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DETERMINANTS OF HEARING IMPAIRMENT IN SWEDISH HUNTERS

- an e-epidemiological approach

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Determinants for hearing impairment in Swedish hunters

an e-epidemiological approach

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Stockholm 2016

To my family

Wisdom is not a product of schooling
but of the lifelong attempt to acquire it.

Albert Einstein

All you need is love.
But a little chocolate now and then doesn't hurt.

Charles M. Schulz

ABSTRACT

Background: 290 236 persons bought a hunting license in Sweden 2014/15. Hunters are spread all over the country and come from all socioeconomic groups. Recreational firearms can produce peak noise levels between 156-164 decibel sound pressure levels (dB SPL). Human challenge experiments in the past have demonstrated that a single gunshot can cause a severe temporary threshold shift (TTS). The recovery period after such acoustic trauma is unpredictable and a permanent threshold shift (PTS) cannot be excluded. Swedish and international surveys have shown a high usage of hearing protection at training but much less so at actual hunting. Although several studies have measured noise levels from different types of weapons, there are only sparse observational data on dose-risk relationships and effect-modifying factors in humans engaged in real-life hunting. For continuous noise we know that different factors, as tobacco use, affect the susceptibility to develop hearing impairment when exposed, but little is known of these factors when exposed to impulse noise as from hunting fire arms.

Aims: To construct and validate an internet-based screening tool for testing hearing ability among large populations; To examine the association between high frequency hearing Impairment (HFHI) among Swedish hunters, and their shooting habits and usage of hearing protection; To examine potential risk modification of HFHI by tobacco-use when exposed to impulse noise from hunting rifle calibers.

Materials and method: A secure website with a questionnaire and an internet-based audiometry test, the InternetAudio test, based on a JAVA platform, was constructed. The combination questionnaire and hearing test was tested for feasibility in a pilot study on 560 members of the Swedish Hunters' Association (Study1). The hearing test was validated using clinical pure-tone air-conducted audiometry as gold standard on 72 participants (79% women) with a mean age of 45 years (range 19-71 years). Twenty participants had impaired hearing according to the gold standard test (Study 2). Finally in 2013 invitations to participate in the main survey was sent to 27 063 e-mail addresses retrieved from the Swedish hunters' association membership roster. In all, 1937 persons started the survey, 1771 of them completed the questionnaire, and 202 also completed the InternetAudio test. In this final survey the InternetAudio test was further validated while used under authentic conditions by 12 participants (Study 3). Associations, between the number of unprotected shooting with hunting rifle calibers (HRC) and HFHI, expressed as prevalence ratio (PR), were multivariately modelled using Poisson regression (Studies 3). In study 4 the possible effect modification of tobacco use in form om cigarettes/snus-use or both was examined with the same methods.

Results and conclusions: In the pilot study 162 out of 560 (29%) had answered the questionnaire, out of which 88 (16%) had completed the hearing test. Those who completed the hearing test were older than the non-participants, and had to a larger extent headphones (p=0.003) and the correct version of the JAVA program (p=0.007) than those who only answered the questionnaire (Study1). In the validation study of the Internet based hearing test the Pearson correlation coefficient was 0.94 (p< 0.0001) for the right ear and 0.93 for the left (p = 0.0001). The sensitivity for hearing loss was 75% [95% CI, 51%-90%], and the specificity was 96% [95% CI, 86%-99%]. The test-retest reproducibility was excellent (Study 2). In Study 3 subjective severe hearing impairment was reported by 195/1771 (11%), while 23/202 (11%) exhibited HFHI upon testing with Internet-based audiometry. As many as 328/1771 (19%)

had never used hearing protection during hunting. In the preceding 5 years, 785/1771 (45%), had fired >6 unprotected gunshots with HRC. The adjusted PR of HFHI when reporting 1-6 unprotected gunshots with HRC, relative to 0, was 1.5 [95% (CI) 1.1-2.1; P = 0.02]. We could not verify any excessive HFHI prevalence among 89 hunters reporting unprotected exposure to such gunshot noise >6 times. In Study 4, current daily use of tobacco was reported by 61 hunters (19 cigarettes, 47 moist snuff, and 5 both. Tobacco users tended to be younger, to fire more shots with HRC weapons, and to report more hunting days. Their adjusted PR (1-6 unprotected HRC shots versus 0) was 3.2 (1.4-6.7), p <0.01. Among non-users of tobacco the corresponding PR was 1.3 (0.9-1.8), p= 0.18. P value for the interaction was 0.01. The importance of ear protection could not be quantified among hunters with HRC weapons because our data suggested that the HFHI outcome had led to changes in the use of such protection. Among hunters using weapons with less sound energy, however, no or sporadic use of hearing protection was linked to a 60% higher prevalence of HFHI, relative to habitual use.
Hearing ability can be accurately screened with a PC, an internet connection, and a pair of headphones among a willing population. The thesis results support the notion of a wide variation in individual susceptibility to impulse noise. Susceptible individuals may sustain long-lasting or possibly irreversible damage to the inner ear from just one or a few shots, furthermore Tobacco use modifies the association between exposure to unprotected impulse noise from HRC weapons and the probability of having HFHI among susceptible hunters. The mechanisms remain to be clarified, but since the effect modification was apparent also among users of smokeless tobacco, combustion products may not be critical.

LIST OF PUBLICATIONS

This thesis is based on the following studies, which will be referred to in the text by their numerals:

1. Bexelius C, Honeth L, Ekman A, et al. Evaluation of an internet-based hearing test--comparison with established methods for detection of hearing loss. J Med Internet Res 2008; 10: e32. http://www.jmir.org/2008/4/e32/

2. Honeth L, Bexelius C, Eriksson M, Sandin S, Litton JE, Rosenhall U, Nyren O, Bagger-Sjoback D. An internet-based hearing test for simple audiometry in nonclinical settings: preliminary validation and proof of principle. Otol Neurotol 2010 Jul;31(5):708-14.

3. Honeth L, Ström P, Ploner A, Bagger-Sjöbäck D, Rosenhall U, Nyrén O. Shooting history and presence of high-frequency hearing impairment in Swedish hunters: A cross-sectional internet-based observational study. Noise and Health 2015 sept-oct, vol 18.

4. Honeth L, Ström P, Ploner A, Bagger-Sjöbäck D, Rosenhall U, Nyrén O. Tobacco-modified association between unprotected exposure to noise from hunting rifle caliber weapons and high frequency hearing loss. A cross- sectional study among Swedish hunters. Submitted for publication.

CONTENTS

LIST OF ABBREVIATIONS

dB	Decibel
Hz	Hertz
kHz	kiloHertz
SPL	Sound pressure level
NIHL	Noise induced hearing loss
TTS	Temporary threshold shift
PTS	Permanent threshold shift
PR	Prevalence ratios
HFHI	High frequency hearing impairment
HRC	Hunting rifle caliber
CVD	Cardio vascular disease
RP-HL	The reference person´s hearing level
TP-HL	The test person´s hearing level
HL	Hearing level
CI	Conficence interval
CO	Carbon Monoxide
TSNA	Tobacco-specific nitrosamines
OAE	Otoacoustic emissions

1 INTRODUCTION

1.1 BASIC ACOUSTICS

Hearing ability among young humans range from the frequencies 20-20 000 Hertz (Hz). The sound pressure is measured in the unit Pascal (Pa) and is related to the changes of the static atmospheric pressure caused by a sound wave. Humans can hear sound pressures as low as 20µPa and at 60 Pa we experience pain. When objectively expressing different sound pressure levels a logarithmic scale and the unit decibel sound pressure level (dB SPL) is used. This is an objective measure, and is not to be confused with loudness, which is connected to our perception, psycho-physical sensation, of how loud a sound is (e.g. the volume of music). The loudness perception varies between individuals. A 3 dB rise in SPL is a doubling of the power level, but it usually takes a 10 dB rise for humans to perceive a sound as twice as high.

The weakest perceptible sound level is called the hearing threshold. The hearing threshold varies with the frequencies and the human ear is most sensitive to sound in the frequency span of 2-4 kHz. Because of this variable sensitivity, different weightings have been established for measuring sounds that might be hazardous for the hearing and to give an approximation of how the human ear perceives the noise. A-weighting is most commonly used when measuring environmental noise.

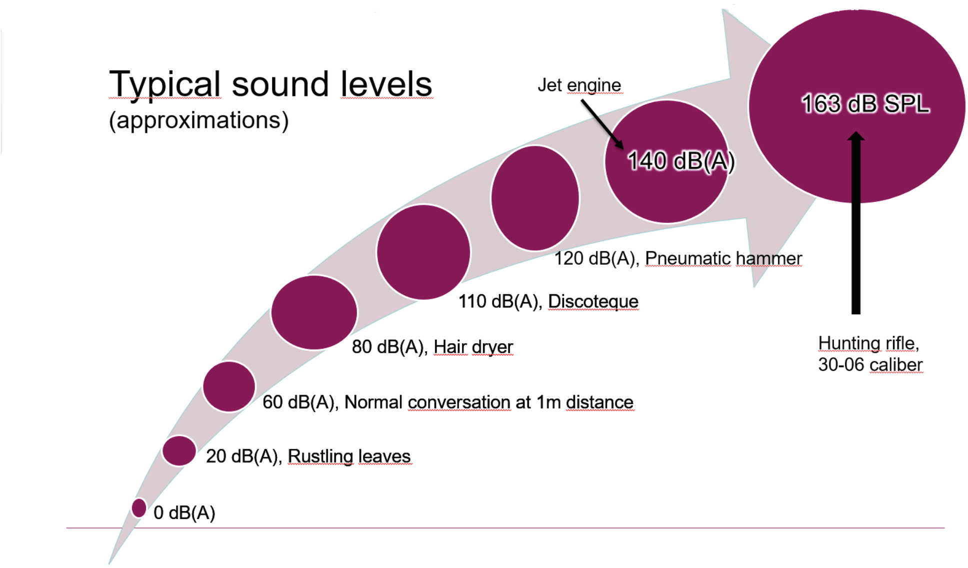

Fig 1. Decibel scale

1.1 HEARING IMPAIRMENT

In this thesis the word hearing impairment instead of loss will be used with one exception, noise induced hearing loss (NIHL). This is according to the WHO International Classification of Functioning, Disability and Health, ICF 2001 (1).

With increasing age there is a progressive hearing impairment, starting at the higher frequencies. Apart from age, hearing can also be affected by noise exposure, genetic factors, diseases, trauma, chemicals and medication.
Sweden has 1.4 million persons with self-assessed hearing problems which affect their ability to hear conversations. About half a million of those have hearing aids (2). Approximately 700 000 with self-assessed hearing problems are in the working ages (16-64 years) (3). During the last 30 years self-assessed hearing problems among the middle-aged has increased without clear reasons. Statistics show that early retirement is twice as common among those with hearing problems, which indicate the medical burden for the individual and society (2).

1.2 NOISE-INDUCED HEARING LOSS (NIHL)

Noise is defined as unwanted sound. Noise with rather small variations in the sound levels and with extended exposure is called continuous. It is occupational or environmental. This type of noise can cause gradually developing NIHL most often affecting both ears; Noise is also sudden intense sounds with short durations, called impulse noise, such as explosions, metallic sounds from sheet-metal work or noise from firearms. High impulse noise can cause acute acoustic trauma and NIHL. This type of NIHL is often one-sided (one ear) and with a steep audiogram curve on one or two frequencies.

There are regulations on the permitted noise levels at work devised to reduce the risk for NIHL. These regulations specify that the daily dB(A) SPL during an 8 hour day at work should not exceed 80-85 (continuous) and the peak levels SPL (impulse) should not exceed 135 (4). If these noise levels are reached or exceeded hearing protection should be used. The risk criteria for noise exposure are specified in the standard ISO 1999 (2013). Leisure-time noise is only subjected to public health recommendations (5, 6) . According to these recommendations the peak dB SPL should not exceed 110 (6).

Both continuous and impulse noise might subject the cochlea in the inner ear to a harmful amount of sound energy and affect the hearing ability in similar pathways by wearing down the fine structures of the cochlea over time (continuous) or instantly (impulse). The effects on the cochlea is both of metabolic and mechanical character, depending on the noise levels and time of exposition.

More specifically, noise reduces the blood flow to the cochlea (7) and activates the mitochondria in the hair cells and induces the excess production of the neurotransmitter glutamate (8) and free radicals (9). This causes toxic swelling, and induces cell death in the organ of Corti. The inner and outer hair cells, their stereocilia, the basilar membrane and even the organ of Corti as a whole can be damaged (10). The outer hair cells are the most vulnerable and are the first to be affected by intense noise levels. The production of free radicals will continue up to 10 days after exposure and the cell death can be seen up to 14 days after exposure (11).

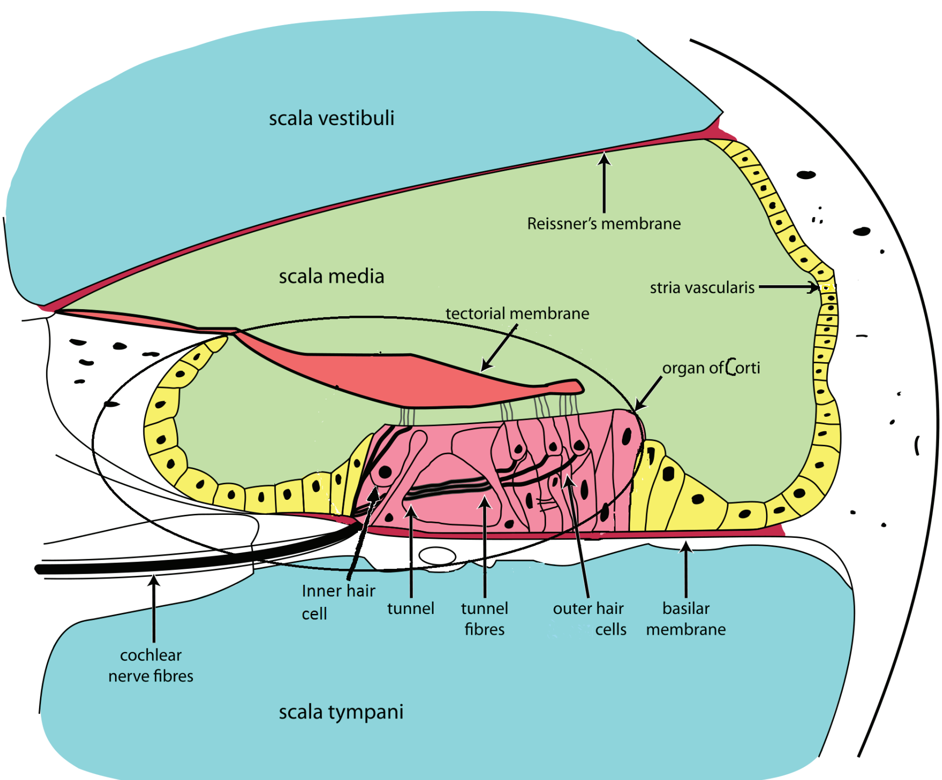

Fig 2. Cochlear cross-section (Fred the Oyster, Wikimedia commons)

Exposure to high intensity noise either of impulse noise or intense continuous noise might result in tinnitus and/ or acute hearing loss, and hyperacusis (12). The acute hearing loss called a temporal threshold shift (TTS) and the other mentioned symptoms might disappear after a period of rest, or be reduced depending on the extent of the damage, the recovery speed and state of the protective antioxidant system of the cells. The recovery might take hours, days or even months (13) and if it is insufficient there will be a permanent threshold shift (PTS) for life, a NIHL.

Typically the development of the NIHL is most evident in the first years of exposure to noise, Changes seen in the audiometry mostly involve the frequencies 4 and 6 kHz (14), but PTS might also affect 3 kHz. In this thesis we study hearing loss of 25 dB or more on 4 or 6 kHz, which we define as a high frequency hearing impairment (HFHI).

After having developed the NIHL can remain quite stable in spite of years of exposure, until it starts aggravating again with older age (14). The effect of ageing on hearing ability has been shown to override the effect of long-time noise exposure of dB levels of 85-90 (15), and this will hamper the possibilities to study small effects on the hearing ability of a specific noise in observational studies.

1.1.1 Individual susceptibility

Different individuals have different susceptibility to develop hearing impairment in response to noise, depending on age, gender, height, pigmentation, medical condition, smoking and environmental factors as chemicals, ototoxic substances, (16). This has been tested in historical provocation experiments where volunteers were exposed to unprotected gun-shot noise (13, 17). Some of the volunteers did not show any TTS when exposed, whereas others exhibited very marked and pronounced TTS. The latter individuals had detectable hearing loss for a long time (>2 month) after exposure, even though the depth of the hearing loss diminished over time (17). This between-individual variation in recovery to impulse noise was also seen by Luz and Hodge (18) and Dancer et al (19) .

In recent years the genetic components in the variability/susceptibility to develop hearing impairment in response to noise, has become more evident (20-22). The research on genetic influence has focused on genes involved in the different pathways regulating features of the cochlea, such as cochlear elements (e.g., stereocilia and cadherins), genes that are involved in the antioxidant recovery system (SOD and Glutation), genes involved in potassium recycling (potassium is very important for sensory transduction in the cochlea) (23), and genes responsible for the production of heat-shock proteins (heat-shock proteins can be protective for the ear, when exposed to noise).

1.3 HUNTERS AND SHOOTING

Recreational firearms can produce peak noise levels between 156-163 dB SPL, depending on type of weapon and ammunition (24). These sound levels may cause irreparable damage to the cochlea, even after just a single shot (25). Hearing protection such as ear plugs and ear muffs are useful and protect the ear (26) (27). They give a sound attenuation in field studies, if used correctly, of respectively 22-35 dB SPL and 18-20 dB SPL(28). However, Swedish and international research have shown low use of hearing protection when hunting compared to training shooting (29) (30).

Cross-sectional Swedish studies among hunters have reported a high percentage of hearing loss on the noise frequencies 3, 4 and 6 kHz, even at rather low ages (31, 32). These studies are also supported by international data reporting a 57-100% increased odds of having a marked high-frequency (4, 6 and 8kHz) hearing loss among hunters, and an increasing prevalence with increasing number of years of shooting (32, 33). In an American study among workers exposed to occupational noise, recreational shooting was associated with an average of 5-10 dB higher decibel hearing level (dBHL) in the 3-6 kHz frequencies (34). Similarly, a Norwegian survey found the same frequencies to be most affected by impulse noise (mostly shooting)(35).

None of these studies examined type of weapon specifically or number of shots and their effect on the hearing ability.

In Sweden 2014/15, there were 290 236 hunting permits sold, of which 6-7% were sold to women (36). The hunters are spread all over the country. To be able to buy a hunting permit you have to have a hunter’s license and to use a hunting-weapon a hunting weapon license is needed. Normally there is an age limit of 18 years to get a hunting weapon license, but there are exceptions.

The measurement of the width/ diameter of a bullet or a gun barrel is called caliber. It is expressed in English measurements like hundredths or thousandths of an inch or in millimeters, depending on where the bullet was developed (Europe or the U.S.). The name of the constructor that first designed the bullet is also often a part of the name of the ammunition/bullet. Shotguns have a different measurement called gauge, which also expresses the inside diameter of the shotgun barrel. In Sweden bullet weapons are divided into weapon classes 1-4, depending on bullet weight and impact energy, but in this thesis, for international reasons, we use the following classification of hunting weapons according to their use and the expected peak SPL emitted when hunting.

Table 1. Examples of hunting rifles in three categories; Hunting rifle calibers; lighter rifle calibers; shotguns, and the names of the most common ammunition used for the different weapon types.

	Hunting rifle calibers	Lighter rifle calibers	Shotguns
Un-weighted	**peak dB SPL**, microphone placed 3 cm from the shooter's left ear		
	163 (30-06)	141 (.22 long rifle (LR))	161 (.12 gauge)
Most common calibers (bullets)			
	6.5 x 55 mm	.22 long rifle (LR)	.20
	7 x 57 mm		
	7 mm Remington Magnum		
	.30-06 Springfield		.16
	.308 Winchester		
	.300 Winchester Magnum		
	.338 Winchester Magnum		.12
	.375 Holland &Holland		
Uses			
	Larger game	Small game, training	Small game (bird, rabbit, hare, badger, fox) and, skeet shooting

1.4 MEASURING HEARING ABILITY

Hearing ability can be graded according to the WHO classification into normal hearing versus slight, moderate, severe and profound hearing impairment based on the average of the hearing threshold of the frequencies 0.5, 1, 2 and 4 kHz of hearing in the better ear, (37).

Table 2. Grading of hearing impairment according to WHO. 4FA = average of hearing threshold levels in dB, of the better ear at 0.5, 1, 2 and 4 kHz.

	WHO
Grade of impairment	**4FA**
Normal	≤25
Slight	26-40
Moderate	41-60
Severe	61-80
Profound	≥80

Hearing ability can be measured with many different methods; both where the subject is rather passive (neurophysiological methods), and where the subject interacts (psychoacoustic methods).

1.4.1 Neurophysiological methods

These methods are used in animal research as well as in the clinic on infants and adults. In auditory brain stem response (ABR), electrodes are placed on the head and the ear is stimulated with clicks or tone pulses. The electric response elicited in the auditory nerve and brainstem are registered. The required sound levels of the clicks or tone pulses which raise a measurable electric response indicates the hearing threshold of the tested frequency. This can be done while the subject is at sleep or under sedation.

Oto acoustic emission, (OAE) is done as a screening test for hearing ability on all newborns in Sweden. The method measures the oto-emissions that are sent from the outer hair cells in response to short emitted sounds. This is preferably done with the subject resting or sleeping. A small soft probe containing both an emitter and a microphone is placed in the ear canal and the oto emissions reproducibility in percentage are measured in several frequencies.

1.4.2 Psychoacoustic methods

The most common way to asses hearing ability and is called clinical pure tone audiometry (38). This technique is widely used both in the clinic, in occupational medicine and in epidemiological studies. The hearing levels in dB, across the frequencies 0,125; 0,25; 0,5; 1; 2; 3; 4; 6 and 8 kHz are measured for each ear separately. The presentation levels vary between -10 dB HL up to a maximum of 100, 110 dB HL. The clinical pure-tone audiometry is done in sound proof booths with calibrated equipment by specially educated and trained personnel. It is possible to measure both air and bone-conducted hearing. Masking of the opposite ear is done when necessary. Clinical pure-tone audiometry is used to diagnose type and severity of the hearing impairment and follows the international standards of ISO 8253-1 (39). With screening audiometry, most often performed with computerized automatic audiometry, air-conducted screening hearing tests can be performed in schools and work places by lay people at a lower cost compared to regular clinical pure-tone audiometry. In screening audiometry the presentation levels varies from the screening levels 0 dB HL (or +10 dB HL) up to 90 dB HL.

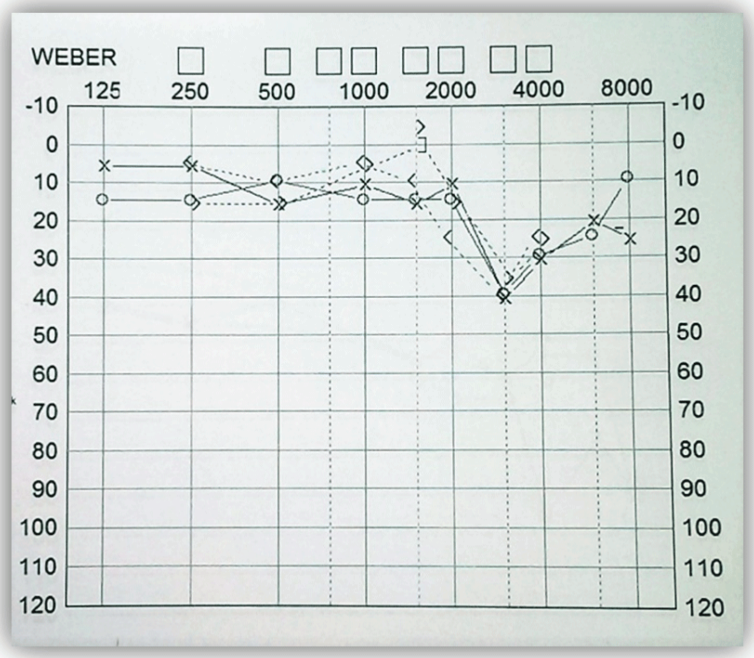

Fig. 3. Clinical pure-tone audiometry with a moderate to mild bilateral NIHL at 3, 4 and 6 kHz.

Speech-test and speech-in-noise test are tests which measures speech intelligibility in quiet and in noise, using sentences as speech material and a fixed signal-to-noise ratio. The result of the test is mostly given as the speech reception threshold (SRTn), which is defined as the signal-to-noise ratio for a person to recognize 50% of the speech material correctly. It was originally developed to test the benefit of hearing aids and is sometimes said to test hearing ability more as a social function.

1.5 E-EPIDEMIOLOGY

E-epidemiology has been defined as the scientific activity "underlying the acquisition, maintenance and application of epidemiological knowledge and information using digital media such as the internet, mobile phones, digital paper, digital TV. E-epidemiology also refers to the large-scale epidemiological studies that are increasingly conducted through distributed global collaborations enabled by the Internet." (40).

1.5.1 Internet surveys

In Sweden in year 2015, 93% of the population have access to the Internet and actually 91% use it (41). Ninety-two percent have access to a computer, 77% has a smartphone. However 200 000 Swedes who have access to Internet at home do not use it-lack of interest is given as the main cause (41).

The use of Internet surveys in research has dramatically changed the conditions for data collection and storage by offering better and quicker data sorting, filing and storage and less data entry errors but it has also introduced new problems. Sample bias as "the digital divide", and lower response rates have been two major concerns (42). The digital divide is the notion that responders in Internet surveys tend to be younger and better educated (42-44). Lower response rates in Internet surveys than other survey types are more debatable, as the response rates in all types of scientific surveys have generally been declining in the last decades (45, 46). In several recent comparisons on the data collection technologies there are no or very slight differences in the response rates between the different study types (Internet-, paper-, combination paper- and Internet studies) (42) (47).

1.5.2 The Internet and hearing tests

The need for more cost-efficient methods to measure hearing ability and offer intervention services at distant geographic locations as well as the wish to embrace new technologies as the Internet and computers have driven the demand/ need for tele-audiology (48) (49).

Today many commercial hearing aid companies offer a wide range of hearing tests on the Internet and on smartphones, however scientifically validated and completely self-administered Internet-based hearing tests are still as scarce today as they were when the planning of this thesis started in 2005-06. Within the clinic there are some scientifically evaluated tele-Internet-audiometry tests (50-52). However, these tests often require a simultaneous Internet and telephone-connected audiologist at the clinical location, and a computer, audiometer and telephone at the patient location, or special sound cards, or modules which still make them demanding in terms of personnel and other resources.

In later years several speech-in-noise tests for the Internet or telephone have emerged. Speech-in-noise tests are easier to adapt to the new technology. As the test result is given as a ratio between the signal and the underlying noise, the need for calibration is not a major concern. They have showed to be cost-efficient and quite accurate methods to assess the general hearing ability for speech (53) (54, 55). This type of tests are better than questionnaires for self-estimation of hearing ability (56), but they give little room for examining dB-differences in specific frequencies as would a regular audiometry test.

The current evolution of NIHL relies to a large extent on exact threshold estimates in the high frequency range. Speech-in-noise-tests are not sensitive enough to capture these small notches, indicative of an impending NIHL. Scientifically validated self-administered Internet-hearing tests are needed if the objective is to more precisely quantify smaller and frequency-specific hearing impairment in large scale epidemiological studies and/ or to quantify intra-individual changes. To at all be able to conduct hearing test investigations on a geographically widespread community at a reasonable cost, e.g. hunters, modern IT-technology is necessary. Apart from all other advantages it makes it possible to test participants in their homes, with electronic equipment that they already possess.

1.1.2 Validity and reliability

Before using a new test-technique, like a screening hearing-test on the Internet, it has to be thoroughly validated to assess the risk of measurement error. This is typically done by using a gold standard test to determine the accuracy of the new test. After dichotomization of the test results, both in the new test and in the gold standard test, into “sick”/ “not sick” and comparing the results it is possible to decide the sensitivity and specificity for the new test (57).

Sensitivity, also called the true positive rate measures the proportion of truly sick people that is correctly detected by the test as being sick. It should be close to 1. Specificity, also called the true negative rate measures the proportion of truly not sick people that is correctly detected as being not sick by the test. This should also be close to 1. The sensitivity and the specificity are relatively stable characteristics of the test, but they can change if the test-population is dramatically different from the population intended for the test concerning e.g. age, co-morbidity or tendency to seek medical advice (58).

To evaluate a new test's ability to predict the true disease status, given a certain result from the test in the tested population, the positive and negative predictive values can be calculated on the same data as the sensitivity and specificity. These values are highly dependent on the prevalence (also called the pre-test probability) of the tested disease in the test population. The predictive values help in determining if a diagnostic test should be used as a screening test or not in a certain population depending on the prevalence of the examined disease (57) (59).

Another important measure when evaluating a new test is the reliability. It is essentially a measure of random error in the test result. It is typically measured as the test's ability to reproduce the same result on the same individual when the person is re-tested (therefore sometimes called "repeatability"). The reliability is expressed as some coefficient of agreement; in the case of dichotomous outcomes, such as sick- not sick, the κ (kappa) statistic is most common. This should be close to 1, indicating high similarity of the test-retest results.

1.6 TOBACCO (CIGARETTES AND SNUS) AND HEARING

Twenty percent of the Swedish population in the ages 16-84, smoke tobacco daily or sporadically (only daily use is 9% among men, and 11% among women) according to the health survey done by the Public Health Agency of Sweden in 2015 (60). Daily Snus use (Swedish moist snuff, typically administered as quids behind the upper lip) among men is more common (19%), than among women (4%).

1.6.1 Cigarettes

Observational research has indicated that cigarette smoking aggravates hearing impairment following continuous noise (61-68). The smoking effect combined with exposure to continuous noise is seen in both high and low frequencies and with a dose-response relationship (64). The effect of smoking combined with exposure to impulse noise on hearing is less studied.

The cause of the potentiated effect of continuous noise on hearing by smoking is debated and probably multifactorial. Tobacco contains, apart from nicotine, different types of tobacco-specific nitrosamines, TSNAs (69) (70-72). TSNAs are mainly known as potent carcinogens (73), by damaging the cell's DNA(74) . This cytotoxic effect can be potentiated in cell cultures by free radicals (75). When smoking there is a production of carbon monoxide (CO), which has been shown to induce hearing loss in high doses (76). CO and nicotine are factors influencing the microcirculation in the organ of Corti, leading to a decrease of the local circulation (77) as well as affecting the formation of free radicals adding to the oxidative stress which is already caused by noise exposure (78). Moreover smoking causes a general inflammatory process in the body which also might involve the cochlea (78). However, human provocation experiments have revealed a smaller TTS upon noise exposure among smokers than among non-smokers after exposure noise but not after chewing a nicotine gum which had an opposite effect on non-smokers (larger TTS)(79). This apparent protective effect by smoking was thought to be attributable to (CO)(79). On the other hand profound and dose-dependent damages in guinea pigs' cochlear hair cells and in the supportive cells have been reported after subcutaneous injections of nicotine and examination with electron microscopy (80).

1.6.2 Snus

Snus use involves exposure to nearly the same chemicals as does tobacco smoking, with the important difference that there is no exposure to combustion products such as CO. The nicotine levels of normal use of snus is similar with normal use of cigarettes (81). The levels of TSNAs in Swedish snus is relatively low, as the snus is based on a low-nitrate tobacco and does not undergo any fermentation process (69). Snus use has been associated with higher blood pressure (82), and endothelial dysfunction (83) which indicates vascular changes, but there are studies that contradict these results (84) (85). To our knowledge there is no published research on the association between snus use and NIHL.

2 AIMS OF THE THESIS

- To evaluate a completely Internet based investigation package for hearing assessment consisting of a questionnaire and a hearing test, in regard of feasibility and face validity. (Study 1)

- To validate the Internet based hearing test with clinical audiometry as gold standard. (Study 2)

- To use the investigation package to study the association of shooting history (rifle calibers, hearing protection, and frequency of shots in total and shots without protection) in relation to the probability of having HFHI among Swedish hunters. (Study 3)

- To investigate differences between hunters with and without HFHI in regard to patterns of exposure to modifiable external factors. (Study 4)

- To test the hypothesis that tobacco use (in the form of cigarette smoking or snus use, or both) effect-modifies the association between a history of unprotected exposure to impulse noise from HRC weapons and probability of having HFHI. (Study 4)

3 MATERIALS AND METHODS

3.1 STUDY DESIGN

Studies 1, 3 and 4 all had the same study design; cross-sectional and completely Internet based. A homepage was constructed for the study. It contained information about the study and instructions for participants. It also contained an extensive questionnaire and an Internet-based audiometry test. In study 1, invitations were sent by regular mail to the participants together with the link to the study homepage, a personal username and a password. In studies 3 and 4 e-mailed invitations were sent with instructions about how to create your personal log-in on the study homepage.

Study 1 aimed at evaluating the feasibility of collecting epidemiological data on hearing ability with this completely Internet-based method among a selected group of Swedish hunters. The willingness to participate and possible reasons for non-participation, including technical obstacles were of special interest. The Internet-based hearing test was also evaluated in terms of comparing the test to a question on self-estimated hearing in the questionnaire.

Study 3 aimed at examining shooting history (rifle calibers, hearing protection, and frequency of shots in total and shots without protection) in relation to the probability of having HFHI. In this study we also examined the potential for selection bias due to non-participation, by sending out e-mails, with 3 questions, to invitees who had not responded or declined participation within 18 months after the initial invitation to participate in the study. Furthermore, the InternetAudio test was further validated under authentic conditions by those participants who had completed the test at home, lived in the vicinity to Stockholm, and had given an affirmative answer to a question in the questionnaire about their willingness to undergo a pure-tone clinical audiometry at the Karolinska University Hospital audiology department.

Study 4, aimed at investigating differences between hunters with and without HFHI in regard to patterns of exposure to modifiable external factors but also to test the hypothesis that tobacco use effect-modifies the association between a history of unprotected exposure to impulse noise from HRC weapons and probability of having HFHI. The last aim in this study was to investigate if the effect modification, if any, by tobacco smoking looks different from that caused by use of snus. Snus use entails exposure to essentially the same chemicals as does tobacco smoking (72), with the important difference that there is no exposure to combustion products.

Study 2 was a cross-sectional validation and repeatability study done using clinical pure-tone air-conducted audiograms as gold standard. The participants were both outpatients with suspected hearing impediments, who were referred for clinical audiometry to the Department of Audiology at the Karolinska University Hospital, Solna, and staff from the same hospital. The patients received a written invitation to the study, enclosed with the letter that confirmed the appointment. The hospital staff was contacted through verbal information and invitation letters handed out at staff meetings. Because the test is primarily designed for people in working ages, we restricted the validation study to individuals younger than 75 years.

3.1.1 Questionnaire

For the purpose of studies 1, 3 and 4 we constructed a questionnaire. The questions in the questionnaire were partly derived from:

- Questions on hearing ability and noise at drafting for military service. (86)
- Tinnitus Handicap inventory-THI. (87)
- H70 questionnaire. (88)
- Handicap and standard audiometric tests in elderly persons. (89)
- SCB:s ULF, undersökning av levnadsförhållanden; ULF 2005:1-2
- Hearing measurement scale. (90)

The questionnaire consisted of 143- 163 questions and subqueries, concerning sociodemographic characteristics, hunting habits, caliber use, use and type of hearing protection, number of shots with and without hearing protection, and self-estimated hearing. The questionnaire also covered possible confounding factors, e.g. exposure to noise at work, during military service or in connection with leisure-time activities, ear diseases, and other diseases possibly affecting hearing ability. We asked about regular use of anticoagulants and analgesics. We further asked about the use of cigarettes and snus. We asked of family histories of hearing impairment, including use of hearing aids. In study 1 there was also a section after the hearing test concerning technical questions on the computer, type of headphones and version of JAVA program. This part of the questionnaire was excluded in study 3 and 4.

To be able to proceed in the survey, all items had to be answered. (Appendix complete questionnaire).

3.1.2 The Hearing test

3.1.2.1 Development

Before the start of this Thesis work an inventory of the available internet-based hearing test was made. At the time, two Internet sites offered interesting examples of completely Internet-based hearing test. The one from www.digital-recordings.com, was evaluated compared to clinical audiometry, by parts of the early research group, in a small clinical test of 20 persons (data not shown). The test-results were promising, but the test needed several general improvements.

Performing a hearing test in the home environment by yourself with your own equipment, entails certain challenges:

- noise from computer fans, and constant background sounds,
- the headphones and theirs sound emitting properties,
- the performance of the computer sound cards,
- sudden noise which will disturb the test person,
- test failure due to inability to follow instructions on how to use the test.

The first 3 obstacles could be reduced by some sort of calibration, and biological calibration became the method of choice. The fourth obstacle was thought to be removed by letting the test person repeat the test as many times as wanted. The problem of failure to follow instructions is difficult to avoid, but efforts were made to make the instructions as clear as possible.

In order not to make the test too time-consuming, which could reduce the willingness to carry on with the test, the level of 60 dB SPL was set as the tested sound limit. This limit was also a consequence of the expected dynamic range from ordinary PC/ headphones, limited by their signal-to-noise ratio. However, our pre-studies had shown that even the most expensive commercial headphones vary in quality of frequency emission. Many of the headphones had “dead points” where the tone had reduced intensity or was distorted at specific frequencies (data not shown). This problem was reduced by using a frequency modulated sinus tone that is a slightly oscillating tone instead of a pure sinus tone, i.e. a narrow band noise centered on a sinus tone. In this way the drawback of poor frequency response of the headphones is reduced.

Before settling with these improvements the hearing test was again evaluated compared to clinical audiometry, in a small clinical test of 19 persons. It had now a specificity of 1 and sensitivity of 0.77 (data not shown). Further, less important improvements were made during the course of this thesis, but mainly before study 2. The final version of the Internet-based hearing test is called the InternetAudio test and is used in studies 3 and 4.

3.1.2.2 The InternetAudio test

The final hearing test which is used in study 3 and 4, is a JAVA program which is downloaded on the computer. The test person had to provide the headphones. “Biological calibration” is needed. The calibration is similar to the clinical zero decibel (dB)-value. It is done by a reference person, between the ages of 14 and 35 with subjective normal hearing. The reference person’s hearing level (RP-HL) is controlled in the calibration test. This calibration test measures the RP-HL within a 30 dB-level of the six tested frequencies, 0.5, 1, 2, 4, 6, 8 kilohertz (kHz). The reference person determines his/her HL with a volume marker on a ruler of 300 units on the computer screen. The tone at each frequency is presented to both ears simultaneously to get the RP-HL from the better ear, and the volume marker has to be adjusted to the level at which the tone is barely audible. The tone is a frequency modulated sinus tone- a slightly vibrating tone. Certain criteria have to be met in the calibration process for it to pass. (1) The RP-HL is not approved if the volume marker has not been moved at any frequency. (2) If the dB-values exceed a range of 15 dB SPL, or (3) if the volume marker has exactly the same dB-value on more than two frequencies. The latter is to rule out visual positioning of the marker. The calibration process is done twice, and if it has similar results it is accepted and the RP-HL is considered to be the zero-level of dB for this computer, headphones and environment. The actual hearing test of the test person can then proceed.

Six frequencies, 0.5,1,2,4,6,8 kHz, are tested. Tones are presented to each ear separately in random order and at random intervals varying between 4-6 seconds. This is to avoid presumption, and is in conformity with the recommendations in the Swedish methodology book for audiometric testing (91, 92). The sound level range between 0-60 dB SPL. The test person determines his/ her hearing level (TP-HL) by pressing the keyboard space bar when a tone is detected. If the tone is not heard, it is presented at a 10 dB higher level. If the tone is heard, it is presented at a 5dB lower level. To avoid ambiguity the space bar must be pressed within a 1 second time interval from when the tone is presented. If more than 10 responses are recorded outside these time limits, the test is automatically classified as being invalid and is not approved or saved on the website server. The test person is asked to do the test again.

The final and approved hearing result of the test person is the decibel difference between the RP-HL and the TP-HL, and is presented on the computer screen as an audiogram curve with a written explanation of how to interpret the results. The number of late/ early space-bar strokes is also presented. The test result (including the reference person's result) is saved on the web-site server. The total time consumption of the actual hearing test is approximately 15-25 minutes, depending on hearing ability.

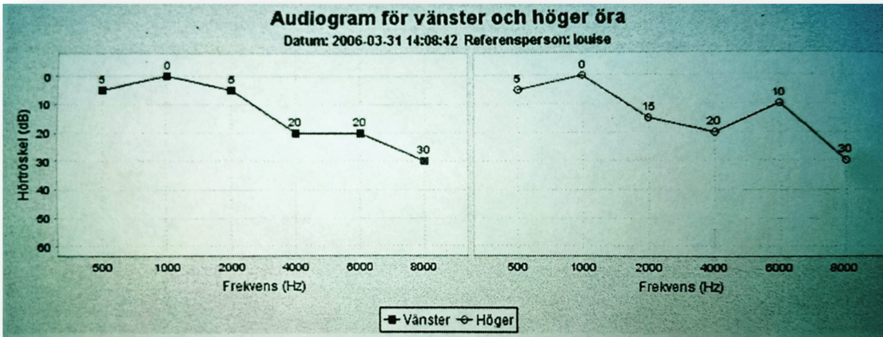

Fig. 4 InternetAudio test result as presented on the computer screen. Here with a mild bilateral hearing loss at 8 kHz.

In study 1 the Internet-based hearing test had the following differences compared to the Internet Audio test. First, the calibration process had no quality check and it was possible to proceed with the actual hearing test, even if the calibration process had failed. This calibration process was only done once. Second, the dB level of the next tone presented, when a tone was registered as not heard, was at a 6 dB higher level and when heard, at a 6 dB lower level, instead of 10 dB as in the other studies.

In the internet-based hearing test used in study 2, the calibration process was not done twice as in studies 3 and 4. The accepted number of late/ early space-bar strokes in study 2 was set to 5 (instead of 10 in studies 3 and 4), before the test was automatically classified as being invalid and not approved or saved.

3.2 STUDY GROUP

3.2.1 Study 1

An invitation letter was sent to 560 members of the Swedish Hunters' Association. Subjects were selected proportionally to the distribution among the members in terms of gender (men=500, women = 60) and age in the age group 20-60. The postal invitation included a description of the study, a personal log-in and password. The invitation also included a prepaid return letter where the participants could decline participation. This letter included a voluntary question about reason for non-participation. The data collection was closed after 6 months. Fig 5 presents the flow chart.

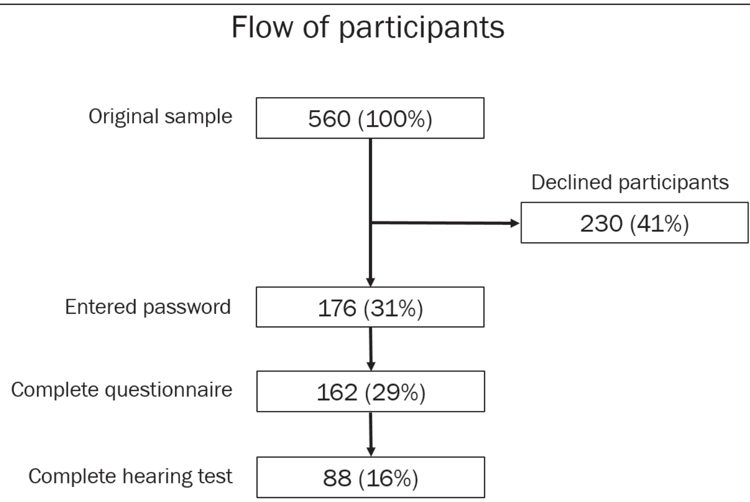

Fig 5. Flowchart of participation in the pilot study, for evaluating an Internet-based hearing test among 560 members of the Swedish Hunters' Association.

3.2.2 Study 2

Seventy-two patients and staff consented to undergo the validation test, 79 % were women, and, 96% were in the working ages between 19 and 65. For the reproducibility test, 12 additional persons, seven of the staff and five non-staff, were asked to do the Internet-based test twice with at least 30 minutes interval between. This test was done for each group in two different locations outside the clinic, with one reference person for each group. Of the 246 patients who were approached, 8% agreed to participate. Fifty-two (22%) of 240 invited staff members participated. Of the 72 patients and staff who consented to undergo the test, 79% (57 persons) were women, and 96% were in the working ages between 19 and 65 years

3.2.3 Studies 3 and 4

E-mail invitations, including 2 e-mail reminders were sent out to 27 063 e-mail addresses received from the Swedish hunters' association membership roster, 9123 did not lead to functioning e-mail accounts and bounced back, while 2119 reached individuals who actively declined participation. In all, 1937 persons started the survey, 1771 of them completed the questionnaire, and 203 also completed the InternetAudio test. One participant was excluded due to technical evidence suggesting invalid performance in the InternetAudio test, leaving 202 valid InternetAudio tests. In paper 3, 13 of the hunters also completed an additional clinical audiometry at Karolinska University Hospital for validation purposes. To examine possible selection bias due to nonparticipation, we sent an e-mail to all 17940 invitees who had not responded within 18 months after the initial invitation. The e-mail contained three simple questions about exposure and outcome. Figure 6 presents the flow-chart

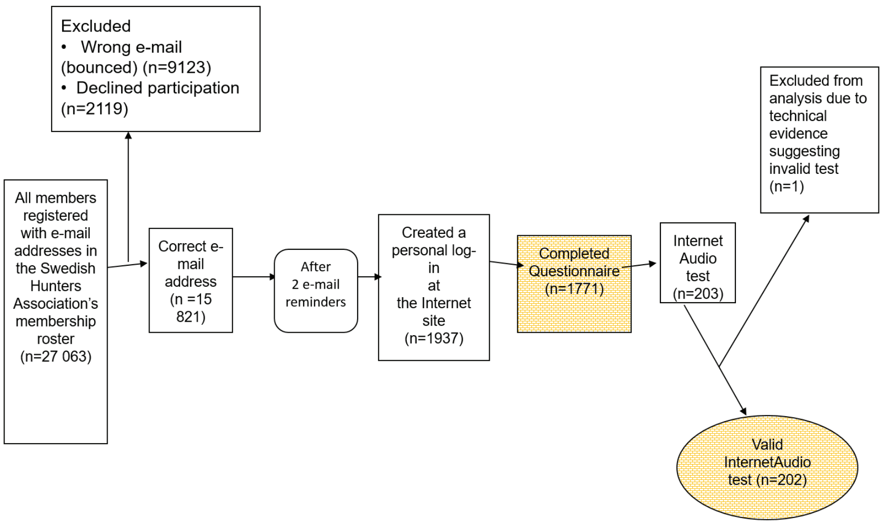

Fig 6. Flow-chart for study 3 and 4

3.3 DATA COLLECTION

3.3.1 Study 1

The audiometric data from the hearing test was in paper 1 classified as normal hearing, minor, moderate, and major hearing impairment according to the definition from WHO(37). Normal hearing is set as between 0 and 25 dB on all frequencies. The cut-off level for minor hearing impairment was one or more frequency-values in the range 26-40 dB, for moderate hearing impairment one or more frequency-values in the range 41-60 dB, and for major hearing impairment one or more frequency-values higher than 60 dB on either frequency (e.g. tone not heard). The result of the hearing test was compared to the self-estimated hearing ability question from the questionnaire prior to the hearing test. The self-assessed hearing status was categorized according to the response categories in the questionnaire (good, slightly reduced, severely reduced, don't want to answer). The analysis is based on the audiometric data from the best ear in the hearing test. The Internet based hearing test and the self-assessed hearing question (within parenthesis) were compared by using a contingency table presenting individuals categorized as normal (good) hearing, minor (slightly reduced), moderate(severely reduced) hearing.

Background data age and gender was provided from the Swedish Hunters' Association for comparison of non-responding invitees, invitees who actively declined participants and respondents. Full respondents were compared with participants who had answered the questionnaire but not completed the hearing test (questionnaire respondents) with regard to different background variables derived from the questionnaire, including age, gender, level of education and number of individuals in household. To evaluate the different technical steps, the full respondents were also compared with the questionnaire respondents by regard of whether or not they had headphones at their home prior to the test, if they had the correct version of JAVA installed on their computers and their willingness of giving their e-mail address for future contact.

3.3.2 Study 2

Of the participants, 89% preferred to do the Internet-based test at the hospital using the same reference person and the same locality. The reference person at the clinic was one of the employed technicians in the preferred age span, considered (and later on tested) with normal hearing. The internet Audio test was done in an office at the clinic. Ordinary headphones were used, bought in a computer store. The results of the calibrations of the Internet-based hearing test were all within defined limits. The clinical pure-tone audiometry, including air conduction thresholds at 0.125 – 8 kHz was performed in soundproof booths by licensed audiologists, unaware of the results of the alternative method. The pure-tone levels of the frequencies were determined in 5 dB steps utilizing the shortened ascending method, ISO 8253-1, Tyler, Wood, 1980 (93). THD 39 earphones were used.

The results of the Internet-based hearing test and the pure-tone audiogram were compared at each frequency. The overall results of the respective tests in the individual participant was further classified as "normal hearing" or "hearing impairment", according to a classification system for noise damage by Heijbel-Lidén, modified after Klockhoff (94). The cut-off level for "hearing impairment" in the frequencies 0.5 kHz, 1 kHz and 2 kHz was ≥25 dB in more than one frequency or ≥30dB in one frequency. The corresponding cut-off in the frequencies 4 kHz and 6 kHz was ≥45dB in either frequencies, or one frequency ≥50 dB. The 8 kHz frequency was not included in the sensitivity and specificity assessment, as hearing impairment affecting only 8 kHz does not interfere with verbal communication.

When negative dB HL values on the pure/tone audiometry, i.e. minus five or minus ten dB HL were obtained, we adjusted all negative dB values from the pure-tone audiograms to zero, as a negative hearing threshold has no practical/clinical consequence for the patient. The Internet-based hearing test cannot detect negative dB values. If, in the Internet-based hearing test, there was no measured response at a given frequency, an arbitrary level of 65 dB was given, as the hearing test does not test higher dB-values. This adjustment was also made in the clinical audiogram when the dB HL surpassed 60.

When a participant had done several approved Internet-based hearing tests we randomly chose one of them for the comparison with the pure-tone audiogram.

3.3.3 Study 3

The questionnaire consisted of 143 questions and subqueries, of which a part was analysed in this study.

3.3.3.1 Graded variables

Participants self-estimated the annual number of fired training shots and hunting shots separately per weapon type. Of particular interest was the number of shots fired without hearing protection in the preceding 5 years. Shots fired with hunting rifle calibers generate the highest dB SPL peak levels. The number of unprotected shots (categorized into 0, 1-6, and >6) with these weapons was the exposure of main interest in this study. This shot categorization was chosen in order to have reasonably equally-sized groups in the statistical analysis.

Self-assessed hearing status was categorized according to the response categories in the questionnaire (good, slightly reduced, severely reduced, don't want to answer), along with a special category for missing answers.

3.3.3.2 Dichotomous variables

Classified as exposed to “non-hunting-related noise” were participants who answered affirmatively to at least one of the following questions: if present or past work was believed to have damaged the hearing ability by noise exposure, or if hearing deteriorated during military service, or if military service or leisure activities had caused tinnitus. Only participants who indicated present use of cigarettes and/or smokeless tobacco were classified as “tobacco users”. Those who self-reported hypertension, previous myocardial infarction, or use of anticoagulants were categorized as positive for (CVD+). If there was a history of Meniére’s disease, sudden deafness, vestibular schwannoma, ear surgery, chronic otitis media, or ear disease as a child, the participant was classified as having “ear disease”. Similarly, the classification for “other disease” was positive if an affirmative answer was given in regard to either of a history of meningitis, migraine, head trauma, epilepsy, diabetes, joint disease, or cancer. If an affirmative answer was given in regard to the use of anticoagulants, painkillers or anti-inflammatory medication at least twice a week in the last month, the participant was classified as “anticoagulant/anti-inflammatory drug user”. When an affirmative answer was given to a question about any family members with a hearing impairment in need of hearing aid before 65 years of age, the participant was classified as having “a family history of hearing impairment”.

3.3.3.3 Measures of outcome

Our primary goal was to capture HFHI, and for this reason we only considered hearing levels for the frequencies 4 and 6 kHz, typically affected by severe acoustic trauma. RP-HL values ≥25 dB for either of these two frequencies on either ear were categorized as HFHI.

3.3.4 Study 4

Study 4 used the same graded and dichotomous variables and outcome measurements as study 3 with a few exceptions:

Tobacco use was the suspected effect-modifying factor. An affirmative answer to the question “do you smoke cigarettes daily? yes/ no/ don’t know”, rendered a classification as “cigarette user”. This class also included participants who reported smoking cessation in the preceding 5 years. Hunters who answered that they had never smoked or had stopped smoking longer than 5 years ago were classified as “cigarette non-users”. The same classification principles were used for snus use. Users of cigarettes, snus, or both were jointly categorized as “tobacco users”.

Because of small numbers in some cells in stratified analyses, the response alternatives to a question about use of hearing protection while hunting (always/ often/ sometimes/ rarely or never) were collapsed into always/often versus sometimes/rarely/never. The responses about the average number of hunting days (0/1-5/6-10/11-20/>20 days) were dichotomized into 0-20 days versus >20 days per year. Similarly, the responses to an open-ended question about the average number of shots overall per year (HRC, Magnum and small caliber weapons, during hunting or training and regardless of ear protection) were categorized as 0-50, 51-100 and >100. For shotguns the response alternatives were already given (0-100/101-300/301-500/ >500). Age, reported as a discrete numerical variable, was first categorized as 11-35, 36-55, 56-75, 76-91 years, but since its relation to HFHI was apparently linear, it was used as a continuous linear term in the final models.

3.4 STATISTICAL METHODS

All four studies have been evaluated in terms of descriptive statistics and calculations have been made using SAS software 9.1.3 (paper 1 and 2), SAS software, version 9.4 (paper 3 and 4). All tests of statistical hypotheses were made on the two-sided 5% level of significance.

3.4.1 Study 1

To test if the distribution of subjects across categories of the demographic variables between full compliers and questionnaire completers was equal and the Pearson Chi2 test was used to compare "full respondents" and "only questionnaire-respondents". This test was also used to calculate the statistical significance of the difference between the hearing test and the self-estimated hearing. Cohen's kappa statistics was used to test the agreement between the latter.

3.4.2 Study 2

To compare the Internet-based hearing test with the pure-tone audiogram Pearson's correlation coefficient was used to estimate agreement for numerical variables such as dB HL and Cohen's Kappa statistic for dichotomous variables such as hearing impairment versus no hearing impairment. The comparisons were made separately for the left and right ear.

3.4.3 Study 3

The probability of having HFHI was modeled as function of the exposure of unprotected gunshot blasts from hunting rifle calibers and the covariates age, sex, number of shots with no ear protection with, respectively, hunting rifle calibers, lighter hunting calibers and shotguns, non-hunting-related noise exposure, education level, tobacco use, CVD, ear disease and family history of hearing impairment. Prevalence ratios (PR) of HFHI associated with different exposure and covariate levels were estimated, as a consistent and conservative alternative to prevalence odds ratios (95). The actual model fit was performed via an approximate Poisson model with robust standard errors(96) which provides less biased estimates than logistic regression (97). The PRs reported were obtained as the exponentiated regression coefficients of the approximating Poisson model. To account for the binary outcome we used the robust sandwich estimator for modeling of the variance. Because of the dominant effect of age on hearing impairment, we performed an extensive goodness-of-fit analysis where we compared the model with a linear term for age to more complex models involving quadratic term, spline function or categorization of age. In the regression analysis we used a model with a linear term for age. In the smaller intra-study validation of the InternetAudio test in study 3 we did the same adjustments of the hearing data versus clinical audiometry as in study 2, and Pearson's correlation coefficient was calculated for each ear separately.

3.4.4 Study 4

In study 4 the same Poisson model as in study 3 is used but the covariates were restricted to age (as a discrete linear variable) and sex as none of the other covariates materially changed the PR of association. Interaction terms (various aspects of tobacco use and shooting noise exposure) were introduced, supplemented by corresponding stratified analyses.

4 ETHICAL CONSIDERATIONS

The regional ethical committe in Stockholm have approved all four studies. In all studies agreement to enrol in the study was considered informed consent, and all participants had the possibility to terminate participation and withdraw all individual data.

5 RESULTS

5.1 STUDY 1

After 3 reminders, 162 out of 560 (29%) had completed the questionnaire (questionnaire respondents), of whom 88 (16%) had completed the hearing test (full respondents). After reminders 1 and 2, 146 had actively declined participation, and an additional 84 declined participation during the telephone reminder (total 230, 41% of the total sample). There were 154 individuals who could not be reached or did not contact the study centre. Fourteen invitees entered the password without completing the study.
The distribution of gender was similar in all groups of respondents as in the original sample, declining invitees and the non-respondents. The full respondents were older than the non-respondents and the questionnaire respondents. The full respondents were not statistically significant different from the questionnaire respondents in terms of socio-demographic characteristics or self-estimated hearing prior the test. When looking at the technical attributes, full respondents were statistically significantly more likely to have headphones at home (p=0.003) and the correct JAVA version in their computers (p=0.007) compared to only questionnaire respondents
Table 3 lists the different reasons for declining participation.

Table 3. Reasons for declining participation in study 1. (n=230).

Reasons for declining	After paper reminders 1 and 2 n=146	After telephone reminder n=84	Total n=230
Have hearing impairment prior to the study	5 (3%)	1 (1%)	6 (3%)
Have no computer	7 (5%)	10 (12%)	17 (7%)
Have no headphones	27 (18%)	4 (5%)	31 (13%)
Have no reference	3 (2%)	1 (1%)	4 (2%)
Don't trust technique	3 (2%)		3 (1%)
Not interested	**62 (42%)**	**19 (23%)**	**81 (35%)**
Have no time	**13 (9%)**	**26 (31%)**	**39 (17%)**
No experience of hunting	17 (12%)	15 (18%)	32 (14%)
Computer problem	5 (3%)	8 (10%)	13 (7%)
Other	4 (3%)		4 (2%)

5.1.1 Hearing test

In total 126 Internet-based hearing tests were executed by 88 participants. When there were several tests done per person, the "best" test (i.e., smallest degree of hearing impairment) result was used in the analysis. After removal of tests with an incorrect reference, 61 internet-based hearing tests remained. Mean age was 45 years. Hearing impairment in the self-estimated hearing question was reported by 52% and 20% had a hearing impairment according to the Internet-based hearing test. The Chi-Square test shows this difference to be statistically significant ($P<0.001$). Six hunters (50%) of 12 hunters with hearing impairment according to the internet-based hearing test had classified their self-estimated hearing differently. After exclusion of severe hearing impairment the Cohen's kappa coefficient was calculated to 0.18 (95%CI 0.005-0.359), indicating low agreement between self-estimated hearing and the internet-based hearing test.

5.2 STUDY 2

5.2.1 Validity

Fifty-two of the participants (72%) had normal hearing according to the pure-tone audiogram performed at the clinic, while 20 (28%) had impaired hearing. Figure 5 shows a scatter-plot of the relationship between averages of the pure-tone HL across frequencies (0.5-8 kHz) obtained with the pure-tone audiogram (X-axis) and the corresponding averages attained with the Internet-based test (Y-axis) for each ear separately. The Pearson's correlation coefficient was 0.94 (p-value < 0.001) for the right ear and 0.93 for the left (p-value=0.001). In the regression of the pure-tone audiogram on the Internet-based test, the intercept (where the regression line meets the y-axis) for the right ear was -0.66 (95% CI -2.43, 1.10) and the slope 0.85 (95% CI 0.77, 0.92); for the left ear, the intercept was 0.00 (95% CI -1.56, 1.57) and the slope 0.84 (95% CI 0.77, 0.92).

In Figure 8, box-and-whisker diagrams depict, for each tested frequency, the distribution of intra-individual differences between the frequency-specific HL result (dB) of the Internet-based hearing test and the corresponding result of the clinical pure-tone audiogram. A negative value indicates that the audiogram dB HL value is higher than the one obtained with the Internet-based test, and positive value the opposite. For the four lowest frequencies, there was a general tendency toward negative differences (i.e., higher dB values in the pure-tone audiogram than in the Internet-based test), while the mean differences were close to zero for the two highest frequencies. Although the most

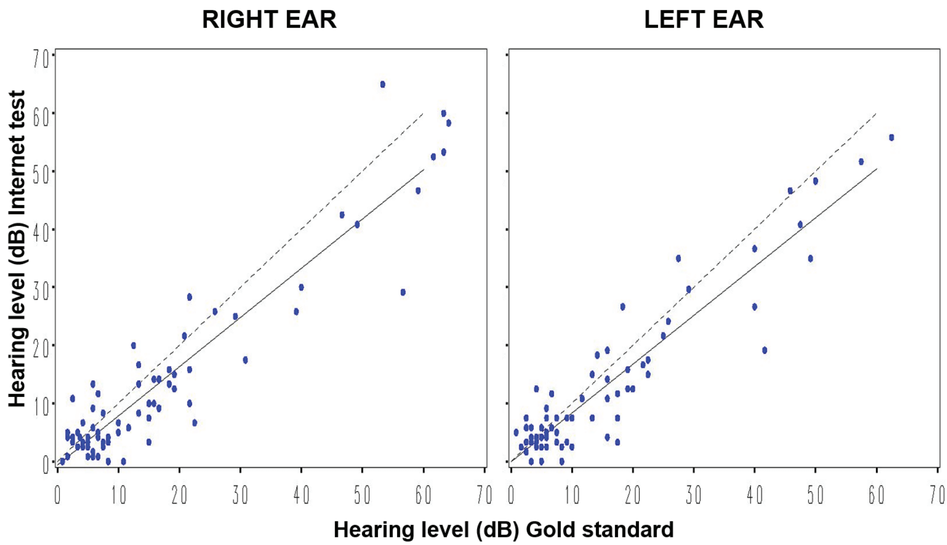

Fig 7. Pure-tone average (0.5-8 kHz). N=72.
Continuous line = regression line, dashed line= perfect concordance

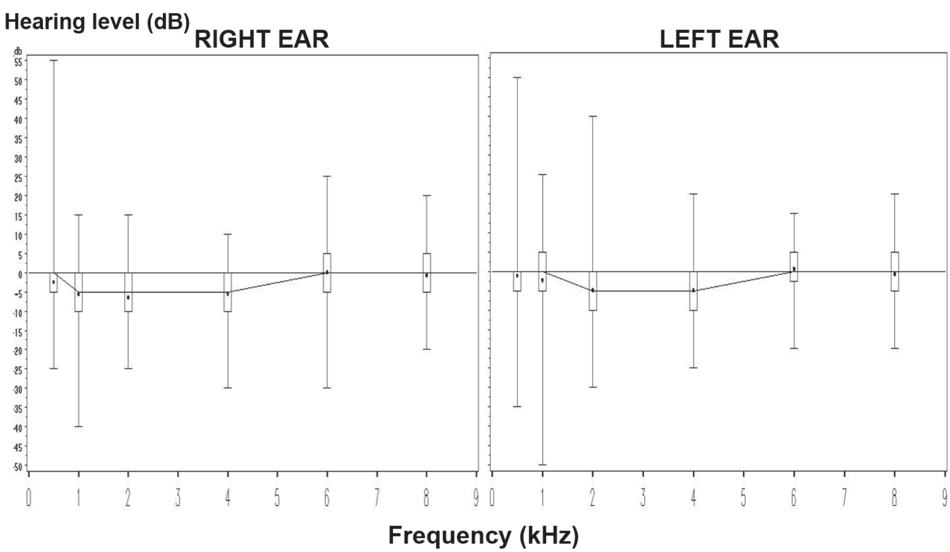

Fig 8. Distribution of intra-individual differences between the frequency-specific hearing-level result in dB, (y-axis) and tested frequency in kHz, (x-axis), of the Internet-based hearing test and the corresponding result of the clinical audiogram. The medians are aligned, dots=mean. N=72

extreme values (the ends of the whiskers) show vast deviations for single individuals, the mean differences were small; the greatest mean differences were seen for the frequencies 2 and 4 kHz, with, respectively, –5.6 dB (standard deviation, [SD] 8.29) and –5.1 dB (SD 6.9). The 25th percentiles of intra-individual differences (the lower borders of the boxes) did not exceed –10 dB (it was –7.5 dB at 25th percentiles) for any of the frequencies. In the 0.5 kHz frequency the 50th and the 75th percentile coincided due to many similar dB-values.

Using the definition of hearing impairment described in section of data collection, 24% of the subjects were classified as having a hearing impairment with the Internet-based and 28% with the pure-tone audiometry. The sensitivity was 75 percent (95%CI 51, 90), and the specificity was 96%, (95% CI 86, 99) (Table 4). The Cohen's Kappa comparing the Internet-based hearing test and the pure-tone audiogram done at the clinic was 0.75, (95% CI 0.57, 0.92) for the right ear, and 0.66, (95% CI 0.43, 0.89) for the left. This indicates good agreement between detecting hearing impairment with our Internet-based hearing test compared to a pure-tone audiogram.

Table 4. Sensitivity, specificity, with 95% confidence intervals of the Internet-based hearing test. Hearing classification according to a modification of the classification suggested by Klockhoff et al, 1973

	Clinical hearing impairment	Clinical normal hearing
Internet-based hearing impairment	15	2
Internet-based normal hearing	5	50
95%CI	Sensitivity = 75% (51-90)	Specificity =96% (86-99)

The eight individuals who conducted the Internet-based test at home were also analysed separately as to correlation between pure-tone average of all frequencies, and showed a Pearson correlation coefficient of 0.90 (p-value = 0.003) for the left ear, and 0.93 (p-value = 0.007) for the right ear.

5.2.1 Reproducibility/ test-retest reliability

The results of the 12 participants showed that the test-retest reproducibility was excellent, with a Pearson's correlation coefficient of 0.99 (p-value < 0.001) for both ears respectively. The Cohen's kappa of the overall test result (normal hearing versus hearing impairment was 1, (95% CI 1, 1) which means complete agreement, for both left and right ear.

5.3 STUDY 3

5.3.1 Responders

Men constituted the overwhelming majority among participants (Table 5). The youngest participant was 11 years and the oldest 91 years of age. Mean age was 52.9 (SD 14.4 years). The overall education level (51% with university education) was higher than in the Swedish population (25% in 2012) (98). Hearing impairments, both own and among relatives, were fairly common; only about one third self-reported "good hearing" (even less among those who completed the hearing test), and 11% reported severely reduced hearing.

Table 5. Characteristics of study 3 and 4 participants, who answered the questionnaire (n=1771), and the subset that completed the InternetAudio test (n=202).

		Questionnaire n/%	InternetAudio test n/%
Overall		1771 / 100	202 / 100
Sex			
	men	1610/ 91	177/ 88
	women	161/9	25/ 12
Age (years)			
	11-35	245/ 14	22/ 11
	36-47	366/ 21	51/ 25
	48-56	366/ 21	44/ 22
	57-65	385/ 22	46/ 23
	66-91	409/ 23	39/ 19
Place of residence			
	city	203/ 11	16/ 8
	suburb	189/ 11	20/ 10
	medium sized town	268/ 15	32/ 16
	municipality	383/ 22	35/ 17
	rural area	728/ 41	99/ 49
Highest education level			
	elementary school	225/ 13	35/ 17
	high school	648/ 37	70/ 35

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	university	898/ 51	97/ 48
Self-assessed auditory status			
	good	628/ 36	58/29
	slightly reduced	940 /53	121/ 60
	severely reduced	195/ 11	23/ 11
	don’t know	2 /1	0
	missing	6	0
Non-hunting related noise*			
	yes	1345 / 76	162/ 80
	no	426/ 24	40/20
A family history of impaired hearing			
	yes	477/ 27	46/ 23
	no	1134/ 64	136/ 67
	don’t know	149/ 8	20/ 10
	missing	11	0
Ear disease**			
	yes	328/ 19	29/ 14
	no	1443/ 81	173/ 86
History of CVD***			
	yes	500/ 28	52/ 26
	no	1271/ 72	150/ 74
Tobacco use			
	yes	418/ 24	47/ 23
	no	1353/ 76	155/ 77
Other disease****			
	yes	669/ 38	71/ 35
	no	1102/ 62	131/ 65
Anti-inflammatory/anticoagulant drug use			
	yes	446/ 25	47/ 23
	no	1325/ 75	155/ 77

* History of noise at work past or present, noise during army service, tinnitus after army service or tinnitus after leisure activities**. History of Menière’s, sudden deafness, vestibular schwannoma, ear surgery, chronic otitis media or ear disease as a child. ***History of myocardial infarction and/or hypertension and/or use of anticoagulants. ****History of meningitis, migraine, head-trauma, epilepsy, joint disease or cancer.

Hunting-related exposures among the 1771 participants who answered the questionnaire and the subset of 202 participants who completed the hearing test are shown in Table 6. The proportions of hunters who had never fired any unprotected shots were around 40% for both hunting rifle calibers and lighter hunting weapons. For shotguns, 58% in both groups had never fired any such shots. Between 36% and 45% in both groups had fired more than six unprotected shots with all types of weapons in the preceding five years. About one fifth of the participants never used hearing protection (Table 6), but among the 1443 who did, electronic level dependent earmuffs (electronic earmuffs) were the most popular (81%).

5.3.2 Non-responders

Of 3746 hunters who answered the three questions posed to non-responders, 42% indicated slightly reduced hearing and 7% severely reduced hearing; 42% had never fired any unprotected shots in the preceding year and 56 percent had fired more than 6 unprotected shots last year. This suggests that the study participants, on average, perceived their hearing ability as slightly worse than the invitees in general, but that their shooting pattern was not dramatically different.

5.3.3 Association between exposure to unprotected noise from hunting rifle caliber weapons and HFHI

Of the 202 participants who completed the InternetAudio test, 97 (48%) were found to have HFHI (Table 7). HFHI was significantly linked to exposure to unprotected shooting noise from hunting rifle caliber weapons in the preceding five years. The HFHI prevalence among participants with 1-6 reported such shots was 50% higher than among those with zero. However, among 89 hunters who reported more than 6 such shots, the prevalence of HFHI was essentially the same as in the reference category with no unprotected shots. Thus, there was no consistent dose-response relationship between the accumulated audio trauma over time and HFHI prevalence. While age was strongly associated with HFHI prevalence (50% increase in HFHI prevalence with each 10-year age increment), age was not linked to the exposure to unprotected noise from hunting rifle caliber weapons (data not shown). Hence, age did not confound the crude association between unprotected hunting rifle caliber noise exposure and HFHI prevalence. Moreover, further multivariable adjustments, as described in a footnote of Table 7, did not materially change the observed crude prevalence ratios, despite suggested crude associations between HFHI and several of the covariates (a reported previous history ear disease, CVD, non-hunting-related noise exposure, highest attained education, and tobacco use – data not shown). Of note, for all investigated calibers, the reported average total yearly number of shots (of which the overwhelming majority were fired with ear protection in use) was unrelated to the HFHI prevalence (data not shown), and this aspect of the shooting habits did not confound the observed association between exposure to unprotected shooting noise from hunting rifle calibers and prevalence of HFHI.

Table 6. Hunting-related characteristics of study 3 and 4 participants; who answered the questionnaire (n=1771), and the subset that completed the InternetAudio test (n=202).

		Questionnaire n/%	InternetAudio test n/%
Overall		1771 / 100	202/ 100
Number of shots without protection past 5 years, per caliber			
	hunting rifle calibers		
	0	733/ 41	96/ 48
	1-6	253/ 14	22/ 11
	>6	785/ 45	84/ 42
	lighter hunting weapons		
	0	655/ 37	69/ 34
	1-6	361/ 20	44/ 22
	>6	755/ 43	89/ 44
	shotguns		
	0	1023/ 58	117/ 58
	1-6	116/ 7	11/ 5
	>6	632/ 36	74/37
Number of shots in total on average per year, per caliber			
	hunting rifle calibers		
	0-50	50/ 3	4 / 2
	51-100	445/ 25	47/ 23
	101-200	422/ 24	48/ 24
	>200	854/ 48	103/ 51
	lighter rifle calibers		
	0	543/ 31	60/ 30
	1-90	495/ 28	49/ 24
	91-400	467/ 27	55/ 27
	>400	266/ 15	38/ 19

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	shotguns		
	0-100	1600/ 90	185/ 92
	101-300	118/ 7	12/ 6
	301-500	24/ 1	2/ 1
	>500	29/ 2	3/ 1
Number of hunting days per year			
	0	18/ 1	2/ 1
	1-5	94/ 5	10/ 5
	6-10	273/ 15	37/ 18
	11-20	457/ 26	45/ 22
	>20	929/ 53	108/ 53
Ear protection use during hunting			
	always	686/ 39	70/ 35
	often	372/ 21	39/ 19
	sometimes	227/ 13	26/ 13
	rarely	158/ 9	25/ 12
	never	328/19	42/21
Type of ear protection used during hunting			
	earplugs	87/ n. a	12/ n. a
	earmuffs	78/ n. a	6/ n. a
	electronic earmuffs	1171/ n. a	134/ n. a
	electronic earplugs	107/ n. a	8/ n. a

Table 7. Associations in study 3, expressed as Prevalence ratios, between exposures of main interest (unprotected shots with, respectively, hunting rifle calibers, lighter rifle calibers, and shotguns) and the probability of HFHI (a hearing impairment ≥25 dB on 4 and/ or 6 kHz). The analysis is restricted to participants who completed the InternetAudio test (n=202).

		Prevalence of HFHI in category n/N (%)	Prevalence ratio with 95% confidence interval (within parentheses)					
			Crude	p-value	Age and sex adjusted	p-value	Fully adjusted*	p-value
Overall		97/202 (48)						
Number of unprotected shots in the preceding 5 years per caliber								
	h**unting rifle calibers**							
	0	30/69 (43)	1(reference)		1(reference)		1(reference)	
	1-6	28/44 (64)	**1.5 (1.0-2.1)**	**0.03**	**1.5 (1.1-2.1)**	**0.02**	**1.5 (1.0-2.2)**	**0.05**
	>6	39/89 (44)	1.0 (0.7-1.4)	0.97	1.1 (0.8-1.4)	0.73	0.9(0,6-1,3)	0.67
	lighter rifle calibers							
	0	59/117 (50)	1(reference)		1 (reference)		1 (reference)	
	1-6	7/11 (64)	1.3 (0.8-2.0)	0.34	1.5 (1.0-2.3)	0.07	1.2 (0.7-2.1)	0.43
	>6	31/74 (42)	0.8 (0.6-1.1)	0.26	0.9 (0.7-1.2)	0.47	0.9 (0.7-1.2)	0.51
	shotguns							
	0	41/96 (43)	1 (reference)		1 (reference)		1 (reference)	
	1-6	10/22 (45)	1.1 (0.6-1.8)	0.81	1.3 (0.8-2.0)	0.28	1.1 ((0.7-1.9)	0.65
	>6	46/84 (55)	1.3 (0.9-1.7)	0.11	1.1 (0.9-1.5)	0.37	1.3 (0.9-1.9)	0.11
Age(continuous)								
	per 10 yrs		**1.5 (1.4-1.7)**	**<0.01**	**1.5 (1.4-1.7)****	**<0.01**	**1.6(1.4-1.8)****	**<0.01**

*Poisson model included age, sex, number of shots with no ear protection with, respectively, hunting rifle calibers, lighter hunting calibers and shotguns, non-hunting-related noise exposure, education level, tobacco use, CVD (history of myocardial infarction and/or hypertension and/or use of anticoagulants), ear disease and family history of hearing impairment.

** Not age adjusted

5.3.4 Associations between exposure to unprotected noise from rifles with other calibers and HFHI

Crude associations between HFHI prevalence and unprotected shooting noise from rifles with other calibers were, at most, only suggested (Table 7). Adjustments for age resulted in moderate changes of the HFHI prevalence ratio estimates, but in the multivariately adjusted full model, which included adjustment for exposure to hunting rifle caliber noise, the associations were statistically non-significant.

5.4 STUDY 4

Table 8 compares the distributions of shooting-related, other noise-related, medical, and socio-demographic exposures among hunters with and without HFHI. The Table is stratified according to whether or not the hunters had been exposed to the unprotected sound blast from at least one shot with an HRC weapon in the preceding 5 years. Within each stratum, age-adjusted PRs express the crude association between each exposure and HFHI. While the only truly conspicuous difference between hunters with and without HFHI was markedly differing age distributions, seen in both strata, few other statistically significant differences emerged.

Among hunters exposed to unprotected noise from HRC weapons, a much larger proportion of those with HFHI than of those without had only fired 1-6 such shots. The former were also more inclined to use ear protection during hunting than the latter. Among those who had apparently endured at least one unprotected sound blast from an HRC weapon without HFHI, we observed a somewhat unexpected accumulation of hunters with high school (9-12 years) as highest attained education, and a corresponding deficit of people with a university education.

Of the hunters who had not been exposed to the unprotected noise from HRC weapons in the past 5 years, those with HFHI were significantly more likely to report many (>20) hunting days per year and to wear ear protection less often during hunting than did those without HFHI. The unexposed hunters with HFHI did also report the existence of a relative with hearing impairment significantly more often than the unexposed hunters without HFHI. In neither of the groups did we observe any direct association between smoking and prevalence of HFHI.

Table 8. Distributions of exposures in study 4 – both related to shooting noise and to other factors suspected of being related to hearing loss – among hunters with and without high-frequency hearing impairment (HFHI), along with prevalence ratios (PR) and 95% confidence intervals (CI) as measures of age-adjusted associations between the exposures and HFHI. The analysis is stratified according to the main exposure, namely having fired at least one shot in the preceding 5 years with a hunting rifle caliber weapon without ear protection. N=202.

		Not exposed to the unprotected sound-blast from a shot with a hunting rifle caliber weapon in the preceding 5 years (n= 69)				**Exposed to at least one unprotected sound-blast from a hunting rifle caliber weapon in the preceding 5 years (n= 133)**			
		HFHI n/ %†	No HFHI n/ %†	Age-adjusted PR (95%CI)	p-value	HFHI n/ %†	No HFHI n/ %†	Age-adjusted PR (95%CI)	p- value
Overall	202	30	39			67	66		
Number of unprotected shots with hunting rifle caliber in the preceding 5 years									
	0	30/100	39/100	n.a.		0	0	n.a.	
	1-6	0	0			28/42	16/24	**1.4 (1.1-1.9)**	**0.02**
	>6	0	0			39/58	50/76	1 (reference)	
Total number of shots, hunting and training, on average per year with hunting rifle calibers regardless of ear protection									
	0-50	12/40	16/39	0.7 (0.5-1.2)	0.23	10/15	8/12	1.1 (0.7-1.5)	0.78
	51-100	7/23	12/31	0.7 (0.3-1.3)	0.21	17/25	11/17	1.1 (0.8-1.6)	0.51
	> 100	11/37	11/28	1 (reference)		40/60	47/71	1 (reference)	
Number of hunting days per year									
	0-20	18/60	29/74	**0.5 (0.3-0.8)**	**<0.01**	28/42	19/29	1.1 (0.8-1.5)	0.62
	>20	12/40	10/26	1 (reference)		39/58	47/71	1 (reference)	
Ear protection use during hunting									
	always/often	24/80	37/95	1 (reference)		27/40	21/32	1 (reference)	
	sometimes/rarely/never	6/20	2/5	**1.6 (1.1-2.5)**	**0.02**	40/60	45/68	**0.7 (0.5-0.9)**	**0.02**
Tobacco use*									
	yes	5/17	13/33	1.0 (0.4-2.2)	0.97	16/24	27/41	1.0 (0.6-1.5)	1.0
	no	25/83	26/67	1 (reference)		51/76	39/59	1 (reference)	
Sex									
	men	25/83	30/77	1 (reference)		63/95	59/89	1 (reference)	
	women	5/17	9/23	0.8 (0.4-1.7)	0.62	4/75	7/11	1.1 (0.5-2.5)	0.82
Age categories (not age-adjusted)									
	11-35	1/3	7/18	0.2 (0.0-1.1)	0.06	2/3	12/18	**0.2 (0.1-0.7)**	**0.02**
	36-55	4/13	23/59	**0.2 (0.1-0.5)**	**<0.01**	23/34	40/61	**0.5 (0.3-0.7)**	**<0.01**
	56-75	22/73	8/21	1 (reference)		38/57	14/21	1 (reference)	
	76-91	3/10	1/3	1.0 (0.6-1.9)	0.94	4/6	0	**1.4 (1.2-1.6)**	**< 0.01**
Non-hunting related noise**									
	yes	22/73	34/87	1.0 (0.6-1.7)	0.88	49/73	57/86	1.0 (0.7-1.4)	0.92
	no	8/27	5/13	1 (reference)		18/27	9/14	1 (reference)	
Ear disease***									
	yes	5/17	9/23	1.2 (0.6-2.5)	0.53	4/6	11/17	0.8 (0.3-1.7)	0.48
	no	25/83	30/77	1 (reference)		63/94	55/83	1 (reference)	
CVD^									
	yes	15/50	8/21	0.9 (0.5-1.6)	0.72	19/28	8/12	1.0 (0.7-1.3)	0.86
	no	15/50	31/79	1 (reference)		48/72	58/88	1 (reference)	
Other disease^^									
	yes	8/27	6/15	1.2 (0.8-1.9)	0.42	20/30	26/39	0.8 (0.5-1.1)	0.21
	no	22/73	33/85	1 (reference)		47/70	40/61	1 (reference)	
Family history of impaired hearing									
	yes	12/40	6/15	**1.8 (1.1-2.9)**	**0.02**	13/19	15/23	0.9 (0.6-1.4)	0.65
	no	16/53	29/75	1 (reference)		48/72	43/65	1 (reference)	
	don't know	2/7	4/10			6/9	8/12		
Place of residence									
	city	4/13	6/15	1.3 (0.6-2.7)	0.47	2/3	4/6	0.8 (0.3-2.3)	0.63
	suburb	6/20	2/5	1.2 (0.6-2.2)	0.67	5/ 7	7/11	0.9 (0.4-1.7)	0.69
	medium sized town	4/13	10/26	0.7 (0.3-1.5)	0.39	11/16	7/11	1.0 (0.7-1.4)	0.80
	municipality	6/20	3/8	1.4 (0.8-2.9)	0.28	14/21	12/18	0.9 (0.6-1.3)	0.59
	rural area	10/34	18/46	1 (reference)		35/52	36/55	1 (reference)	
Highest education level									
	primary school	5/17	7/18	0.7 (0.3-1.3)	0.25	17/25	6/9	0.9 (0.6-1.2)	0.44
	high school	10/33	9/23	1.5 (0.9-2.5)	0.10	13/21	37/56	**0.6 (0.4-1.0)**	**0.03**
	university	15/50	23/59	1 (reference)		36/54	23/35	1 (reference)	

†Column % *Use of cigarettes or snuff or both currently or in the preceding 5 yrs. **History of noise at work past or present, noise during army service, tinnitus after army service or tinnitus after leisure activities. *** History of Menière's, sudden deafness, vestibular schwannoma, ear surgery, chronic otitis media or ear disease as a child. ^History of myocardial infarction and/or hypertension and/or use of anti-coagulants. ^^ History of meningitis, migraine, head trauma, epilepsy, joint disease or cancer.

Table 9 particularizes the distributions of relevant exposure variables in categories of tobacco use. Eighty-four hunters had stopped using tobacco (60 cigarettes, 24 snus) more than 5 years ago and were thus classified as non-users. Additionally, 18 went from cigarettes to snus, 1 went from snus to cigarettes. Of the 19 current cigarette smokers, 5 also used snus daily. The prevalence of snus use was more than twice as high as that of cigarette smoking, but the age distributions were similar in the two groups. Compared to non-users, tobacco users tended to be younger (mean age 46.2 versus 55.5 years, $p< 0.001$), fire more shots with HRC weapons (both unprotected [$p=0.07$]) and overall [$p=0.02$]), and to report more hunting days ($p<0.001$), compared to non-users. There were no important differences between the groups in regard to reported frequency of hearing protection utilization during hunting, nor was there any difference in type of protection; 86% in both groups used electronic earmuffs (data not shown).

Table 9. Distributions of shooting-related exposure variables as well as sex and age in categories of tobacco use

		No tobacco use n/%†	Tobacco use		
			Cigarettes n/%†	Snuff n/%†	Any tobacco use (cigarettes and/or snuff) n /%†
Overall	202	141/100	19/100	47/100	61*/100
Exposure to shooting noise from hunting rifle caliber weapons					
Number of **unprotected** HRC shots in the preceding 5 years					
	0	51/36	9/47	10/21	18/29
	1-6	35/25	1/5	9/19	9/15
	>6	55/39	9/47	28/60	34/56
Total number of HRC shots (hunting and training), on average per year in the preceding 5 years, regardless of hearing protection preceding 5 years, regardless of hearing protection					
	0-50	39/28	4/21	3/6	7/12
	51-100	34/24	3/16	10/21	13/21
	>100	68/48	12/63	34/72	41/67
Number of hunting days per year					
	0-20	78/55	6/32	10/21	16/26
	>20	63/45	13/68	37/79	45/74
Ear protection use during hunting					
	always/ often	77/55	12/63	22/47	32/52
	sometimes/ rarely/ never	64/45	7/37	25/53	29/48
Sex					
	Men	123/87	14/74	45/96	54/89
	Women	18/13	5/26	2/4	7/11
Age categories					
	11-35	14/10	2/11	7/15	8/13
	36-55	52/37	12/63	29/62	38/62
	56-75	67/47	5/26	11/23	15/25
	76-91	8/6	0	0	0

†Column % *5 hunters used both cigarettes and snuff

Table 10 addresses effect modification, by tobacco use, of the PR relationship between self-reported unprotected exposure to the sound blast from an HRC weapon in the preceding 5 years and the probability of having HFHI. Without any stratification for tobacco use, the age- and sex-adjusted PR was 1.5 (1.0-2.1; p=0.02), in the 1-6 unprotected shots category, relative to the unexposed reference category (study 3). Stratification according to any tobacco use (upper third of the table) disclosed a lower PR estimate (1.3) among non-users of tobacco, but a considerably higher estimate (3.2) among tobacco users, however based on only 9 hunters in the exposed category. Despite the small sample size, the interaction term attained statistical significance (p=0.01). Our attempt to estimate effect modification by cigarette use and snus use separately (lower two thirds of the Table 10) was hampered by small numbers. Snus use was associated with a higher PR point estimate (2.3) among users than among non-users (1.3), but the snus * shooting interaction term was non-significant (p=0.09). The PR estimate for the 1-6 unprotected shots category in the cigarette smoking stratum was based on only one exposed hunter, prohibiting any meaningful statistical inference. A supplementary analysis of the possible effect modification by snus, excluding the 19 smokers (current and/or stopped smoking in the preceding 5 years), somewhat reinforced the difference between the non-user and user strata; the PR estimate for the 1-6 unprotected shots category in the non-user stratum was 1.3 (95% CI 0.9-1.9) and in the user stratum it was 2.7 (95% CI 1.1-6.7) (p for the interaction term=0.05) (data not shown).

Table 10. Relative risks (prevalence ratios) of having HFHI (a hearing impairment ≥25 dB on 4 and/ or 6 kHz) by hunting noise exposure status (unprotected sound blast from hunting rifle caliber weapons in the preceding 5 years), stratified according to self-reported tobacco use.

			Hunters with HFHI in category n / %	Hunters without HFHI in category n/ %	Prevalence ratio with 95% confidence interval (within parentheses): Age and sex adjusted	p-value	p-value for interaction term
Overall		202	97	105			
Any tobacco use							
	No	141	76/54	65/46			
		Number of unprotected shots with hunting rifle caliber in the preceding 5 years					
		0	25/49	26/51	1(reference)		
		1-6	21/60	14/40	1.3 (0.9-1.8)	0.18	
		>6	30/56	25/45	1.1 (0.8-1.5)	0.57	
							0.01
	Yes	61	21/ 34	40/66			
		Number of unprotected shots with hunting rifle caliber in the preceding 5 years					
		0	5/28	13/72	1(reference)		
		1-6	7/78	2/22	**3.2 (1.4-6.7)**	**<0.01**	
		>6	9/26	25/74	1.0 (0.4-2.3)	0.95	
Cigarettes							
	No	183	92/50	91/50			
		Number of unprotected shots with hunting rifle caliber in the preceding 5 years					
		0	28/47	32/53	1(reference)		
		1-6	27/63	16/37	**1.4 (1.0-2.0)**	**0.03**	
		>6	37/46	43/54	1.1 (0.8-1.4)	0.69	
							0.04
	Yes	19	5/26	14/74			
		Number of unprotected shots with hunting rifle caliber in the preceding 5 years					
		0	2/22	7/78	1(reference)		
		1-6	1/100	0	4.2*		
		>6	2/22	7/78	0.9 (0.2-4.8)	0.91	
Snus							
	No	155	77/49	78/50			
		Number of unprotected shots with hunting rifle caliber in the preceding 5 years					
		0	26/44	33/56	1(reference)		
		1-6	21/60	14/40	1.3 (0.9-1.9)	0.11	
		>6	30/50	31/51	1.1 (0.8-1.5)	0.63	
							0.09
	Yes	47	20/43	27/57			
		Number of unprotected shots with hunting rifle caliber in the preceding 5 years					
		0	4/40	6/60	1(reference)		
		1-6	7/78	2/22	**2.3 (1.0-5.0)**	**0.04**	
		>6	9/32	19/68	0.9 (0.4-2.0)	0.78	

*Too few a cases to estimate CI or p-value.

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Causal interference

An observed association between an exposure and an outcome is not equivalent to causality for the outcome. First the association can be prone to different types of errors, random or systematic (bias). Errors interfere with the probability that the observed association is due to a true association in the studied population and not to chance or bias. Second, even if chance and bias have been ruled out and the association truly exists in the population, the attribution of causality may be incorrect due to confounding or reverse causation.

6.1.1.1 Random errors and precision

Random error are variability in the data which are caused by unknown factors in the measuring equipment, the individual, or the environment. They affect the precision of the study. Precision can be elevated by increasing the study sample size, and be reduced if the study sample is small -as when there is a low response rate in a survey. The response rate is a problem in study1, 3 and 4. Precision will also decline if the study is unbalanced, with very few observations in some subgroups, e.g. only few of the participants exposed. The precision is reflected by width of the confidence intervals of the measures of exposure. The 95% CI represents the range within which the true value lies with 95% probability. Wide CI: s indicate low precision, and the opposite with narrow CI:s.

Random errors can be reduced by using better instruments with less variation, and/or by collecting more data.

6.1.1.2 Systematic errors and validity

Systematic errors affect the internal and external study validity. The internal validity is high if the observed association between the exposure and the outcome faithfully represents the true association in the target population and thus is unaffected by systematic errors.

The external validity concerns the generalizability of the results from the study; if it is reasonable to apply the results to other populations.
Systematic errors comprise the two main types (a)selection bias and (b) information bias but also include erroneous attribution of causality through (c) confounding and (d) reverse causality(58). Some systematic errors can be

prevented through a rigorous study design, while others, notably confounding can be eliminated or reduced in the statistical analysis if they are expected and the appropriate covariate data have been accurately collected. Although the cross-sectional design in study 1, 3 and 4 is open to various confounding, the questionnaire used in this thesis includes 143-163 questions and subqueries concerning a wide array of possible confounding factors that are an important part of the statistical analysis to reduce and prevent systematic errors.

6.1.1.2.1 Selection bias

Selection bias is a systematic error and occurs if the inclinations to participate in the study/ refuse participation/ drop out, is determined jointly by the studied exposure and the studied outcome (99). Similarly, stratification on any variable that is under the influence of both the exposure (or a cause of the exposure) and the outcome (or a cause of the outcome) will also result in selection bias. Further, a distorted magnitude of the exposure-outcome association may occur if the inclination to participate is determined by the outcome alone (or a cause of it), but if an association is indeed observed, it is very likely to exist also in the target population, but not necessarily with the same strength. If these links with the reasons for non-participation do not exist, non-participation – even if it is substantial – will not entail any selection bias.

In e-epidemiology, with electronic communication via the Internet, and where the only contact information is the e-mail address, a new aspect of selection is introduced - Who were reached by the invitations and who had the required equipment and the know how to participate? With an internet access as high as 93 % in Sweden in 2015 (41), the prerequisites for successful contact are better than in most other countries in the world.

However, when launching the e-mail invitations in studies 3 and 4 the first question - "who were reached by the invitations?" showed to become a major set-back. As much as one third of the e-mail invitations bounced back because of low accuracy in the e-mail address- databases of the Swedish Hunters Association. This in turn activated various spam-filters which also stopped invitations with the correct addresses. Furthermore, in study 1, where regular postal invitations were used, the main reason for non-participation was lack of time or interest.

Full participation in this type of study admittedly demand both motivation and time. To participate several different steps had to be passed- completing an extensive questionnaire, installing the correct JAVA version on the PC, acquire headphones, find a normal hearing individual who could perform the calibration-test, and then finally execute the hearing-test. The low response rate was not a surprise, and in study 3 and 4, the successive attrition between entry into the study (n=1937) and the successful completion of the hearing test (n=202) clearly demonstrates just how demanding the study procedure was.

In general the Internet-users are alleged to be younger, wealthier, and better educated. This phenomenon is called the “digital divide”(44) and is perhaps a transient phase before the technology has become fully disseminated in the population, such as in Sweden today. In study 1, our data indicated that “full-responders” (both the questionnaire and the internet hearing test) were not only slightly older but more well-equipped in terms of computer technology compared to only questionnaire-responders. This result does in part contradict the digital divide at least in terms of age. In study 3 and 4 our responders exhibited a higher average education level than the Swedish population (98), which again support the “digital divide” theory, but it is also possible that people who engage in sports hunting – a rather expensive and exclusive hobby – may be better off than the average Swede. It is important to point out that our target population was hunters, not the general Swedish population.

In study 3 and 4 data showed no important differences between responders who only answered the questionnaire and those who also successfully carried out the InternetAudio test However responders in general indicated slightly more problems with their hearing ability, but only a marginally higher incidence of unprotected shooting compared to the non-responders who answered our simple e-mail questionnaire with 3 questions. This seems to indicate that the outcome (or a cause of the outcome) may have influenced the decisions to participate, while any important influence from the exposure seems less likely. Therefore, the non-participation in study 3 and 4 is unlikely to have caused full-fledged selection bias, whereas some distortion of our indices of association between exposure and outcome might have occurred due to the link between the outcome and non-participation. Since the latter link was rather weak, the distortion was probably moderate, but it must be emphasized that the proportion of all non-responders who provided this information was small.

In study 4, the prevalence of cigarette smoking and snus use among participants was well in line with Swedish statistics (100)

Regardless of whether or not non-participation has introduced selection bias, the low participation rate in all the studies and partial non-response pose a threat to the external validity of our results, i.e., the generalizability to other populations. The more distinct the remaining participant group, the more caution is needed when the results are to be translated to other populations and contexts. And it seems wise to admit that the possibility of selection bias is an important caveat in study 1, 3 and 4.

6.1.1.2.2 Information bias

Information/observation errors (misclassification) arise in the collection of information from the subjects once they have entered the study, either because of incorrect reporting, misuse of equipment or equipment error (101). If the error/misclassification is differential, information bias might arise.

Differential misclassification of the exposure is when this misclassification is affected by the outcome, and differential misclassification of the outcome is when this misclassification is affected by the exposure. In our case the cross-sectional design, where the outcome has already occurred when we ask about the exposure of interest, differential misclassification – both of the exposure and the outcome - is a realistic concern. For instance, a hunter with hearing impairment probably tends to remember the past number of unprotected shots (exposure) better than a normal hearing hunter. Or when concerning misclassified outcome- if a hunter with many hunting days and many fired shots per year asks a fellow hunter with equally bad hearing to be his/her reference person in the InternetAudio test. The calibration criteria in the InternetAudio test are designed to reduce this risk, as well as the information to the participant of using a normal hearing reference person in the calibration process. Even so the results from the hearing tests must be interpreted with caution. Differential misclassification can either exaggerate or underestimate the association between exposure and outcome.

Non-differential misclassification is not related to the outcome or exposure (of e.g. number of shots per caliber, use of hearing protection, and number of hunting days). It occurs randomly and does not affect the association between exposure and outcome, but might dilute the estimated effect (101).

6.1.1.2.3 Erroneous attribution of causality

6.1.1.2.3.1 Reverse causation

Reverse causation is a bias that concerns the interpretation of an observed association between two variables. If other biases such as confounding (see below) are excluded, one must assume that one variable (referred to as the exposure) has affected the other (referred to as the outcome), or in other words, that A has affected B. But a similar association would be seen if B had affected A. If the latter scenario would be true, and the association is misjudged to represent an effect of A on B, this represents reverse causation. This is probably seen in study 3, where the use of hearing protection among susceptible hunters during hunting compared to the resistant hunters, lead to a false impression that sporadic use of ear protection is more protective than frequent use. The susceptible hunters are those who experience gunshot sounds as ototraumatic and this affects their use of hearing protection, but the damage to their hearing is already done, i.e., the perceived outcome had, in fact, affected the perceived exposure. This paradox – reverse causation – was not observed in the group of hunters who were not exposed to unprotected shooting with HRC weapons in the preceding 5 years. The reverse causation in studies 3 and 4 interferes with how to interpret the protective effect of use of ear protection.

6.1.1.2.3.2 Confounding

A simple explanation of confounding is that an observed association (or absence of an association) between the exposure and the outcome is totally explained by (or the causal effect of the exposure is mixed together with) the causal effect of a third variable. This third variable is a common cause of both the exposure and the outcome. As opposed to bias due to selection or measurement error, the observed association between the studied exposure and outcome truly exists in the target population, but the causal structure is misinterpreted. It should be pointed out that even if confounding is demonstrated it does not exclude a causal effect by the exposure on the outcome, but the observed association is either amplified, attenuated, or even reversed by the confounding factor.

There are different ways to deal with possible confounding. In our cross-sectional internet study it is not possible to randomize, which is the safest way to eliminate all possible confounding. Restriction of the study to a narrow stratum of a key confounding factor is another way to reduce confounding, but it would have been unwise to restrict participation as there was already a low response rate. Matching participants on a key confounding factor, yet another way to reduce confounding, would be very demanding both in terms of the current size of the study population and economically. Confounding can be minimized in the statistical analysis by stratification or multivariable regression modelling. A major disadvantage with stratification is its inability to control simultaneously for multiple confounding variables because you have to subdivide the study population in a growing number of new substrata with each introduced variable for every introduced variable. This would not have been possible with preserved precision in our studies due to the small study population. The rich availability of possible covariates in study 3, however, allowed us to control for most conceivable confounding factors by multivariable regression modelling. In the fully adjusted model in study 3, in table 7, the PR has a slightly wider CI and the p-value is slightly higher, as an expression of the lower precision when introducing more variables. In study 4, on tobacco use, we were hampered by the small study sample, and had to be stricter on inclusion of covariates in the statistic model from the beginning, in order to not lose the precision. This did not, however, pose a major problem as the results from study 3 had already revealed that none of the covariates but age was a confounder.

In general, age is the strongest and most predictable determinant of hearing impairment, and if age is also a determinant of the studied exposure in studies concerning causes of hearing impairment, the possible causal effect of the studied exposure on the hearing ability will be mixed with the effect of age. Thus, age must always be considered as a potential confounding factor. This is supported in studies 3 and 4. The association between age and HFHI prevalence was clearly evident in our data, and the effect of age has been shown to easily override the effect of long-time noise exposure with dB-levels of 85-90(15). As the relationship was essentially linear we used age as a continuous variable in the model to remove the confounding effect, and it proved to be efficient. To use ISO 7029:2000, Acoustics -- Statistical distribution of hearing thresholds as a function of age, which was an initial suggestion was ruled out. Hunters is a non-otologically screened population as in ISO 7029:2000, and they are exposed to hunting noise. Comparing hearing thresholds from clinical audiometry with screening hearing thresholds from the InternetAudiotest was deemed not to be fair.

Nevertheless, residual confounding due to insufficiently fine categorization, or confounding from unmeasured causal factors (e.g., genetic factors) cannot be totally excluded and must be taken account when interpreting the results.

6.1.2 Effect Measure Modification /effect modifiation

Effect measure modification is when the level of association between exposure and outcome varies by levels of a third factor. When there is effect modification by a third factor, analysis of the pooled data can be misleading, but in stratified analyses, however, you must be careful to ensure that the sample size is adequate to provide a meaningful analysis (102). In our case this can be seen in study 3 versus study 4 where we study tobacco users and the probability of having a HFHI when being exposed to unprotected sound blast from HRC weapons. Categorization as tobacco-users in study 3 was restricted to current use (23%) (those who gave an affirmative answer to the question do you smoke or use snus?), but in study 4 we also included previous users who had smoked or used snus in the past 5 years but had quitted at the time of the data collection (30%). The reason for including the previous 5 years in study 4, was because the exposure of interest, (unprotected shots with HRC weapons) was examined during a period of the previous 5 years. Even so the small number of participants effectively prevented any deeper statistical analysis on subgroups of tobacco users and also to include more covariates in the model.

6.1.3 InternetAudio test

Study 1 revealed a large part of measurement errors in the Internet hearing test and the need for further improvement of the test-procedure. Many of the performed hearings tests and even accepted calibrations results showed unreasonable values and had to be discarded.

The weakest link in our Internet-based hearing test is the reference person which is the only workable method for calibration of the testing system. Other Internet-based hearing tests have used a reference tone or a specific program for calibrating the zero level (103) (52). This is problematic as noise levels of computers and the surrounding environments, as well as the quality of headphones, vary for each individual and setting.

The hearing of the reference person is crucial, as the hearing result of the test person is the actual difference between the HL of the reference person and the HL of the test person. Our possibilities to test the hearing of the reference person are very limited. Before study 2 we designed mandatory approval criteria to detect reference persons with hearing loss estimated to be more than 15 dB, but there is a gray zone that will affect the hearing results of the test person. Some participants might also be tempted to use themselves as reference person, not understanding the importance of the calibration procedure.
This possible inadequacy of the RP-HL might be a major problem in cross-sectional studies of the prevalence of hearing impairment in the population, but it will probably have less impact on longitudinal epidemiologic studies of intrapersonal change, that is, rate of deterioration of hearing ability over time. In study 2, the main validation study, was for efficacy reasons not done in the general population, where the prevalence of hearing impediments is low. Further, we did not evaluate this method among elderly persons. The participation rate was low in all of the subpopulations that were approached with our invitations and this might have led to selection of particularly motivated, computer-practiced individuals. This may have led to some overestimation of the accuracy. The small sample size, and particularly the small number of

subjects with hearing impediments, limited the precision of our accuracy and reliability estimates. The smaller intra-study validation in study 3 does however give the same results as in study 2.

Both study 2 and the smaller intra-study validation in study 3 showed that the Internet-based hearing test tended to underestimate the hearing threshold levels. It was also difficult, in the way the internet hearing test was constructed, to rule out failure of having passed all calibration tests and then leaving the test running while leaving the room and so giving a false result, indicating a severe hearing loss (65dB HL) for all frequencies. Or despite clear instructions some participants may have used themselves as reference-persons and by doing so produced an erroneous perfect hearing result with 0dB HL on all frequencies. Such errors might have contributed to the risk of misclassification – most probably non-differential – and might thus have diluted the true associations investigated in studies 3 and 4.

The Internet-based hearing test is concurrently improved during the course of this thesis based on the results from study 1 and 2. The final test-tool is named the Internet Audio test and is used in study 3 and 4.

6.2 FINDINGS AND IMPLICATIONS

6.2.1 Study 1

Our study had a response rate of 29% to the questionnaire and 16% to the hearing test, which is low for an epidemiological study. There were no differences between questionnaire respondents and full respondents in terms of sociodemographic characteristics and self-estimated hearing. When looking at non-respondents, the primary reason for non-participation was lack of interest. The survey engaged highly motivated individuals and this affected both the precision and the external validity of the results, but on the other hand it was feasible to use the tool-kit on motivated individuals.

The comparison, between self-assessed hearing by a question and measured hearing by the Internet-based hearing test showed low agreement (Cohens kappa coefficient= 0.18 (95% CI 0.005-0.359)). The Internet-based hearing test indicated a hearing loss in 20% of the tested individuals, compared to 52% in the self-estimated question. These results could reflect the difficulty in self-assessing one's own hearing problems, or are a result of difficulties in the calibration procedure of the internet-based hearing test. These results could also reflect the low correlation between the decided cut-off levels of one or more frequencies compared to a single question. In any case based on the results from only the questionnaire it seems as if the hunters had a higher prevalence of self-assessed hearing problems compared to the Swedish population (2).

Study 1 resulted in the following improvements of the hearing test, which were implemented in study 2: (1) the calibration process is made mandatory to be able to proceed testing, (2) an automated quality check is performed in the java-program before the calibration is accepted, (3) the dB level of the next tone presented, when a tone was registered as not heard, was changed to a 10 dB higher level and, when heard at a 5 dB lower level. The first two changes would probably reduce the willingness to proceed and perform the hearing test, but on the other hand the hearing tests performed would hopefully be of an acceptable quality.

6.2.2 Study 2

The reformed Internet based hearing test from study 1 was validated. The validation showed an acceptable agreement with the pure-tone audiometry. The two methods showed little difference for all hearing frequencies (0.5, 1, 2, 4, 6, and 8 kHz) and it did not vary with greater hearing impairment (dB). The sensitivity for hearing impairment according to a classification system for noise damage by Heijbel-Lidén, modified after Klockhoff (94) was reasonable and it had high specificity, and high repeatability. The moderate sensitivity makes the test less suited for ruling out hearing impairment, but the high specificity makes it useful for ruling in hearing impairment.

Study 2 resulted in the following improvements of the hearing test, which were implemented in study 3: The reference person has to repeat the calibration twice with acceptable similarity before approval. The accepted number of late/early space-bar pressures was elevated from five, to 10, which might help those who complain of tinnitus, and/or have a major hearing impairment.

6.2.3 Study 3

In study 3 we observed a significant 50% higher prevalence of HFHI among hunters who reported having fired 1-6 shots from HRC weapons without any hearing protection compared to those who reported not having fired any such shots without protection in the past 5 years. The observed result persisted after multivariable control for a large number of conceivable confounding factors, including age. In the fairly large group of hunters who reported more than 6 unprotected shots with HRC weapons (twice as many hunters as those who reported 1-6 shots) the HFHI prevalence was, however, close to that observed among those reporting no such shots. There was no linear relationship between the accumulated number of such shots and the outcome of the InternetAudio test. Neither exposure to unprotected shooting noise from weapons that generate less sound energy, nor the reported total annual number of shots, the vast majority of them fired with hearing protection in place, did seem to be related to HFHI, regardless of caliber.

The observation that one or a few unprotected high-energy impulse sound blasts from HRC weapons seemed to have an effect on the hearing test, in contrast to a history of no unprotected shots, is expected and is supported in the previous literature (13, 19, 25, 32-35, 104, 105). It is, however, difficult to explain why the prevalence of HFHI was lower among hunters who reported >6 unprotected shots than among those with less exposure in other ways than by individual differences in the susceptibility to ototraumatic events such as intense exposure to impulse noise. We believe that many susceptible hunters who experienced acute subjective hearing loss likely stopped such unprotected shooting, when the adverse effects of impulse noise like temporary hearing loss and tinnitus become apparent, while those with resistant ears continued shooting unprotected.

In general, the prevalence of HFHI was rather high also in the reference categories, the members of which were not exposed to unprotected shots. This could, in part, be explained by the age structure among participating hunters but also by our study being restricted to hunters. The association between age and HFHI prevalence was clearly evident in our data, but the removal of confounding ought to have been rather efficient in our analyses with age as a continuous variable. This notwithstanding, the crude prevalence ratios did not change materially after adjustment for age because age was basically unrelated to the shooting exposures.

The absence of a relationship between HFHI prevalence and the use of other types of firearms in this study should not be taken as evidence of their audiological safety and could be partly explained by the fact that all participants had a positive shooting history, and those serving as the reference categories, who were not exposed to unprotected shots in analyses of one type of weapon, were invariably exposed to other types of shooting noise. This lack of contrast could have impeded our ability to verify small effects of shooting noise. Also, the InternetAudio test, and possibly even the pure-tone audiogram, may be too blunt an instrument to expose the full inner ear damage caused by the noise from these types of weapons. This notion is supported by Pawlaczyk-Luszczynska et al, who despite normal clinical audiometry were able to demonstrate effects on the cochlea caused by unprotected shooting noise from small caliber firearms with OAE (105).

6.2.4 Study 4

The results from study 3 lead us to the conclusions that the wide variation in individual susceptibility also concerns exposure to high-energy impulse noise from HRC weapons and that susceptible individuals may sustain long-lasting or possibly irreversible damage to the inner ear from just 1 or a few shots. This variation in susceptibility to impulse noise might be genetically determined, as seems to be the case when the noise is occupational (20, 21, 106), but there might also be external factors that modify the harmful impact as tobacco smoking that aggravates hearing impairment following continuous noise (61-67). Can this effect also be seen for impulse noise?

6.2.4.1 Effect measure modification by tobacco use

In study 4 we did fact observe evidence for a modifying role of tobacco in the inner ear's ability to withstand extreme impulse noise, like unprotected sound blasts from HRC weapons. Among tobacco users, a history of 1-6 unprotected shots was associated with a significant 220% increase in the age-adjusted HFHI prevalence ratio, relative to 0 such shots, while among tobacco non-users the corresponding excess was no more than a non-significant 30%. As in study 3, we in study 4 again saw evidence of individual susceptibility as the effect modification by tobacco use was only seen among those hunters who were in the 1-6 shot stratum, and the hunters in the >6 shot stratum seemed unaffected.

Small numbers prohibited us from statistically verifying any effect modification by cigarette smoking alone, but we noted that the effect modification of the PR was nearly significant also among exclusive snus users. Since only a minority among the tobacco users smoked, the effect modification was likely driven to a large extent by snus use. Tobacco use itself was not associated with an increased prevalence of HFHI, neither among hunters exposed to unprotected noise from HRC weapons, nor among those who were unexposed to such noise.

The scientific evidence of oxidative stress in the cochlea as an effect of noise (9), can easily be transferred to the notion that current tobacco-use might increase this effect among susceptible individuals. A risk increase for NIHL among smokers is well published (61-67, 107), but available data concerns continuous noise. In study 4 it was found that the modifying effect of tobacco also includes impulse noise. Research has demonstrated that it is possible to "protect" your ears from "blast-induced cochlear damage" with an anti-oxidant cocktail. This will enhance the TTS recovery and reduce the PTS (108) (109). Possibly tobacco-use could have an opposite effect compared to the antioxidant cocktail, and increase the oxidative stress, and then make the cochlea even more vulnerable among susceptible individuals when exposed to hazardous noise. Our present results where the effect modification seen likely is driven by snus (as the majority of the tobacco users where sniffers), seem to contradict that combustions products as CO, are responsible for the tobacco-associated vulnerability to high-energy impulse noise. Maybe nicotine alone or in collaboration with TSNAs should be more interesting for coming research within the field.

6.2.4.2 Hearing protection and number of hunting days

A much larger proportion of exposed hunters *with* HFHI than exposed hunters without HFHI fell in the 1-6 shots category. The majority of hunters exposed to the unprotected noise from HRC weapons reported more than 6 shots in the preceding 5 years – some substantially more, but *less than half* of them showed evidence of HFHI upon InternetAudio testing.

Apart from the adverse effect on hearing of the unprotected shooting experience among susceptible hunters we also suspected a greater inclination among these hunters (with HFHI) to use hearing protection during hunting compared to non-susceptible hunters (with no HFHI), leading to a false impression that sporadic use of ear protection is more protective than frequent use. This possible reverse causation was not observed in the group of hunters who were not exposed to unprotected shooting with HRC weapons in the preceding 5 years. Those hunters who were unexposed to unprotected shooting with HRC weapons with sporadic use of hearing protection during hunting had a significant 60% higher prevalence of HFHI than unexposed hunters who used protection always or often. Likewise, the prevalence of HFHI among unexposed hunters reporting >20 hunting days per year was twice as high as among those reporting 0-20 hunting days. As shooting without protection occurs only during hunting and essentially never during training, this suggests that unprotected shooting with non-HRC weapons may also entail some risk for HFHI.

7 CONCLUSION AND FUTURE STUDIES

- The low response rates, mainly because of technical problems, but also because of lack of interest and time threaten both the validity and precision of the results in this thesis. The actual participation is, although somewhat sociodemographic biased, not deemed to be associatedwith neither the studied exposure and to a very small extent to the studied outcome. Therefore the risk of selection bias affecting results is small.

- The InternetAudio test can assess hearing with the help of a PC with an Internet connection. Among motivated individuals it is a valid alternative to questionnaires about self-perceived hearing loss in epidemiological studies or at out-patient clinics with pre-calibrated equipment.

- HFHI can be the result of unprotected shooting with HRC weapons for susceptible individuals.

- The individual susceptibility to develop HFHI seems highly variable. The factors that influence this susceptibility needs to be further investigated in order to give better the advice to the public.

- The susceptibility to develop HFHI, when exposed to unprotected shooting with HRC seems to be much greater among tobacco users than among non-users. The exact mechanisms remain to be clarified in future studies, but since the effect modification was apparent also among users of smokeless tobacco, combustion products may not be critical.

- In this thesis the importance of ear protection could not be quantified among hunters using HRC weapons because our data suggested that the HFHI outcome had led to changes in the use of such protection. Among hunters using weapons with less sound energy, however, no or sporadic use of hearing protection was linked to a 60% higher prevalence of HFHI, relative to habitual use.

- Even though there is a wide variation in individual susceptibility to high-energy impulse noise all hunters and others exposed to gunshot noise, should be encouraged to always use hearing protection, and stop using tobacco products, as the individual susceptibility is unknown.

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