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Occupational Noise Exposure and Individual Risk Factors for Hearing Loss and Tinnitus

PhD dissertation

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PREFACE

This dissertation concludes the PhD project "*Occupational Noise Exposure and Individual Risk Factors for Hearing Loss and Tinnitus"*. The project was carried out between 2012 and 2016 as a part time PhD study at the Danish Ramazzini Centre, Department of Occupational Medicine, Aarhus University Hospital, in collaboration with the Department of Otology, Head and Neck Surgery, Regionshospitalet Holstebro. The project was funded by the Danish Working Environment Research Fund.

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Finally, I wish to thank my family for moral support and assistance - without you, I would not have finished this dissertation. Special thoughts go to my dear sister, who tragically died of cancer during this PhD project. Your courage and joy of life taught me more than words can ever express.

Thomas Winther Frederiksen
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ORIGINAL PAPERS

This PhD dissertation is based on the following original papers:

Paper I: Thomas W. Frederiksen, Cecilia H. Ramlau-Hansen, Zara Ann Stokholm, Matias B. Grynderup, Åse Marie Hansen PhD, Jesper Kristiansen, Jesper M. Vestergaard, Jens P. Bonde, Henrik A. Kolstad. **Noise Induced Hearing Loss – a preventable disease? Results of a 10-year longitudinal study of occupationally noise exposed workers.** (Under review in "Noise and Health").

Paper II: Thomas W. Frederiksen, Cecilia H. Ramlau-Hansen, Zara A. Stokholm, Matias B. Grynderup, Åse Marie Hansen, Søren Peter Lund, Jesper Kristiansen, Jesper M. Vestergaard, Jens P. Bonde, Henrik A. Kolstad **Atherogenic Risk Factors and Hearing Thresholds.** (Published in "Audiology and Neurotology" 2014;19:310–318).

Paper III: Thomas W. Frederiksen, Cecilia H. Ramlau-Hansen, Zara A. Stokholm, Matias B. Grynderup, Åse Marie Hansen, Søren Peter Lund, Jesper Kristiansen, Jesper M. Vestergaard, Jens P. Bonde, Henrik A. Kolstad **Occupational Noise Exposure, Psychosocial Working Conditions and the Risk of Tinnitus.** (Published in "International Archives of Occupational and Environmental Health", December 2016).

Paper IV: Thomas W. Frederiksen, Cecilia H. Ramlau-Hansen, Zara A. Stokholm, Matias B. Grynderup, Åse Marie Hansen, Søren Peter Lund, Jesper Kristiansen, Jesper M. Vestergaard, Jens P. Bonde, Henrik A. Kolstad **Salivary Cortisol and Tinnitus.** (Under review in "International Journal of Audiology").

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
AUC	Area under curve with respect to ground
BL	Baseline
BMI	Body mass index
CAR	Cortisol awakening response
CMDQ	Common Mental Disorders Questionnaire
CRH	Corticotrophin releasing hormone
dB(A)	Decibel, A-weighted
dB	Decibel
dBHL	Decibel Hearing loss
DBP	Diastolic blood pressure
FU	Follow-up
h	hour
HbA1c	Glycosylated haemoglobin
HDL	High density lipoprotein
HFHL-better	High frequency hearing loss better ear
HFHL-worse	High frequency hearing loss worse ear
HFHT-better	High frequency hearing threshold better ear
HFHT-worse	High frequency hearing threshold worse ear
HL	Hearing loss
HPA	Hypothalamic-Pituitary-Adrenal
HPD	Hearing protection device
HTL	Hearing threshold level
Hz	Hertz
ISO	International Organisation for Standardisation
kHz	Kilohertz
L_{Aeq}	A-weighted equivalent sound levels
$L_{Aeq, work}$	A-weighted equivalent noise levels for full work shift
LDL	Low density lipoprotein
LFHL-better	Low frequency hearing loss better ear
LFHT-better	Low frequency hearing threshold better ear
LFHL-worse	Low frequency hearing loss worse ear
LFHT-worse	Low frequency hearing threshold worse ear
NEM1	Noise Exposure Matrix 1
NEM2	Noise Exposure Matrix 2
nmol/L	Nanomol per litre
OR	Odds ratio
RIA	Radioimmunoassay
SBP	Systolic blood pressure
TG	Triglyceride
THI	Tinnitus Handicap Inventory
TFI	Tinnitus Functional Index
WHO	World Health Organisation

CONTENTS

1. INTRODUCTION

Millions of people all over the world suffer from hearing loss. Accordingly, the World Health Organisation (WHO) estimates that 250 million people worldwide have a moderate-to-severe or greater hearing loss – a number that more than doubles if people with mild hearing loss are included[1]. Hearing loss is probably the most important risk factor for tinnitus (commonly referred to as ringing in the ears or head), and tinnitus is often one of the first signs of potential damage to hearing[2]. Fortunately, for many, tinnitus is a temporary phenomenon lasting for only a short period but one in 10 adults has clinically significant tinnitus, and for around one in 100 adults, tinnitus severely affects their ability to live a normal life[2]. The British Royal National Institute for Deaf People recently estimated that 13 million people in Western Europe and the USA, seek medical advice for their tinnitus[3]. Therefore, both hearing loss and tinnitus currently rank among the most common chronic conditions especially in the older population[4], and this has serious social and economic implications[5].

Contrary to conductive hearing loss, sensorineural hearing loss is most often irreversible and no cure is currently available, meaning that prevention is of paramount importance to avoid it. Twin studies have revealed that around half of the variance in sensorineural hearing loss in the middle-aged and older is derived from genetic factors and the other half from environmental factors[6]. This gives the potential to prevent many cases of sensorineural hearing loss (and related tinnitus) if we learn more about the underlying environmental factors that may cause sensorineural hearing loss.

A number of exposures in the working environment are known to cause hearing loss and tinnitus, of which noise is probably the most recognised. In an ever-changing working environment, the distribution of exposures, however, also changes. Prior research may have helped identifying risk factors and brought these into focus for prevention, and new unacknowledged risk factors may have been introduced.
To set priorities for future preventive efforts against hearing loss and tinnitus, ongoing evaluation of established risk factors and identification of new risk factors are therefore important. The primary aim of this PhD project was to explore the relation between a number of primarily occupational exposures and tinnitus and hearing loss to facilitate this process by contributing with new knowledge.

2. AIMS

The primary aims of this dissertation were to (1) describe current, Danish, occupational noise levels and to examine their association with changes in hearing thresholds and tinnitus status, and (2) to identify other occupational and environmental risk factors for hearing loss and tinnitus in a cohort of primarily industrial workers. Four studies were undertaken with the following specific aims:

Study I: To describe current Danish industrial noise levels and use of hearing protection devices (HPDs) over a 10-year follow-up period, and to evaluate the association between occupational noise exposure and hearing threshold shift in the same period.

Study II: To examine the association between atherogenic risk factors (high levels of LDL, TG, total cholesterol and low levels of HDL, elevated systolic- and diastolic ambulatory blood pressure, smoking habits, high levels of glycosylated haemoglobin, and high BMI) and hearing thresholds.

Study III: To examine the influence of occupational noise (current and cumulative doses) and psychosocial work factors (psychological demands and decision latitude) on tinnitus occurrence among workers.

Study IV: To examine whether salivary cortisol, as an objective proxy for stress, is associated with tinnitus.

3. BACKGROUND

3.1 Hearing loss

General perspectives

Worldwide, around 1.3 billion people are currently affected by hearing loss[7], defined by the WHO as hearing thresholds greater than 25 dB averaged at frequencies 0.5, 1, 2, and 4 kHz in one or both ears[8]. Of the 1.3 billion people affected by hearing loss, around 250 million people have disabling hearing impairment, defined by the WHO as hearing loss greater than 40 dB in the better hearing ear in adults[8]. As the prevalence of hearing loss increases with age, the extent of the problem is expected to increase due to both an increasing world population and a general lengthening of life expectancy. Thus, by the year 2040, the number of people over 65 years, worldwide, is estimated to increase to 1.3 billion, and in this population group, around one-third are currently affected by disabling hearing impairment[9].

The impact of disabling hearing loss on the individual is often high. According to a recent review, it may have severe consequences for interpersonal communication (relationships with partners, colleagues and children), psychosocial well-being (loneliness, social isolation and depression) and quality of life[10]. Hence, adult-onset hearing loss currently ranks 15th amongst the leading causes of the Global Burden of Disease, and 2nd among the leading causes of Years Lived with a Disability[7].

Hearing loss is categorised into two basic types: conductive hearing loss and sensorineural hearing loss. Conductive hearing loss occurs when sound is not transmitted adequately through the outer or middle ear, and it therefore most often results from disease in the middle ear or outer ear structures[11]. This type of hearing loss can often be treated either medically or surgically.

Sensorineural hearing loss occurs from damage to the inner ear or its central neural pathways, and, most often, it has no potential for medical or surgical correction meaning that it is permanent[12]. Prevention is therefore paramount. Twin studies indicate that around half of the variance in sensorineural hearing loss in the middle-aged and older is due to genetic factors and the other half is due to non-genetic factors[6]. This indicates that many cases of sensorineural hearing loss are preventable if we identify the underlying non-genetic risk factors.

3.2 Risk factors for sensorineural hearing loss

Non-genetic (environmental) risk factors for sensorineural hearing loss include both occupational and extra-occupational factors. So far, research has identified several occupational risk factors, including chemicals such as styrene, toluene, xylene[13], heavy metals[14], vibration[15], and occupational noise exposure[16]. The last is among the primary exposures evaluated in the studies included in this dissertation and will be discussed in detail below. Extra-occupational risk factors include age[17], ototoxic medication (aminoglycosides, salicylates, cisplatin, and loop diuretics)[18] and leisure time noise exposure[19]. Lately, atherogenic risk factors have also been suggested as potential risk factors for sensorineural hearing loss which will also be discussed below.

Occupational noise as a risk factor for sensorineural hearing loss

In industrialised countries, occupational noise exposure is among the leading occupational health risks, and, currently, noise induced hearing loss ranks as the second most common form of sensorineural hearing impairment, after age-related hearing impairment[20]. This has led to extensive research into the pathophysiological mechanisms leading to noise induced hearing loss, and numerous epidemiological studies have evaluated the effect of noise exposure to establish evidence of safe limits for exposure[16]. The ISO 1999 (International Organization for Standardization) standard is based on multiple exposure-response studies, and current regulation in Europe is based on this standard, which also provides procedures for estimating the hearing impairment due to noise exposure. According to the ISO 1999, the effect of noise below 85 dB(A) is limited. However, when exposure exceeds this level over longer periods, there is a risk of hearing loss[16].

For practical reasons, noise (sound) levels are measured on a logarithmic scale (the decibel scale), as the human ear has a large dynamic range in sound perception. This means that a 3 dB increase in sound pressure level represents a power ratio of approximately 2. The ISO 1999 therefore recommends that the sound level averaged over an 8 hour working period should not exceed 85 dB(A), and since every increase of 3 dB(A) doubles the effect of the noise, the time spent in noise should be cut in half for every 3 dB(A) increase. This is called “the 3 dB(A) exchange rate” and means that, at an average noise level of 87 dB(A), a worker is only allowed to work for 4

hours and the time is further reduced to 2 hours if the mean noise level increases 90 dB(A).

Sensorineural hearing loss following noise exposure is most often caused by continuous and regular exposure to noise but can also result from a single or repeated acoustic trauma. Accordingly, the destruction of the organ of Corti seems to be due to two mechanisms: metabolic decompensation after noise exposure over a longer period of time or mechanical destruction by short exposure to very high noise intensities (>140 dB(A))[21]. The process of metabolic damage to the cochlear hair cells appears to be mediated by a heavy production of reactive oxygen species, leading to oxidative cell death[22]. This is supported by animal studies in which administration of antioxidant vitamins has been shown to reduce hearing loss after noise exposure[23].

A large variability in hearing loss is seen after identical levels of noise exposure. According to the ISO 1999 database, an 8-hour daily exposure of 100 dB(A) for 30 years gives a median sensorineural hearing loss at 4 kHz of 45 dBHL, but with a range of 60 dB between the 10th and 90th percentiles. Thus, after 30 years, an individual may end up with a normal hearing or profound hearing loss following the same cumulative noise exposure[16]. This indicates that noise induced hearing loss is a complex disease caused by an interaction between both genetic and environmental factors[24].

Since the harmful effect of noise on hearing has been documented through many years, much has been done to implement preventive measures. Engineering solutions have been developed to minimise noise emission and reflection, and legislation in most industrialised countries today limits the time of work-related noise exposure and obligates the use of hearing protection devices (HPDs)[25,26]. HPDs include earmuffs and earplugs that can be worn either separately or in combination, theoretically providing the wearer with 10 to 40 dB of attenuation depending on correct and consequent use[27]. Unfortunately, this is often not the case and effective protection of less than 3 dB has been reported in studies observing worker's actual behaviour at their worksite[28].

Both noise regulations and noise protection in industrialised countries over the last decades may have changed current industrial noise levels and individual occupational noise exposure, giving good reasons for continued assessment of occupational noise levels and the impact they have on worker's hearing status. Searching PubMed for literature on "Noise Induced Hearing Loss" identified more than 7,000 studies. Thus,

the amount of research within this field of research is extensive, and the association between high noise exposure levels and hearing loss seems well documented. The noise-related studies included in this dissertation were therefore not performed to confirm this association, but rather to assess the potential effect of the above-mentioned regulations of both occupational noise levels and noise protection. This has also been done by other research groups, and, interestingly, there seems to be a trend towards a declining prevalence of noise induced hearing loss in industrialised countries although results differ somewhat between industries[29]. Thus, relatively recent studies involving industrial workers in general tend to show decreasing hearing loss from occupational noise exposure[30-34], whereas certain occupational groups such as construction workers[35-37] and the military[38-40] are still at risk. Results from these studies and others will be discussed in more detail and in light of our own results in section 6.3.

Atherogenic risk factors and sensorineural hearing loss

Atherosclerosis is a disease in which plaques containing fat, cholesterol and white blood cells build up inside arteries. This gradually narrows the affected arteries, thereby limiting the flow of oxygen and nutrients to the organs they supply[41]. The prevalence of atherosclerosis is high in industrialised countries. Thus, among subjects above 50 years of age, ultrasonography will reveal atherosclerosis in more than 85%[42].

Most recognised risk factors include dyslipidaemia, diabetes, cigarette smoking, family history, sedentary lifestyle, obesity, and hypertension[41].

Metabolically, the cochlea is a very active organ depending on a steady flow of oxygen and nutrients to maintain homeostasis. Hypothetically, atherosclerosis could therefore be associated with sensorineural hearing loss. Prior literature is rather scarce, and methodologies as well as results are not concordant. Most studies are cross-sectional[43-45], some both cross-sectional and longitudinal[46] and some strictly longitudinal[47]. Results for smoking were the most consistent with four studies[43,45-47] finding statistically significant associations with poorer hearing and one study finding no association[44]. For the other risk factors like BMI, hypertension, serum lipids, and diabetes-related measures, results were conflicting. This is further discussed in relation to our results in section 6.3.

3.3 Tinnitus

Tinnitus is the perception of sound arising without corresponding auditory stimulation and affected individuals often describe it as high or low pitched ringing or buzzing in one or both ears[48]. Tinnitus is a frequent complaint with a prevalence ranging from 10 to 15%, depending on study population and criteria applied. Most people affected by tinnitus are able to live normal lives, but for around 0.5% of the population, tinnitus may be accompanied by debilitating symptoms such as concentration difficulty, insomnia and annoyance[49]. Tinnitus can be intermittent or continuous, and particularly in the latter case, it may lead to disabling symptoms[50]. There are two types of tinnitus: subjective tinnitus and objective tinnitus. Subjective tinnitus is by far the most common type of tinnitus and is only perceived by the person affected. Another, much rarer, type is called objective tinnitus, which may be heard by an observer using a stethoscope. This condition is most often caused by vascular disease or myoclonus of the middle ear or palatal muscles[2].

3.4 Risk factors for tinnitus

The exact pathophysiological mechanisms behind tinnitus are still not clarified. As hearing loss is the major risk factor for tinnitus, it has been suggested that tinnitus represents a homeostatic response of the central dorsal cochlear nucleus auditory neurons to loss of auditory input[2]. The strong association between hearing loss and tinnitus means that many of the known risk factors for hearing loss also represent risk factors for tinnitus, including age, middle and inner ear diseases, ototoxic medications, and (occupational) noise exposure[48], of which the latter will be described in more detail below. Psychological factors including mental stress, depression, and anxiety have also been suggested to cause tinnitus or to exacerbate tinnitus symptoms[51-53].

Occupational noise as a risk factor for tinnitus

As previously mentioned, hearing loss and tinnitus are strongly associated. As noise exposure is a well-established risk factor for hearing loss, noise exposure may also be expected to be a potential risk factor for tinnitus, which has been observed in prior studies[54]. However, in about one-third of cases, tinnitus occurs without concurrent hearing loss, meaning that non-auditory factors may also be involved[55]. Among seven

identified studies that investigated the association between occupational noise exposure and tinnitus, all found statistically significant associations between current or prior occupational noise exposure and tinnitus[56-62]. Strikingly, all studies were based on self-reported noise exposure information, which will be discussed in section 6.3.

Psychosocial working conditions and tinnitus

The association between psychopathology and tinnitus is complex. Associations between tinnitus and depression and anxiety have been reported[2,50], and mental stress has also been suggested to be associated with tinnius[63]. Also, tinnitus sufferers seem to report significantly more strain during stress tests when compared to healthy controls[64], and, furthermore, the psychosocial stress level seems to increase with severity of tinnitus[65]. Psychosocial work factors may therefore be associated with tinnitus. Two studies have evaluated the association between work-related stress and tinnitus, both finding associations between more work stress and more tinnitus[66,67], but otherwise, evidence is scarce. Moreover, evidence is based on self-reported stress levels, which may be problematic. The main disadvantage is that perception (and reporting) of a given psychosocial working environment may not only be influenced by the working conditions but also by distressing health conditions and personality traits, increasing the risk of reporting bias and exposure misclassification[68]. Averaging self-reported exposure information across work units has been suggested as a potential way of obtaining information less affected by reporting bias[69].

Cortisol and tinnitus

Cortisol is a steroid hormone produced in the cortex of the adrenal gland in response to emotional and physiological stressors and regulates the metabolic system and anti-inflammatory pathways. The release of cortisol is mediated by the hypothalamic-pituitary-adrenal (HPA) axis through corticotrophin releasing hormone (CRH) secreted from the hypothalamus and adrenocorticotropic hormone (ACTH) secreted from the pituitary gland[70].

When physically or psychologically threatening situations (stressors) affect an individual, CHR is secreted from the hypothalamus. This causes an increased production of ACTH in the pituitary gland, which then leads to increased production of cortisol from the adrenal cortex. In response to the increased level of cortisol, the

secretion of CRH and ACTH is inhibited via a negative feedback system[71]. This delicate feedback system integrates physical and psychosocial influences to allow the organism to adapt effectively to its environment, and if the system is fatigued by prolonged stress, this can lead to disease[72]. Salivary cortisol is a well-established neuro-endocrine marker of the acute stress-response and possibly also reflects prolonged stress-conditions[73].

As mentioned above, previous studies with self-reported exposure have suggested a link between stress and tinnitus[63,64,74], but, as with psychosocial working conditions, there is a potential risk of reporting bias. Using salivary cortisol as an objective biomarker of stress instead of subjective reports is a potential way to circumvent reporting bias[75].

Cortisol is still relatively new in tinnitus research, and, only few studies have been published. Most has been published by a Canadian group that found significantly altered cortisol secretion patterns in tinnitus patients (with related distress from tinnitus) in four studies[76-79]. Three other studies found no association between tinnitus and cortisol measures[80-82]. These studies will be discussed in relation to ours in section 6.3.

4. MATERIALS AND METHODS

4.1 Study overview

An overview of the materials and methods in the four studies in the dissertation is given in table 1, with additional information available in the following sections and appended papers (I-IV).

Table 1. Overview of materials and methods used in the four studies included in this dissertation

	Study I	**Study II**
Topic	Noise surveillance and use of HPDs. Association between occupational noise and hearing	Association between well-established risk factors for atherosclerosis and hearing thresholds
Design	Follow-up study also containing cross-sectional analyses	Cross-sectional study
Population	539 persons (baseline) 424 persons (follow-up) 207 persons (both rounds)	559 persons
Outcome	Low- and high-frequency hearing thresholds	Low- and high-frequency hearing thresholds
Outcome assessment	Hearing threshold shift. Hearing loss	Questionnaire
Exposure	Occupational noise exposure. Time since first occupational noise exposure >80 dB(A)	Atherogenic risk factors (blood lipids, glycosylated hemoglobin, smoking habits, body mass index (BMI), and ambulatory blood pressure)
Exposure assessment	Noise dosimetry. Noise exposure matrix	Blood samples, questionnaire, biometry, sphygmanometry
Confounders	Age, sex, baseline hearing, prior noise exposure	Age, sex, education, income, family history of hearing loss before age 70, military service, cumulative occupational noise exposure, leisure-time noisy activities
Main statistical analyses	Multiple linear and logistic regression	Multiple linear regression

Table 1 (continued). Overview of materials and methods used in the four studies included in this dissertation

	Study III	**Study IV**
Topic	Association between occupational noise and psychosocial work factors and tinnitus	Association between cortisol measures and tinnitus
Design	Cross-sectional study	Cross-sectional study
Population	534 persons	632 persons
Outcome	Tinnitus (yes/no)	Tinnitus (yes/no)
Outcome assessment	Questionnaire	Questionnaire
Exposure	Occupational noise exposure (current ad cumulative). Psychosocial work factors (psychological demands and decision latitude)	Cortisol measures (awakening cortisol, awakening+30 cortisol, CAR, evening cortisol, cortisol slope and AUC)
Exposure assessment	Noise dosimetry. Noise exposure matrix. Copenhagen Psychosocial Questionnaire	Analysis of workers' saliva sampled at awakening, awakening+30 min and evening time
Confounders	Age, sex, depression, anxiety, somatisation disorder, education and income	Age, sex, worst ear hearing threshold, anxiety and depression
Main analyses	Multiple logistic regression	Multiple logistic regression

4.2 General methods applied in all studies

Population

All four studies in this dissertation are based on a field study initiated in 2001-03 (baseline) with the primary purpose of monitoring occupational noise exposure, auditory function and preventive measures (use of HPDs) among noise-exposed workers. In total, 819 workers from Aarhus, Denmark, were recruited from 86 randomly selected companies within 12 trades: children day care, financial services and the 10 manufacturing trades with the highest reporting of noise induced hearing loss according to the Danish Working Environment Authority. Children's day care units were chosen as prior measurements in Denmark had indicated full-shift equivalent noise levels around 80 dB(A) in these units, and finance (bank and insurance workers with suspected low noise exposure) was chosen as a reference group. In 2009-10, the same companies and workers were asked to participate again to repeat measurements. This time the focus of data collection was extended to also encompass extra-auditory effects of noise and psycho-social work factors. We were able to re-identify 756 participants. Due to time and economic restraints, 27% (n = 202) were not contacted (at random), leaving 554 eligible for follow-up. Of these 554 workers, a total of 271 workers (49%) responded and agreed to participate again. Furthermore, 394 workers within the 12 trades were recruited *de novo* to include new workers with expected shorter noise exposed working histories, making a total of 665 participants in the follow-up cohort.

Quantification of occupational noise exposure

At baseline and follow-up, individual dosimeters (Bruel & Kjær, model 4443, Nærum, Denmark) measuring A-weighted equivalent sound levels (L_{Aeq}) at 5 second intervals were handed out to the participants. Microphones were fitted at the right side collar if right handed and vice versa if left handed. Measuring range was set to 70-120 dB(A). Individual A-weighted equivalent noise levels were computed for the full work shift ($L_{Aeq,\ work}$). Based on individual measurements, we, subsequently, calculated workplace and trade-specific mean noise levels.

For the estimation of retrospective and cumulative occupational noise exposure, we created two noise exposure matrices (NEM1 and NEM2), as described below:

NEM1 was based on 1268 full-shift noise exposure recordings from the 2001-02 study and the 2009-10 study. We predicted noise exposure levels for each combination of trade, occupation (blue vs. white collar worker), and calendar year (1980-2010) by mixed regression analyses, including these as fixed effects and the participants as random effect. The predicted noise exposure levels were linked with participants' employment histories by trade, occupation, and calendar year. Information on employment histories (1980-2010) was retrieved from the Danish Supplementary Pension Fund. Using information from the resulting matrix, we calculated cumulative occupational noise exposure levels for each participant as the product of estimated noise exposure level (L_{AEq} in dB(A)) and duration of employment (T) using the formula: 10 x log [$\Sigma(10^{dB(A)/10}$ x T)], resulting in "dB(A)-year" on a logarithmic scale.

NEM2 was based on: (1) questionnaire information on current and previous employment including trade, period, and the workers' subjective judgment of whether any previous jobs had involved comparable or higher noise exposure levels than their current job, and (2) workplace average L_{Aeq} levels at baseline and follow-up. Each individual employment year was given a noise exposure level based on the following criteria: (1) if the "noise year" was a part of an employment period in a company included in the study, the average workplace level was applied. (2) for employment periods outside the companies included in the study, noise exposure was classified from the company level of the following employment in a company included in the study. This means that if the worker reported that noise levels in the prior job were comparable to or higher than the level of the current job, these years were given the same level as in the current workplace. If the noise level was judged to be substantially lower than the exposure at the current company, this employment period was not included in the noise exposure matrix.

Finally, we calculated cumulative occupational noise exposure levels for each participant in the follow-up period as the product of estimated noise exposure level (L_{AEq} in dB(A)) and duration of employment (T) using the formula: 10 x log [$\Sigma(10^{dB(A)/10}$ x T], resulting in "dB(A)-year" on a logarithmic scale.

The same model was used to estimate the first year of occupational noise exposure >80 dB(A) and the duration of exposure > 80 dB(A) and > 85 dB(A).

Questionnaire

At both rounds, all participants were asked to fill in a questionnaire that was handed out at the time of the audiometric examination. Information retrieved for each study will be described below.

Audiometry

Air-conduction thresholds were determined for each ear at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz by pure tone audiometry at the workplaces, using a Voyager 522 audiometer equipped with TDH-39 headphones (Madsen Electronics, Taastrup, Denmark). The audiometer was installed in a mobile examination unit equipped with a sound proof booth (model AB-4240, Eckel Noise Control Technologies, Bagshot, UK). Audiometry was performed by trained examiners, using a standardised protocol.

To avoid temporary threshold shifts from possible noise sources, all participants were asked to wear hearing protection from the beginning of the day until the audiometry was done. Otoscopy was performed initially to verify that ears were free of wax and the tympanic membrane was visible. The audiometer was calibrated every 6 months according to ISO standards.

Tinnitus

Tinnitus was defined in the questionnaire as “ringing or buzzing in one or both ears”. Related questions included frequency of tinnitus (1. almost never experiencing tinnitus, 2. experiencing periods of tinnitus at least monthly, 3. experiencing periods of tinnitus at least weekly or 4. experiencing tinnitus daily), frequency of annoyance when experiencing tinnitus (1. almost never or never, 2. rarely, 3. sometimes, 4. often, or 5. always) and frequency of insomnia due to tinnitus (1. almost never or never, 2. rarely, 3. sometimes, 4. often, or 5. always). A person was classified as having tinnitus if experiencing tinnitus daily accompanied by either annoyance (sometimes, often, or always) or insomnia (sometimes, often, or always).

4.3 Study I

Design

Study I was a longitudinal study.

Population

For cross-sectional analyses of the base-line population (n = 819), we excluded 76 workers with incomplete questionnaire exposure information or no noise dosimetry, 16 workers with incomplete audiometry, 109 white-collar workers (typically managers and office workers considered to differ considerably from the remaining population with respect to extraneous predictors of hearing loss), 65 workers reporting current or prior chronic middle-ear infection or tympanic membrane perforation (possible conductive hearing loss), and 14 workers with asymmetrical hearing loss (possible hearing loss from other causes than noise), resulting in 539 eligible workers for base-line cross-sectional analyses. Correspondingly, for cross-sectional analyses on the follow-up population, we excluded 38 workers with incomplete questionnaire exposure information or no noise dosimetry, 98 white-collar workers, 75 workers reporting current or prior chronic middle-ear infection or tympanic membrane perforation and 30 workers with asymmetrical hearing loss, resulting in 424 eligible workers. For longitudinal analyses, we focused on the 271 workers participating in both rounds. Of these, 262 had complete audiometries from both rounds. We excluded two workers with incomplete questionnaire exposure information, 48 white-collar workers and workers reporting either chronic middle ear infection (n = 2), tympanic membrane perforation (n = 2), scull fracture (n = 0) concussion (n = 1), meningitis (n = 0) or Méniere's disease (n = 0) in the follow-up period, resulting in a final study population of 207 persons.

Audiometric measures

Audiometry was performed as described in section 4.2. Based on pure tone air-conduction thresholds, we calculated an average binaural hearing threshold level for the critically noise-sensitive frequencies at baseline and follow-up (3-6 kHz-HTL-BL or 3-6 kHz-HTL-FU). Correspondingly, a baseline and a follow-up hearing loss variable (3-6 kHz-HL-BL and 3-6 kHz-HL-FU) was defined if 3-6 kHz-HTL-BL or 3-6 kHz-HTL-FU was above 20 dB. Threshold shift from baseline to follow-up (Δ3-6 kHz-HTL) was calculated by subtracting baseline hearing thresholds (3-6 kHz-HTL-BL) from follow-up hearing thresholds (3-6 kHz-HTL-FU). Thus, worsened hearing was reflected by a positive threshold shift. We regarded an inter-aural difference of 20 dBHL or more in two consecutive frequencies from 3-6 kHz as asymmetrical hearing loss.

Questionnaire information

Information on age, sex, professional history (current and prior employment, duration, industry, collar, use of HPDs, and the workers' judgment whether noise levels in prior jobs were higher, comparable, or lower) was retrieved from the questionnaire.

Occupational noise exposure assessment

Occupational noise was measured as described in section 4.2. As sound levels were expected to vary more from day to day for the individual worker than between different workers[83], we estimated the most efficient grouping strategy based on the highest contrast in mean exposure level between the groups by modelling noise exposure with two mixed effect models including either worker and industry or worker and company as random effects. The highest contrast was found using company means, and thus worker's noise exposure was classified by the average L_{Aeq} calculated for their workplace and not by his or her individual measurement.

The estimation of cumulative occupational noise exposure in study I was based on NEM2 (described in section 4.2)

Statistical analyses

Logistic regression was used to estimate the association between first year of occupational noise exposure >80 dB(A) and hearing loss in the critically noise-sensitive frequencies for the baseline and the follow-up population, adjusting for age and sex.

Among the workers participating in both surveys, crude and adjusted associations between noise exposure variables and hearing threshold shift in the follow-up period were examined, using linear regression. Stratified analyses were performed to evaluate possible effect modification from prior occupational noise exposure and baseline hearing loss on the association between cumulative noise exposure and hearing threshold shift in the follow-up period. A Wald test was performed to test the hypothesis of no effect modification.

HPD use at baseline and follow-up was cross-tabulated with age and sex to identify possible changes in use over the follow-up period. Finally, to look for changes in noise emission from the industries included in this study, we calculated mean industry noise levels based on all individual blue-collar noise recordings at baseline and follow-up.

4.4 Study II

Design

Study II was a cross-sectional study.

Participants

As data on atherogenic risk factors were only collected in the 2009-10 survey, study II included only the follow-up population (n = 665). From this population, we excluded 88 participants who reported middle ear disease on the questionnaire and therefore possibly had conductive hearing loss. Furthermore, we excluded one participant reporting Ménière's disease. In total, 576 workers were included.

Audiometric measures

Audiometry was performed as described in section 4.2. Based on air-conduction thresholds, we calculated low- and high-frequency hearing thresholds for each ear. Low frequency hearing thresholds were calculated as the average of pure-tone hearing thresholds at 0.25, 0.5, and 1 kHz, and high frequency hearing thresholds were defined as the average of pure-tone hearing thresholds at 4, 6, and 8 kHz. Since analyses were performed on both the better and worse hearing ear, we defined four hearing thresholds: low frequency hearing threshold better ear (LFHT-better), low frequency hearing threshold worse ear (LFHT-worse), high frequency hearing threshold better ear (HFHT-better), and high frequency hearing threshold worse ear (HFHT-worse). Better and worse hearing ear were defined as the ear with the lowest and highest average thresholds in the given spectrum, respectively. If hearing levels were equal in both ears, the same threshold value was used for statistical analysis of better and worse ear. Correspondingly, low and high frequency hearing loss for better- and worse ear (LFHL-better, LFHL-worse, HFHL-better, and HFHL-worse) were defined if LFHT-better, LFHT-worse, HFHT-better, or HFHT-worse were above 25 dBHL.

Occupational noise exposure assessment

Occupational noise was measured as described in section 4.2, and for quantification of cumulative occupational noise exposure NEM1 was used.

Questionnaire information

Information on socioeconomic status (personal income and educational level), middle ear disease (perforated ear drum, recurrent aural discharge, and chronic otitis), family history of hearing handicap before age 70 years, military service, leisure time noise exposure (hunting, use of fire arms, heavy use of portable music player, motor sport, playing electrically amplified musical instruments), smoking habits (ever, never, or current smoker, and smoking intensity) and medication (lipid lowering medication, antidiabetic agents and antihypertensive medications) from the questionnaire was used. Number of smoking pack-years was calculated as number of cigarettes smoked per day divided by 20 and multiplied by number of years smoking.

Biometry, biochemical data and blood pressure monitoring

For each worker, height and weight were measured and non-fasting venous blood was sampled by a medical laboratory technologist. Equipment for 24-hour ambulatory blood pressure monitoring (Space Labs 90217) was fitted together with the noise dosimeter and worn by the participant until the next day. Blood pressure was measured every 20 minutes during daytime (7 am to 11 pm) and every 30 minutes during night time (11 pm to 7 am), and average 24-hour systolic and diastolic blood pressure values were calculated.

Statistics

We computed percentage differences in LFHT and HFHT for both better- and worse ear by atherogenic risk factors using linear regression analysis. Analyses were adjusted for age, sex, educational level (none, short courses, skilled worker, short range training, middle range training, long range training), personal income (≤299,999 DDK, 300,000-499,999 DDK, ≥500,000 DDK), family history of hearing loss (yes/no), ear disease (yes/no), military service (yes/no), noisy leisure time activities (yes/no), hunting and shooting (yes/no).

4.5 Study III

Design

Study III was a cross-sectional study.

Population

Only participants from the 2009-10 survey (n = 665) were included in study III, as psychosocial work factors were not accounted for in the 2001-2 survey. To restrict the analyses to potentially noise-exposed workers, 67 financial workers and 64 workers from the original 2001 cohort now either unemployed (n = 44) or no longer working in noise-exposed industries (n = 20), were excluded, leaving 534 participants eligible for this study.

Occupational noise exposure assessment

Occupational noise was measured as described in section 4.2, and for quantification of cumulative occupational noise exposure NEM1 was used.

Audiometric measures

Audiometry was performed according to section 4.2. We defined two hearing measures: 0.5-4.0 kHz hearing threshold (0.5-4.0 kHz HT) was computed as the average of pure-tone hearing thresholds at 0.5, 1, 2, 3, and 4 kHz in the worse ear. 0.5-4.0 kHz hearing handicap was defined if 0.5-4.0 kHz HT > 25 dBHL (according to WHO hearing impairment definition). Worse ear hearing ability was chosen over better ear, as we assumed that hearing levels in the worse ears were the most predictive of tinnitus status.

Questionnaire information

For study III, we retrieved the following data from the questionnaire: psychosocial work factors, mental symptoms, use of hearing protection device, income and education (see details below).

Measures of psychosocial working conditions

Psychosocial working conditions were measured according to Karasek's and Theorell's demand-control model[84] with scales from the Copenhagen Psychosocial Questionnaire[85]. Psychological demands, decision authority, and skill discretion were each measured by four items on a scale from "always" (1) to "never" (5). For each scale, a mean value of the four items was calculated. Decision latitude was computed as the mean value of decision authority and skill discretion.

Furthermore, we calculated mean values of decision latitude and psychological demands for each work unit after exclusion of participants with tinnitus. Participants with tinnitus were excluded from the calculation of the mean scores as tinnitus distress could influence their assessment of the psychosocial work environment, thus introducing reporting bias. The mean values were then assigned to all employees at the particular work place.

Measures of mental symptoms

Symptoms of depression, anxiety, and somatoform disorder (illness worries) were assessed using the Common Mental Disorders Questionnaire[86] (CMDQ). All questions referred to the last 4 weeks and were measured on a 5-point response scale from "not at all" (0 points) to "extremely" (4 points). We used the six-question subscale for depression, the four-question subscale for anxiety, and the seven-question subscale for somatoform disorder. Participants were classified as depressive if scoring ≥3 on ≥3 of the 6 depressive symptom questions. Anxiety was classified if the score was ≥3 on ≥3 of the four anxiety symptom questions and somatoform disorder was classified if the score was ≥3 on ≥3 of the seven somatoform disorder symptom questions. These selection criteria were chosen to obtain optimal validity[86].

Use of hearing protection devices

Of the 534 workers, 333 reported using a HPD. Among HPD users, 140 participants completed a detailed log-book specifying when they used a HPD during the day of noise measurements.

Income and education

Participants were asked about gross household income (<299,999 DDK, 300,000–499,999 DDK, >500,000 DDK) and educational level (none, short courses, skilled worker, short-range training, middle range training, long-range training).

Tinnitus

Tinnitus was defined as described in section 4.2.

Statistics

Odds ratios of tinnitus according to noise exposures were analysed by logistic regression and performed using both continuous-scale exposure information (if

available) and exposure divided into relevant groups or tertiles. These analyses were adjusted for age and sex. Associations between psychosocial working conditions and tinnitus were analysed by logistic regression with robust clusters based on the work unit of the participants and adjusted for: (1) age and sex, and (2) age, sex, depression, anxiety, somatisation disorder, income, and education. These potential confounders were decided upon a priori. Analyses were performed using both continuous-scale exposure information and exposure divided into tertiles. We analysed for interaction between psychological demands and decision latitude. The interaction term was calculated based on both continuous and trichotomised data.

4.6 Study IV

Design

Study IV was a cross-sectional study.

Participants

Only participants from the 2009-10 study (n = 665) were included in study IV, as salivary cortisol measurements were not performed in the 2001-2 survey. We excluded one participant who reported Ménière's disease (as tinnitus is an integral part of this syndrome) and six night-workers (due to potentially disturbed diurnal cortisol secretion[87]. Of the remaining 658 participants, 633 collected saliva samples. Participants with any cortisol measurement >100 nmol/L were considered outliers[88] and also excluded (n=1), resulting in a final study population of 632 participants.

Salivary cortisol sampling and cortisol determination

Participants were instructed to provide three saliva samples, the first at approximately 8 p.m. the first day (evening sample), the second at awakening (awakening sample), and the third 30 min later (awakening+30 sample). Participants were instructed not to smoke, drink, or eat within 30 minutes before sampling. Saliva was collected in Salivette® tubes and refrigerated until collected the next day. Awakening samples were considered valid if they were collected within 30 min after waking up (all except eight samples). Awakening+30 samples were considered valid if they were collected within 60 min after the awakening sample (all except 17 samples), and evening samples were considered valid if they were collected after 5 p.m. (all except 10 samples). For this study, we only included valid samples.

Determination of cortisol in saliva

Cortisol level was determined by radioimmunoassay (RIA) as described by Hansen et al.[89]. The RIA for cortisol determination was designed for quantitative in vitro measurement of cortisol in serum, plasma, urine, and saliva. We used The Spectria Cortisol Coated Tube RIA, purchased from Orion Diagnostica, Espoo, Finland, and used it according to the manufacturer's specifications. The sample volume was 150 µL, the range of the standard solutions prepared was 1.0-100.0 nmol/L, and the incubation time was 30 min at 37°C. The specifications given by the manufacturer were a sensitivity of twice the standard deviation of the zero-binding value in saliva (0.8 nmol/L), a bias of 10% (3-15%), an intra-assay variation of 5.4%, and an inter-assay variation of 7.3%. Limit of detection was 1.59 nmol/L. Between-run coefficients of variation were 19% at 11.5 nmol/L and 16% at 49.2 nmol/L[90].

Equivalence between different runs

To show equivalence between different runs, natural saliva samples (5.9 nmol/L and 24.4 nmol/L) were used as control samples and analysed together with the test samples. Westgard control charts were used to document that the trueness and the precision of the analytical methods remained stable[91].

Cortisol measures

The cortisol measures analysed were: (1) awakening cortisol, (2) awakening+30 cortisol, (3) evening cortisol, (4) cortisol awakening response (CAR) calculated as the difference between cortisol levels at awakening and after 30 min, (5) slope of cortisol calculated as the change in cortisol from the maximum morning to the evening sample, and (6) area under curve with respect to ground (AUC) calculated as:

[(awakening conc. + awakening+30 min conc.) / 2) × time difference between the awakening conc. and the awakening+30 conc.] + [(awakening+30 min + evening concentration) / 2) × time difference between the awakening +30 and the evening concentration]

Tinnitus

Tinnitus was defined as described in section 4.2.

Covariates

Information on participants' age and sex was retrieved from their personal civil registration number. Information on awakening time, time of saliva sampling, and common mental disorders (depression and anxiety) was retrieved from the questionnaire. Symptoms of depression and anxiety were assessed using the Common Mental Disorders Questionnaire (CMDQ)[85]. Noise exposure was assessed according to NEM1 and audiometry was performed according to section 4.2.

0.5-4.0 kHz hearing threshold (0.5-4.0 kHz HT) was computed as the average of pure-tone hearing thresholds at 0.5, 1, 2, 3, and 4 kHz in the worse ear. Worse ear hearing ability was chosen over better ear, as we assumed that hearing levels in the worse ears were the most predictive of tinnitus status.

Statistics

Odds ratios of tinnitus according to cortisol measures were analysed by logistic regression using both continuous-scale exposure information and tertile categorisations. Cortisol measures were log-transformed to reduce skewness and variances. We analysed (1) awakening cortisol, (2) awakening+30, (3) evening cortisol, (4) CAR, (5) slope and (6) AUC in separate models.

The crude associations between cortisol and tinnitus were followed by two sets of adjustment. The basic adjusted model included sex and age (continuous) and the fully adjusted model also included worst ear hearing threshold (continuous dB HL), depression (yes/no) and anxiety (yes/no).

5. RESULTS

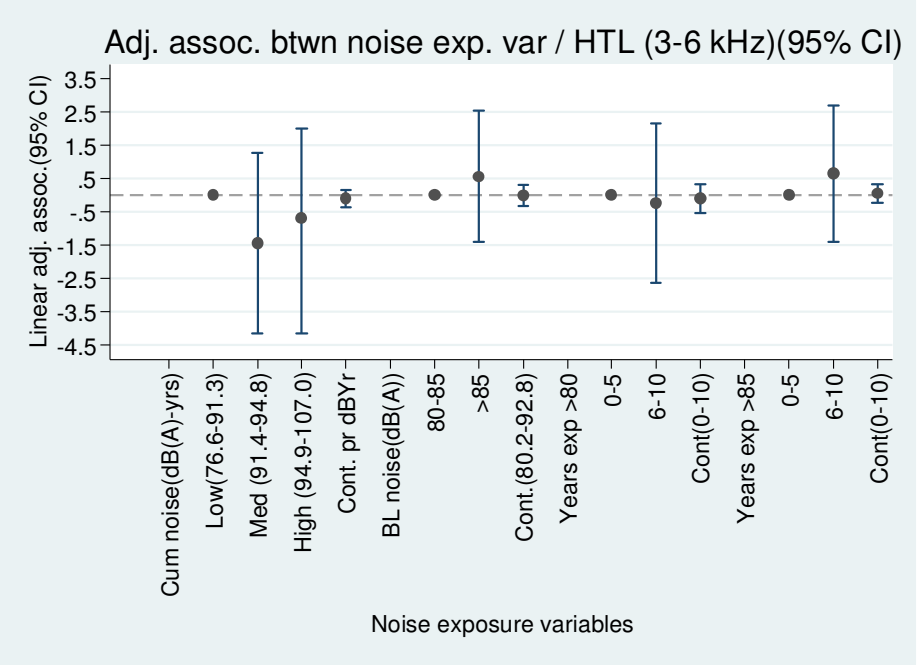
The following section summarises the main results of each study (I-IV).

5.1 Study I

Main findings

Longitudinal analyses showed no statistically significant associations between occupational noise exposure variables and workers' hearing threshold shifts in the period from 2001 to 2010 (figure 1). We quantified noise exposure in the follow-up period in four ways and found the following adjusted associations with hearing threshold shift (2001 to 2010) in the critically noise-sensitive frequencies: Cumulative occupational noise exposure (cum noise): -0.10 dB hearing threshold shift per additional noise-year (95% CI 0.36; 0.16). Baseline occupational noise exposure (BL noise): -0.01 dB hearing threshold shift per additional dB(A) (95% CI -0.33; 0.31). Years exposed >80 dB(A): -0.09 dB hearing threshold shift per additional year (95% CI -0.52; 0.34). Years exposed >85 dB(A): 0.06 dB hearing threshold shift per additional year (95% CI -0.22; 0.34).

Figure 1. Association between noise exposure variables (cumulative noise exposure (cum noise), baseline noise exposure (BL noise), years exposed to work day mean noise >80 dB(A), years exposed to work day mean noise >85 dB(A)) and hearing threshold change in the critically noise-sensitive frequencies (3, 4, and 6 kHz).



Among the baseline population (figure 2), we observed a statistically significant increased risk of hearing loss among workers with first noise exposure more than 20 years prior to baseline compared to the reference group (OR: 1.90 (95% CI 1.11; 3.22). The risk of hearing loss also increased with earlier first noise exposure among the follow-up population, but not statistically significantly (figure 3).

Figure 2. Adjusted odds ratios (OR) of hearing handicap in the critically noise sensitive frequencies (HL) according to year of first noise exposure among the baseline population.

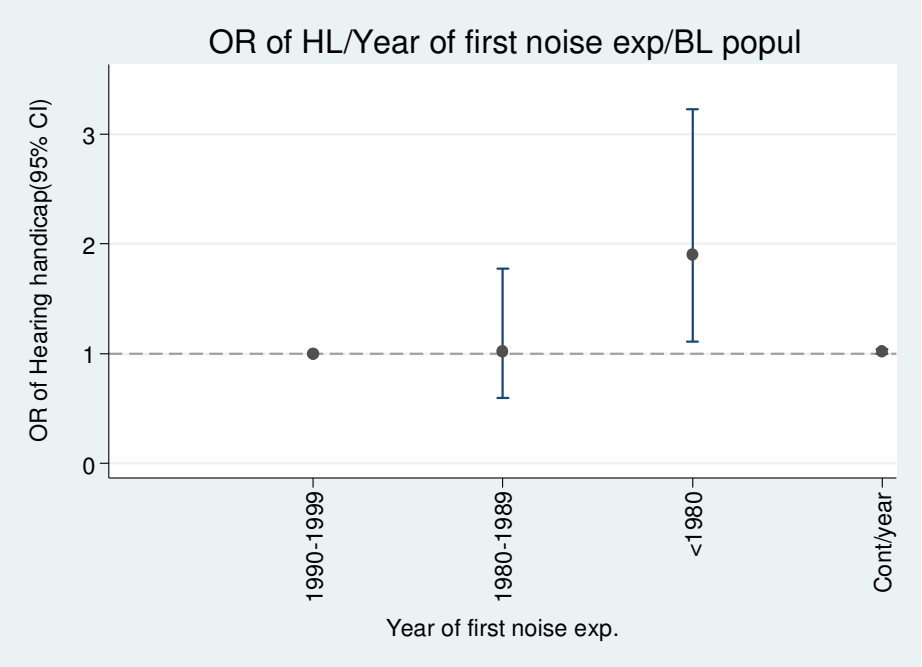
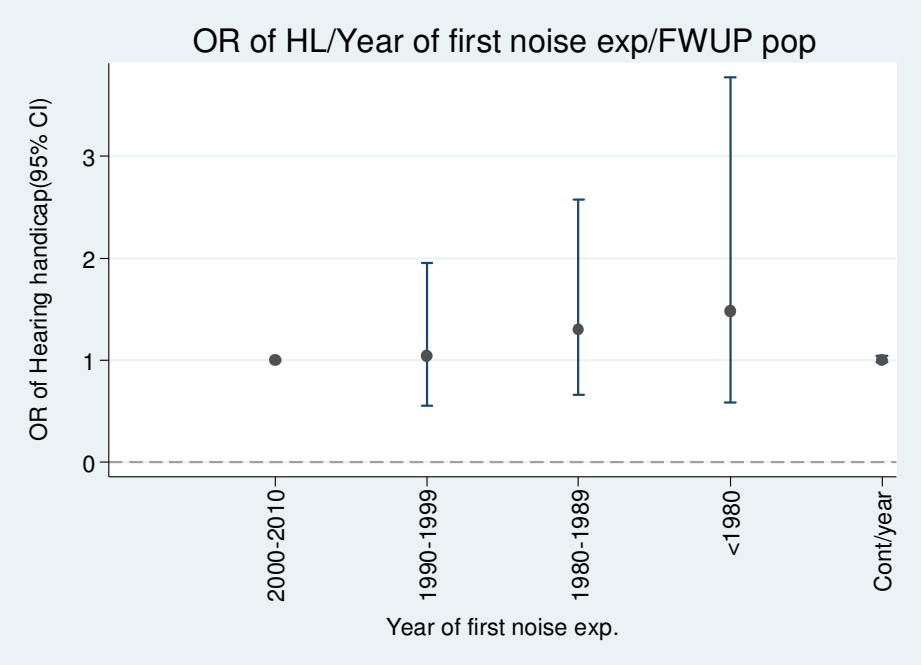


Figure 3. Adjusted odds ratios (OR) of hearing handicap in the critically noise sensitive frequencies (HL) according to year of first noise exposure among the follow-up population.



When exposed to daily mean occupational noise levels >85 dB(A), 70 % of participants used a HPD at baseline. This number rose to 76% among the workers participating in the follow-up survey (results not illustrated).

From 2001-2010, we observed a decline in noise exposure levels in all industries except "manufacture of machinery". Thus, mean noise exposure levels for all industries decreased with 1.1 dB(A) during the follow-up period.

Additional analyses

To account for potential effect modification from baseline hearing status and prior occupational noise exposure, we analysed the association between cumulative noise exposure and hearing threshold shift in the follow-up period stratified by these two variables. As we found no significant differences in results between the strata, we presented results without stratification.

We identified 12 workers participating in both surveys who changed from high to low noise exposure industries during the follow-up period. Although the low number in itself makes healthy survivor bias unlikely, we still analysed whether the change was associated with their baseline hearing status, which was not the case.

Adjusting analyses for HPD use by subtracting 10 dB(A) from participants' noise exposure levels if they reported daily use of a HPD did not change results for the association between cumulative noise exposure and hearing threshold shift in the follow-up period.

5.2 Study II

Main findings

As demonstrated in figure 5, we found statistically significant associations between low HDL (18.4% lower LFHT-worse for each unit increase in HDL (CI -32.2; -1.9)), high TG (8.3% higher LFHT-worse for each unit increase in TG (CI 2.4; 15.2)), high BMI (1.8% higher LFHT-worse for each unit increase in BMI (CI 0.2; 3.4)) and former smoking (21.6% higher LFHT-worse for former smokers (CI 2.9; 43.6)). Comparable but weaker associations were found in the better ear (figure 4). Borderline significant results were observed for the association between the atherogenic risk factor score (see section 4.4) and low frequency hearing loss in the worse ear (3.4 % higher LFHT-worse for each unit increase in atherogenic risk score (CI -0.2; 7.1).

No significant associations were observed between atherogenic risk factors and high frequency hearing thresholds (figures 6 and 7).

Figure 4. Adjusted percentage differences in the better ear low frequency hearing threshold according to atherogenic risk factors.

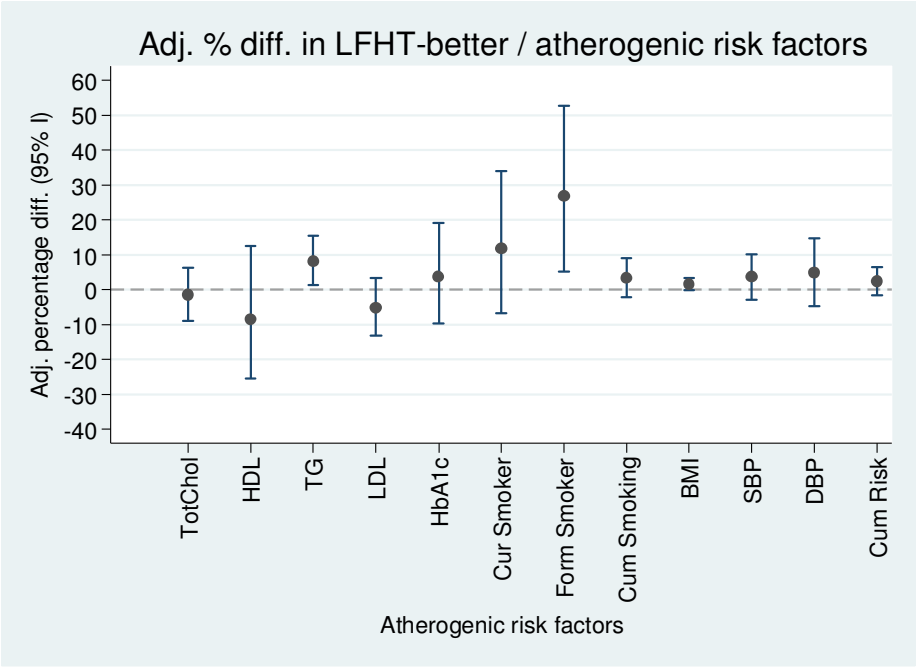


Figure 5. Adjusted percentage differences in the worse ear low frequency hearing threshold according to atherogenic risk factors.

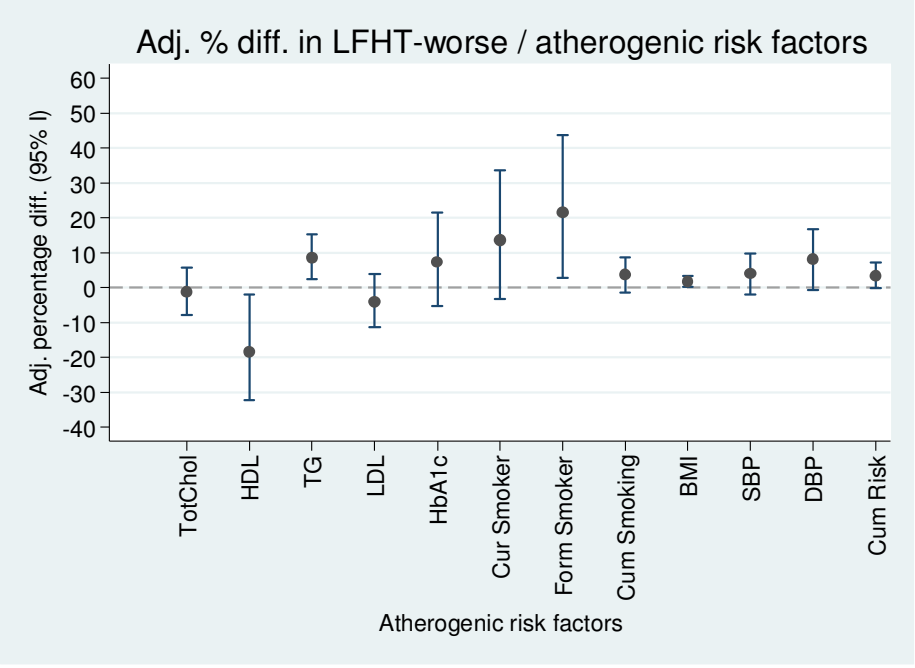


Figure 6. Adjusted percentage differences in the better ear high frequency hearing threshold according to atherogenic risk factors.

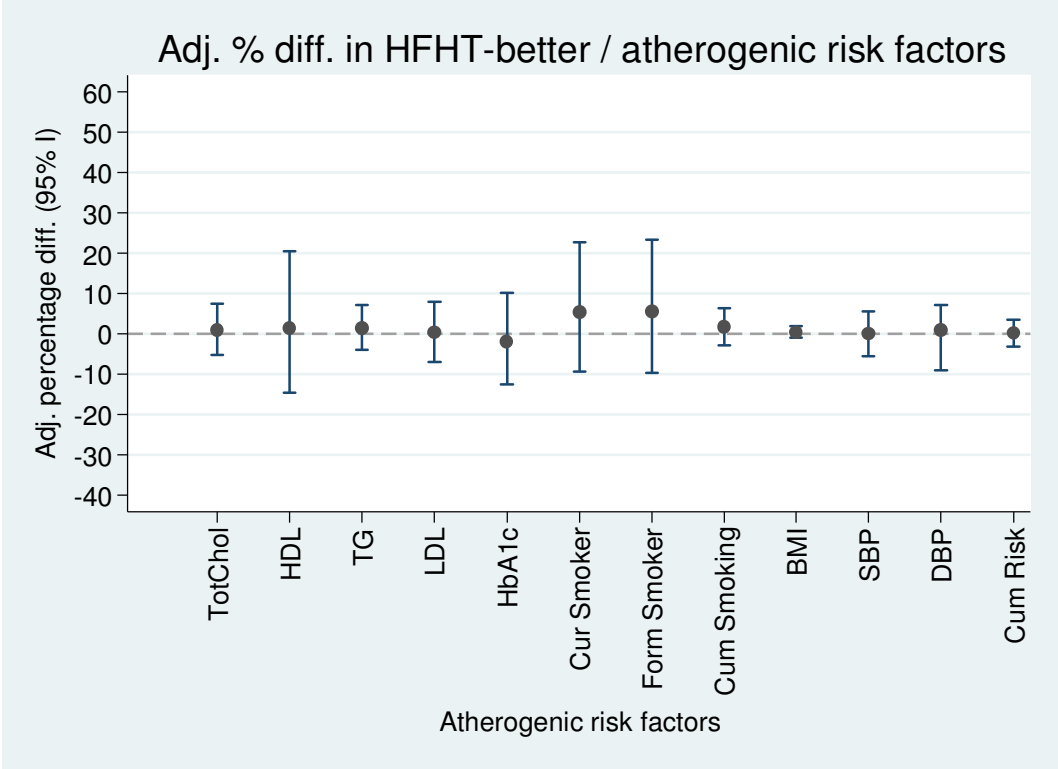
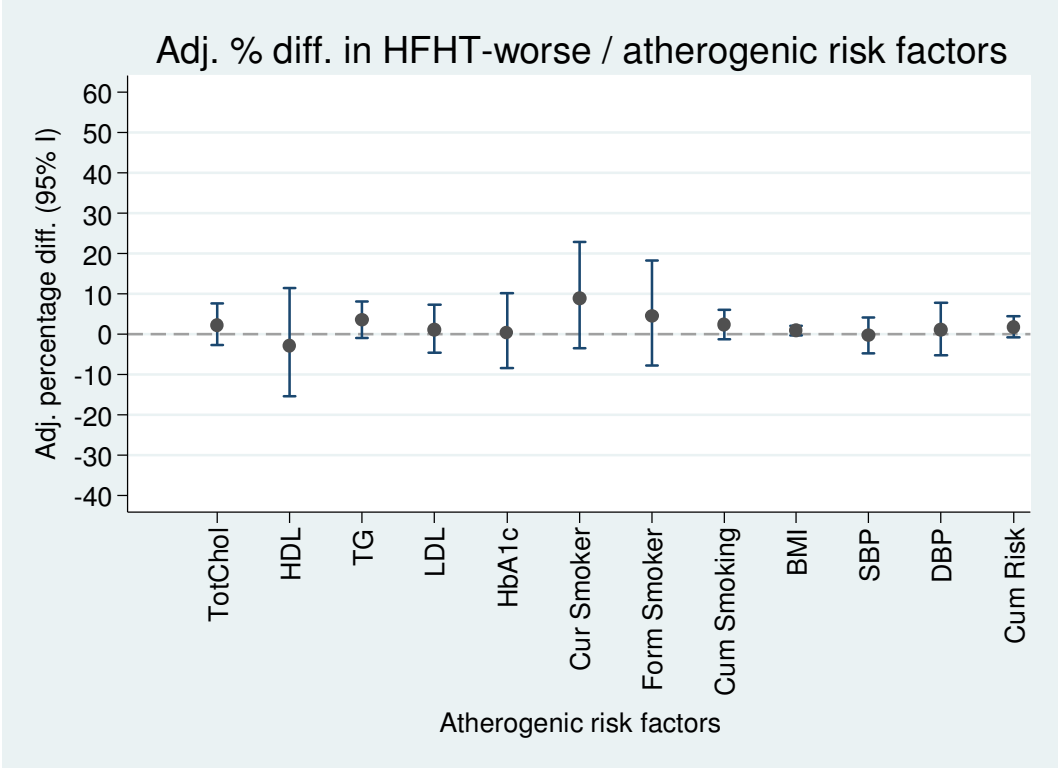


Figure 7. Adjusted percentage differences in the worse ear high frequency hearing threshold according to atherogenic risk factors.



Additional analyses

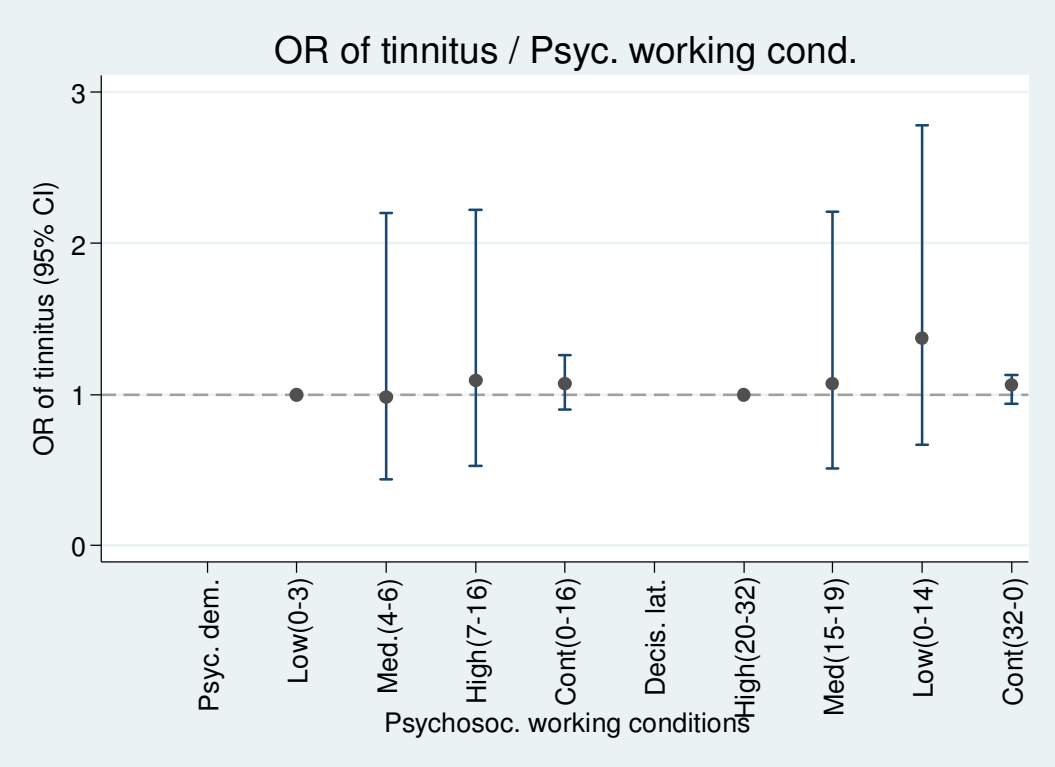
Exclusion of participants reporting to take antihypertensive medications, antidiabetic agents or lipid-lowering medication did not significantly alter the main results.

5.3 Study III

Main findings

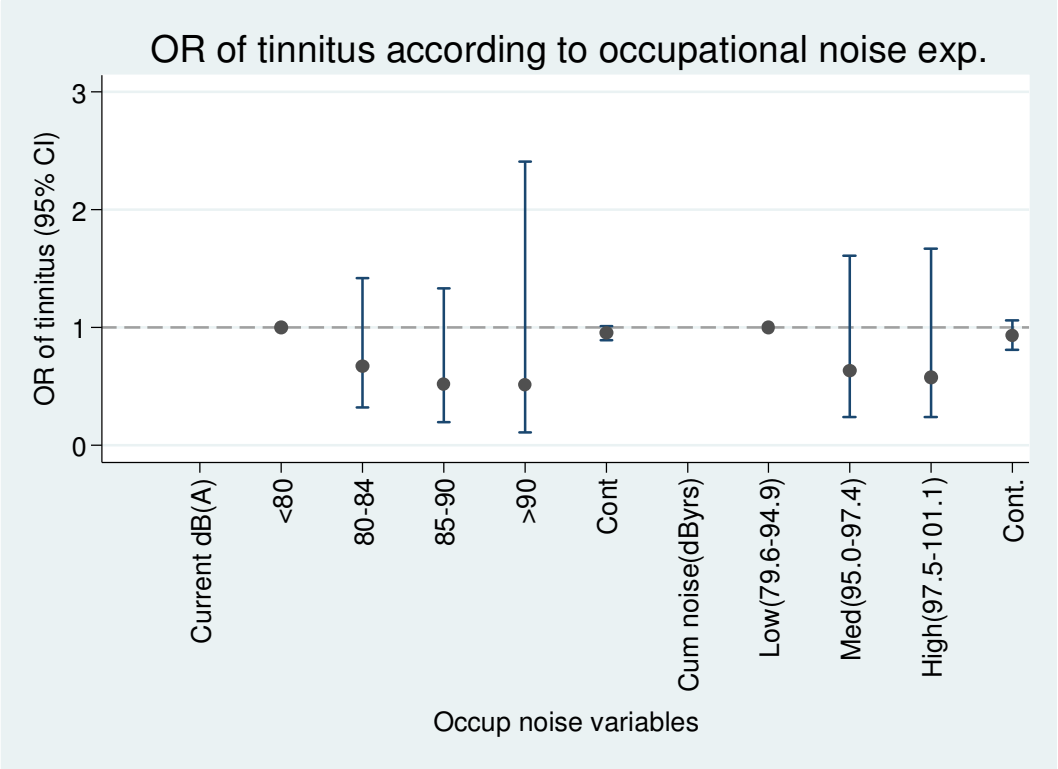
Psychosocial work factors were not statistically significantly associated with tinnitus (figure 8). Thus, for psychological demands we observed an adjusted OR of 1.07 (95% CI 0.90; 1.26) for one unit increase on the 16-point scale. Correspondingly, for decision latitude, we found an OR of 1.06 (95% CI 0.94; 1.13) for one unit decrease on the 32-point scale.

Figure 8. Adjusted odds ratios (OR) of tinnitus according to psychosocial working conditions (psychological demands and decision latitude).



We found no statistically significant associations between either current (OR: 0.95 (95% CI 0.89; 1.01 for each 10 dB(A) increase in noise level) or cumulative occupational noise exposure (OR: 0.93 (95% CI 0.81; 1.06 for each additional dB(A)year)) and tinnitus. For a graphical presentation of results, see figure 9.

Figure 9. Adjusted odds ratios (OR) of tinnitus according to occupational noise exposure variables (current and cumulative noise exposure).



Additional analyses

In this study, we performed a sub-analysis to evaluate the effect of HPD use by subtracting 10 dB(A) from participants' noise exposure if they reported using a HPD daily. This did not change results for the association between occupational noise exposure and tinnitus noticeably.

Furthermore, we analysed whether the associations between the noise exposure variables and tinnitus were independent of participants' hearing status by adjusting analyses for worse ear hearing levels, which resulted in only marginal changes (results not shown).

5.4 Study IV

Main findings

As indicated in figures 10 to 15, we observed no statistically significant associations between cortisol measures (awakening cortisol, awakening+30 cortisol, cortisol awakening response, evening cortisol, cortisol slope, and area under the curve) and

tinnitus. A weak association between steeper slope of cortisol across the day and tinnitus was indicated. Thus, the OR of tinnitus showed a discreetly decreased risk of tinnitus, with a flatter diurnal slope of cortisol (fully adjusted OR: 0.76 (95% CI 0.54; 1.06) per 1.0 nmol/L flatter slope on the logarithmic scale

Figure 10. Adjusted odds ratios (OR) of tinnitus according to awakening cortisol level.

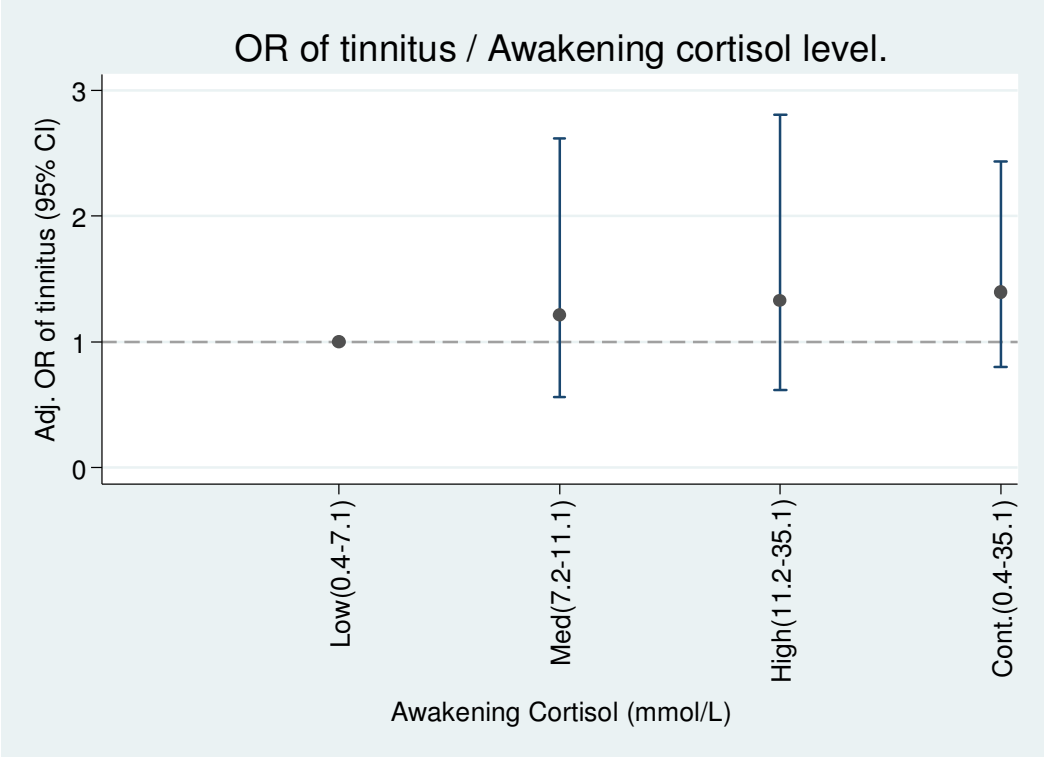


Figure 11. Adjusted odds ratios (OR) of tinnitus according to awakening+30 cortisol level.

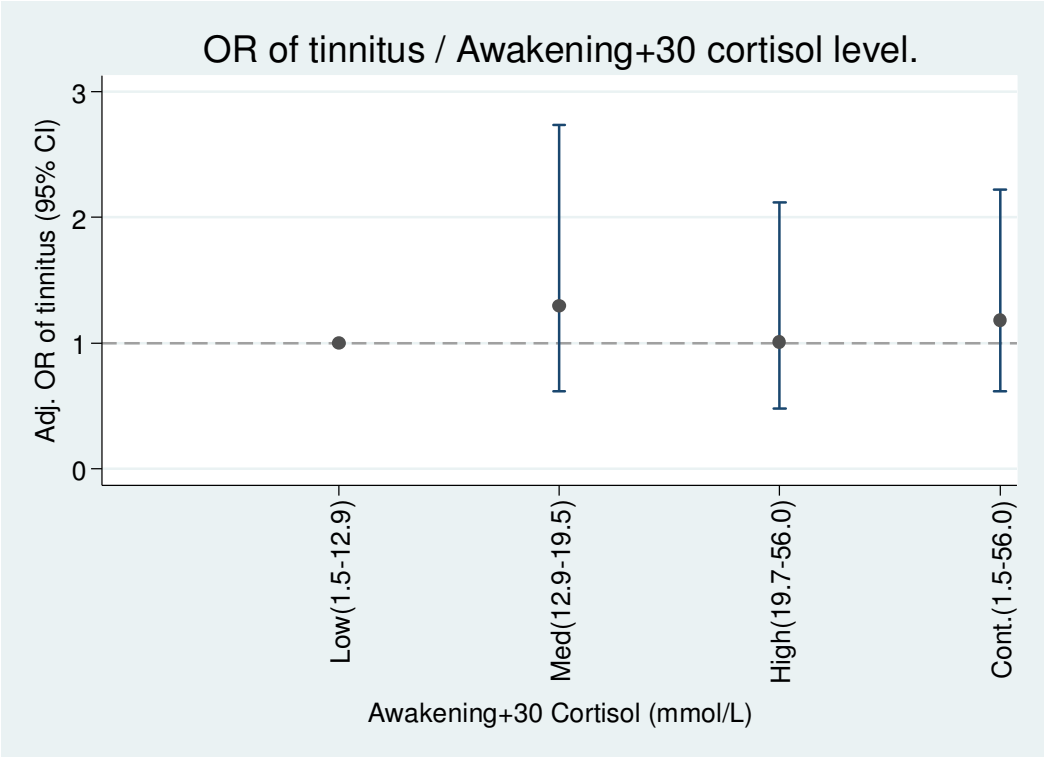


Figure 12. Adjusted odds ratios (OR) of tinnitus according to cortisol awakening response.

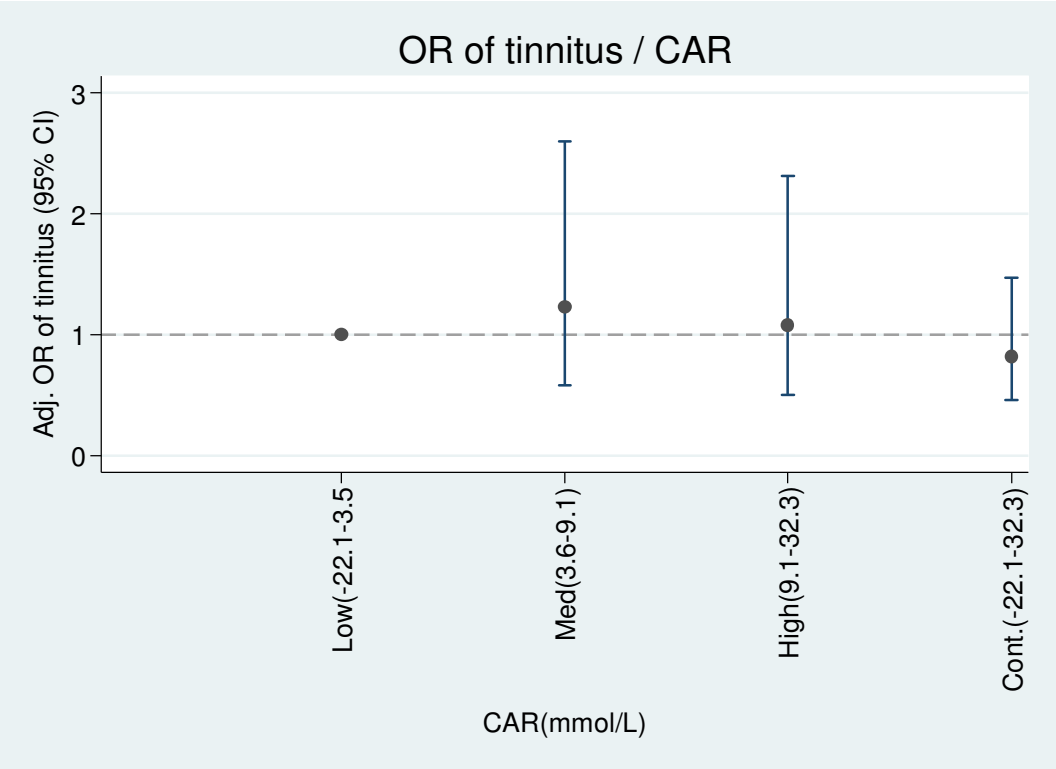


Figure 13. Adjusted odds ratios (OR) of tinnitus according to evening cortisol level.

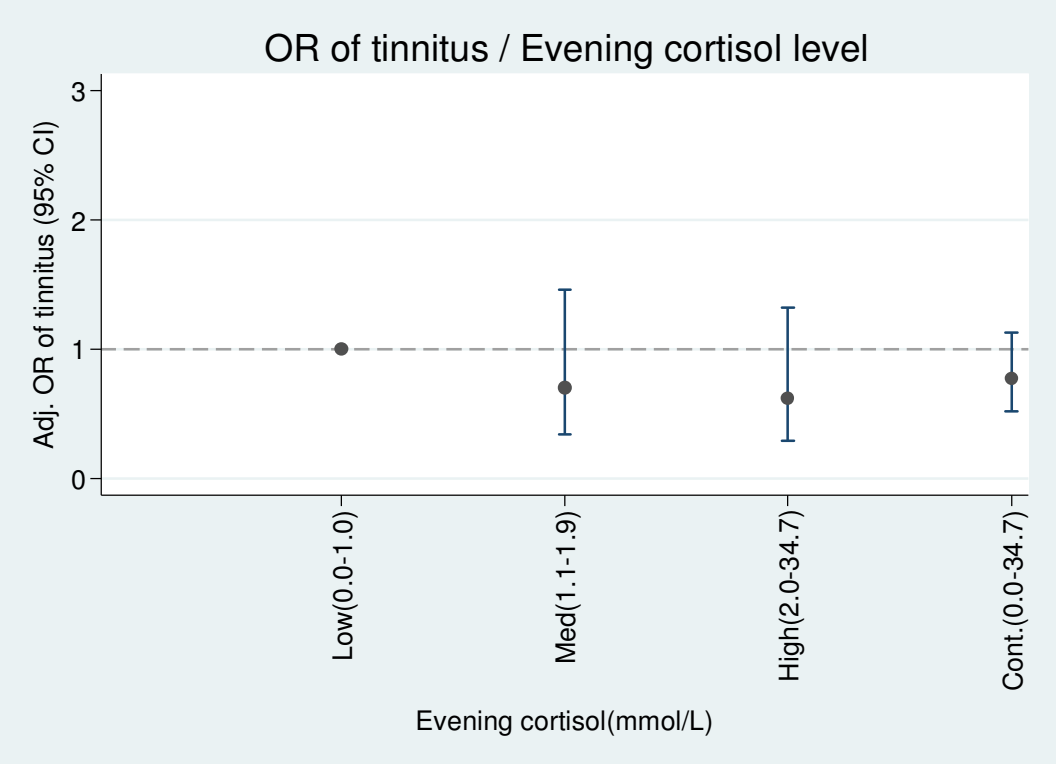


Figure 14. Adjusted odds ratios (OR) of tinnitus according to slope of cortisol.

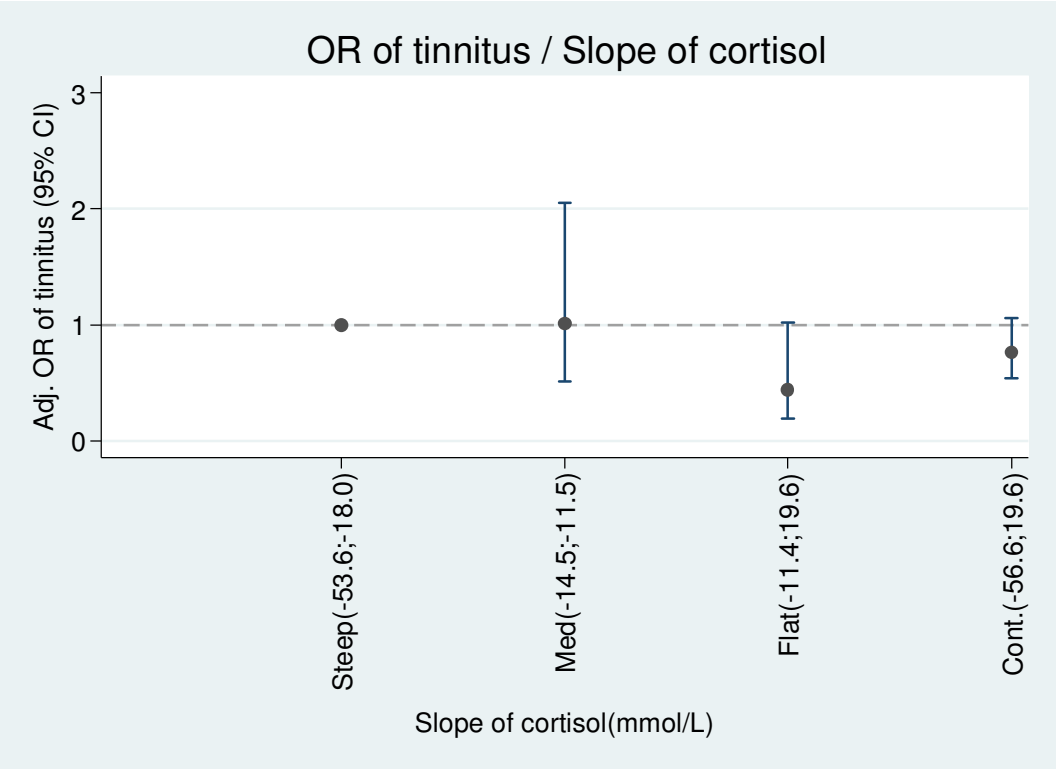
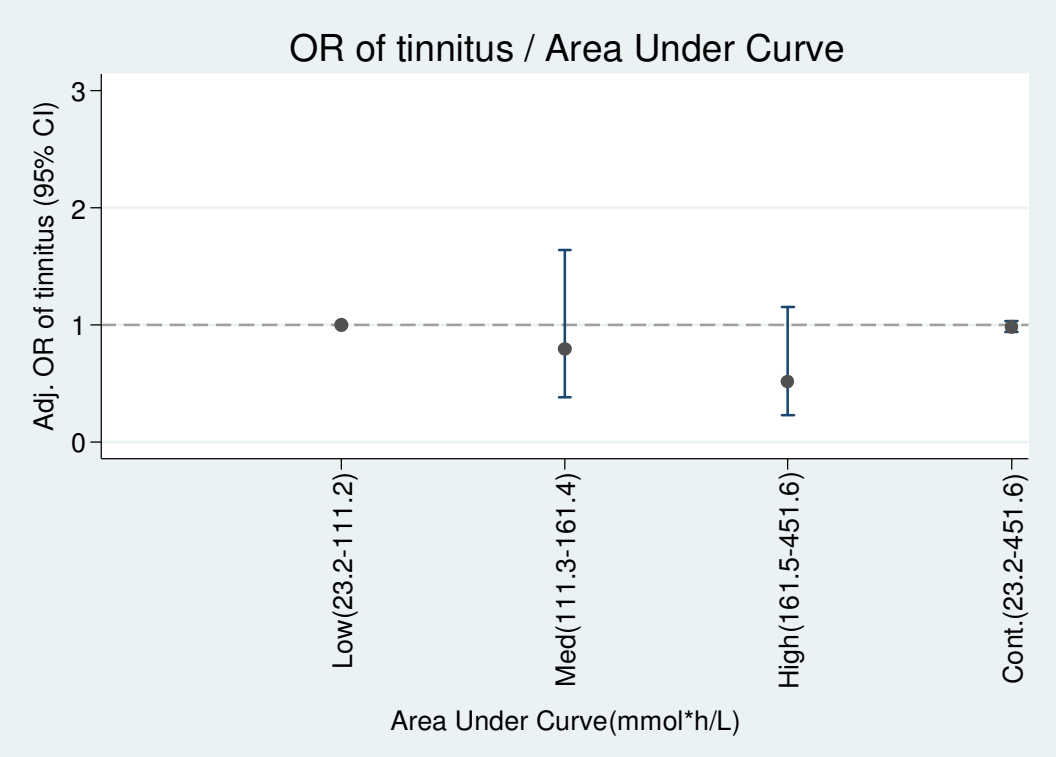


Figure 15. Adjusted odds ratios (OR) of tinnitus according to area under curve (AUC).



Additional analyses

We performed supplementary analyses in which we adjusted for occupational noise levels which did not change results markedly (results not shown).

Finally, we adjusted for time of awakening and time of cortisol sampling to see whether this would affect results. Results were practically unchanged (results not shown).

6. DISCUSSION

6.1 Key findings

In study I, we found decreasing industrial noise levels in the period 2001-2010 across all noisy industries except manufacture of machinery. Furthermore, we observed an increasing use of HPDs (from 2001-2010) when participants worked in mean noise levels exceeding 85 dB(A). No association between noise exposure during the follow-up period and hearing loss in the critically noise-sensitive frequencies was observed. Evaluating the association between first year of occupational noise exposure >80 dB(A) and hearing loss in the noise sensitive frequencies showed that the earlier workers had been exposed, the higher OR of hearing loss. Particularly exposure before the 1980s seemed connected with hearing loss.

In study II, we found significant associations between smoking, high BMI, and triglyceride level and low high density lipoprotein level and increased low frequency hearing thresholds (average of pure tone hearing thresholds at 0.25, 0.5, and 1 kHz), whereas no associations between atherogenic risk factors and high frequency hearing thresholds were observed.

Studies III and IV revealed no associations between occupational noise exposure, psychosocial working conditions, or salivary cortisol levels and tinnitus.

6.2 Methodological considerations

6.2.1 Outcome definitions

In study I, mean hearing levels in the critically noise sensitive frequencies (3, 4, and 6 kHz) were chosen as the outcome variable since the exposure was occupational noise known to exert most damage in this frequency spectrum[16]. These frequencies were also chosen to facilitate comparison of our results with corresponding studies, as this outcome variable appears to be the most commonly applied[29,30,32,33,37,92].

In study II, we decided to use both low and high frequency hearing levels as outcome variables, as the causal pathway from atherosclerosis to hearing loss was unclear and results from prior equivalent studies did not give any clear answer as to which specific frequencies to choose.

In studies III and IV, tinnitus (dichotomous) was defined from the three tinnitus-related questions in the questionnaire. These questions concerned frequency, level of annoyance, and insomnia, due to tinnitus. Using standard tinnitus questionnaires like the Tinnitus Functional Index (TFI) or the Tinnitus Handicap Inventory[93] (THI) would have allowed for a more detailed tinnitus classification and easier comparison with other studies. Tinnitus-related symptoms were, however, among many other questions in the questionnaire and therefore had to be simplified to avoid a too lengthy and laborious questionnaire.

6.2.2 Exposure assessment

To circumvent the problem of biased self-reporting of exposure, we sought to assess exposures as objectively as possible in all four studies. Thus, noise exposure was objectively measured with personal dosimetry, all atherogenic risk factors except smoking were assessed using objective data, and we made use of saliva cortisol as an objective indicator of stress. To avoid the problem of biased self-reporting of psychosocial work factors, we used group-based exposure and excluded patients with tinnitus from the calculation of work-unit mean exposure scores, thereby avoiding that distressing tinnitus would influence the assessment of working conditions.

We estimated cumulative occupational noise in two exposure models (NEM I and II) described in section 4.2. These were both based on grouped (trade and workplace) mean noise levels, as we expected occupational noise levels to have high day-day variability within workers. Grouped mean values should therefore provide a better estimate for the individual, long-term noise exposure[94].

A general advantage of using group based exposure assessment for psychosocial working conditions is that - compared to individual level exposure assessment - it reduces the risk of classical error and thereby attenuation bias[95]. The disadvantage, however, is an increased imprecision of the exposure-response slope coefficients[96] (reduced power).

6.2.3 Selection bias

Participation in our study was voluntary for both companies and workers. Knowing that the study concerned potentially harmful effects of occupational noise and stress, company managers with poor control of psychological working conditions, noise exposure and noise protection and perhaps knowing that they had many workers with, e.g. hearing loss and tinnitus, may have rejected participation. Also, participants with poor health in terms of, e.g. hearing loss or psychological disorders, may have either rejected or shown more interest in participation to either hide or expose their problems to the management. An over-sampling of both healthy companies and healthy workers in relation to exposures represents a potential risk of sampling bias in our study and would result in an attenuation of our results towards the null and an undermining of the internal validity of our results. This problem is, however, less likely in studies II and IV, since exposures in these studies (except BMI and smoking) were most likely unknown by both managers and the participant in advance.

In study I, the risk of selecting healthy workers at follow-up (no hearing loss) was also a concern, particularly if loss to follow-up or change of job during the follow-up period was related to both hearing loss and noise exposure. This would attenuate the association. We were not able to analyse whether workers who were lost to follow-up did so because of a particularly susceptible hearing, as no longitudinal data were available for drop-outs. Among the workers participating in both rounds, we were able to analyse how many changed from high to low noise exposure jobs and whether this change was associated with base-line hearing. We identified 12 workers (4%) who potentially changed from high to low exposure jobs during follow-up. This low number in it self contradicts a significant healthy worker selection among the workers participating in both rounds. However, we analysed whether potential change of job from high to low exposure was associated with baseline hearing status and found no association.

6.2.4 Information bias

Occupational noise exposure measurements were obtained using personal dosimetries that provided better personal noise exposure assessments than stationary measurements. The pros and cons of using group-based noise exposure assessment are addressed in section 6.2.2. The potential misclassification arising from this strategy is expected to be non-differential and should therefore bias our results towards the null.

We made two retrospective noise exposure matrices to estimate cumulative retrospective occupational noise exposure. These were based on noise recordings at baseline and follow-up and on information on prior employment. The resulting estimate is therefore a rough estimate of the true cumulative noise exposure and misclassification is a potential problem. Again, we do not expect this misclassification to be related to worker's hearing or tinnitus status and should therefore be non-differential, biasing results towards the null.

Another potential source of noise exposure misclassification is the use of HPDs that were worn by many of our workers – particularly when exposed to high noise levels. As use of a HPD is most probably also associated with both hearing loss and tinnitus status this could potentially lead to differential misclassification and an underestimation of the true association. In studies I and III, we therefore performed sub-analyses accounting for the use of HPDs by subtracting 10 dB from noise exposure levels if workers reported daily use of a HPD. This level was chosen since previous studies had observed HPD attenuation of noise between 5-20 dB(A) depending on HPD type[97]. This did not alter results significantly. However, differential misclassification is still possible if HPDs are used more consistently at higher than at lower noise levels.

Concerning atherogenic risk factors, all measures except smoking habits were objective, thereby reducing the risk of misclassification. Information on smoking was self-reported and could potentially be under-reported due to stigmatisation. Again, we do not believe that this misclassification is related to the outcome (hearing levels) and the misclassification should therefore also be non-differential.

To circumvent reporting bias, we chose to use aggregated self-reported information on psychosocial work factor (as described in section 4.5). One may argue that working conditions may vary significantly between workers joining the same work-unit and that this variance is not captured by our work-unit average exposure measurement. However, we find it reasonable to assume that this misclassification is non-differential and results in Berkson type error. From this, estimates obtained from grouped exposures are not expected to be attenuated, but this is at the cost of reducing the precision of the estimates[96].

Using a biomarker like saliva cortisol as an objective correlate of stress has an advantage over self-reported stress, because reporting bias is not an issue of concern. Due to the diurnal cortisol variation, correct sampling time is, however, important to

avoid misclassification. We instructed workers to collect saliva samples at awakening, 30 minutes after awakening, and at 8 p.m. Even if instruct to do so, many workers did not collect their samples at this exact time, which could be a possible source of misclassification. A sensitivity analysis was therefore carried out to observe whether adjusting for awakening time and sampling time affected our results, which was not the case.

Our tinnitus classification is described in section 4.2 and commented on in section 6.2.1. We chose to dichotomise the outcome into tinnitus and non-tinnitus cases, and the classification can be argued to be either too strict or too loose. A too loose classification would over-diagnose tinnitus, whereas a too strict definition would under-diagnose tinnitus. This misclassification is expected to be non-differential across workers and would bias results towards the null.

Air-conduction thresholds were measured in a sound proof box using a standardised protocol and was preceded by otoscopy. Air-conduction thresholds are not equal to sensorineural hearing thresholds if a conductive hearing loss is present. Measuring bone conduction thresholds would have allowed us to isolate the sensorineural component of hearing thresholds, but due to time and financial restrictions, this was not possible. Instead, to avoid this misclassification from conductive losses, we excluded workers reporting current or prior chronic middle ear infection or tympanic membrane perforation in studies I and II.

Audiometry was performed during the working day, meaning that some workers were potentially noise exposed before audiometry. To avoid possible temporary threshold shifts from such exposure, we instructed workers to wear a HPD until audiometry was over.

Another theoretical possibility of misclassification of hearing thresholds is non-organic hearing loss in workers who may be eager to demonstrate substantial hearing loss to the management. Such hearing losses would probably be related to both the exposure and outcomes of studies I and III, i.e. workers exposed to high noise levels would exaggerate their hearing losses as a silent protest. This would lead to differential misclassification and inflated results.

6.2.5 Confounding and effect modification

The selection of potential confounders in the four studies was based on a review of previous literature and was also guided by peer reviewers' comments and restricted by

the limited number of tinnitus and hearing loss cases and accompanying limited ability to adjust.

In study I, we adjusted for age and sex. Analyses on the association between cumulative noise exposure and hearing threshold shift in the follow-up period were initially stratified on prior occupational noise exposure and baseline hearing loss. Results indicated no effect modification from these factors. We also performed analyses adjusted for these factors which did not change results noticeably.

In study II, we also adjusted for age and sex together with a number of other potential risk factors that we had cross-sectional data on (socioeconomic status, family history of hearing loss, military service, noisy leisure time activities, and hunting and shooting).

As prior studies most often adjusted associations for only age and sex, we also performed analyses adjusted for these factors only, which did not change the overall results.

In study III, we adjusted analyses in two steps: (1) adjusting for only age and sex, and (2) included also depression, anxiety, somatisation disorder, education, and income. Again, extended confounder adjustment changed results only marginally.

In study IV, a comparable stepwise adjustment was made, and worst ear hearing threshold was also included in the extended adjustment. Adjusted results did not differ significantly from crude results

6.3 Main findings in the light of other studies

Study I

As described in the background section, studies on noise-induced hearing loss are very numerous, although high quality studies with objective measurements of both noise exposure and hearing thresholds and a longitudinal design are not as frequent.

In study I, we found no statistically significant association between occupational noise exposure variables and hearing threshold change in the critically noise-sensitive frequencies in the period from 2001 to 2010. Actually, our regression coefficient was slightly negative. In a comparable study by Rabinowitz et al. from 2006[32], a statistically significant inverse association between 10-year hearing loss rates and cumulative occupational noise exposure was found (ß = -0.18 in the highest exposure group). This study was, in many aspects, similar to ours: study design was

longitudinal and covered 10 years, noise exposure was assessed using personal dosimetries and outcome was measured with pure-tone audiometry focusing on the 3, 4 and 6 kHz frequencies. Rabinowitz et al. also performed sub-analyses to rule out a healthy worker bias to be the reason for the inverse association and thereafter speculated whether the inverse association was potentially due to hearing conservation measures. Specifically the use of HPDs may have managed to reduce hearing loss, especially among those with the highest exposure. The inverse association could therefore be due to differential use of HPDs as Rabinowitz et al. identified the main part of large threshold shifts among workers exposed to mean noise levels < 85 dB where HPDs may not be used as consistently. In study I, we tabulated use of HPDs according to mean occupational noise exposure level over or under 85 dB(A) and found a more consistent use of HPDs at levels above 85 dB(A). As explained in section 6.2.4, this may lead to differential misclassification and attenuation of our results as use of HPD is probably not only related to noise exposure but also to hearing.

In a retrospective study by Brühl et al.[34], the hearing loss of Swedish male metal workers in the years 1964, 1972, 1980, 1987, and 1989 was compared to ISO 1999 A. Results indicated that during the period from 1964 to 1989, the hearing loss decreased from about 20 to 5 dB in the age group 20-29 years and from 30 to 10 dB in the age group 50-59 years. Authors ascribed this to better hearing protection and lower exposure levels during that period. By categorising our baseline and follow-up workers by their year of first noise exposure >80 dB(A), we discovered the highest risk of hearing loss among workers with first exposure before the 1980s in the baseline as well as the follow-up population. Although this analysis is different from Brühl et al.'s, it also indicates that working in noise before noise legislation was effectively enforced comprised a higher risk of hearing loss.

Other longitudinal studies evaluating the association between recent industrial noise and hearing loss include railway workers[92], automobile company workers[30], and steelworkers[31], all showing a limited effect of contemporary industrial noise levels.

Certain industries, however, still seem to lag behind in terms of protecting workers against noise induced hearing loss. Thus, Engdahl & Tambs[35], Leensen et al[36], and Seixas[37] all find significant associations between construction noise and hearing loss. Paradoxically, in the study by Leensen et al., low exposure levels again seem to have the largest impact (7 dB hearing loss in the 55-64 year group compared with an

internal control group), which is also explained by differential use of HPDs at high and moderate exposure levels. Seixas also emphasises HPDs as a potential source of noise misclassification and observes a poor compliance and inaccurate reporting of HPD use. In his cohort of construction workers, every 10 dB increase in noise exposure results in a 2-3 dB hearing loss after 10 years. Due to lack of statistical power, we were not able to analyse construction workers separately, but, interestingly, we found the most substantial fall in noise exposure levels over the follow-up period in this group, which may indicate better results for this group in the future.

Study II

In study II, we analysed the association between atherogenic risk factors (blood lipids, glycosylated haemoglobin, smoking habits, body mass index (BMI), and ambulatory blood pressure) and hearing thresholds. We found associations between smoking, high BMI, and triglyceride level and low high density lipoprotein level and increased low frequency hearing thresholds. Associations were generally strongest with hearing levels in the worst hearing ear. No significant observations were observed between atherogenic risk factors and high frequency hearing thresholds. Comparing these results to five corresponding studies[43-47], results for smoking seem to be the most consistent, although Gates et al.[44] did not find significant results for smoking and Fransen et al.[43] only found an association between smoking and high frequency hearing loss, which is contradictory to our results because we found that smoking was associated with only low frequency hearing thresholds. BMI is associated with hearing thresholds in two other studies[43,46], whereas Shargorodsky[47] finds no association. Concerning blood pressure, there also seems to be controversy. Two studies find an association with hearing variables[44,45] and two do not[46,47]; the latter two being in line with the results of our study.

Concerning diabetes-related measures, we find no association with neither of our hearing outcomes, which is in accordance with Shargordsky's prospective study[47], but in disagreement with results from Friedland[45] and Engdahl[46].

Results for blood lipids are only in accordance concerning results for HDL. Thus, neither Engdahl[46] nor Gates[44] or our study finds any risk of poorer hearing from high HDL. In fact, Gates finds higher HDL a protective factor in relation to hearing thresholds, but only among women. Likewise, we found that low HDL was associated with increased low frequency hearing thresholds. Total cholesterol, which is the only

cholesterol measure taken into account in most of the studies, is found to be associated with poorer hearing in two of the studies[45,47], whereas two studies[44,46] together with ours find no association.

In conclusion, prior studies generally find rather weak associations between atherogenic risk factors and hearing loss, results are contradictory and the studies have low levels of evidence.

Study III

In study III, we analysed the association between two occupational exposures (noise and psychosocial work factors) and tinnitus and found no statistically significant associations.

Regarding occupational noise exposure, this is remarkable, since other studies we identified on this issue consistently find statistically significant associations[56,58-61,98,99]. Each of these studies, however, assesses occupational noise exposure based on self reports, which potentially carries a risk of reporting bias. It is possible in these studies that subjects selectively report higher prior noise exposure if affected by tinnitus (potentially thought of as caused by their prior occupation), which would inflate results. Publication bias is another possible explanation. We measured current noise exposure objectively, and assessed current and cumulative exposure based on these measurements together with databases containing information on prior employment to eliminate the risk of reporting bias. As also discussed in study I, occupational noise exposure levels were, however, rather low and HPDs were worn by more than three-quarters of workers when exposed to "toxic" noise (>85 dB(A)) which could also explain our findings.

With regard to psychosocial work factors and tinnitus, we found no indications of an association between the two factors. Four previous studies are of interest[66,67,100,101], although psychosocial work factors were not quantified precisely as we did. One study evaluated the association between "job stress" and tinnitus among call centre operators in Taiwan and found significant associations for the group reporting high job stress[67]. In a second study "occupational stressors" (e.g risk of being moved to another job or getting fired) were also significantly associated with tinnitus[66], whereas a third study found an inverse association between work place organisational justice and tinnitus[100]. In the fourth study, no association between "pressure and social stress

at work" and tinnitus was found[101]. All studies used self-reported individual exposure information which again creates potential for reporting bias as individuals with negative effects (like tinnitus distress) may perceive their work environment more unfavourably, potentially generating an artificial correlation between exposure and outcome. Taking the discrepancy of results and heterogeneity of exposure assessment taken into account, evidence is still needed before conclusions may be drawn within this field.

Study IV

In study IV, we found no significant associations between cortisol measures (awakening cortisol, awakening+30 cortisol, cortisol awakening response, evening cortisol, cortisol slope, and area under the curve) and tinnitus.

Prior studies in this research field are rather heterogeneous with respect to design, cortisol measures, and the classification of tinnitus, and are therefore not readily comparable to our results: In a study by Hébert et al.[78], basal cortisol levels in a group of tinnitus patients with high tinnitus-related distress were found to be significantly elevated compared with the reference group. We also included tinnitus-related distress into our tinnitus classification by only categorising workers with tinnitus if they met a criterion of either accompanying annoyance or insomnia. Still, the conflicting results could be due to inclusion criteria and tinnitus classification, as Hébert's participants with tinnitus were patients with hospital-diagnosed tinnitus and the subgroup with "high tinnitus distress" was classified from a standard tinnitus questionnaire (Tinnitus Reaction Questionnaire). This potentially leaves less room for misclassification and more contrast between groups, making it easier to demonstrate a possible association.

In three other related studies from the same research group, differences in cortisol secretion among tinnitus and non-tinnitus cases were analysed after exposure to noise[76], social stress[77], and a dexamethasone test[79]. All studies showed altered cortisol secretion in tinnitus sufferers compared to controls.

In a recent study by another group, salivary cortisol secretion in tinnitus patients and a control group was compared before and after a stress inducing task. No significant differences between the groups were observed[82]. This result contradicts the results from the Canadian group[77]. The results from these "intervention studies" are, however, interesting and may suggest that it requires an active provocation of the HPA axis and consequtive cortisol measurements to identify an association between

cortisol measures and tinnitus. Merely observing the habitual daily cortisol secretion between tinnitus and non-tinnitus cases reveals no association.

7. CONCLUSION

The findings in this dissertation show that mean industrial noise levels in Denmark in the years 2001-2010 declined from 83.9 dB(A) to 82.8 dB(A). For workers exposed to mean noise levels >85 dB(A), HPDs were used by more than three-quarters. We found no indications of an association between cumulative occupational noise exposure in the follow-up period and changes in hearing threshold in the critically noise-sensitive frequencies (3-4-6 kHz). However, the risk of hearing loss seemed to increase with earlier first year of noise exposure, particularly if exposure was before the 1980s. In addition, we analysed whether the levels of occupational noise exposure measured for this cohort were associated with tinnitus and found no evidence of this.

According to prevailing studies, emotional stress is associated with tinnitus. We therefore hypothesised that the stress hormone cortisol and psychosocial working conditions assessed according to Karasek's and Theorell's demand-control model would be associated with tinnitus. Our results confirmed neither of these hypotheses.

Finally, we analysed whether atherogenic risk factors in terms of blood lipids, glycosylated haemoglobin, smoking habits, BMI, and ambulatory blood pressure were associated with low- and high-frequency hearing threshold. Results suggested associations between smoking, high BMI, and triglyceride level and low high density lipoprotein level and increased low frequency hearing thresholds, whereas no association was found with high frequency hearing threshold.

8. PERSPECTIVES

It is interesting and important that we, in line with other recent studies, found no association between current industrial noise levels and changes in workers' hearing thresholds, as this could indicate that preventive measures have borne fruit. Often this "good news" is lost in the crowd of new epidemiological studies with more "exotic" results. If we want the public and politicians to keep on supporting research in the working environment, it is important not only to report when potential hazards are identified but also when the resulting preventive measures may have reduced a problem. According to our results for tinnitus and hearing loss, the reason for the low impact of occupational noise can be ascribed to either lower occupational noise levels, better protection, or both. Future studies should focus on identifying which of the preventive factors are the most important by better quantification of HPD use and the true attenuation provided by them. This will provide better estimates of which noise levels actually reach the ear-drum. Also, noise surveillance studies should continue to be conducted in the future since noise exposure levels and protection in some industries still seem to be inappropriate with respect to hearing conservation.

The exact mechanisms explaining subjective tinnitus are still not clarified. We tried to add a small piece to the puzzle by evaluating the association between cortisol and psychosocial working conditions and tinnitus. Our results indicated no association, but corresponding studies are still quite scarce, and results are too conflicting to draw conclusions. Our studies were observational, cross-sectional, and with a low number of tinnitus cases. If we expect the association to be modest, future observational studies should focus on including more participants and refining the tinnitus classification, if possible, by using standardised questionnaires, although these are more time consuming.

Concerning atherogenic risk factors, our results indicated an association between smoking, high BMI, and TG level and low HDL level and low frequency hearing. Like our study, most other studies are cross-sectional and results are rather conflicting. Future studies should be longitudinal to better clarify causality. Since our included exposure variables are only proxies for atherosclerosis, it would also be interesting if future studies measured atherosclerosis more directly, e.g. by a non-invasive examination like arterial ultrasonography.

9. ENGLISH SUMMARY

Introduction: Hearing loss and tinnitus represent frequent disorders with potentially debilitating symptoms. Hearing loss due to occupational noise exposure is well documented, and noise is also known to cause tinnitus. In industrialised countries, this knowledge has led to legislation and new procedures that seek to reduce the impact of occupational noise. Accordingly, a number of recent studies have suggested that the impact of occupational noise on hearing is decreasing, but further surveillance and evidence are needed.

Lifestyle has changed significantly over the last century, increasing the incidence of conditions like atherosclerosis. As the cochlea is, metabolically, a very active organ, depending on its vasculature, atherosclerosis may be involved in the pathogenesis of hearing loss. Evidence for this is scarce and still inconsistent.

Hearing loss is probably the most important risk factor for tinnitus, but self-report studies suggest that psychological factors such as stress also play a role.

Self-report studies, however, have validity problems due to reporting bias. One way to circumvent this is to use an objective biomarker for stress such as cortisol.

As psychological work factors are associated with stress, an association between psychological work factors and tinnitus could also be hypothesised.

Methods: This dissertation takes advantage of a survey of 819 workers conducted between 2001 and 2002 in Aarhus, Denmark, with the purpose of monitoring occupational noise exposure and hearing levels among primarily industrial workers. In 2009-10, the same companies and workers were asked to participate again. This time the purpose was extended to also include psychosocial work factors and stress-related disorders. Exposures included in the four papers include occupational noise (current and cumulative), atherogenic risk factors (LDL, TG, total cholesterol, and low levels of HDL, elevated systolic and diastolic ambulatory blood pressure, smoking habits, high levels of glycosylated haemoglobin and high BMI), psychosocial work factors (psychological demands and decision latitude), and saliva cortisol.

Outcome measures (hearing levels and tinnitus) were estimated by audiometry and questionnaire information. In one longitudinal study and three cross-sectional studies,

associations between exposures and outcome were evaluated using linear and logistic regression analyses.

Results: During the follow-up period (2001-2010), we found decreasing ocupational noise exposure levels. Among workers participating in both rounds, we found no association between occupational noise exposure in the follow-up period and changes in hearing threshold in the critically noise-sensitive frequencies (binaural average of pure tone hearing thresholds at 3, 4, and 6 kHz). Hearing protection devices appeared to be used adequately.

We found statistically significant associations between high BMI and triglyceride level and low high density lipoprotein level and increased speech frequency hearing thresholds (binaural average of pure tone hearing thresholds at 0.5, 1, 2, and 3 kHz).

No associations between psychological work factors, occupational noise exposure, or cortisol and tinnitus were demonstrated.

Conclusion: These results indicate that current, Danish, industrial noise levels, in combination with relevant protection, are safer than they once were with respect to hearing loss and tinnitus. Our hypotheses that psychological working conditions and cortisol were associatied with tinnitus were not confirmed in this study. For atherogenic risk factors, we found statistically significant associations between smoking, high BMI, and triglyceride level and low high density lipoprotein level and increased low frequency hearing thresholds. This warrants further studies, preferably based on longitudinal data.

10. DANISH SUMMARY (DANSK RESUMÉ)

Baggrund: Høretab og tinnitus er begge hyppige lidelser med potentielt invaliderende symptomer. Høretab på baggrund af erhvervsmæssig støjbelastning er veldokumenteret og støj er også en mistænkt årsag til tinnitus. I industrialiserede lande, har denne viden ført til lovgivning og nye procedurer, der har til hensigt at reducere skadevirkningen af erhvervsmæssig støj. En række nyere studier har antydet, at skadevirkningerne af erhvervsmæssig støj på hørelidelser er faldende, men der er stadig behov for yderligere overvågning og dokumentation.

Livsstil har også ændret sig væsentligt i det sidste århundrede, og dermed øget forekomsten af visse livsstilsrelaterede sygdomme som åreforkalkning. Cochlea er, metabolisk set, et meget aktiv organ og er derfor afhængigt af en velfungerende karforsyning. Aterosklerose kunne derfor være medvirkende årsag til høretab. Eksisterende studier indenfor dette område er få og resultaterne er usammenhængende.

Høretab er sandsynligvis den vigtigste risikofaktor for tinnitus, men studier der har anvendt selvrapporteret eksponering antyder, at psykologiske faktorer som stress også spiller en rolle. Anvendelse af selvrapporterede data indebærer en risiko for reporting bias, hvilket kan omgås ved at anvende en objektiv biomarkør for stress, såsom cortisol.

Eftersom belastende psykosociale arbejdsforhold kan være er forbundet med stress, kunne man også forestille sig en mulig sammenhæng med tinnitus.

Metoder: Denne afhandling tager udgangspunkt i en undersøgelse af 819 arbejdere gennemført mellem 2001 og 2002 i Aarhus, med det formål at overvåge erhvervsmæssig støjeksponering og høretærskler blandt primært industriarbejdere. I 2009-10 blev de samme virksomheder og arbejdstagere bedt om at deltage igen. Denne gang blev formålet udvidet til også at omfatte psykosociale arbejdsforhold og stress-relaterede lidelser. Eksponeringerne omfatter erhvervsmæssig støj (nuværende og kumulativ), risikofaktorer for atherosclerose (LDL, TG, total kolesterol og lave niveauer af HDL, forhøjet systolisk- og diastolisk døgn-blodtryk, rygevaner, høje niveauer af glykosyleret hæmoglobin og højt BMI), psykosociale arbejdsforhold (høje krav og lav kontrol) og spyt-cortisol.

Udfaldene omfatter høretærskler og tinnitus, målt via audiometri og spørgeskema-oplysninger. Sammenhængen mellem eksponeringer og udfald blev undersøgt via lineær- og logistisk regression.

Resultater: I løbet af opfølgningsperioden (2001-2010) fandt vi faldende erhvervsmæssige støjeksponerings-niveauer. Vi fandt ingen sammenhæng mellem erhvervsmæssig støjbelastning og ændringer i høretærskelen i de kritisk støjfølsomme frekvenser (3, 4 og 6 kHz). Høreværn syntes at blive anvendt adækvat. Vi fandt statistisk signifikante sammenhænge mellem højt BMI og TG-niveau og lavt HDL-niveau og højere høretærskler i talefrekvenserne (0,5, 1, 2 og 3 kHz). Derimod fandt vi ingen associationer mellem hverken psykosociale arbejdsforhold, arbejdsstøj eller cortisol og tinnitus.

Konklusion: Resultaterne indikerer, at nuværende danske industrielle støjniveauer (i kombination med relevant beskyttelse) er mindre skadelige end tidligere i forhold til høretab og tinnitus. Hypotesen om at psykosociale arbejdsforhold og cortisol er associeret med tinnitus blev ikke bekræftet i denne undersøgelse.

Blandt risikofaktorer for aterosklerose fandt vi statistisk signifikante associationer mellem rygning, højt BMI og TG og lavt HDL og dårligere lavfrekvent hørelse.

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12. ORIGINAL PAPERS

Paper I: Thomas W. Frederiksen, Cecilia H. Ramlau-Hansen, Zara Ann Stokholm, Matias B. Grynderup, Åse Marie Hansen PhD, Jesper Kristiansen, Jesper M. Vestergaard, Jens P. Bonde, Henrik A. Kolstad. **Noise Induced Hearing Loss – a preventable disease? Results of a 10-year longitudinal study of occupationally noise exposed workers.** (Under review in 'Noise and Health').

Paper II: Thomas W. Frederiksen, Cecilia H. Ramlau-Hansen, Zara A. Stokholm, Matias B. Grynderup, Åse Marie Hansen, Søren Peter Lund, Jesper Kristiansen, Jesper M. Vestergaard, Jens P. Bonde, Henrik A. Kolstad. **Atherogenic Risk Factors and Hearing Thresholds.** (Published in 'Audiology and Neurotology' 2014;19:310–318).

Paper III: Thomas W. Frederiksen, Cecilia H. Ramlau-Hansen, Zara A. Stokholm, Matias B. Grynderup, Åse Marie Hansen, Søren Peter Lund, Jesper Kristiansen, Jesper M. Vestergaard, Jens P. Bonde, Henrik A. Kolstad. **Occupational Noise Exposure, Psychosocial Working Conditions and the Risk of Tinnitus.** (Published in International Archives of Occupational and Environmental Health, December 2016).

Paper IV: Thomas W. Frederiksen, Cecilia H. Ramlau-Hansen, Zara A. Stokholm, Matias B. Grynderup, Åse Marie Hansen, Søren Peter Lund, Jesper Kristiansen, Jesper M. Vestergaard, Jens P. Bonde, Henrik A. Kolstad. **Salivary Cortisol and Tinnitus.** (Under review in International Journal of Audiology).

PAPER I

Noise Induced Hearing Loss – a Preventable Disease? Results of a 10-year Study of Occupationally Noise Exposed Workers

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ABSTRACT

Objectives

To survey current Danish industrial noise levels and use of hearing protection devices (HPD) over a 10-year period and to characterise the association between occupational noise and hearing threshold shift in the same period. Furthermore, the risk of hearing loss among the baseline and the follow-up cohort according to year of first occupational noise exposure is evaluated.

Methods

In 2001-2003 we conducted a baseline survey of noise and hearing related disorders in 11 industries with suspected high noise levels. In 2009-2010 we were able to follow up on 271 out of the 554 baseline workers (49%). Mean noise levels per industry and self-reported HPD use are described at baseline and follow-up. The association between cumulative occupational noise exposure and hearing threshold shift over the 10-year period was assessed using linear regression, and the risk of hearing loss according to year of first occupational noise exposure was evaluated with logistic regression.

Results

Over the 10-year period mean noise levels declined from 83.9 dB(A) to 82.8 dB(A) and for workers exposed > 85 dB(A) the use of HPD increased from 70.1% to 76.1%. We found a weak, statistically insignificant, inverse association between higher ambient cumulative noise exposure and poorer hearing (-0.10 dB hearing threshold shift pr dB-year (95% CI: -0.36; 0.16). The risk of hearing loss seemed to increase with earlier first year of noise exposure, but odds ratios were only statistically significant among baseline participants with first exposure before the 1980s (OR: 1.90 (95% CI: 1.11; 3.22)

Conclusions

We observed declining industrial noise levels, increased use of HPD and no significant impact on hearing thresholds from current ambient industrial noise levels, which indicate a successful implementation of Danish hearing conservation programs.

1. INTRODUCTION

Occupational noise exposure is recognised as a substantial risk factor for hearing loss and, world-wide, it remains the most frequent cause of preventable sensorineural hearing loss (1, 2). This has led to extensive research into the auditive effects of occupational noise and, in consequence, preventive measures have been implemented. These include engineering solutions minimizing noise emission and reflection, and legislations limiting the time of work-related noise exposure and obliging the use of hearing protection devices (HPD) (3)(4)(5). This means that industrial noise levels and individual occupational noise exposure have potentially changed over the last decades, at least in developed countries. There are therefore good reasons to continue assessing the burden of auditive disease from occupational noise at national or sub-national levels to follow-up on the possible effect of preventive initiatives. A recent systematic review on occupational noise exposure and hearing concluded that hearing loss due to workplace noise was a significant problem in the 1960s and 1970s in industrialized countries, but the impact seemed to have decreased since that period (6). This was suggested to be due to preventive measures, improved regulation or decreased noise exposure. The evidence, however, was still limited mainly du to blunt or incomplete exposure data. Hearing data was concluded to be generally good. Looking through results from recent studies, results also seem to differ between industries and studies are often based on one specific profession, limiting generalisation of results (7-9).

Based on cross-sectional data collected in 2001-2003, we found a three-fold increased risk of hearing handicap among workers with first exposure to occupational noise before the 1980s (10). However, workers starting in noisy work during later years showed no increased risk. We interpreted these findings as the result of successful preventive programs enforced during 1980-1990. To follow-up on these results, we conducted an equivalent survey in 2009-10.

The main objectives of this study were to describe trends in industrial noise exposure levels and use of hearing protection devices over a 10-year period. Furthermore, we evaluate the association between current, Danish, industrial noise levels and hearing threshold shift in the same period and analyse if the year of first occupational noise exposure is associated with hearing loss.

2. MATERIALS AND METHODS

2.1 Participants

This study takes advantage of an initial survey of 819 workers conducted between 2001 and 2003 in Aarhus, Denmark, with the purpose of monitoring occupational noise exposure, auditory function and preventive measures (use of hearing protection devices (HPD)) among noise exposed workers. Participants were recruited from randomly selected companies within 12 trades: children day care (due to reports indicating high noise levels in these units), financial services (expected to have low-level noise exposure) and the 10 manufacturing trades in Denmark with the highest reporting of noise induced hearing loss according to the Danish Working Environment Authority. In 2009-10, the same companies and workers were asked to participate again. We were able to re-identify 756 participants. Due to time and economic restraints, 202 participants (27%) were not contacted (at random) leaving 554 eligible for follow-up. A total of 271 workers (49%) responded and agreed to participate again. At follow-up, 394 workers within the 12 trades were recruited *de novo* to include new workers first to have been noise exposed during later years, making a total of 665 participants in the follow-up cohort.

For cross-sectional analyses of the baseline population we excluded 76 workers with incomplete questionnaire exposure information or no noise-dosimetry, 16 workers with incomplete audiometry, 109 white-collar workers (typically managers and office workers considered to differ considerably from the remaining population with respect to extraneous predictors of hearing loss), 65 workers reporting current or prior chronic middle-ear infection or tympanic membrane perforation (possible conductive hearing loss), and finally 14 workers with asymmetrical hearing loss (possible hearing loss from other causes than noise, defined in section 2.2) resulting in 539 eligible workers for baseline cross-sectional analyses.

Correspondingly, for cross-sectional analyses on the follow-up population, we excluded 38 workers with incomplete questionnaire exposure information or no noise-dosimetry, 98 white-collar workers, 75 workers reporting current or prior chronic middle-ear infection or tympanic membrane perforation and 30 workers with asymmetrical hearing loss resulting in 424 eligible workers.

For longitudinal analyses, we focused on the 271 workers participating in both surveys. Of these, 262 had complete audiometries from both surveys. We excluded two workers with incomplete questionnaire exposure information, 48 white-collar workers and workers reporting either chronic middle ear infection (n=2), tympanic membrane perforation (n=2), scull fracture (n=0) concussion (n=1), meningitis (n=0) or menieres disease (n=0) in the follow-up period, resulting in a final study population of 207 persons.

The local ethical scientific committee approved the study (M.20080239). All participants gave written, informed consent to participate.

2.2. Audiometric measures

Air-conduction thresholds were determined for each ear at 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kHz by pure tone audiometry at the workplaces, using a Voyager 522 audiometer equipped with TDH-39 headphones (Madsen Electronics, Taastrup, Denmark). The audiometer was installed in a mobile examination unit equipped with a sound proof booth (model AB-4240, Eckel Noise Control Technologies, Bagshot, UK). Audiometry was performed by trained examiners using a standardized protocol.

To avoid temporary threshold shifts from possible noise sources, all participants were asked to wear hearing protection from the beginning of the day until the audiometry was performed. Otoscopy verified that ears were free of wax and the tympanic membrane was visible. The audiometer was calibrated every 6 months according to ISO standards. Based on pure tone air-conduction thresholds, we calculated an average binaural hearing threshold level for the critically noise-sensitive frequencies at baseline and follow-up (3-6 kHz-HTL-BL or 3-6 kHz-HTL-FU). Correspondingly, a baseline and a follow-up hearing loss variable (3-6 kHz-HL-BL and 3-6 kHz-HL-FU) was defined if 3-6 kHz-HTL-BL or 3-6 kHz-HTL-FU were above 20 dB. Threshold shift from baseline to follow-up (Δ3-6 kHz-HTL) was calculated subtracting baseline hearing thresholds (3-6 kHz-HTL-BL) from follow-up hearing thresholds (3-6 kHz-HTL-FU). Thus, worsened hearing was reflected by a positive threshold shift. We regarded an inter-aural difference of 20 dBHL or more in two consecutive frequencies from 3-6 kHz as asymmetrical hearing loss.

2.3 Questionnaire information

All participants filled in a questionnaire providing information on medical and professional history. For the purpose of this study, information on age, sex,

professional history (current and prior employment, duration, industry, occupation (blue vs. white collar), use of HPD and the workers judgment whether noise levels in prior jobs were higher, comparable or lower) was retrieved.

2.4 Occupational noise exposure assessment

At baseline and follow-up, individual dosimeters (Bruel & Kjær, model 4443, Nærum, Denmark) measuring A-weighted equivalent sound levels (L_{Aeq}) in 5 second intervals were handed out to the participants. Microphones were fitted at the right side collar if right handed and vice versa if left handed. Measuring range was set to 70-120 dB(A). Individual A-weighted equivalent noise levels were computed for the full work shift ($L_{Aeq, work}$).

Subsequently, workplace and trade specific mean noise levels were calculated based on the individual dosimetries. As noise levels were expected to vary more from day to day for the individual worker than between different workers (11, 12), we estimated the most efficient grouping strategy based on the highest contrast in mean exposure level between the groups. This was done by modeling noise exposure with two mixed effect models including either worker and industry or worker and company as random effects. The highest contrast was found using company-means and thus worker's noise exposure was classified by the mean L_{Aeq}-value calculated for their workplace and not by his or her individual measurement.

The estimation of cumulative occupational noise exposure in the follow-up period was based on: (1) questionnaire information on current and previous employment including trade, period, and the workers' subjective judgment of whether any previous jobs had involved comparable or higher noise exposure levels than their current job, and (2) workplace average L_{Aeq} levels at baseline and follow-up. Each individual employment year was given a noise exposure level based on the following criteria: (1) if the year was within an employment period in a company included in the study, the average workplace level was applied (2) for employment periods in companies not included in the study, noise exposure was classified from the company level of the following employment in a company included in the study, i.e: (a) if the worker reported that noise levels in the prior job were comparable to or higher than the level of the current job, these years were given the same level as in the current workplace or (b) if the noise level was judged to be substantially lower than the exposure at the current company this employment period was classified as non-exposed.

Finally, we calculated cumulative occupational noise exposure levels for each participant in the follow-up period as the product of estimated noise exposure level (L_{AEq} in dB(A)) and duration of employment (T) using the formula: 10 x log [$\Sigma(10^{dB(A)/10}$ x T], resulting in "dB(A)-year" on a logarithmic scale.

The same model was used to estimate the first year of occupational noise exposure >80 dB(A) and the duration of exposure > 80 dB(A) and > 85 dB(A).

2.5 Statistics

We tabulated sex, age and industry across decade of first year of occupational noise exposure above 80 dB(A) for the baseline and follow-up populations (Table 1). For workers that participated in both surveys we tabulated sex, age, 3-6 kHz-HL-BL, occupational noise exposure before baseline and HPD use across 3 categories of cumulative occupational noise in the follow-up period (Table 2).

Logistic regression was used to estimate the association between first year of occupational noise exposure > 80 dB(A) and hearing loss in the critically noise sensitive frequencies for the baseline and the follow-up population, adjusting for age and sex (Table 3).

Among the workers participating in both surveys, crude and adjusted associations between noise exposure variables and hearing threshold shift in the follow-up period were examined, using linear regression with the lowest exposure group as a reference (Table 4). Outcome variables as well as residuals were assessed and found normally distributed. Stratified analyses were performed to evaluate possible effect modification from prior occupational noise exposure and baseline hearing loss on the association between cumulative noise exposure and hearing threshold shift in the follow-up period. A Wald test was performed to test the hypothesis of no effect modification.

HPD use at baseline and follow-up was cross-tabulated with age and gender to identify possible changes in use over the follow-up period (table 5). To look for changes in noise emission from the industries included in this study, we calculated mean industry noise levels based on all individual blue-collar noise recordings at baseline and follow-up (Table 6).

In a sub-analysis we subtracted 10 dB(A) from company noise levels if workers reported to use HPD and repeated the analyses between the cumulative noise exposure variable and hearing threshold shift in the follow-up period as described above.

The STATA statistical package (version 13, StataCorp, College Station, TX, USA) was used for all analyses.

3. RESULTS

As shown in Table 1, the women-to-man-ratio was lower with earlier first noise exposure in baseline and follow-up populations. Also, mean age was higher with earlier first noise exposure in both populations.

Among the 207 workers participating in both surveys, we observed a tendency toward a higher prevalence of males among workers exposed to higher cumulative noise levels and more frequent use of HPD, but no difference in the prevalence between baseline and follow-up (Table 2). Conversely, mean age seemed to be lower with higher cumulative noise exposure (Table 2).

Table 3 shows adjusted odds ratios (ORs) of hearing loss in the critically noise sensitive frequencies (as defined in section 2.2) by year of first occupational noise exposure for baseline and follow-up populations. For the baseline population we observed no increased risk of hearing loss among those with first exposure after the 1980s compared to the reference group (adjusted OR: 1.02 (95% CI 0.59; 1.77). For baseline workers with first exposure before the 1980s we found a statistically significantly increased risk of hearing loss (adjusted OR: 1.90 (95% CI 1.11; 3.22) compared to the reference group. For each extra year since first exposure we found an OR of 1.02 for hearing loss (95% CI 1.00; 1.04) among the baseline workers.

For the follow-up population we also observed a tendency toward increased risk of hearing loss with longer time since first exposure, but results were statistically insignificant. Thus, the adjusted OR for hearing loss for the group with the earliest exposure (before the 1980s) was 1.48 (95% CI 0.58; 3.77).

In the longitudinal analyses of the 207 workers participating in both surveys, we initially performed analyses on the association between cumulative noise exposure and hearing threshold shift in the follow-up period stratified by baseline hearing status and prior noise exposure, to account for possible effect modification from these factors (Table 4). Results showed only marginal differences between the strata and Wald tests indicated no effect modification by these variables.

Therefore, we proceeded with the main longitudinal analyses without stratification for baseline hearing status and prior noise exposure. Adjusted results showed a weak, statistically insignificant, inverse association between higher cumulative noise exposure and hearing threshold shift during the 10-year period. Thus, average hearing threshold shift in the period was -0.09 dB for each extra noise-year (95% CI -0.35;

0.17) (adjusted for age and sex). A vague inverse association was also found between higher number of years exposed > 80 dB (-0.06 dB threshold shift per extra year exposed > 80 dB(A) (95% CI -0.57; 0.29) (adjusted for age and sex) but this association turned weakly positive when analyzing number of years exposed > 85 instead (0.08 dB threshold shift per extra year exposed > 85 dB) (adjusted for age and sex). No association was found between occupational noise level measured at baseline and hearing threshold shift.

Accounting for the use of HPD by adjusting analyses for HPD-use or subtracting 10 dB(A) from company noise levels for the sub-group reporting daily use of HPD did not noticeably change the association between cumulative occupational noise and hearing threshold shift in the follow-up period (association when adjusting for HPD: -0.11 dB per noise-year (95% CI 0(-0.38; 0.16), and association when subtracting 10 dB if HPD was used: -0.09 (95% CI -0.26; 0.10).

According to Table 5, 70% of the baseline population exposed to noise levels >85 dB(A) used HPD, raising to 76% among the follow-up population. Around 75% of men and 50% of women used HPD when exposed > 85 dB(A) at both surveys. No distinctive differences in HPD use between age groups were observed at either baseline or follow-up.

Table 6 shows a general decline in noise levels from baseline to follow-up across the noisy industries included in this study. Only ‘manufacture of machinery’ shows an increasing noise level from 81.3 dB(A) at baseline to 81.8 dB(A) at follow-up. The most prominent fall in noise level over the follow-up period was seen in ‘construction’ (-4.5 dB(A)). Average decline for all included industries from baseline to follow-up was 1.1 dB(A).

4. DISCUSSION

Main results from this study indicate that worker's cumulative occupational noise exposure during the follow-up period from 2000-2010 was not associated with statistically significant changes in hearing in the critically noise sensitive frequencies. Categorizing the baseline and follow-up workers by their year of first noise exposure >80 dB(A), we found the highest risk of hearing loss among workers with first exposure before the 1980s in the baseline as well as the follow-up population.

The prevalence of HPD use among workers exposed to average occupational noise levels > 85 dB(A) increased from 70.1% in 2001-2003 to 76.1% in 2009-2010, whereas mean noise levels in the included industries decreased with 1.1 dB(A).

An average decline in noise level of 1.1 dB(A) over 10 years may appear minute, but remembering that 1 dB represents a power ratio of approximately 1.26 (the decibel is a logarithmic unit), the effect on hearing preservation may be significant. Also, some of the largest declines in mean noise levels are found among the industries with the highest baseline levels, meaning that no mean industry levels exceeded 85 dB(A) in 2009-10. However, mean company noise levels used to classify worker's noise exposure, still exceed 85 dB(A) for a substantial part of workers and in this case around three quarters of workers reported to use HPD. Accordingly, the finding of no association between recent occupational noise levels and hearing threshold shift among our participants was not unexpected.

In a longitudinal cohort study from 2006 (13) an inverse association between 10-year binaural hearing loss rates in the noise-sensitive frequencies (3, 4, and 6 kHz) and higher occupational noise exposure was found among 6217 noise exposed employees. The authors found no indication of a healthy worker bias in their analyses and, therefore, speculated if the result could be related to differential use of HPD as they found the majority of large threshold shifts among workers exposed to average noise levels < 85 dB where HPDs may not be used as consistently. Unfortunately, data on HPD use was not available in that study. We asked workers if they used HPD in their current job and found that among workers exposed to average noise levels <85 dB(A) the use of HPD was in deed substantially lower than at higher levels (Table 5). Misclassification of actual noise at the ear from differential use of HPD could therefore also have introduced a similar bias in our study explaining the null findings.

Another 10-year longitudinal study recently conducted on construction workers in the USA (14) demonstrated that noise levels in this particular industry still constitute a risk for hearing loss in the noise sensitive frequencies (3, 4 and 6 kHz), even though the average estimated noise exposure L(EQ) for the workers was only 2 dB(A) above 85 dB(A). The study population included only newly hired construction apprentices (mean age 27.6 years) assumed to have limited prior noise exposure and good hearing at inception. Interestingly, they found a poor compliance of HPD use among the workers. Thus, only 50% of the construction workers reported to use HPD and when observed, the fraction of exposure time in which HPDs were used was only 17% to 24% (15). Including newly hired apprentices is an advantage to the study as effect modification otherwise may occur from prior noise exposure and poor baseline hearing (16). We included also workers with prior noise exposure and workers from a broader age spectrum (mean age at baseline: 39.9 years) and therefore also performed stratified analyses.

A review from 2015 on occupational noise exposure and hearing concluded that industrial noise levels in general had been reduced over the last few decades and that this had led to improved hearing in noise exposed groups in recent years (6). Only among construction workers, results showed that noise is still a substantial problem with regard to hearing. Our population was too small to allow for trade-specific sub-analyses but in general the conclusions of the review are in line with our findings and, interestingly, we observed the largest fall in noise exposure level from base line to follow-up among construction workers (4.4 dB(A))

Among the strengths of our study is the longitudinal design. Much of prior literature in this field is derived from cross-sectional studies lacking temporal specificity (17-19). Furthermore, our exposure quantification derived from individual dosimetries gives objective measures instead of subjective questionnaire information as often used to classify noise level. We did not have capacity to measure bone conduction thresholds which would have been a better measure of sensorineural hearing threshold. Instead, we excluded participants with possible conductive hearing loss and asymmetric hearing loss from analyses to avoid misclassification. As white-collar workers were considered to differ considerably from the remaining population with respect to covariates (e.g. leisure time noise) that we were not able to adjust for, we decided to restrict the population to occupationally noise exposed workers. Exposure

contrast in this group were considered sufficient, with individual exposure levels ranging from 67.5 to 106.0 dB(A).

A lower loss to follow-up than 51% in our study would have been desirable, but in our selected industries with expected low job tenancy, we find a follow-up of 49% reasonable.

Among the workers participating in both surveys we identified 12 workers (4.4 %) who moved from high to low exposure jobs. If this shift was made because of a higher susceptibility to noise exposure among the 12 workers it could potentially introduce a 'healthy worker bias' by attenuating the exposure response relationship. By regression analysis we therefore analysed if there was an association between change from high to low exposure job during the 10-year period and baseline hearing levels. We found no significant association, indicating that this was not an issue of concern.

Another possibility of bias in our study is misclassification of noise exposure due to HPD use. Information on HPD use was retrieved from the questionnaire and was not controlled by observation of actual behaviour. As mentioned above, prior studies have revealed a large discrepancy between self-reported use and actual behaviour (15) which could also be the case in our study. To analyse if (self-reported) HPD use had impact on our results, we performed sub-analyses subtracting 10 dB from company noise exposure levels for workers reporting HPD use and also tried to adjust regression analyses for use of HPD. Both sub-analyses revealed only slight changes of the main results. However, as pointed out above, differential misclassification of actual 'noise at the ear' by more consistent use of HPD at noise levels above 85dB(A) is still a possibility and could have biased our results by attenuating the exposure response relationship.

Using average company noise levels to classify worker's exposure could furthermore add to noise misclassification. We expected sound levels to vary more from day to day for the individual workers than between different workers and chose it over industry means because analyses of variance showed most exposure contrast using company levels. Misclassifications is, however, still a possibility but should be non-differential across noise exposure levels and would therefore bias results towards the null.

CONCLUSION

This study demonstrates a fall in recent industrial noise levels, increasing use of HPD and no association between the current occupational noise levels and hearing threshold shift.
We interpret these findings as an indication of a successful implementation of preventive measures enforced in Denmark during the last decades to prevent noise induced hearing loss.

CONFLICTS OF INTEREST

All authors state that they have no conflicts of interest.

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TABLES

Table 1. Characteristics of 539 workers from baseline population and 424 workers from the follow-up population by year of first occupational noise exposure >80 dB(A), Aarhus, Denmark

	Baseline population Year of first noise exposure						Follow-up population Year of first noise exposure							
	1990-1999		1980-1989		<1980		2000-2010		1990-1999		1980-1989		<1980	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Sex, no (%)														
Women	52	19.5	22	15.1	14	11.1	29	29.9	38	25.7	20	19.2	10	13.3
Men	215	80.5	124	84.9	112	88.9	68	70.1	110	74.3	84	80.8	65	86.7
Age (years), mean (SD)	267	38.1 (9.4)	146	36.9 (7.0)	126	47.1 (7.0)	97	34.6 (10.0)	148	42.6 (9.2)	104	45.0 (6.1)	75	54.8 (5.5)
		%		%		%		%		%		%		%
Industry (NACE-codes)														
Manufacture of food (15)	33	12.4	19	13.1	18	14.3	20	20.6	16	10.8	14	13.5	5	6.7
Manufacture of wood products (20)	38	14.2	13	9.0	16	12.7	9	9.3	12	8.1	8	7.8	6	8.0
Publishing and printing (22)	33	12.4	25	17.2	16	12.7	8	8.3	17	11.5	9	8.7	7	9.3
Manufacture of non-metallic mineral prod. (26)	25	9.4	12	8.3	15	11.9	7	7.2	7	4.7	5	4.8	7	9.3
Manufacture of basic metals (27)	16	6.0	8	5.5	8	6.4	4	4.1	12	8.1	12	11.5	6	8.0
Manufacture of fabricated metals (28)	34	12.7	21	14.5	17	13.5	11	11.3	24	16.2	13	12.5	8	10.7
Manufacture of machinery (29)	25	9.4	16	11.0	10	7.9	11	11.3	11	7.4	12	11.5	11	14.7
Manufacture of motor vehicles (34)	25	9.4	16	11.0	13	10.3	8	8.3	12	8.1	8	7.7	6	8.0
Manufacture of furniture (36)	7	2.6	6	4.1	4	3.2	1	1.0	2	1.4	2	1.9	0	0.0
Construction (45)	14	5.2	7	4.8	5	4.0	2	2.1	7	4.7	5	4.8	2	2.7
Day care (85)	17	6.4	2	1.4	4	3.2	16	16.5	16	10.8	8	7.7	4	5.3
Other industries	-	-	-	-	-	-	0	0.0	5	3.4	6	5.8	4	5.3
Retired or unemployed	-	-	-	-	-	-	0	0.0	7	4.7	2	1.9	9	12.0

Table 2. Characteristics of the 207 workers participating at both baseline and follow-up by tertiles of cumulative occupational noise exposure (dB(A)-years) in the follow-up period, Aarhus, Denmark, 2009

	Cumulative occupational noise exposure (dB(A)-years)					
	67.7 – 91.8		91.9 – 94.6		94.7 – 107.0	
	n	%	n	%	n	%
Sex, no (%)						
Women	21	45.7	11	13.9	13	15.9
Men	25	54.4	68	86.1	69	84.2
3-6 kHz-HL-BL*						
No	33	71.7	51	64.6	56	68.3
Yes	13	28.3	28	35.4	26	31.7
Duration of daily occupational noise exposure > 80 dB(A) before baseline						
<10 years	24	52.2	28	35.4	44	53.7
≥10 years	22	47.8	51	64.6	38	46.4
Reporting daily use of HPD at baseline						
Yes	21	47.7	46	60.5	56	71.8
No	23	52.3	30	39.5	22	28.2
Reporting daily use of HPD at follow-up						
Yes	22	47.8	47	59.5	55	67.1
No	24	52.2	32	40.5	27	32.9
Age in 2009 (years), mean (SD)	46	50.9 (8.2)	79	48.6 (8.7)	82	46.0 (8.4)

* defined as an average binaural hearing threshold > 20 dB in the noise sensitive frequencies (3, 4 and 6 kHZ)
Eller * as defined in section 2.2

Table 3. Age and sex adjusted odds ratios (OR) of hearing handicap in the critically noise sensitive frequencies* according to year of first noise exposure among the baseline and follow-up populations

Year of first noise exposure > 80 dB	Subjects	Cases	OR	95% CI
Baseline population				
1990-1999	265	70	ref	
1980-1989	148	32	1.02	0.59; 1.77
<1980	126	79	1.90	1.11; 3.22
Continuous pr. year	539	181	1.02	1.00; 1.04
Follow-up population				
2000-2010	97	30	ref	
1990-1999	147	69	1.04	0.55; 1.95
1980-1989	105	62	1.30	0.66; 2.57
<1980	75	61	1.48	0.58; 3.77
Continuous pr. year	424	222	1.00	0.98; 1.04

* defined as an average binaural hearing threshold > 20 dB in the noise sensitive frequencies (3, 4 and 6 kHZ)

Table 4. Crude and adjusted associations between noise exposure variables and bilateral hearing threshold shift in the critically noise sensitive frequencies (3-6 kHz) among 207 workers followed from baseline to follow-up

	n	Crude Δ3-6 kHz-HTL-BI	Adjusted[1] Δ3-6 kHz-HTL-BI	Ajusted[2] Δ3-6 kHz-HTL-BI
Cumulative occupational noise exposure, dB(A)-years				
Low (76.6-91.3)	46	reference	reference	reference
Medium (91.4-94.8)	79	-1.14 (-3.79; 1.52)	-1.34 (-4.04; 1.35)	-1.44 (-4.15; 1.27)
High (94.9-107.0)	82	-0.88 (-3.51; 1.76)	-0.51 (-3.29; 2.20)	-0.70 (-4.15; 2.01)
Continuous		-0.13 (-0.39; 0.13)	-0.09 (-0.35; 0.17)	-0.10 (-0.36; 0.16)
Baseline occupational noise exposure (L_{Aeq})				
80-85 dB(A)	99	reference	reference	reference
>85 dB(A)	106	1.08 (-0.92; 3.08)	0.77 (-1.20; 2.74)	0.56 (-1.41; 2.54)
Continuous (80.2-92,8)		0.01 (-0.32; 0.33)	0.00 (-0.32; 0.32)	-0.01 (-0.33; 0.31)
Years exposed > 80 dB(A) from baseline to follow-up				
0-5	43	reference	reference	reference
6-10	166	-0.42 (-2.76; 1.91)	-0.14 (-2.53; 2.26)	-0.24 (-2.64; 2.15)
Continuous (0-10)		-0.25 (-0.68; 0.17)	-0.06 (-0.50; 0.37)	-0.09 (-0.52; 0.34)
Years exposed > 85 dB(A) from baseline to follow-up				
0-5	133	reference	reference	reference
6-10	76	0.75 (-139; 2.89)	0.64 (-1.41; 2.68)	0.65 (-1.41; 2.70)
Continuous (0-10)		0.07 (-0.21; 0.35)	0.08 (-0.20; 0.36)	0.06 (-0.22; 0.34)

[1] Adjusted for sex and age
[2] Adjusted for sex, age, baseline hearing threshold and prior noise exposure >10 years

Table 5. HPD use at baseline and follow-up according to occupational noise level, sex and age group

	HPD use among baseline participants (n=539)								HPD use among follow-up participants (n=424)							
	<85 dB(A)				≥85 dB(A)				<85 dB(A)				≥85 dB(A)			
	yes		no		yes		no		yes		no		yes		no	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Sex, no (%)																
Female	22	37.3	37	62.7	12	44.4	15	55.6	15	20.0	60	80.0	9	56.3	7	43.8
Male	98	50.3	97	49.7	171	73.1	63	26.9	119	71.7	47	28.3	109	78.4	30	21.6
Age, no (%)																
< 40	66	52.8	59	47.2	101	71.6	40	28.4	46	56.8	35	43.2	48	71.6	19	28.4
40-50	43	47.8	47	52.2	45	63.4	26	36.6	53	55.8	42	44.2	33	80.5	8	19.5
>50	11	28.2	28	71.8	37	75.5	12	24.5	35	53.9	30	46.2	37	78.7	10	21.3
All	120	47.2	134	52.8	183	70.1	78	29.9	134	55.6	107	44.4	118	76.1	37	23.9

Table 6. Mean noise levels per industry at baseline and follow-up, Aarhus, Demnark

	No. of noise measurements	Mean noise level at baseline ($L_{Aeq, work}$), min, max (dB(A))	No. of noise measurements	Mean noise level at follow-up ($L_{Aeq, work}$), min, max (dB(A))	Difference (dB(A))
Industry (NACE code)					
Manufacture of food (15)	79	84.7 (74.0-99.1)	58	84.5 (76.6-91.6)	-0.2
Manufacture of wood products (20)	72	85.3 (76.5-96.3)	40	84.9 (72.8-96.2)	-0.4
Publishing and printing (22)	87	81.9 (64.7-90.7)	53	81.7 (67.8-89.4)	-0.2
Manufacture of non-metallic prod. (26)	64	85.2 (74.8-97.2)	40	84.0 (75.4-106.0)	-1.2
Manufacture of basic metals (27)	44	85.6 (75.4-100.0)	24	83.0 (74.9-93.0)	-2.6
Manufacture of fabricated metals (28)	84	85.4 (73.7-97.4)	58	83.2 (71.7-94.9)	-2.2
Manufacture of machinery (29)	55	81.3 (73.3-90.7)	65	81.8 (67.5-91.3)	+0.5
Manufacture of motor vehicles (34)	65	83.8 (70.2-96.2)	44	82.6 (72.3-100.0)	-1.2
Manufacture of furniture (36)	18	81.0 (73.4-88.0)	7	80.6 (73.7-85.7)	-0.4
Construction (45)	27	84.6 (73.7-91.3)	22	80.1 (70.9-88.3)	-4.5
Day care (85)	32	82.2 (68.4-92.5)	56	81.9 (76.0-103.0)	-0.3
All noisy trades	627	83.9 (64.7-100.0)	467	82.8 (67.5-106.0)	-1.1

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PAPER II

Atherogenic Risk Factorsand Hearing Thresholds

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Atherogenic Risk Factors and Hearing Thresholds

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Key Words
Sensorineural hearing loss · Blood lipids · Glycosylated hemoglobin · Smoking habits · Body mass index · Ambulatory blood pressure

Abstract
The objective of this study was to evaluate the influence of atherogenic risk factors on hearing thresholds. In a cross-sectional study we analyzed data from a Danish survey in 2009–2010 on physical and psychological working conditions. The study included 576 white- and blue-collar workers from children's day care units, financial services and 10 manufacturing trades. Associations between atherogenic risk factors (blood lipids, glycosylated hemoglobin, smoking habits, body mass index (BMI), and ambulatory blood pressure) and hearing thresholds were analyzed using multiple linear regression models. Adjusted results suggested associations between smoking, high BMI and triglyceride level and low high-density lipoprotein level and increased low-frequency hearing thresholds (average of pure-tone hearing thresholds at 0.25, 0.5 and 1 kHz). Furthermore, an increasing load of atherogenic risk factors seemed associated with increased low-frequency hearing thresholds, but only at a borderline level of statistical significance. Associations were generally strongest with hearing levels of the worst hearing ear. We found no statistically significant associations between atherogenic risk factors and high-frequency hearing thresholds (average of pure-tone hearing thresholds at 4, 6 and 8 kHz).

Introduction

Sensorineural hearing thresholds generally increase with age due to a gradual degeneration of the cochlea and its central neural pathways [Schuknecht, 1964; Schuknecht and Gacek, 1993]. A complex interplay of environmental and genetic factors is thought to be the reason for this [Van Eyken et al., 2007].

Twin studies suggest that around half of the variance in sensorineural hearing thresholds in the middle-aged and older age groups is derived from genetic factors and the other half from environmental factors [Karlsson et al.,

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1997]. This allows for marked variation in median hearing thresholds within age groups [International Organization for Standardization, 2000] and the potential for the prevention of hearing loss if we learn more about the underlying nongenetic risk factors.

Occupational and leisure-time noise exposure [Daniel, 2007], ototoxic medication [Schacht et al., 2012] and industrial chemicals such as styrene and toluene [Hoet and Lison, 2008; Sliwinska-Kowalska, 2008] are among the already known risk factors for sensorineural hearing loss. In industrialized countries, this knowledge has led to legislation and new procedures intending to reduce the impact of these factors [Osguthorpe and Klein, 1991; Rybak and Whitworth, 2005]. This makes it relevant to look for other risk factors for sensorineural hearing loss as the composition of environmental exposures may have changed.

Smoking [Katsiki et al., 2013], hypertension [Chobanian, 1988], impaired blood sugar regulation [Selvin et al., 2006], high body mass index (BMI) [Van Gaal et al., 2006] and dyslipidemia [Koba and Hirano, 2011; Talayero and Sacks, 2011] are known to cause atherosclerotic vascular disease, leading to narrowing of arteries and decreased blood flow. As the cochlea is metabolically a very active organ depending on a steady supply of nutrients and oxygen from its vasculature to maintain homeostasis, atherosclerosis may be involved in the pathogenesis of sensorineural hearing loss.

In the Framingham cohort, cardiovascular disease events were associated with low-frequency hearing loss [Gates et al., 1993] and a more recent study has supported this finding [Friedland et al., 2009]. As atherosclerosis is intimately related to cardiovascular disease events, this may represent the common cause of both cardiovascular disease events and hearing loss, explaining the association found in the Framingham study. However, studies exploring the direct association between atherogenic risk factors and hearing loss show inconsistent findings: dyslipidemia in terms of elevated levels of total cholesterol, low-density lipoprotein (LDL), triglycerides (TG) and low levels of high-density lipoprotein (HDL) have shown mainly adverse effects on hearing ability [Gates et al., 1993; Suzuki et al., 2000; Shargorodsky et al., 2010] but a gainful effect of high total cholesterol level has also been reported [Jones and Davis, 2000]. A relation between hearing ability and diabetes-related measures has been reported in several studies [Austin et al., 2009; Jang et al., 2011; Akinpelu et al., 2014], which is also the case for smoking [Fransen et al., 2008; Shargorodsky et al., 2010] and high BMI [Fransen et al., 2008; Lalwani et al., 2013]. Other studies, however, have shown conflicting results for these factors [Gates et al., 1993; Shargorodsky et al., 2010]. The possible effect of hypertension has been evaluated both independently and in combination with noise exposure, showing both increased risk of hearing loss [Gates et al., 1993; Toppila et al., 2000] and no association [Shargorodsky et al., 2010]. These conflicting results could indicate weak associations that may have to act in combination to significantly affect hearing.

The aim of this study was to evaluate the association between well-established risk factors for atherosclerosis (high levels of LDL, TG and total cholesterol, low levels of HDL, elevated systolic and diastolic ambulatory blood pressure, smoking habits, high levels of glycosylated hemoglobin and high BMI) and hearing thresholds.

Materials and Methods

Participants

This cross-sectional study takes advantage of a survey of 819 workers conducted between 2001 and 2002 in Aarhus, Denmark, with the purpose of monitoring occupational noise exposure and hearing levels among blue- and white-collar workers. The cohort was recruited from children's day care units, financial services and 10 manufacturing trades. In 2009–2010, the same companies and workers were asked to participate again. This time the purpose was extended to also include psychosocial work factors, stress-related disorders, medical risk factors and parameters concerning auditory function. A total of 271 workers agreed to participate again and a further 394 workers were recruited de novo, making a total of 665 participants in 2009–2010. At the company level all participants were as far as possible selected at random. However, to avoid disruption of workflow, selection in some cases had to be done in accordance with the local manager.

In the present study, we excluded 88 participants with possible conductive hearing loss due to questionnaire-reported middle ear disease. Furthermore, we excluded 1 participant reporting Ménière's disease. In total, 576 workers were included in the present study. The age range was 20–73 years (mean 44.1). Workers only participating in 2001–2002 were not included in the present study due to lack of information on atherogenic risk factors as these were only measured in 2009–2010. Eight participants reported to be on antidiabetic medication, 22 participants were on high cholesterol medication and 53 participants took antihypertensives. The local scientific ethics committee approved the study (M.20080239). All participants gave written, informed consent to participate.

Variables

Audiometric Measures

Air conduction thresholds were determined for each ear at 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kHz by pure-tone audiometry at the workplaces, using a Voyager 522 audiometer equipped with TDH-39 headphones (Madsen Electronics, Taastrup, Denmark). The audiometer was installed in a mobile examination unit equipped with a sound-proof booth (model AB-4240; Eckel Noise Control Technologies, Bagshot, UK). Audiometry was performed by trained examiners using a standardized protocol.

To avoid temporary threshold shifts from possible noise sources, all participants were asked to wear hearing protection from the beginning of the day until the audiometry was done. Otoscopy was performed initially to verify that ears were free of wax and the tympanic membrane was visible. The audiometer was calibrated every 6 months according to the standards of the International Organization for Standardization. Based on air conduction thresholds we calculated low- and high-frequency hearing thresholds for each ear. Low-frequency hearing thresholds were calculated as the average of pure-tone hearing thresholds at 0.25, 0.5 and 1 kHz and high-frequency hearing thresholds were defined as the average of pure-tone hearing thresholds at 4, 6 and 8 kHz. As analyses were performed on both the better and the worse hearing ear, we defined 4 hearing thresholds: low-frequency hearing threshold better ear (LFHT-better), low-frequency hearing threshold worse ear (LFHT-worse), high-frequency hearing threshold better ear (HFHT-better) and high-frequency hearing threshold worse ear (HFHT-worse). The better and worse hearing ear were defined as the ear with the lowest and highest average thresholds in the given spectrum, respectively. If hearing levels were equal in both ears, the same threshold value was used for statistical analysis of the better and the worse ear. Correspondingly, low- and high-frequency hearing loss for the better and the worse ear (LFHL-better, LFHL-worse, HFHL-better and HFHL-worse) were defined if LFHT-better, LFHT-worse, HFHT-better or HFHT-worse were above 25 dB hearing level, respectively.

Occupational Noise Exposure Assessment

Individual dosimeters (model 4443; Bruel & Kjær, Nærum, Denmark) measuring A-weighted equivalent sound levels (L_{Aeq}) in 5-second intervals were handed out to the participants. Microphones were fitted at the right-side collar if right-handed and vice versa if left-handed. The measuring range was set to 70–120 dB(A). Individual A-weighted equivalent noise levels were computed for the full work shift ($L_{Aeq, work}$).

Based on 1,268 noise exposure recordings from the 2001–2002 study and the 2009–2010 study, we predicted noise exposure levels for each combination of trade, occupation (blue- vs. white-collar worker) and calendar year (1980–2010) by mixed regression analyses, including these as fixed effects and the participants as random effect. The predicted noise exposure levels were linked with the employment histories of the participants by trade, occupation and calendar year. Information on employment histories (1980–2010) were retrieved from the Danish Supplementary Pension Fund. Using information from the resulting noise exposure matrix we calculated cumulative occupational noise exposure levels for each participant as the product of estimated noise exposure level [L_{Aeq} in dB(A)] and duration of employment (T) using the formula: $10 \times \log [\Sigma(10^{dB(A)/10} \times T)]$, resulting in 'dB(A)-year' on a logarithmic scale.

Questionnaire Information

A questionnaire was handed out to the participants at the time of the audiometric examination to provide information on medical and professional history. For the purpose of this study, information on socioeconomic status (personal income and educational level), middle ear disease (perforated ear drum, recurrent aural discharge and chronic otitis), family history of hearing handicap before the age of 70 years, military service, leisure-time noise exposure (hunting, use of fire arms, heavy use of portable music player, motor sport, playing electrically amplified musical instruments), smoking habits (ever-, never- or current smoker and smoking intensity) and medication (lipid-lowering medication, antidiabetics and antihypertensives) was used. The number of pack-years was calculated as the number of cigarettes smoked per day divided by 20 and multiplied by the number of years smoking.

Biochemical Data, Biometry and Ambulatory Blood Pressure Monitoring

For each worker, height and weight were measured and non-fasting venous blood was sampled by a medical laboratory technologist. Equipment for 24-hour ambulatory blood pressure monitoring (Space Labs 90217) was fitted together with the noise dosimeter and worn by the participant until the next day. Blood pressure was measured every 20 min during daytime (7 a.m. to 11 p.m.) and every 30 min during nighttime (11 p.m. to 7 a.m.) and average 24-hour systolic and diastolic blood pressure values were calculated. As some participants removed the equipment during nighttime, only measurements containing at least 4 nighttime observations were accepted as '24-hour ambulatory blood pressure'.

BMI was calculated as weight in kilograms divided by height in meters squared. Venous blood was refrigerated immediately after extraction, separated and frozen after being returned to the hospital. Biochemical analyses were done at the Department of Biochemistry, Aarhus University Hospital, Denmark, after all samples were collected at the work site. LDL levels were estimated using the Friedewald equation: estimated LDL = total cholesterol – HDL – (TG/5). A total of 15 participants (2.6%) had TG levels over 4.5 mmol/l, making the calculation unreliable. These values were excluded in the analysis of associations between LDL and hearing levels.

Statistics

We tabulated possible confounders according to LFHL-better, LFHL-worse, HFHL-better and HFHL-worse status.

We computed percentage differences in low- and high-frequency hearing thresholds for both the better and the worse ear by atherogenic risk factors using linear regression analysis. For these analyses, hearing threshold values were log transformed to normalize distribution of residuals. As LFHT-better for 82 workers (14.2%), LFHT-worse for 23 workers (4.0%), HFHT-better for 21 workers (3.6%) and HFHT-worse for 4 workers (0.7%) were zero or negative (minimum −5 dB hearing level) these values were replaced with a value of 1 dB hearing level before log transformation.

We adjusted for age, sex, educational level (none, short courses, skilled worker, short-range training, middle-range training, long-range training), personal income (DDK ≤299.999, DDK 300.000–499.999, DDK ≥500.000), family history of hearing loss (yes/no), ear disease (yes/no), military service (yes/no), noisy leisure-time activities (yes/no), hunting and shooting (yes/no). Crude results and results adjusted only for age and sex were also calculated, but were presented only in the text.

As atherosclerosis may result from the combined effect of several risk factors, we calculated an atherogenic risk factor score to examine the combined effect on hearing levels. For this purpose, the highest tertile of total cholesterol, TG, LDL, glycosylated hemoglobin, cumulative smoking, BMI, and 24-hour systolic and diastolic blood pressures was given a score of 1; otherwise a score of 0 was given. For HDL we reversed the scoring. The total atherogenic risk factor score was then calculated as the sum of the individual scores, ranging from 0 to 9 (i.e. a higher score indicated a higher risk of atherosclerosis). All statistical analyses were performed using Stata version 13.

We performed subanalyses in which participants reporting lipid-lowering medications, antidiabetics and antihypertensives were excluded.

Results

The characteristics of participants according to low- and high-frequency hearing loss in the better and the worse ear are presented in table 1. Participants with hearing loss in both low and high frequencies were generally around 10 years older than participants without hearing loss. Among participants with low-frequency hearing loss in the better and the worse ear, the prevalence was higher of males and participants reporting a family history of early hearing loss and former military service than among those with no low-frequency hearing loss. Among participants with high-frequency hearing loss in the better and the worse ear, the prevalence was higher of males, participants with a family history of early hearing loss, former military service, leisure-time hunting or shooting activities and blue-collar work compared with those with no high-frequency hearing loss. Cumulative occupational noise exposure was slightly higher for participants with high- and low-frequency hearing loss for the better as well as the worse ear.

Table 2 shows adjusted percentage differences in low-frequency hearing thresholds in the better and the worse ear by atherogenic risk factors. Results for the better ear showed statistically significant associations between TG ($p = 0.02$), status as former smoker ($p = 0.01$) and low-frequency hearing threshold. Also, there seemed to be a strong association between BMI and LFHT-better, but only at a borderline level of statistical significance ($p = 0.08$). Results for the worse ear showed statistically significant associations between HDL ($p = 0.03$; inverse association), TG ($p = 0.01$), status as former smoker ($p = 0.03$), BMI ($p = 0.03$) and low-frequency hearing threshold. Associations between average 24-hour diastolic blood pressure ($p = 0.07$), the atherogenic risk factor score ($p = 0.07$) and LFHT-worse also appeared strong, albeit only at a borderline level of statistical significance. For the remaining atherogenic risk factors, we generally observed weak positive associations with both better and worse ear low-frequency hearing thresholds. In general, the atherogenic risk factors showed stronger associations for the worse ear than the better ear at low frequencies.

Table 3 gives results for high-frequency hearing thresholds for the worse and the better ear as those presented for low frequencies in table 2. Adjusted results showed no statistically significant results for any of the atherogenic risk factors. As with results for low frequencies, we did, however, observe a general trend of weak positive associations (except for HDL) and the associations were in general stronger with worse ear than better ear thresholds.

To test if the association between BMI and low-frequency hearing threshold in the worse ear was mediated through high TG and low HDL, we performed a multivariable analysis that included BMI, TG and HDL in addition to the other confounders and LFHT-worse. The association between BMI and LFHT-worse decreased substantially. Thus, the mean percentage difference in LFHT-worse was 1.1% (95% CI: –0.6 to 2.8, $p = 0.22$) per unit of BMI, when TG and HDL were included in the model and 1.8% (95% CI: 0.2–3.4, $p = 0.03$) when not included.

Excluding participants taking lipid-lowering medication ($n = 22$, 3.8%), antidiabetics ($n = 8$, 1.4%) and antihypertensives ($n = 53$, 9.2%), respectively, from the statistical analyses testing for associations between blood lipids, glycosylated hemoglobin and blood pressures and hearing thresholds did not alter results noticeably. For example, the adjusted percentage difference in worse ear low-frequency hearing threshold changed from 8.6 (95% CI: 2.4–15.2) per mmol/l of TG to 8.9 (95% CI: 2.6–15.6) when excluding participants on lipid-lowering medications. Comparable differences were observed when testing associations between glycosylated hemoglobin and ambulatory blood pressures and hearing thresholds, excluding participants on antidiabetics and antihypertensives, respectively.

We also analyzed associations between atherogenic risk factors and hearing levels adjusted only for sex and age. This was done to enable a comparison of results to most previous studies that only adjusted for these factors. As expected, adjusting for only sex and age resulted generally in moderately stronger associations, but not to such an extent that the overall results were changed.

Discussion

The main findings of our analyses suggest an association between high BMI, high TG level, low HDL level and smoking and increased worse ear low-frequency hearing threshold. Comparable associations were observed for LFHT-better but these were generally weaker and only at a statistically significant level for TG and status as former smoker. Associations between atherogenic risk factors and high-frequency hearing thresholds for the better and the worse ear were, for the most part, weakly positive, but

Table 1. Characteristics of 576 industrial, financial and day care workers with or without low- and high-frequency hearing loss in the better and the worse ear, Aarhus, Denmark, 2009–2010

Characteristic	Low-frequency hearing loss				High-frequency hearing loss			
	better ear		worse ear		better ear		worse ear	
	yes (n = 9)	no (n = 567)	yes (n = 28)	no (n = 548)	yes (n = 153)	no (n = 423)	yes (n = 233)	no (n = 343)
Sex								
Female	1 (11.1)	151 (26.6)	5 (17.8)	147 (26.8)	18 (11.7)	134 (31.7)	25 (10.7)	127 (37.0)
Male	8 (88.9)	416 (73.4)	23 (82.1)	401 (73.2)	135 (88.2)	289 (68.3)	208 (89.3)	216 (63.0)
Missing	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Age, years	52.1±10.5	44.0±10.7	53.4±9.6	43.6±10.6	52.8±8.6	41.0±9.6	50.5±9.2	39.8±9.5
Missing	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Education								
None	3 (33.3)	66 (11.6)	6 (21.4)	63 (11.5)	20 (13.1)	49 (11.6)	29 (12.5)	40 (11.7)
Short courses	1 (11.1)	59 (10.4)	4 (14.3)	56 (10.2)	20 (13.1)	40 (9.5)	33 (14.2)	27 (7.9)
Skilled worker	4 (44.4)	308 (54.3)	15 (53.6)	297 (54.2)	89 (58.2)	223 (52.7)	132 (56.6)	180 (52.5)
Short-range training	0 (0.0)	35 (6.2)	0 (0.0)	35 (6.4)	6 (3.9)	29 (6.9)	11 (4.7)	24 (7.0)
Middle-range training	1 (11.1)	85 (15.0)	2 (7.1)	84 (15.3)	16 (10.5)	70 (16.6)	24 (10.3)	62 (18.1)
Long-range training	0 (0)	14 (0)	1 (3.6)	13 (2.4)	2 (1.3)	12 (2.8)	4 (1.7)	10 (2.9)
Missing	0 (0)	0 (0)	0 (0.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Personal annual income, DDK								
<299,999	2 (22.2)	247 (43.6)	10 (35.7)	239 (43.6)	53 (34.6)	196 (46.3)	89 (38.2)	160 (46.7)
300,000–499,999	5 (55.6)	282 (49.7)	13 (46.4)	274 (50.0)	90 (58.8)	197 (46.6)	129 (55.4)	158 (46.1)
>500,000	1 (11.1)	32 (5.64)	4 (14.3)	29 (5.3)	8 (5.2)	25 (5.9)	12 (5.2)	21 (6.1)
Missing	1 (11.1)	6 (1.1)	1 (3.6)	6 (1.1)	2 (1.3)	5 (1.2)	3 (1.3)	4 (1.2)
Family history of early hearing loss								
No	1 (11.1)	264 (46.6)	6 (21.4)	259 (47.3)	52 (34.0)	213 (50.4)	86 (36.9)	179 (52.2)
Yes	5 (55.6)	161 (28.4)	10 (35.7)	156 (28.5)	52 (34.0)	114 (27.0)	75 (32.2)	91 (26.5)
Do not know	3 (33.3)	135 (23.8)	12 (42.9)	126 (23.0)	45 (29.4)	93 (22.0)	67 (28.8)	71 (20.1)
Missing	0 (0)	7 (1.2)	0 (0)	7 (1.3)	4 (2.6)	3 (0.7)	5 (2.2)	2 (0.6)
Military service								
Yes	5 (55.6)	163 (28.8)	13 (46.4)	155 (28.3)	66 (43.1)	102 (24.1)	95 (40.8)	73 (21.3)
No	3 (33.3)	391 (69.0)	14 (50.0)	380 (69.3)	83 (54.3)	311 (73.5)	133 (57.1)	261 (76.1)
Missing	1 (11.1)	13 (2.3)	1 (3.6)	13 (2.4)	4 (2.6)	10 (2.4)	5 (2.2)	9 (2.6)
Leisure-time hunting or shooting								
Yes	1 (11.1)	83 (14.6)	5 (17.9)	79 (14.4)	29 (19.0)	55 (13.0)	44 (18.9)	40 (11.7)
No	8 (88.9)	484 (85.4)	23 (82.1)	469 (85.6)	124 (81.1)	368 (87.0)	189 (81.1)	303 (88.3)
Missing	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Leisure-time noisy activities								
Yes	2 (22.2)	141 (24.9)	4 (14.3)	139 (25.4)	32 (20.9)	111 (26.2)	57 (54.5)	86 (25.1)
No	7 (77.8)	426 (75.1)	24 (85.7)	409 (74.6)	121 (79.1)	312 (73.8)	176 (75.5)	257 (74.9)
Missing	0 (0.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Trade								
Manufacture	8 (88.9)	453 (79.9)	25 (89.3)	436 (79.6)	135 (88.2)	326 (77.1)	207 (88.8)	254 (74.1)
Day-care	0 (0)	49 (8.64)	1 (3.6)	48 (8.8)	3 (2.0)	46 (10.9)	7 (3.0)	42 (12.2)
Finance and other services	1 (11.1)	65 (11.5)	2 (7.1)	64 (11.7)	15 (9.8)	51 (12.1)	19 (8.2)	47 (13.7)
Missing	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Occupation								
White-collar worker	1 (11.1)	126 (22.2)	3 (10.7)	124 (22.6)	20 (13.1)	107 (25.3)	30 (12.9)	97 (28.3)
Blue-collar worker	8 (88.9)	441 (77.8)	25 (89.3)	424 (77.4)	133 (86.9)	316 (74.7)	203 (87.1)	246 (71.7)
Missing	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cumulative occupational noise exposure, dB-years	96.6 [90.2–99.8]	96.1 [87.8–99.1]	97.3 [91.8–99.8]	96.0 [87.6–99.0]	97.6 [91.8–99.5]	95.5 [87.2–98.3]	97.4 [91.8–99.5]	95.0 [86.6–98.1]
Missing	0	0	0	0	0	0	0	0

Values are presented as numbers (with percentages), means ± SD or medians (with percentiles p10–p90 in square brackets), where appropriate. Low-frequency hearing loss was defined if the average of pure-tone hearing thresholds at 0.25, 0.5 and 1 kHz were above 25 dB. High-frequency hearing loss was defined if the average of pure-tone hearing thresholds at 4, 6 and 8 kHz were above 25 dB.

Table 2. Adjusted percentage differences in the better and worse ear low-frequency hearing threshold according to atherogenic risk factors

Exposure	Number	Better ear		Worse ear	
		adjusted percentage difference	p value	adjusted percentage difference	p value
Total cholesterol (per 1 mmol/l)	555	–1.4 (–8.8 to 6.4)	0.71	–1.2 (–7.8 to 5.7)	0.72
HDL (per 1 mmol/l)	555	–8.5 (–25.5 to 12.5)	0.40	–18.4 (–32.2 to –1.9)	0.03
TG (per 1 mmol/l)	555	8.1 (1.3 to 15.4)	0.02	8.6 (2.4 to 15.2)	<0.01
LDL (per 1 mmol/l)	540	–5.2 (–13.2 to 3.4)	0.23	–4.1 (–11.4 to 3.8)	0.30
Glycosylated hemoglobin (per 1%)	554	3.7 (–9.7 to 19.0)	0.61	7.3 (–5.2 to 21.4)	0.26
Smoking status					
Never	253	Ref.		Ref.	
Current	169	11.7 (–6.8 to 33.9)	0.23	13.6 (–3.4 to 33.6)	0.12
Former	152	26.8 (5.3 to 52.7)	0.01	21.6 (2.9 to 43.6)	0.02
Cumulative smoking (per 10 pack-years)	564	3.4 (–2.2 to 9.1)	0.23	3.7 (–1.4 to 8.7)	0.15
BMI (per 1 kg/m^2)	576	1.6 (–0.2 to 3.4)	0.08	1.8 (0.2 to 3.4)	0.03
Average 24-hour ambulatory systolic BP (per 10 mm Hg)	565	3.6 (–2.9 to 10.2)	0.28	3.9 (–2.0 to 9.8)	0.20
Average 24-hour ambulatory diastolic BP (per 10 mm Hg)	565	5.0 (–4.7 to 14.8)	0.32	8.1 (–0.7 to 16.9)	0.07
Atherogenic risk factor score (per 1 point)	576	2.4 (–1.6 to 6.5)	0.25	3.4 (–0.2 to 7.1)	0.07

Values in parentheses are 95% CI. Adjusted percentage differences: adjusted for age, sex, education, income, family history of hearing loss before age 70, military service, cumulative occupational noise exposure, leisure-time noisy activities (heavy use of portable music player, playing electrically amplified instrument, doing motor sports, shooting and hunting). Atherogenic risk factor score (0–9 points): 1 point for each atherogenic risk factor belonging in the high tertile (lowest tertile for HDL, as this factor is assumed to protect against atherosclerosis).

Table 3. Adjusted percentage differences in the better and worse ear high-frequency hearing threshold according to atherogenic risk factors

Exposure	Number	Better ear		Worse ear	
		adjusted percentage difference	p value	adjusted percentage difference	p value
Total cholesterol (per 1 mmol/l)	555	0.9 (–5.3 to 7.5)	0.782	2.3 (–2.7 to 7.7)	0.371
HDL (per 1 mmol/l)	555	1.5 (–14.6 to 20.5)	0.867	–2.9 (–15.4 to 11.4)	0.676
TG (per 1 mmol/l)	555	1.4 (–4.0 to 7.1)	0.608	3.5 (–0.9 to 8.1)	0.123
LDL (per 1 mmol/l)	540	0.3 (–6.9 to 8.0)	0.944	1.2 (–4.6 to 7.3)	0.687
Glycosylated hemoglobin (per 1%)	554	–1.9 (–12.6 to 10.1)	0.747	0.4 (–8.4 to 10.1)	0.928
Smoking status					
Never	253	Ref.		Ref.	
Current	169	5.4 (–9.4 to 22.7)	0.494	8.9 (–3.5 to 22.9)	0.166
Former	152	5.5 (–9.7 to 23.4)	0.494	4.5 (–7.7 to 18.3)	0.487
Cumulative smoking (per 10 pack-years)	564	1.7 (–2.9 to 6.4)	0.465	2.4 (–1.3 to 6.1)	0.203
BMI (per 1 kg/m^2)	576	0.4 (–1.0 to 1.9)	0.579	0.9 (–0.3 to 2.1)	0.137
Average 24-hour ambulatory systolic BP (per 10 mm Hg)	565	0.0 (–5.5 to 5.5)	0.995	–0.3 (–4.7 to 4.1)	0.894
Average 24-hour ambulatory diastolic BP (per 10 mm Hg)	565	1.0 (–9.1 to 7.2)	0.815	1.2 (–5.3 to 7.8)	0.718
Atherogenic risk factor score (per 1 point)	576	0.1 (–3.2 to 3.5)	0.958	1.8 (–0.8 to 4.5)	0.175

Values in parentheses are 95% CI. Adjusted percentage differences: adjusted for age, sex, education, income, family history of hearing loss before age 70, military service, cumulative occupational noise exposure, leisure time noisy activities (heavy use of portable music player, playing electrically amplified instrument, doing motor sports, shooting and hunting). Atherogenic risk factor score (0–9 points): 1 point for each atherogenic risk factor belonging in the high tertile (lowest tertile for HDL, as this factor is assumed to protect against atherosclerosis).

none were at a statistically significant level. Generally, associations between atherogenic risk factors and hearing thresholds were strongest at low-frequency hearing levels in the worse ear.

In a large European multicenter study on risk factors for age-related hearing impairment, an association between high BMI and hearing thresholds was also observed [Fransen et al., 2008]. The effect was equally distributed over all frequencies and not predominantly restricted to specific frequencies as observed in our study. Regrettably, for the comparison with this study, lipids were not accounted for. In the same study, a dose-dependent association between smoking and high-frequency hearing thresholds was observed. In our study, we were only able to demonstrate weak positive associations between cumulative smoking and hearing thresholds. In contrast, we demonstrated statistically significant associations between status as former smoker and low-frequency hearing thresholds. We would have expected significant results for cumulative smoking as well. A suggested explanation for our finding may be that participants had quit smoking due to adverse health effects, including cardiovascular disease, which we expected to be associated with hearing loss.

One of the rare prospective studies in this research field, including 26,917 participants, demonstrated a higher risk of hearing loss in participants with hypercholesterolemia and a past history of smoking [Shargorodsky et al., 2010]. BMI ≥30, a history of hypertension or diabetes were not associated with hearing loss in that study. All exposures and outcomes were, however, self-reported and frequency-specific analyses were thus not conducted.

Apart from cardiovascular disease events, cardiovascular risk factors in relation to hearing were also analyzed in the Framingham study [Gates et al., 1993]. In brief, the authors observed associations between blood pressure, blood glucose level and HDL and hearing levels, whereas no association with smoking, relative weight, serum cholesterol or TG were observed. Some of the associations were restricted to women and most associations were strongest for worse ear low-frequency thresholds, as also demonstrated in our study.

This seemingly 'low frequency- and worse ear-specific effect' of cardiovascular risk factors (in our study synonymous with atherogenic risk factors) also observed in our study is interesting. From studies investigating the relative contribution of genetic and nongenetic factors to hearing thresholds, we know that that the proportion of variance in hearing levels accounted for by nongenetic factors are higher in the worse hearing ear, particularly at low frequencies [Gates et al., 1999; Viljanen et al., 2007]. However, this still offers no explanation of the possible causal pathway. A hypothetical causal pathway, also suggested by others [Gates et al., 1993; Friedland et al., 2009], is that atherosclerosis causes microvascular disturbances in the mainly terminal vessels of the cochlea. This, subsequently, results in the ischemic degeneration of inner ear structures responsible for the detection and propagation of auditory signals. As apical parts of the cochlea (where blood supply is most distal and low-frequency sound is transmitted) are, theoretically, the most vulnerable to ischemia, this would explain the higher effect of atherogenic risk factors on low-frequency thresholds.

According to Schuknecht et al., who correlated audiometric patterns with cochlear histopathology [Schuknecht and Ishii, 1966; Schuknecht and Gacek, 1993], the hallmark of strial presbycusis (characterized by the degeneration of the stria vascularis) compared to the more common sensory presbycusis (characterized by loss of hair cells in the base of the cochlea) is a flattened audiogram, showing a relatively higher impact on low frequencies compared to the more common high-frequency sloping audiogram of, for example, the sensory presbycusis. If, hypothetically, the stria vascularis due to its highly vascular structure is susceptible to atherosclerotic vascular changes, this could also explain why atherogenic risk factors affect low frequencies most in the present and corresponding studies.

Finally, we cannot exclude that the apparent lack of impact on high-frequency thresholds is due to masking from additional risk factors for high-frequency hearing loss that were not taken into account in the present study. The background prevalence of high-frequency hearing loss in our study is about 10 times the background prevalence of low-frequency hearing loss (table 1), indicating that frequent risk factors are involved. We carefully adjusted for age, various sources of leisure-time noise and occupational noise, but additional noise exposure and other unknown factors may still have influenced our results.

Concerning blood lipids, we observed the strongest associations with BMI, TG and HDL and no association with LDL. This is interesting from a clinical point of view because recent studies found high BMI strongly associated with high TG and low HDL but not with LDL [Nicholls et al., 2006; Shamai et al., 2011]. This could indicate a causal pathway from high BMI through elevated TG and lowered HDL to hearing loss. We tested this, and results showed a substantial attenuation in the effect of BMI when adjusting for TG and HDL, supporting a link between obesity and low-frequency hearing levels that is partially mediated by high TG and low HDL.

Atherosclerotic vascular disease is often the result of a joint effect of multiple risk factors. We, therefore, assessed whether an increasing load of the risk factors included in this study affected hearing thresholds by an atherogenic risk factor score. This score showed a borderline statistically significant association with low-frequency hearing threshold in the worse ear and weaker positive associations with low-frequency hearing threshold in the better ear and high-frequency hearing threshold in the worse ear, indicating that the effects of the individual risk factors sum up.

Our study has a number of strengths. Firstly, we have analyzed hearing thresholds on a continuous scale, allowing us to keep as detailed information on individual hearing levels in the analyses as possible. Further, we analyzed different frequency hearing levels, as we assumed from previous studies [Gates et al., 1993; Friedland et al., 2009] that atherogenic risk factors would have frequency-specific effects.

Audiometric data were complete for all participants, and missing data on explanatory variables were limited. Furthermore, we had objective measures of most variables, leaving little room for differential misclassification. As hazardous noise levels are frequent in manufacturing industries, from which many of our participants were recruited, this was a potential source of bias to our study. To address this problem we carefully evaluated cumulative noise exposure for each participant back to 1980 and adjusted for this in our analyses.

If the effect of our exposure variables is mediated through atherosclerotic vascular changes, we suppose that the effect will be on the cochlea and thus affect sensorineural hearing thresholds. Testing bone conduction thresholds would have made evaluation of sensorineural thresholds more precise. However, due to time constraints in this epidemiological field study, we refrained from this. Instead we excluded participants with questionnaire information indicating conductive hearing loss due to middle ear disease.

The high number of tests for possible associations between exposures and outcomes is a possible limitation of our study as it increases the risk of obtaining significant results just by chance. However, we find our results consistent and in line with prior studies and suggested mechanisms, speaking against the risk of observing spurious significant associations.

A substantial part of our study population consisted of blue-collar workers from manufacturing industries, the rest being day care workers and employees from the financial sector. The general population is more heterogeneous, but this should not have conflicted with the external validity of our study because the effect of atherogenic risk factors is not expected to depend on population characteristics.

Another possible limitation of our study is the potential risk of a healthy worker survivor effect as this was a cross-sectional study that consisted not only of newly recruited participants but, supposedly, also included the healthiest earlier hired workers (little hearing loss and healthy lifestyle causing few atherogenic risk factors). This would be a possible source of selection bias in our study. The result of this would, however, be an underestimation of associations. Furthermore, it is unlikely that, for example, the level of TG, which is unknown to most subjects, predicts employment status conditional on hearing threshold.

Individual sensorineural hearing level varies due to a complex interplay of environmental exposures over time and genes determining individual susceptibility to these exposures. This study has been an attempt to uncover the possible contribution from atherogenic risk factors which, in contrast to the irreversibility of sensorineural hearing loss, are potentially reversible if treated through either modification of lifestyle or pharmacological intervention. According to our findings, lifestyle intervention will not only have preventive effects on cardiovascular disease but also on low-frequency sensorineural hearing loss.

Conclusion

We observed that low HDL, high TG, high BMI and history of former smoking were associated with increased low-frequency hearing thresholds, particularly in the worst hearing ear. Moreover, we found that as the number of risk factors for atherosclerosis increased, so did hearing levels. Generally, associations were strongest for low-frequency hearing thresholds.

In this study, we found no statistically significant associations between atherogenic risk factors and high-frequency hearing thresholds.

According to these results, atherogenic risk factors represent a potential risk for increased low-frequency hearing thresholds and lifestyle intervention is therefore a relevant target for hearing protection.

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PAPER III

Occupational Noise Exposure, Psychosocial Working Conditions and the Risk of Tinnitus

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ORIGINAL ARTICLE

Occupational noise exposure, psychosocial working conditions and the risk of tinnitus

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Abstract
Purpose The purpose of this study was to evaluate the influence of occupational noise (current and cumulative doses) and psychosocial work factors (psychological demands and decision latitude) on tinnitus occurrence among workers, using objective and non-self-reported exposure measures to prevent reporting bias.
Methods In a cross-sectional study, we analyzed data from a Danish survey from 2009 to 2010 that included 534 workers from children day care units and 10 manufacturing trades. Associations between risk factors (current noise exposure, cumulative noise exposure and psychosocial working conditions) and tinnitus were analyzed with logistic regression.
Results We found no statistically significant associations between either current [OR 0.95 (95% CI 0.89; 1.01)] or cumulative [OR 0.93 (95% CI 0.81; 1.06)] occupational noise exposure and tinnitus. Likewise, results for psychosocial working conditions showed no statistically significant association between work place decision latitude [OR 1.06 (95% CI 0.94; 1.13)] or psychological demands [OR 1.07 (95% CI 0.90; 1.26)] and tinnitus.
Conclusions Our results suggest that current Danish occupational noise levels (in combination with relevant noise protection) are not associated with tinnitus. Also, results indicated that the psychosocial working conditions we observed in this cohort of mainly industrial workers were not associated with tinnitus. Therefore, psychosocial working conditions comparable to those observed in this study are probably not relevant to take into account in the evaluation of workers presenting with tinnitus.

Keywords Tinnitus · Noise · Psychological demands · Decision latitude · Psychosocial work factors

Introduction

Tinnitus is the perception of sound in the absence of an external sound. It represents a frequent disorder with a prevalence of around 10–15% depending on study population and criteria applied (Henry et al. 2005). Though tinnitus is a frequent complaint, only around 0.5% of the population have debilitating symptoms such as severe annoyance, concentration difficulty and insomnia (Baguley et al. 2013).

The etiology of tinnitus is heterogeneous and includes both somatic and psychological factors. Among somatic factors, hearing loss is probably the most important risk factor for tinnitus (Henry et al. 2005; Axelsson and Prasher 2000). Factors contributing to hearing loss therefore also represent potential risk factors for tinnitus including age, acute and long-term (occupational) noise exposure, middle- and inner ear diseases, and ototoxic medications (Baguley

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et al. 2013). However, many people without hearing loss also experience tinnitus and often no obvious cause is found in the individual patient (Meikle and Griest 1989).

In spite of stricter occupational noise legislation, millions of workers worldwide are still exposed to occupational noise levels that increase the risk of hearing loss and tinnitus (Axelsson and Prasher 2000; Verbeek et al. 2012). Prevention programs in developed countries that include noise assessment, noise controls, audiometric monitoring of workers' hearing, worker education and appropriate use of hearing protection have, however, resulted in a decreasing incidence of hearing loss in this part of the World (Nelson et al. 2005).

Psychological factors such as mental stress, psychosocial strain, anxiety and depression have been suggested to either cause tinnitus or exacerbate tinnitus symptoms (Oishi et al. 2011; Holgers et al. 2005; Salviati et al. 2014; Evered and Lawrenson 1981). As high job strain is associated with increased mental stress (Nieuwenhuijsen et al. 2010; de Jonge et al. 2000), the risk of tinnitus may be affected by psychosocial working conditions.

A Taiwanese study from 2008 (Lin et al. 2009) found a statistically significant association between feeling stressed at work and tinnitus. Correspondingly, a Swedish study from 2011 revealed a relationship between work-related stressors and hearing problems (tinnitus and hearing complaints) (Hasson et al. 2011), but otherwise epidemiologic evidence of a possible association between job-related stress factors and tinnitus is scarce.

Retrospective evaluation of noise exposure and psychosocial factors often causes problems in epidemiological studies, especially if evaluation relies on self-reported data. People suffering from tinnitus may search their memory more thoroughly for explanatory factors than unaffected individuals, potentially leading to differential misclassification of exposure levels and inflated results. This problem is potentially circumvented by using work unit-aggregated levels of psychosocial exposures (Kolstad et al. 2011), objective noise measurements and construction of noise exposure matrices (Seixas and Checkoway 1995; Davies et al. 2009).

The two main objectives of this study were: (1) to evaluate the association between objective measures of occupational noise (based on noise dosimetries) and tinnitus and (2) to evaluate the association between work unit-aggregated measures of psychosocial work factors (psychological demands and decision latitude) and tinnitus.

Materials and methods

Participants

This cross-sectional study takes advantage of an initial survey of 819 workers conducted between 2001 and 2002 in Aarhus, Denmark, with the purpose of monitoring occupational noise exposure and auditory function among noise-exposed workers. Participants were recruited from randomly selected companies within 12 trades: children day care, financial services and the 10 manufacturing trades in Denmark with the highest reporting of noise-induced hearing loss according to the Danish Working Environment Authority. Financial workers were selected as a reference group. In 2009–2010, the same companies and workers were asked to participate again. This time the purpose was extended to also include psychosocial work factors and medical risk factors. A total of 271 workers (33.1%) agreed to participate again, and further 394 workers were recruited de novo, making a total of 665 participants in 2009–2010. At the company level, all participants were as far as possible selected at random. However, to avoid disruption of workflow, selection in some cases had to be done in accordance with the local manager. Only participants from the 2009–2010 study were included in the present study, as psychosocial work factors were not accounted for in the 2001–2002 survey. To restrict the analyses to potentially noise-exposed workers, 67 financial workers and 64 workers from the original 2001 cohort now either unemployed ($n = 44$) or no longer working in noise-exposed industries ($n = 20$) were excluded, leaving 534 participants eligible for this study. The local ethical scientific committee (Central Region Denmark) approved the study (M.20080239), and informed consent was obtained from all individual participants included.

Occupational noise exposure

Individual dosimeters (Bruel & Kjær, model 4443, Nærum, Denmark) measuring A-weighted equivalent sound levels (L_{Aeq}) in 5-s intervals were handed out to the participants. Microphones were fitted at the right side collar if right handed and vice versa if left handed. Measuring range was set to 70–120 dB(A). Individual A-weighted equivalent noise levels were computed for the full work shift ($L_{Aeq,\,work}$).

Based on 1268 noise exposure recordings from the 2001–2002 study and the 2009–2010 study, we predicted noise exposure levels for each combination of trade, occupation and calendar year (1980–2010) by mixed regression analyses including these as fixed effects and the participants as random effect resulting in a noise exposure matrix. Based on information on historical employment status (1980–2010) retrieved from the Danish Supplementary Pension Fund and the noise exposure matrix, we calculated cumulative occupational noise exposure levels for each participant as the product of estimated noise exposure level [L_{Aeq} in dB(A)] and duration of employment (T) using the formula: $10 \times \log [\Sigma(10^{dB(A)/10} \times T]$, resulting in "dB(A)-year" on a logarithmic scale.

Audiometric measures

Air conduction thresholds were determined for each ear at 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kHz by pure tone audiometry at the workplaces, using a Voyager 522 audiometer equipped with TDH-39 headphones (Madsen Electronics, Taastrup, Denmark). The audiometer was installed in a mobile examination unit equipped with a soundproof booth (model AB-4240, Eckel Noise Control Technologies, Bagshot, UK). Audiometry was performed by trained examiners using a standardized protocol.

To avoid temporary threshold shifts from possible noise sources, all participants were asked to wear hearing protection from the beginning of the day until the audiometry was done. Otoscopy was performed initially to verify that ears were free of wax and the tympanic membrane was visible. The audiometer was calibrated every 6 months according to ISO standards. We defined two hearing measures: 0.5–4.0 kHz hearing threshold (0.5–4.0 kHz HT) was computed as the average of pure tone hearing thresholds at 0.5, 1, 2, 3 and 4 kHz in the worse ear. 0.5–4.0 kHz hearing handicap was defined if 0.5–4.0 kHz HT >25 dBHL (according to WHO hearing impairment definition). Worse ear hearing ability was chosen over better ear, as we assumed that hearing levels at the worse ears were the most predictive of tinnitus status.

Questionnaire information

A questionnaire was handed out to the participants at the time of the audiometric examination to provide information on tinnitus and its related symptoms, psychosocial work factors, mental symptoms, use of hearing protection device, income and education (as described below).

Tinnitus

Tinnitus was defined in the questionnaire as "ringing or buzzing in one or both ears." Related questions included frequency of tinnitus [(1) almost never experiencing tinnitus, (2) experiencing periods of tinnitus at least monthly, (3) experiencing periods of tinnitus at least weekly or (4) experiencing tinnitus daily], frequency of annoyance when experiencing tinnitus [(1) almost never or never, (2) rarely, (3) sometimes, (4) often or (5) always] and frequency of insomnia due to tinnitus [(1) almost never or never, (2) rarely, (3) sometimes, (4) often or (5) always]. A person was classified as having tinnitus if experiencing tinnitus daily accompanied by either annoyance (sometimes, often or always) or insomnia (sometimes, often or always).

Measures of psychosocial working conditions

Psychosocial working conditions were measured according to Karasek and Theorell's demand-control model (Karasek 1990) with scales from the Copenhagen Psychosocial Questionnaire (Kristensen et al. 2005). Psychological demands, decision authority and skill discretion were each measured by four items on a scale from "always" (1) to "never" (5). For each scale, a mean value of the four items was calculated. Decision latitude was computed as the mean value of decision authority and skill discretion.

Furthermore, we calculated mean values of decision latitude and psychological demands for each work unit after exclusion of participants with tinnitus. Participants with tinnitus were excluded from the calculation of the mean scores as tinnitus distress could influence their assessment of the psychosocial work environment, thus introducing reporting bias. The mean values were then assigned to all employees at the particular work place. This method was recently used in a study of depression (Grynderup et al. 2012).

Measures of mental symptoms

Symptoms of depression, anxiety and somatoform disorder (illness worries) were assessed using the Common Mental Disorders Questionnaire (CMDQ) (Christensen et al. 2005). The CMDQ is a brief case finding instrument designed to screen for mental symptoms in general practice. All questions referred to the last 4 weeks and were measured on a 5-point response scale from "not at all" (0 points) to "extremely" (4 points). We used the six-question subscale for depression, the four-question subscale for anxiety and the seven-question subscale for somatoform disorder. Participants were classified as depressive if scoring $\geq$3 on $\geq$3 of the 6 depressive symptom questions. Anxiety was classified if the score was $\geq$3 on $\geq$3 of the 4 anxiety symptom questions, and somatoform disorder was classified if the score was $\geq$3 on $\geq$3 of the 7 somatoform disorder symptom questions. These selection criteria were chosen to obtain optimal validity (Christensen et al. 2005).

Use of hearing protection devices

Of the 534 workers, 333 reported to use HPD. Among HPD users, 140 participants completed a detailed log-book specifying when they used HPD during the day of noise measurements.

Income and education

Participants were asked about gross household income (<299,999 DDK, 300,000–499,999 DDK, >500,000 DDK) and educational level (none, short courses, skilled worker, short-range training, middle-range training, long-range training).

Statistics

We tabulated possible confounders and information on occupational background according to presence of tinnitus. Odds ratios of tinnitus according to noise exposures were analyzed by logistic regression and performed using both continuous-scale exposure information (if available) and exposure divided into relevant groups or tertiles. These analyses were adjusted for age and sex.

Associations between psychosocial working conditions and tinnitus were analyzed by logistic regression with robust clusters based on the work unit of the participants and adjusted for: (1) age and sex, and (2) age, sex, depression, anxiety, somatization disorder, income and education. These potential confounders were decided upon a priori. Analyses were performed using both continuous-scale exposure information and exposure divided into tertiles. We analyzed for interaction between psychological demands and decision latitude. The interaction term was calculated based on both continuous and trichotomized data.

To test whether associations were independent of auditory function, we performed additional analyses adjusting for mean hearing levels at worse hearing ear (mean of 0.5, 1, 2, 3 and 4 kHz HL). Another subanalysis was conducted to investigate whether the use of hearing protection devices (HPD) influenced the observed associations between current noise exposure and tinnitus. In this analysis, we subtracted 10 dB(A) from each 5-s noise recording obtained at work while using HPD. This analysis was restricted to the 342 workers with valid information on HPD use (140 workers returning the HPD log-book and 202 workers reporting not to use HPD at work).

All analyses were conducted using Stata 13 statistical software (StataCorp LP, College Station, TX, USA).

Results

A total of 41 (8%) participants were classified as suffering from tinnitus according to our criteria. Characteristics of participants according to tinnitus status are presented in Table 1. Of the 534 participants, 126 were women (23.6%). Age range was 20–64 years (mean 43.0 years). Among participants with tinnitus, we observed a tendency toward higher prevalence of males, workers above 45 years of age, workers with anxiety and somatization disorder and workers with hearing impairment compared with participants without tinnitus. Median speech frequency hearing thresholds (0.5–4 kHz) were on average 7.5 dB higher in the tinnitus group. The highest number of tinnitus cases was found among workers manufacturing fabricated metals.

For each 10 dB(A) increase in current occupational noise exposure level, we observed an age-and-gender-adjusted OR_{adj1} of 0.95 (95% CI 0.89; 1.01) for tinnitus, and the association seemed to decrease with higher noise levels (Table 2). Further adjustment for mental disorders, education and income did not change this result markedly. Results for cumulative occupational noise exposure showed no statistically significant association with tinnitus [OR_{adj1} 0.94 (95% CI 0.82; 1.07 for each dB(A)-year)]. Again, further adjustment for mental disorders, education and income did not change this result.

For psychosocial working conditions, we observed no statistically significant associations between either low decision latitude [OR_{adj1} 1.09 (95% CI 1.02; 1.16) for one unit increase on a 32-level scale] or psychological demands and tinnitus [OR_{adj1} of 1.04 (95% CI 0.91; 1.91) for one unit increase on a 16-level scale]. Results for decision latitude and psychological demands did not change noticeably when further adjusting for mental disorders, education and income.

We observed no interaction between psychological demands and decision latitude (all p values >0.05 for both continuous and trichotomized exposure variables).

Accounting for the use of HPD by subtracting 10 dB(A) from every 5-s noise recording obtained at work for the subgroup with valid HPD information did not change the OR for the association between current occupational noise and tinnitus [OR_{Adj2} after 10 dB(A) subtraction: 0.97 (95% CI 0.91; 1.05)].

Testing whether associations were independent of participant's hearing levels by further adjusting analyses for hearing levels at worse hearing ear, resulted in minimal changes in the association between current occupational noise exposure and tinnitus. Thus, the OR_{Adj2} changed from 0.95 (95% CI 0.89; 1.01) to 0.96 (95% CI 0.89; 1.05) for the association between continuous current noise exposure and tinnitus. Associations between cumulative noise exposure and psychosocial working conditions and tinnitus were practically unchanged.

Discussion

The objectively measured current and cumulative occupational noise levels observed in this study were not statistically significantly associated with tinnitus. Moreover, for psychosocial working conditions, we found no association with tinnitus.

In previous epidemiological studies on risk factors for tinnitus such as "The Blue Mountain Hearing Study" and "The Beaver Dam Offspring Study," rather strong associations between both cumulative and current occupational noise and tinnitus have been reported (Nondahl et al. 2011; Sindhusake et al. 2003). Both current and historical exposure assessment, however, relied on self-reported noise

Table 1 Characteristics of 534 noise-exposed workers aged 20–64 years, Åarhus, Denmark, 2009–2010

Characteristic	Tinnitus ($n = 41$)				No tinnitus ($n = 493$)			
	n	%	Median	p10; p90	*n*	%	Median	p10; p90
Sex, no (%)								
Female	6	(14.6)			120	(24.3)		
Male	35	(85.4)			373	(75.7)		
Age, no (%)								
<35 years	5	(12.2)			99	(20.1)		
35–44 years	9	(22.0)			174	(35.3)		
45–54 years	17	(41.5)			158	(32.1)		
≥55 years	10	(24.4)			62	(12.6)		
Education								
None	3	(7.3)			62	(12.6)		
Short courses	7	(17.1)			61	(12.4)		
Skilled worker	26	(63.4)			273	(55.4)		
Short-range training	1	(2.4)			22	(4.5)		
Middle-range training	3	(7.3)			72	(14.6)		
Long-range training	1	(2.4)			3	(0.6)		
Annual income								
0–299,999 DDK	18	(43.9)			232	(47.3)		
300,000–499,999 DDK	23	(56.1)			242	(49.4)		
>500,000 DDK	0	(0)			16	(3.3)		
Hearing thresholds at 0.5–4 kHz (dB HL),			20.0	6.3; 33.8			12.5	3.8; 28.8
Hearing impairment[a]								
No	24	(58.5)			424	(86.0)		
Yes	17	(41.5)			69	(14.0)		
Depression								
No	35	(85.4)			416	(84.4)		
Yes	6	(14.6)			77	(15.6)		
Anxiety								
No	35	(85.4)			445	(90.3)		
Yes	6	(14.6)			48	(9.7)		
Somatoform disorder								
No	33	(80.5)			416	(84.4)		
Yes	8	(19.5)			77	(15.6)		
Industry								
Manufacture of food	5	(12.2)			76	(15.4)		
Manufacture of wood products	4	(9.8)			41	(8.3)		
Publishing and printing	5	(12.2)			61	(12.4)		
Manufacture of non-metallic mineral products	2	(4.9)			35	(7.1)		
Manufacture of basic metals	3	(7.3)			37	(7.5)		
Manufacture of fabricated metals	7	(17.1)			58	(11.8)		
Manufacture of machinery	5	(12.2)			58	(11.8)		
Manufacture of motor vehicles	3	(7.3)			42	(8.5)		
Manufacture of furniture	1	(2.4)			5	(1.0)		
Construction	2	(4.9)			24	(4.9)		
Day care	4	(9.8)			56	(11.4)		

[a] WHO definition. See "Audiometric measures" section

Tabel 2 Odds ratios (OR) of having tinnitus according to occupational noise exposure and psychosocial working conditions

Exposure	Tinnitus ($n = 41$)	No tinnitus ($n = 493$)	OR_{Crude}	95% CI	OR^{a}_{Adj1}	95% CI	OR^{b}_{Adj2}	95% CI
Occupational noise exposure								
Current occupational noise ($L_{Aeq, work}$), dB(A)								
<80	17	142	1		1		1	
80–84	15	192	0.65	0.31; 1.35	0.67	0.44;1.33	0.67	0.32; 1.42
85–90	7	107	0.55	0.22; 1.36	0.52	0.53; 2.92	0.52	0.20; 1.33
>90	2	40	0.42	0.51; 2.71	0.46	0.10; 2.15	0.51	0.11; 2.41
Missing	0	12						
Continuous pr. 10 dB(A)			0.95	0.89; 1.01	0.95	0.89;1.01	0.95	0.89; 1.01
Cumulative occupational noise (dB(A)-years)								
Low (79.6–94.9)	11	167	1		1		1	
Medium (95.0–97.4)	12	166	1.10	0.47; 2.56	0.67	0.27;1.68	0.63	0.24; 1.61
High (97.5–101.1)	18	160	1.71	0.78; 3.73	0.63	0.23; 1.76	0.58	0.24; 1.67
Missing	0	0						
Continuous			1.08	0.96; 1.19	0.94	0.82; 1.07	0.93	0.81; 1.06
Psychosocial working conditions (exposure range[c])								
Psychological demands								
Low (0–3)	12	157	1		1		1	
Medium (4–6)	12	158	0.99	0.47; 2.07	1.08	0.50; 2.36	0.98	0.44; 2.20
High (7–16)	15	176	1.12	0.54; 2.07	1.18	0.57; 2.45	1.09	0.53; 2.22
Missing	2	2						
Continuous (0–16)			1.06	0.88; 1.28	1.09	0.90; 1.30	1.07	0.90; 1.26
Decision latitude								
High (20–32)	11	166	1		1		1	
Medium (15–19)	13	165	1.18	0.53; 2.64	1.13	0.51; 2.52	1.07	0.51; 2.21
Low (0–14)	15	160	1.41	0.65; 3.06	1.46	0.67; 3.16	1.37	0.67; 2.78
Missing	2	2						
Continuous (32-0)			1.05	0.97; 1.15	1.05	0.96; 1.15	1.06	0.94; 1.13

[a] Adjusted for age and gender

[b] Adjusted for age, gender, depression, anxiety, somatization disorder, education and income

[c] According to method described in "Measures of psychosocial working conditions" section

levels, and results could therefore potentially be biased. Moreover, these studies were conducted a decade or two before this study, and participants were generally older, meaning that both historical and current occupational noise exposure levels for participants in these studies were probably higher.

We analyzed objective measures of occupational noise exposure in relation to tinnitus and were not able to reproduce comparable risk estimates. As long-term exposure to high noise levels [>85 dB(A)] is generally accepted to cause hearing loss (International Organization for Standardization (ISO) 1990) which is a well-established risk factor for tinnitus, we found our negative results surprising. As described in "Occupational noise exposure" section, we calculated individual "dB(A)-years" as a sum-measure of the average daily occupational noise exposure through each year of employment back to 1980. Tabulating the number of years exposed to average daily occupational noise levels >85 dB(A) for each participant revealed that, with regard to the risk of inner ear damage, the retrospective noise exposure for our participants was generally low. Thus, 62.9% of the population had never been exposed to more than one year with average daily occupational noise exposure above 85 dB(A) and only 21.7% had been exposed for more than 5 years above this level. No participants had been exposed to a full year of average daily occupational noise exposure above 90 dB(A). If the causal pathway from noise to tinnitus is through hearing loss, the low historical noise exposure levels in this cohort may therefore partly explain our finding.

Potential selection bias from a healthy worker effect is another possible explanation for our results: As tinnitus is often accompanied by hearing loss and hypersensitivity to noise (Gilles et al. 2014; Nelson and Chen 2004b), this

Tabel 3 Reported use of hearing protection device according to current occupational noise exposure levels

	Using hearing protection device at work			
	No ($n = 202$)		Yes ($n = 332$)	
	n	%	*n*	%
Current occupational noise($L_{Aeq, work}$), dB(A)				
<85	166	45.4	200	54.6
85–90	27	23.7	87	76.3
>90	5	11.9	37	88.1
Missing	4	33.3	8	66.7

may exclude workers with low thresholds for developing tinnitus and hearing loss from noise-exposed employment. If this argument holds true, our noise-exposed population may represent a selection of workers with a high resistance to noise in terms of developing tinnitus and hearing loss. Indeed, this cross-sectional study may have been particularly vulnerable to this type of bias as it consisted not only of newly recruited participants but also of "survivors" from the original study group from 2001.

Non-differential misclassification of historical noise levels which is an inherent limitation of exposure matrices is another possible source of bias affecting our results for cumulative occupational noise exposure.

Concerning current noise exposure, only 114 (21%) and 42 (8%) of workers were exposed to current average occupational noise levels >85 and >90 dB(A), respectively (Table 2). Table 3 shows that in these two groups there were many HPD users (76 and 88%, respectively). Again, if noise-related tinnitus is the result of either temporary or permanent threshold shifts [neither of which should occur at noise levels <85 dB(A)], we would not expect to observe strong associations between the observed current noise levels in this study and tinnitus, especially if HPD use was as adequate, as indicated in Table 3.

Furthermore, we performed additional regression analyses to see whether current or cumulative noise levels were associated with participant's hearing levels. Indeed, no significant association was observed, which again supports that the cumulative and current noise exposure levels we observed were not large enough to cause tinnitus, through a pathway including hearing loss.

The causal pathway from noise exposure to tinnitus could also, potentially, be mediated through mental stress resulting from noise exposure as suggested in some studies (Ising and Kruppa 2004; van Dijk et al. 1987). In this case, noise should only cause annoyance and would not have to be at deleterious levels to also cause tinnitus. Our results, however, do not support this hypothesis either, at least at the given noise levels.

Based on noise recordings and questionnaire data from 752 workers from the original 2001–2002 cohort (see "Participants" section), Rubak et al. conducted a study published in 2008, analyzing the association between occupational noise exposure and tinnitus with and without concomitant hearing handicap (Rubak et al. 2008). In Rubak's study, current occupational noise levels were higher than we measured in 2009–2010, and cumulative occupational noise was calculated from partly self-reported levels. The authors found no association between occupational noise exposure and tinnitus without concomitant hearing handicap, but interestingly an increased risk of tinnitus was observed if hearing handicap was also present.

According to Karasek & Theorell's job strain model, mental strain is the result of the interaction of high psychological demands and low decision latitude (Karasek 1990). Traditionally, the combined effect of the two factors has therefore been analyzed as a quadrant term with median splits of psychological demands and decision latitude. In this study, we found no statistically significant interaction effects between psychological demands and decision latitude and therefore decided to report associations separately, as this method, in our opinion, would give us more detailed information on the effect of each component (Mikkelsen et al. 2011).

Due to distressing tinnitus symptoms possibly affecting the individual's perception and reporting of the work environment, the association between self-reported psychological working conditions and tinnitus may be affected by reporting bias. This is potentially circumvented using work unit-aggregated measures as we did in the present study. These measures are independent of a specific worker's appraisal of his or her working conditions and thus provide a more objective description of the working environment (Kolstad et al. 2011; Kasl 1998).

Making use of the above-mentioned method in the analysis of our data for psychological working conditions, we found no statistically significant associations with tinnitus of either psychological demands or decision latitude. Prior studies have studied the association between self-reported (not job-related) mental stress and tinnitus; the majority finding positive associations (Canlon et al. 2013) (Heinecke et al. 2008; Horner 2003). We were able to find two studies evaluating the effect of job-related stress factors on tinnitus (Lin et al. 2009; Hasson et al. 2011) (both reporting positive associations with self-reported occupational stress factors), but no studies analyzing the association between non-self-reported psychosocial working conditions and tinnitus as performed in the present study. This study therefore offers a new perspective on this issue and indicates that current psychosocial working conditions in Danish industrial trades are not associated with tinnitus.

In our main analyses, we did not adjust for hearing level as it was our assumption that it was in the causal pathway from noise to tinnitus. Hearing disabilities can, however, cause mental distress and may also cause participants to avoid noise exposure (Nelson and Chen 2004a). In that regard, hearing ability could be a potential confounder. We therefore performed a sensitivity check by further adjusting for hearing levels. This resulted in practically unchanged results.

We also subtracted 10 dB(A) from every 5-s noise recording obtained during work while using HPD for those providing a log-book (Park and Casali 1991). This was done to investigate the potential effect of noise attenuation from HPD's on our results for current noise exposure. This did not alter results substantially.

This study has a number of strengths. Firstly, we used objective and non-self-reported exposure measures with little missing information, leaving little room for reporting biased results. Also, we had detailed information on potential confounders enabling us to perform analyses adjusted for other potential risk factors.

Among limitations is the cross-sectional nature of this study, preventing us from drawing strong conclusions regarding causality. Also, our definition of tinnitus may have led to misclassification of tinnitus status. Using a standardized tinnitus questionnaire as the Tinnitus Handicap Inventory (Zeman et al. 2012) possibly could have refined our tinnitus classification, but due to pressure of space in the questionnaire, we refrained from this. Regarding noise exposure, higher exposure levels (current and cumulative) and contrast would have enabled us to also explore the association between noise and tinnitus status at higher levels than given in this study. As occupational noise levels in other countries worldwide are possibly higher than what we measured, this limits the external validity of our results.

Conclusion

Overall, our results suggest that occupational noise exposure at the levels given in this study is not associated with tinnitus. However, our results do not rule out a possible increased risk of tinnitus from occupational noise levels exceeding the levels measured in this population.

Likewise, we found no indication of an association between psychosocial working conditions (in terms of high psychological demands and low decision latitude) and tinnitus, suggesting that psychosocial working factors comparable to those observed in this study are probably not relevant in the evaluation of a worker presenting with tinnitus.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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PAPER IV

Salivary Cortisol and Tinnitus

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ABSTRACT

Objectives Stress is a suspected cause of tinnitus and studies relying on self-reported stress measures have supported this hypothesis. Self-report studies may, however, have validity problems. The objective of this study was to investigate if salivary cortisol, as an objective indicator of stress activation of the HPA axis, was associated with tinnitus.

Methods In a cross-sectional study, we analyzed data from a Danish survey from 2010, including 632 white- and blue-collar workers from 10 manufacturing trades, children day care units and financial services. Associations between cortisol measures (awakening cortisol, awakening+30 cortisol, cortisol awakening response, evening cortisol, cortisol slope and area under the curve) and tinnitus were analyzed using logistic regression.

Results Overall, no statistically significant associations were observed between cortisol measures and tinnitus. Weak associations between a steeper cortisol slope across the day (reflecting higher awakening cortisol and lower evening cortisol) and tinnitus were indicated.

Conclusions This observational study did not support the hypothesis that salivary cortisol, as a reflection of HPA axis activity, is associated with tinnitus. Weak indications of an association between a steeper slope of cortisol and tinnitus warrants further study.

1. INTRODUCTION

Tinnitus is the perception of sound in one or both ears with no external sound source. Depending on study population and criteria applied, the prevalence ranges around 10-15 %. Disabling symptoms such as severe annoyance, interference with sleep and impaired concentration, however, only affect around 0.5 % of people (Baguley, McFerran & Hall, 2013; Henry, Dennis & Schechter, 2005).

Tinnitus can have many causes, including both physical and psychological factors. Among physical factors, hearing loss is probably the most important risk factor and therefore most factors causing hearing loss also carry a risk of tinnitus, including age, noise exposure, middle ear diseases and ototoxic medications (Henry, Dennis & Schechter, 2005). Among psychological factors, previous studies have found associations between depression, anxiety, and mental stress and tinnitus (Canlon, Theorell & Hasson, 2013; Gilles, Goelen & Van de Heyning, 2014b; Holgers, Erlandsson & Barrenas, 2000; Oishi et al., 2011; Udupi et al., 2013). These studies mostly used self-reported exposure information and could therefore have validity problems, as participants with a distressing condition like tinnitus may search their memory more thoroughly for explanatory factors than unaffected individuals, potentially leading to differential misclassification of exposure levels and inflated results. Classifying exposure from an objectively measured biomarker instead of self-reports would enable us to circumvent reporting bias. Furthermore, it will provide insight into the causal mechanisms possibly linking stress and tinnitus.

Salivary cortisol is an established neuro-endocrine marker of the acute stress-response and possibly also reflect prolonged stress-conditions (Biondi & Picardi, 1999). Finding an association between cortisol and tinnitus therefore has the potential of validating a possible association between stress and tinnitus.

Prior studies evaluating the association between cortisol and tinnitus are scarce and designs and findings are heterogeneous. A Korean study from 2013 (Kim et al., 2014) found no difference in basal cortisol levels between tinnitus patients and a control group. In a Canadian study from 2004, Hébert et al. found elevated cortisol levels among tinnitus patients reporting high tinnitus related distress compared to controls (Hebert, Paiement & Lupien, 2004) and later, in 2006, the same group found blunted cortisol reactivity to psychosocial stress in tinnitus sufferers (Hebert & Lupien, 2007).

These results are interesting but yet too few and contradictory to draw causal conclusions.

The objective of this study was to investigate if increased cortisol levels and changes in the dynamics of cortisol secretion are associated with tinnitus.

2. MATERIALS AND METHODS

2.1 Participants

This cross-sectional study takes advantage of a survey of 819 workers conducted between 2001 and 2002 in Aarhus, Denmark, with the original purpose of monitoring occupational noise exposure and hearing levels among blue- and white collar workers. The cohort was recruited from children day care units, financial services and 10 manufacturing trades. In 2009-10, the same companies and workers were asked to participate again. This time the purpose was extended to also include psychosocial work factors, stress-related disorders, medical risk factors and parameters concerning auditory function. A total of 271 workers agreed to participate again and further 394 workers were recruited de novo, making a total of 665 participants in 2009-10. At the company level all participants were as far as possible selected at random. However, to avoid disruption of workflow, selection in some cases had to be done in accordance with the local manager. Only participants from the 2009-10 study (n=665) were included in the present study, as salivary cortisol measurements were not done in the 2001-2 survey. We excluded one participants reporting Ménière's disease (as tinnitus is an integral part of this syndrome) and six night-workers (due to potentially disturbed diurnal cortisol secretion(Mirick et al., 2013)). Of the remaining 658 participants, 633 collected saliva samples. Participants with any cortisol measurement > 100 nmol/L were considered outliers and also excluded (n=1) (Hansen et al., 2012), resulting in a final study population of 632 participants. All 632 participants from the final study population returned the questionnaire.

The study protocol was approved by the local ethics committee (M20080239) and the Danish Data Protection Agency (2009-41-3072).

2.2 Salivary cortisol sampling and cortisol determination

Participants were instructed to provide three saliva samples, the first at approximately 08.00 p.m. the first day (evening sample), the second at awakening (awakening sample), and the third 30 min later (awakening+30 sample). Participants were instructed not to smoke, drink or eat within 30 minutes before sampling. Saliva was collected in Salivette® tubes and refrigerated until collected the next day. Saliva samples were then stored at -20 °C until analysis within 6 months. Awakening samples were considered valid if they were collected within 30 min after waking up

(all except eight samples). Awakening+30 samples were considered valid if they were collected within 60 min after the awakening sample (all except 17 samples) and evening samples were considered valid if they were collected after 5 pm (all except 10 samples). In this paper, we only included valid samples.

2.2.1 Determination of cortisol in saliva

Cortisol level was determined by radioimmunoassay (RIA) as described by Hansen et al. (Kristenson M, Garvin P, Lundberg U, 2011). The RIA for cortisol determination was designed for quantitative in vitro measurement of cortisol in serum, plasma, urine, and saliva. We used The Spectria Cortisol Coated Tube RIA, purchased from Orion Diagnostica, Espoo, Finland and used it according to the manufacturer's specifications. The sample volume was 150 μL, the range of the standard solutions prepared was 1.0-100.0 nmol/L, and the incubation time was 30 min at 37 °C. The specifications given by the manufacturer were a sensitivity of twice the standard deviation of the zero binding value in saliva (0.8 nmol/L), a bias of 10% (3-15%), an intra-assay variation of 5.4%, and an inter-assay variation of 7.3%. Limit of detection was 1.59 nmol/L. Between-run coefficients of variation were 19% at 11.5 nmol/L and 16% at 49.2 nmol/L. (Hansen et al., 2003).

2.2.2 Equivalence between different runs

To show equivalence between different runs, natural saliva samples (5.9 nmol/L and 24.4 nmol/L) were used as control materials and analysed together with the samples. Westgard control charts were used to document that the trueness and the precision of the analytical methods remained stabled (Westgard et al., 1981).

2.2.3 Cortisol measures

The cortisol measures analysed were: (1) awakening cortisol, (2) awakening+30 cortisol, (3) evening cortisol, (4) cortisol awakening response (CAR) calculated as the difference between cortisol levels at awakening and after 30 min), (5) slope of cortisol calculated as the change in cortisol from the maximum morning to the evening sample, and (6) area under curve with respect to ground (AUC) calculated as:

[(awakening conc. + awakening+30 min conc.) / 2) × time difference between the awakening conc. and the awakening+30 conc.] + [(awakening+30 min + evening

concentration) / 2) × time difference between the awakening +30 and the evening concentration]

2.3 Tinnitus

Tinnitus related questions included frequency of tinnitus (1. almost never experiencing tinnitus, 2. experiencing periods of tinnitus at least monthly, 3. experiencing periods of tinnitus at least weekly or 4. experiencing tinnitus daily), frequency of annoyance when experiencing tinnitus (1. almost never or never, 2. rarely, 3. sometimes, 4. often or 5. always) and frequency of insomnia due to tinnitus (1. almost never or never, 2. rarely, 3. sometimes, 4. often or 5. always). A person was classified as having tinnitus if experiencing tinnitus daily accompanied by either annoyance (sometimes, often or always) or insomnia (sometimes, often or always).

2.4 Covariates

The covariates selected for the analyses were known to be associated with cortisol or tinnitus and decided upon a priori based on a review of the literature (Hasson et al., 2011; Kudielka, Hellhammer & Wust, 2009).

Information on participants' age and sex was retrieved from their personal civil registration number. Information on awakening time, time of saliva sampling, and common mental disorders (depression and anxiety) was retrieved from the questionnaire. Symptoms of depression- and anxiety were assessed using the Common Mental Disorders Questionnaire (CMDQ) (Kristensen et al., 2005). Noise exposure was assessed using individual dosimeters (Bruel & Kjær, model 4443, Nærum, Denmark) measuring A-weighted equivalent sound levels (L_{Aeq}) in 5 second intervals during working hours and leisure time. Measuring range was set to 70-120 dB(A). Individual A-weighted equivalent noise levels were computed for the full work shift ($L_{Aeq, work}$).

Air-conduction thresholds were determined for each ear at 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kHz by pure tone audiometry at the workplaces, using a Voyager 522 audiometer equipped with TDH-39 headphones (Madsen Electronics, Taastrup, Denmark).

0.5-4.0 kHz hearing threshold (0.5-4.0 kHz HT) was computed as the average of pure-tone hearing thresholds at 0.5, 1, 2, 3 and 4 kHz in the worse ear. Worse ear hearing ability was chosen over better ear, as we assumed that hearing levels at the worse ears were the most predictive of tinnitus status.

2.5 Statistics

We tabulated the potential confounders according to presence of tinnitus. Odds ratios of tinnitus according to cortisol measures were analyzed by logistic regression using both continuous-scale exposure information and tertile categorizations. Cortisol measures were log-transformed to reduce skewness and variances. We analysed (1) awakening cortisol, (2) awakening+30, (3) evening cortisol, (4) CAR, (5) slope and (6) AUC in separate models.

The crude associations between cortisol and tinnitus were followed by two sets of adjustment: The basic adjusted model included sex and age (continuous) and the full adjusted model also included worst ear hearing threshold (continuous dB HL), depression (yes/no) and anxiety (yes/no).

As we did not find any association between the occupational noise exposure levels measured for this cohort and tinnitus in a prior study (unpublished data), this variable was not included in the main model. However, we performed a sub-analysis, adjusting for occupational noise exposure.

Another sensitivity analysis was performed due to concerns that time of awakening and cortisol sampling time would affect our results. In this analysis, time of awakening and time of cortisol sampling was included in the fully adjusted model.

3. RESULTS

A total of 51 (8.1%) participants were classified as suffering from tinnitus according to our criteria. Of the 632 participants, 171 were women (27.1 %). Age range was 20-73 years (mean 44.2 years). Characteristics of participants according to tinnitus status are presented in Table 1. Among participants with tinnitus, we observed a tendency toward higher age, higher worst ear hearing threshold, and a higher prevalence of males, compared with participants without tinnitus.

Table 2 presents crude and adjusted odds ratios for tinnitus by a one-unit increase of the log-transformed cortisol measures also categorized into three levels. For the awakening sample, we observed weak indications of an association between higher cortisol concentrations and higher adjusted risk of tinnitus, whereas higher evening cortisol concentrations seemed to be associated with a lower adjusted risk of tinnitus. Thus, the fully adjusted odds ratios for 1.0 nmol/L increase on the logarithmic scale for awakening and evening cortisol were 1.40 (95% CI: 0.80; 2.44) and 0.77 (95% CI: 0.52; 1.13), respectively. This was also reflected in the morning to evening slope where we found indications of an inverse association between a flatter cortisol slope and tinnitus (fully adjusted OR: 0.76 (95% CI 0.54; 1.06) per 1.0 nmol/L flatter slope on the logarithmic scale). However, none of these associations were statistically significant. We found no indication of associations between awakening+30 cortisol, CAR and AUC and tinnitus.

Adjusting analyses for full shift occupational noise exposure resulted in a slightly weaker association between awakening cortisol and tinnitus, whereas the remaining associations were practically unchanged. Thus, fully adjusted odds ratios for the association between awakening cortisol and tinnitus changed from 1.40 (95% CI: 0.80; 2.44) to 1.20 (95% CI: 0.65; 2.20).

For each cortisol measure, the possible effect of measuring time and awakening time was examined in sub-analyses where the two time variables were included in the fully adjusted logistic regression analyses for each cortisol measure. To account for the assumed non-linear association between cortisol concentration and time in the morning, due to the morning cortisol peak, sampling time for awakening+30 was

included as continuous and squared variables. For the association between CAR and tinnitus, this resulted in a moderately higher odds ratio for tinnitus (OR *before* adjusting for awakening time and measuring time: 0.82 (95% CI 0.46; 1.47), OR *after* adjusting for awakening time and measuring time: 1.02 (95% CI 0.52; 2.00). The odds ratio for the association between awakening cortisol and tinnitus also decreased moderately from 1.40 (95% CI 0.80; 2.44) to 1.21 (95% CI 0.64; 2.27). The remaining associations were essentially unchanged.

4. DISCUSSION

We examined the association between single time point saliva cortisol measures (awakening, awakening+30 and evening cortisol), dynamic cortisol measurements (CAR and slope of cortisol) and a time adjusted measure of total cortisol exposure (AUC) and tinnitus. In general, no statistically significant associations were observed and our hypothesis that increased cortisol levels and changes in the dynamics of cortisol secretion (as a reflection of an activated HPA axis from prolonged stress) is associated with tinnitus is thus not supported.

We observed weak associations between increased awakening cortisol and decreased evening cortisol levels and tinnitus, which were also reflected in the results for slope of cortisol, showing a discreetly decreased risk of tinnitus with flatter diurnal slope of cortisol, or, in other words – an increased risk of tinnitus with steeper slope of cortisol. These findings could indicate an increased HPA axis activation around awakening and a corresponding deactivation in the evening among participants classified with tinnitus compared to participants without tinnitus. This finding should, however, be interpreted with caution due lack of statistical significance and stronger results should be obtained in future studies before conclusions are drawn.

Prior studies on the association between cortisol and tinnitus are scarce, and study designs are heterogeneous. Authors generally agree that, theoretically, cortisol is a useful objective measure of stress, but there is doubt about the direction of causality between cortisol and tinnitus. Is tinnitus a stressor causing changes in the cortisol secretion or is stress (reflected by altered cortisol secretion) causing tinnitus? Regrettably, no longitudinal studies have been performed to clarify this.

A recent Korean study compared the levels of stress hormones in a large group of tinnitus patients (n = 344) with a healthy control group (n = 87) (Kim et al., 2014). Results for cortisol (single time point measure in blood sampled between 9 and 11 a.m.) showed no difference in basal cortisol between tinnitus and control groups ($p = 0.976$). Compared to our results for awakening and awakening+30 concentrations of cortisol, which are the closest we temporally get to a 9 – 11 a.m. measurement, results are not contradictory, though we do find a weak tendency toward a higher risk of

tinnitus with higher levels of awakening cortisol. Regrettably, for the comparison with this study, dynamic cortisol measurements were not performed.

In a Canadian case-control study from 2004 by Hébert and colleagues, 18 chronic tinnitus patients with high (n = 9) and low (n = 9) tinnitus-related distress were compared with a healthy control group (n = 18) with respect to diurnal cortisol variation and chronic basal cortisol levels (Hebert, Paiement & Lupien, 2004). For the measure of diurnal cortisol variation no significant changes were found across the three groups, but results for chronic basal cortisol levels showed significantly higher levels above the median in the high tinnitus-related distress group compared to both the low distress group and controls. On the basis of these results, the Canadian authors suggested a dysregulated HPA axis in tinnitus patients and therefore performed a second study in 2006 (Hebert & Lupien, 2007). In this study, the integrity of the HPA axis in tinnitus patients was tested by measuring saliva cortisol during and following exposure to an acute stressful situation (the Trier Social Stress Test) in tinnitus patients (n = 18) and controls (n = 18). This study revealed a blunted cortisol response to acute stress in chronic tinnitus patients compared to controls. To further test the hypothesis of HPA axis dysregulation in tinnitus patients, Hébert and Simoens performed a Dexamethasone suppression test on patients with chronic tinnitus (n=21) and healthy controls (n=21) (Simoens & Hebert, 2012). Both groups displayed similar basal cortisol levels (this time measured as AUC) and diurnal secretion pattern, but tinnitus patients showed stronger and longer-lasting cortisol suppression after Dexamethasone administration, indicating an abnormally strong glucocorticoid receptor mediated feedback in these patients.

The studies by Hébert and Simoens differ from ours in both tinnitus classification (patients diagnosed with severe chronic tinnitus vs. workers classified from a questionnaire) and design (mainly intervention studies vs. a strictly observational study), and results are therefore not readily comparable. Including results from the Korean observational study, it, however, seems reasonable to suggest that it takes an active provocation of the HPA axis and subsequent cortisol measurements to find an association between cortisol secretion and tinnitus. Merely observing the habitual daily cortisol secretion and pattern between individuals with and without tinnitus reveals no association.

Among the strengths of our study is the detailed information on relevant factors that could possibly confound our results. Compared to only adjusting for age and sex (as done in most prior tinnitus studies), we were able to extend adjustment to also include hearing level, depression and anxiety – known risk factors for tinnitus that are possibly also associated with cortisol levels (Canlon, Theorell & Hasson, 2013; Gilles, Goelen & Van de Heyning, 2014a; Holgers, Erlandsson & Barrenas, 2000; Oishi et al., 2011). Extended adjustment did, however, not change results markedly for any of the cortisol measures.

Furthermore, to our knowledge, our population was the largest so far, which should also compensate for the fact that cortisol was only collected for 24 h, even if it has been suggested to include data for two or more days, due to high variability within individuals (Hellhammer et al., 2007).

From occupational noise dosimetries performed on each participant, we knew that some participants were exposed to high noise levels at work. In a sub-analysis, we therefore adjusted for A-weighted full work shift noise levels. This resulted in a moderate weakening of the association between awakening cortisol and tinnitus, while the remaining associations were unaffected. This indicates that noise exposure could affect our analyses, but it is speculative why only awakening cortisol is affected, and this result could therefore be a chance finding.

Earlier cortisol studies have emphasized the importance of awakening time and cortisol sampling time (Edwards et al., 2001). We therefore performed a sub-analysis in which we adjusted for awakening time and sampling time to account for the fact that all participants did not wake up at the same time and that saliva samples were not collected at the exact time they were instructed to. This sub-analysis showed no changes in the overall results though associations for CAR and awakening cortisol and tinnitus both became moderately weaker which furthermore supports our main finding of no association between cortisol measures and tinnitus.

Among limitations is the cross-sectional nature of this study, preventing us from drawing conclusions regarding causality. Also, our definition of tinnitus may have led to misclassification of tinnitus status. Using a standardized tinnitus questionnaire as

the Tinnitus Handicap Inventory (Zeman et al., 2012) possibly could have refined our tinnitus classification. However, due to pressure of space in the questionnaire that also contained issues unrelated to hearing, tinnitus related questions had to be limited.

Many of our participants were recruited from industrial trades and thus exposed to noise. As tinnitus is often accompanied by hypersensitivity to noise (Gilles, Goelen & Van de Heyning, 2014a; Nelson & Chen, 2004), this may have excluded tinnitus sufferers generally more sensitive to any stressors from participating in our study, because they would avoid employment in such industries. If this argument holds true, our population may represent a selection of workers less affected by stress. This healthy worker effect would introduce selection bias to our study, and possibly bias results towards the null.

Though our population was probably the largest so far, the limited number of tinnitus cases (n = 51) which is reflected in the wide confidence intervals also represents a limitation to this study.

To conclude, this observational study did not support our hypothesis that increased cortisol levels and changes in the dynamics of cortisol secretion, as indicators of stress activation of the HPA axis, were associated with tinnitus. We did observe weak indications of associations between higher awakening and lower evening cortisol and tinnitus, but these results should be confirmed in future studies before conclusions are drawn.

CONFLICTS OF INTEREST

All authors state that they have no conflicts of interest

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Table 1. Characteristics of 632 participants with or without tinnitus, Aarhus, Denmark 2009-10.

Characteristic	Tinnitus (n=51)		No tinnitus (n=581)		OR	CI
Sex, no (%)						
Female	5	(9.8)	166	(28.6)	1	
Male	46	(90.2)	415	(71.4)	3.68	(1.43; 9.42)
Age (years), mean (SD)	49.1	(10.8)	43.8	(10.5)	1.05	(1.02; 1.08)
Worst ear hearing threshold (dbHL), median (p10-p90)	25.0	(7.5; 36.3)	12.5	(5.0; 28.8)	1.04	(1.03; 1.06)
Occupational noise (dB(A)), mean, SD	79.5	(6.4)	80.6	(6.8)	0.98	(0.94; 1.02)
Awakening time, median h (p10-p90)	5:30	(4:54; 6:18)	5:36	(4:42; 6:48)	0.87	(0.62; 1.22)
Sampling time, median h (p10-p90)						
Awakening	5:50	5:00; 7:00	5:40	4:40; 7:00	1.12	(0.88; 1.41)
Awakening +30	6:25	5:30; 7:50	6:15	5:10; 7:30	1.13	(0.91; 1.41)
Evening	20:15	19:45; 21:30	20:04	18:20; 21:15	1.07	(0.98; 1.17)
Depression						
No	42	82.4	488	84.0	1	
Yes	9	17.7	93	16.0	1.12	(0.53; 2.39)
Anxiety						
No	44	86.3	525	90.4	1	
Yes	7	13.7	56	9.6	1.49	(0.64; 3.47)
Education						
Middle- or long range training	5	9.8	115	19.8	1	
Skilled worker or short range training	36	70.6	340	58.5	2.44	(0.93; 6.35)
No education or short courses	10	19.6	126	21.7	1.83	(0.61; 5.50)

Tabel 2. Odds ratios (OR) of having tinnitus according to cortisol measures in a population of 632 participants.

Exposure	Tinnitus (n=51)	No tinnitus (n=581)	OR_{Crude}	95 % CI	OR_{Adj1}	95 % CI	OR_{Adj2}	95 % CI
Awakening, mean (range) nmol/L								
Low 5.0 (0.4; 7.1)	14	187	1		1		1	
Medium 9.1 (7.2; 11.1)	16	190	1.12	0.53; 2.37	1.23	0.58; 2.62	1.21	0.56; 2.62
High 16.0 (11.2; 35.1)	19	190	1.34	0.65; 2.74	1.43	0.69; 2.96	1.33	0.62; 2.81
Continuous 10.1 (0.4; 35.1)			1.41	0.82; 2.43	1.47	0.84; 2.54	1.40	0.80; 2.44
Awakening +30, mean (range) nmol/L								
Low 9.3 (1.5; 12.9)	17	184	1		1		1	
Medium 16.1 (12.9; 19.5)	17	183	1.01	0.50; 2.03	1.19	0.58; 2.45	1,30	0.62; 2.74
High 25.2 (19.7; 56,0)	16	187	0.93	0.45; 1.89	0.95	0.46; 1.97	1.01	0.48; 2.12
Continuous 16.9 (1.5; 56.0)			1.11	0.61; 2.03	1.13	0.61; 2.10	1.18	0.62; 2.22
CAR, mean (range) nmol/L								
Low 0.3(-22.1; 3.5)	14	182	1		1		1	
Medium 6.3 (3.6; 9.1)	19	179	1.07	0.52; 2.18	1.13	0.55; 2.34	1.23	0.58; 2.60
High 14.5 (9.1; 32.3)	15	182	0.93	0.45; 1.94	0.95	0.68; 1.97	1.08	0.50; 2.31
Continuous 7.0 (-22.1; 32.3)			0.75	0.42; 1.35	0.76	0.43; 1.34	0.82	0.46; 1.47
Evening, mean (range) nmol/L								
Low 0.7 (0.0; 1.0)	19	184	1		1		1	
Medium 1.5 (1.1; 1.9)	15	175	0.81	0.40; 1.63	0.68	0.33; 1.38	0.70	0.34; 1.46
High 3.8 (2.0; 34.7)	15	205	0.71	0.35; 1.46	0.66	0.31; 1.38	0.62	0.29; 1.32
Continuous 2.1 (0.0; 34.7)			0.87	0.61; 1.23	0.81	0.55; 1.180	0.77	0.52; 1.13
Slope, mean (range) nmol/L								
Steep -23.5 (-53.6; -18.0)	20	183	1		1		1	
Medium –14.5 (-17.9; -11.5)	13	190	1.13	0.58; 2.20	1.03	0.52; 2.05	1.01	0.51; 2.05
Flat -7.0 (-11.4; 19.6)	16	188	0.58	0.27; 1.27	0.53	0.24; 1.18	0.44	0.19; 1.02
Continuous -15.0 (-53.6; 19.6)			0.83	0.62;1.14	0.79	0.58; 1.10	0.76	0.54; 1.06
AUC, mean (range) nmol ×h/L								
Low 83.6 (23.2; 111.2)	16	175	1		1		1	
Medium 134.4 (111.28; 161.4)	15	176	0.94	0.47; 1.88	0.81	0.40; 1.66	0.79	0.38; 1.64
High 212.1 (161.5; 451.6)	15	177	0.59	0.27; 1.28	0.54	0.24; 1.19	0.51	0.23; 1.15
Continuous 143.5 (23.2; 451.6)			0.99	0.94; 1.03	0.98	0.93; 1.03	0.98	0.94; 1.03

[1] Adjusted for age and sex

[2] Adjusted for age, sex, worst ear hearing threshold, anxiety and depression.

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Declaration of co-authorship

Full name of the PhD student: Thomas Winther Frederiksen

This declaration concerns the following article/manuscript:

Title:	Noise Induced Hearing Loss – a Preventable Disease? Results of a 10-year Longitudinal Study of Occupationally Noise Exposed Workers
Authors:	Thomas W. Frederiksen, Cecilia H. Ramlau-Hansen, Zara Ann Stokholm, Matias B. Grynderup, Åse Marie Hansen, Jesper Kristiansen, Jesper M. Vestergaard, Jens P. Bonde, Henrik A. Kolstad

The article/manuscript is: Published ☐ Accepted ☐ Submitted ☒ In preparation ☐

If published, state full reference:

If accepted or submitted, state journal: Noise & Health

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

No ☒ Yes ☐ If yes, give details:

The PhD student has contributed to the elements of this article/manuscript as follows:

A. No or little contribution
B. Has contributed (10-30 %)
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D. Has done most of the work (70-90 %)
E. Has essentially done all the work

Element	Extent (A-E)
1. Formulation/identification of the scientific problem	D
2. Planning of the experiments and methodology design and development	C
3. Involvement in the experimental work/clinical studies/data collection	B
4. Interpretation of the results	D
5. Writing of the first draft of the manuscript	E
6. Finalization of the manuscript and submission	E

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3. Involvement in the experimental work/clinical studies/data collection	B
4. Interpretation of the results	D
5. Writing of the first draft of the manuscript	E
6. Finalization of the manuscript and submission	E

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Authors:	Thomas Winther Frederiksen, Cecilia Høst Ramlau-Hansen, Zara Ann Stokholm, Matias Brødsgaard Grynderup, Åse Marie Hansen, Søren Peter Lund, Jesper Kristiansen, Jesper Medom Vestergaard, Jens Peter Bonde, Henrik Albert Kolstad

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D. Has done most of the work (70-90 %)
E. Has essentially done all the work

Element	Extent (A-E)
1. Formulation/identification of the scientific problem	D
2. Planning of the experiments and methodology design and development	C
3. Involvement in the experimental work/clinical studies/data collection	B
4. Interpretation of the results	D
5. Writing of the first draft of the manuscript	E
6. Finalization of the manuscript and submission	E

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Authors:	Thomas Winther Frederiksen, Cecilia Høst Ramlau-Hansen, Zara Ann Stokholm, Matias Brødsgaard Grynderup, Åse Marie Hansen, Søren Peter Lund, Jesper Kristiansen, Jesper Medom Vestergaard, Jens Peter Bonde, Henrik Albert Kolstad

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C. Has contributed considerably (40-60 %)
D. Has done most of the work (70-90 %)
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1. Formulation/identification of the scientific problem	D
2. Planning of the experiments and methodology design and development	C
3. Involvement in the experimental work/clinical studies/data collection	B
4. Interpretation of the results	D
5. Writing of the first draft of the manuscript	E
6. Finalization of the manuscript and submission	E

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Declaration of co-authorship

Full name of the PhD student: Thomas Winther Frederiksen

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Authors:	Thomas Winther Frederiksen, Cecilia Høst Ramlau-Hansen, Zara Ann Stokholm, Matias Brødsgaard Grynderup, Åse Marie Hansen, Søren Peter Lund, Jesper Kristiansen, Jesper Medom Vestergaard, Jens Peter Bonde, Henrik Albert Kolstad

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III

Declaration of co-authorship

Full name of the PhD student: Thomas Winther Frederiksen

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Authors:	Thomas Winther Frederiksen, Cecilia Høst Ramlau-Hansen, Zara Ann Stokholm, Matias Brødsgaard Grynderup, Åse Marie Hansen, Søren Peter Lund, Jesper Kristiansen, Jesper Medom Vestergaard, Jens Peter Bonde, Henrik Albert Kolstad

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