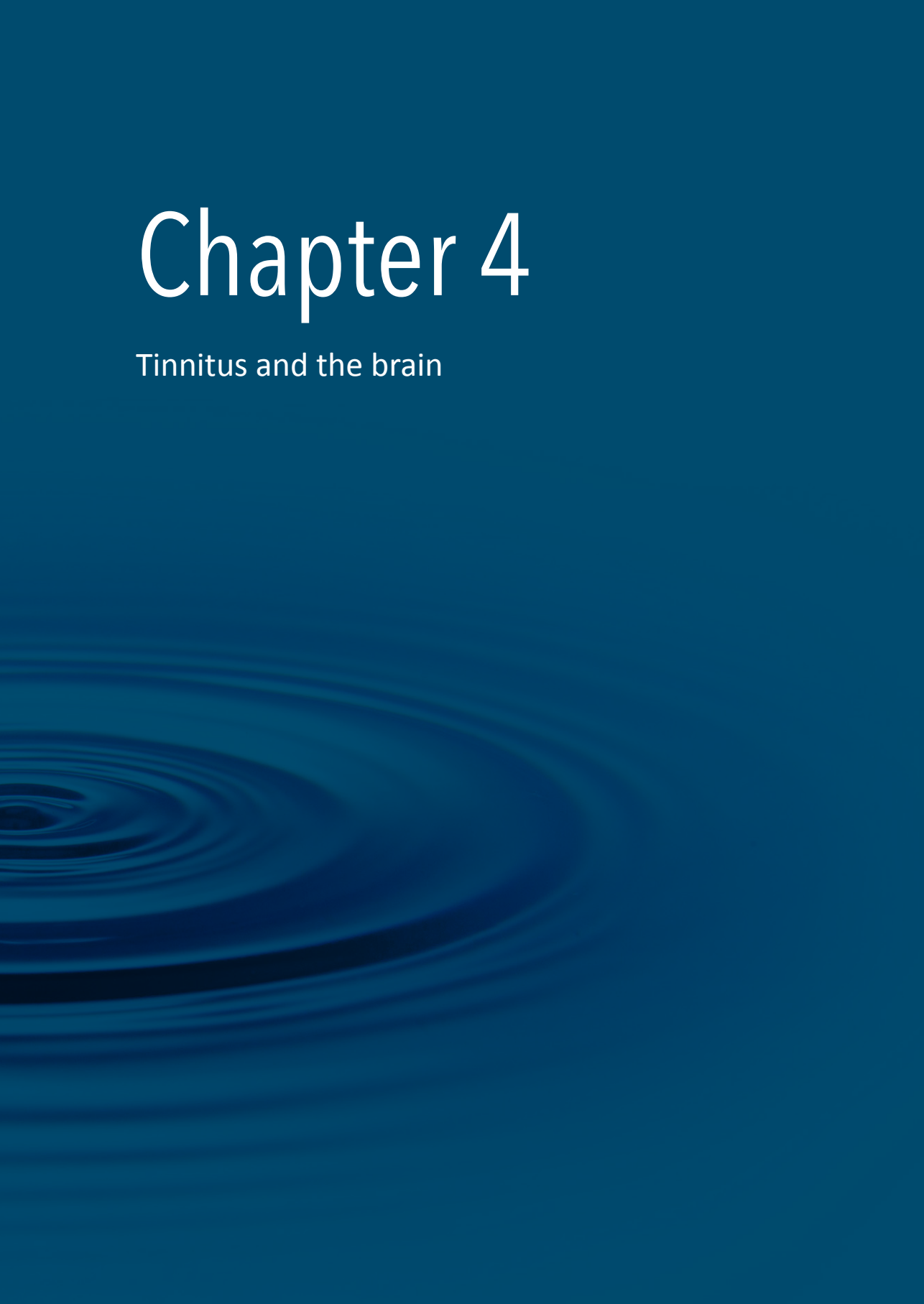
Supplementary Table 2. Sensitivity analysis; Effect estimates of one or more ototopical antibiotic prescriptions compared to no ototopical prescriptions in participants with no conductive hearing loss.

		≥1 prescription	1 prescription	2 prescriptions	≥3 prescriptions
		Effect estimate (95% CI)	**Effect estimate (95% CI)**	**Effect estimate (95% CI)**	**Effect estimate (95% CI)**
Any antibiotic	PTA_{2-8}	**3.88 (1.96, 5.80)**	**2.55 (0.36, 4.74)**	**3.04 (0.68, 5.41)**	**6.41 (3.50, 9.32)**
	$PTA_{0.25-8}$	**3.04 (1.61, 4.47)**	1.49 (-0.09, 3.07)	1.99 (0.24, 3.74)	**5.46 (3.32, 7.60)**
	Tinnitus	0.98 (0.71, 1.36)	0.84 (0.54, 1.31)	1.45 (0.75, 2.80)	0.98 (0.55, 1.73)
Neomycin/ Polymyxin B/ Corticosteroid	PTA_{2-8}	2.02 (-0.19, 4.23)	0.98 (-1.69, 3.65)	2.14 (-2.24, 6.53)	4.48 (-0.51, 9.48)
	$PTA_{0.25-8}$	1.59 (-0.06, 3.23)	0.62 (-1.33, 2.58)	2.39 (-0.85, 5.63)	**3.98 (0.26, 7.69)**
	Tinnitus	0.89 (0.61, 1.30)	0.86 (0.54, 1.38)	0.99 (0.48, 2.06)	0.87 (0.39, 1.93)
Framycetin/ Gramicidin/ Dexamethasone	PTA_{2-8}	2.09 (-2.47, 6.66)	3.98 (-2.32, 10.28)	-4.06 (-12.92, 4.81)	4.80 (-4.03, 13.62)
	$PTA_{0.25-8}$	1.20 (-2.20, 4.59)	2.14 (-2.53, 6.82)	-4.19 (10.76, 2.39)	4.97 (-1.60, 11.54)
	Tinnitus	1.42 (0.68, 2.94)	1.90 (0.73, 4.96)	0.40 (0.05, 3.26)	1.81 (0.46, 7.16)
Oxytetracyclin/Polymyxin B/Hydrocortisone	PTA_{2-8}	**7.64 (3.30, 11.98)**	**10.31 (4.48, 16.14)**	8.70 (-1.10, 18.49)	2.08 (-5.75, 9.90)
	$PTA_{0.25-8}$	**7.33 (4.10, 10.55)**	**9.54 (5.21, 13.87)**	**9.39 (2.15, 16.62)**	2.15 (-3.62, 7.91)
	Tinnitus	0.98 (0.48, 2.02)	1.17 (0.46, 2.98)	2.51 (0.56, 11.18)	0.22 (0.03, 1.74)
Bacitracin/ Colistin/ Hydrocortisone	PTA_{2-8}	3.56 (-1.13, 8.24)	-1.49 (-7.24, 4.25)	**13.44 (0.98, 25.90)**	**12.08 (2.66, 21.48)**
	$PTA_{0.25-8}$	3.25 (-0.24, 6.74)	-1.28 (-5.53, 2.98)	8.55 (-0.69, 17.78)	**12.78 (5.77, 19.78)**
	Tinnitus	0.75 (0.33, 1.69)	1.01 (0.39, 2.65)	0.46 (0.05, 4.38)	0.44 (0.08, 2.43)

Effect estimates for ≥1 prescription of auricular antibiotics as compared to none represent the decibel (dB) difference in hearing threshold for a pure tone average (PTA) over 2, 4 and 8 kilohertz (kHz) (PTA_{2-8}) and 0.25, 0.5, 1, 2, 4, 8 kHz ($PTA_{0.25-8}$), averaged over both ears, or the likelihood (odds ratio) for tinnitus. Additionally, a trend analysis for cumulative exposure was done. All models are adjusted for usage of the other investigated oral antibiotics and for age, age^2, highest level of education achieved and smoking status. Significant estimates ($p<0.05$) are indicated in **bold**.

Chapter 4

Tinnitus and the brain



Chapter 4.1

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

Ear and Hearing, 2021

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ABSTRACT

Objective

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

Design

2,616 participants (mean age 65.7 years [SD: 7.5]; 53.9% female) of the population-based Rotterdam Study underwent tinnitus assessment (2011-2014) and magnetic resonance imaging of the brain (2011-2014). Associations between tinnitus (present vs absent) and total, gray, 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, Mini-Mental State Examination score and hearing loss. Finally, potential regional gray matter density and white matter microstructural volume differences were assessed on a voxel-based level again using multivariable linear regression.

Results

Participants with tinnitus (21.8%) had significantly larger brain tissue volumes (difference in SD: 0.09 [95% CI: 0.06, 0.13]), driven by larger white matter volumes (difference: 0.12 [95% confidence interval: 0.04, 0.21]) independent of hearing loss. There was no association between tinnitus and gray 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 gray matter volume. Voxel-based results did not show regional specificity.

Conclusion

We found that tinnitus in older adults was 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 potentially has a neurodevelopmental origin in earlier life independent of aging processes.

INTRODUCTION

Tinnitus is a poorly understood and common disorder, often debilitating in the daily life of people with tinnitus[1]. The disorder can be characterized by the perception of a sound while there is no objective corresponding external sound source[2,3].

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[1,2,4,5]. However, several observations indicate that tinnitus also has a central component to its pathogenesis, regardless of the peripheral damage that might trigger it[6]. Aging is accompanied by neurodegeneration, i.e. cerebral structural and functional cell loss[7]. Recently, hearing loss, a chronic condition which is highly prevalent within the elderly[8], has been related to smaller brain volumes and compromised white matter microstructural integrity[9-11]. As such, it could be hypothesized that tinnitus, with hearing loss being its most important risk factor, is also related to impaired brain health. 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[3-6,12-17]. More specifically, volume reductions in both cortical areas (such as the prefrontal cortex, temporal lobe and Heschl's gyrus) and the subcortical areas (such as the limbic system) were reported in individuals with tinnitus[3,4,12,14,18]. Moreover, decreased white matter microstructural integrity of the brain (as indicated by lower levels of fractional anisotropy and higher levels of mean diffusivity) have been related to tinnitus[19,20]. Contrary, different studies also found gray matter increases in the temporal lobe in individuals with tinnitus, or no differences in brain volumetry and/or underlying brain microstructure in relation to tinnitus presence at all[13,14,17,18,21,22]. These inconsistencies might be explained by small sample sizes, high heterogeneity of individuals with tinnitus, predominant use of clinical samples possibly introducing selection bias, differences in imaging methodology and data analysis, and a predominant interest in auditory regions in the brain or the limbic system, disregarding potential whole brain associations[2]. Importantly, several studies did not adjust for potential confounding effects of hearing loss, which may be present due to the high prevalence of hearing impairment in elderly populations[8]. As such, it remains unclear if and how tinnitus is related to altered neurodegeneration independent of important confounding factors.

Therefore, the aim of this study was to explore the association between tinnitus and brain tissue volumes, white matter microstructural integrity and regional gray and white matter density on a voxel-based level 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 aging[23]. 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 center at study entry and subsequent visits every 3 to 4 years.

For this study, 4,773 participants who visited the study center 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 magnetic resonance imaging (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.

Tinnitus assessment

Tinnitus was assessed during a home interview. Participants were asked if they experienced or recently had 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[23]. 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 using a 1.5-tesla MRI scanner with a dedicated 8-channel head coil (software version 11x; General Electric Healthcare, Milwaukee, WI)[24]. The entire scan protocol and sequence details have been described elsewhere[24].

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 gray matter, white matter, cerebrospinal fluid (CSF), and white matter hyperintensities[25]. Total brain tissue volume was the sum of gray matter, normal-appearing white matter, and white matter hyperintensity volume. Supratentorial intracranial volume was estimated by summing gray matter and white matter (normal-appearing white matter and white matter hyperintensity volume) and CSF volumes[24]. A multi-atlas approach was used to obtain lobar brain volumes (frontal, parietal, temporal, occipital) from all participants[25].

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 1000 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[26]. From this (in combination with the tissue segmentation), global mean fractional anisotropy (FA) and mean diffusivity (MD) in the normal-appearing white matter was derived. FA is a measure of the directional constraint placed on water molecules in the normal-appearing white matter and is given as a ratio ranging from 0 (isotropic or non-directonal) to 1 (uni-directional). MD is the directionality averaged diffusivity of the water molecules, expressed in square millimeters per second. Lower white matter microstructure is reflected by lower levels of FA and higher levels of MD.

Voxel based morphometry of white matter tracts

We performed a voxel-based analysis of diffusion tensor MRI data using FSL software (FSL-VBM v1.1, including FSL fnirt and FSL flirt software) for preprocessing[27]. All 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 to register diffusion characteristics between individuals, i.e. the evaluation of 23 white matter tracts across subjects[27]. White matter tract segmentation masks of every participant were registered to the Montreal Neurological Institute (MNI) template in the same way as FA and MD maps. These were then merged to one tract probability atlas image[27]. To map voxels from voxel-based analysis, a 10% probability cut-off was used to define tract boundaries microstructure.

Voxel based morphometry of gray matter density

Using an optimized protocol with FSL software, voxel-based analysis of the gray matter was performed[27]. Gray matter density maps derived from T1-weighted images were

nonlinearly registered to the MNI template. A spatial modulation procedure was applied to preserve local gray 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[28].

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 zero 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[23]. 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[23]. 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[23]. The MMSE was administered during home interview to assess global cognitive functioning[23]. A score below 24 was considered as indicative of probable cognitive impairment[29]. To assess depressive symptoms, the Center for Epidemiological Studies Depression scale was used. A score of 16 or greater was considered as indicative of probable depressive symptoms[30]. To determine hearing levels in decibel (dB), pure tone audiometry was used according to the ISO-standard 8253-1[23], measured on different air conduction frequencies (0.25-8 kilohertz). Clinically relevant cut-offs were defined: normal hearing 0 – 20 dB; mild hearing loss 20 – 40 dB; moderate/severe hearing loss > 40 dB.

Statistical analysis

First, we explored whether continuous variables were distributed normally. Subsequently, we investigated whether characteristics differed between participants with and without tinnitus, using T-tests (for continuous variables that were normally distributed), χ^2-tests (for dichotomous variables) and Mann-Whitney U-Tests (for continuous variables that were non-normally distributed). Second, we explored the association of tinnitus (present versus absent) with brain tissue volume (total, white matter, gray 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 (present versus absent) and lobar gray and white matter volume (frontal, temporal, parietal, occipital lobe) for the left and right hemisphere separately, to assess potential region specific associations. Fourth, we performed the same multivariable linear models for the association between tinnitus and every voxel of the brain measures in a VBM analysis (gray matter density and white matter microstructural volume), in order to further disentangle potential region specific associations. Using multivariable linear regression analysis, in the above statistical analyses, enabled us to infer on potential average differences in brain measurements in those with tinnitus, as compared to those without tinnitus.

For the current paper, we have presented our analyses and results with tinnitus as the determinant and brain measures as the outcome. It is important to realize though, that it remains unknown whether tinnitus onset starts before differences in brain structure or vice versa. To infer on causality, longitudinal study designs are warranted. Nevertheless, 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 (present versus absent) 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 address heterogeneity related to tinnitus presence ('daily' versus 'more than once a week, but not daily') we re-ran analyses, excluding those reporting tinnitus more than once a week, but not daily (N = 200) and only considered those reporting daily tinnitus as 'present tinnitus'. Moreover, 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 gray 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 (males: 51.8%; females: 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).

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). No statistically significant associations were found between tinnitus and gray matter volume and white matter microstructural integrity (table 2). In a sensitivity analysis including solely those reporting tinnitus on a daily basis, we found slightly larger effect estimates as compared to the main analysis which included those reporting tinnitus more than once a week, but not daily. More specifically, we found that those with tinnitus had on average larger total brain tissue volume (difference: 0.10 [95% CI: 0.05, 01.5]), again driven by larger white matter volumes (difference: 0.16 [95% CI: 0.05, 0.26]) (supplementary table e-1, model 2) as compared to those without tinnitus.

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 gray matter volume on a lobar level (table 3).

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	
Sex, %				0.002
Female, %	53.9	48.2	55.5	
Male, %	46.1	51.8	44.5	
Hearing loss, dB	27.0 (15.3)	28.8 (SD: 17.3)	25.8 (SD: 14.6)	<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-score < 24, %	1.5	1.8	1.4	0.073
Depressive symptoms ≥ 16, %	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.

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**.

Voxel-based morphometry

We conducted exploratory voxel-based analysis to identify if tinnitus was associated with regional white matter integrity and gray 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 fiber bundles (figure 1). However, these associations did not show regional specificity and were not statistically significant (figure 1, supplementary table e-2). No statistically significant associations were found between tinnitus and voxel based white matter MD and gray matter density (figure 1, supplementary tables e-3 and e-4).

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 appeared to be associated with larger brain tissue volumes, fully driven by larger white matter volumes (supplementary table e-5). Associations did not differ between males and females (supplementary table e-6).

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**.

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**.

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 gray 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 is associated with smaller brain tissue volumes and decreased white matter microstructural integrity[9,11], we had expected similar results: an association between

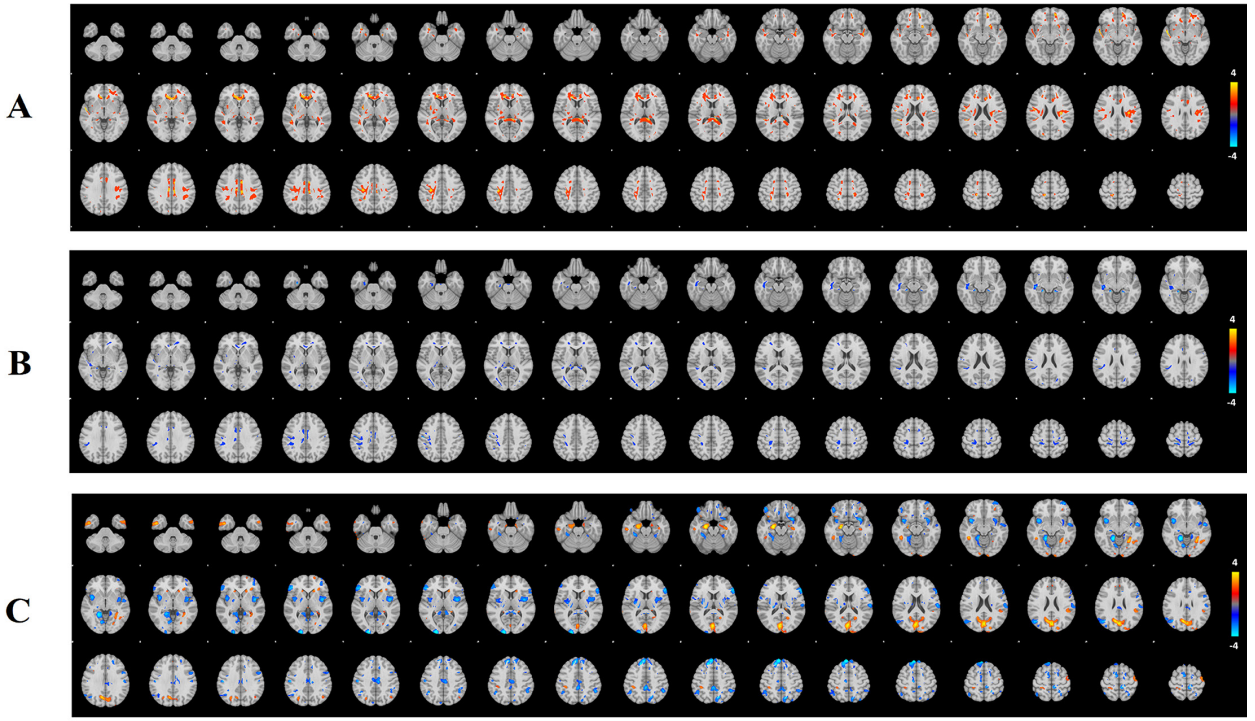

Figure 1. A: Axial projection of white matter voxels: fractional anisotropy associated with tinnitus. Colors reflect the tendency of the association: blue for a negative direction (decrease of white matter fractional anisotropy in tinnitus), red for a positive direction (increase of white matter fractional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

prevalent tinnitus (as compared to those without tinnitus) and smaller brain volumes and compromised white matter microstructural integrity. Conversely, we found that individuals with tinnitus had larger white matter volumes relative to those without tinnitus, which was also independent of hearing loss. These results suggested that tinnitus might not be related with aging processes in the brain such as neurodegeneration. Indeed, another study reports no associations between tinnitus and white matter volume changes. They suggest that decreased white matter volume may be explained by comorbid hearing loss, which is again largely determined by age[16]. In line with this, several other studies propose that gray matter changes, which is also known to decrease with age[7], are attributable to the age-related hearing loss, rather than tinnitus per se[16,17]. In light of our results, it may be hypothesized that tinnitus is associated with neurodevelopmental aspects in earlier life. 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]) reports larger gray 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[14]. Interestingly, these larger gray matter volumes are reported in both participants with prevalent tinnitus as well as comorbid hearing impairment and those with prevalent tinnitus but without comorbid hearing impairment[14]. In context of these and our findings, it might be hypothesized that there is an impact of neurodevelopment before tinnitus incidence. In other words, people with relatively larger brain volumes could be at a higher risk of developing tinnitus in older ages. Though, it is important to note that such longitudinal evidence does not yet exist. To elucidate whether this is indeed a potential pathway towards tinnitus incidence, longitudinal studies are warranted in populations with a wide age range and data on repeated measurements of brain health and tinnitus.

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[31]. Interestingly, we found that prevalent tinnitus was associated with larger white matter volumes in every lobe relative to participants without tinnitus, 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 in those with prevalent tinnitus as compared to those without 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[3], reduced white matter volumes[4] and decreased white matter integrity[3]. 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 fail to appropriately adjust for effects of aging, which may have led to residual confounding by age and its associated neurodegeneration[7].

We should also consider the possibility that the relatively larger white matter volumes in those with tinnitus, as compared to those without tinnitus, might be explained by other factors. For example, it is known that a higher intelligence is correlated with larger brain volumes[32]. Or that a healthy lifestyle indicated by high levels of physical activity, absence of smoking, none or moderate alcohol consumption and a healthy bodyweight is related to better brain health[33-36]. In that sense, larger white matter volumes in those with tinnitus, might be explained by a higher educational level and/or adhering to a healthier lifestyle, as compared to those without tinnitus. However, we could not confirm such differences in characteristics between groups. Moreover, we tried to limit confounding by adding those factors into our statistical models. Nevertheless, due to the current cross-sectional design, we cannot exclude the possibility of residual confounding.

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[37]. As such, our results confirm that central processes also play a substantial role in prevalent tinnitus. Nevertheless, to elucidate if and how neurodevelopment is associated with the incidence of tinnitus, longitudinal studies are needed.

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. Some limitations of the current study should also be acknowledged. First, there is no gold standard for the definition of tinnitus, with the main difference between studies being the frequency of tinnitus being present[38]. As such, it might be complicated to compare results between studies. To address this potential heterogeneity, we conducted a sensitivity analysis: only considering those with daily tinnitus as 'present tinnitus'. Interestingly, we saw that effect estimates became larger when compared to the primary analysis. This might suggest that those with daily tinnitus have, on average, even larger brain tissue volumes than those with tinnitus more than once a week, but not daily. Nevertheless, the actual difference between effect estimates may be considered as relatively small and the corresponding confidence interval widens. Therefore, care must be taken when interpreting these results. Future studies are needed to further unravel heterogeneity in terms of tinnitus presence and potential differences in health related determinants or outcomes in those reporting daily tinnitus versus those reporting weekly tinnitus, but not daily. Second, due to incomplete data we could not investigate the severity of the tinnitus complaints. Third, we did not have information on time of tinnitus onset and which ear was affected by the tinnitus.

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 potentially has a neurodevelopmental origin in earlier life independent of aging processes. Future (longitudinal) population-based studies with data on tinnitus onset are warranted to elucidate the role of peripheral damage and central processes in the pathophysiology of tinnitus.

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SUPPLEMENTAL

Supplementary table e-1. The association between tinnitus and brain tissue volume and white matter microstructural integrity: a sensitivity analysis in participants only reporting daily tinnitus versus absent tinnitus

		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	0.08 (0.04, 0.13)	-0.03 (-0.11, 0.04)	0.16 (0.07, 0.24)	0.04 (-0.07, 0.14)	-0.02 (-0.11, 0.07)
	Model 2	0.10 (0.05, 0.15)	-0.00 (-0.09, 0.08)	0.16 (0.05, 0.26)	0.04 (-0.08, 0.16)	-0.05 (-0.15, 0.05)

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**.

Supplementary table e-2. Associations of tinnitus with tract-specific fractional anisotropy in voxel-based morphometry

White matter tract left hemisphere	p-value
Projection fibers	
Corticospinal tract	0.0002769
Anterior thalamic radiation	0.0002216
Superior thalamic radiation	0.0043458
Posterior thalamic radiation	0.0005035
Association fibers	
Superior longitudinal fasciculus	0.0013536
Inferior longitudinal fasciculus	0.0004202
Inferior fronto-occipital fasciculus	0.0001839
Uncinate fasciculus	0.0001659
Limbic system fibers	
Cingulate gyrus part of cingulum	0.0000991
Parahippocampal part of cingulum	0.0070433
Fornix	0.0017664
Tracts in brainstem	
Medial lemniscus	1.0

White matter tract right hemisphere	p-value
Projection fibers	
Corticospinal tract	0.0000572
Anterior thalamic radiation	0.0001282
Superior thalamic radiation	0.0007524
Posterior thalamic radiation	0.0004496
Association fibers	
Superior longitudinal fasciculus	0.0000005
Inferior longitudinal fasciculus	0.0000149
Inferior fronto-occipital fasciculus	0.0003376
Uncinate fasciculus	0.0008778
Limbic system fibers	
Cingulate gyrus part of cingulum	0.0004230
Parahippocampal part of cingulum	0.0002652
Fornix	0.0318250
Tracts in brainstem	
Medial lemniscus	0.0077708

White matter tract	p-value
Tracts in brainstem	
Middle cerebellar peduncle	0.031924
Callosal fibers	
Forceps minor	0.0000393
Forceps major	0.0000159

Corresponding p-values in the association between tinnitus and white matter fractional anisotropy of the white matter tracts. Adjusted for age, education, hearing loss, intracranial volume, smoking, alcohol intake, physical activity, body mass index, hypertension, hypercholesterolemia, depressive symptoms, and MMSE-score. P-values < 5.91264162686e-08 were considered statistically significant.

Supplementary table e-3. Associations of tinnitus with tract-specific mean diffusivity in voxel-based morphometry

White matter tract left hemisphere	p-value
Projection fibers	
Corticospinal tract	0.0000571
Anterior thalamic radiation	0.0026413
Superior thalamic radiation	0.0004335
Posterior thalamic radiation	0.0001128
Association fibers	
Superior longitudinal fasciculus	0.0007278
Inferior longitudinal fasciculus	0.0059493
Inferior fronto-occipital fasciculus	0.0094840
Uncinate fasciculus	0.0212059
Limbic system fibers	
Cingulate gyrus part of cingulum	0.0000922
Parahippocampal part of cingulum	0.0000071
Fornix	1.0
Tracts in brainstem	
Medial lemniscus	1.0

White matter tract right hemisphere	p-value
Projection fibers	
Corticospinal tract	0.0000249
Anterior thalamic radiation	0.0029212
Superior thalamic radiation	0.0008157
Posterior thalamic radiation	0.0000066
Association fibers	
Superior longitudinal fasciculus	0.0000533
Inferior longitudinal fasciculus	0.0001037
Inferior fronto-occipital fasciculus	0.0001037
Uncinate fasciculus	0.0027297
Limbic system fibers	
Cingulate gyrus part of cingulum	0.0001363
Parahipocampal part of cingulum	0.0000077
Fornix	0.0394213
Tracts in brainstem	
Medial lemniscus	0.0206689

White matter tract	p-value
Tracts in brainstem	
Middle cerebellar peduncle	0.0143508
Callosal fibers	
Forceps minor	0.0042957
Forceps major	0.0002741

Corresponding p-values in the association between tinnitus and white matter mean diffusivity of the white matter tracts. Adjusted for age, sex, education, hearing loss, intracranial volume, smoking, alcohol intake, physical activity, body mass index, hypertension, hypercholesterolemia, depressive symptoms, and MMSE score. P-values < 6·.488710564411804e-08 were considered statistically significant.

Supplementary table e-4. Associations of tinnitus with gray matter density in voxel-based morphometry

Gray matter left hemisphere	p-value	Gray matter right hemisphere	p-value
Hippocampus	0.0103876	Hippocampus	0.0000886
Amygdala	0.0427975	Amygdala	0.0000816
Anterior temporal lobe medial part	0.0059244	Anterior temporal lobe medial part	0.0083105
Anterior temporal lobe lateral part	0.0008550	Anterior temporal lobe lateral part	0.0011697
Gyri parahippocampalis et ambiens	0.0312791	Gyri parahippocampalis et ambiens	0.0174751
Medial and inferior temporal gyri	0.0141835	Medial and inferior temporal gyri	0.0011733
Lateral occipitotemporal gyrus (gyrus fusiformis)	0.0135958	Lateral occipitotemporal gyrus (gyrus fusiformis)	0.0185210
Cerebellum	1.0	Cerebellum	1.0
Insula	0.0088903	Insula	0.0015206
Lateral remainder of occipital lobe	0.0001004	Lateral remainder of occipital lobe	0.0001005
Cingulate gyrus anterior (supragenual) part	0.0061398	Cingulate gyrus anterior (supragenual) part	0.0034751
Cingulate gyrus posterior part	0.0125124	Cingulate gyrus posterior part	0.0044029
Middle frontal gyrus	0.0039459	Middle frontal gyrus	0.0114366
Posterior temporal lobe	0.0000432	Posterior temporal lobe	0.0005596
Remainder of parietal lobe (including supramarginal and angular gyrus)	0.0084933	Remainder of parietal lobe (including supramarginal and angular gyrus)	0.0070487
Caudate nucleus	0.0049052	Caudate nucleus	0.0156250
Nucleus accumbens	1.0	Nucleus accumbens	1.0
Putamen	0.0401653	Putamen	0.0204337
Thalamus	0.0299732	Thalamus	0.0221727
Pallidum (globus pallidus)	1.0	Pallidum (globus pallidus)	1.0
Lateral ventricle frontal horn central part and occipital horn	0.0008908	Lateral ventricle frontal horn central part and occipital horn	0.0008908
Lateral ventricle temporal horn	0.0114824	Lateral ventricle temporal horn	0.0000618
Precentral gyrus	0.0011105	Precentral gyrus	0.0049792
Straight gyrus (gyrus rectus)	1.0	Straight gyrus (gyrus rectus)	1.0
Anterior orbital gyrus	0.0083985	Anterior orbital gyrus	0.0063398
Inferior frontal gyrus	0.0008239	Inferior frontal gyrus	0.0059445
Superior frontal gyrus	0.0004006	Superior frontal gyrus	0.0000762
Postcentral gyrus	0.0008130	Postcentral gyrus	0.0051213
Superior parietal gyrus	0.0000813	Superior parietal gyrus	0.0000984
Lingual gyrus	0.0000319	Lingual gyrus	0.0023954
Cuneus	0.0000205	Cuneus	0.0000219

Supplementary table e-4. Continued

Gray matter left hemisphere	p-value	Gray matter right hemisphere	p-value
Medial orbital gyrus	0.0042782	Medial orbital gyrus	0.0079841
Lateral orbital gyrus	0.0237166	Lateral orbital gyrus	0.0048728
Posterior orbital gyrus	0.0093572	Posterior orbital gyrus	0.0024671
Substantia nigra	1.0	Substantia nigra	1.0
Subgenual anterior cingulate	1.0	Subgenual anterior cingulate	1.0
Subcallosal area	1.0	Subcallosal area	1.0
Pre-subgenual anterior cingulate gyrus	0.0417909	Pre-subgenual anterior cingulate gyrus	1.0

Gray matter	p-value
Brainstem	1.0
Corpus callosum	0.0198651
Third ventricle	0.0440902

Corresponding p-values in the association between tinnitus and gray matter. Adjusted for age, sex, education, hearing loss, intracranial volume, smoking, alcohol intake, physical activity, body mass index, hypertension, hypercholesterolemia, depressive symptoms, and MMSE score. P-values < 2.9920688308080145e-07 were considered statistically significant

Supplementary table e-5. The association between tinnitus and brain tissue volume and white matter microstructural integrity – subgroup with no frequency threshold >20 dB

		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	-0.02 (-0.09, 0.05)	-0.02 (-0.09, 0.04)	0.01 (-0.05, 0.08)	0.01 (-0.05, 0.08)	-0.02 (-0.09, 0.06)	-0.01 (-0.09, 0.06)	0.02 (-0.05, 0.09)	-0.02 (-0.09, 0.05)
	Model 2	0.02 (-0.06, 0.10)	0.01 (-0.06, 0.09)	0.06 (-0.02, 0.14)	0.06 (-0.02, 0.14)	0.05 (-0.06, 0.14)	0.05 (-0.04, 0.13)	0.06 (-0.03, 0.14)	0.02 (-0.06, 0.11)
	White matter								
Tinnitus present versus absent	Model 1	**0.11 (0.04, 0.19)**	**0.11 (0.04, 0.19)**	**0.08 (0.01, 0.16)**	**0.11 (0.04, 0.18)**	**0.10 (0.02, 0.18)**	**0.12 (0.04, 0.20)**	**0.11 (0.03, 0.20)**	**0.09 (0.01, 0.17)**
	Model 2	**0.10 (0.00, 0.19)**	**0.10 (0.01, 0.19)**	**0.09 (0.00, 0.18)**	**0.12 (0.03, 0.21)**	**0.11 (0.02, 0.21)**	**0.13 (0.04, 0.22)**	**0.12 (0.01, 0.22)**	**0.09 (-0.00, 0.19)**

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 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**.

Chapter 4.2

Tinnitus and mental health: cross-sectional and longitudinal associations in a population-based sample of middle-aged and elderly persons

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ABSTRACT

Importance

Tinnitus is a common disorder, but its impact on daily life varies widely in population-based samples. It is unclear whether this interference in daily life is associated with mental health problems that are commonly detected in clinical populations.

Objective

To investigate the association of tinnitus and its interference in daily life with symptoms of depression and anxiety and poor sleep quality in a population-based sample of middle-aged and elderly persons in a cross-sectional analysis and during a 4-year follow-up.

Design, Setting, and Participants

This cohort study evaluated data from the population-based Rotterdam Study of individuals 40 years or older living in Rotterdam, the Netherlands. Between 2011 and 2016, data on tinnitus were obtained during a home interview at least once for 6128 participants. Participants with information on depressive and anxiety symptoms and self-rated sleep quality, with Mini-Mental State Examination scores indicating unimpaired cognition, and with repeatedly obtained tinnitus and mental health outcome data were included. Data analyses were conducted between September 2019 and April 2020.

Main Outcomes and Measures

The presence of tinnitus and its interference with daily life were assessed during a home interview. Depressive symptoms were assessed with the Center for Epidemiologic Studies–Depression, anxiety symptoms with the Hospital Anxiety and Depression Scale, and sleep quality with the Pittsburgh Sleep Quality Index. Linear regression analyses and linear mixed models adjusted for relevant confounders were used to assess the cross-sectional and longitudinal association of tinnitus with mental health.

Results

Of 5418 complete-case participants (mean [SD] age, 69.0 [9.8] years; 3131 [57.8%] women), 975 (mean [SD] age, 71.7 [4.5] years; 519 [53.2%] women) had repeated measurements available for follow-up analyses. Compared with participants without tinnitus and participants with nonbothersome tinnitus, participants with tinnitus interfering with daily life reported more depressive (difference, 0.20; 95% CI, 0.11-0.28) and anxiety (difference, 0.15; 95% CI, 0.08-0.22) symptoms and poorer sleep

quality (difference, 0.10; 95% CI, 0.03-0.16). Compared with participants without tinnitus, participants with nonbothersome tinnitus also reported more depressive (difference, 0.06; 95% CI, 0.03-0.09) and anxiety (difference, 0.05; 95% CI, 0.02-0.07) symptoms and poorer sleep quality (difference, 0.05; 95% CI, 0.03-0.08). Individuals indicating more interference with daily life reported having more mental health problems. During a mean follow-up of 4.4 years (range, 3.5-5.1 years), participants with tinnitus reported more anxiety symptoms and poorer sleep quality than those without tinnitus.

Conclusions and Relevance

Findings of this population-based cohort study indicate that tinnitus was associated with more mental health problems in middle-aged and elderly persons in the general population, in particular when tinnitus interfered with daily life but not solely. Over time, more severe tinnitus was associated with an increase in anxiety symptoms and poor sleep quality. This outcome suggests that mental health problems may be part of the burden of tinnitus, even among individuals who do not report their tinnitus interfering with daily life.

INTRODUCTION

Tinnitus, commonly defined as a sound that is heard in absence of an objective sound-source, is a common condition in the general population, with a prevalence between 9 and 40%[1]. For most individuals, tinnitus is not bothersome, but for about 5 to 20% of the people experiencing tinnitus the condition significantly impairs their daily life, also regarded as severe tinnitus[1].

Tinnitus has been frequently associated with a range of psychological conditions, such as depression, anxiety, irritability, sleep disturbances, subjective distress and intense worrying[2]. These associations have been extensively researched in clinical studies[3,4], including highly selected populations containing those with the highest tinnitus-associated burden of disease. More recently, cross-sectional epidemiological studies in population-based cohorts have confirmed associations of the prevalence of tinnitus with depression and anxiety[5-13], as well as associations between tinnitus and sleep disorders[14,15]. Because most of these studies did not take into account the interference of tinnitus on daily life, it remains unclear whether these associations are mainly observed in the high-severity tinnitus group, with high resemblance to the clinical tinnitus populations, or are also applicable to the sub-clinical tinnitus population.

The association between tinnitus and mental health is potentially affected by the grade of hearing loss. Hearing loss commonly co-occurs with tinnitus (43.2%,[16]), is known as an accelerating factor for tinnitus[17], and has also been suggested to be associated with mental health[18]. To date, clinical studies suggest that tinnitus, hearing loss and mental health problems co-exist[19], but no population-based studies of tinnitus have taken hearing loss into account. Lastly, due to the lack of longitudinal studies, the temporality of the association of tinnitus with mental health is still under debate. For example, tinnitus may precede mental health problems, mental health problems may precede tinnitus, or there may be a bidirectional relationship between mental health problems and tinnitus. In addition, the current recommended therapy for tinnitus is cognitive behavioral therapy, which is a psychological treatment that also entails components that may benefit mental health[20-22].

To gain more insight into the association between tinnitus and mental health problems and the possible underlying mechanisms, we investigated the association between tinnitus and depressive symptoms, anxiety symptoms and self-rated sleep quality in a relatively large population of middle-aged and older adults, focusing on its interference with daily life and the presence of hearing loss. Furthermore, we aimed to gain insight into the effect of tinnitus on the development of mental health complaints over time by analyzing longitudinal data over a period of four years.

METHODS

Setting and study population

This study was embedded in the Rotterdam Study, a prospective population-based cohort of middle-aged and elderly persons. The Rotterdam Study was initiated in 1990 and investigates determinants and consequences of aging and age-related disease. The study population consists of individuals aged ≥ 40 years living in the well-defined Ommoord district of Rotterdam, the Netherlands. All participants were invited to undergo extensive examinations at study entry and subsequently every 3 to 6 years. Further details of the study have been described elsewhere[23]. 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). The Rotterdam Study has been entered into the Netherlands National Trial Register (www.trialregister.nl) and into the World Health Organization International Clinical Trials Registry Platform (who.int/ictrp/network/primary/en/) under shared catalog number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Tinnitus and hearing assessments were introduced into the core study protocol in 2011. Between 2011 and 2016, data on tinnitus were obtained during a home interview at least once for 6128 participants. Of those, we excluded 158 participants who had no information on depressive symptoms, anxiety symptoms, or self-rated sleep quality available from the same home interview. In addition, because poor cognitive status may impair the ability to complete questionnaires validly, participants were also excluded if their Mini-Mental State Examination score was lower than 23 (n = 143) or if these data were missing (n = 409). Of the remaining 5418 participants, repeatedly obtained data on tinnitus and mental health outcomes were obtained for 1140 participants, with a mean follow-up time of 4.4 years (range, 3.5-5.1 years). Participants were excluded from the longitudinal analysis when there was incident tinnitus (n = 96) or when they no longer reported tinnitus (n = 69) at follow-up. Thus, 5418 complete-case participants were included in the cross-sectional analyses, and 975 complete-case eligible participants were included in the longitudinal analysis.

Measurements

Tinnitus

Tinnitus presence was assessed during the home interview. Participants were asked if they experienced sounds in their head or in (one of) their ears, such as whizzing, beeping, or humming, without an objective external sound source being present.

Possible answers to this question were “no, never,” “yes, less than once a week,” “yes, more than once a week but not daily,” and “yes, daily.” Participants who reported having symptoms at least once a week were considered to have tinnitus. Because of the heterogeneity and often temporary character of tinnitus, experiencing symptoms less than once a week was not considered having tinnitus. Participants who indicated experiencing tinnitus were additionally asked whether the tinnitus interfered with their daily life (yes or no). If participants reported daily tinnitus independent of whether they reported interference with daily life, they were asked to complete the simplified Tinnitus Handicap Inventory (THI-s), a validated questionnaire to assess impairments in daily life caused by tinnitus. This 10-item inventory uses possible scores of 0 (no), 2 (sometimes), or 4 (yes) per item, and a score of 16 or higher represents a moderate to severe impairment[24].

Based on the questions described above, we identified 3 groups at baseline: (1) participants without tinnitus, (2) participants with tinnitus but not interfering with daily life (nonbothersome tinnitus), and (3) participants with tinnitus interfering with daily life (bothersome tinnitus). The THI-s scores were assessed separately for anyone experiencing daily tinnitus.

Depressive Symptoms

The Center for Epidemiologic Studies–Depression (CES-D) scale was used to assess depressive symptoms. The CES-D is a validated instrument that is widely used to estimate self-reported depressive symptoms.[25]. The CES-D is a 20-item questionnaire, where each item is scored on a four-point scale from 0 (low) to 3 (high). The cut-off value for clinically relevant depressive symptoms was set at ≥16 points[25,26]. If not all but more than 75% of the items were completed a weighted total score was calculated, if less than 75% of the questions were answered CES-D was set to missing.

Anxiety symptoms

The anxiety subscale of the Hospital Anxiety and Depression scale (HADS-A)[27] was used to assess anxiety symptoms. The HADS-A is a 7-item questionnaire, where each item is scored on a four-point scale from 0 (never) to 3 (usually). A weighted global score was calculated by multiplying by 7/6 when 6 components were available. If less than 6 component scores were available, the HADS-A was set to missing. The total score range between 0 and 21, where the cut-off value for clinically relevant anxiety symptoms was set at ≥8 points[27].

Sleep outcomes

The Pittsburgh Sleep Quality Index (PSQI) was used to measure self-rated sleep quality. The PSQI is a 19-item questionnaire, covering sleep-associated problems. The PSQI

covers 7 compoments, reported on a four-point scale from 0 (never) to 3 (daily)[28]. A weighted global score was calculated by multiplying by 7/6 when 6 components were available. If less than 6 components were available, PSQI was set to missing. The final global score ranges between 0 and 21. The cut-off value for clinically relevant poor sleeping quality was set at ≥5 points[28].

Other variables

Highest achieved educational level was scored using the UNESCO classification[29]. To calculate Body Mass Index (BMI) (kg/m^2), length and weight were assessed on calibrated scales at the research center without heavy clothing and shoes. Smoking data was collected through self-report and categorized into never, former, or current smoking. Alcohol consumption (glasses/day) was assessed through self-report. Pure tone audiometry was performed by a trained health care professional in a soundproof booth. Air conduction thresholds for both ears were obtained on frequencies 0.25, 0.5, 1, 2, 4, 8 kilohertz (kHz)[23]. Pure tone average hearing thresholds (PTA), averaged over 0.5, 1, 2 and 4 kHz, were determined from the best hearing ear, as proposed by the WHO[30]. Hearing loss was defined as a PTA ≥25 decibel hearing level (dB HL) or higher[30]. The Mini-Mental State Examination (MMSE) was administered during home interview to screen cognitive status[31].

Statistical analysis

Demographic Analyses

The demographic characteristics of the population were described. Descriptive statistics were used to assess and compare differences in characteristics among participants with or without tinnitus. For continuous variables with a normal distribution, we described the mean (SD) value and used a t test for statistical testing. For continuous variables with a nonnormal distribution, we described the median value and the interquartile range and used a Mann-Whitney test to compare groups. For categorical variables, we described the number (%) and used the $\chi 2$ test to compare the groups. Depressive symptoms, anxiety symptoms, and sleep quality scores were all log transformed (score +1) to achieve a normal distribution of the residuals.

Cross-sectional Analyses

To estimate the cross-sectional association of tinnitus with depressive symptoms, anxiety symptoms, and sleep quality at baseline, we used linear and logistic regression analyses. Participants with bothersome tinnitus and participants with nonbothersome tinnitus were compared with participants without tinnitus (reference group) and with each other (nonbothersome tinnitus as the reference group). To account for

confounding, 2 models were used: model 1 was adjusted for sex and age, and model 2 was additionally adjusted for the highest achieved educational level, BMI, alcohol use, smoking, and hearing threshold.

In addition, all cross-sectional regression analyses were repeated with the entire data set stratified for hearing loss (PTA≥25 dB HL) because this variable is the most important risk factor for tinnitus. In a subgroup of 625 participants with daily tinnitus symptoms and an available THI-s score, we also assessed the associations of tinnitus impairment with mental health, comparing participants with a relevant tinnitus impairment (THI-s score ≥16) to those with no tinnitus impairment (THI-s score <16).

Longitudinal Analyses

We used linear mixed models with random intercepts and slopes to explore the longitudinal association between tinnitus and depressive symptoms, anxiety symptoms, and sleep quality over time. In each model, we entered follow-up time in years after baseline measurement to use as a time variable and added an interaction term of tinnitus and follow-up time in all models to allow for slope differences in the association between mental health outcomes and time explained by the presence of tinnitus. The linear tinnitus term (intercept difference) and the interaction term between tinnitus and follow-up time (slope difference) are the main outcomes in this longitudinal analysis. Confounder adjustment was performed per model 1 and model 2 as fixed effects. The control variables were included as fixed effects.

Data analyses were conducted between September 2019 and April 2020 using SPSS statistics, version 24.0 (IBM Corp) for data handling and cross-sectional analyses and R statistical software, version 4.0.4 (The R Foundation for Statistical Computing) with the lme package for longitudinal analyses. A 2-sided $P < .05$ was considered statistically significant.

RESULTS

Cross-sectional association of tinnitus with mental health

For the cross-sectional analyses, 5418 participants were included, with a mean (SD) age of 69.0 (9.8) years, and 3131 (57.8%) were women. In total, 4245 participants (78.4%) reported no prevalent tinnitus, 1063 participants (19.6%) reported nonbothersome tinnitus, and 110 participants (2.0%) reported bothersome tinnitus (Table 1). Of 110 participants with bothersome tinnitus, 94 experienced tinnitus daily, and 16 experienced tinnitus less frequently.

Table 1. Demographic characteristics

	Total sample (N =5,418)	Participants without tinnitus (N = 4,245)	Participants with bothersome tinnitus (N = 110)	Participants with non-bothersome tinnitus (N = 1,063)	p-value
Age, years (SD)	69.0 (9.8)	69.0 (9.8)	68.6 (8.7)	69.3 (9.7)	0.664
Female, N (%)	3,131 (57.3)	2,476 (58.3)	59 (53.6)	561 (52.8)	0.004
Education level, N (%)					0.291
Primary	394 (7.3)	297 (7.0)	12 (10.9)	85 (8.0)	
Lower	2,103 (38.8)	1,625 (38.3)	44 (40.0)	434 (40.8)	
Middle	1,619 (29.9)	1,282 (30.2)	33 (30.0)	304 (28.6)	
Higher	1,251 (23.1)	999 (23.5)	21 (19.1)	231 (21.7)	
THI-s ≥16*, N (%)	88 (13.4)	-	41 (51.3)	47 (8.6)	<0.001
Hearing threshold, dBHL (SD)	23.6 (13.4)	22.6 (12.9)	30.8 (14.9)	26.8 (14.5)	<0.001
Hearing loss, N (%)	1,742 (32.2)	1,245 (29.3)	56 (50.9)	441 (41.5)	<0.001
Depressive symptoms					
Weighted score‡	3 (1 - 8)	3 (1 - 7)	6 (2 - 12)	4 (1 - 9)	<0.001
Clinically relevant, N (%)	500 (9.2)	371 (8.7)	17 (15.5)	112 (10.5)	0.014
Anxiety symptoms					
Weighted score‡	2 (0 - 4)	2 (0 - 4)	3 (1 - 6)	2 (0 - 5)	<0.001
Clinically relevant, N (%)	453 (8.4)	328 (7.7)	14 (12.7)	111 (10.4)	0.004
Sleep quality					
Weighted score‡	3 (1 - 6)	3 (1 - 5)	4 (2 - 7)	3 (2 - 6)	<0.001
Clinically relevant, N (%)	1,361 (25.1)	1,013 (23.9)	38 (34.5)	310 (29.2)	<0.001
Body mass index, kg/m^2 (SD)	27.5 (4.4)	27.4 (4.4)	27.8 (3.9)	27.7 (4.2)	0.297
Smoking, N (%)					0.020
Never	1,728 (31.9)	1,398 (32.9)	34 (30.9)	296 (27.8)	
Past	2,809 (51.8)	2,156 (50.8)	60 (54.5)	593 (55.8)	
Current	798 (14.7)	622 (14.7)	14 (12.7)	162 (15.2)	
Alcohol units/day, N (%)					0.603
Never	792 (14.6)	631 (14.9)	16 (14.5)	145 (13.6)	
1-2	3,526 (65.1)	2,766 (65.2)	70 (63.6)	690 (64.9)	
3-4	862 (15.9)	665 (15.7)	22 (20.0)	175 (16.5)	
>5	229 (4.2)	177 (4.2)	2 (1.8)	50 (4.7)	
MMSE‡	28 (27 - 29)	28 (27 - 29)	28 (27 - 29)	29 (27 - 29)	0.932

Values are mean (standard deviation (SD)) for normally distributed continuous variables, a t-test was used to compare groups. *% of those who filled out the THIS-s. ‡Median (interquartile range (IQR)) for non-normally distributed continuous variables, a Mann-Whitney U test was used to compare groups. Dichotomous variables are given as N, (%), a X^2-test was used to compare groups. THI-s: Tinnitus Handicap Inventory – screening version. dB HL: decibel hearing level. Hearing loss was averaged over the 0.5, 1, 2, 4 kHz frequencies in the best ear. Depressive symptoms were measured with the CES-D list, a score of ≥16 was considered clinically relevant. Anxiety symptoms were measured with the HADS- anxiety subscale, a score of ≥8 was considered clinically relevant. Sleep quality was self-reported with the PSQI, a score of ≥6 was considered as clinically relevant lower sleep quality. MMSE: Mini-Mental State Examination.

Participants with bothersome tinnitus scored significantly higher on depressive symptoms (all differences are log transformed [score +1]) (difference, 0.20; 95% CI, 0.11-0.28), anxiety symptoms (difference, 0.15; 95% CI, 0.08-0.22), and sleep quality (difference, 0.10; 95% CI, 0.03-0.16) compared with participants without tinnitus as well as compared with participants with nonbothersome tinnitus when adjusted for confounders (Table 2). Participants with nonbothersome tinnitus also scored significantly higher on depressive symptoms (difference, 0.06; 95% CI, 0.03-0.09), anxiety symptoms (difference, 0.05; 95% CI, 0.02-0.07), and sleep quality (difference, 0.05; 95% CI, 0.03-0.08) compared with participants without tinnitus (Table 2). When using cutoffs to indicate clinically relevant symptoms for these mental health outcomes, we found effect sizes indicating similar associations (eTable 1).

Because the presence of hearing loss may affect the association between tinnitus and mental health outcomes, we repeated the analyses in a data set stratified on hearing loss (≥25 dB HL). These analyses suggested that associations of tinnitus with mental health were found in both groups of participants with or without hearing loss (Table 3). Yet, when using a cutoff to distinguish between clinically relevant mental health outcomes, the results were mixed and apparently more pronounced for the group without hearing loss (eTable 2).

Table 2. Association between participants with (non-) bothersome tinnitus and depressive symptoms, anxiety symptoms and self-reported sleep quality.

		Depressive symptoms	**Anxiety symptoms**	**Sleep quality**
		Difference (95% CI)	**Difference (95% CI)**	**Difference (95% CI)**
No tinnitus	Model 1	Ref	Ref	Ref
	Model 2	Ref	Ref	Ref
Bothersome tinnitus	Model 1	**0.21 (0.11, 0.28)**	**0.16 (0.09, 0.23)**	**0.09 (0.02, 0.15)**
	Model 2	**0.20 (0.11, 0.28)**	**0.15 (0.08, 0.22)**	**0.10 (0.03, 0.16)**
Non-bothersome tinnitus	Model 1	**0.07 (0.04, 0.10)**	**0.05 (0.02, 0.07)**	**0.05 (0.03, 0.07)**
	Model 2	**0.06 (0.03, 0.09)**	**0.05 (0.02, 0.07)**	**0.05 (0.03, 0.08)**

Difference represents the difference in the log-transformed (raw score +1) of the CES-D (for depressive symptoms), HADS – anxiety subscale (for anxiety symptoms) or PSQI (for self-reported sleep quality) in participants with non-bothersome or bothersome tinnitus. CI: confidence interval. Model 1: adjusted for sex and age. Model 2: additionally adjusted for highest achieved education, hearing loss, body mass index, alcohol use and smoking status. Significant effect estimates ($p<0.05$) are indicated in **bold**.

Information on tinnitus impairment was available for 625 participants with daily tinnitus. Of those, 88 participants (14.1%) reported relevant tinnitus impairment. Participants with relevant tinnitus impairment had higher scores on all 3 mental health outcomes compared with participants with daily tinnitus but no tinnitus impairment (Table 4; eTable 3). Median scores on all 3 mental health outcomes were higher per more severe tinnitus impairment category (eTable 4).

Table 3. Association between participants with (non-) bothersome tinnitus and depressive symptoms, anxiety symptoms and self-reported sleep quality stratified analyses for hearing impairment.

		Depressive symptoms	Anxiety symptoms	Sleep quality
		Difference (95% CI)	**Difference (95% CI)**	**Difference (95% CI)**
No hearing loss (<25 dB HL), N=3,676				
No tinnitus	Model 1	Ref	Ref	Ref
	Model 2	Ref	Ref	Ref
Bothersome tinnitus	Model 1	0.21 (0.07, 0.35)	0.13 (0.02, 0.24)	0.06 (-0.04, 0.16)
	Model 2	0.20 (0.05, 0.34)	0.12 (0.01, 0.24)	0.06 (-0.04, 0.16)
Non-bothersome tinnitus	Model 1	0.05 (0.00, 0.09)	0.02 (-0.01, 0.06)	0.05 (0.02, 0.09)
	Model 2	0.04 (-0.00, 0.09)	0.02 (-0.02, 0.06)	0.06 (0.03, 0.09)
Hearing loss (≥25 dB HL), N=1,742				
No tinnitus	Model 1	Ref	Ref	Ref
	Model 2	Ref	Ref	Ref
Bothersome tinnitus	Model 1	0.23 (0.12, 0.34)	0.19 (0.09, 0.28)	0.12 (0.03, 0.21)
	Model 2	0.22 (0.11, 0.33)	0.18 (0.09, 0.28)	0.13 (0.04, 0.21)
Non-bothersome tinnitus	Model 1	0.09 (0.04, 0.13)	0.08 (0.04, 0.11)	0.05 (0.01, 0.08)
	Model 2	0.08 (0.04, 0.13)	0.08 (0.04, 0.11)	0.05 (0.01, 0.08)

Difference represents the difference in the log-transformed (raw score +1) of the CES-D (for depressive symptoms), HADS – anxiety subscale (for anxiety symptoms) or PSQI (for self-reported sleep quality) in participants with non-bothersome or bothersome tinnitus, stratified for hearing loss. OR: Odds ratio for the cut-off value for a clinically relevant score on either the CES-D (≥16), HADS (≥8) or PSQI (≥6). CI: confidence interval. dB: decibel. Model 1: adjusted for sex and age. Model 2: additionally adjusted for highest achieved education, hearing loss, body mass index, alcohol use and smoking status. Significant estimates ($p<0.05$) are indicated in **bold**.

Table 4. Association between participants with daily tinnitus and a relevant tinnitus handicap (vs. daily tinnitus and low tinnitus handicap) and depressive symptoms, anxiety symptoms and self-reported sleep quality.

		Depressive symptoms	Anxiety symptoms	Sleep quality
		Difference (95% CI)	**Difference (95% CI)**	**Difference (95% CI)**
No relevant tinnitus handicap	Model 1	Ref	Ref	Ref
	Model 2	Ref	Ref	Ref
Relevant tinnitus handicap	Model 1	0.22 (0.12, 0.33)	0.15 (0.07, 0.23)	0.12 (0.05, 0.19)
	Model 2	0.21 (0.11, 0.31)	0.15 (0.06, 0.23)	0.12 (0.05, 0.20)

In participants with daily tinnitus (N=662), tinnitus handicap was assessed by the Tinnitus Handicap Inventory – screening version (THI-s). A relevant handicap was defined as a THI-s score ≥16. Difference represents the difference in the log-transformed (raw score +1) of the CES-D (for depressive symptoms), HADS – anxiety subscale (for anxiety symptoms) or PSQI (for self-reported sleep quality) in participants with tinnitus with and without interference with daily life. CI: confidence interval. Model 1: adjusted for sex and age. Model 2: additionally adjusted for highest achieved education, hearing loss, body mass index, alcohol use and smoking status. Significant effect estimates ($p<0.05$) are indicated in **bold**.

Associations of tinnitus with mental health over time

Repeated data were available for 975 participants (mean [SD] age, 71.7 [4.5] years; 519 [53.2%] women), with a mean (SD) follow-up of 4.4 (0.2) years (range, 3.5-5.1 years). At baseline, no tinnitus was reported by 792 participants (81.2%), nonbothersome tinnitus by 163 participants (16.7%), and bothersome tinnitus by 20 participants (2.1%).

Participants with any tinnitus (ie, bothersome or nonbothersome) had higher scores on all 3 mental health outcomes compared with participants with no tinnitus (Figure; eFigure and eTable 5). The change in mental health symptoms during follow-up was not significantly different for participants with tinnitus, nor was the change different for bothersome vs nonbothersome tinnitus, compared with participants without tinnitus.

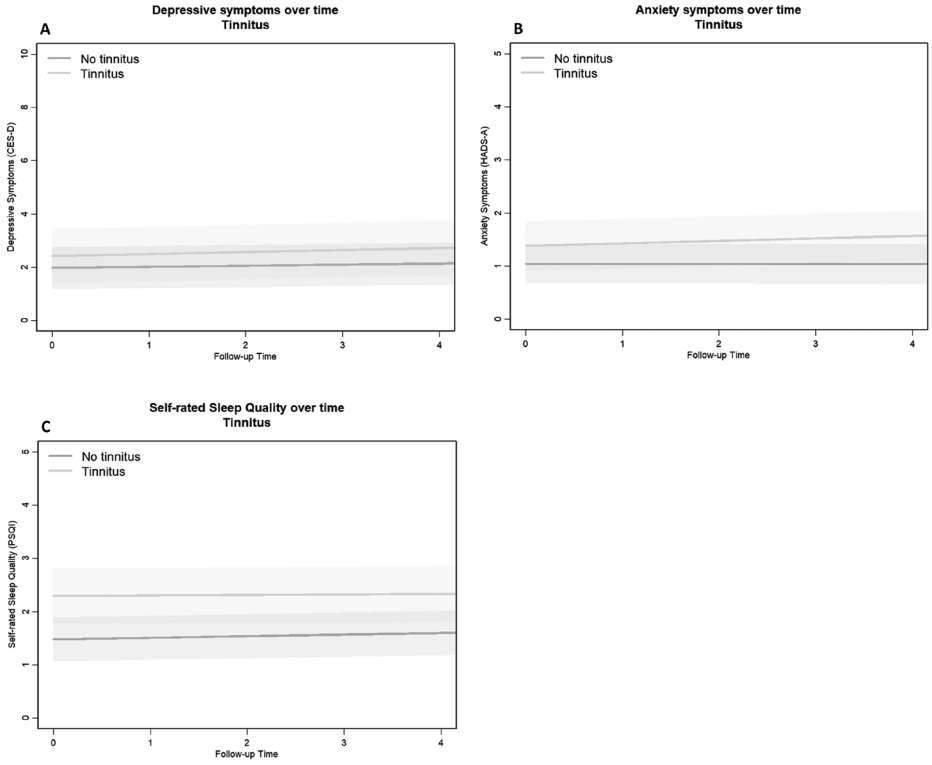

Figure 1 a-c. Association between participants with bothersome and non-bothersome tinnitus and depressive symptoms, anxiety symptoms and self-reported sleep quality over time.
Results of the linear mixed models, showing the average change in log(raw score+1) over time for the panel A: CES-D (for depressive symptoms), panel B: HADS – anxiety subscale (for anxiety symptoms) or panel C: PSQI (for self-reported sleep quality).

DISCUSSION

In this study, we confirmed that both bothersome and nonbothersome tinnitus were associated with having more depressive and anxiety symptoms and poorer sleep quality in participants with vs without tinnitus, although associations were stronger in those indicating bothersome tinnitus. Associations were present in individuals with or without hearing loss. Furthermore, longitudinal analyses were suggestive (although not statistically significant) of more depressive and anxiety symptoms and poorer sleep quality after 4 years of follow-up in participants with bothersome tinnitus.

The association of tinnitus with psychopathology is in line with previous cross-sectional studies, reporting tinnitus to be associated with depressive symptoms, anxiety and poorer sleep quality[3-11,32,33]. We found that the effect size of the association for bothersome tinnitus over nonbothersome tinnitus was nearly 3 times as large for depressive and anxiety symptoms and twice as large for sleep quality compared with no tinnitus. However, the absolute values for the differences in effect size cannot be determined from the reported values because they represented a transformed value. Moreover, we found that the group with daily tinnitus and severe tinnitus impairment (high THI-s score) had a 2 to 4 times higher likelihood for psychopathology than the group with daily tinnitus and mild tinnitus impairment (low or moderate THI-s score). This outcome is in line with the results reported by Bhatt et al[11], who found even higher odds ratios but who compared participants with tinnitus vs those without tinnitus. Yet, the association between tinnitus and mental health outcomes was not detected only in a small group with severe tinnitus. We also observed a similar association in the subgroup of participants with nonbothersome tinnitus. Although the effect sizes were smaller than in the bothersome tinnitus group, the associations were consistently found for each of the 3 investigated mental health outcomes. Mental health problems can thus also be observed in milder manifestations of tinnitus. A plausible explanation for the consistent association between tinnitus and mental health outcomes, even for nonbothersome tinnitus, could be that people with tinnitus generally develop negative thoughts about their tinnitus, stress arousal, and hyperawareness.[34]. Those negative thoughts and hyperawareness have been suggested to be a mechanism towards developing mental health problems[35,36]. Yet, equally, it could be speculated that mental health problems lead to a negative focus which worsens the experience of tinnitus[37] or that a tendency towards negative thoughts, stress and hyperawareness could be a shared common cause. Further research is needed to determine the exact pathways.

Another potential cause for the association of tinnitus with mental health problems is the presence of hearing loss because it is a strong risk factor for tinnitus[16] and also because it is associated with mental health problems[18], depression in particular[38]. However, we observed associations between tinnitus and mental health outcomes in both subgroups, that is, with and without hearing loss, suggesting that hearing loss is not a common cause that explains these associations in full. We unexpectedly found a higher likelihood for clinically relevant mental health problems with severe tinnitus for the subgroup without hearing loss. Even though it is counterintuitive that absence of hearing loss appeared to strengthen the association of tinnitus with more psychopathology, it may be that in individuals without hearing loss, different neural pathways are involved in tinnitus generation, and that these neural pathways in turn have a stronger association with mental health problems[39]. In the presence of hearing loss, tinnitus pathophysiology is more likely to be initiated by hearing-related factors, whereas in the absence of hearing loss, it is thought that changes in the brain are responsible for the occurrence of tinnitus[17].

Several other causal pathways have been posed to explain the association between tinnitus and mental health[11]. To gain more insight in the directionality of the association between tinnitus and mental health problems, we used longitudinal analyses to explore how mental health problems evolve over time in the presence or absence of tinnitus. Our results shown in the Figure appeared to suggest an increase in anxiety symptoms and poorer sleep quality during a period of 4 years for patients owing to more severe tinnitus, albeit the associations did not reach statistical significance because of limited power. In addition, because we were able to study only the association of tinnitus with mental health, we cannot infer that the temporality of the association is solely from tinnitus to mental health and not the other way around. The association may also be bidirectional, with more severe tinnitus increasing mental health problems and mental health problems inducing greater concern about tinnitus. To our knowledge, there are no other longitudinal epidemiological studies devoted to exploring the association between tinnitus and mental health over time. Future population-based studies with repeated measurements over time are therefore urgently needed.

Major strengths of this study are the presence of both cross-sectional and longitudinal data in a relatively large population-based sample and the large number of confounding variables taken into account, including hearing loss. Several limitations of this study should be noted. As with all tinnitus research, a lack of a uniform definition of this subjective disorder hampers the ability to compare our results with other studies. Nevertheless, we asked a frequently used question to assess tinnitus.1

In addition, we investigated tinnitus severity both by asking an additional question and through the use of the THI-s. Regarding our longitudinal analyses, a follow-up time of 4 years may be too long to investigate an association between the presence of tinnitus and mental health outcomes. It would also be interesting to investigate the longitudinal association between mental health problems and incident tinnitus; however, tinnitus incidence was too low in the present study to provide these results. We believe that the results of this study extend current knowledge and are valuable because we not only investigated cross-sectional associations between tinnitus and mental health in relevant subgroups of a large population-based sample of middle-aged and elderly individuals but also explored possible associations over time.

In conclusion, we showed that tinnitus was strongly associated with having more depressive and anxiety symptoms and having poorer sleep quality, even when tinnitus did not interfere with daily life (nonbothersome tinnitus). Hearing loss did not appear to play a primary role in these associations. Moreover, any tinnitus at baseline was associated with an increase in anxiety symptoms and poorer sleep quality over time. These results underline the importance of increasing awareness of the association of tinnitus with mental health problems among affected individuals. Primary health care professionals should monitor mental health in patients with tinnitus, even in patients who do not report significant impairments to their daily life.

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eTable 1. Association between participants with (non-) bothersome and clinically relevant depressive symptoms, anxiety symptoms and self-reported sleep quality.

		Clinically relevant depressive symptoms	Clinically relevant anxiety symptoms	Clinically relevant lower sleep quality
		OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**
No tinnitus	Model 1	Ref	Ref	Ref
	Model 2	Ref	Ref	Ref
Bothersome tinnitus	Model 1	**2.08 (1.15, 3.76)**	1.71 (0.89, 3.28)	**1.94 (1.24, 3.03)**
	Model 2	**2.02 (1.10, 3.69)**	1.68 (0.87, 3.24)	**2.03 (1.29, 3.19)**
Non bothersome tinnitus	Model 1	1.27 (0.98, 1.64)	**1.52 (1.18, 1.96)**	**1.31 (1.10, 1.56)**
	Model 2	1.19 (0.91, 1.55)	**1.46 (1.13, 1.89)**	**1.32 (1.11, 1.58)**

Odds ratio (OR) represents the odds of having a clinically relevant score on either the CES-D (≥16), HADS (≥8) or PSQI (≥6) in participants with mild or bothersome tinnitus. CI: confidence interval. Model 1: adjusted for sex and age. Model 2: additionally adjusted for highest achieved education, hearing loss, body mass index, alcohol use and smoking status. Significant effect estimates ($p<0.05$) are indicated in **bold**.

eTable 2. Association between participants with (non-) bothersome tinnitus and clinically relevant depressive symptoms, anxiety symptoms and self-reported sleep quality, stratified analyses for hearing impairment.

		Clinically relevant depressive symptoms	Clinically relevant anxiety symptoms	Clinically relevant lower sleep quality
		OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**
No hearing loss (<25 dB HL), N=3,676				
No tinnitus	Model 1	Ref	Ref	Ref
	Model 2	Ref	Ref	Ref
Bothersome tinnitus	Model 1	**3.48 (1.54, 7.87)**	1.59 (0.55, 4.61)	**2.71 (1.35, 5.45)**
	Model 2	**3.58 (1.57, 8.17)**	1.59 (0.55, 4.62)	**2.69 (1.34, 5.43)**
Non bothersome tinnitus	Model 1	0.94 (0.64, 1.40)	**1.53 (1.09, 2.15)**	**1.37 (1.07, 1.71)**
	Model 2	0.89 (0.60, 1.33)	**1.47 (1.03, 2.08)**	**1.36 (1.06, 1.77)**
Hearing loss (≥25 dB), N= 1,742				
No tinnitus	Model 1	Ref	Ref	Ref
	Model 2	Ref	Ref	Ref
Bothersome tinnitus	Model 1	1.45 (0.59, 3.53)	1.92 (0.83, 4.44)	1.55 (0.86, 2.81)
	Model 2	1.43 (0.57, 3.55)	2.04 (0.87, 4.78)	1.64 (0.90, 2.99)
Non bothersome tinnitus	Model 1	**1.63 (1.13, 2.33)**	**1.49 (1.01, 2.20)**	1.26 (0.98, 1.63)
	Model 2	**1.61 (1.11, 2.33)**	1.48 (1.00, 2.19)	1.28 (0.99, 1.66)

Odds ratio (OR) represents the odds of having a clinically relevant score on either the CES-D (≥16), HADS (≥8) or PSQI (≥6) in participants with with mild or bothersome tinnitus, stratified for hearing loss. CI: confidence interval. dB: decibel. Model 1: adjusted for sex and age. Model 2: additionally adjusted for highest achieved education, hearing loss, body mass index, alcohol use and smoking status. Significant estimates ($p<0.05$) are indicated in **bold**.

eTable 3. Association between participants with daily tinnitus and a relevant tinnitus handicap (vs. daily tinnitus and low tinnitus handicap) and clinically relevant depressive symptoms, anxiety symptoms and self-reported sleep quality.

		Clinically relevant depressive symptoms	Clinically relevant anxiety symptoms	Clinically relevant lower sleep quality
		OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**
Relevant tinnitus handicap versus no relevant tinnitus handicap	Model 1	3.44 (1.84, 6.43)	2.22 (1.18, 4.18)	1.96 (1.19, 3.24)
	Model 2	3.87 (1.99, 7.54)	2.38 (1.24, 4.58)	1.98 (1.19, 3.31)

In participants with daily tinnitus (N=625), tinnitus handicap was assessed by the Tinnitus Handicap Inventory – screening version (THI-s). Odds ratio (OR) represents the odds of having a clinically relevant score on either the CES-D (≥16), HADS (≥8) or PSQI (≥6) for a relevant tinnitus handicap (THI-s score ≥16) versus no relevant tinnitus handicap (THI-s score <16). CI: confidence interval. Model 1: adjusted for sex and age. Model 2: additionally adjusted for highest achieved education, hearing loss, body mass index, alcohol use and smoking status. Significant effect estimates (p<0.05) are indicated in **bold**.

eTable 4. Median scores for the mental health outcomes per tinnitus handicap category

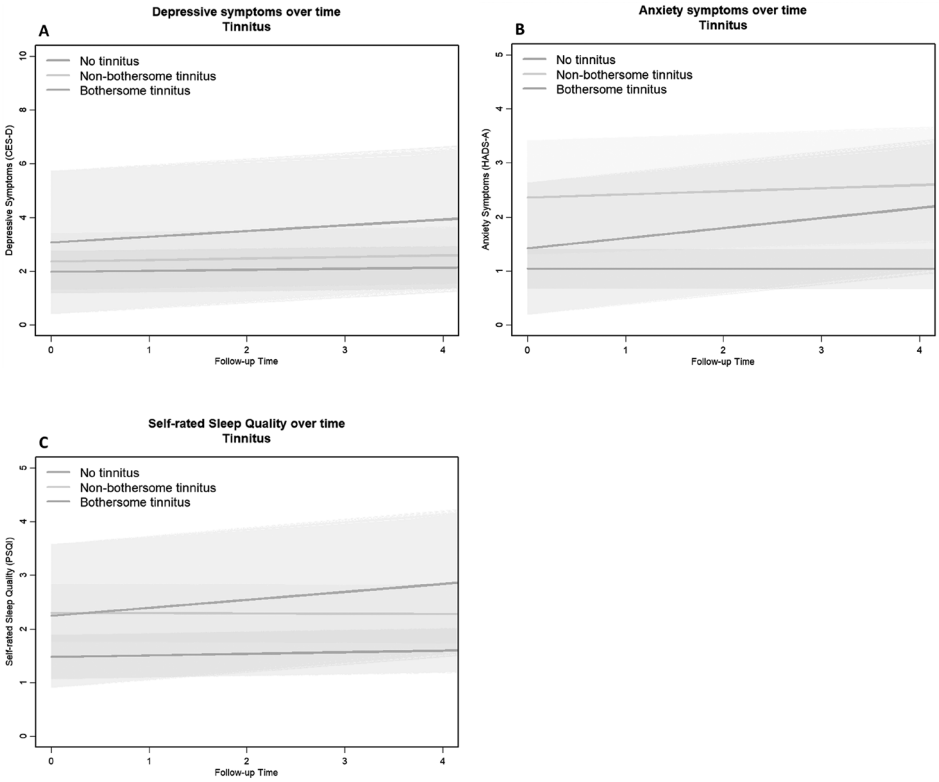
		CES-D	HADS	PSQI
Slight handicap (THI-s 0-6)	N=444	3 (1 - 8)	2 (0 – 4)	3 (1 – 5)
Mild handicap (THI-s 8-14)	N=123	5 (1 – 10)	3 (0 – 5)	4 (2 – 8)
Moderate handicap (THI-s 16-22)	N=60	7 (2 – 16)	3 (1 – 7)	4 (3 – 8)
Severe handicap (THI-s 24-30)	N=20	7 (3 – 13)	6 (2 – 7)	6 (3 – 10)
Catastrophic handicap (THI-s 32-40)	N=8	10 (0 – 26)	5 (1 – 8)	6 (2 – 14)

Overview of median (IQR) scores of the CES-D, HADS and PSQI for all with a THI-s score per THI-s handicap category.

eTable 5. Association between participants with (non-) bothersome tinnitus at baseline and depressive symptoms, anxiety symptoms and self-reported sleep quality over time.

		Depressive symptoms		Anxiety symptoms		Sleep quality	
		Intercept difference (95% CI)	**Slope difference (95% CI)**	**Intercept difference (95% CI)**	**Slope difference (95% CI)**	**Intercept difference (95% CI)**	**Slope difference (95% CI)**
Bothersome tinnitus versus no tinnitus	Model 1	0.30 (-0.08, 0.68)	-0.00 (-0.09, 0.09)	0.13 (-0.18, 0.45)	0.03 (-0.04, 0.10)	0.21 (-0.09, 0.51)	-0.00 (-0.06, 0.05)
	Model 2	0.34 (-0.04, 0.72)	-0.00 (-0.09, 0.09)	0.15 (-0.16, 0.47)	0.03 (-0.05, 0.10)	0.20 (-0.09, 0.50)	-0.01 (-0.06, 0.05)
Mild tinnitus versus no tinnitus	Model 1	**0.16 (0.03, 0.30)**	-0.01 (-0.05, 0.02)	0.10 (-0.02, 0.21)	-0.00 (-0.03, 0.02)	**0.17 (0.07, 0.28)**	-0.00 (-0.02, 0.02)
	Model 2	**0.17 (0.03, 0.31)**	0.00 (-0.03, 0.04)	0.10 (-0.02, 0.21)	-0.00 (-0.03, 0.02)	**0.18 (0.07, 0.28)**	-0.00 (-0.02, 0.02)
Tinnitus versus no tinnitus	Model 1	**0.18 (0.04, 0.31)**	-0.01 (-0.05, 0.02)	0.10 (-0.01, 0.21)	0.00 (-0.02, 0.03)	**0.18 (0.08, 0.28)**	-0.00 (-0.02, 0.02)
	Model 2	**0.19 (0.05, 0.32)**	-0.01 (-0.04, 0.02)	0.10 (-0.01, 0.21)	0.00 (-0.02, 0.03)	**0.18 (0.08, 0.28)**	-0.00 (-0.02, 0.02)

Intercept difference represent the difference in log (raw score+1) at baseline for the CES-D (for depressive symptoms), HADS – anxiety subscale (for anxiety symptoms) or PSQI (for self-reported sleep quality) in participants with mild or bothersome tinnitus versus participants without tinnitus. Slope difference represents the additional change in log(raw score+1) per year increase in follow-up time. CI: confidence interval. Model 1: adjusted for sex and age. Model 2: additionally adjusted for highest achieved education, hearing loss, body mass index, alcohol use and smoking status. Significant effect estimates ($p<0.05$) are indicated in **bold**.

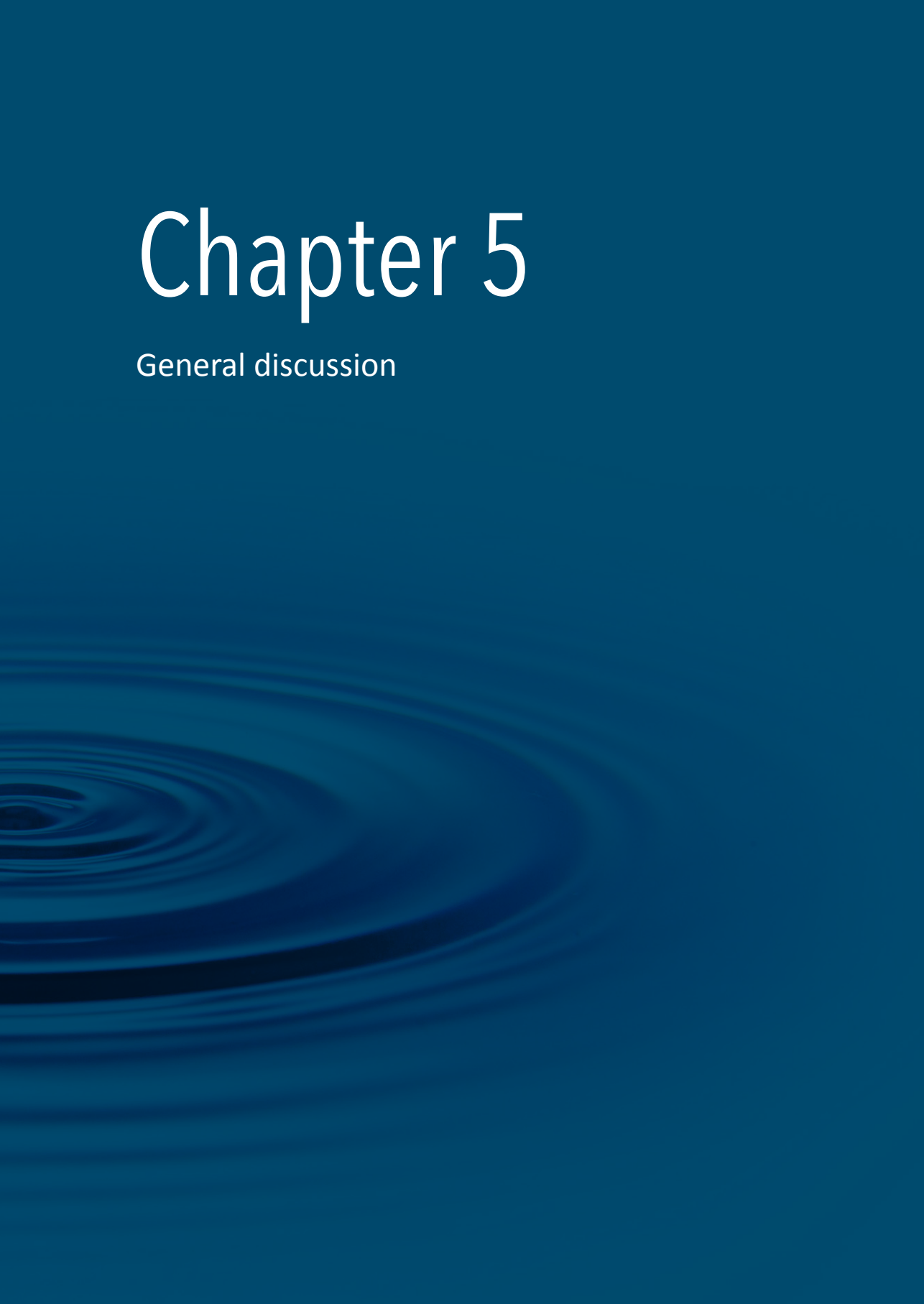


eFigure 1. Association between participants with and depressive symptoms, anxiety symptoms and self-reported sleep quality over time.

Results of the linear mixed models, showing the average change in log(raw score+1) over time for the CES-D (for depressive symptoms), HADS – anxiety subscale (for anxiety symptoms) or PSQI (for self-reported sleep quality).

Chapter 5

General discussion



It is estimated that around 1 million people in the Netherlands have tinnitus[1], which would represent around 5.7% of the total population. The interest in tinnitus in mainstream media has been increasing in recent years. Social media appear to play an important role, with many public stories of people suffering from tinnitus, even to the extreme of requesting euthanasia. The first euthanasia case for tinnitus in The Netherlands was documented in 2014 and it has been given several times since. Fortunately, most people learn to live and cope with their tinnitus. Scientific interest in tinnitus has increased as well. Between 2000 and 2020, scientific output per annum for tinnitus on Pubmed increased 3.57 fold, which is above the average of 3.25 across all research fields. Despite the increase in scientific output, we still don't have a good understanding of tinnitus pathophysiology nor are able to causally treat it.

In this thesis, we have ascertained tinnitus prevalence in a population-based study of middle-aged and older adults and we have aimed to further specify and identify determinants for tinnitus. The ultimate goal of this research is to contribute to the establishment of a risk profile for tinnitus, in order to personalize treatment strategies and work towards prevention.

Is tinnitus an ageing disease?

One can argue that tinnitus is largely an age-related disease similar to hearing loss. The basis for this hypothesis is that tinnitus prevalence increases with age and that tinnitus has a strong association with hearing loss, which is also strongly related to age[2,3]. A meta-analysis from 2016 showed that the reported prevalence of tinnitus is lowest among of young adults (<25 years), and steadily increases towards older age groups and was highest in adults above 65 years of age[4]. It should be noted though that sample sizes of most of these studies were limited, especially at high ages. In contrast, we could not establish a relationship between tinnitus prevalence and increasing age in our study population of 50 years and above (**chapter 2.1**). Second, hearing loss was equally prevalent in participants with and without tinnitus. These are both arguments that tinnitus is not an age-related condition per se. As literature reports an association with age and we did not find a relationship between tinnitus and hearing loss above the age of 50, it might be that a plateau is reached at 50-60 years.

This was further supported by the findings of our neuro-imaging study (**chapter 4.1**). Brain-tissue volumes decreases due to age-related degeneration of brain tissue[5] and this has also been demonstrated in people with age-related hearing loss[6]. If age would induce tinnitus or contribute to the incidence of tinnitus, one would expect an association between tinnitus and lower brain tissue volumes. We found the contrary:

in participants with tinnitus, brain volumes – especially white matter - were actually larger compared to participants without tinnitus. To date, only one other study investigated brain-tissue volumes in participants with tinnitus. This study showed larger grey tissue volumes of the left auditory cortex in a middle-aged population[7], which again is contradictory to the principle of age-related decrease of brain volume. A little more is known from functional-MRI studies. A meta-analysis (of nine studies) on tinnitus found that mainly non-auditory regions showed aberrant neural activity[8]. Brain imaging in populations with tinnitus is not often done, therefore most studies to date are conducted in small populations, resulting in a high degree of uncertainty. Longitudinal studies including self-reported tinnitus and imaging are needed to determine whether incident tinnitus is subject to structural changes of the brain and what specific areas play a role.

To investigate whether tinnitus is an ageing disease incidence data are needed, i.e. how many develop tinnitus over a certain period of time. There are only a few studies that report on the incidence of tinnitus. In England, a study of case records from general practitioners and linked hospital data, showed a tinnitus incidence of 5.4 new cases (in the second line of care) per 100,000 person years[9]. A recent UK Biobank study found a 8.7% four-year incidence of tinnitus[10]. These exact numbers are, however, unknown for the Dutch population. Therefore, longitudinal population-based studies would help to clarify tinnitus incidences and reveal the possible underlying factors of incident tinnitus, including age. Future studies are warranted into the development or deterioration of brain tissue volumes of older adults with incident tinnitus, to further investigate the association between tinnitus and age-related changes of the brain. To conclude, we did not find a strict relationship between aging and tinnitus in older adults. So tinnitus is unlikely to be age dependent in older adults, in contrast to the strong age-dependency of hearing loss. Further studies, including longitudinal data, should shed more light on whether and if so, how age-related hearing loss is connected to tinnitus development.

Does hearing loss cause tinnitus?

Tinnitus and hearing loss are often mentioned in the same breath. It is true that some people with hearing loss have tinnitus, but not all. And vice versa; many people with tinnitus have hearing loss, but not all (**chapter 2.1**). It is thought that tinnitus occurs through similar processes as phantom limb pain or perception[11]. For many, the frequency of their tinnitus, or the tone they hear, equals the worst measured frequency in hearing[12]. Some people with tinnitus with normal audiometry may have hidden hearing loss - a form of hearing loss that cannot be detected with common audiometry[13]. Tinnitus can even be induced by hearing loss simulation[14]. Both brain

and cochlea may initiate tinnitus[15], although the cochlea, the peripheral hearing organ, is thought to be the main source of tinnitus generation. However, central neural pathways in the brain encompassing both the auditory and non-auditory parts are needed for tinnitus maintenance[16]. It can be argued that different pathophysiological mechanisms can be at play for tinnitus initiation between those with and without hearing loss. There may be similar central mechanisms at play for tinnitus maintenance irrespective of the site of initiation[17].

Tinnitus with and without hearing loss may reflect two different pathophysiological mechanisms. In our studies, we decided to stratify for hearing loss , to see whether the investigated associations were mediated or confounded by the presence of hearing loss. The study described in **chapter 2.2** is an example of this. In participants with hearing loss, speech in noise performance was worse when tinnitus was present, compared to no tinnitus. This effect was not seen in participants without hearing loss, where speech in noise understanding was similar between those with and without tinnitus. This indicates a mediating role of hearing impairment in the effect of tinnitus on speech understanding. None of the other existing studies that have investigated speech in noise intelligibility in tinnitus patients adjusted for the role of hearing loss in this association and may therefore have reported contradictory results. Adjustment for hearing loss is not often performed in other studies investigating tinnitus, which raises the question whether the existing associations would change when adjusted for hearing loss.

And what about other determinants of tinnitus?

In this thesis, we investigated several other determinants that have been associated with tinnitus in the past, including cardiovascular health status[18-20]. We studied cardiovascular health as a composite outcome, as there may be an additive effect when more than one component is suboptimal. A poor cardiovascular health as a composite outcome has been associated with worse hearing[21,22], and we also found an association with an increased risk of prevalent tinnitus (**chapter 3.1**). But, after stratification for hearing loss, the association of poor cardiovascular health remained only present in participants without hearing loss. This suggests a mediating role of hearing loss. The fact that we found an association for the group without hearing loss strongly suggests that, next to the cochlea, the brain also plays a role in the pathophysiological pathways, although the exact mechanism still need to be elucidated. The mechanism of the association between poor cardiovascular health and tinnitus may be found in brain damage and poorer cochlear function due to microangiopathy. Several individual components of the cardiovascular health outcome are known to cause microangiopathy of the brain, including hypertension,

hypercholesterolemia and diabetes[23]. Longitudinal studies are needed to investigate whether the deterioration of cardiovascular health, or specific components of cardiovascular health, is associated with tinnitus incidence. This could in turn help shed light on possible pathophysiological pathways.

Another determinant investigated in this thesis is the use of ototoxic medication. This type of medication includes platinum-based chemotherapeutics, aminoglycoside antibiotics, quinines, salicylates or loop diuretics and are known to cause high-frequency loss that in turn is often associated with tinnitus[24,25]. Especially for platinum-based chemotherapeutics or highly dosed intravenously administered aminoglycoside antibiotics, the ototoxic capacity is well known[26]. For some other frequently used medication groups, the ototoxic effects are less clear[27]. In the present thesis we investigated the possible ototoxic effect of oral macrolide antibiotic treatment (**chapter 3.2**) and antibiotic ear drops (**chapter 3.3**). Macrolide antibiotics are commonly used but their possible ototoxic effect is not very clear. We found that a history of macrolide antibiotic treatment is associated with tinnitus but not with hearing loss (**chapter 3.2**), although we cannot elaborate on the exact mechanism of action. More research is needed to quantify the ototoxic capacity of some commonly and often prescribed drugs.

Next, we found increased hearing thresholds in participants with a history of antibiotic ear drops, but no association with tinnitus. Although the ototoxicity of these ear drops is known, we performed the first population-based study on this subject (**chapter 3.3**). By excluding all participants with a conductive hearing loss, we minimalized the risk of confounding by indication. Each drug has its own mechanism of action and therefore its own way of causing ototoxicity and might even work in a synergistic manner with other risk factors for hearing loss or tinnitus[28]. It is thought that, similar to other subtypes of tinnitus, ototoxic tinnitus may be caused by either cochlear damage or involvement of complex neural networks, depending on the specific drug[28]. Further research investigating tinnitus-initiating mechanisms of medication should be able to help find causal pathways of tinnitus development.

Another commonly investigated and reported determinant of tinnitus is mental health status. A poor mental health and tinnitus often coexists and may lead to a downward spiral[29]. For the association between mental health outcomes and tinnitus, the main question remains which causes which. In **chapter 4.2** we investigated this association in two ways. First, we wanted to know whether depressive and anxiety symptoms or poor (self-reported) sleep were present in participants with non-bothersome tinnitus, as it is already known that this association exists in participants with more severe

tinnitus, which we confirmed. Additionally we indeed found this association also to exist in participants with non-bothersome tinnitus. One of the key mechanisms involved in the association between tinnitus and mental health problems is the limbic system. The limbic system is known to be part of the emotional control system in the brain[30] and is reportedly often overactive in patients with mental health problems[31]. Additionally, the limbic system appears to play a large role in the tinnitus burden[32]. Features of tinnitus burden are closely related to that of anxiety or depression. This adds to the idea that the limbic system might be overactive in all with tinnitus, even in those without significant burden/complaints.

This has been confirmed in our results as the group with high tinnitus burden show a strong association with psychopathology outcome measures on depression and anxiety.

The association between mental health and tinnitus may be confounded by hearing loss, as hearing loss and mental health problems are known to be associated[33,34]. It can be hypothesized that mental health problems may have a more prominent role in tinnitus initiation in the absence of hearing loss[35]. However, we did not find hearing loss to confound the association between mental health problems and tinnitus. Next, we investigated whether tinnitus affects sleep, depressive and anxiety symptoms over a longer period of time. We found that tinnitus at baseline is associated with poorer mental health outcomes four years later, but we were unable to investigate whether this association was also there the other way around due to lack of incident tinnitus in the follow-up data. Therefore, the question still remains whether tinnitus causes a poorer mental health or vice versa, and this association may also be present in both ways. Investigating a large population and following up on the data may give more certainty on the direction of association.

Tinnitus risk reduction?

Hearing loss is the most prominent risk factor for tinnitus, but not always preventable. Two highly prevalent types of hearing loss are age-related and noise-induced hearing loss. Age-related hearing loss has a multifactorial origin, which is partly unpreventable. The risk of severe age-related hearing loss may be reduced by preventative lifestyle interventions, so the contribution of modifiable environmental risk factors is minimized[36]. Noise-induced hearing loss is a preventable risk factor for tinnitus[19,20,37]. Especially in the young, noise-induced tinnitus is a rising problem[38].Through noise hygiene and education of the young, the role as a risk factor for tinnitus may be minimized. We were, unfortunately, unable to investigate noise exposure as a risk factor in this population as we did not have information on (lifetime) noise exposure.

Ototoxicity-associated tinnitus may be prevented by wisely choosing medication, although in many cases it has been a deliberate choice. Still, it would be worthwhile for prescribers to assess the likelihood for tinnitus amongst other side-effects and choose another drug whenever possible.

No literature exists on preventing or reducing tinnitus risk through a healthy life style, although one can argue that this may work. On the one hand, cardiovascular health status would improve, on the other hand mental health may benefit as well. Both of these actions can have a beneficial effect on tinnitus.

Methodological considerations

The studies in this thesis were conducted within the Rotterdam Study. The specific strengths and limitations per study are described in the respective chapters. Some general methodological considerations are written below.

Tinnitus definition

Tinnitus is generally defined as a beep/buzz/ring heard in the head or ears, in absence of an objective sound source. In absence of a gold standard, the exact definition differs per study[4]. By nature of its definition, tinnitus is a subjective measure, which makes it hard to compare outcomes between studies. Still, most studies use a similar question to assess tinnitus to the question we use in the Rotterdam Study: 'Do you experience sounds in the head or in (one of) the ears (such as whizzing, peeping, or humming) without an objective external sound source being present'. Possible answers to this question were: 'no, never'; 'yes, less than once a week'; 'yes, more than once a week but not daily' and 'yes, daily'. The fact that the question used in the Rotterdam Study is similar to that in other studies, should add to the external validity of the results reported in this thesis. In all of our studies we investigated tinnitus as a binary outcome in which we defined prevalent tinnitus as being present more than one day per week. We think this is a valid definition, as even tinnitus patients who visit the Erasmus MC outpatient clinic for their tinnitus, do not all report daily tinnitus (data not shown in this thesis).

Next to the challenges in defining what constitutes tinnitus, the severity of tinnitus is also a hard concept to grasp. Severity can be interpreted in two ways. First, as the loudness of tinnitus - however this cannot be objectified or described in all patients. Second, the severity can be defined as the extent to which the tinnitus disturbs someone in their daily life, which is most commonly used. The impact on daily life was assessed in two ways in the Rotterdam Study. Firstly with a question ('does the tinnitus interfere with your daily life?') for all that report any tinnitus (whether daily

or less frequently). Secondly, in the subgroup of participants with daily tinnitus, the screening version of the tinnitus handicap inventory (THI-s) was used[39]. There are several different validated questionnaires to assess tinnitus severity, including the Tinnitus Questionnaire, Tinnitus Functional Index, Tinnitus Handicap Questionnaire[40]. They are all able to identify the patients or participants with a high tinnitus burden although the domains covered in the different questionnaires can differ. This may be of influence in comparing results between studies.

Study design

The Rotterdam Study is an ongoing population-based cohort study. This type of study is a neat design to investigate large range of diseases and exposures, resulting in findings that in theory are generalisable to a large population. Apart from the up-sides of this study design, there are some limitations that should be mentioned. Most importantly, results from a population-based study should be interpreted with care as associations do not equal causation[41]. It generally cannot be used to investigate precise pathophysiological pathways. Next, population-based studies can be subject to several types of bias - specifically confounding, selection and information bias. We have aimed to minimize the risk for bias in all studies included in this thesis. This was achieved through random sampling from the general population, maintaining high response rates over several rounds of follow-up, blinded measurements and thorough adjustment in statistical analyses. Despite these efforts, residual bias or confounding is always possible.

Cross-sectional analyses

Almost all studies in this thesis have a cross-sectional design. Although the Rotterdam Study has been running since 1989, outcomes and measurements related to hearing loss and tinnitus were only added to the study's core protocol in 2011. Follow-up data is therefore still limited, which means we were unable to investigate a temporal aspect within most of the associations we found. Assessment of such a temporal effect would be very interesting, as this might elucidate on the causal pathway of the determinant and the outcome. However, there are no published longitudinal studies on tinnitus incidence and determinants. As tinnitus may also disappear, this is another interesting aspect to study in a longitudinal design.

Future perspectives and clinical implication

Even though tinnitus research has taught us much about the disease, a lot still needs to be investigated. Epidemiological studies are not ideal to show causal relationships or help determine pathophysiological pathways. The ultimate goal would be to determine a risk profile which would show whether someone is likely to develop

tinnitus and, more importantly, which treatment strategy would be appropriate for this person.

The studies in this thesis have a twofold implication. First, to provide more insight in associated pathways in developing tinnitus. And second, to create personal risk profiles for initiating and maintaining tinnitus. A major step in a personal risk profile is someone's genetic make-up. Genetics of hearing-related conditions are an ever developing field[42]. Genome-wide association studies (GWAS) are a specific type of genetic studies commonly done in larger populations. This type of study generally investigates whether some variations in the genome are more frequent in people with a certain disease than in those without this disease. This could be seen as a first step in finding genes related to a disease. For tinnitus, a recent study reported 3 loci and 8 genes associated with tinnitus, which led to the conclusion that tinnitus is a polygenic disorder[43]. At the moment we are involved in an even larger GWAS for tinnitus, which will hopefully help to find more genes and possible genetic pathways to determine tinnitus risk. When genetic pathways are known, they can be used for possible diagnostic criteria and eventually therapy development. Personalized therapy will be the future of medicine in general. As genetics help us determine a specific (risk) profile per person, its application in clinical practice may involve the development of a pharmacogenetic passport that can be developed. This special passport helps determine the choice of medication as well as the optimal dosage of a certain medication, as metabolism known to differ between persons[44]. At the moment, the backbone of tinnitus treatment is cognitive behavioural therapy[45], although many people would prefer daily oral medication[1]. Hopefully, personalized therapy will be able to optimize tinnitus treatment in the future.

Future clinical studies should focus on the development and clinical implementation of both a profile from known risk factors (as described in this thesis) as well as a polygenic risk score. These steps will probably help towards the development of more targeted therapeutic strategies and ultimately towards the prevention of tinnitus, or at least a reduction of its burden.

Conclusion

There are several conclusions that can be drawn from this thesis. First, tinnitus has no strict linear relationship with age in our study population of adults > 50 years of age, although it is closely related to ageing disorders like hearing loss and cardiovascular health. When available, proper adjustment for hearing loss should therefore be a standard procedure in tinnitus research. This will help to make a distinction between different mechanisms that are at play in tinnitus pathophysiology.

Second, we found new risk factors for tinnitus, including impaired cardiovascular health and macrolide antibiotic usage. In addition, we provided new insights in the association between tinnitus and the known risk factors hearing loss and mental health problems. Third, we are still a long way from being able to assemble a tinnitus risk profile. Studies on the genetic background can be of specific importance in achieving that goal. Fourth, there is a lack of longitudinal studies in tinnitus research. They may shed more light on the direction of the associated risk factors and help determine cause and consequence.

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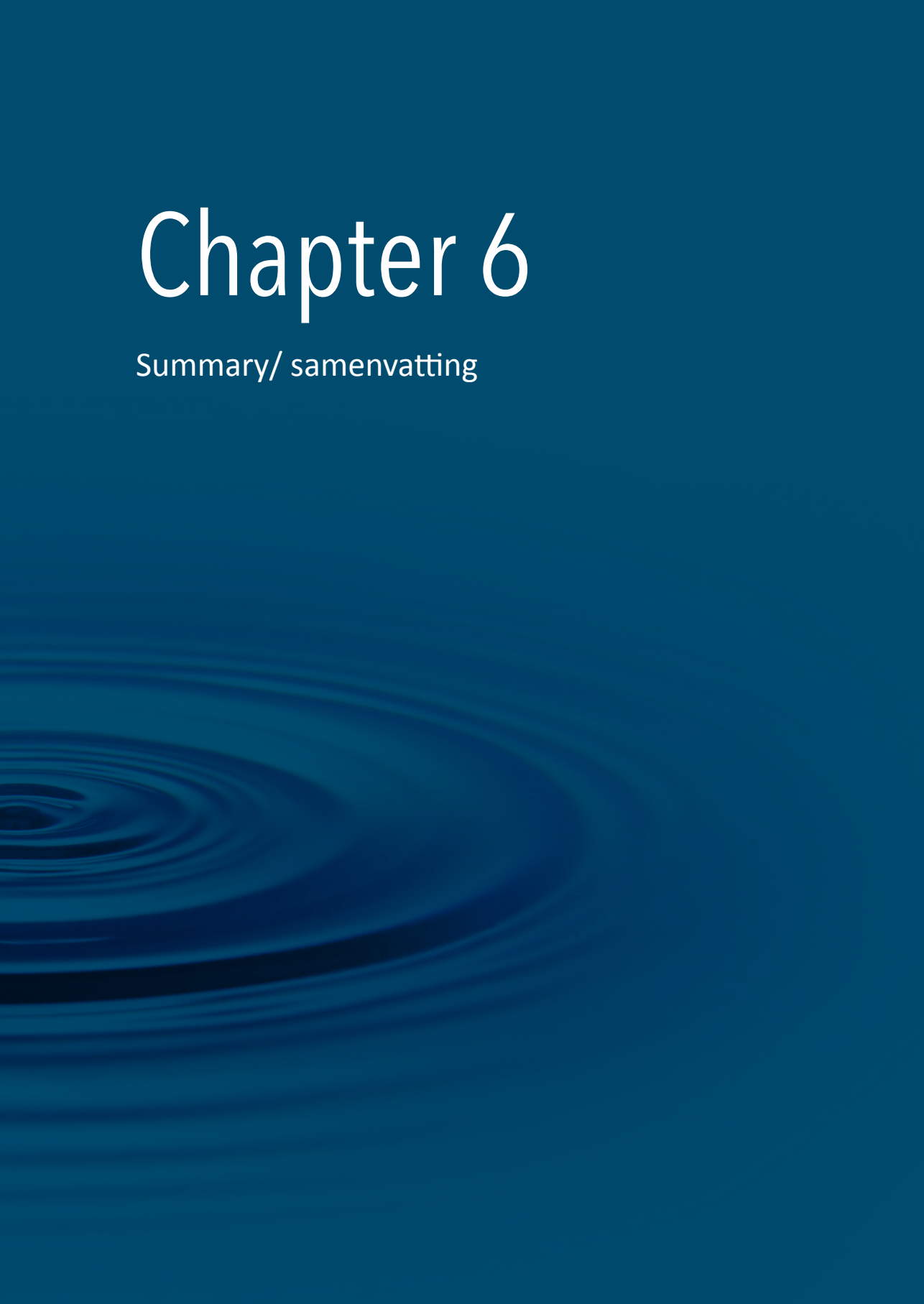
Chapter 6

Summary/ samenvatting



SUMMARY

Tinnitus is defined as a beep/buzz/sound in the head or ears. If there is an external sound source, which can be heard by an observer this is considered objective tinnitus, this is in the minority of cases the origin. In case of an absent external sound source it is called 'subjective tinnitus' which we is the subject of this thesis. Fortunately, for most people with tinnitus, the burden is so little they can live well with it. On the other hand, there is a small subset with considerable complaints, that enters the health care system as a result. The main hypothesis on the pathophysiology is that alterations in both cochlea and brain are necessary for tinnitus generation and maintenance. However, the question remains why one person does develop tinnitus and another does not. Epidemiological studies can help determine risk factors for tinnitus. The main goal for this thesis has been to further specify and identify determinants for tinnitus in a population of older adults. All research in this thesis is embedded within the population-based Rotterdam Study.

In **chapter 2** we investigated the prevalence of tinnitus and its relation with hearing loss in this population of older adults. In **chapter 2.1** we found that the prevalence of tinnitus in participants above 50 years of age is similar in all following 5-year age-groups, whereas the prevalence of hearing loss was higher in each age-group. In contrast to what we expected, we found that the likelihood for tinnitus was double in presence of hearing loss but similar in each age-group. This resulted in the conclusion that tinnitus is not a strict age-dependent disorder, in contrast to the strong age-dependency of hearing loss. Next, we investigated whether hearing function would be affected by tinnitus. We found in **chapter 2.2** that speech-in-noise understanding is similar in participants with and without tinnitus. Only in participants with hearing loss we found that speech-in-noise understanding was reduced in those with tinnitus as compared to no tinnitus. This was the first study to investigate speech-in-noise understanding in participants with tinnitus in which the mediating role of hearing loss was accounted for.

In **chapter 3** we investigated modifiable risk factors for tinnitus. Reduced cardiovascular health is associated with hearing loss, which led to the question whether this was similar for tinnitus. Some individual components of cardiovascular health were shown to increase the risk for tinnitus. In **chapter 3.1** we investigated the association between cardiovascular health as a composite outcome and tinnitus. We found that a poor cardiovascular health increased the likelihood to have tinnitus, but only in those without hearing loss. In the next two chapters (**chapter 3.2, chapter 3.3**) we investigated the possible role of ototoxic medication in the risk for tinnitus. We were

the first to report an increased likelihood of tinnitus prevalence and incidence after macrolide antibiotic prescriptions (**chapter 3.2**). In this study we did not find that the use of macrolide antibiotics resulted in higher hearing thresholds. However, in **chapter 3.3** we found that the use of antibiotic eardrops increased hearing thresholds, which may contribute to the presence of hearing loss. However, these antibiotic eardrops did not increase the risk for tinnitus.

In the next chapter (**chapter 4**) we focused on the interplay between tinnitus and the brain. Changes in the brain are thought to be essential in tinnitus generation and maintenance. In **chapter 4.1** we reported that participants with tinnitus had larger white matter brain volumes in all lobes as compared to participants without tinnitus. Whether tinnitus increases white matter volumes or that larger white matter volumes increase the risk for tinnitus still needs to be elucidated. On a more functional level, we found in **chapter 4.2** that participants with tinnitus, both bothersome and non-bothersome, had more depressive and anxiety symptoms and reported poorer sleep quality. Reduced psychosocial well-being is recognized as an important factor in tinnitus as a disease. It is new that we found that participants with a low tinnitus burden, or non-bothersome tinnitus, also report lower psychosocial well-being. We do not know whether tinnitus causes the psychosocial problems or that they cause tinnitus, but we think that both directions are plausible.

It should be noticed that in both **chapter 3 and chapter 4** most studies are cross-sectional, resulting in a point estimate. To investigate whether this potential risk factor that is investigated in these studies is likely to be a causal risk factor longitudinal studies are needed. This gives room for future studies to help us elucidate on the direction of the associations investigated.

The general discussion, **chapter 5**, describes the main findings, how these of the different studies are related. The results and conclusions are put side by side with current literature and subsequently, methodological considerations, clinical implications and directions for future research are discussed.

SAMENVATTING

Tinnitus wordt gedefinieerd als een piep/suis/geluid in het hoofd of in de oren. Wanneer er voor dit geluid een externe geluidsbron aan te wijzen is, die ook door een buitenstaander gehoord kan worden noemen we dit objectieve tinnitus. Echter is dit in de minderheid van de gevallen zo. Wanneer er voor het geluid dat gehoord wordt geen extern te horen geluidsbron is noemen we dat subjectieve tinnitus. Deze vorm van tinnitus hebben we verder onderzocht in dit proefschrift. Gelukkig is de tinnitus last voor de meerderheid zo klein dat ze er goed mee kunnen leven. Daar staat wel een kleine groep met behoorlijke klachten tegenover die zorg zal zoeken voor hun tinnitus klachten.

De meest gangbare hypothese van de pathofysiologie is dat er veranderingen in zowel de cochlea als het brein nodig zijn om tinnitus te ontwikkelen en het te laten bestaan. De vraag blijft echter waarom de ene persoon tinnitus ontwikkelt en de ander niet. Epidemiologisch onderzoek draagt bij aan het identificeren van risicofactoren voor tinnitus. Het doel voor het schrijven van dit proefschrift was om determinanten van tinnitus te identificeren en definiëren in een oudere populatie. Alle onderzoeken in dit proefschrift zijn gedaan met data verzameld in de Rotterdam studie, een populatie studie.

In **hoofdstuk 2** onderzochten we de prevalentie van tinnitus en de relatie met gehoorverlies in deze oudere populatie. In **hoofdstuk 2.1** beschrijven we dat de prevalentie van tinnitus bij deelnemers van 50 jaar en ouder is vergelijkbaar in alle 5-jaars leeftijdsgroepen daarboven, terwijl de prevalentie van gehoorverlies in elke leeftijdsgroep hoger was. Tegengesteld aan wat we verwachtten, vonden we dat kans om tinnitus te hebben twee keer zo groot was als iemand ook gehoorverlies heeft, maar dat dit hetzelfde was in elke onderzochte leeftijdsgroep. Hieruit concludeerden we dat tinnitus niet persé een leeftijd gerelateerde aandoening is, zoals gehoorverlies dat wel is. Vervolgens hebben we onderzocht of de luisterfunctie door tinnitus beïnvloed wordt. In **hoofdstuk 2.2** vonden we dat spraak-in-ruis verstaan vergelijkbaar is tussen deelnemers met en zonder tinnitus. Wel bleek dat wanneer er sprake is van gehoorverlies en tinnitus het spraak-in-ruis verstaan verminderd is ten opzichte van de deelnemers zonder tinnitus. Dit onderzoek was de eerste waarin het spraak-in-ruis verstaan bij mensen met tinnitus werd onderzocht waarin ook gecorrigeerd werd voor de mediërende rol van gehoorverlies in de associatie.

In **hoofdstuk 3** onderzochten we modificeerbare risicofactoren van tinnitus. Een verminderde cardiovasculaire gezondheid is geassocieerd met gehoorverlies. Dit

leidde tot de vraag of dit ook zo zou zijn voor tinnitus. Van verschillende individuele componenten van cardiovasculaire gezondheid is in andere studies laten zien dat ze het risico op tinnitus kunnen vergroten. In **hoofdstuk 3.1** onderzochten we de associatie tussen cardiovasculaire gezondheid als een gecombineerde uitkomst en tinnitus. We vonden dat een verminderde cardiovasculaire gezondheid de kans op tinnitus vergroot, maar alleen in de deelnemers met gehoorverlies. In de volgende twee hoofdstukken (**hoofdstuk 3.2, hoofdstuk 3.3**) onderzochten we mogelijke rol van ototoxische medicijnen in het risico op tinnitus. Ons onderzoek waarin we een vergrote kans op tinnitus prevalentie en incidentie laten zien na recepten voor macrolide antibiotica (**hoofdstuk 3.2**) was de eerste om dit zo te laten zien. In ditzelfde onderzoek vonden dat recepten voor macrolide antibiotica niet geassocieerd was met verhoogde gehoordrempels. Terwijl we in **hoofdstuk 3.3** wel verhoogde gehoordrempels vonden na voorschriften voor antibiotische oordruppels, wat kan bijdragen aan het optreden van gehoorverlies. We vonden geen verhoogd risico op tinnitus na antibiotische oordruppels.

In het volgende hoofdstuk (**hoofdstuk 4**) verlegden we de focus naar het samenspel tussen tinnitus en het brein. Veranderingen in het brein worden essentieel geacht voor het ontstaan van tinnitus en het blijven voortbestaan van de tinnitus. In **hoofdstuk 4.1** laten we zien dat deelnemers met tinnitus grotere witte stof volumina in alle loben van het brein hadden ten opzichte van deelnemers zonder tinnitus. De vraag of tinnitus de witte stof volumina vergroot of dat grotere witte stof volumina het risico op tinnitus vergroten moet nog beantwoord worden. Op een meer functioneel niveau, vonden we in **hoofdstuk 4.2** dat deelnemers met tinnitus, zowel belemmerend als niet belemmerend, meer depressieve en angst symptomen hadden en aangaven slechtere slaap kwaliteit te ervaren. Een verminderd psychosociale gezondheid wordt gezien als een belangrijke factor voor tinnitus als aandoening. Het is nieuw dat we hebben laten zien dat deelnemers met tinnitus met weinig klachten, ook wel niet belemmerende tinnitus, ook een verminderde psychosociale gezondheid hebben. We weten niet of tinnitus deze psychosociale problemen veroorzaakt of vice versa, maar we denken dat het beide kanten op kan gaan.

Een opmerking die geplaatst moet worden is dat zowel **hoofdstuk 3** als **hoofdstuk 4** bestaan uit voornamelijk cross-sectionele onderzoeken die een waarde geven van alleen dat moment. Als je zou willen onderzoeken of die specifieke risicofactor in een studie waarschijnlijker een causale risicofactor is zou je een longitudinale studie moeten gebruiken. Dat dit nog niet is gedaan biedt ruimte voor onderzoeken in de toekomst om dit uit te zoeken.

In **hoofdstuk 5**, de algemene discussie, worden de hoofdbevindingen beschreven en hoe deze van de verschillende studies aan elkaar gerelateerd zijn. De resultaten en conclusies worden afgezet tegen de bestaande literatuur en vervolgens worden methodologische aandachtspunten, klinische implicaties en mogelijke toekomstige onderzoeken besproken.

Chapter 7

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PhD portfolio

List of publications

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PhD training	Year	ECTS
Research skills		
Master of Science in Clinical epidemiology, NIHES	2018-2019	70.2
Integrity in Science, Erasmus MC	2019	0.3
Basic course on regulations and organization for clinical investigators (BROK)	2016	1.5
BROK reregistration	2020	0.1
Conferences		
233th Scientific meeting of Dutch society for ENT: oral	2018	1.0
CANmeeting, Nicosia, Cyprus: oral/poster	2018	1.6
234th Scientific meeting of Dutch society for ENT: oral	2019	1.0
European Federation of Audiology Societies, Lisbon, Portugal: oral	2019	1.6
In depth courses, seminars, workshops and research visits		
Weekly research seminars, department of epidemiology, Erasmus MC	2018-2021	3.5
Biweekly research meeting, department of otorhinolaryngology, Erasmus MC	2019-2021	2.5

Teaching activities

Lectures		
Supervising various 3th year medical student workgroups for	2018-2021	1.0
Supervising various ER, OR and anaesthesiology nurses workgroups in training	2018-2021	1.0
Supervising students		
Junior MedSchool student: Tinnitus and the brain	2018	1.5
Master thesis of N. Ly: Ototoxicity of aminoglycoside eardrops	2019-2020	4.0
Master thesis of MH Duong: Ototoxicity of statin medication	2021	4.0

Other

Peer review	2021	0.5
Coaching		
Coaching 1st-3rd year medical students	2018- 2021	2.0

LIST OF PUBLICATIONS

**authors contributed equally*

ENT-related publications

Trpchevska N*, **Oosterloo BC***, Freidin MB, Broer L, Goedegebure A, Williams FMK*, Cederroth CR*, Nagtegaal AP*. Genome-wide association meta-analysis identifies 12 novel loci for age-related hearing loss. *In preparation*

De Feijter M, **Oosterloo BC**, Gangapersad IJS, Goedegebure A, Ikram MA, Luik A. The cross-sectional association between tinnitus and acitgraphy-estimated sleep in a population based cohort of middle aged and elderly persons. *Submitted*

Oosterloo BC, Nagtegaal AP, Ly FN, Visser LE, Baatenburg de Jong RJ, Goedegebure A, Stricker BHC. Ototoxicity of ototopical antibiotic prescriptions. *Submitted*

Oosterloo BC, Voortman T, Baatenbug de Jong RJ, Kavousi M, Goedegebure A. Poor cardiovascular health increases risk for tinnitus; results from the Rotterdam Study. *Submitted*

Vanoverschelde A, **Oosterloo BC**, Ly FN, Goedegebure A, Stricker BCh, Lahousse L. Macrolide-associated ototoxicity: a cross-sectional and longitudinal study to assess the association of macrolide use with tinnitus and hearing loss. *J Antimicrob Chemother. 2021 Sep 15;76(10):2708-2716*

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ABOUT THE AUTHOR

Berthe Cornélie (Neelke) Oosterloo was born on December 29th 1990, in Berkel & Rodenrijs, the Netherlands. She grew up in Rotterdam. In 2008 she graduated from the Marnix Gymnasium in Rotterdam and started medical school at the Erasmus University Rotterdam. In 2011-2012 she did her medical school related research internship at the Pediatric Intensive Care Unit under the supervision of dr. Koen Joosten. In her thesis she investigated the role of nutritional status at PICU admission and outcome. After she obtained her BSc in 2012, she did a year-long extra-curricular research internship at the Burrin Lab at the Baylor College of Medicine, Children's Nutrition Research Centre in Houston, Texas (USA). Here she investigated different feeding strategies for premature babies. In 2013 she started working on her MSc in Medicine in Rotterdam, which she graduated in 2016.

Even though Neelke started her research career in Paediatrics, she subsequently shifted her interest to Otorhinolaryngology. After medical school she started as a researcher at the Amsterdam Medical Centre working with prof. dr. Ruurd van Elburg and dr. Arine Vlieger on the INCA study, investigating the consequences (development of atopic disorders, different growth patterns) of antibiotic treatment in the first week of life. Subsequently, in 2018 Neelke got the opportunity to start a PhD research project at the ENT-department of the Erasmus MC under supervision of Prof. dr. Rob Baatenburg de Jong and dr. André Goedegebure and dr. Paul Nagtegaal. Parallel to working on her PhD research within the Rotterdam Study, in 2019 she obtained a MSc degree in Health Sciences, with a specialisation in Clinical Epidemiology. The PhD research within the ENT and epidemiology departments resulted in this thesis. Since May 2021 she works as a medical doctor at the ENT-department of the Erasmus MC. Neelke lives with her husband, Tim Smulders, and their daughter Maithe (September 2020) in Rotterdam.

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