Hypertension, atherosclerosis, and fracture risk

Observational investigations with a focus on antihypertensive therapy

Tove Bokrantz

Department of Molecular and Clinical Medicine

Institute of Medicine

Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

Gothenburg 2022

Cover illustration: Lisa Modica/Knowable Magazine

Hypertension, atherosclerosis, and fracture risk
Observational investigations with a focus on antihypertensive therapy

tove.bokrantz@gmail.com

ISBN 978-91-8009-759-8 (PRINT)
ISBN 978-91-8009-760-4 (PDF)
http://hdl.handle.net/2077/70548

Printed in Borås, Sweden 2022
Printed by Stema Specialtryck AB

Hypertension, atherosclerosis, and osteoporotic fractures are all major public health problems that account for significant morbidity and mortality in the aging population.

ABSTRACT

Aim: To investigate associations between hypertension, arteriosclerotic disease, and fracture risk in adults and the elderly, with a certain focus on how specific antihypertensive drug treatments in patients with high blood pressure might affect the risk for fractures.

Methods and results: In Study I and II, the associations between different antihypertensive treatments and fracture risk were investigated in a cohort of about 60.000 adult women and men diagnosed with hypertension in primary health care. A primary health care database from southern Sweden (SPCCD) linked with national registers was used to identify the cohort, exposure status and fracture outcome from 2006 to 2012. Multivariate Cox proportional hazards models were used to estimate fracture risk across users of different antihypertensive drugs. We found ongoing treatment with thiazide diuretics to be associated with a decreased risk of osteoporotic fractures including hip fractures, compared to other antihypertensive drug therapies. In contrast, treatment with loop diuretics was associated with an increased risk of hip fractures, while the use of beta-blockers, ACE inhibitors, angiotensin receptor blockers, aldosterone receptor blockers, or calcium channel blockers revealed no significant association with fracture risk. The overall findings were similar across men and women, but the decreased fracture risk with thiazides was only statistically significant in men. Study III and IV were based on data from the MrOS Sweden study, a prospective study of elderly men in Gothenburg, Malmö, and Uppsala. Men aged 69-81 years were randomly selected from population registers and invited to participate during the years 2001–2004. Fracture outcomes have been collected since then. Multivariate Cox proportional hazards models were used to estimate the association between (1) peripheral arterial disease and (2) the diagnosis of hypertension at baseline, and hip fractures during follow-up. In Study III, peripheral arterial disease at baseline, defined as an ankle-brachial index <0.9, was found to be associated with an increased risk of hip fractures independently of age and BMD. In Study IV, no association between hypertension and hip fracture risk during follow-up was found, despite some differences regarding both risk factors and protective factors for fractures between elderly men with and without hypertension.

Conclusions: In summary, fracture risk seems to differ across users of different blood-pressure-lowering drugs. The results support the hypothesis of antihypertensive treatment with thiazide diuretics as a drug that might be beneficial for fracture risk, at least in men. Elderly men with peripheral arterial disease are generally a fragile group of patients with high morbidity, where the study results indicate that these men also have an increased risk of suffering from hip fractures. However, we found no indications that hypertension itself is associated with fracture risk in elderly men.

Keywords: epidemiology, hypertension, antihypertensive agents, fractures, peripheral arterial disease, bone density
ISBN 978-91-8009-759-8 (PRINT), ISBN 978-91-8009-760-4 (PDF)
http://hdl.handle.net/2077/70548

SAMMANFATTNING PÅ SVENSKA

Bakgrund och syfte

Kardiovaskulär sjukdom är en av de ledande orsakerna till sjuklighet och död världen över, där högt blodtryck (hypertoni) är en av de viktigaste riskfaktorerna. Hypertoni är väldigt vanligt och förekomsten ökar med åldern. Upp emot 60% av män och kvinnor över 60 år bedöms ha högt blodtryck. Hypertoni samvarierar därmed även ofta med andra åldersrelaterade sjukdomar, såsom benskörhet och ökad frakturrisk. I likhet med hypertoni är benskörhet vanligt hos äldre, framförallt hos kvinnor. Benskörhet leder till frakturer som bidrar till stort lidande och stora kostnader för samhället. Men många frakturer hos äldre sker även oberoende av benskörhet. Det har uppskattats att varannan kvinna över 50 år någon gång under resten livet kommer att drabbas av en fraktur, medan 1 av 4 män kommer drabbas. Detta avhandlingsarbete tar utgångspunkt i samvariationen mellan denna frakturrisk och kardiovaskulär sjukdom, där hypertoni och åderförkalkning är starka riskfaktorer. Målet har varit att undersöka om det kan finnas mer direkta samband mellan hypertoni, åderförkalkning (arteriosklerос) och frakturrisk, samt att särskilt studera hur olika blodtryckssänkande läkemedel kan påverka risken att drabbas av fraktur.

Metod och resultat

Alla delarbeten i denna avhandling utgörs av longitudinella observationsstudier. Delarbete I och II baseras på data från primärvårdsregistret SPCCD och inkluderar närmare 60 000 patienter som diagnostiserats med hypertoni i primärvården i södra Sverige. Kopplat till SPCCD finns även uppgifter från Nationella slutenvårdsregistret med diagnoser från sjukhusvistelser, samt Läkemedelsregistret med uppgifter över alla uthämtade förskrivna läkemedel i Sverige. Under en uppföljningstid på 6 år jämfördes risken att drabbas av benskörhetsrelaterade frakturer mellan individer som behandlats med olika blodtryckssänkande läkemedel. Delarbete I visade att behandling med läkemedelsklassen tiaziddiuretika var associerat med sänkt risk för benskörhetsrelaterade frakturer, jämfört med andra blodtryckssänkande läkemedel. Detta bekräftades i delarbete II, där både subklasserna hydroklortiazid och bendroflumetiazid var för sig visade sig vara associerat med sänkt risk för höftfraktur. När män och kvinnor undersöktes separat var dock resultaten starkare för män och mer osäkra för kvinnor. I delarbete II undersöktes även ett större antal läkemedelsklasser, där loopdiuretika i motsats till tiaziddiuretika visade sig vara associerat med ökat risk för höftfraktur. Övriga läkemedelsgrupper var neutrala (ACE-hämmare, angiotensinreceptorhämmare, betablockerare, kalciumkanalblockerare och mineralkortikoidantagonister).

Delarbete III och IV baseras på data från MrOS Sverige, en studie med äldre män boende i Göteborg, Malmö och Uppsala. Män i åldrarna 69–80 år blev slumpmässigt utvalda och inbjudna att delta i MrOS under åren 2001–2004, där de fick genomgå ett stort antal undersökningar och svara på frågor i frågeformulär. Syftet var att undersöka och identifiera riskfaktorer för benskörhet och frakturer hos män. Männen genomgick bl.a. bentäthetsmätning med så kallad DXA och undersöktes med blodtrycksmätning både i arm och ben vilket kunnat användas för beräkning av arteriosklerotiska kärl i benen. Information om frakturer som inträffat sedan studiestarten har följts upp ända fram till 2018. I delarbete III undersöktes sambandet mellan arteriosklerotisk kärlsjuka i benen och risken att drabbas av höftfraktur på sikt. Denna kärlsjuka visade sig vara associerat med ökad risk för höftfraktur oberoende av bentäthet, men också att sambandet till viss del tycks påverkas av annan sjuklighet och skörhetsfaktorer. I delarbete IV undersöktes istället sambandet mellan hypertoni och risken för höftfraktur. Detta arbete är ännu ej helt färdigställt, men resultaten hittills visar att hypertoni i sig inte är kopplat till frakturrisk hos dessa äldre män. Männen med hypertoni som får höftfraktur ser dock ut att ha både en del skyddsfaktorer och riskfaktorer för fraktur som skiljer sig från männen utan hypertoni.

Slutsatser

Sammanfattningsvis bidrar denna avhandling med ökad kunskap kring hur samvariationen mellan hypertoni, arterioskleros och frakturrisk ser ut, samt hur vanligt förekommande blodtryckssänkande läkemedel kan påverka risken att drabbas av fraktur hos män och kvinnor under behandling för högt blodtryck. I valet av blodtryckssänkande behandling förefaller tiaziddiuretika ha en fördelaktig effekt genom att minska risken för vanligt förekommande benskörhetsrelaterade frakturer, åtminstone höftfraktur, men möjligen endast hos män. Underlaget för kliniska rekommendationer är således fortfarande knapphändigt. Män med perifer kärlsjuka i benen är överlag en skör patientgrupp med stor sjuklighet, där studieresultaten indikerar att denna patientgrupp även har en ökad risk att drabbas av höftfrakturer.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Bokrantz T., Ljungman C., Thomas Kahan T., B. Boström K., Jan Hasselström J., Hjerpe P., Mellström D., Schiöler L., Manhem K.
Thiazide diuretics and the risk of osteoporotic fractures in hypertensive patients. Results from the Swedish Primary Care Cardiovascular Database.
Journal of Hypertension 2017; 35(1): 188-197

II. Bokrantz T., Schiöler L., B. Boström K., Thomas Kahan T., Mellström D., Ljungman C., Hjerpe P., Jan Hasselström J., Manhem K.
Antihypertensive drug classes and the risk of hip fracture: results from the Swedish primary care cardiovascular database
Journal of Hypertension 2020, 38(1): 167-175

III. Bokrantz T., Manhem K., Lorentzon M., Karlsson M., Ljunggren Ö., Ohlsson C., Mellström D.
The association between peripheral arterial disease and risk for hip fractures in elderly men is not explained by low hip mineral density. Results from the MrOS Sweden study.
Submitted to Osteoporosis International

IV. Bokrantz T., Manhem K., Lorentzon M., Karlsson M., Ljunggren Ö., Ohlsson C., Mellström D.
Hypertension and fracture risk in elderly men, with focus on hip fractures. Observational results from the MrOS Sweden study.
Manuscript

CONTENT

ABBREVIATIONS

ABI	Ankle brachial index
ACEi	Angiotensin-converting enzyme inhibitor
ARB	Angiotensin II receptor blocker
ATC-code	Anatomical Therapeutic Chemical code
BB	Beta-blocker
BMD	Bone mineral density
BMI	Body mass index
BP	Blood pressure
CCB	Calcium channel blockers
CRP	C-reactive protein
DXA	Dual energy X-ray absorptiometry
eGFR	Estimated glomerular filtration rate
ESC	European Society of Cardiology
ESH	European Society of Hypertension
FEV1	Forced expiratory volume in 1 second
FGF23	Fibroblast growth factor 23
FRAX	Fracture assessment tool
Hb	Hemoglobin
HDL	High-density lipoprotein
HR	Hazard ratio
ICD	International Classification of Diseases, 10th Revision
LDL	Low-density lipoprotein
MOF	Major osteoporotic fractures
MRA	Mineralocorticoid receptor antagonists (also referred to as Aldosterone receptor antagonists)
MrOS	The Osteoporotic Fractures in Men study
OR	Odds ratio
PAD	Peripheral arterial disease
RAAS	Renin-angiotensin-aldosterone-system
RCT	Randomized controlled trial
PINP	Propeptide of type I collagen
PTH	Parathyroid hormone
SBP	Systolic blood pressure
SD	Standard deviations
SPCCD	The Swedish Primary Care Cardiovascular Database

1 INTRODUCTION

Cardiovascular disease is one of the leading causes of morbidity and mortality worldwide, with high blood pressure (hypertension) being one of the most important risk factors. Hypertension is very common, and the incidence increases with age. As such, hypertension often co-exists with other age-related diseases, such as osteoporosis and increased fracture risk. This thesis is based on this covariation, with the aim to further investigate whether there may be more direct links between hypertension, arteriosclerosis, and fracture risk, with a special focus on how treatment with different blood-pressure-lowering drugs may affect fracture risk.

Structure of the introduction chapter

This thesis is based on four studies, two of which are published in scientific journals and two are still in the working process. All studies are based on epidemiological research with quantitative measurements. When summarizing the research presented in this thesis, my ambition has been to describe and explain the scientific knowledge that I have gained during my years as a doctoral student. Not only a summary of the specific topics that my research has addressed but also the process of conducting scientific studies and critically evaluating my own research results as well as the results of others.

Therefore, the introduction section of this thesis is structured in three distinct chapters. The first chapter is written mainly for myself and includes an introduction to epidemiological research. For me, this has been a way to put in print some of the basic methods, terminology, and challenges in conducting epidemiological studies that I have encountered and gradually learned more about during my scientific journey. The second chapter includes a broader background and understanding of the topics addressed in this thesis. It contains a brief review of hypertension, atherosclerotic disease, antihypertensive drugs, bone, and fractures. Finally, the third chapter specifically describes the background of why these conditions may coexist and underpins the research questions addressed in each of the four studies.

To enable a broad audience to read the thesis, I provide the following reading recommendations: if the concept of epidemiology is not your main interest, you may jump right on to subchapter two of the introduction, or vice versa.

1.1 EPIDEMIOLOGICAL STUDIES

Epidemiology can be described as the "study of the occurrence of illness" in different groups of people [1]. Epidemiological studies constitute a cornerstone in public health decisions and include a vast range of study designs that can be either observational (where the cause is observed) or experimental (where the cause is manipulated). This thesis is based on observational studies, usually categorized in *descriptive* or *analytical* studies.

Descriptive observational studies aim to estimate disease occurrence in a population (i.e., a specific group of people) and focus on the questions *to whom, what kind, where, and when* diseases occur. Descriptive studies are usually used for establishing disease frequency and pattern in a population, such as estimating the prevalence of infected people during a specific week of a virus pandemic. Descriptive studies are also important for generating *hypotheses* that can be tested in analytic observational studies.

Analytical observational studies aim to further dive into the question of *why* diseases occur. This is done by investigating statistical associations between exposures (risk factors) and outcomes (diseases). All studies in this thesis have an analytical approach, at least in part. The goal in analytic observational studies is often to ultimately provide information about potential causal effects that could be of interest in clinical practice. However, casual conclusions should always be cautiously considered based on observational data, which much of this chapter is dedicated to addressing further in the upcoming sections.

In analytical studies, the first step is to formulate a suitable *hypothesis*. This includes specifying (1) the exposure, (2) how the exposure is expected to affect the outcome compared to the absence of exposure, and (3) the population of interest. An example from the present thesis could be: "Antihypertensive treatment with thiazide diuretics is associated with a decreased risk for osteoporotic fractures in men and women above 50 years of age with hypertension, compared to men and women above 50 years of age with hypertension who is not treated with thiazide diuretics." The next step is to choose a proper study population to test the hypothesis in. Since it is often unrealistic to include the entire population in the study (for example, all men and women above 50 years of age with hypertension in Sweden), epidemiologists typically select a smaller group from the population, a *sample*, to conduct the study on. The goal then is to extrapolate the conclusions from the sample to the population of interest (see Figure 1). However, this requires overcoming several methodological and statistical challenges. In the following sections, some of these challenges and ways to deal with them are described.

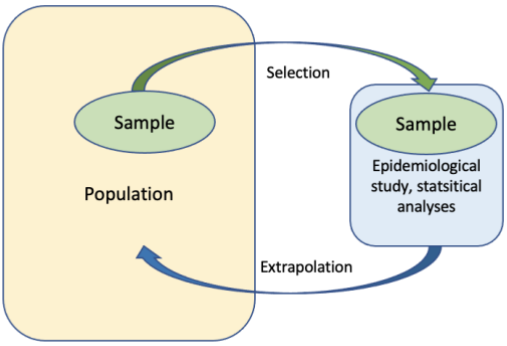


Figure 1. *In an epidemiological study, a sample is selected from the population on which the study is conducted. Statistical analyses are then performed on the data generated, with the aim to extrapolate the results from the sample to the population.*

Cross-sectional studies versus cohort studies

Two common types of research designs in epidemiology are cross-sectional and cohort studies. Cross-sectional studies are common among descriptive observational studies. Here, data on health status, including both the exposure (risk factor) and the outcome (disease), are obtained during a single medical care visit or point in time. It is then possible to establish if the disease is more common in the group with or without the specific risk factor. However, since data on exposure and outcome are obtained at the same time, it cannot be certain that the exposure preceded the outcome. Hence, it is difficult to assess any causality. In contrast, the cohort study is a longitudinal study where the study sample is characterized at the study start (the baseline) according to the exposure and then followed for a specific time to observe the occurrence of outcomes. A cohort is, to put simply, a group of individuals with shared characteristics. Thus, it is possible to ascertain that the exposure precedes the outcome and thereby estimate the difference in outcome rates between the exposed and the unexposed in the cohort. This is one step closer to establishing a causal relationship. A prospective longitudinal study collects all information about disease occurrence prospectively, while a retrospective longitudinal study looks back at the exposures preceding the disease. All studies in this thesis are cohort studies, which is regarded as one of the most powerful types of observational studies [2].

Causal inference in epidemiological studies

As mentioned above, most analytical studies aspire to say something about cause and effect between an exposure and outcome, which in epidemiological studies is referred to as *causal inference*. However, true causality is in fact rather impossible to study. Regardless of study design, when we conduct clinical studies in humans, we always deal with a group of individual people who inherently differ from each other

in a myriad of ways. Hence, when we compare the occurrence of an outcome between a group of people with a specific risk factor and a group of people without the risk factor, we cannot be certain that any differences in outcome occurrence between the groups the facto is attributable to the risk factor. Instead, the true cause of different outcomes could be something else, such as differences in genetics, lifestyle, other diseases, age, sex etcetera, if these things are *unevenly distributed* between the exposed and unexposed people.

So, how do we conduct a study where we can ascertain causality? An ideal study would be the one of a hypothetical universe, where we had two Planet Earth, one exact replicate of the other, including all people living there (which would be incarnations of themselves). Assume that we give a toxic agent to all people on Earth 1, but not on Earth 2, and then followed the people simultaneously for a specific period of time. At the end of this ghastly study, we could say that any excess in mortality rate on Earth 1 was due to the toxin. However, this is just an experiment of thought, impossible to conduct.

The closest you may come to such a study is by an interventional epidemiological study, the randomized controlled study (RCT). In the RCT, the participants are carefully selected and randomly assigned to the exposure, for example, to a specific blood pressure-lowering drug. The random assignment will also ensure that characteristics like age, sex, physical activity, smoking, comorbidity, and baseline blood pressure levels will be evenly distributed between the comparison groups, as long as the study sample is large enough. However, if the randomization is not successful, we might end up with older participants or more smokers in the group without active treatment, hence, the lower blood pressure levels achieved in the treatment group might be caused by the absence of smoking or old age, not the treatment itself. In such a case, age and smoking *confound* the results (see the upcoming topic *Confounding* below). In observational studies, the presence of some such differences in characteristics between exposed and unexposed individuals is more the rule than the exception. If we study a certain effect of hypertension, people with and without hypertension will inevitably differ across several characteristics, which can introduce confounding if we do not address it properly.

According to the reasoning above, RCTs are the golden standard for interventional studies. However, such studies also have limitations. RTCs are time-consuming, resource-intensive, and expensive. As such, they rarely last longer than a few years, limiting the opportunity to study outcomes that are rare or occur long after the exposure. Neither can we study exposures that can not be assigned to a person, such as height, shoe size, or conditions such as hypertension. Furthermore, ethical considerations are essential. For instance, the initial example of an experiment with a toxin given to the people on Earth 1 would never be allowed. This is the same reason why experimental studies on pregnant women are very rare, or why giving intellectually impaired individuals high doses of sugary caramels with the aim of

following its effect on tooth health became such a scandal in Swedish medical research (referring to the experiments at Vipeholm in the 50s [3]). Also, in situations where we have effective treatment options, such as well-documented antihypertensive drugs for lowering blood pressure, it would be unethical to conduct a study where participants did not receive such treatment with the aim to follow the natural course of hypertension. Consequently, observational studies are still the preferable option in many situations.

Dealing with bias in observational studies

The main limitation and concern in epidemiological studies are the risk of *bias*. *Bias* in epidemiology has been defined as "an error in the conception and design of a study – or in the collection, analysis, interpretation, reporting, publication, or review or data – leading to results or conclusions that are systematically (as opposed to randomly) different from truth" [4].

Errors occur, in daily life as well as in research. Importantly is to differentiate random errors from systematical ones. For example, in a study that investigates the blood lowering effect of two different antihypertensive drugs, random errors could occur in the form of inaccurate measurements due to an anxious patient, a stressed staff that happened to use the wrong sized armed cuff, or an erroneous measurement noted down just by mistake. In contrast, systematic errors could occur if two different measuring devices were used in each treatment group, where one device always obtained higher measurements than the other. Then the effect of the blood pressure-lowering drug in that latter group will be estimated to be lesser than it in fact was. While the impact of random errors reduces with the size of the study, the effect of systematical errors is not. In short, the term bias in epidemiological studies refers to all kinds of systematic errors that distort the results of a study from the truth.

Bias is linked to a study's *internal validity*, which simply refers to how well a study measures what it intends to measure. A high degree of bias will always weaken the internal validity. Bias can also affect *external validity,* the extent to which the study results also hold in other contexts, i.e., how well the results can be generalized to populations beyond the study sample. Another important concept is *reliability,* the extent to which measurements are consistent when repeated. For instance, if measuring the weight of an object on an electronic scale, the reliability is high if the scale shows identical weight over and over again when we put the same object on the scale. In the bigger picture, reliability refers to reproducibility, the ability to reproduce an experiment or a study. High reliability is essential for high validity, but reliability is not a guarantee for validity. The following section discusses common sources of biases that may affect the validity of studies and the ability to interpret causal inference.

Selection bias

When selecting a study sample (see figure 1), one needs to be aware of the risk of *selection bias*, the risk that the study sample does not reflect the intended target population. Any significant differences between the participants in the study sample compared to the individuals in the population may be a source of selection bias, which primarily affects the study's external validity. For example, voluntary recruitment through social media advertisement will only include participants inclined to respond to such ads. Suppose the intended population is overall men and women >70 years of age. In that case, the ones responding to those ads will most probably differ in many aspects from those who do not, risking rendering study results that are far from the truth in the general population. Importantly, selection biases cannot be overcome with statistical adjustments. Therefore, epidemiological studies need to ensure the use of proper sampling techniques. The investigator and the reader of such research need to be aware of how the study sample was collected to draw reasonable conclusions.

Confounding

Confounding is one of the main causes of bias in epidemiological studies. Confounding could simply be described as the "confusion of effects".

A classic example of confounding is the association between Downs syndrome and birth order, described by Rothman [1]. In this case, a distinct correlation between birth order and Downs syndrome was found, with the last child having a considerably increased risk of being born with Downs syndrome compared to the firstborn. However, as the number of siblings increases, the mother inevitably gets older. In fact, if the mother's age is taken into account, the association between birth order and Downs syndrome vanishes completely. Hence, the association was caused by the mother's age, not the child's birth order, i.e., age confounded the initial results. To make this a little more complicated, age is probably not the direct cause of Downs syndrome but a proxy for a diversity of biological alterations due to aging that affects the egg cells and the meiosis, leading to trisomy 21 and Downs syndrome. However, some of these biological processes are still unknown or impossible to measure systematically. Hence, a confounding factor (also referred to as a *confounder*) is a factor or phenomenon that has been measured (as age) or a factor that represents a proxy for something we have not or cannot measure (the biological processes that cause aging). To act as a confounder, the factor has to meet the following criteria:

a) be associated with the exposure (either as a true cause of the exposure or a proxy for an unmeasured cause, or merely unevenly distributed between the exposed and unexposed)
b) be a true cause of the outcome or a proxy for an unmeasured cause of the outcome
c) not a mediating factor in the causal link between the exposure and outcome

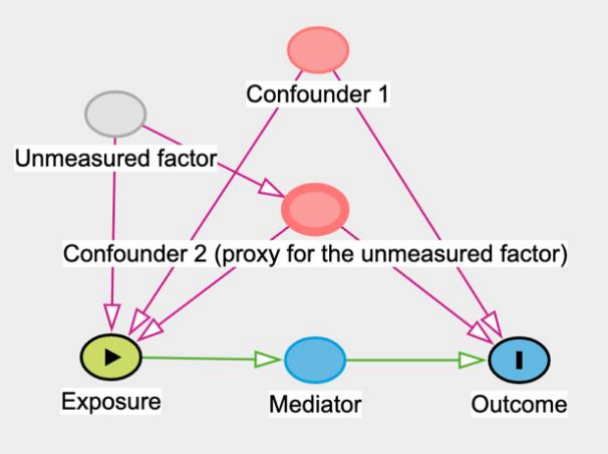


Figure 2. *Illustration of the relationship between exposure, outcome, and confounders.*

Confounding by indication is a particular case of confounding that may be present in studies investigating the effect of a given treatment in an observational setting. In an RCT, the treatment is randomly assigned to the participants, but in an observational study, the treatments are given based on the indication and current guidelines. For instance, when the effect of two first-line antihypertensive drugs is compared in a clinical setting, it is assumed that the physician's choice between prescribing one or the other drug to a patient with hypertension is sufficiently random. However, suppose one of the drugs is routinely given to individuals with specific comorbidity due to a preferable additional effect on the comorbidity. In that case, the drug's effect (or lack of effect) on blood pressure level could be caused by the comorbidity rather than the medication itself. In this case, the indication for choosing the particular drug caused bias.

Dealing with confounding in cohort studies

As mentioned previously, the risk of confounding has to be thoroughly addressed in observational studies to minimize the risk of bias. In this thesis, three different methods to deal with confounding have been used, which will be presented below.

Restriction (or exclusion) is one way to reduce the risk of confounding [1]. As a confounder only can be present if it is unevenly distributed between the exposed and unexposed individuals, it can be eliminated by excluding all participants that express the potential confounder. For example, if age is a potential confounder, restriction can be achieved by only including individuals of the same age. In this case, age cannot be unevenly distributed and therefore cannot cause confounding. However, such restriction may result in poor external validity, as the results can only be generalized to individuals of that specific age.

Instead, *stratification* can be used, where individuals of all ages are included, but the effect of interest is measured across different age groups [1]. If the effect differs significantly between different age groups, age could be an important confounder. Stratification does not affect external validity. Another advantage of stratification is the possibility to identify the magnitude of the effect by age in each category on the studied association. This can help identify and highlight factors that induce *effect modification* rather than confounding. Such variables affect the magnitude of the effect between the exposure and outcome depending on the level of this third variable. Unlike confounding, effect modification is a biological phenomenon, implying that exposure has different impacts in different circumstances. An example is the effect of smoking on lung cancer under the impact of simultaneous exposure to asbestos. Both smoking and asbestos are risk factors for lung cancer, but when exposed to both, the risk of lung cancer is enhanced not only additively but also multiplicative [5]. The drawback of stratification is that it usually limits the statistical power since separate analyses are conducted for each age category. Statistical power is described further below, see *statistical inference*.

Finally, statistical *adjustments* in multivariate analyses can be used to reduce the effect of confounding [1]. When using this method, data for potential confounders (such as age) is added to the statistical model where we investigate the relationship between the primary exposure and the outcome. The model then yields an individual estimate of the effect on the outcome from the primary exposure and each potential confounder. The advantage of such a model is the possibility to account for several confounders simultaneously, which will keep the statistical power high. However, before adding multiple variables into a multivariate analysis, it is crucial to identify proper potential confounders that fulfill the criteria in Figure 2. Otherwise, bias might be introduced into the model rather than being adjusted for. For example, if we include a pure mediator in the model, we "take away" the association between the exposure and outcome mediated through this particular factor, which could be referred to as unnecessary overadjustment [6]. In such a case, we risk underestimating the total effect of the exposure on the outcome. There are statistical methods that can be used for helping to identify which variables should be treated as confounders. Various statistical methods can be used to determine which variables should be treated as confounders. However, these methods have the disadvantage of not taking into account any biological process and might therefore produce incorrect confounders or miss important ones. Another helpful tool to identify the relationship between exposure, outcome, and other factors is to use the illustrative tool DAG (directed acyclic graphs). Here, biological and temporal knowledge is used to identify confounders and distinguish them from factors such as mediators or effect modifications, see Figure 3.

It is important to note that restriction, stratification, and statistical adjustments can only compensate for known and appropriately measured

confounders, implying the need for thoroughly obtaining data for such factors when conducting a study. Still, there is always a risk of some remaining unknown and unmeasured confounders in any observational study, which cannot be accounted for, so-called *residual confounding* [1]. This is an inherent limitation in all observational studies.

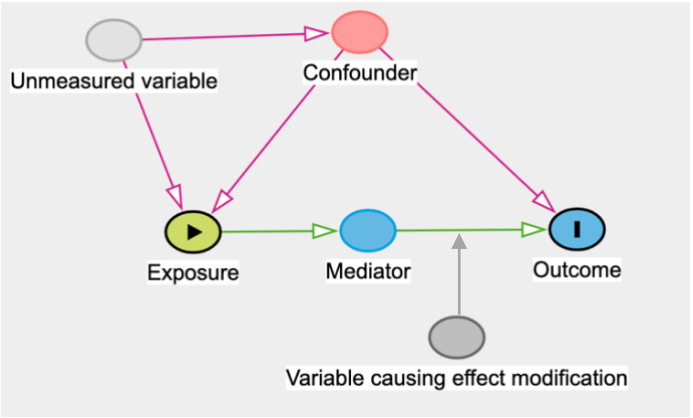


Figure 3. *An example of a DAG (directed acyclic graphs) used to visualize confounders and other important factors affecting the relationship between an exposure and outcome.*

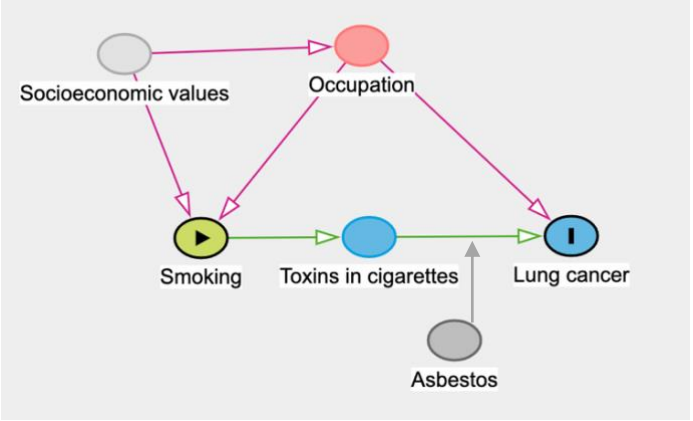


Figure 4. *An example of how DAG (directed acyclic graphs) can be used when investigating the association between smoking and lung cancer.*

Information bias – Recall bias, misclassification, and missing values

An epidemiologist relies on data (measurements) to make interpretations of reality. However, the way we collect and categorize our data might end up with systematic errors, referred to as *information bias*.

For example, we intend to study the association between hypertension and quality of life by letting our participants fill out questionnaires about diagnoses of disease, how they feel about life, and relevant potential confounders. We define hypertension by self-reported disease from at least five years back, and quality of life by combining the answers from several questions regarding their current experience of life. But how well does self-reported hypertension capture what we indeed intend to measure? First, we know that discontinuation of antihypertensive medications is common. Hence, people might have forgotten that they were diagnosed with hypertension several years ago, rendering a "no" on the question of whether they have hypertension. This can be categorized as a *recall bias*, referring to the risk of false information when we obtain a posteriori information about the exposure, in the worst case, obtained after the outcome has occurred. We also know that hypertension is underdiagnosed. Hence, several people might give us a negative answer simply because they are unaware of their high blood pressure. In both the former and the latter case, we will end up with individuals that, in reality, have hypertension but, in our study, incorrectly are categorized as individuals without hypertension. This we refer to as *misclassification*. A proportion of the participants might as well just skip the question for reasons we have no clue about, rendering *missing data*. More profound, we must question ourselves as researchers whether the measurement itself really translates to the phenomenon we ought to investigate. In this case: can the biological effect of elevated blood pressure be translated into a question on a questionary?

Recall bias, misclassification, and missing values can all render more or less information bias, depending on whether it occurs randomly or is related to either the exposure or the outcome. Regarding misclassification, this is referred to as *non-differential* versus *differential misclassification* [1]. If the question in the questionary results in random misclassification across both groups of comparison, e.g. we misclassify just as many individuals as hypertensive when they have normal blood pressure, as individuals with elevated blood pressure as normotensive, we have a *non-differential misclassification.* Such misclassification will result in a diluted estimate of the effect in our statistical analysis. In this case, we will *underestimate* the negative consequences of hypertension on quality of life. In other words, non-differential misclassification will decrease the study's possibility of detecting differences between the exposed and non-exposed, and as such, we might miss

interesting clinical associations. This is unfortunate, but at least we know which direction the effect will be distorted. On the other hand, differential misclassification can give biased results in either direction, and there is no way we can foresee how it will distort the effect estimation. In the example above, we certainly would end up with differential misclassification, as people with hypertension tend to report no hypertension mistakenly, but patients without hypertension rarely would report having high blood pressure.

Missing data exists in all kinds of observational studies. Even though we do our best to minimize its extent, it is fair to regard it as unavoidable. Yet again, depending on the size of missing data, the type of missing data, and how we deal with it in our analyses, we will end up with more or less biased study results. Missing data are usually categorized as *missing completely at random*, *missing at random, or missing not at random* [7]. When data is *missing completely at random,* the probability of rendering a missing value is the same for all study subjects. For instance, we lack information on blood pressure for some participants as the measuring device happened to be broken that day, or an incident fracture was forgotten to be registered as the physician had to run at an emergency call. These things can happen randomly to anyone at any time. Data *missing at random* occurs when the missingness of a variable depends on other variables that we observe. For example, if men are less likely to show up for the intended blood pressure measurement, we will have a larger amount of missing values for blood pressure among men than women. Then the missing value of blood pressure depends on gender. On the other hand, when data is *missing not at random,* the cause of missingness is related to the variable itself. Suppose individuals without hypertension are less likely to show up for a blood pressure measurement than individuals with known hypertension. In that case, we will have fewer blood pressure values among normotensive individuals. Another example is if a physician is more prone to obtain height and weight measurements in obese patients than in patients with seemingly normal weight.

Like the problem with non-differential misclassification described above, data missing not at random causes the highest risk of bias, whereas data missing completely at random causes lesser problems. There are methods that can indicate whether data are missing completely at random or not, but unfortunately, there is no way to ensure what kind of missing data we are dealing with [7]. At least, several statistical approaches have been proposed for dealing with missing data. The most straightforward way is to exclude all individuals who have any missing data from the analysis. But if the extent of missing data is large, the number of included individuals (and statistical power) will shrink substantially, and those who remain might not be representative of the initial study sample. Another approach is to replace all missing values with either the worst or the best value in the observed data, and then compare the results of these two analyses. If the analyses produce similar

estimates, the risk for bias from the missing values is low. The problem is when the analyses produce opposing results, as we have no clue which of these estimates are most close to the truth. Hence, this method only works well if the data are missing completely at random. A third approach is to create a certain category for the missing values in the analysis, the *missing indicator method*. For example, smoking can be categorized as either *smoker, non-smoker,* or *missing*. For continuous variables, such as BMI or blood pressure, missing values are set to a fixed value (usually zero), and an extra dummy variable (1/0) is added to the analysis to indicate whether the value for that variable is missing. As with the best/worst-case scenario, we then keep the full dataset as no observations or individuals are excluded, which is an advantage. However, this method only works fine if data is missing completely at random, and is unfortunately known to most often produce biased estimates [8]. Today, more advanced statistical methods using *multiple imputations* are considered the most valid approach to deal with missing values [7]. Multiple imputations cause no loss of power and can produce unbiased estimates with both data missing completely at random and missing at random. However, multiple imputations still can not handle data missing not at random without the risk of biased results.

Statistical inference

Statistical inference is the process when we use statistical analyses and models to make estimates of the findings in the study sample that can be extrapolated to the population the study sample was drawn from. Statistical inference together with a critical mind regarding potential bias lays the foundation for causal inference. Some of the basics for assessing statistical inference are described below.

Merely a result of random chance?

The very essence of an analytical observational study, including the studies in this thesis, is the objective to identify true associations between a risk factor and an outcome. To do so, we need to rule out the risk for the association to be merely a result of random chance, at least assess how considerable the risk is for such a situation. This can be accomplished by statistical hypothesis testing, which will produce a value of the likelihood for our findings to be a result of chance. We begin with formulating a *null hypothesis*, which postulates that there is no effect or difference between the comparison groups. The *null hypothesis* is the statistical hypothesis, which usually is the opposite of the research hypothesis that motivated the study. For instance, the null hypothesis would state that there is no difference in mean BMI between individuals with or without hypertension, or there is no difference in the quality of life between individuals with or without depression. The

counterpart is the *alternative hypothesis*, stating that there is a difference between the comparison groups. Let us now use the first hypothetical example with BMI. We have collected data showing a mean BMI of 26.8 among individuals with hypertension and a mean BMI of 25.2 among normotensive individuals. The groups differ according to BMI, favoring the alternative hypothesis, right? But is the difference *statistically significant*?

Before we can answer that question, we need to determine a level of significance, known as alpha (α), which is usually set to 5% in epidemiological studies. Then we can run the statistical hypothesis test. There as several different tests to choose between, depending on the distribution of the data and the nature of the variables (if they are binary, continuous, or categorical), but the details regarding these tests are beyond the scope of this present section. In this case, we have normally distributed data, and BMI is a quantitative continuous variable. Hence, we will use the standard *t-test*. The test will yield a *p-value*, which shows the probability of obtaining the current test result or a more extreme result when the null hypothesis is true. Let us say that our test yields a p=0.002 (or 0.2%). This means that if the null hypothesis is true, there is a 0.2% risk that the difference in BMI observed between individuals with versus without hypertension was merely a consequence of random chance. This risk is no doubt lower than the specified significant level of 5%. Hence, we can confidently reject the null hypothesis and conclude that the mean BMI in individuals with hypertension is significantly higher than the mean BMI in normotensive individuals. However, as there is still a chance that the null hypothesis is true (even though it is very small), we can never entirely rule out that the difference we measured occurred by random chance.

Significance level versus statistical power

As mentioned previously, the custom in epidemiological research is to set the significance level to 5%. It is believed to be a reasonable level of risk where we confidently can reject the null hypothesis. However, with a p-value just below 0.5, there is still almost a risk of 1 in 20 that the difference between groups that we observed is merely a random finding (if the null hypothesis is true). For example, in certain genetic research such as the field of hereditary genetic diseases, this risk is often regarded as too high, and the level of significance might be set much stricter. Hence, the p-value is arbitrary and should be interpreted as such.

When rejecting the null hypothesis when it is true, we commit a *type 1 error*. Conversely, when not rejecting the null hypothesis when it is false, we commit a *type 2 error*. These two are closely related. When we decrease the level of significance to further protect us from type 1 errors (as in the field of genetics), the risk for type 2 errors increases, and vice versa. In general, we are more cautious of committing type 1 errors, as we do not want to advocate for associations that do not

exist. On the other hand, type 2 error means that we can miss clinically relevant differences between comparison groups. Encouraging, we can decrease the risk of type 2 errors just by increasing the study sample, without changing the level of significance and the subsequent risk for type 1 error. By doing so, we enhance the *power* of the study, i.e., increase the possibility of detecting relevant differences between comparison groups.

Confidence intervals

Implying statistical inference by p-values is predominately done when using statistical tests to investigate the difference in mean values between two groups, as in the example above with BMI. However, when available, presenting a *confidence interval* (CI) instead of a p-value is preferable as it gives us more specific information about the uncertainty of a measured value. In other words, a better description of the precision of the results in our study.

The differences between comparisons groups that we obtain in a statistical test or the magnitude of an association between a risk factor and an outcome the statistical model yields, is always correct only for that particular study sample. However, what we really wish to know is what these values truly are in the population from where we collected our sample; the population that we wish to extrapolate our findings to. That we can never know for sure, but the CI can be helpful. Using a 95% CI is the general rule in epidemiological research. However, one can set the level of confidence at any level that one finds appropriate, analogous to the significance level (α). The 95% CI is defined by the mean value or the point estimate of a measure of effect, plus or minus two standard errors. This renders a range within which the true value is likely to lie with 95% certainty. To be a little more exact, if we identically repeat the study a hundred times, 95 of these times will yield a 95% CI expected to cover the true value in the population [9]. This is commonly interpreted as a 95% probability that the CI that we obtained in our one study contains the true value in the population, but should probably better be described as "we are 95% confident that the true value lies between these limits".

To illustrate with an example, we estimate the difference in mean BMI between individuals with and without hypertension in a sample from my hometown in northern Sweden. This yields a difference in mean of 1.12 (a 12% higher mean BMI in individuals with hypertension), including a 95% confidence interval of 1.05-1.30. With 95% certainty, we can say that the true difference in mean BMI in the citizens of my hometown lies in the range of 1.05-1.30, with the most probable value being 1.2 (the *point estimate*). As all values in the confidence interval lie above 1, we interpret this as a statistically significant higher BMI in individuals with hypertension. However, suppose the confidence interval in this example

included values both below and above 1. In that case, the true estimate might be either a higher or lower BMI in individuals with hypertension, which usually is interpreted as a non-significant result equivalent to a p-value >0.05. Noteworthy, a confidence interval of 0.95-1.65 still tells us that a higher mean value is more likely than the reverse. In contrast, a confidence interval of 0.45-1.05 is more likely to include a true value of a lower rather than a higher mean value. This illustrates the advantage of the confidence interval compared to the p-value.

A narrow confidence interval means a better precision, a wider confidence interval a higher degree of uncertainty of where the true value might be. Different factors affect the width of the confidence interval. Larger sample sizes reduce the general uncertainty by decreasing the influence of random errors, which produces narrower confidence intervals. On the contrary, if the variability in the sample is high (for example, the BMI values differ vastly between individuals), the confidence interval will be wider.

Clinical significance and evidence

Statistical significance is not the same as clinical significance. This means that whatever statistically significant results we get, these do not automatically translate to a difference in effect between treatment options, or an increased risk for the onset of a disease due to a specific risk factor, that is of a clinically relevant magnitude. Likewise, statistical inference is not the same as causal inference. When implying the latter, we need to consider the whole design of the study and its risk for bias.

In summary, the findings in a study can be either due to bias, due to chance, or represent a true relationship between the exposure and the outcome. As we never fully can rule out the risk of bias in observational studies, and we cannot completely rule out the risk of the statistical results being due to chance, a wise rule when drawing conclusions from observational epidemiological studies is to be cautious with claiming causality. This is the reason for the more humble and frequent use of the word *association* when describing findings of a relationship between exposures and outcomes from observational data. Finally, due to the inherent limitations in one single study, the foundation of evidence lies in the collective effort of the research community considering the reports from several studies. It is the gradual accumulation of evidence from several studies with results pointing in the same direction that eventually leads to an emerging consensus.

Measuring disease

Incidence

The fundamental of epidemiology is to observe and measure disease occurrence, including all kinds of illnesses and health outcomes. Two basic measurements are *incidence* and *prevalence* which will be described further in this section.

Incidence is the number of new people who get afflicted by a disease during a given time, or the rate of occurrence of new cases of the disease. Thus, incidence can be described as either a proportion or a rate.

The *incidence proportion*, also known as the cumulative incidence, is synonymous with the *risk* of getting afflicted by the disease**.** For example, hypothetically, 10 of the 200 patients with hypertension that I care for at my health care center, were afflicted by a cardiovascular event during the last year. In other words, 5% of my patients with hypertension suffered from a cardiovascular event last year, which could be interpreted as a 5% risk of a cardiovascular event per hypertensive patient, per year.

The *incidence rate*, on the other hand, is an instantaneous concept; a momentary rate at which diseases or events occur within a group of people. The advantage of the incident rate is that it considers when people are lost to follow-up in a study. Some of my hypertensive patients might have been very old and died before the year was ended, and some might have moved from town or preferred to be followed by another physician at another health care center. Hence, I would not have had the privilege to follow the entire population of 200 patients during that whole year. As such, it would be wiser to calculate the exact time that each patient contributed with during my little one-year study, which will render a total amount of *person-time* during follow-up. Person-time is the product of the total number of people multiplied by the total time these people were observed, implying the actual time when the study sample is at risk of experiencing the event. Person-time can be converted to any appropriate unit, such as *person-days*, or, more commonly, *person-years*. When calculating the incidence rate, the person-time is used as the denominator instead of the total number of people at the start of the study that we used when calculating the incident proportion (see Table 1). As seen in Table 1, the incidence rate is slightly larger than the incidence proportion due to the difference in the denominator. The incidence proportion tends to underestimate the risk of an outcome if several study subjects are lost to follow-up, since these subjects can no longer contribute to an outcome in our study.

While the incidence proportion can be described as a risk of 10 in 200 to suffer from a cardiovascular event, the incidence rate will be described as 0.06 cardiovascular events/person-years (i.e., 6 cardiovascular events per 100 person-

years). Let us compare this with speed while driving. If driving at 50km/h, one will travel 50 km every one hour as long as the speed is kept steady. Accordingly, with an incident rate of 6 cardiovascular events/100 person-years, 6 people with hypertension will suffer from a cardiovascular event for every 100 person-years that pass. How long 100 person-years are measured in calendar years depends on the size of the population. A population of one person will need to be followed for 100 whole years to complete 100 person-years, but a population of 200 people will take only half a year to complete this time (100/200 = 0.5) since each person contributes to the accumulated time.

In summary, although the incidence proportion is more intuitively understood, it is preferable to calculate the incidence rate in situations where a significant amount of lost to follow-up is anticipated in a study.

Table 1.

Incidence proportion		Incidence rate	
Population	200	Time at risk	62400 person-days ≈ 171 person-years
Events	10	Events	10
Incidence proportion	10/200 = 0.05	Incidence rate	10/171 = 0.05847 ≈ 0.06 person-years ≈ 6 per 100 person-years

Prevalence

Prevalence is a measure of disease status, i.e, the proportion of people affected by a disease at a certain time. It is calculated by dividing the number of people in the study who are affected by the disease at a specific time with the total study sample. The prevalence of the disease is highly affected by the incidence and the duration of the disease. For instance, an infectious epidemic such as the coronavirus in 2020, spread fast and affect many people, but only for a short while as people normally recovered after a few days or weeks. Hence, the prevalence was high during the winter but rapidly declined during summer when the incidence rate decreased markedly. On the other hand, hypertension, which has both a high incidence and is a chronic disease, will have a high and stable prevalence as long as the condition cannot be cured. Diseases with a high prevalence, such as hypertension, diabetes, obesity, and osteoporosis, tend to cause a high burden in society, both with regards to the individuals affected and the economic burden related to high costs for the health care system.

Measuring differences in risk between groups

Estimates of prevalence and incidence are the core of descriptive studies. Analytic studies, however, aim to compare how these measurements differ between groups of individuals with different exposures. For instance, not only is it desirable to know the proportion of patients with hypertension that risk to suffer from a cardiovascular event, but also if and how much the risk differs from patients without hypertension. In the following section, the basics of some of the measurements that can answer these questions will be described.
Table 2 presents an illustration of such measurements for the hypothetical sample of hypertensive patients at my primary health care center.

First, there is the *risk ratio*, commonly referred to as *relative risk*. The risk (incidence proportion) of suffering a cardiovascular event in the hypertensive patients at my primary health care center was 5%, and the equivalent risk in patients without hypertension was 2%. By dividing the risk in the exposed group by the risk in the unexposed group, the relative risk is obtained. In this case, the relative risk would be 2.5. This means that patients with hypertension had 2.5 times the risk of a cardiovascular event compared to patients without hypertension.

Secondly, there is the *incident rate ratio,* or simply *rate ratio*. This is also a relative measurement, but now comparing the incident rate between patients with and without hypertension. By dividing the incident rate in hypertensive patients by the incident rate in patients without hypertension, an incident rate ratio of 3 is obtained. As seen in Table 2, the incident rate ratio is higher than the risk ratio, reflecting the fact that the former has taken into account the amount of loss of person-time in each group due to lost to follow-up. Two other relative risk measurements are used in the studies in this thesis: *odds ratio* and *hazard ratio*. These will be described further in the methodological section ahead, but the concept of these is comparable with the measures of ratio described here.

Third, there is the *risk difference.* In contrast to the relative risk measurements, this is a measurement of absolute risk. The relative risk can be very high although the absolute risk for an outcome is very small. Hence, it can be wise to calculate and present both relative and absolute measurements. The risk difference (or the absolute risk reduction) is calculated by subtracting the risk (incidence proportion) among the exposed with the risk among the unexposed. In the illustrative example (Table 2), a risk difference of 3% is obtained. This means that patients with hypertension have a 3% higher risk per year of experiencing a cardiovascular event compared to patients without hypertension. This may appear less intimidating than the 2.5 times higher relative risk. However, according to the high prevalence of hypertension globally, such an increase in absolute risk could rapidly translate into a significant amount of cardiovascular events each year. An important advantage of the risk difference measurement is that it can be converted into an estimate of the

absolute number of people that can be prevented from a cardiovascular event if hypertension could be cured. By inverting the risk difference, the *number needed to treat* (NNT) is achieved. In the illustration, an NNT of 33 (1/0.03=33) is obtained, meaning that hypertension needs to be cured, or the blood pressure normalized, in 33 patients to prevent one cardiovascular event in one year.

Table 2.

	Hypertension YES	Hypertension NO
Number of patients	200	750
Number of cardiovascular events	10	15
Incidence proportion (risk)	10/200 = 0.05 = 5%	15/750 = 0.02 = 2%
Incidence rate	10/171 person-years = 0.05847 ≈ 0.06 person-years ≈ 6 per 100 person-years	15/698.6 person-years = 0.02147 ≈ 0.02 person-years ≈ 2 per 100 person-years
Risk ratio (relative risk) $Risk^{exposed}/risk^{unexposed}$	0.05/0.02 = 2.5	0.02/0.05 = 0.4
Incidence rate ratio Incident $rate^{exposed}$/incident $rate^{unexposed}$	0.06/0.02 = 3	0.02/0.06 ≈ 0.33
Risk difference Risk ^{exposed} - risk ^{unexposed}	0.05-0.02 = 0.03 = 3%	0.02-0.05 = -0.03 = -3%
Number needed to treat (NNT) 1/ risk difference	1/0.03 = 33	1/-0.03 = -33

1.2 BACKGROUND: HYPERTENSION, ATHEROSCLEROSIS AND FRACTURES

Hypertension: definition and prevalence

Hypertension is generally defined as blood pressure (BP) repeatedly ≥140/90 mmHg. In 2017, the American guidelines changed this definition to a BP ≥130/80 mmHg, whereas the 2018 European guidelines kept the former definition [10, 11]. However, the treatment goals and strategies in these guidelines are similar, and both recommend a systolic BP target of 130 mmHg for most patients. Most patients with elevated BP (~90%) have primary hypertension, where no specific cause can be identified. Nevertheless, hypertension is highly related to aging, and besides predisposing genetic factors several modifiable risk factors such as sedentary lifestyle, overweight, smoking, and alcohol, contribute to the presence of hypertension [10].

Specific etiology apart, hypertension is immensely common. In 2019, hypertension was estimated to globally affect around one-third of all adult men and women aged 30-79 years [12]. More strikingly is the prevalence in the elderly, where it is more common to have hypertension than not, estimated to affect more than 60% of the people over the age of 60 years [13]. In Sweden, the estimated age-adjusted prevalence of hypertension in 2019 was 24,5% in women and 35,6 % in men, similar to other high-income European countries [12]. As these numbers illustrate, in high-income countries, hypertension is still more common in men than in women, but the gender differences decrease with age, and in low-income countries, the differences between gender are even lower [14].

Elevated BP is mainly an asymptomatic condition. Therefore, around half of the people affected by hypertension are estimated to be unaware of their high BP, and less than half of the people who receive treatment reach target BP [12]. Although people with hypertension may feel perfectly fine, the long-term consequences are the more detrimental, with a substantially increased risk for stroke, myocardial infarction, heart failure, peripheral arterial disease, and renal failure. Hypertension was ranked as the leading risk factor of premature death in 2019 [15] and has been estimated to cause more than 40% of worldwide deaths from cardiovascular diseases (CVD) as well as chronic kidney disease and diabetes [12, 16].

The diagnosis and management of hypertension are primarily conducted in primary health care and constitute a substantial part of the daily work of the general practitioner.

Antihypertensive drugs

Lifestyle interventions should always constitute a foundation when treating hypertension and preventing cardiovascular events. Such interventions include smoking cessation, recommendations for physical activity, a healthy diet, and weight control. Nevertheless, due to the widespread heredity for elevated BP, the aging of the population, and the common failure of keeping to a healthy lifestyle, most patients will still require drug therapy to achieve optimal BP control. Hence, in line with the high prevalence of hypertension, antihypertensive drugs are among the most prescribed drugs globally. The most common classes of drugs that decrease BP are described below.

Thiazide diuretics (thiazides)

Thiazide diuretics (from here on referred to as *thiazides*) were developed in the 1950s and have since then played an important role in treating hypertension. Thiazides are inexpensive and proven in several large RCTs to effectively reduce cardiovascular morbidity and mortality [10, 17, 18]. In the "thiazide family", there are two major thiazide compounds; hydrochlorothiazide and bendroflumethiazide, which share pharmacodynamics and pharmacokinetics, yet not the same generics. In addition, there are a few "thiazide-like" drugs, the most common one being chlorthalidone, which mainly shares the exact mechanism of action as other thiazides but also affects some additional biological pathways. Thiazide-like drugs were used in some of the well-known RCTs, but as of today are prescribed more seldom (at least in Sweden).

Thiazides inhibit the Na^+/Cl^- cotransporters in the distal convoluted tubule of the kidney. The immediate effect is reduced sodium uptake and increased urine volumes, reducing extracellular fluid and cardiac output, causing a decline in BP. However, this initial effect is short-lived, and the mechanism behind the long-term decrease in BP is still unclear [19, 20]. Thiazides also affect the uptake of other electrolytes, which might lead to elevated serum levels of potassium, magnesium, and calcium. As such, thiazides can lead to hyponatremia or hyperpotassemia, which might have serious consequences if severe and not discovered in time. Also, thiazides are ineffective in moderate to severe renal impairment (eGFR<30) [10]. Hence, patients are often structurally followed with blood tests to assure normal levels of electrolytes and sufficient renal function. Otherwise, thiazides are regarded as a safe medication with minor side effects.

Inhibitors of the renin-angiotensin-aldosterone system

Today, two of the most prescribed antihypertensive drugs are the angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB), which became increasingly popular after their broad introduction in the 1990s. These drug classes affect the renin-angiotensin-aldosterone system, a hormonal feedback loop that effectively increases BP when sensitive receptors in the kidney react to a physiologically threatening decrease in blood flow. In short, when blood flow drops, the kidneys produce renin which leads to increased levels of angiotensin II, which in turn causes vasoconstriction, enhanced levels of antidiuretic hormone (which stimulates thirst and appetite for salt), and release of the hormone aldosterone from the adrenal glands. Aldosterone then stimulates sodium reabsorption in the kidney, which results in water retention and increased blood volume. However, in hypertensive individuals, this hormonal pathway could be said to be overactive compared to physiological demands, contributing to a constantly inappropriate high BP. [21, 22]

ACEi decreases the levels of angiotensin II and by doing so, blocks the cascade of events described above, which leads to normalized BP. ARB on the other hand, directly block the receptors of angiotensin II and, thus, inhibit the similar cascades of biological responses as ACEi. In addition, both ACEi and ARB improve heart failure, are renoprotective in patients with diabetes, decrease proteinuria, and slow disease progression in patients with chronic kidney disease. Hence, although these drugs are primarily used to manage hypertension, they are also prescribed for further indications. Treatment with ACEi and ARB can lead to high serum potassium levels, and in rare cases, cause renal failure. Hence, patients should be followed with blood tests like those under thiazide treatment. [10, 22]

Aldosterone receptor antagonists, also known as mineralcorticoid receptor antagonists (MRA), effectively reduce the effect of aldosterone. By doing so, sodium reabsorption is reduced and water excretion enhanced, leading to a diuretic effect that lower BP. As these drugs are potent diuretics, they are also used to treat edema and heart failure. An evident downside is that electrolyte disturbances are relatively frequent and more pronounced than from thiazides, ACEi and ARB. Importantly, MRA is the drug of choice when treating secondary hypertension due to primary aldosteronism [10].

Beta-blockers

In the early 1960s, James Black synthesized the first beta-blockers (BB), which he later achieved the Nobel prize for. In 1975, the Swedish company AstraZeneca launched the first cardio-selective BB called “Seloken”. After that, BB had a long

period among the first-line antihypertension drugs, maybe even more so in Sweden. BB inhibit β-adrenergic receptors, thus blocking the sympathetic nervous system's stress hormones adrenaline and noradrenaline. Cardio-selective BBs (or simply "selective BB") primarily inhibit the β_1-adrenergic receptors, located mainly in the heart. By doing so, both the heart rate and the inotropy and chronotropic effect of the heart are reduced, leading to lower heart rhythm and cardiac output. Also, a subsequent decrease in BP is achieved (additionally mediated by the effect of blocking β_1-adrenergic receptors in the kidney, leading to decreased renin levels). Less selective BB also affect other types of β-adrenergic receptors found in several tissues, such as vascular smooth muscle cells. Cardio-selective drugs are the most commonly used BB today, mainly for controlling heart rate in tachyarrhythmias such as atrial fibrillation and as a cornerstone in treating heart failure and coronary heart disease. Although the blood lowering effect of BB is effective, later RCTs have revealed a less preventive effect on stroke than other first-line drugs. Hence, BB is no longer a first-line choice for treating isolated hypertension. [10, 22]

Calcium channel blockers

Calcium channel blockers (CCB) have a relatively simple mechanism of action. They disrupt the movement of calcium through calcium channels which cause smooth muscle cells in the vascular wall to relax, leading to vasodilation that lower BP [22]. Like BB, CCB are a heterogeneous class of agents, but the far most used type are the dihydropyridine CCB. The non-dihydropyridine CCB slows the heart rate in addition to the reduction of BP and may be used to relieve symptoms of angina or tachyarrhythmias if BB is not suitable. CCB have, like thiazides, a long history, and a BP-lowering effect equal to thiazides, ACEi, and ARB [10]. An advantage is that the drug does not affect serum levels of electrolytes. In contrast, pedal edema is a relatively common side-effect that can cause substantial discomfort for the patient.

Loop diuretics

The most potent diuretics available are the loop diuretics, with a rapid onset and action within an hour. As such, these drugs constitute a foundation in the treatment of conditions with edemas, such as symptomatic heart failure. Loop diuretics inhibit sodium/potassium/chloride transporters in the thick ascending limb of the loop of Henle (anatomically before the distal tubule where thiazides act), resulting in free water excretion from the interstitium, eliminated by enhanced urinary volumes [23]. The blockage of the ion-transporters also results in urinary loss of sodium, potassium, magnesium, and calcium, which may lead to problematic electrolyte disturbances [22, 23]. Serious side effects are generally more common from loop

diuretics than several other antihypertensives. Therefore, although the reduced fluid volumes lead to reduced BP, loop diuretics are only recommended for the treatment of hypertension when other drugs are insufficient. For example, when thiazides can no longer be used due to impaired renal function (loop diuretics are much less dependent on renal function). Although widely used and recommended for the treatment of heart failure, current evidence indicates that the use of these drugs, especially in high doses, is associated with increased all-cause mortality and hospitalization rates, also when assessing adjustments for bias related to the severity of heart failure [23, 24].

Pharmacological guidelines for managing hypertension

Current ESC/ESH Guidelines (2018) recommend using either an ACEi, ARB, CCB, or thiazide as the initial drug for the management of uncomplicated hypertension [10]. This recommendation is based on evidence from placebo-controlled studies and confirming meta-analyses, proving comparable ability to reduce BP, cardiovascular events, and overall cardiovascular morbidity and mortality [10]. In resistant hypertension (commonly defined as elevated BP despite treatment with three different antihypertensives), the recommendation is to add an MRA, another diuretic, or BB. Although BB is no longer considered a first-line drug for isolated hypertension, it should be considered during any step of treatment if there is a specific indication besides hypertension, such as heart failure, coronary heart disease, or atrial fibrillation. Likewise, loop diuretics can be used when complementary indications exist, or insufficient effects of other drugs.

Some of the challenges related to antihypertensive treatment are the low adherence to medication and the fact that more than one drug often is needed to reach target BP [25]. This might be frustrating for the physician in clinical practice and leads to poor BP control for the individual, but it also causes methodological challenges when conducting observational studies, such as the risk for misclassification and difficulties to compare different treatments groups when several treatments are used simultaneously.

Arteriosclerosis versus atherosclerosis

Arteriosclerosis is synonymous with arterial stiffening, strongly associated with aging [26]. Diabetes mellitus and renal failure have been postulated as specific risk factors, whereas hypertension acts as both a risk factor and a consequence of arteriosclerosis [26-28]. Arteriosclerosis arises due to alterations in the media layer of the arterial wall, with loss of elasticity and ultimately calcification [26]. Already in the 16th century, arteriosclerosis was described as “a degeneration of arteries into

bone" [26]. Although strongly associated with an increased risk for CVD, the mechanism underlying this association is not yet fully understood.

Atherosclerosis, on the other hand, refers to the specific condition of accumulation of fatty and fibrous material in the intima layer of the vascular wall that constructs atherosclerotic plaques (Figure 6) [29]. This can cause critical narrowing of the arteries (stenosis) or a sudden thrombosis due to a ruptured plaque leading to an acute blockage of the blood flow, commonly revealed in the clinic as a myocardial infarction or stroke. Calcification of the atherosclerotic plaques eventually emerges in the disease's late stages, like for arteriosclerosis, which may increase the risk of a plaque rupturing [26]. Inflammatory cells are important mediators in forming atherosclerotic plaques, and biomarkers of inflammation such as C-reactive protein (CRP) prospectively predict atherosclerotic diseases [29]. Other main risk factors are high levels of low-density lipoprotein (LDL) cholesterol, smoking, visceral obesity, hypertension, and diabetes mellitus. Atherosclerosis is a systemic condition affecting several vascular beds. Hence, if you have atherosclerosis at one site, you most often have it at other locations.

Although distinct definitions, atherosclerosis can be seen as a type of arteriosclerosis, and atherosclerosis often accompanies arteriosclerosis. Both vascular alterations contribute to the significant burden of CVD worldwide, which still comprises the number one cause of death from a global perspective [30].

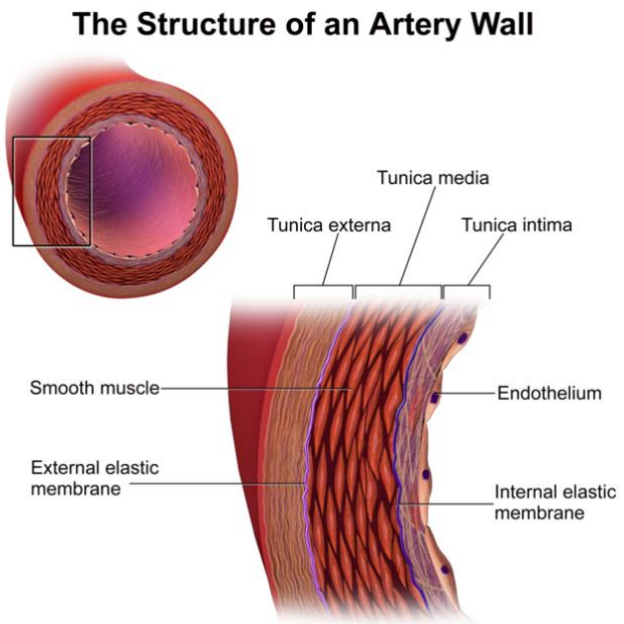


Figure 5. *Microscopic anatomy of the artery wall*

Illustration: Blausen.com staff (2014).
https://commons.wikimedia.org/wiki/File:Blausen_0055_ArteryWallStructure.png

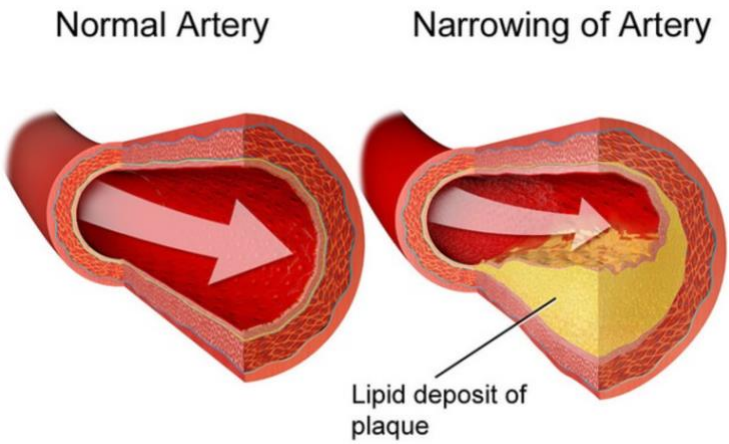


Figure 6. *Atherosclerotic plaque narrowing an artery, causing a blockage of blood flow.*

Illustration: Blausen.com staff courtesy of Oregon State University, CC BY-SA 2.0
https://commons.wikimedia.org/wiki/File:Atherosclerosis.jpg

Peripheral arterial disease – epidemiology and burden of disease

Peripheral arterial disease (PAD) (also termed peripheral artery disease or lower extremity artery disease) is the atherosclerotic occlusive disease of the lower extremities. PAD usually appears late in adult life, with a sharply increased prevalence with advancing age, from about 5% in middle-aged men and women to about 20% by the age of 85 [31]. As atherosclerosis is a systemic disease, other atherosclerotic manifestations are highly present in individuals with PAD. For instance, it has been estimated that 25-70% of individuals with PAD have concomitant coronary artery disease [32]. Other cardiac conditions, such as heart failure and atrial fibrillation, are also associated with PAD [32]. In addition, PAD has been associated with an increased risk of all-cause mortality, also in the absence of concomitant CVD [33].

Diagnosis and symptoms

The recommended diagnostic tool for PAD is the measurement of ankle-brachial-index (ABI), see Figure 7. ABI measurements are non-invasive, inexpensive, and require little time to perform, but adequate training is essential for carrying out the examination correctly. PAD is defined as an ABI $\leq$0.9 or <0.9, depending on the literature (Figure 8). An ABI < 0.90 has been proposed to be approximately 72% sensitive and nearly 99% specific for angiographically significant PAD [34]. An ABI $\geq$1.40 is also considered abnormal, representing incompressible arteries due to arterial stiffening, especially common in patients with concomitant diabetes [32]. In such cases, or when an ABI measurement is difficult to obtain, toe pressure can be used.

PAD leads to decreased blood flow in the lower limbs, causing pain and discomfort. The most typical symptom is intermittent claudication, defined as exertional calf pain that resolves within 10 min of rest. However, only about 10% of individuals with PAD have these classic symptoms [35]. It seems as atypical symptoms might be even more common, with more diffuse pain or discomfort in the leg, buttock, or thigh during exercise or rest, with figures ranging up to 50% of individuals with PAD depending on study design and sample selection [35-37]. The most severe stage of PAD is chronic limb-threatening ischemia, defined by ischemic pain also at rest, with or without ulcers, gangrene, or infection in the lower limb, which significantly increases the risk for amputation. It is a clinical diagnosis, but the risk often increases with decreasing ABI or toe pressure.

Most individuals with PAD, however, are asymptomatic. Noteworthy, among these, symptoms might be masked due to a reduced physical

capacity secondary to other CVD such as heart failure, or reduced pain sensitivity secondary to diabetic neuropathy, leading to an incapacity to reveal any leg symptoms while walking.

Both symptomatic and asymptomatic PAD have been associated with poorer physical function (chair stand, walking speed, handgrip strength, standing balance), adverse calf muscle characteristics, and poorer quality of life [37-39]. These findings have also been confirmed in comparison with a sedentary, asymptomatic, age-matched group of non-PAD persons [39] and independently of concomitant CVD [38].

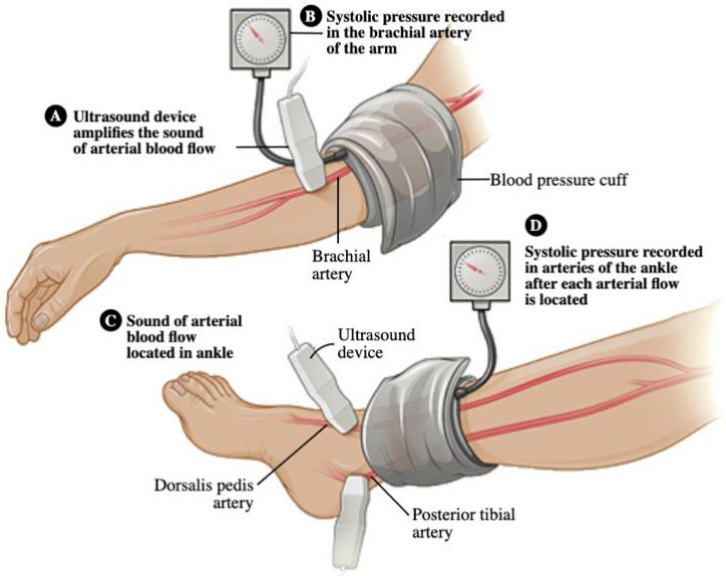


Figure 7. *The illustration shows the ankle-brachial index (ABI) test, comparing blood pressure in the ankle to blood pressure in the arm. ABI = ankle systolic blood pressure / brachial systolic blood pressure.*
Illustration: National Heart Lung and Blood Insitute (NIH)
https://www.nhlbi.nih.gov/health/health-topics/topics/pad/diagnosis

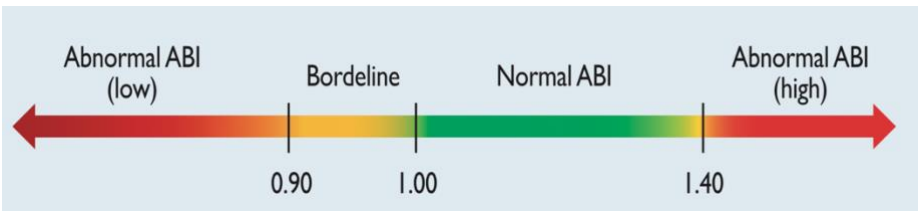


Figure 8. How to interpret ABI? *PAD diagnosis should be based on the lowest ABI after measurements in both legs, interpreted as illustrated.*
Illustration: ESC Guidelines 2017 [32].

Pathophysiology

The atherosclerotic lesions in the peripheral arteries cause decreased blood flow with subsequent ischemia in the lower limbs. This reduced blood flow is most evident during walking or similar exercise when the working muscles have higher demands for oxygen supply, causing symptoms like intermittent claudication described above. Individuals with PAD also present an abnormal vasodilator response, where the vessels cannot dilate appropriately during the demand of exercise, worsening the effect of the atherosclerotic stenoses on the blood flow. Furthermore, the muscle ischemia during exercise with the following reperfusion during rest seems to trigger a cascade of physiological responses resulting in further tissue injuries, such as increased levels of inflammatory mediators, endothelial dysfunction, and muscle atrophy. Hence, although the leading cause of impaired exercise capacity in individuals with PAD is limited blood flow due to atherosclerotic lesions, the pathophysiology seems to be more complex. This might explain some of the heterogeneity in symptoms in this population, regardless of the ABI value. [40]

Risk factors

The most well-documented and strongest risk factors for PAD are hypertension, diabetes, and smoking [31, 41, 42], but other risk factors for atherosclerosis mentioned earlier are risk factors for PAD as well. Noteworthy, kidney dysfunction is ranked number four on the list of risk factors for PAD [42]. Smoking is a well-known risk factor for atherosclerotic disease in general, but a particularly strong risk factor for PAD, with about a 3-fold risk to develop PAD among current smokers compared to non-smokers [31, 41]. Although the effect of smoking slowly declines after cessation, former smoking remains a risk factor for PAD.

Treatment

Regardless of symptoms, individuals with PAD are at high risk for cardiovascular events [32, 42]. Hence, when identifying PAD, the main target is to decrease the overall burden of atherosclerotic disease and prevent cardiovascular events. According to the 2017 ESC guidelines, all patients with PAD should be recommended treatment with statins, and antihypertensive medication if elevated BP [32]. Statins improve the cardiovascular prognosis but have also been shown to significantly improve walking distance and reduce pain. Antiplatelet therapy is recommended only in patients with symptomatic PAD, where clopidogrel may be preferable over ASA [32]. Lifestyle interventions including smoking cessation, should of course, be promoted.

Specific treatment options for PAD are, however, limited. Physical exercise, preferable supervised, is the golden standard in symptomatic patients. Exercise does not improve ABI but significantly improves pain-free and maximum walking distance and quality of life [32]. The next step in treatment is revascularization by endovascular methods or open surgery. These interventions are often effective in the short run but not without risks of complications, and the durability of the effect is limited. Hence, only patients with significant symptoms that interfere with daily life despite standard treatment are recommended for these interventions [32].

Bone

Bone is a highly dynamic tissue with several functions. The skeleton provides structural support to the body, protects vital organs, and constitutes the foundation to which muscle and tendons attach, allowing us to move efficiently. Bone is also a reservoir for minerals such as calcium and phosphate, and the skeleton has a central role in regulating calcium balance in the body. In addition, the bone marrow is the primary site for hematopoiesis (the formation of blood cells), and bone tissue are highly vascularized. Bone is divided into two major types: cortical bone and trabecular bone. Cortical bone comprises compact calcified tissue and forms the outer layer of bone, accounting for about 80% of the skeleton [43]. Trabecular bone is the interior part, made up of a large sponge-like network. The proportion of cortical versus trabecular bone differs between different sites. For instance, the vertebrae of the spine are mostly made of trabecular bone, whereas the femoral neck of the hip mainly consists of cortical bone [44].

Bone metabolism

Bone continuously modifies and regenerates during life according to hormonal regulation and the mechanical load put on the skeleton. This remodeling process involves bone cells that deteriorate bone: osteoclasts, leaving room for cells to form new appropriate bone structures: osteoblasts [43]. The interplay between these cells is intricate and involves serval signaling pathways. Dysregulation of this system plays a critical role in the development of osteoporosis [45]. However, the major part of bone cells is osteocytes. Osteocytes form a network of sensory channels in the bone matrix, detecting and reacting to physiological changes by conducting biochemical signals to promote bone growth and remodeling processes [43].

Parathyroid hormone (PTH) and Vitamin D are essential for calcium regulation in the body and hence, also for bone remodeling. PTH is released from the parathyroid glands as a reaction to low serum levels of calcium. PTH has three main

effects: 1) to stimulate bone resorption, which releases calcium and phosphate from bone, 2) to increase calcium and phosphate reabsorption in the kidney, and 3) to activate vitamin D to its active form, which in turn stimulates the absorption of calcium and phosphate from the intestine [46]. By doing so, serum calcium homeostasis is restored. High continuous levels of PTH inevitably lead to increased bone resorption and enhance the risk of osteoporosis. Vitamin D, on the contrary, indirectly promotes the strengthening of bone by maintaining serum calcium and phosphate in adequate concentrations to allow mineralization of bone matrix [44]. Hence, Vitamin D deficiency enhances the risk of osteoporosis by leading to lower calcium levels, which in turn stimulates the release of PTH, and the vicious cycle is on. Vitamin D, which we mainly get from sunlight but also from the food we consume, needs to be activated through two steps in the body, first by the liver (to 25-hydroxy (25-OH) vitamin D) and then by the kidney (to 1,25 dihydroxy (1,25-OH) vitamin D). Hence, adequate renal function is essential to keep the levels of active vitamin D normal. In fact, renal failure leads to decreased levels of 1,25-(OH)-D, affects the normal regulation of calcium and phosphate absorption and leads to continuously increased levels of PTH, which all have detrimental effects on bone tissue [46]. Noteworthy, although PTH promotes bone resorption when constantly secreted, it stimulates bone formation when given intermittently, which can be used in the treatment of osteoporosis [47].

Osteoporosis

Osteoporosis is defined as “a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture“ [48]. The prevalence of osteoporosis increases progressively with age and is more common in women than men, affecting about 21% of women aged 50-84 years, compared to about 6% of men [49].

The diagnosis of osteoporosis relies on the quantitative assessment of bone mineral density (BMD). The current golden standard for BMD measurements is dual X-ray absorptiometry (DXA) [43]. The DXA uses two low-dose x-ray beams with different energy levels that attenuate differently in bone and soft tissue. By measuring how much of the beams that pass through a particular area, BMD is calculated and expressed in g/cm^2. BMD is commonly measured as a total amount in the body and specifically at hip sites and the lumbar spine. DXA also distinguishes between fat mass and lean mass (muscles) and can be used to evaluate body composition. A limitation of DXA is the two-dimensional method which cannot differentiate between trabecular and cortical bone and evaluate the microarchitecture in the bone tissue. The current diagnosis of osteoporosis is defined as a BMD-value 2.5 standard deviations (SD) or more below the mean value in a young adult female

reference group, also referred to as a T-score of ≤2.5 SD [49]. Notably, although this definition was conducted for osteoporosis in postmenopausal women, the same thresholds are still used for men. This has been motivated by the fact that for any given BMD, the age-adjusted fracture risk does not differ too much between women and men [49, 50].

Osteoporosis is an asymptomatic condition, and the clinical significance of the disease lies in the fractures that arise. In this respect, osteoporosis is similar to hypertension, which is diagnosed by BP measurements, whereas the clinical important consequence is a cardiovascular event such as stroke.

Risk factors for osteoporosis and gender differences

The main risk factor for primary osteoporosis is age. Bone deteriorates with age, also with normal aging. In both men and women, BMD starts to slowly decrease from around the age of 40. Women lose bone mass rapidly after menopause, as a consequence of the abrupt decline in estrogen levels. This rapid bone loss continues for about 8-10 years before it slows down to a degree more like the age-related bone loss in elderly men. Low estrogen levels increase bone resorption by lengthening the life span of osteoclasts and decrease bone formation by shortening the life span of osteoblasts. The initial rapid decline in BMD primarily affects trabecular bone, with typical locations in the vertebrae, pelvis, and ultra-distal radius. Men on the other hand, which generally have a higher peak bone mass during young adult life (especially trabecular bone) and no abrupt drop in gonadal sex steroid secretion, have a slower and steadier rate of bone loss. Men lose about half as much bone with aging as women. However, men also seem to experience a decline in bioactive sex steroid levels in old age, both estrogen and testosterone, which probably act as an accelerating force of bone loss also in men. Increased levels of PTH during aging have also been observed in both men and women, which further could contribute to primary osteoporosis. [45, 50]

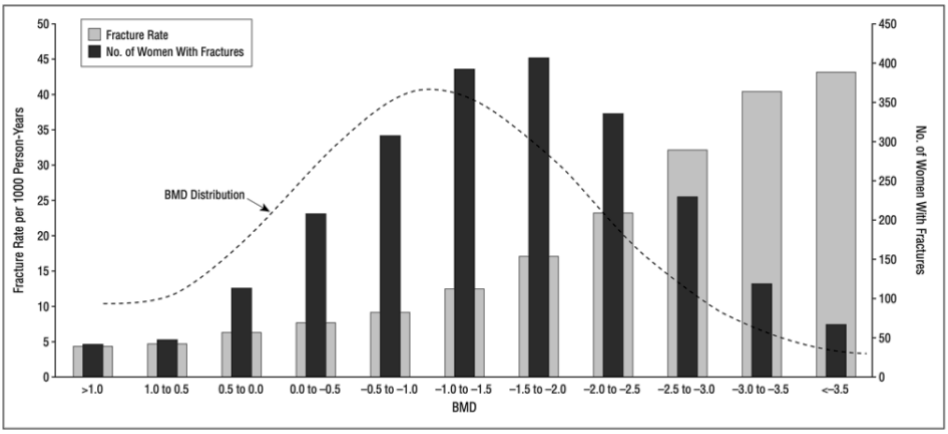
Nonetheless, osteoporosis is a multifactor condition, where both genetics and behavioral factors contribute. Immobility and smoking are examples of important risk factors for impaired bone health. Finally, osteoporotic fractures are predominantly a consequence of accidental injury, although the forces are small. Factors that affect the risk of falling are many: low functional performance, dizziness, and impaired vision are just a few examples. [51, 52]

Osteoporotic fractures

The most classical osteoporotic fractures are fractures of the hip, spine, distal forearm, and proximal humerus, together often referred to as major osteoporotic fractures (MOF). Women are particularly susceptible to distal forearm fractures during the decade after menopause, in line with the rapid loss of trabecular BMD at this site [49]. The risk of vertebral fractures increases more slowly, but instead exponentially during later life. Hip fractures mainly occur at older ages, both in women and men, and are the predominant osteoporotic fractures from the age of 75 years [49]. Men generally begin to suffer from vertebral and hip fractures about 10 years later than women, and distal forearm fractures are overall much rarer [51, 52]. The risk for fractures is overall higher among Caucasian men and women, and Sweden has among the highest incidence of osteoporotic fractures in the world. It has been estimated that about 46% of Swedish women aged 50 years and older will suffer from any kind of MOF sometime during their remaining life [53]. In men, the equivalent risk is about 22% [53].

Although certain sites of fracture are more strongly associated with osteoporosis, the disease is a systemic condition, and individuals with low BMD are at higher risk for almost all types of fractures [49]. At the same time, although low BMD is the number one risk factor for fractures (apart from age) [54], many individuals that experience a fracture at any of the classical osteoporotic sites do not have osteoporosis. In a large cohort study of postmenopausal women not using any osteoporotic treatment, 82% of those who experienced a MOF during follow-up had normal BMD (Figure 9) [55]. Hence, defining osteoporotic fractures might not be straightforward, and you may find alternative nomenclature for these problematic fractures among older individuals, such as “low energy fractures” or simply “fragility fractures”.

Other established risk factors for osteoporotic fractures are low BMI, smoking, alcohol, use of oral glucocorticoid steroids, low physical function, inflammatory diseases such as rheumatoid arthritis, diabetes, severe renal failure, previous fractures, and parental history of fracture [49, 56]. Based on such risk factors, predictive tools for assessing fracture risk have been developed. The most well-established tool is FRAX®, which is a web-based algorithm that calculates the 10-year risk for MOF and hip fractures, with or without BMD values [57]. FRAX® is widely used to guide treatment decisions in the clinical setting.



Bone mineral density (BMD), osteoporotic fracture rate, and number of women with fractures.

Figure 9. *The incidence rate and absolute numbers of osteoporotic fractures in relation to BMD level, in 149 524 postmenopausal white women (mean age 64.5 ± 9.3 years). [55] Reproduced with permission from American Medical Association, 2004.*

The burden of osteoporotic fractures

In 2010, it was estimated that almost 9 million osteoporotic fractures occurred annually worldwide (approximately 1000/hour)[49]. The incidence differs a lot between regions, so despite the lower population, about 34% of these fractures occur in Europe [49]. Osteoporotic fractures cause acute pain and disability and have even been associated with increased mortality. Hip fractures are the most severe. It has been reported that up to 20% of patients die during the first year following a hip fracture, with higher mortality rates among men [49, 58]. Although the mortality rates most often result from serious comorbidity, almost a third of these deaths have been estimated to be causally related to the fracture [49].

In other cases, fractures cause permanent loss of function and the need for assistance at home or in nursing homes, resulting in lower quality of life for the individual and major costs for society. Less than half of the survivors after a hip fracture regain the functional level they had prior to the fracture [49]. Concurrent with the demographic shift towards an aging population, this morbidity and economic burden from fractures will probably continue to increase.

Osteoporosis and fracture prevention – treatment options

Earlier, hormone replacement therapy had an important role as a treatment for osteoporosis in postmenopausal women, but as major risks for CVD and breast cancer are involved with this treatment, it is no longer recommended. Today, the main treatment for osteoporosis is bisphosphonates, which are given together with calcium and vitamin D supplements. Bisphosphonates reduce the activity of osteoclasts and increase their apoptosis. Additional agents are raloxifene (a selective estrogen receptor modulator), denosumab, strontium ranelate, and parathyroid hormone peptides. Most of these agents reduce fracture risk fairly similar, with around 30-70% for vertebral fractures and 15-30% for non-vertebral fractures [49, 59]. However, studies have reported that only a minority of men and women that are susceptible to fractures are receiving any of these medications. The adherence to treatment is also poor, about 50% discontinue treatment within 1 year [49].

1.3 PREVIOUS STUDIES AND KNOWLEDGE GAPS

Hypertension, atherosclerosis, bone, and fractures

Hypertension, atherosclerosis, osteoporosis, and frailty fractures, share several risk factors (as described in the former chapter 1.2). By affecting adults, especially the elderly, and the relationship with shared lifestyle factors, these conditions intuitively coexist. Whether these conditions also share pathophysiological pathways is more uncertain. At least, the calcification process in arterial stiffness and atherosclerosis resembles the bone formation process [60-62], and hypertension has been associated with alterations in calcium homeostasis with increased urinary losses of calcium and elevated levels of serum PTH [63, 64].

Hypertension and fracture risk

Let us begin with hypertension and its relationship with fractures. Studies have reported an association between hypertension and osteoporosis or reduced BMD in postmenopausal women [64-66]. Also, an association between increased risk for any fractures and hip fractures in both women and men has been observed [67-70]. However, also opposing results exist [71]. In addition, some of the confirming studies were limited by the lack of data on potential confounders which might contribute to biased results [67, 68]. According to the differences in the epidemiology of hypertension and osteoporotic fractures in men and women, hypertension might impact fracture risk differently across gender. The few studies that have analyzed fracture risk stratified by gender, found a significantly increased risk only in women [72, 73]. But as the study samples overall in the above-mentioned studies predominately comprised women, the gap in the evidence regarding men is larger. Thus, this thesis aimed to address this issue further, with respect to plausible explanation factors for an association between hypertension and fracture risk, and assess the question of whether hypertension is associated with fracture risk in elderly men (see Study IV in this thesis).

Peripheral arterial disease and fracture risk

An association between subclinical as well as clinical atherosclerotic diseases and bone loss or osteoporosis has been established previously [74-79]. A number of studies have also reported increased fracture risk associated with manifest CVD, especially stroke and heart failure [70, 74, 80-82].

In this context, we found PAD to be of certain interest, since it not only is a marker of general atherosclerosis but also represents a localization of vascular alterations that might affect bone tissue specifically in the hip region and lower limbs, of certain interest for the occurrence of hip fracture. In addition, there is a limited number of previous studies investigating PAD and hip fracture and they show mixed results [70, 83-87]. This implies room for further evidence. Since I was lucky to be granted access to data from a study of elderly men that are uniquely well-documented regarding both PAD status, bone measurements, and fracture outcome, we wanted to take the opportunity to investigate this relationship further (Study III).

Antihypertensive drugs – bone and fractures

In contrast to the limited research regarding hypertension as a risk factor for fractures, there are numerous prior studies assessing how antihypertensive drug treatment might affect bone tissue and the risk of fracture. This might reflect a general concern of long-term effects beyond the impact on BP and CVD outcomes due to the wide and chronic use of these drugs; a concern that also this thesis aspires to dive deeper into. Moreover, due to the risks that come with polypharmacy in the elderly, identifying additional bone-protective or fracture-reducing effects of already well-established and relatively safe and inexpensive antihypertensive treatment options could be valuable for the large number of people with high BP worldwide. With this in mind, a brief overview of available studies and research gaps that sparked the curiosity, hypotheses, and research questions for the two first studies in this thesis are provided below.

Thiazides – a bone-tissue preserving drug?

Thiazides modulate the reabsorption and excretions of electrolytes in the kidney. The waste of sodium is a fundamental effect, and as the thiazide-sensitive electrolyte transporters regulate calcium reabsorption inversely with sodium reabsorption, increased calcium absorption follows [88]. This is already utilized as a treatment for preventing the recurrence of calcium-containing urinary stones [89]. This knowledge has also raised the hypothesis that thiazides could act as a calcium-preserving drug that prevents bone mineral loss and osteoporosis. In addition, results from in vitro

studies and mice models have suggested that thiazides directly stimulate mineral formation by the expression of thiazide-sensitive Na^+/Cl^- co-transporters in bone cells [90, 91] and increase intestinal calcium absorption [90].

Already in 1983, Wasnich et al. found that individuals using thiazides had significantly higher bone mineral content compared to non-users [92]. Since then, several observational studies have confirmed these findings [93-98], but also conflicting results exist [99, 100]. A few small RCTs have also been performed, identifying a small but significant reduction of BMD loss with thiazide treatment compared to placebo among healthy postmenopausal women [101-104]. But how well is this beneficial effect on bone tissue translated into fewer fractures?

A known negative side-effect of thiazides is hyponatremia. Even though primarily mild and asymptomatic, hyponatremia could cause impaired cognitive function and impaired gait stability, associated with an increased number of falls and risk for subsequent fractures [105, 106]. Also, chronic hyponatremia is associated with osteoporosis [107]. Unfortunately, the incidence of thiazide-induced hyponatremia is poorly investigated [108]. However, in a case-control study including both men and women, hyponatremia in thiazide users was associated with an increased risk for hip and vertebral fractures compared to thiazide users with normal sodium levels, implicating that such side-effect might attenuate any beneficial effect on fracture risk from thiazide treatment [109].

Another risk is hypotension or aggravation of orthostatic symptoms, which might lead to falls and subsequent fractures. A relatively wide held belief is that antihypertensive drugs can increase the risk of falls, at least among the elderly and especially with diuretic treatment. However, the results in available studies have been somewhat conflicting and there is no current conclusive evidence for such an association. In a case-crossover study in a nursing home, a new prescription or dose-change of thiazides or loop diuretics was associated with a two-fold risk of falling early after drug initiation. However, only statistically significant for loop-diuretics [110]. Also, an association between long-term use of antihypertensive drugs and fall injuries has been reported [111]. However, recent meta-analyses have not confirmed these findings [112-115]. Kahlaee et al., conducting a meta-analysis of 29 observational studies, found an increased risk of falls during the first 24 hours after initiation of antihypertensive treatment, which was prolonged up to 21 days for diuretic users [113]. However, no association between longer antihypertensive treatment and falls was seen, regardless of drug class. Notably, only five of the included studies assessed time-dependency and could be used in the analysis based on days with treatment, and the effect estimates were rather heterogeneous between these studies. Hence, the results for long-term use are more robust. In addition, Albasri et al. recently (2021) conducted a meta-analysis of seven RCTs investigating outcomes from different antihypertensive treatments compared to placebo, with the primary endpoint being cardiovascular outcomes but additionally assessed the

occurrence of falls as adverse events [115]. Again, the pooled risk ratio revealed no association between antihypertensive treatment and risk of falls, regardless of drug class [115]. Hence, although side effects from treatment with thiazides might use might alter the risk for injurious falls in vulnerable individuals, a positive effect on BMD might be more prominent.

When the first study of this thesis was initiated, there was still some inconsistency in the results from previous studies investigating thiazide use and fracture risk. Three available meta-analyses based on observational studies reported decreased fracture risk with thiazide treatment, with pooled relative risk measurements ranging from 12-24% depending on fracture site [116-118]. However, the individual studies included in these analyses were not all concurrent. Moreover, there might be a relevant time-dependency of fracture outcomes with thiazide treatment since an effect on BMD probably takes some time, whereas negative side effects occur rather immediately.

With this background, we wanted to further investigate the association between thiazide treatment and fracture risk. This did not only lay the ground for the first study of this thesis, but also a ground for an interest in clinical research that led me through the years as a doctoral student.

Can RAAS-blockage decrease fracture risk?

Studies in animal models have suggested that inhibition of the angiotensin II signaling pathway may increase bone mass and bone strength [119], and improve fracture healing [120, 121]. However, angiotensin II has also been shown to exert stimulatory effects on osteoblastic cell lines which could promote bone development and remodeling [122], emphasizing a potentially complex role in bone tissue formation. Indeed, studies in humans have had difficulties confirming the hypothesis of RAAS-blockage as a pathway for improving bone composition and preventing fractures. Instead, previous studies have been quite inconsistent. Some studies have demonstrated a reduced risk for composite or hip fracture with either an ACEi or an ARB [123, 124] others have failed [125, 126]. A meta-analysis of 11 cohort studies showed no association between the use of ACEi or ARB and the risk of composite fractures, as well as no association with the risk of hip fracture in the pooled analysis of 4 cohort studies explicitly investigating the hip fracture risk [127]. Only two studies considered in this report investigated ACEi and ARB separately with hip fracture as an outcome [128, 129]. In the pooled analysis of these two studies, both the use of ARB and ACEi was associated with a reduced risk for hip fracture (RR 0.80; 95% CI 0.75–0.85 and RR 0.91; 95% CI 0.86–0.95 respectively) [127]. However, at least one of these studies was limited by no possibility to adjust for important factors such as comorbidity and concomitant drug use that might impact

the risk of falls and fracture [128]. Conversely, a meta-analysis based on six case-control studies assessing ACEi treatment for fracture risk, reported an increased risk for any fracture in ACEi users (pooled RR 1.27; 95% CI 1.01-1.60), with an even higher risk among individuals > 65 years of age [130]. However, as pointed out by the authors, the heterogeneity among studies was high and the results should therefore be cautiously interpreted. The overall inconsistent results in these previous studies might also reflect the fact that ACEi and ARB, despite their similar effects on the renin-angiotensin system and reduction of BP, might exhibit different impacts on bone tissue. Hence, further large studies investigating these drugs in relation to fracture risk, especially stratified between ACEi and ARB, are motivated.

Even less is known about the potential effects of aldosterone on bone. Lower BMD has been seen among patients with primary aldosteronism compared to controls [131], but others did not find such an association [132]. A longitudinal study with over 2500 patients with primary aldosteronism found an association with elevated fracture risk compared to hypertensive controls, but treatment with MRA only seemed to reduce the elevated fracture risk in men [133]. As the use of these drugs slowly increases, also in patients without diagnosed primary aldosteronism, any association with fracture risk would be relevant to evaluate.

Beta-blockers – does selectivity matter?

β-adrenergic receptors are found in several tissues. In vitro studies have identified expressions of these receptors also in bone tissue [134, 135], and activation of the sympathetic nervous system has been suggested to impair bone microstructure in mice models [136]. Hence, inhibition of sympathetic nervous system activity by BB could be beneficial for bone structure. Indeed, an interventional study in hypertensive rats reported preserved bone mass and microarchitecture in ovariectomized rats receiving low but not high doses of the non-selective BB propranolol, compared to controls [137]. In line with these findings of a dose-depended effect, a clinical study in postmenopausal women assigned to the relatively high dose of propranolol (160mg/day) for 3 months, failed to identify any favorable effect on serum markers of bone turnover compared to placebo [138]. As an answer to these findings, an ambitious study in 2018 showed that predominately β_1-adrenergic receptors are present in human bone cells, and reported better trabecular bone microarchitecture in men and women clinically treated with β_1-selective (cardio-selective) BB compared to those who did not (adjusted for age and sex) [135]. Those investigators also conducted a complementary randomized trial in 155 postmenopausal women assigned to treatment with three different BB for 20 weeks. The results showed that only the use of β_1-selective BB had favorable effects on bone turnover markers and

trabecular BMD compared to placebo, whereas the use of non-selective BB did not [135]. Noteworthy, these women were all normotensive at baseline. Hence, if these results are transferable to individuals with hypertension is unclear.

Regarding fracture outcomes, previous studies have identified a small effect of BBs on fracture risk [139, 140], but others have not [123, 141]. In 2014, a meta-analysis based on 9 case-control studies and 7 cohort studies, reported a 15% lower fracture risk in patients treated with BB compared to controls independent of gender, fracture site, and dose [142]. Interestingly, in line with the abovementioned studies investigating BB and bone structure, secondary analyses showed that β_1-selective BB were significantly more effective than non-selective BB in reducing the risk of any fracture. However, the significant heterogeneity between the included studies was an obvious limitation, highlighted by the authors themselves [142]. In that light, the evidence of a clinically significant fracture-reducing effect of BB is still controversial, leaving room for further studies.

Calcium channel blockers – a neutral drug or not?

In contrast to other antihypertensive drugs, there has been no convincing explanation of why treatment with CCB should affect bone tissue. CCB have improved bone mass in an ovariectomized rat model [143], but no effect on calcium or bone metabolism markers was found in humans [144]. In addition, a small case-control study with men treated with a CCB for an average of three years failed to report any effect on BMD [145]. In a Danish nationwide study, the use of CCB was associated with a small but significant (6-7%) reduced risk of any fracture and hip fractures compared to controls [146], in line with the findings in another large nationwide study from Norway [128]. Similar findings by Thorell et al., were, however, not maintained after adjustments of age, sex, and comorbidity [126]. Finally, a rather sizeable population-based study, including both a case-control and case-crossover design, did not find any association between the use of CCB and risk for hip fracture [123].

Still, due to the widespread use of CCB, even a small effect on fracture risk could be clinically interesting. Furthermore, when investing the effect of other antihypertensive medication on fracture risk in a clinical setting, the use of CCB could easily end up as the reference group. Hence, establishing better evidence regarding any association between the use of CCB and fracture risk could be valuable.

Loop diuretics – a recipe for losing calcium and suffering from injurious falls?

Opposite to thiazides, loop diuretics increase urinary calcium excretion and have been shown to promote bone loss of the hip in both men and women [147-149]. Another main concern with the use of loop diuretics is the assumed increased risk of falling, especially among the elderly. Besides electrolytic disturbances that could induce neurological symptoms and dizziness, the rapid onset of the diuretic effect and decline in BP after taking the medication might enhance orthostatic hypotension and cause urinary urgency with an immediate need to hurry to the toilet, which presumably could promote the risk of injurious falls. In line with the earlier discussion regarding thiazide diuretics and other antihypertensive drugs for the risk of falls, only a few studies have specifically investigated the relationship between loop diuretics and falls. Although the results mostly coherently have pointed towards an increased risk for falls, studies have been small, adjustments for confounders lacking, or the methods used have overall been rather diverse [150-153]. Hence, although a meta-analysis including five observational studies assessing loop-diuretics concluded an increased risk for falls with an adjusted OR of 1.36 (95% CI 1.17-1.57), these findings might be interpreted with caution. However, as the studies in this thesis did not address incident falls as any primary outcome but the fractures that can arise from falling, further diving into these questions is beyond the scope of this thesis. Specific etiology apart, previous studies have confirmed the concern that treatment with loop diuretics might be associated with increased fracture risk, summarized in a meta-analysis in 2015 [154] and later studies [128, 155, 156].

2 AIM

The overarching aim of this thesis is to investigate associations between hypertension, arteriosclerosis, and fracture risk in adults and the elderly, with a certain focus on how specific antihypertensive drug treatments in patients with high blood pressure might affect the risk for fractures. The specific aims for each study that constitute the foundation of this thesis are listed below:

Study I

To investigate if treatment with thiazides is associated with a reduced risk of osteoporotic fractures compared to other antihypertensive drug therapy in adult men and women. First, we aim to investigate the impact of duration of thiazide use on fracture risk, and the consequences of discontinuation of treatment. Second, we aim to investigate possible differences in effect between men and women.

Study II

To investigate the associations between exposure to different antihypertensive drug classes and the risk of hip fracture in adult men and women diagnosed with hypertension.

Study III

To examine if peripheral arterial disease is associated with an increased risk for hip fracture in elderly men independent of hip BMD.

Study IV

To describe differences regarding risk factors and protective factors for fractures in a well-characterized sample of elderly men with hypertension compared to men without hypertension. Furthermore, to investigate if hypertension is associated with an increased risk for hip fractures or other fractures in this population.

3 METHODS

Personally, I find statistics to be the most challenging part of epidemiological research. Therefore, this methodological chapter will begin with a description of some of the basic statistical methods used in epidemiological studies, including the specific statistical methods used in this thesis. This section is written mainly as a way for me to put difficult subjects into words and is not necessary to read for further understanding of the study designs in this thesis. Thereafter, descriptions of the specific study designs for each study in this thesis will be given.

3.1 INTRODUCTION TO STATISTICAL METHODS

Correlation

In the present thesis, the aim of all studies is primarily to estimate associations between exposure and outcome. A simple way to investigate whether two variables are related to each other is by investigating the *correlation* between these two. This can be done by visually plot the data or by conducting a statistical analysis of correlation.

One of the most common analyses used to calculate correlation is the *Pearson correlation coefficient,* an analysis for linear correlation. In such analysis both variables are treated the same, e.g., neither regarded as exposure nor outcome, we just test whether these two are related. Let us illustrate with a hypothetical example. We have obtained a large amount of data including age and systolic blood pressure levels (SBP) in a prospective observational study and wonder if SBP is correlated with age. When adding data for age and SBP into a Pearson correlation analysis, we obtain an estimate of the strength and direction of the association by the correlation coefficient (r). The correlation coefficient can range from -1 to 1. $R = 1$ represents a perfect correlation between the variables in the positive direction. Hence, when age increases, SBP increases. In contrast, $r = -1$ represents a perfect correlation in a negative direction (when age increases, SBP decreases). In the case of $r = 0$, there is no linear correlation between the two variables at all. See illustrations in Figure 10 below. Hence, if we obtain an $r = 0{,}45$, we could argue that there is a moderately strong positive correlation between age and SBP.

However, the covariance between the two variables in a Pearson correlation analysis needs to have a linear distribution for the analysis to calculate the correlation. But associations can take several different forms, such as a U-shaped association where SBP levels initially decrease during adolescence, but later in life increases with age. In such a case, the Person correlation coefficient would indicate no correlation between the variables, despite the fact that they do have a sort of correlation. In addition, the Pearson correlation analysis is sensitive to outliers (extreme values) in a dataset and works best when data is normally distributed. By excluding the outliers or logarithm transforming the values, a better normally distribution can be achieved. Another way is to use a correlation analysis that better handles such data. The most common alternative is the *Spearman correlation coefficient*, which calculates the correlation based on the rank between the variables rather than the distance between them as the Person correlation does. Hence, in the Spearman analysis, the correlation coefficient does not get distorted by outliers, and it can deal with non-linear correlations. The downside is that we lose some precision

in the calculation. Thus, if the data allows you to use the Pearson analysis that should still be the first choice. Based on these differences, the Pearson correlation analysis is a so-called *parametric* analysis whereas the Spearman correlation analysis is a *non-parametric* analysis (a nomenclature used for other types of statistical analyses as well).

In summary, with a correlation test, one can estimate the strength and direction of an association, commonly used in descriptive cross-sectional studies. However, we gain no information about the effect size and no adjustments for confounding are possible.

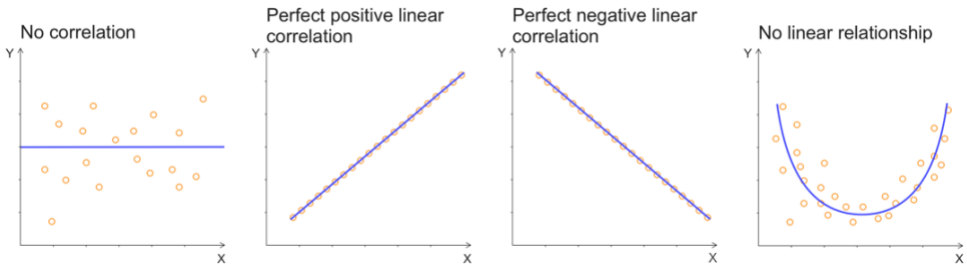


Figure 10. *Illustrations of different kinds of correlations between a variable X and a variable Y. For example, the variable Y could be age and X systolic blood pressure.*

Regression models

In contrast to the analysis of correlation, in a regression model, one can investigate both if an association exists between variables and the effect size of the association. In other words, regression models further estimate *how* one variable affects another. In addition, you can study more than two variables at the same time, adjusting for confounders. Regression models can be used for both predictive and explanatory modeling. In this thesis regression models have been used for explanatory purposes, hence, this approach is what will be described below.

One of the most recognized regression models is *linear regression*. Again, I would like to use the variables age and SBP to make an example, now with the aim to investigate how increasing age after 30 years (measured in years) affects SBP (measured in mmHg). In the regression model, one specifies which variable is the exposure and which is the outcome. In this case, SBP is the outcome of interest and age the exposure, as we investigate how the former is affected by the latter. In all regression models, the outcome is called the *dependent variable* and the exposure is

called the *independent variable*, *predictor,* or *covariate.* When modeling the data for the dependent and independent variables in the linear regression, the model yields a regression coefficient (β). When adding age and SPB, let us say, we get a β=0.6, meaning that SBP is expected to increase by 0.6 mmHg for every year of increasing age. Hence, we now know how much the SBP will increase with each year of aging.

However, there might be further variables affecting SBP, such as gender, body weight, alcohol intake etcetera. As we anticipated these to be confounding factors, we have collected data for these variables as well, and can now include them in the regression model. First, we include gender as a second covariate besides SBP. We have now expanded the initial *univariate regression model* to a *multiple regression model*. We then yield two β-coefficients, 0.4 for SBP and 2.9 for gender. In multiple models, the β-coefficients represent the change in the dependent variable per unit change in the independent variable, when all other covariates are held constant. In this case, SBP will increase by 0.4 mmHg for every year of increasing age when gender is held constant. Similarly, SBP will increase by 2.9 mmHg when being male, when age is kept constant. As you might have noted, the β-coefficient for SBP decreased slightly when adding gender to the model, as expected when we thought the univariate model was confounded by gender. The model is now *adjusted* for age. Hence, the latter multiple regression gives us a more true estimate of the association between age and SBP, as we, in this case, want to know how age, and not age and gender together, is associated with the increase in SBP.
Before using a statistical method, you need to ensure that your data fulfill the necessary criteria to fit the statistical analysis, otherwise, the results of the analysis may be unreliable or even misleading.

As with the Pearson correlation analysis, linear regression is a parametric analysis where the relationship between the two main variables (here: exposure and outcome) needs to linear. The most necessary criteria/assumptions for the statistical analyses used in this thesis are summarized at the end of this chapter.

Logistic regression

In the example above, the dependent variable (SBP) was a quantitative, continuous variable. However, the outcome of interest is often binary, as in the studies in this thesis that address fracture outcomes (fracture yes/no). Then we need to use a *logistic regression* model instead. However, the principle for the model is the same, with a univariate regression model that can be expanded to a multiple regression model adjusting for potential confounders. [157]

Distinctive for the logistic regression is that the regression coefficient yield by the model is the *odds* for the event to occur. Specifically, when the model gets a β-coefficient of, let us say 0.5, this means that when the independent

variable (let us keep the example with age as exposure) increases with 1 year, the logarithm of odds for the dependent variable (fracture) to occur increases by 0.5. What this actually means is for most of us (at least for me) rather tricky to understand. Hence, the β-coefficients from a logistic regression model are rarely presented. Instead, we look at the exponentiated coefficient; the *odds ratio* (OR). If we use the example above with a β=0.5, the model will yield an OR of 1.65. This means that when age increases by 1 year, the odds of experiencing a fracture increase by 65%. Or if we change the exposure age into having hypertension or not; when having hypertension the odds of experiencing a fracture increase by 65%. An OR can be interpreted as a relative risk when the outcome we investigate is rare in the population or the OR is relatively small, which suddenly makes the OR much easier to understand. However, if the outcome is very common or the OR gets very high, the interpretation is more complicated and uncertain. The OR is normally presented with a confidence interval and a p-value to ease statistical inference.

Cox regression

In all referred studies in this thesis, we used Cox regression models (frequently referred to as Cox proportional hazard models, or simply Cox model) to estimate the association between exposure and outcome and to adjust for confounding factors. Like the logistic regression, the Cox proportional hazard model is used when the outcome is binary, or more specific: an event of some sort. In contrast to the regression models described earlier, the Cox regression also incorporates time in the model. This is an important advantage when the follow-up time differs between participants in an observational study. The Cox model is commonly mentioned as a survival analysis (which estimates the difference in survival time until death between groups), but the time to any kind of event can be estimated. In fact, the Cox model has become the most commonly multivariate approach for analyzing *time to event* data in medical research [158].

In the studies in this thesis, we followed the study participants until the occurrence of the event we are interested in (fracture), the participant dies, is lost to follow up, or reaches the end date of the study. This will render different follow-up times for several participants. When the individual can no longer be followed and the event of interest has yet not occurred, it is called *censoring*. Censoring cannot be handled in a logistic regression, which might lead to unreliable measures of effect, but this is exactly what the Cox model can. If we like to assess the association between the use of a certain antihypertensive medication and hip fracture, the time span between the first dispensed medication of interest and the first hip fracture is the *time to event*. The cox model will compare the cumulative use of the antihypertensive medication during the time to event, at the exact time (day) when a patient experiences a hip fracture, with all other patients in the study who are alive and still

at risk of fracture at the same day. In short, the model estimates how specified factors affect the rate of a particular event happening at a certain point in time, which is referred to as the *hazard rate*.
In other words, the *hazard* could be described as the probability that an individual who is under observation at a certain time has an event at that time. However, like the logistic regression model, we are primarily interested in comparing the hazards between the exposed and unexposed groups. So, we do not present the regression coefficient (the hazard) yield in a Cox model, but the exponentiated coefficient, the *hazard ratio* (HR). The HR is usually interpreted the same way as the OR from logistic regression, or as the incident rate ratio, i.e. similar to a relative risk. Additional covariates are frequently included in cox models for adjustments of confounding factors.

Importantly, the major assumption of the Cox model is that the hazards are proportional over time [159, 160]. This implies that the HR of a given covariate between the unexposed and exposed group is constant over time (Figure 11). In other words, if an individual has a risk of fracture at a specific time point that is twice as high as that of another individual, then at all later times the risk of fracture remains twice as high. If this assumption is highly violated, the Cox regression model might produce a non-statistically HR although a clinically relevant relationship between exposure and outcome exists during certain periods of the study. Therefore, the proportional hazard assumption should be tested for the main covariate included in the model, which is commonly done with the statistical test of scaled Schoenfeld residuals. Non-significant results from the Schoenfeld residuals test are regarded as an indication that the proportional assumption is sufficiently met.

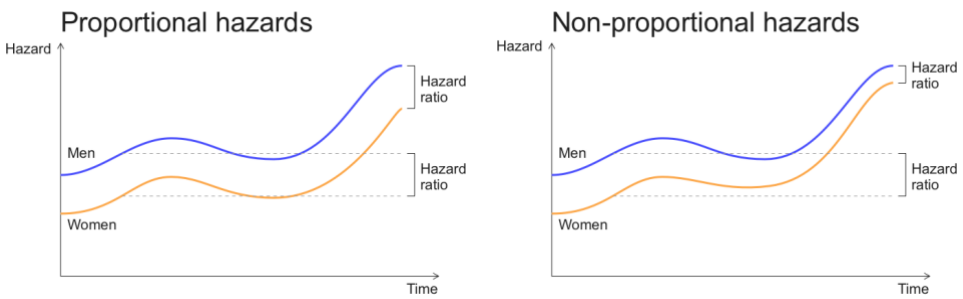


Figure 11. *Illustration of the proportional hazards assumption.*

Poisson regression

The Poisson regression can be used for survival analysis similar to the Cox regression model. Both models can handle different follow-up times and both models assume proportional hazards. However, unlike the Cox model, the Poisson model assumes that the event rate of the outcome is constant within specified intervals of time. Instead of the instant measurement in the Cox model at the index date, the Poisson regression computes the event rate by dividing the total number of events within each time interval by the total number of patient years at risk within the same time interval, and then compare the rate between the exposed and unexposed group. As such, if the effect of time is of interest, or we have non-proportional hazards, it might be favorable to use the Poisson regression divided into specific time intervals, instead of the Cox model. With that said, if we chop the time axis as finely as possible in a Poisson regression model, we will get the same results as in the Cox model. On the other hand, a Cox-regression model can also be divided into specific timeframes, which was done in studies I and II in this thesis. Hence, which model to use might not be obvious and is best discussed with a statistician well acquainted with the pros and cons of the different models.

Multicollinearity and collider bias

If two covariates in a multivariate regression model are highly correlated, the variables express *collinearity*. This makes it difficult for the model to separate the individual effect of the covariates from each other, which results in imprecise estimates (wider confidence intervals). Collinearity does not need to be a problem if we only wish to predict an outcome based on the whole model. However, when we attempt to determine how a specific covariate (normally the exposure of interest) is associated with the dependent variable, collinearity can cause a higher risk for type II error due to the wider confidence interval. When we have more than two correlated covariates in the model causing wider confidence intervals and less statistical precision, we have *multicollinearity*.

One rule of thumb that has been proposed to diminish the risk for multicollinearity is to allow at least 10 observations (number of outcomes/events) for each included variable. Another is to statistically identify variables that highly correlate and then remove all but one of them or to choose between similar variables based on empirical knowledge. In this thesis, we have considered a combination of these approaches in situations where we found multicollinearity to be a potential problem. Noteworthy, it may not always be easy to predict multicollinearity before observing its effects on the regression model, because the correlation between two of the covariates may be quite low, but together with a third (or more) covariate, the total correlation is high.

A high degree of multicollinearity enhances the risk of type II error. We may also have an opposite situation, where the presence of certain covariates introduces an association between the exposure and the outcome that was not present at the start. Meaning that if we have a covariate x (the exposure of interest) and a dependent variable y (the outcome) with no association between those two in a univariate model, but an association suddenly emerges when we also include the covariate z in the model. Then the association between x and y only exists in the presence of z. This is called *collider bias*. If we are not aware of this, we might conclude that an independent relationship exists between x and y when it is not true (commencing a type 1 error). Therefore, it is wise to stepwise include covariates into a multivariate model, to see what each covariate contributes with. Finally, when presenting data, it is recommended to show both crude results from univariate models and adjusted results from multivariate models, making it possible for the reader to follow how the point estimate and confidence interval change during the process.

Competing risk analyses

A distinctive feature of survival data analyses such as the Cox regression model is the concept of censoring. To clarify, in a study where the event of interest is an incident fracture, all study subjects who do *not* experience a fracture before the end of the study are *censored.* Hence, censoring occur when participant is still alive and free from the event at the end of the study or is lost to follow-up before an event have occurred (due to emigration, death, or dropout from the study). A significant assumption is that the probability of censoring is independent of the event of interest (conditional on the level of the covariate), also known as non-informative or independent censoring.

A *competing risk* is an event that, if it occurs, precludes the primary event of interest from happening. For instance, in a study where the primary outcome is time to death due to a cardiovascular cause, death attributable to a non-cardiovascular cause such as cancer is a competing event, since a subject dying from cancer can no longer die from a cardiovascular cause. Similarly, when the primary outcome is an incident fracture, the event of death serves as a competing event, as the subject who is dead no longer can be at risk of experiencing a fracture. However, the conventional Cox model assumes that competing risks are absent. Hence, if death is regarded as a competing risk and the occurrence of death is high, the competing risk from dying may lead to inappropriate conclusions from the results of a statistical analysis. Competing risk from death is particularly common in geriatric research and is wise to consider in studies with elderly subjects. One problem with the presence of competing risks is that we may overestimate the probability of the occurrence of

events in a population due to a certain risk factor based on the HR from a standard Cox model. For example, in a study with subgroups of individuals ≥ 80 years of age or with high fracture probability, failure to account for competing mortality overestimated fracture probability by 20–29 % with the standard Cox model [161]. In another study of older men, the association between the risk factor age ≥75 years and incident hip fracture, attenuated from HR 2.30 (95% CI 1.57, 3.36) to HR 2.01 (95% CI 1.35, 2.99) when modeling for competing risks in addition to a conventional Cox regression model [162]. It does not imply that the HR from the standard Cox model itself is incorrect, but we need to be cautious of how we interpret the results.

Statistical methods for competing risk analyses include the *cause-specific hazard regression* and the *subdistribution hazard function*, the latter also known as the Fine-Gray methodology. Both methods are based on the Cox regression but handle censoring from competing risks differently [163]. The cause-specific hazard models specifically censor individuals after events regarded as competing risks (such as death) and have been suggested as superior when studying the etiology of diseases [164]. In contrast, the subdistribution hazard method keep the individuals sustaining the competing risk in the model as still alive and at risk for the primary endpoint, which instead should be used when the focus is to assess prognostic questions. In fact, although the latter method has been frequently used in geriatric research, it has been proposed to be even harmful if not used and interpreted correctly [163, 165]. This means that when the research question is about the association between a risk factor and an outcome in a population still alive and at risk, such as "does elderly men with a CVD (still alive) have an increased risk of hip fractures (during the time when they live) compared to living men without a CVD?", the cause-specific hazard regression has been recommended [165]. Another attempt to describe this further could be with the following example: we have conducted a study investigating the exposure of heavy smoking for the risk of developing dementia, using a Cox model yielding a significant HR of 1.62 for dementia in heavy smokers compared to non-smokers. This can be interpreted as a 62% increased risk of dementia in heavy smokers compared to non-smokers. However, this only accounts for the heavy smokers still alive and at risk and should not without reflection be translated into a prediction that 62% more individuals among heavy smokers will suffer from dementia. This is since a substantial part of heavy smokers might die of other causes before they develop dementia, due to the overall higher mortality risk in smokers. If the intention is to make such predictions, one should use the subdistribution hazard-based method instead.

In summary, in the presence of a substantial degree of competing risks, it is important to differentiate between the question (and thus the interpretation of the result): "does smoking increase the risk of dementia?", versus; "will a higher proportion of smokers suffer from dementia compared to non-smokers?". At its extreme, the answer might be yes to the first question and no to the second.

Overview: assumptions and measures of effect in the statistical analyses used in the included papers.

If the assumptions are significantly violated (when including data in the model that does not fulfill these criteria), the results of the analysis may be unreliable or even misleading.

Model	Variables	Assumptions	Measure of effect
Linear regression (parametric model)	*Dependent variable:* Continuous *Independent variables (predictor/covariate):* Continuous, binary or ordinal	**– Linear relationship** between the independent (x) and the dependent variable (y) **– Independent observations**: Observations should not come from repeated or paired data; each observation is not influenced by or related to the rest of the observations **– Normally distributed residuals**: The residual is the difference between the actual value and the predicted value of a variable: the distance from the observed values and the regression line – **Homoscedasticity:** The residuals have constant variance at every level of the independent variable x. – Little or **no multicollinearity** between covariates (since it makes the model unstable)	**Regression coefficient (*r*)** Determination coefficient (R^2)
Logistic regression (parametric model)	*Dependent variable:* Binary or ordinal (If more than two ordinal outcomes, multinominal or ordinal logistic regression should be used) *Independent variables (predictor/covariate):* Continuous, binary or ordinal	**– Linear relationship** between the logit (logarithm for the odds) of the outcome and each of the covariates. **– Independent observations** – Little or **no multicollinearity** **– A sufficiently large sample size** (a rule of thumb is a minimum of 10 cases with the outcome for each covariate) – **Absence of strongly influential outliers**	**Odds ratio (OR)** OR = 1: No assoctaion/effect OR > 1: positive association (increasig odds) OR < :1 negative association (decreasig odds)
Cox regression (semi-parametric model)	*Dependent variable:* A binary event *Independent variables (predictor/covariate):* Continuous, binary or ordinal	**– The hazards are proportional over time** **– Independent observations** **– Independent censoring**: The probability of censoring is independent of the event of interest (conditional on the covariates)	**Hazard ratio (HR)** HR = 1: No assoctaion/effect HR > 1: positive association HR < :1 negative association
Poisson regression	*Dependent variable:* A number (count), ex number of events *Independent variables (predictor/covariate):* Continuous, binary or ordinal	**– Poisson distribution**: The distribution of counts follows a Poisson distribution **– Independent observations**	**Hazard ratio (HR)**

3.2 OVERVIEW – METHODS IN INCLUDED STUDIES

	Paper I	Paper II	Paper III	Paper IV
Data source	SPCCD	SPCCD	MrOS Sweden	MrOS Sweden
Design	Cohort study	Cohort study	Cohort study	Cohort study
Follow-up time	2006(2008)–2012	2006(2008)–2012	2001(2004)–2013	2001(2004)–2018
Sample size	57822 individuals	59246 individuals	2893 men	3014 men
Population	Individuals >45 years of age (55% women), with a diagnosis of hypertension in primary health care. Mean age 66 (+/-11) years.	Individuals ≥ 50 years of age (57% women) with a diagnosis of hypertension in primary health care. Mean age 69 (+/-10.7) years.	Randomly assigned men aged 69-81 years at baseline. Mean age 75.4 (+/-3.2) years.	Randomly assigned men aged 69-81 years at baseline. Mean age 75.4 (+/-3.2) years.
Exposure	Treatment with thiazide diuretics (based on dispensed prescriptions)	Treatment with seven different classes of antihypertensive drugs (thiazides, loop diuretics, BB, ARB, ACEi, CCB, MRA)	Baseline peripheral arterial disease, based on an ABI <0.9	Baseline hypertension (self-reported diagnosis together with antihypertensive treatment)
Outcome	Osteoporotic fractures (hip, spine, forearm, proximal humerus), based on the code of diagnosis I nationwide register	Hip fracture, based on the code of diagnosis I nationwide register	Main outcome: – Hip fracture Secondary outcomes: – Any fracture – Major osteoporotic fractures – Vertebral fractures (from x-ray archives)	Main outcome: – Hip fracture Secondary outcomes: – Any fracture – Major osteoporotic fractures – Vertebral fractures (from x-ray archives)
Statistics	Chi^2-test ANOVA Cox proportional regression models	Chi^2-test ANOVA Cox proportional regression models	T-test Mann-Whitney U Cox proportional regression models	T-test Fischer's exact test Mann-Whitney U Logistic regression Cox proportional regression models Poisson regression

Abbreviations: SPCCD = The Swedish Primary Care Cardiovascular Database, MrOS = The Osteoporotic Fractures in Men study, ABI=Ankle brachial index, ACEi = angiotensin-converting enzyme inhibitors, ARB = angiotensin II receptor blockers, BB = beta-blockers, CCB= calcium channel blockers, MRA = mineralcorticoid-receptor blockers

3.3 METHODS STUDY I

THIAZIDE DIURETICS AND THE RISK OF OSTEOPOROTIC FRACTURES IN HYPERTENSIVE PATIENTS

The Swedish Primary Care Cardiovascular Database (SPCCD)

Study I and II is based on data from the Swedish Primary Care Cardiovascular Database (SPCCD). SPCCD comprises medical records from 74751 individuals diagnosed with hypertension between 1 January 2001 to 31 December 2008, at 48 different primary healthcare centers in the southern part of Sweden. Twenty-four primary health care centers (both public and private) were selected from the mixed urban area of the southwestern part of Stockholm County, and 24 (out of 25) public primary healthcare centers from the more rural area of Skaraborg. In 2008, the total population of these areas was approximately 592.000 inhabitants, hence, SPCCD covers almost 13 % of the population in these areas. All patients ≥30 years documented with the diagnosis of hypertension at any of the primary healthcare centers during the years of inclusion were incorporated in the database.

All primary healthcare centers used the same computerized medical record system (Profdoc Journal III, Pofdoc AB, Uppsala, Sweden), from which a purpose-built software extracted selected medical data of interest for the condition of hypertension. By using the unique personal identification number assigned to all Swedish residents, the data retrieved from primary care was linked to data from large national medical registers and registers of socioeconomic and demographic data, see Figure 12.

Ethics

Written informed consent to data extraction was obtained from all directors of the primary healthcare centers. SPCCD was approved by the Regional Ethical Review Board in Gothenburg (Dnr 569-08).

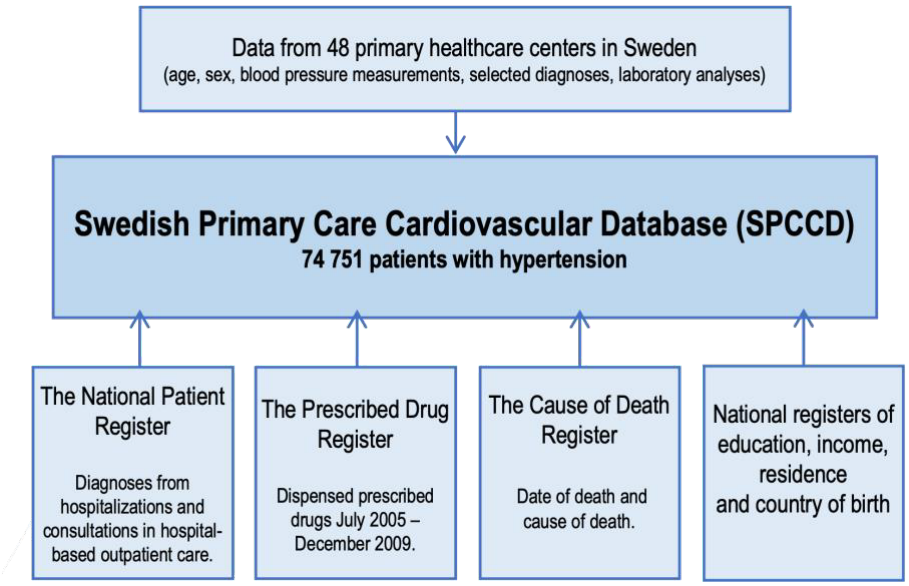


Figure 12. *The Swedish Primary Care Cardiovascular Database (SPCCD) contains medical data from primary healthcare, linked to national registers held by the Swedish Board of Health and Welfare, and demographic and socioeconomic data provided by Statistics Sweden.*

Assessment of hypertension, fractures, and potential confounders

The diagnosis of hypertension was based on the clinical decision of the physician during their daily practice in primary care and registered according to the Swedish primary care version of the International Classification of Diseases, 10th Revision, (ICD-10). According to current national guidelines, hypertension was defined by a SBP of ≥140 mmHg, or a diastolic BP of ≥90 mmHg, measured in a supine or seated position after at least 5 min of rest, at a minimum of three different occasions, alternatively by any current use of antihypertensive treatment. Measurements were obtained by either a physician or a nurse, using auscultatory or oscillometric devices. In 2010, a validation of the coding of diagnoses was made in Skaraborg, showing that the diagnosis of hypertension had an average sensitivity of 83% [166]. In both Study I and II we defined baseline BP by the last recorded BP before or at baseline (lowest value). Previously sensitivity analyses in SPCCD found no major differences between the last recorded BP and the average pressure of the last three measurements

within the preceding 12 months [167]. Hence, we regarded the last recorded BP to be representative of BP levels over time.

Besides hypertension, the following diagnoses were retrieved from primary healthcare; atrial fibrillation/flutter, heart failure, diabetes mellitus, ischemic heart disease, ischemic and hemorrhagic stroke, and transient ischemic attack (TIA). In addition, data on body height, weight, and smoking habits were obtained. BMI was calculated as body weight (to the nearest 0.1 kg) divided by the square of height in meter (to the nearest cm), based on the last recorded values. Also, laboratory values of fasting glucose, HbA1c, cholesterol, creatinine, and microalbuminuria were collected, but not used in the studies in this thesis.

In addition to the diagnoses from primary healthcare, we used the information from the National Patient Register to identify prevalent comorbidity at baseline that might act as confounders in the association between the exposure to thiazide and fracture risk. The National Patient Register comprises information on the date of admission, discharge, and consultation in all Swedish hospitals and hospital-based outpatient care, including up to eight recorded ICD-10 diagnoses per occasion. Furthermore, all information on incident fractures during follow-up was obtained from this register. The fractures of interest in Study I included the common sites for osteoporotic fractures, i.e., fractures of the hip, spine, forearm, and proximal humerus (see Table 3).

Data on socio-economic factors at baseline were retrieved from the national registers held by Statistics Sweden. The Cause of Death Register was used to retrieve the date of death, used for censoring in the statistical analysis. The register covers more than 99% of all deaths in Sweden.

Assessment of drug use including thiazide diuretics

All data of drug use was obtained from the Swedish Prescribed Drug Register, which comprises information of prescription, the date of dispensation, the total amount of pills, dosage, product name, substance, and the Anatomical Therapeutic Chemical (ATC) code. To achieve the most valid data on drug use, we used data of dispensed drugs only, since solely the prescription from a physician is less guarantee that the patient actually collects and uses the drug.

Regarding antihypertensive drugs, it is well-known that patients relatively often discontinue or switch their treatment [168]. Hence, as thiazide diuretics were the primary exposure of interest, we aimed to estimate when and how long each patient used these drugs during the course of the study. Based on the information in the Prescribed Drug Register, the duration of thiazide use was calculated by estimating the number of days that each dispensation would last. When a patient had dispensed a prescription prior to the end of supply, the remaining tablets

from the first dispensation were added to the latter. The drugs of interest included plain thiazides and fixed drug combinations containing a thiazide. The thiazide-like drugs chlortalidone and metolazone were included as well (increasing renal sodium loss and decreasing calcium loss similar to thiazides [96, 104]), and amiloride as it affects the reabsorption in the distal convoluted tubules similar to thiazides [169]. See Table 3 for an overview of these drugs including the corresponding ACT-codes. In addition to dispensed prescriptions of thiazides, any use of drugs that might be potential confounders was obtained, but only to estimate the prevalence of use across the cohort at baseline. Since drug use from automated multi-dose drug dispensing was more difficult to calculate, patients using that system were excluded from the analyses.

Table 3.

Exposure – thiazide diuretics (with corresponding ACT-code)	**Outcome – Osteoporotic fractures** (with corresponding ICD-code)
Plain thiazides (C03AA)	Hip fractures (S72.0-S72.2)
Fixed drug combinations: Thiazide + potassium (C03AB) Thiazide + potassium-sparing agents (C03EA) Thiazide + ARB (C09DA) Thiazide + ACE inhibitor (C09DA)	Vertebral fractures (S22.0, S22.1, S32.0, S32.7, M48.5) Fractures of the forearm (distal radius/ulna) (S52.2-S52.6)
Thiazides-like drugs: Chlortalidone and metolazone (C03BA) Amiloride (C03DB)	Fractures of the proximal humerus (S42.2, S42.3)

ARB: angiotensin receptor blockers, ACE inhibitor: angiotensin-converting enzyme inhibitors

Study sample and follow-up specific for Study I

In Study I, patients in SPCCD <45 years of age were excluded since the outcome was osteoporotic fractures. In total, the study sample included 57822 individuals (Figure 13). As this study specifically aimed to use treatment information from pharmaceutical dispensations, the baseline was not set to the beginning of the inclusion to SPCCD, but to a date after the introduction of the Swedish Prescribed Drug Register, which came into practice a little later, 1 July 2005. In addition, to avoid misclassification of medical exposure at baseline, we wanted to obtain information of medical exposure 6 months prior to baseline. Hence, the baseline was set to 1 January 2006 (or the date the patient received their first diagnosis of hypertension and was included in SPCCD if that date came later).

All patients were followed from baseline until they had an incident fracture, died, or reached the end of the follow-up on December 31, 2012, whichever came first. When the first fracture occurred in a patient, that date was defined as the index date. If the same individual experienced fractures at different sites during follow-up, an index date was defined for the first fracture in each category. The risk of fracture at the index date was then compared between patients exposed to thiazides with patients using other antihypertensive treatments but never been exposed to thiazides. Current exposure to thiazides was defined as the use of thiazides on the index date, by a maximum of 60 days since the last drug supply. In further analyses, the current users were divided into 4 groups based on the duration of use (by days); ≤ 6 months, >6-12 months, >12-24 months, and >24 months. We determined these categories based on the results of a prior RCT, showing an effect of thiazides on BMD first after 6 months [101]. In a similar manner, we intended to examine the duration of discontinuation for the loss of effect on fracture risk. Former exposure to thiazides was defined as the use of thiazides at baseline but not on the index date (>60 days since the end of drug supply), divided into the following sub-groups; ≤4 months, >4-6 months, and >6 months, without drug supply since index date.

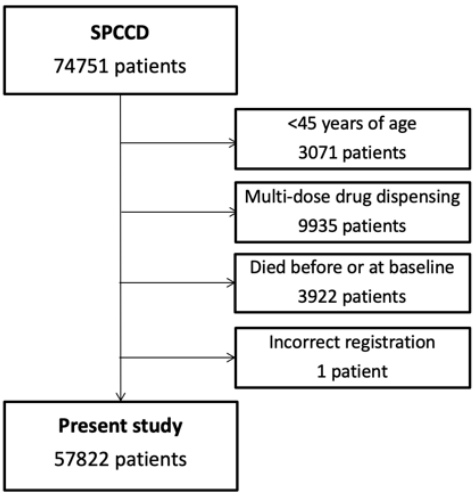


Figure 13. *Flow chart of the study population in study I.*

Statistical analyses

Differences in baseline characteristics between groups were analyzed using the Chi-square test for categorical variables and the analysis of variance (ANOVA) for continuous variables. Missing data were overall not considered a significant problem and was handled as missing completely at random. The variables BMI and smoking, however, showed a substantial number of missing values, with available data for merely about one-third of the study sample. In this case, we used the missing indicator method to handle the lack of data. As this method may produce biased estimates, we made an additional final model without BMI to compare with the model including BMI. The estimates in these two models were virtually the same, which can be interpreted as unlikely that the missing indicator method caused any major bias in our analysis.

The risk of osteoporotic fractures was calculated with Cox proportional hazards models, presented with 95% confidence intervals (CI). As treatment with thiazides was expected to vary over time, modeling was done with thiazides as a time-varying covariate. The Cox model was initially adjusted for age and sex. We then tested all other variables presented in the baseline table as potential confounders, by including them one by one in the initial model. If the variable caused a change in the point estimate of 5% or more, it was considered a confounder in the present cohort and therefore included in the final model. In additional analyses, the risk for each fracture outcome was estimated separately. Finally, secondary analyses stratified by gender were performed, to reveal any difference in the effect of treatment with thiazides between men and women. All analyses were performed in SAS Version 9.4 (SAS Institute, North Carolina, USA).

3.4 METHODS STUDY II

ANTIHYPERTENSIVE DRUG CLASSES AND THE RISK OF HIP FRACTURE

Study population

Study II also used data from the Swedish Primary Care Cardiovascular Database (SPCCD), described in the methodological section of study I. However, this time we only included men and women 50 years and older, but also allowed patients using automated multi-dose drug dispensing to be included, rendering a total study sample of 59246 individuals. Again, patients were followed from 1 January 2006, until their first incident fracture, death, emigration, or the end of the follow-up period on 31 December 2012. The risk of fracture was analyzed across users of different antihypertensive drugs.

Assessment of outcome – hip fractures

Unlike in Study I, we only considered hip fractures as an outcome in this study. As described previously, all information of incident fractures were obtained from the National Patient Register comprising diagnoses only from visits at a hospital or hospital-based outpatient care, registered according to ICD-10. The decision to restrict the outcomes to hip fractures was based on the higher probability that these fractures are being registered and properly coded in hospitals, compared to other less dramatic fracture types. Hence, aiming for better validity and less misclassification due to unrecorded fractures. In addition, fractures of the hip are commonly regarded as the most devastating fracture among common osteoporotic- or fragility-related fractures, making it a clinically relevant outcome as well. To further ensure that we only included the first incident fracture, rather than complications from a former one, the diagnosis had to be linked with a code for surgical treatment (NFJ or NFB), otherwise excluded. Pathological fractures due to malignancy, or fractures of an undefined or distal part of the femur, were also excluded.

Assessment of exposure – antihypertensive drugs

We aimed to assess the risk for hip fracture across seven major classes of BP-lowering drugs, which are specified with corresponding ATC-codes in Table 4. In contrast to Study I, thiazides were now categorized into three different groups: hydrochlorothiazide, bendroflumethiazide, or fixed drug combinations containing a thiazide. As only 35 individuals dispensed a thiazide-like drug (chlorthalidone or

metolazone) during follow-up, they were included in the group of bendroflumethiazide users. A similar number of patients ever dispensed a fixed drug combination of CCB together with an ACE inhibitor or an ARB, thus included among ACE inhibitor users or ARB users. Finally, selective and nonselective beta-blockers were analyzed together.

Similar to Study I, the date of the first hip fracture was defined as the index date, and the duration of treatment with each antihypertensive drug on this date was calculated for each patient. Calculation of the duration of drug use was done exactly as in Study I. However, as we did not want to exclude the more vulnerable individuals this time, also calculations from automated multidose drug dispensing (MDD) were made. These dispensations were estimated to last for 14 days, as the vast majority followed this routine. If dispensing antihypertensive medications besides the MDD, these drugs were accounted for in the same way as other dispensed prescriptions. Unfortunately, the dose in the MDD could not be estimated in about half of the patients (n=4345), and 566 patients had dispensed antihypertensive medications in other forms than tablets, hence, both categories were excluded from the analyses.

Current drug treatment was defined in the same way as in Study I, as use of the drug of interest on the index date with no more than 60 days since the last drug supply. We also added a minimum of treatment duration to be included as a "current user", with a treatment duration of at least 60 days, with the motivation that an effect of a drug on fracture risk other than immediate side effects, most probably would take time. Users of each category of antihypertensive drugs were analyzed separately, and individuals treated with more than one antihypertensive drug class were considered once in each category. In contrast to Study I, no subgroup analyses according to time of duration or discontinuation were analyzed this time.

Table 4.

Drug	ATC-code
Angiotensin-converting enzyme inhibitors (ACE inhibitors)	C09AA, C09BB
Angiotensin II receptor blockers (ARB)	C09CA, C09DB
Aldosterone receptor blockers (incl the potassium-sparing agent Amiloride)	C03D
Beta-blockers (BB)	C07
Calcium channel blockers (CCB)	C08
Loop diuretics	C03C
Hydrochlorothiazide (incl combinations with potassium or the potassium-sparing drug Amiloride)	C03AA03, C03EA01
Bendroflumethiazide (incl combinations with potassium), and thiazide-like drugs chlorthalidone and metolazone	C03AA01, C03AB01, C03BA04
Fixed drug combinations containing a thiazide (ACE inhibitor + thiazide, ARB + thiazide)	C09BA, C09DA

Assessment of covariates – potential confounding factors

All baseline characteristics registered in primary care, diagnoses from hospital care, dispensed prescriptions of drugs, and socioeconomics, were retrieved in the same way as in Study I. In contrast to Study I where we used statistical methods to determine confounders to be included in the final analyses, covariates were now assessed by the use of DAGs (direct acyclic graphs). In the end, all baseline characteristics believed to affect fracture risk were handled as potential confounders, since an uneven distribution of these factors across exposure groups could not be ruled out, (see published paper I for specific variables). One could argue that we could have measured the prevalence of these factors across the users of different antihypertensive drugs at baseline, but since drug use was expected to vary over the study period, such an estimate might not have been valid over the course of the study.

Statistical analyses

The same statistical methods were used in Study II as in Study I. Chi-square tests and ANOVA was used to estimate differences in baseline characteristics between individuals who would suffer an incident hip fracture during follow up, compared to those who would not. Missing data for BMI and smoking was again handled by the missing indicator method. Finally, the risk of hip fracture was calculated with Cox proportional hazards models with time-varying exposure status, for the whole cohort and for men and women separately. Analyses were adjusted for the assessed risk factors for hip fractures considered as potential confounders, by including them one by one in the sex and age-adjusted Cox proportional hazards model.

3.5 METHODS STUDY III

THE ASSOCIATION BETWEEN PERIPHERAL ARTERIAL DISEASE AND RISK FOR HIP FRACTURES IN ELDERLY MEN

Study population

The Osteoporotic Fractures in Men (MrOS) study is an international, multicentre, prospective, observational study, with the primary objective to establish risk factors for osteoporosis and fractures in elderly men. The total cohort comprises men in the United States (n=5994), Hong Kong (n=2000), and Sweden (n=3014).

In Study III and IV in this thesis, we used data from the Swedish MrOS, consisting of participants from three study sites in large Swedish cities (Gothenburg n=1010, Malmö n=1005, and Uppsala n=999). During the years 2001-2004 men aged 69-81 years were recruited and investigated. The participants were randomly identified from national population registries and invited by letter to participate. To be eligible for the study, the participants had to be able to walk unassisted and answer questionnaires in Swedish and were not allowed to have bilateral hip prostheses. The attendance rate was 45%.

Ethics

Written informed consent was obtained from all study participants in MrOS. Ethics committees at the Universities of Gothenburg, Lund, and Uppsala approved the study (Dnr Gbg 014-01, Ups 01-057 och LU 693-00).

Assessment of baseline variables

At baseline, medical information was obtained by standardized questionnaires, both self-administered questionnaires and questioning by interview. An extensive number of physical examinations and blood analyses were collected. In the following section, only the survey-questions and measurements used in studies III and IV in this thesis will be described.

The self-administered questionnaires included questions about previous fractures (fracture location and the age of the participant when the fracture occurred), history of fractures among close relatives, falls during the last 12 months preceding the baseline visit (yes/no), ongoing or history of smoking, and medical history (ever been diagnosed by a physician with conditions such as diabetes, hypertension, myocardial infarction, heart failure, stroke, chronic obstructive

pulmonary disease (COPD), arthritis (yes/no)). Further information was gathered with assistance from study personnel, including physical activity (walking every day for exercise (yes/no) and amount of km/day), alcohol consumption (days/month and "ever a period of use of more than 5 drinks/day"), and current drug use. Participants were encouraged to bring packages of ongoing medications, including both prescribed and over-the-counter drugs. All medications were registered by the personnel according to the Anatomical Therapeutic Chemical (ATC) Classification System. The obtained information was not verified in medical records.

Height and weight were measured using the same standard equipment for all participants, of which BMI was calculated (kg/m^2). Total BMD, and BMD at hip sites and lumbar spine (L1-L4), as well as lean and fat mass, were assessed by DXA. The Hologic QDR 4500/A-Delphi (Hologic, Waltham, MA, USA) was used in Gothenburg, and the Lunar Prodigy DXA (GE Lunar Corp., Madison, WI, USA) was used in Lund and Uppsala. Since results from the Lunar devices are slightly higher than those from the Hologic devices, standardized BMD was calculated. Muscle strength was assessed by measurement of handgrip strength, using a Jamar® hand dynamometer (Jackson, MI, USA). The best value from two measurements of each hand was used. Physical performance was assessed by walking speed (meter/seconds on a 6-meter walk at a normal pace). The fastest of two performances was used. Lung function was assessed with a spirometer, measuring vital capacity and forced expiratory volume during the first second (FEV1). Visual acuity was estimated on a standard eye chart at a 5m distance (with own glasses).

Serum and plasma samples were obtained around 8:00 a.m. after an overnight fast and non-smoking, frozen within 1 hour, and stored at –80°C until analyzed. The estimated glomerular filtration rate (eGFR) was calculated with serum cystatin C [170].

Assessment of peripheral arterial disease

At baseline, SBP was estimated by the mean value of two measurements in the right arm after 10 min of rest. The left arm was used if the right arm was not appropriate. Diastolic BP was only measured in the Gothenburg cohort, hence, not reported in the studies included in this thesis. Ankle-brachial index (ABI) was obtained by specifically trained personnel. Peripheral BP was measured twice at the posterior tibial artery of each ankle (if the signal was not audible, the dorsalis pedis artery was used). Then, the average of the two readings of the ankle pressures was divided by the average of the two systolic brachial artery pressures. PAD was defined as ABI <0.90 in at least one leg. Subjects with an ABI >1.40 were excluded (28 cases), as it might represent falsely high values due to incompressible arteries. Of the 3014

participants in MrOS Sweden, 2893 had a valid ABI measurement and were included in the analysis.

Assessment of fractures and follow-up time

All incident fractures occurring after the baseline visit were identified in computerized X-ray archives in Gothenburg, Lund, and Uppsala. By using the Swedish personal registration number, these archives were screened regularly for new fractures. The date and type of fracture were registered according to the ICD-10 code (Table 5). All radiographic reports eligible for MrOS were confirmed a second time by the same orthopedist to avoid misclassification.

Study subjects were followed until the date of their first assessed fracture type, death, emigration, or the end of the study period, whichever occurred first. In study III, the end of follow-up was December 31, 2013. In total, 1246 men died during the study period (41 %), rendering a median follow-up time of 10 years. Only the first fracture of each site was considered for analysis. However, the same person could sustain fractures at different sites at different times during follow-up, rendering risk times for more than one fracture category.

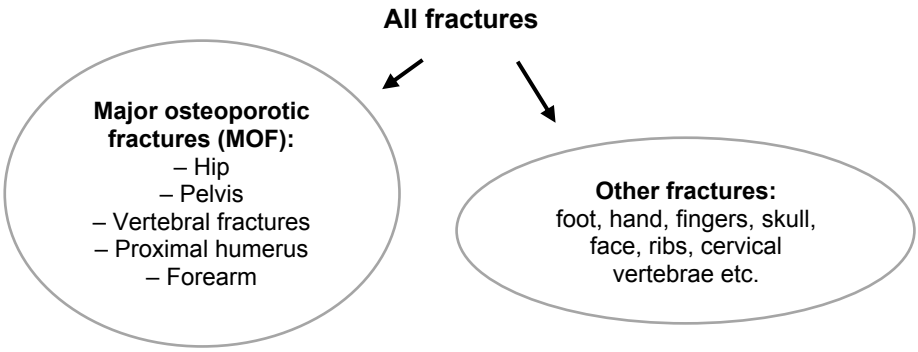


Table 5.

Fracture sites with corresponding ICD 10-codes	
All fractures	S02, S12, S22, S32, S42, S52, S62, S72, S82, S92
MOF (hip, pelvis, proximal humerus, forearm, vertebral fractures)	S32.1, S32.4-5, S42.2, S52.5, S56.6, S72.0-2
Hip fractures	S72.0-2
Vertebral fractures	S22.0-1, S32.0

Statistical analyses

Differences in means between two groups regarding baseline data were tested with a double-sided t-test if the dependent variable was either ordinal or continuous. The test of O'Brien was used to test whether both groups had equal mean and variance. If data were skewedly distributed, the non-parametric Mann-Whitney U test was used. A p-value <0.05 was considered statistically significant. The risk for hip fractures was calculated using Cox proportional hazard models and the hazard ratios (HR) are presented with 95% confidence intervals (CI). The Schoenfeld Residuals Test was performed to ascertain the model assumption of proportional hazard. Since we expected men with PAD to have a higher degree of overall morbidity and higher risk of death, the Cox models were assured to censor for events of death, considering death as a competing risk factor.

Probable confounders were included in multivariate models based on the assumption of being causally related to the outcome (fracture) and associated with the exposure (PAD), but not an obvious mediating factor between those two. Established risk factors for hip fractures were based on findings in previous studies. However, to lower the risk of multicollinearity, we left out some variables from the analyses based on the assumption of being too highly correlated with other eligible variables. This ended up with including current smoking but not COPD and including CVDs but not CRP. Handgrip strength was included as it represents the primary parameter of sarcopenia and is regarded as the most reliable measure of muscle strength [171], whereas appendicular lean mass was left out due to its high correlation with handgrip strength. None of the factors collected only in the Gothenburg cohort was used in the Cox models due to the limited sample size. Covariates were included stepwise in the Cox proportional hazard models. All analyses were made using Stata for Windows, version 15 (Stata Corp, Texas 77845 USA), and a statistics program package developed at the Department of Community Medicine and Public Health, Gothenburg University.

3.6 METHODS STUDY IV

HYPERTENSION AND FRACTURE RISK IN ELDERLY MEN

Study population

In study IV we also used data from MrOS Sweden, described in detail in the methodological section for study III.

Assessment of exposure and baseline variables specific for study IV

Hypertension was defined as a statement of hypertension in the self-administered questionnaire together with the use of any antihypertensive drug (beta-blockers/thiazides/loop-diuretics/ACEi/ARB/MRA). Participants who reported yes for hypertension but reported no use of antihypertensive medication (n=86), were included in the normotensive group.

In addition to clinical tests and measurements described earlier, we used several of the serum analyses obtained in MrOS in Study IV. These include, amongst others, important factors for calcium homeostasis such as vitamin D and PTH, different markers of bone formation and sex steroids. Serum calcium and 25(OH)D were analyzed according to routine laboratory methods. Detailed methods for determining fasting serum or plasma concentrations of glucose, lipids, hemoglobin (Hb), intact fibroblast growth factor-23 (iFGF23), serotonin, erythropoietin (EPO), leptin, high-sensitive C-reactive protein (hsCRP), intact parathyroid hormone (iPTH), as well as osteocalcin and N-terminal propeptide of type I collagen (P1NP) (markers of bone formation), have been described previously [172-175]. FGF23 is secreted by osteocytes and acts on the kidneys to decrease the activation of Vitamin D, but also increases the reabsorption of calcium and excretion of phosphate. Excessive levels of circulating FGF23 could lead to hypophosphatemia and inhibition of bone mineralization due to inappropriately low levels of activated vitamin D [176]. Specific analytic methods for estrogen, testosterone and sex hormone-binding globulin (SHGB) are described in the attached manuscript for Study IV.

Assessment of fractures and follow-up time

All incident fractures until the end of 2018 were considered, but the primary outcome was hip fractures. Participants were followed until the date of the assessed fracture type, death, or the end of the study on December 31, 2018. As in Study III, only the

first fracture of each site was considered for analysis, but the same person could render risk times for more than one fracture category if sustaining fractures at different sites during follow-up.

Statistical analyses

Differences in means between two groups regarding baseline data were tested by the same procedure and statistical tests as in Study III. Double-sided tests were used throughout and a p-value <0.05 was considered statistically significant. Variables that were obtained only in the Gothenburg cohort are presented separately. The odds ratio (OR) for selected risk factors and protective factors for fractures in men with hypertension were computed by multivariate logistic regression. In those analyses, skewed variables were analyzed in the log scale to better fit the criteria of the model. The association between hypertension at baseline and fractures during follow-up was calculated by Cox proportional hazard models and presented as hazard ratios (HR) with 95% confidence intervals (CI). The Schoenfeld Residuals Test was performed to ascertain the model assumption of proportional hazard. If proportional hazard assumptions were violated, Poisson regression with time intervals was used.
We used the same statistics software as in study III, described previously.

4 RESULTS

4.1 RESULTS STUDY I

THIAZIDE DIURETICS AND THE RISK OF OSTEOPOROTIC FRACTURES IN HYPERTENSIVE PATIENTS

Baseline characteristics

In total, 2345 patients experienced an osteoporotic fracture during follow-up. As some patients experienced more than one fracture (at different sites), the number of osteoporotic fractures slightly exceeded the number of patients. In total, 2565 fractures occurred, including 1210 hip fractures, 531 vertebral fractures, 422 forearm fractures, and 402 humerus fractures.

At baseline, patients that later would experience an osteoporotic fracture were older (74 vs. 66 years), more often women (71%), had slightly higher BP, and overall, more comorbidity and dispensed medications, including more antihypertensive drugs other than thiazides. These patients also had lower socioeconomic values and were more often born in Sweden. For specifics, see Table 1 in the appended published paper I.

Facture risk according to thiazide exposure

Current treatment with thiazides was associated with a significantly decreased risk of osteoporotic fractures as compared to never use of thiazides, with an adjusted of HR 0.89 (95% CI 0.81–0.98) (Figure 14). The association appeared to get stronger with the duration of treatment, reaching a 13% decreased risk for patients exposed to thiazides for more than two consecutive years. In contrast, former treatment with thiazides was associated with an increased risk of osteoporotic fractures, which tend to diminish with a longer duration since discontinuation. These analyses were performed both with and without adjustment for baseline BP, with no differences between the results. Additional adjustments for baseline CVD (heart failure, atrial fibrillation, and ischemic heart disease) neither influenced the results. When stratifying for gender, current treatment with thiazides appeared to be associated with a decreased osteoporotic fracture risk in both women and men, but only statistically significant in men (Figure 15). In contrast, any former treatment with thiazides was associated with a significantly increased fracture risk across both genders.

Separate analyses according to the fracture site, showed similar results for hip fractures as for any osteoporotic fracture, with a decreased fracture risk in current thiazide users and an increased risk in former users. Regarding vertebral fractures, no association between current thiazide use and fracture risk was seen, but increased risk in former users. No significant association was found

between treatment with thiazides and fractures of the forearm or humerus. Due to the relatively low rate of fractures in each category, we decided not to highlight these findings further.

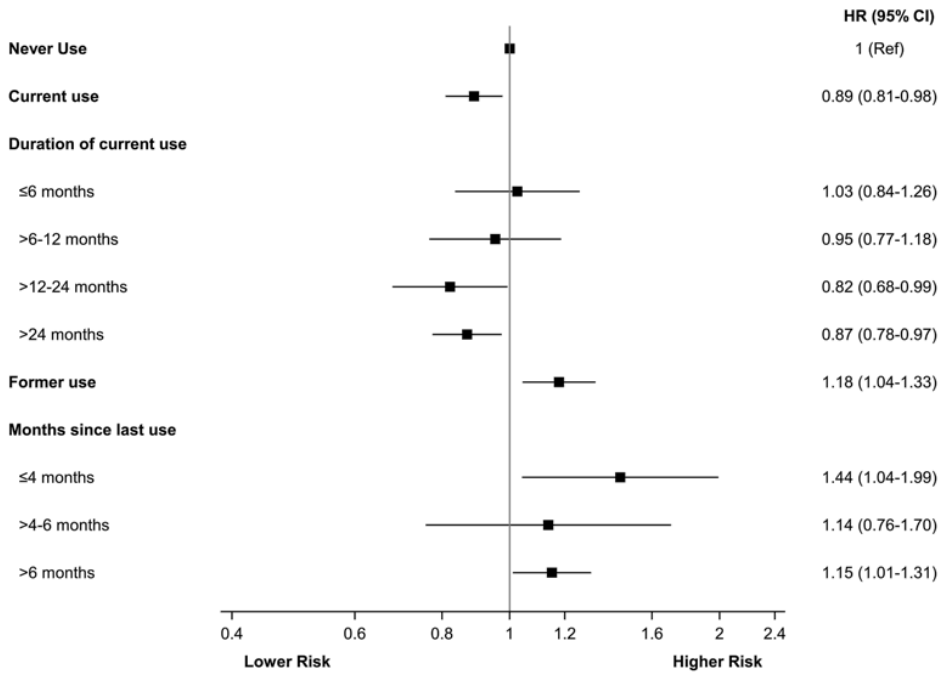


Figure 14. Hazards ratio and 95% CIs for osteoporotic fractures in relation to dispensed prescriptions of thiazides.

All estimates were adjusted for age, sex, previous fracture, smoking, diabetes mellitus, cerebrovascular disease, chronic obstructive pulmonary disease, Parkinson's disease, alcoholism, antihypertensives other than thiazides, antiosteoporotic treatment, glucocorticosteroids, antidepressants/anxiolytics/sedatives, neuroleptics, antiepileptics, hormone replacement therapy, ethnicity, and educational level.
Additional adjustments for baseline BP level or cardiovascular comorbidity did not change the estimates. CI, confidence interval; HR, hazards ratio; BP, blood pressure

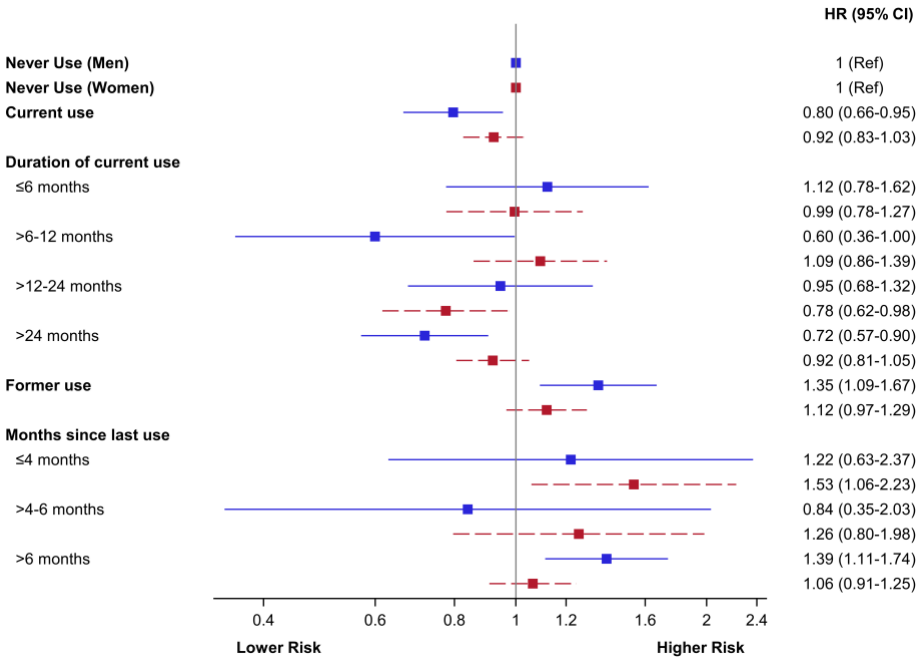


Figure 15. The risk of osteoporotic fractures in men versus women with hypertension, in relation to dispensed prescriptions of thiazides.

All estimates were adjusted for age, sex, previous fracture, smoking, diabetes mellitus, cerebrovascular disease, chronic obstructive pulmonary disease, Parkinson's disease, alcoholism, antihypertensives other than thiazides, antiosteoporotic treatment, glucocorticosteroids, antidepressants/anxiolytics/sedatives, neuroleptics, antiepileptics, hormone replacement therapy, ethnicity, and educational level.
CI, confidence interval; HR, hazards ratio.

4.2 RESULTS STUDY II

ANTIHYPERTENSIVE DRUG CLASSES AND THE RISK OF HIP FRACTURE

Baseline characteristics

During the study, 2593 patients experienced an incident hip fracture (4.4% of the study sample). As expected, at baseline these patients were older (mean age 79.2 vs 68.5 years), mostly women (72%), had slightly higher BP, and a higher frequency of comorbidities including CVD and previous fractures. Medical treatments were also more frequent overall, apart from hormone replacement therapy and statins. Factors indicating lower socioeconomic status were also more prevalent among patients who would experience a hip fracture. Regarding antihypertensive drugs, BB were the most common treatment overall, followed by ACEi and CCB. For specifics, see published paper II.

The risk of hip fracture according to different antihypertensive treatments

Consistent with the results in Study I, current treatment with thiazides was associated with a decreased risk of hip fracture, compared to patients with hypertension not using a thiazide (Figure 16). The effect size was similar across treatment with bendroflumethiazide (HR 0.86; 95% CI 0.75–0.98) and hydrochlorothiazide (HR 0.84; 95% CI 0.74– 0.96). Also, treatment with fixed drug combinations containing a thiazide was associated with a decreased fracture risk. On the contrary, current treatment with loop diuretics was associated with an increased risk of hip fracture compared to nonusers (HR 1.23; 95% CI 1.11–1.35). No significant associations were found between incident hip fractures and current treatment with neither BB, ACEi, ARB, CCB, or aldosterone receptor blockers.

The crude Cox models adjusted only for age and gender showed slightly stronger associations overall (data not shown). However, despite the many covariates, the significant associations remained in the fully adjusted model without any signs of collider bias. Additionally, we conducted an analysis where we divided the reference group of non-users into the two categories *never users* (never dispensed the drug of interest) and *former users* (dispensed the drug previously, but more than 60 days ago). When analyzing the association between current use of the different antihypertensive therapies and incident hip fractures and never users only (with former users as a third group in the model), the associations were overall marginally stronger (data not shown).

When stratifying the analyses between men and women, the decreased risk for hip fracture in users of plain hydrochlorothiazide and bendroflumethiazide only remained statistically significant in men. Otherwise, no major differences in the associations (or lack of associations) between different antihypertensive drug treatments and hip fracture risk were seen between the total cohort and men and women separately (Figure 17).

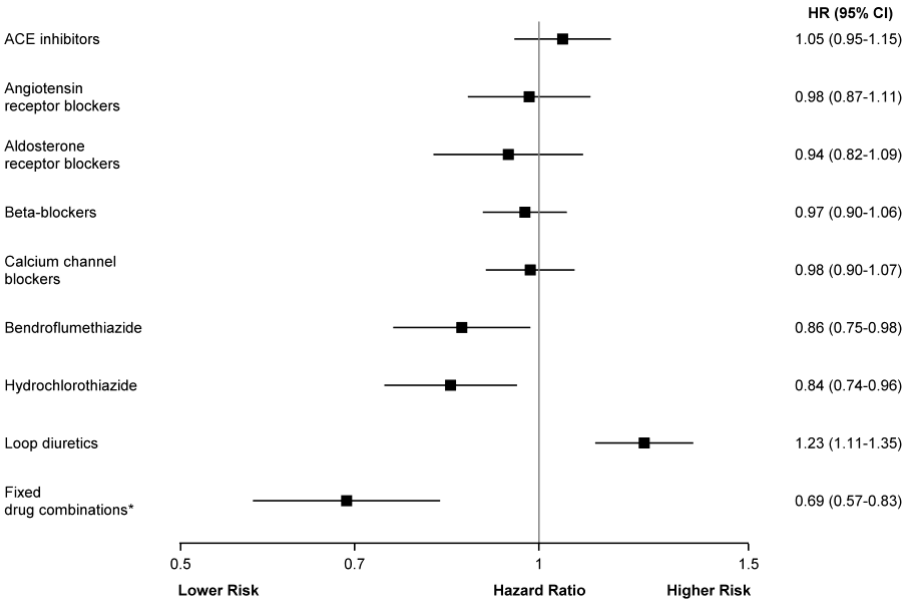


Figure 16. Hazard ratios for hip fracture in relation to dispensed prescriptions of different antihypertensive drug classes.
All estimates were adjusted for age, sex, BMI, smoking, SBP, previous fracture after 50 years of age, COPD, chronic heart failure, ischemic heart disease, cerebrovascular disease, dementia, diabetes mellitus, peripheral atherosclerosis, atrial fibrillation, orthostatic symptoms, rheumatoid arthritis, kidney insufficiency, alcoholism, hyperthyroidism, malignancy, antiosteoporotic drugs, calcium and vitamin D supplements, corticosteroids, hormone replacement therapy, antidepressants, anxiolytics and/or sedatives, antiepileptic drugs, neuroleptics, statins, ethnicity/origin, educational level and level of income.
SBP: systolic blood pressure, COPD: chronic obstructive pulmonary disease, ACE: angiotensin-converting enzyme, CI: confidence interval, HR: hazards ratio.
**Fixed drug combinations represent combinations containing a thiazide.*

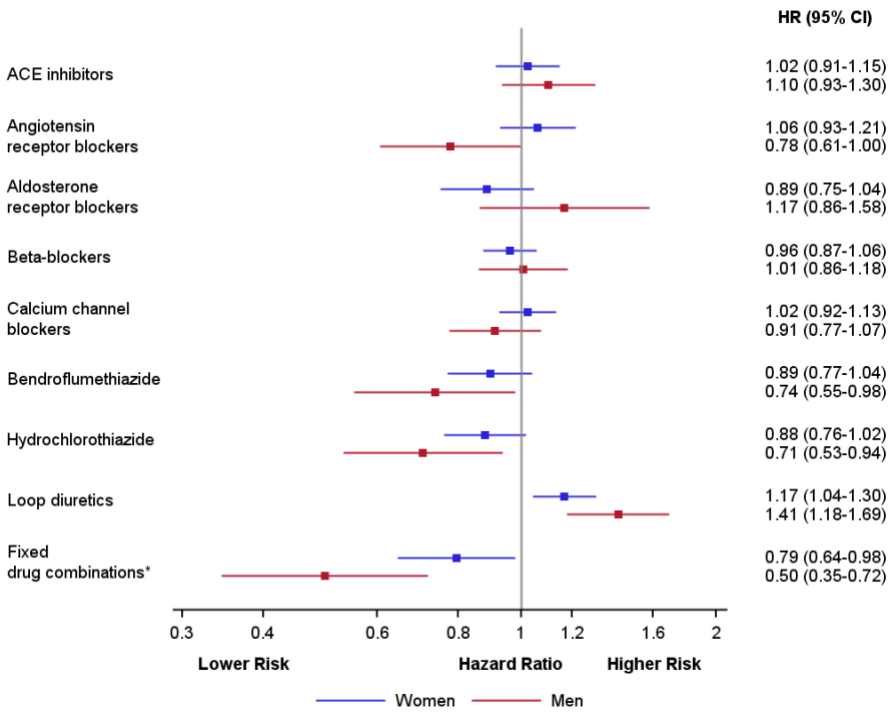


Figure 17. The risk of hip fracture in men and women and men in relation to dispensed prescriptions of different antihypertensive drug classes.
All estimates were adjusted for age, sex, BMI, smoking, SBP, previous fracture after 50 years of age, COPD, chronic heart failure, ischemic heart disease, cerebrovascular disease, dementia, diabetes mellitus, peripheral atherosclerosis, atrial fibrillation, orthostatic symptoms, rheumatoid arthritis, kidney insufficiency, alcoholism, hyperthyroidism, malignancy, antiosteoporotic drugs, calcium and vitamin D supplements, corticosteroids, hormone replacement therapy, antidepressants, anxiolytics and/or sedatives, antiepileptic drugs, neuroleptics, statins, ethnicity/origin, educational level and level of income.
SBP: systolic blood pressure, COPD: chronic obstructive pulmonary disease, ACE: angiotensin-converting enzyme, CI: confidence interval, HR: hazards ratio.
**Fixed drug combinations represent combinations containing a thiazide.*

4.3 RESULTS STUDY III

THE ASSOCIATION BETWEEN PERIPHERAL ARTERIAL DISEASE AND RISK FOR HIP FRACTURES IN ELDERLY MEN

At baseline, 314 men had PAD (10.9% of the cohort). During follow-up, 186 men experienced an incident hip fracture (29 of those had PAD). According to baseline measurements, men with PAD were older, had lower BMD at hip sites and lower lean mass than men without PAD, but BMI did not differ significantly. As expected, men with PAD were more often current smokers and had a higher burden of concurrent diseases including COPD, diabetes, and CVD. Estimated GFR was also lower, CRP was higher, and the number of previous falls and hip fractures tended to be higher in men with PAD. In addition, men with PAD had lower educational level. Results from the Gothenburg part of the MrOS study indicated higher serum cortisol, higher urinary cadmium, and lower lung function in men with PAD. In line with the overall burden of morbidity in men with PAD, also muscle strength (handgrip) and physical function (walking speed) were poorer. Severe sarcopenia defined by EWGSOP2 (handgrip strength <27kg, appendicular lean mass <7kg/m^2, walking speed <1m/s) [171] was, however, very uncommon (<1% of the cohort). For details regarding baseline characteristics, see Table 1 and 2 in appended submitted paper III. Finally, mortality during follow-up was higher in men with PAD compared to men without PAD, with an age-adjusted HR 2.05 (95% CI 1.77-2.39).

PAD at baseline was associated with an increased risk of hip fracture compared to men without PAD (HR 1.70; 95% CI 1.14-2.54), adjusted for age and study site (Table 6). Additional adjustment for total hip BMD marginally affected this association (HR 1.67; 95% CI 1.12-2.48). In a final multivariate model including several comorbidities and functional measurements, the HR for hip fracture in men with PAD attenuated to a non-significant HR 1.38 (95% CI 0.91-2.11). Our power was limited to investigate the severity of PAD for fracture risk, but a sub-analysis showed an almost 3 times higher risk for hip fracture in men with an ABI <0.5, compared to men without PAD (HR 2.83; 95% CI 1.05-7.62), adjusted for age, study site and hip BMD. However, this estimate should be interpreted with caution since only 25 men had an ABI <0.5.

In secondary analyses, we additionally investigated the association between PAD and other fracture outcomes (Table 7). PAD was associated with a significantly increased risk for all fractures and MOF, independent of age, site, BMI, and hip BMD, but in the final multivariate models the results became non-significant. No significant association between PAD and vertebral fractures was seen.

Table 6.

Cox regression models for the association between PAD (ABI<0.9) and hip fractures			
Model	**Hazard ratio (95% CI)**	**Events (hip fracture)**	**Cases[f]**
Model 1[a]	1.70 (1.14-2.54)	186	2893
Model 2[b]	1.67 (1.12-2.49)	186	2868
Model 3[c]	1.55 (1.04-2.32)	181	2796
Model 4[d]	1.52 (1.01-2.29)	178	2761
Model 5[e]	1.38 (0.91-2.11)	181	2718

[a)] Adjusted for age and site.
[b)] Adjusted for age, site and hip BMD.
[c)] Adjusted for age, site, hip BMD, BMI, falls and hand grip strength.
[d)] Adjusted for age, site, hand grip strength, walking speed and appendicular lean mass (continuous variables)
[e)] Adjusted for age, site, hip BMD, BMI, falls, hand grip strength, walking speed, current smoking, eGFR, antihypertensive treatment, diabetes, former hip fracture, education, history of cardiovascular disease
[f)] Total number of cases included in the analysis, after exclusion due missing values for covariates or risk time

Table 7.

Cox regression models for the association between PAD (ABI<0.9) and different fracture outcomes			
Model	**Hazard ratio (95% CI)**	**Events (hip fracture)**	**Cases[f]**
All incident fractures			
Model 1[a]	1.46 (1.16-1.83)	647	2893
Model 2[b]	1.35 (1.07-1.70)	642	2868
Model 3[c]	1.17 (0.92-1.49)	612	2718
Major osteoporotic fractures			
Model 1[a]	1.51 (1.16-1.97)	461	2892
Model 2[b]	1.43 (1.09-1.86)	461	2867
Model 3[c]	1.24 (0.94-1.65)	442	2718
Vertebral fractures			
Model 1[a]	1.24 (0.94-1.65)	225	2893
Model 2[b]	1.35 (0.92-1.98)	225	2868
Model 3[c]	1.14 (0.76-1.72)	216	2718

[a)] Adjusted for age and site.
[b)] Adjusted for age, site and hip BMD, BMI
[c)] Adjusted for age, site, hip BMD, BMI, falls, hand grip strength, walking speed, current smoking, eGFR, antihypertensive treatment, diabetes, former hip fracture, education, history of cardiovascular disease
[d)] Total number of cases included in the analysis, after exclusion due missing values for covariates or risk time

4.4 RESULTS STUDY IV

HYPERTENSION AND FRACTURE RISK IN ELDERLY MEN

Notably, Study IV is still under working process and the results can be viewed as preliminary.

At baseline, one-third of the participants in MrOS had hypertension (n=1001), with no difference in mean age compared to those without hypertension. The most common antihypertensive drug was BB, reflecting the current tradition in Sweden by that time, closely followed by ACEi and ARB. As one could expect, men with hypertension had higher BMI, trunk fat mass, and considerably higher levels of serum leptin compared to men without hypertension. Also, BMD at all sites was higher in men with hypertension. Handgrip strength was slightly superior in men with hypertension but walking speed marginally poorer. Current smoking was slightly more common in men with hypertension, but no difference was seen regarding ever smoking or average alcohol intake compared to men without hypertension. Cardiovascular manifestations such as stroke, ischemic heart disease, PAD, and heart failure were clearly overrepresented in men with hypertension, in line with the expected consequences of high BP. Also, diabetes and arthritis were more common. Men with hypertension had lower renal function and lower levels of testosterone and serotonin. In contrast, serum levels of EPO, PTH, and FGF23 were higher. Triglycerides were slightly higher in men with hypertension, but LDL levels were slightly lower compared to men without hypertension, possibly reflecting the twice as often use of statins in this group. Specifics of all baseline measurements are found in Table 1A and 1B in the appended manuscript for study IV.

During follow-up, 287 men sustained a hip fracture. When comparing the baseline measurements of men with hypertension that experienced a hip fracture during follow-up (n=90) with those without hypertension experiencing a hip fracture (n=197), the overall picture described earlier was confirmed. For instance, men with hypertension sustaining a hip fracture had a mean BMI of 27.2 compared with a mean BMI of 25.5 in men without hypertension, and BMD values were significantly higher in men with hypertension sustaining a hip fracture. To further investigate the differences in baseline data according to hypertension status, BMD values and serum analyses that significantly differed between those with and without hypertension were analyzed in multivariate logistic regression models (Table 8 and 9). When adjusting for BMI, the increased odds ratio (OR) for total BMD and hip BMD in men with hypertension were no longer significant, only for lumbar spine BMD. In contrast, the positive OR for leptin levels, FGF23, PTH, and EPO, and a negative OR for total testosterone, SHGB, and LDL remained, independent of BMI, eGFR, and diabetes. In summary, these results reveal both some risk factors for

fractures (lower testosterone and SHGB, and higher leptin, FGF23, PTH, and EPO) and protective factors for fractures (higher BMI and BMD) associated with hypertension in elderly men. However, when separately analyzing the men with hypertension, the only variable that differed between those who would suffer from a hip fracture and those who would not, was baseline BMD at all sites, being significantly lower in hypertensive men suffering a hip fracture.

Despite some differences in baseline characteristics between men with and without hypertension, no association between hypertension and any fracture type was found in Cox regression models (Table 10). When stratifying for hypertension with and without baseline cardiovascular comorbidity, still no association with fractures was seen compared with men without hypertension (Table 11). Interestingly, in men with hypertension without cardiovascular comorbidity, the proportional hazard assumption was violated according to the Shoenfeld residual test. Hence, we conducted a Poisson regression model with time intervals for this group (see appended manuscript). In those analyses, a significantly lower risk for both hip fracture, MOF, and all fractures was found in men with hypertension free from cardiovascular comorbidity during the first 4 years of the study, compared to men without hypertension. Later, a trend towards an increased fracture risk with longer exposure to hypertension compared with men without hypertension could be hinted but not significantly.

Table 8.

Multivariate logistic regression models for the association between hypertension and selected characteristics in 3104 men in MrOS Sweden

Characteristics	Odds Ratio (95% CI)			
	Adjusted for age, site	Adjusted for age, site, BMI	Adjusted for age, site, BMI, eGFR	Adjusted for age, site, BMI, eGFR, diabetes
Total body BMD (g/cm2)	1.16 (1.07–1.27)	1.01 (0.93–1.11)	1.02 (0.93–1.12)	1.02 (0.93-–1.12)
Total hip BMD (g/cm2)	1.22 (1.13–1.32)	1.06 (0.98–1.16)	1.09 (1.00–1.19)	1.08 (0.99–1.18)
Lumbar spine BMD (g/cm2)	1.23 (1.14–1.32)	1.12 (1.03–1.21)	1.12 (1.03–1.21)	**1.11 (1.02-1.20)***
Testosteron, total (nmol/l)	0.75 (0.69–0.82)	0.84 (0.77–0.92)	0.86 (0.79–0.95)	**0.88 (0.81-0.97)***
Testosterone, free (nmol/l)	0.82 (0.75–0.89)	0.88 (0.80–0.96)	0.90 (0.82–0.98)	0.92 (0.84–1.01)
SHBG (nmol/l)	0.79 (0.72–0.86)	0.87 (0.79–0.95)	0.88 (0.80–0.96)	**0.88 (0.81-0.97)***
FGF23 (pg/ml) (log)	1.35 (1.24–1.48)	1.30 (1.19–1.42)	1.20 (1.09–1.31)	**1.19 (1.08-1.31)***
CRP (mg/L) (log)	1.11 (1.03–1.19)	1.06 (0.98–1.15)	0.99 (0.91-1.07)	0.99 (0.91–1.08)
iPTH (pmol/L) (log)	1.21 (1.17–1.32)	1.19 (1.09–1.30)	1.13 (1.03–1.23	**1.15 (1.05-1.26)***
Leptin (ng/ml) (log)	1.59 (1.46–1.73)	1.41 (1.26–1.58)	1.31 (1.17–1.47)	**1.29 (1.15-1.45)***

*Significant associations in fully adjusted multivariate model
BMD = bone mineral density, SHBG = sex hormone-binding globulin, FGF23 = fibroblast growth factor-23, iPTH = intact parathyroid hormone

Table 9.

Multivariate logistic regression models for the association between hypertension and selected characteristics in 1010 men in MrOS Gothenburg

Characteristics	Odds Ratio (95% CI)			
	Adjusted for age, site	Adjusted for age, site, BMI	Adjusted for age, site, BMI, eGFR	Adjusted for age, site, BMI, eGFR, diabetes
Serotonin (þg/L)	0.84 (0.73–0.97)	0.87 (0.76–1.00)	0.88 (0.77–1.02)	0.89 (0.77–1.02)
Erythropoietin (EPO) (log)	1.33 (1.15–1.54)	1.29 (1.12–1.50)	1.28 (1.10–1.49)	**1.26 (1.08-1.47)***
Interleukin 6 (IL-6) (log)	1.18 (1.03–1.34)	1.14 (1.0–1.30)	1.10 (0.96–1.26)	1.08 (0.94–1.24)
Low density lipoprotein (LDL)	0.72 (0.62–0.82)	0.71 (0.62–0.82)	0.71 (0.62–0.82)	**0.73 (0.64-0.84)***
High density lipoprotein (HDL)	0.77 (0.67–0.88)	0.85 (0.73–0.98)	0.87 (0.75–1.01)	0.89 (0.77–1.03)

*Significant associations in fully adjusted multivariate model

Table 10.

Cox regression models for the association between hypertension and fractures			
Model	**Hazard ratio (95% CI)**	**Events (fractures)**	**Cases**
Hip fracture			
Model 1	0.98 (0.76-1.26)	287	3014
Model 2	1.03 (0.80-1.34)	282	2851
Vertebral fracture			
Model 1	0.98 (0.77-1.24)	323	3013
Model 2	1.04 (0.81-1.33)	310	2850
Major osteoporotic fracture			
Model 1	0.99 (0.84-1.17)	662	3013
Model 2	1.05 (0.89-1.25)	639	2850
All fractures			
Model 1	0.97 (0.84-1.12)	866	3013
Model 2	1.02 (0.88-1.19)	833	2850

Model 1 = Adjusted for age and site
Model 2 = Adjusted for age, site, BMI, hip BMD, eGFR

Table 11.

Cox regression models for the association between hypertension and fractures, stratified by cardiovascular comorbidity* at baseline			
Model	**Hazard ratio (95% CI)**	**Events (hip fracture)**	**Cases**
Men with hypertension and cardiovascular comorbidity (n=957)			
Hip fracture			
Model 1	0.88 (0.59-1.30)	100	957
Model 2	0.93 (0.61-1.39)	99	901
Major osteoporotic fracture			
Model 1	1.06 (0.81-1.37)	230	956
Model 2	1.09 (0.84-1.43)	227	900
All fractures			
Model 1	1.00 (0.80-1.26)	298	956
Model 2	1.04 (0.82-1.32)	291	900
Men with hypertension without cardiovascular comorbidity (n=2037)			
Hip fracture			
Model 1	0.93 (0.67-1.30)	186	2037
Model 2	0.99 (0.70-1.40)	182	1933
Major osteoporotic fracture			
Model 1	0.84 (0.67-1.05)	427	2037
Model 2	0.89 (0.70-1.12)	409	1933
All fractures			
Model 1	0.85 (0.70-1.03)	563	2037
Model 2	0.88 (0.72-1.08)	539	1933

* Including stroke, heart failure, ischemic heart disease, peripheral arterial disease, diabetes
Model 1 = Adjusted for age and site, Model 2 = Adjusted for age, site, BMI, hip BMD, eGFR

5 DISCUSSION

5.1 DISCUSSION STUDY I AND II

ANTIHYPERTENSIVE DRUGS AND FRACTURE RISK

Main findings

In a large cohort study of patients diagnosed with hypertension in primary health care, we found antihypertensive treatment with thiazides to be associated with a decreased risk of osteoporotic fractures including hip fractures, compared to other antihypertensive drug therapies. The association appeared to be stronger with longer treatment duration, while former use of thiazides was associated with an increased risk for fractures. Treatment with loop diuretics was associated with an increased risk of hip fractures, while the use of BB, ACEi, ARB, aldosterone receptor blockers, or CCB revealed no significant association with fracture risk. The overall findings were similar across men and women, but the decreased fracture risk with thiazides was only statistically significant in men.

Blood pressure-lowering drugs and fracture risk

Thiazide diuretics

The current evidence that treatment with thiazides is beneficial for bone tissue is rather well established, with a prospect to decrease fracture risk. Our findings of an association with a relative risk reduction for osteoporotic fractures (at least hip fractures) of about 10-20% with thiazide treatment are consistent with several previous observational studies [128, 129, 177-181] although contrary findings have been presented [96, 125, 182]. Moreover, in a post hoc analysis from the RCT "Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial" (ALLHAT) the thiazide-like diuretic chlorthalidone was associated with a significantly lower risk of hip and pelvic fractures compared to treatment with the ACE inhibitor lisinopril or the CCB amlodipine (HR 0.79; 95% CI, 0.63-0.98)[179]. Interestingly, further secondary analyses from the same RCT showed no differences in orthostatic symptoms, syncope, or falls during follow-up between the assigned drugs [183]. Those findings together could imply that the differences in fracture outcome across those treatments were at least not obviously driven by side effects known to be associated with falling.

Before and after the publication of Study I and II, four different meta-analyses have summarized the findings from observational studies assessing the effect of thiazides on fracture risk. The first one was done in 1995 and the most recent in 2019, the last two including Study I in this thesis. Three of these meta-

analyses reported rather consistent pooled estimates of relative-risk measurements in users of thiazides compared to non-users, ranging from a reduced risk of 12% for osteoporotic fractures [117], 14% for any fracture [118, 184], and 18-24% for hip fractures [116, 117, 184]. The most recent meta-analysis including a total of 40 observational studies, however, could only establish a significantly lower overall fracture risk in thiazide users based on case-control studies (n=19) (RR 0.87; 95% CI 0.70-0.99), as the separate analysis limited to cohort studies (n=21) showed no significant association (RR 0.95; 95% CI 0.85-0.1.08) [185].

A novel finding from Study II is that hydrochlorothiazide and bendroflumethiazide both have a similar association with hip fracture risk. This result strengthens the hypothesis that this association represents a class effect related to the mechanism of action across all thiazides, rather than a specific drug, of importance in clinical practice. Furthermore, our data suggest that fixed drug combinations containing a thiazide might be even superior to single pills regarding fracture outcome. Fixed drug combinations might contribute to better adherence to antihypertensive medication, highlighted in the 2018 ESC/ESH guidelines [10]. This might result in better BP control as well as a more continuous dose of thiazide over time, factors that tentatively could contribute to even lower fracture risk. However, these figures should be taken with precautions since the number of users was few.

Duration of drug use and effect of discontinuation

Our findings of a stronger association between long-term users of thiazides (>2 years) and decreased fracture risk are consistent with several previous studies [177, 180, 186-189]. The trend toward a greater risk reduction with increasing duration of treatment also supports the hypothesis that thiazides reduce the risk of osteoporotic fractures by improving BMD, since such a process would take time. A more pessimistic explanation for the delayed effect seen in observational studies could be unpleasant side effects among new drug users, possibly resulting in increased fracture risk. Those individuals would probably discontinue treatment, leaving only patients tolerating the medication among the long-term users.

How can we explain our findings of increased fracture risk in former users of thiazides? Intuitively, if there truly is a beneficial effect of thiazides on fracture risk, this effect would merely disappear when the patient ends treatment. However, that does not imply that the fracture risk simultaneously would increase in relation to other antihypertensive treatments. An appealing explanation would be a rebound effect with a rapid rise in urinary calcium related to treatment discontinuation which gradually declined with time, as found in the small RCT by Trasbøl et al. [98]. Such rapid loss of calcium could decrease BMD and increase the fracture risk compared to other antihypertensive therapies. However, despite the initial rise in urinary calcium levels after drug discontinuation seen in the study by

Trasbøl et al., they found no significant loss of BMD compared to the placebo group during that time [98]. Hence, I find it somewhat difficult to understand our findings with a trend towards a more pronounced increase in fracture risk shortly after discontinuation and the sustained elevated risk among any former user of thiazide. When a patient discontinues thiazide treatment, he or she probably either switches to another BP-lowering drug due to side effects from thiazides, or simply just ends treatment without switching to an alternative drug. The former patient might end up with a drug that instead increases the risk of fracture, such as loop diuretics, whereas the latter one could end up with uncontrolled hypertension which might lead to increased fracture risk related to the cardiovascular consequences that eventually might arise. Another explanation could be that former users of thiazides might have had difficulties tolerating the treatment due to frailty or comorbidities such as impaired renal function, which are established risk factors for fractures. Such causes could translate into a higher background risk for fractures among former thiazide users, a risk that significantly decreased with thiazide treatment but was revealed after discontinuation. This is merely tentative reasoning, but in an ambitious observational study looking thoroughly at different time-frames with thiazide treatment, Kruse et al found a significantly higher background risk for fractures among thiazide users compared to non-users [186]. The reason for that background risk remains unclear, but the higher risk compared to users of other antihypertensive medications declined and disappeared with continuous thiazide treatment [186]. Finally, other studies have estimated fracture risk among former users of thiazides, with mixed results. A nationwide case-control study from Denmark reported an association with reduced risk for hip fractures in both current and former users of thiazides [180], and a cohort study found a non-significant trend of protection against hip fractures up to 4 months after discontinuation of treatment [181]. However, another prospective cohort study reported an increased risk of hip fractures in former thiazide users, consistent with our findings [177].

Noteworthy, all our analyses were adjusted for age, SBP, and an extensive number of diseases and medications including other antihypertensive drugs, which should lower the risk of confounding from such factors. However, adjustments were done on information from baseline and we could not take into account changes in morbidity during follow-up. Importantly, the statistical power is smaller in the sub-group analyses regarding treatment duration and duration of discontinuation, and the precision in the estimates is more uncertain, illustrated by the wide confidence intervals. Hence, although these findings deserve some attention, they should overall be interpreted more cautiously.

Finally, is there any reasonable explanation for the gender differences regarding thiazide use and fracture risk, with a statistically significantly reduced fracture risk only in men? Observational data have indicated that women might be more susceptible to thiazide-related hyponatremia than men [190] which

possibly could diminish the beneficial effect of thiazides [109]. Moreover, since women have a more prominent overall risk for osteoporosis and fractures than men, a protective effect from thiazide treatment may simply not be sufficient to significantly prevent fracture occurrence in women.

Inhibitors of the renin-angiotensin-aldosterone system

Although experimental studies in animal models have suggested pathways for improved bone composition by blocking the renin-angiotensin-aldosterone system [119] we did not find any association between treatment with either ACEi or ARB and the risk of hip fracture. Nor did we find any association between aldosterone receptor antagonists (MRA) and hip fracture risk. This discordance might imply that the physiology and regulation of bone mass in animals are not referable to the human body. Moreover, as described in the introduction chapter 1.3, several of the previous studies investigating renin-angiotensin-aldosterone modulators and fracture risk have reported rather inconsistent results. Since the publication of Study II in this thesis, at least three new observational studies have been conducted. Two of those are very similar, comprising cohorts based on information from the same nationwide registers in Taiwan, including approximately 57000 individuals diagnosed with hypertension. These studies reported a significantly decreased risk for osteoporotic fractures in users of either ACEis or ARBs compared to non-users (adjusted HRs 0.66-0.77) [191, 192]. The most significant risk reduction was found in ARB-users [191] and the associations were stronger with a longer duration of use (>1 year) [192]. Finally, a retrospective analysis based on routinely collected health data in Scotland, found similar estimates of risk reduction in users of ACEi or ARB as the Taiwanese studies. However, in that study, no confirmation of a hypertensive diagnosis was made. In contrast, as mentioned earlier, in a post hoc analysis from the large RCT ALLHAT, treatment with an ACEi was inferior compared to treatment with a thiazide with regard to hip and pelvic fractures as an adverse event [179].

In summary, some results from other investigators are consistent with ours and some are not. A possible explanation for these divergent results is the differences between study populations and study designs. Another interpretation of the inconsistency is that if a relationship between these drugs and fracture risk exists, then it is weak. Thus, to establish any conclusive evidence regarding the effect of these drugs on fracture outcomes in individuals with hypertension, further studies are warranted to confirm or disprove the findings in ours' and others' studies. At least there is no convincing evidence for an increased fracture risk while using these drugs, which is uplifting regarding the wide prescription for the management of hypertension.

Beta-blockers

We did not find any association between the use of BB and hip fracture risk, in line with some previous studies [123, 141, 193] but in contrast to others [128, 189, 194, 195]. In our study, a large proportion of the patients were treated with BB (62% ever used a BB during follow-up). Almost all of those used β_1-selective drugs. Since the results in a meta-analysis from 2014 (mentioned in the introduction chapter) indicated that the association between use of BB and a decreased fracture risk mainly was driven by the use of β_1-selective drugs [142], our findings was somewhat unexpected. Prior studies have also indicated that the dose of medication might be important, with low but not high doses of BB to have a favorable effect on bone structure [137, 138]. Since we did not assess the dose of medication in our study, we cannot rule out that any dose-dependency could have influenced our results.

Importantly, BB are prescribed for further reasons than hypertension (mainly heart failure and chronic ischemic heart disease), which per se might influence fracture risk [196]. We adjusted all analyses for several potentially important confounders including such comorbidities, but as with all observational studies, we cannot exclude the possibility of residual confounding.

Loop diuretics

Our finding of an increased risk of hip fracture associated with the use of loop diuretics confirms the findings in most previous studies [123, 128, 154, 155, 197]. In addition, this is in line with known side effects of loop diuretics including enhanced urinary loss of calcium, other electrolyte disturbances, and orthostatic symptoms that could alter the risk for falls and fractures. Since our findings are well in line with current evidence, it may strengthen the other findings in our study as well, indicating a less likelihood that the overall study results would be highly biased (assuming that the consistency in prior studies relatively well reflects a true association).

Calcium channel blockers

The few previous studies that have investigated the use of CCB and fracture risk have shown mixed results, described in the introduction chapter 1.3. Hence, the lack of association between the use of CCB and hip fracture risk found in our study is neither controversial nor plainly confirmatory. However, our results together with others [123, 125, 126, 128, 146] suggest that we can continue to prescribe CCB for the treatment of hypertension without major concern for negative effects on hip fracture risk.

General strengths and limitations

The SPCCD comprises a large study sample including both men and women, from both urban and rural areas. Hence, the SPCCD can be regarded as well reflecting the Swedish population attending primary health care, at least in the southern region of Sweden, and the results from Study I and II can be extrapolated accordingly. A strength with register-based studies such as SPCCD is that all data is obtained from electronic records that are routinely filled in during normal daily practice, causing minimal risk of selection bias or recall bias. In addition, we ascertained that information on exposure was obtained before the index date to avoid the risk of reverse causality. Although we cannot know the particular indication for prescribing each BP-lowering drug to patients in SPCCD, at least we can be certain of their diagnosis of hypertension. This is important since the aim was to address the association between different treatment options and fracture risk in hypertensive individuals, not as medications that could be used solely for the purpose of reducing fracture risk.

However, SPCCD and the studies we have conducted also have important limitations. First, some information is not very well and systematically collected in clinical practice, such as BMI, smoking habits, alcohol consumption, and physical activity, which all might be important for both BP and fracture risk. In SPCCD we lack information on several of such variables, and the ones we have (BMI and smoking) suffer from many missing values. We dealt with those missing values by the missing indicator method, while multiple imputations would have been a more valid method. Hence, although we have no obvious reason to believe that these factors would significantly differ across users of different antihypertensive medications, we cannot rule out such bias. In addition, DXA measurements are not performed routinely in primary care, yet only for individuals highly susceptible to osteoporosis and fractures. Hence, we have no information on BMD values and we cannot take into consideration any baseline values of BMD across users of different antihypertensive drugs, nor any changes during follow-up. However, this is something we share with the vast majority of previous studies. The same goes for incident falls, which potentially could be a more or less important mediating factor between different antihypertensive therapies and fractures. Since we do not have any sufficiently reliable or valid data on falls in SPCCD, we cannot make any estimates of how much the events of falling have influenced our results across different antihypertensive drugs.

Secondly, the accuracy of the diagnoses we used for outcome measurements and morbidities included as potential confounders in the multivariate models, might vary. As a clinician, I am well aware that diagnoses both might be missed, erroneously made, or incorrectly recorded, which would lead to misclassifications. For example, the diagnoses of osteoporotic fractures were

obtained from hospital care. However, not all fractures come to clinical attention, especially not vertebral fractures. Vertebral fractures can be difficult to see and define on standard X-rays, and the symptoms can be rather non-specific and pass by as unharmful back pain. In Sweden, it has been estimated that only about 23% of vertebral deformities in women come to clinical attention [49]. In addition, vertebral fractures seldom need surgery and can be handled in primary care. Hip fractures, on the other hand, cause distinct symptoms and are rarely missed. Also, these patients will end up in hospitals as surgery is needed. This was a major reason to limit the outcome to hip fractures in Study II and to combine the fracture code with a code of surgery to further minimize the risk of misclassification. Additionally, since hip fractures account for such high degree of morbidity, it is also a clinical interesting outcome.

Third, when submitting these manuscripts to the Journal of Hypertension, the concern was raised that our results might be confounded by BP level or cardiovascular comorbidities that might differ between the users of different antihypertensive drugs. Indeed, as described in the introduction, the severity of hypertension may be associated with the risk of osteoporotic fractures and CVD even more. In support of this, the proportion of CVD and the level of BP were higher at baseline among those patients who would experience a fracture during follow-up (see Table 1 in each study). Note, these cross-sectional measurements are not adjusted for age. Furthermore, the reason for choosing one antihypertensive drug before another may have been based on comorbidities such as diabetes or coronary heart disease, which might cause biased results due to confounding by indication. With these limitations in mind, we adjusted for these possible confounders in the multivariate models, including BP levels and CVD, lowering the risk for such bias. However, since we only had reliable data for these variables at baseline, we cannot take into account changes in BP or new onset comorbidity during follow-up.

Finally, inherent to an observational study, we cannot rule out residual confounding or assume causal conclusions. However, in the absence of RCTs, these studies might improve the evidence regarding fracture risk associated with antihypertensive treatment, giving the longitudinal study approach and extensive adjustments for possible confounders.

5.2 DISCUSSION STUDY III

THE ASSOCIATION BETWEEN PERIPHERAL ARTERIAL DISEASE AND RISK FOR HIP FRACTURES IN ELDERLY MEN

Main findings

In this prospective study in Swedish elderly men, PAD based on an ABI <0.9 was associated with an increased risk of hip fracture, independent of age and hip BMD. However, after further adjustments for comorbidity, medications, physical function, and educational level, the association was slightly attenuated and no longer statistically significant.

Prior studies - similarities and differences

In this section, we dive deeper into prior studies investigating the association between PAD and hip fracture risk. The aim is to highlight strengths and weaknesses as well as differences compared to the present study in this thesis that might explain some of the inconsistent results.

First, a population-based cohort study from Australia including 4321 men reported an increased risk for hip fracture in men with PAD compared to men without PAD (HR 1.69; 95 % CI 1.08-2.63) [87], independent of age, BMI, smoking, alcohol consumption, previous stroke, coronary heart disease, and abdominal aortic aneurysm. A large register-based cohort study from Taiwan including 1464 incident hip fractures showed similar results with HR 1.57 (95 % CI 1.45–1.71) for hip fractures in women with PAD, and HR 1.50 (95 % CI 1.44–1.78) for men with PAD, independent of age-group, income, numerous medical conditions, and medications [85]. These estimates are rather consistent with our results. However, none of those studies had any information on previous falls and physical function, factors that seemed to impact the results in our analyses. Also, no BMD measurements were made. A more striking association between PAD and hip fracture risk was found in a study of more than 30000 twins from the Swedish twin registry, reporting an increased risk of hip fracture in men and women with a hospital diagnosis of PAD with an HR of 3.20 (95% CI 2.28-4.50), independently of age and several diseases [70]. Further adjustments for lifestyle factors were described to marginally change the estimate, but no data were presented. In contrast, in a register-based study of nearly 190000 Spanish men ≥ 65 years of age, Reyes et al. found a significantly increased risk of hip fracture in men with PAD in an age-adjusted model (RR 1.45; 95 % CI 1.20-1.74), but when adding BMI, smoking status, medical use and comorbidities including other CVD, the association declined significantly (RR 1.13;

95% CI 0.87-1.48) [198]. Moreover, Collins et al, who conducted a similar study to ours but in the US cohort of MrOS, found an increased risk for non-spine fractures in men with PAD independently of hip BMD, but no association with hip fracture risk was observed [83]. The latter lack of association might, however, have been influenced by the limited number of hip fractures during follow-up (n=89).

Finally, a few smaller studies assessing this topic found no association between PAD and fracture risk. A study of 72 residents in a nursing home confirmed increased mortality in residents with an ABI <0.9 or ≥ 1.4, but the incidence of hip fractures ended up being too low to study as an outcome [84]. Likewise, a cohort study with 1332 men and women reported no association between PAD and fracture risk, which again could be explained by the limited sample size, but also an ABI cut-off at ≤0.9 instead of <0.9 [86]. Furthermore, a longitudinal study with 3626 men and women did not find any association between ABI values and hip fracture risk [81]. However, they analyzed the ABI per unit increase in the index, which is dubious to compare with the clinically more relevant cut-off value of ABI <0.9 [81].

In summary, several prior studies support an increased risk for hip fractures in men with PAD, but conflicting results exist. The diversity in study design, especially the inconsistent choice of covariates included in the multivariate analyses, might explain some of the differences in estimates of risk as well as limit the possibility to interpret possible mechanisms. A similar concern was raised by Ungpraset et. al. in their meta-analysis from 2018, including 6 of the studies described above. The authors reported a pooled RR of 1.64 (95% CI 1.17–2.29) for incident hip fracture in patients with PAD compared to patients without PAD, but highlighted the high between-study heterogeneity and possibility of publication bias [199]. Unlike most of these prior studies, we included both comorbidity and functional measurements in our multivariate analysis, which can explain the final non-significant association between PAD and hip fracture risk, despite the relatively large sample size and fracture incidence. However, the risk for type II error should also be considered, since an even larger study sample might be needed to confirm small differences between groups. Moreover, we cannot rule out the possibility of multicollinearity or overadjustment by the inclusion of non-important factors as contributing explanations for the non-significant result in our final model.

BMD in the relationship between PAD and fractures

Only three of the abovementioned previous studies had information on BMD. Two of those find no association between fracture risk and PAD in unadjusted analyses, and further adjustments for BMD measurements were not done [81, 86]. The findings from the third one, the study from Collins et al., did not show any significant indications that hip BMD would be of importance in the relationship between PAD and non-spine fractures, despite the lower BMD in men with PAD at baseline [83].

Consistently, in our study men with PAD had lower baseline total body and hip BMD than men without PAD. Although this is a cross-sectional observation not adjusted for age, it is still in line with studies reporting an association between imaging and clinical manifestations of atherosclerosis and lower BMD, mostly in women but also in men [74, 77]. Despite these findings and the fact that low BMD is one of the most important risk factors for fragility fractures, including BMD in our multivariate analysis did not alter the association between PAD and fracture risk in any significant way. To our knowledge, our study is the first one to identify an association between PAD and increased hip fracture independent of hip BMD.

Increased fracture risk – potential mechanisms and contributing factors

Our results suggest a low probability for the association between PAD and hip fracture risk to significantly be mediated by BMD. Hence, although individuals with PAD might suffer from poor BMD, our results suggest that the impact of impaired hip BMD might be of minor importance for the overall fracture risk in men with PAD. Although we cannot ascertain any mechanistic explanation from our observational study, a number of possible contributing factors are discussed below.

First, the hypothesis of a positive association between PAD and hip fractures might be intuitive, given the number of overlapping risk factors, such as sedentary lifestyle, smoking, renal impairment, and diabetes mellitus [49, 75, 200, 201]. Low vitamin D levels and inflammation are other factors that have been linked to both impaired bone health and peripheral atherosclerosis [202-204], consistent with our findings of a higher level of baseline CRP in men with PAD. Smoking is a major risk factor for fractures [205] and a particularly strong risk factor for PAD [206]. Concurrently, in our cohort men with PAD were almost 3 times more often current smokers than men without PAD. Cadmium exposure has been proposed to account for a substantial part of the atherosclerotic effect of smoking [207], but other sources of cadmium exposure could be contributing as well, as urine cadmium levels have been associated with increased risk for new-onset PAD independently of smoking status [208]. Accordingly, in a sub-analysis from the Gothenburg part of MrOS where urine cadmium levels were obtained, we found urine cadmium to be higher among men with PAD. Increased urinary cadmium has also been associated with increased risk for low BMD and fractures, even at relatively low exposure [209]. Diabetes mellitus is another established risk factor for both PAD and fractures, including hip fractures [32, 210]. Interestingly, the increased fracture risk attributed to diabetes has not been explained by loss of BMD or bone quality, but instead yet not fully known mechanisms [211, 212]. Although our multivariate analyses showed no clear indication that any of those factors alone explain the association between PAD and fracture risk, the sum of the influence from several of those attenuated the

point estimates and precision in our multivariate analyses, indicating that these factors might be of importance for the observed relationship between PAD and fracture risk.

Secondly, baseline measurements confirmed overall more morbidity and signs of frailty in men with PAD, a burden that generally could enhance the risk of injurious falls and fractures. PAD itself might cause frailty by limiting the ability to physical activity, directly due to lower limb symptoms, and indirectly through its close relationship with ischemic heart disease and stroke [32]. Indeed, observational evidence suggests that at least stroke is an independent risk factor for hip fractures [80]. Also, PAD has independently been associated with lower physical function also among asymptomatic individuals [37, 38] (further described in chapter 1.2 of this thesis). In this case, the lower physical function might serve both as a potential confounder in the relationship between PAD and fracture risk (being a cause of a long-term sedentary lifestyle contributing to the development of PAD), or a mediating factor (as a result of PAD itself). Despite whether any of those assumptions is more true than the other, when including measures of handgrip strength and walking speed in the multivariate model, the association between PAD and hip fracture weakened. This strengthens the hypothesis that those variables are of importance in the relationship between PAD and hip fracture risk. With this said, it could be interesting to further explore the magnitude of each of the factors described in this section as mediators in the relationship between PAD and hip fracture risk, which our results, unfortunately, cannot give any proper answer to.

Finally, the findings of a similar pattern for all fractures and major osteoporotic in men with PAD as for hip fractures supports the main results observed for hip fracture risk (with an increased fracture risk independent of age and hip BMD, but a non-significant association in the multivariate model). The fact that the association with vertebral fractures was substantially more uncertain strengthens the hypothesis that the increased fracture risk in men with PAD probably is a consequence of injurious falls rather than low BMD. This since the latter to a higher extent leads to more spontaneous compressions of the vertebrae whereas other fracture sites, especially at the hip, ultimately are a consequence of a fall or other trauma (although the energy often is low).

Death as a competing risk

Men with PAD have an increased risk for cardiovascular and all-cause mortality [32, 33]. Indeed, the age-adjusted mortality rate was higher in men with PAD also in MrOS Sweden. Therefore, death should be considered as a competing risk for fracture outcomes. As such, although our results suggest an increased risk for hip fractures in men with PAD, this only implies to men that are still alive and at risk. Hence, although events of death were censored in our Cox models, we cannot assume

that the results suggesting an increased fracture risk ultimately leads to the same extent of a higher proportion of fractures in men with PAD, as they might die due to something else before they experience a fracture.

Strengths and limitations

The MrOs Sweden study has several strengths. With respect to the extensive study protocol at baseline, this study comprises a relatively large cohort with a long prospective follow-up time, generating good opportunities to study fracture outcomes with rather good statistical power. The ambitious number of clinical tests and measurements also enables taking into account confounders and mediating factors that rarely are obtained in larger register-based studies solely comprising data routinely obtained in clinical practice. In general, the Swedish public health care system and the well-established tradition of collecting data in nationwide and regional registers, enable a valid coverage of fractures diagnosed by both public and private health care providers. Additionally, all fractures considered in MrOS were verified by x-ray which was reviewed an additional time specifically for the purpose of the study, to ensure correct diagnosis and reduce the risk of misclassification.

However, studies like MrOS also have important limitations. Although men were randomly selected from population-based registers, which is an important strength, the attendance rate was only 45%. As we do not know the particular reason for declining participation in the study, it is difficult to know how well the study sample reflects the average population of men in those age spans during that time. However, we do believe that men with a high degree of morbidity or frailty may have declined participation, according to inclusion criteria and the extensive baseline exams. Hence, MrOS is assumed to comprise a larger proportion of healthier men than the reference population, and the results from this study should be interpretant with caution to the frailest part of elderly men. In addition, MrOS consists almost entirely of Caucasian men. This is a highly relevant study population, as Caucasians generally are more susceptible to hip fractures [49], but also limits the generalizability of our findings. Finally, the questionnaires relied on self-reported data which might cause recall bias, and as with all observational studies; although we adjusted for several relevant confounders in our analyses, residual confounding cannot be ruled out.

5.3 DISCUSSION STUDY IV

HYPERTENSION AND FRACTURE RISK IN ELDERLY MEN

Main findings

In a prospective cohort study in elderly men, originally initiated to identify risk factors for osteoporosis and fractures, we did not find any association between hypertension and fracture risk. However, our data suggest some differences regarding both established risk factors and protective factors for fractures in men with hypertension compared to men without hypertension, which deserves to be addressed further.

Hypertension and fracture risk

The lack of association between hypertension and fracture risk in our study is in line with another well-design cohort study, including 1032 men and 1701 women aged ≥50 years from the Dubbo Osteoporosis Epidemiology Study (DOES) [72]. Interestingly, in that study hypertension was associated with an increased risk for any fracture in women (HR 1.49, 95% CI 1.13-1.96), independent of femoral neck BMD, body weight and height, prior CVD, medications for CVD (incl hypertension) and other relevant confounders, but not statistically significant in men (HR 1.53, 95% CI 0.94-2.48). The non-significant result and wider confidence interval in men might have been influenced by the lower incidence of fractures compared to women. Moreover, while women with hypertension had lower baseline femoral neck BMD, men with hypertension had higher femoral neck BMD than men without hypertension. The higher BMD in men with hypertension is consistent with the findings in our study, which may further have contributed to the gender differences in fracture risk found in the abovementioned study. Similar gender differences were seen in a case-control study by Perez-Castrillion et al., identifying a significant association between hypertension and increased fracture risk in women, but not in men [73]. The increased risk in women vanished in a group of thiazide users in stratified analyses across the use of different antihypertensive drugs. Unfortunately, no measurements of BMD were available in that study. Finally, a recent large cohort study investigating the components of metabolic syndrome and fracture risk did not report any association with hip fracture risk and BP (mean arterial pressure), neither in women nor in men [71]. However, as one could expect, fracture risk decreased with increasing BMI across both genders.

In contrast, results from a few large case-control studies based on nationwide registers have suggested an association between hypertension and increased hip fracture risk in both men and women [67-69]. Noteworthy, two of these studies lack

information on valuable confounding factors, only adjusting the analyses for age and sex [68], or age, sex, and the number of comorbid diagnoses (irrespective of the nature of the diagnosis) [67], which might contribute to biased results. However, in the study by Vestergaard et al., analyses were adjusted for several relevant comorbidities, medication incl antihypertensive drugs, and social factors known to affect fracture risk, and still a significant association between hypertension and increased fracture risk in both men and women was found (OR 1.27 (95% CI, 1.20-1.34) in the short term and OR 1.11 (95% CI, 1.00- 1.23) in the long term) [69]. A twin study including 24550 twins from Sweden confirmed these findings, where the diagnosis of hypertension was associated with an increased hip fracture risk in men and women with a multivariable-adjusted HR of 1.59 (95% CI, 1.36- 1.85) [70]. Nevertheless, none of these two latter studies presented separate figures for men and women.

Further studies have been conducted on women only, confirming an association between hypertension and increased fracture risk [64, 213]. However, our analyses from the MrOS Sweden study are to our knowledge the first ones assessing this question in a study conducted specifically on elderly men. Hypothetically, according to the findings in the present and former studies, publication bias may contribute to such gender disparity. On the other hand, the point estimates in prior studies, especially in the study by Vestergaard et al., indicated minor differences in fracture risk between individuals with and without hypertension, suggesting that extensive study samples might be needed to confirm group differences. In such a setting, we cannot rule out the risk for type II error in a study sample with the size of ours.

Noteworthy, we found the association between hypertension and fractures to be time-dependent in "healthier" men with hypertension without baseline CVD comorbidity. During the first 4 years of follow-up, hypertension was associated with decreased fracture risk in this group, compared to men without hypertension. This implies the importance of how we handle follow-up time in future studies investigating this topic.

Finally, when investigating a risk factor such as hypertension it is difficult to distinguish which effect we actually are measuring – the effect from a high BP or the composite effect from the drugs used to treat hypertension.

Risk factors and protective factors associated with hypertension

We found higher baseline BMD at all sites in men with hypertension compared to men without hypertension. However, after adjusting for BMI, only a slightly positive OR for lumbar spine BMD remained. Thus, suggesting that the higher BMD in men with hypertension mainly is driven by the mechanical load of a relatively heavier

body. Increasing BMI and BMD are both strong protective factors for fractures. Apart from the effect of increased mechanical load on the skeleton, higher BMI may result in better "padding" while falling and hence prevent a hazardous fracture. However, how severe obesity in hypertensive individuals affects fracture risk would be interesting to explore further.

In contrast to these protecting factors associated with hypertension, we also found an association with several risk factors for fractures, independent of BMI, renal function, and diabetes. Firstly, our findings of an inverse association between hypertension and testosterone and SHGB is in line with previous studies reporting hypertension to be associated with lower levels of testosterone [214], and high levels of testosterone to be associated with lower prevalence of hypertension in non-obese men [215]. Although conflicting studies exist, testosterone has been associated with both low BMD and increased fracture risk in men [216], and has been suggested to be one of the causes of the declining BMD in aging men [45, 50]. Notably, low levels of estrogen have been proposed to have a larger impact on age-related bone loss also in men [45]. However, the levels of estrogen did not differ between men with and without hypertension in our cohort, another possible factor contributing to the maintained BMD. Secondly, leptin is a hormone directly related to the amount of adipose tissue [217], but higher levels of leptin have also been suggested to possibly be associated with lower BMD, at least at the lumbar spine [218]. Interestingly, we found a positive association between leptin and hypertension, independent of BMI, consistent with a few prior studies [219-221]. This could indicate that there might be further pathways between leptin and hypertension beyond the number and size of adipocytes. Thirdly, the positive association we found between PTH and hypertension independent of eGFR confirms findings from previous studies indicating that elevated levels of circulating PTH directly or indirectly may contribute to elevated BP [222, 223]. This might be of interest as the relationship between elevated levels of PTH and low BMD is well established [224]. Fourthly, the positive association between EPO and hypertension independently of eGFR might be another risk factor for fractures in men with elevated BP. Recently, another study from the Swedish MrOS reported high levels of EPO to be independently associated with increased risk for incident fractures in men with normal renal function [174].

In summary, in this population of elderly men, we identified both protective factors as well as risk factors for incident fractures in men with hypertension. Hence, a merely tentative explanation for the lack of association between hypertension and fracture risk could be suggested due to an interplay between these factors.

Strengths and limitations

The major strengths of this study are the prospective approach with a follow-up time up to 18 years, and the extensive baseline measurements that enable a good characterization of the study participants. A more thorough discussion regarding strengths and limitations connected to the MrOS cohort is given in the discussion section for Study III. However, specifically for this study is the assessment of hypertension as exposure. Studying the effects of hypertension is often a delicate matter, since only half of the people affected by elevated BP are diagnosed accordingly [12] and several BP measurements are required for correct diagnosis [10]. Hence, there might be a risk of misclassification where some men in this study are defined as not having hypertension when they in fact do have hypertension. As the diagnosis is based on self-reported data prone to recall bias, the risk might be even larger. However, this is a problem that this study shares with numerous others. Noteworthy, during the twenty years that have passed since the start of MrOS, the awareness and recommendations regarding how to treat hypertension in the elderly may have changed a bit as well. Today, accumulated evidence suggests that also the elderly benefit from more active treatment to reach lower target BP, and although no major changes have occurred, also the recommendations on which type of antihypertensive medications to use have shifted some. Finally, the risk factors for fractures identified among men with hypertension are cross-sectional observations, which means that there is no information on whether the risk factor preceded the high BP, or whether the high BP might have contributed to the development of the risk factor, only that they seem to relate to one another. However, these findings could possibly contribute to further research assessing causal mechanisms of interest in clinical practice.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

Study I and II

In summary, our findings from a large register-based cohort study confirm the hypothesis that the risk for hip fracture differs across users of different blood-pressure-lowering drugs. Long-term treatment with thiazides seems to have favorable effects that reduce the risk of hip fractures in patients with hypertension, compared to other commonly used blood-pressure-lowering drugs. In contrast, treatment with loop diuretics seems to significantly increase the risk of hip fracture, whereas treatment with BB, ACEi, ARB, aldosterone receptor blockers, or CCB revealed no significant association with fracture risk. These findings are applicable to older individuals from 50 years of age, and overall, in line with several other observational studies. However, as we found the association with thiazides to be overall modest and more pronounced in men and more uncertain in women, the evidence is still scarce for drawing clear conclusions on how to use this knowledge in clinical decisions. On the other hand, as the prevalence of hypertension and the risk of hip fracture in older adults is high, also small differences in effect between drug classes could possibly be translated into many hip fractures. Hence, it would be valuable for physicians treating these patients to be aware of the associations between treatment options and fracture risk highlighted in this thesis.

From a future perspective, since these studies did not address whether any differences in the dose of medication could be of importance for fracture outcomes, it would be valuable to investigate this further. Additionally, in the light of an aging society and accumulating evidence that BP-lowering interventions can be valuable also in the very old, it would be interesting to specifically address the association between fracture risk and different BP-lowering drugs in the elderly. If thiazides still seem to have a favorable effect on fracture risk in the elderly, would it depend on when we initiate therapy and for how long it continues? It would also be interesting, although probably quite complex, to investigate if certain combinations of antihypertensive drugs could be particularly preferable or unfortunate with respect to fracture outcomes.

Study III

The results of this prospective cohort study suggest that PAD is associated with an increased risk for hip fracture independently of hip BMD in elderly Swedish men. However, the high frequency of comorbidity and lower physical performance among

these men are factors that together seem to explain some degree of the observed association. Based on these results, in a clinical setting, it could be valuable to assess the overall fracture risk in elderly men with PAD such as optimizing cardiovascular prevention, encouraging physical activity, and preventing injurious fall accidents. But according to our results, BMD measurements should be done in men with PAD based on the same risk assessment as for any other older man. Future research is warranted to confirm or contradict these findings and to further address how PAD according to fracture risk best should be handled in clinical practice. Finally, as fractures highly afflict women and PAD affects women similar to men, it would be valuable to also investigate this association in a cohort of elderly women.

Study IV

The findings in this study suggest no association between hypertension and fracture risk in Swedish elderly men. However, we identified several risk factors as well as protective factors for fractures in men with hypertension that differed from men without hypertension. For future research in the field of fracture and hypertension, knowledge concerning these factors might be of interest.

ACKNOWLEDGEMENTS

First, I like to acknowledge the people behind the idea and the work constructing the database SPCCD, and the important contribution from the participating primary health care centers that made the project possible. Likewise, a huge acknowledgment to the staff and participants of the MrOS Sweden study for their important contributions to medical research.

Funding

The Swedish primary care cardiovascular database

The SPCCD is endorsed by the Swedish Society for Hypertension, Stroke and Vascular Medicine. Grants have been received from the Swedish Heart Lung Foundation, Karolinska Institutet Research Foundations, the Health & Medical Care Committee of the Regional Executive Board of the Region Västra Götaland, the Swedish Society of Medicine (Lagerströms and Lysanders Foundations), the Skaraborg Research and Development Council, the Stockholm County Council (Stockholm County Drug and Therapeutics Committee, the Department of Drug Management and Informatics, and the EK-group of Centre for Family and Community Medicine), and by grants from the Swedish state through the ALF-agreement (ALFBGB 721311, 139241 and 965452).

MrOS Sweden

This study was supported by grants from the Swedish state (LUA/ALF). A special thanks to Göteborgs Kontoristförening (Dan Mellström).

TACK

Först och främst tack till Karin Manhem, min handledare och förebild. Vilken pedagog, forskare, underbar kvinna och medmänniska. Tack för din oupphörliga uppmuntran och tro på förmågor hos mig som jag ofta tvivlat på. Du öppnade mina ögon för forskning, någonting jag aldrig trodde att jag skulle bedriva. Du har stöttat mig på ett varmt och hjärtligt sätt genom hela denna resa med dess toppar och dalar, vilket jag är evigt tacksam för. Tack för alla samtal och skratt. Jag kunde inte haft en bättre handledare. Tack för allt.

Dan Mellström, min bihandledare och en till synes outtömbar källa på kunskap inom osteoporos och frakturer. Ditt engagemang och din entusiasm har varit ovärderlig och verkligen satt prägel på denna avhandling.

Charlotta Ljungman och Kristina Bengtsson Boström, ytterligare två av mina bihandledare, ert stöd, kloka tankar, idéer och positiva attityd inspirerar. Utan dig Lotta hade mitt första arbete aldrig skrivits, stort tack för idén som var startskottet till allt.

Linus Schiöler, bihandledare och statistiker, som tålmodigt besvarat mina förvirrade frågor om statistik och bidragit med de långa och tidskrävande statistiska analyserna till delarbete I och II. Likaså Valter Sundh, din hjälp med de statistiska analyserna från MrOS och utförliga pedagogiska svar på mina många frågor har verkligen uppskattats.

Även stort tack till mina medförfattare som bidragit med konstruktiv feedback och förbättrat slutprodukten av studierna i denna avhandling. Särskilt tack till SPCCD-gänget: databaskonstruktörerna och medförfattarna Karin Manhem, Kristina Bengtsson Boström, Charlotta Ljungman, Thomas Kahan, Per Hjerpe, Björn Wettermark och Jan Hasselström. Thomas, du har särskilt bidragit till bra diskussion i delarbete I.

Extra tack till två av Karins övriga doktorander som jag fått äran att inspireras av och skratta tillsammans med: Lina Dahlén Holmqvist och Johan-Emil Bager.

Kerstin Landin, du uppmärksammade mitt examensarbete, utan dig hade det kanske inte blivit någon doktorand av mig. Även tack Anna-Clara Collén, verksamhetschef för akutmedicin och geriatrik på Sahlgrenska, för hjälp i sista stund med att genomföra disputationen.

Gunnar Steinbeck och Maria Hedelin, tack för den mycket bra Kliniska Forskarskolan. Avgångsklass HT 2018, tack för fin gemenskap och givande diskussioner.

Kollegorna på Arvidsjaur Hälsocentral, ni förgyller min kliniska vardag i glesbygden på ett unikt och glädjefyllt sätt, ni är underbara. Särskilt tack till Peter Öhman, min första chef i Region Norrbotten, för att du tog emot mig med så öppna armar och den stöttning du gett mig från första stund, både som doktorand och kliniker.

Mina vänner, i vått och torrt. I bergen, på skidor, klätterklippan, skogsstigarna och hemma i ert kök. Ni vet vilka ni är. Utan er, inga äventyr, utan er, ingen självkänsla. Med er delar jag det viktiga här i livet.

Naturen, bergen, skogen, havet – tack för all den energi och återhämtning ni ger mig.

Slutligen, största tack till min fantastiska familj. Till er som stöttat och hejat på mig när det behövts som mest. Tack Gustav för hur du alltid inspirerar mig och visar hur lite som är omöjligt. Tack mamma och pappa för ert oupphörliga stöd. Tack mina inspirerande och smarta bröder Rasmus och Jon, som bland annat bidragit med korrekturläsning i sista stund. Särskilt tack till dig Jon, för långa och givande forskningsdiskussioner och för att du alltid ställer upp när jag behöver dig. Tack också till min växande mage, till dig som snart ska få komma ut i denna värld. För att du hjälpt mig att fokusera och glädjas det lilla extra under arbetet med denna avhandling. Ser fram emot livet med dig.

REFERENCES

1. Rothman KJ (2012) Epidemiology: An Introduction. Oxford University Press (OUP), USA

2. Thiese MS (2014) Observational and interventional study design types; an overview. Biochem Med (Zagreb) 24:199-210.

3. Gustaffson BE, Quensel CE, Lanke LS (1954) The Vipeholm dental caries study. Acta Odontol Scand 11:232-364.

4. Porta M (2014) A Dictionary of Epidemiology. Oxford University Press (OUP), USA

5. Berry G, Newhouse M, Turok M (1972) Combined effect of asbestos exposure and smoking on mortality from lung cancer in factory workers. Lancet 300:476-479.

6. Schisterman EF, Cole SR, Platt RW (2009) Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology 20:488-495.

7. Pedersen AB, Mikkelsen EM, Cronin-Fenton D, et al. (2017) Missing data and multiple imputation in clinical epidemiological research. Clin Epidemiol 9:157-166.

8. Groenwold RHH, White IR, Donders ART, et al. (2012) Missing covariate data in clinical research: when and when not to use the missing-indicator method for analysis. CMAJ 184:1265-1269.

9. Andrade C (2019) The P Value and Statistical Significance: Misunderstandings, Explanations, Challenges, and Alternatives. Indian J Psychol Med 41:210-215.

10. Williams B, Mancia G, Spiering W, et al. (2018) 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension. J Hypertens 36:1953-2041.

11. Whelton PK, Carey RM, Aronow WS, et al. (2018) 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: Executive summary: A report of the American college of cardiology/American Heart Association task force on clinical practice guidelines. Hypertension 71:1269-1324.

12. Zhou B, Carrillo-Larco RM, Danaei G, et al. (2021) Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. Lancet 398:957-980.

13. Chow CK, Teo KK, Rangarajan S, et al. (2013) Prevalence, awareness, treatment, and control of hypertension in rural and urban communities in high-, middle-, and low-income countries. JAMA 310:959-968.

14. Zhou B, Perel P, Mensah GA, Ezzati M (2021) Global epidemiology, health burden and effective interventions for elevated blood pressure and hypertension. Nature Reviews Cardiology 18:785-802.

15. (2020) Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 396:1223-1249.

16. Danaei G, Lu Y, Singh GM, et al. (2014) Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: A comparative risk assessment. Lancet Diabetes Endocrinol 2:634-647.

17. (1991) Prevention of Stroke by Antihypertensive Drug Treatment in Older Persons With Isolated Systolic Hypertension: Final Results of the Systolic Hypertension in the Elderly Program (SHEP). JAMA 265:3255-3264.

18. Furberg CD, Wright Jr JT, Davis BR, et al. (2002) Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). JAMA 288:2981-2997.

19. Ellison DH, Loffing J (2009) Thiazide effects and adverse effects: Insights from molecular genetics. Hypertension 54:196-202.

20. Hughes AD (2004) How do thiazide and thiazide-like diuretics lower blood pressure? JRAAS - Journal of the Renin-Angiotensin-Aldosterone System 5:155-160.

21. Guyton AC (1991) Blood pressure control--special role of the kidneys and body fluids. Science 252:1813-1816.

22. (2004) Måttligt förhöjt blodtryck, en systematisk litteraturöversikt. SBU Rapport (The Swedish Council on Technology Assessment in Health Care) Vol. 1:kap 7

23. Antonietta CM, Calvi E, Faggiano A, et al. (2022) Impact of Loop Diuretic on Outcomes in Patients with Heart Failure and Reduced Ejection Fraction. Curr Heart Fail Rep 19:15-25.

24. Kapelios CJ, Bonou M, Malliaras K, et al. (2022) Association of loop diuretics use and dose with outcomes in outpatients with heart failure: a systematic review and meta-analysis of observational studies involving 96,959 patients. Heart Fail Rev 27:147-161.

25. Qvarnstrom M, Wettermark B, Ljungman C, et al. (2011) Antihypertensive treatment and control in a large primary care population of 21 167 patients. J Hum Hypertens 25:484-491.

26. Tölle M, Reshetnik A, Schuchardt M, Höhne M, van der Giet M (2015) Arteriosclerosis and vascular calcification: causes, clinical assessment and therapy. Eur J Clin Invest 45:976-985.

27. Mitchell GF, Powell JT (2020) Arteriosclerosis: A Primer for "In Focus" Reviews on Arterial Stiffness. Arterioscler Thromb Vasc Biol 40:1025-1027.

28. Wilkinson IB, McEniery CM (2012) Arteriosclerosis: inevitable or self-inflicted? Hypertension 60:3-5.

29. Libby P, Buring JE, Badimon L, et al. (2019) Atherosclerosis. Nat Rev Dis Primers 5:56.

30. Li Z, Lin L, Wu H, et al. (2021) Global, Regional, and National Death, and Disability-Adjusted Life-Years (DALYs) for Cardiovascular Disease in 2017 and Trends and Risk Analysis From 1990 to 2017 Using the Global Burden of Disease Study and Implications for Prevention. Front Public Health 9: 559751.

31. Song P, Rudan D, Zhu Y, et al. (2019) Global, regional, and national prevalence and risk factors for peripheral artery disease in 2015: an updated systematic review and analysis. Lancet Glob Health 7:e1020-e1030.

32. Aboyans V, Ricco JB, Bartelink MLEL, et al. (2018) 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS). Eur Heart J 39:763-816.

33. Pande RL, Perlstein TS, Beckman JA, Creager MA (2011) Secondary prevention and mortality in peripheral artery disease: National Health and Nutrition Examination Study, 1999 to 2004. Circulation 124:17-23.

34. McDermott MM, Criqui MH (2018) Ankle-Brachial index screening and improving peripheral artery disease detection and outcomes. JAMA 320:143-145.

35. Bauersachs R, Debus S, Nehler M, et al. (2020) A Targeted Literature Review of the Disease Burden in Patients With Symptomatic Peripheral Artery Disease. Angiology 71:303-314.

36. Alahdab F, Wang AT, Elraiyah TA, et al. (2015) A systematic review for the screening for peripheral arterial disease in asymptomatic patients. Journal of Vascular Surgery 61:42S-53S.

37. McDermott MM, Greenland P, Liu K, et al. (2001) Leg symptoms in peripheral arterial disease associated clinical characteristics and functional impairment. JAMA 286:1599-1606.

38. Matsushita K, Ballew SH, Sang Y, et al. (2017) Ankle-brachial index and physical function in older individuals: The Atherosclerosis Risk in Communities (ARIC) study. Atherosclerosis 257:208-215.

39. McDermott MM, Guralnik JM, Ferrucci L, et al. (2008) Asymptomatic peripheral arterial disease is associated with more adverse lower extremity characteristics than intermittent claudication. Circulation 117:2484-2491.

40. Hiatt WR, Armstrong EJ, Larson CJ, Brass EP (2015) Pathogenesis of the Limb Manifestations and Exercise Limitations in Peripheral Artery Disease. Circ Res 116:1527-1539.

41. Joosten MM, Pai JK, Bertoia ML, et al. (2012) Associations between conventional cardiovascular risk factors and risk of peripheral artery disease in men. JAMA 308:1660-1667.

42. Liu W, Yang C, Chen Z, et al. (2022) Global death burden and attributable risk factors of peripheral artery disease by age, sex, SDI regions, and countries from 1990 to 2030: Results from the Global Burden of Disease study 2019. Atherosclerosis 347:17-27.

43. Hart NH, Newton RU, Tan J, et al. (2020) Biological basis of bone strength: anatomy, physiology and measurement. J Musculoskelet Neuronal Interact 20:347-371.

44. Clarke B (2008) Normal bone anatomy and physiology. Clin J Am Soc Nephrol 3 Suppl 3:S131-139.

45. Clarke BL, Khosla S (2010) Physiology of Bone Loss. Radiologic Clinics of North America 48:483-495.

46. Yuen NK, Ananthakrishnan S, Campbell MJ (2016) Hyperparathyroidism of Renal Disease. Perm J 20:15-127.

47. Hadjidakis DJ, Androulakis II (2006) Bone remodeling. Ann N Y Acad Sci 1092:385-396.

48. Peck WA, Burckhardt P, Christiansen C, et al. (1993) Consensus development conference: Diagnosis, prophylaxis, and treatment of osteoporosis. Am Journal Med 94:646-650.

49. Hernlund E, Svedbom A, Ivergard M, et al. (2013) Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos 8:136.

50. Ferlin A, Migliaccio S (ed) (2020) Male Osteoporosis: Gender Differences in Pathophysiology, Clinical Aspects, Diagnosis and Treatment. 1st edition. Springer

51. Lane NE (2006) Epidemiology, etiology, and diagnosis of osteoporosis. Am J Obstet Gynecol 194:S3-11.

52. Kanis JA (2002) Osteoporosis III: Diagnosis of osteoporosis and assessment of fracture risk. Lancet 359:1929-1936.

53. Kanis JA, Johnell O, Oden A, et al. (2000) Long-term risk of osteoporotic fracture in Malmo. Osteoporos Int 11:669-674.

54. Johnell O, Kanis JA, Oden A, et al. (2005) Predictive value of BMD for hip and other fractures. J Bone Miner Res 20:1185-1194.

55. Siris ES, Chen YT, Abbott TA, et al. (2004) Bone mineral density thresholds for pharmacological intervention to prevent fractures. Arch Intern Med 164:1108-1112.

56. Cummings SR, Melton Iii LJ (2002) Osteoporosis I: Epidemiology and outcomes of osteoporotic fractures. Lancet 359:1761-1767.

57. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E (2008) FRAX™ and the assessment of fracture probability in men and women from the UK. Osteoporos Int 19:385-397.

58. Veronese N, Maggi S (2018) Epidemiology and social costs of hip fracture. Injury 49:1458-1460.

59. Kanis JA, McCloskey EV, Johansson H, et al. (2013) European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporos Int 24:23-57.

60. Thompson B, Towler DA (2012) Arterial calcification and bone physiology: Role of the bone-vascular axis. Nat Rev Endocrinol 8:529-543.

61. Lampropoulos CE, Papaioannou I, D'Cruz DP (2012) Osteoporosis - A risk factor for cardiovascular disease? Nat Rev Rheumatol 8:587-598.

62. Karwowski W, Naumnik B, Szczepański M, Myśliwiec M (2012) The mechanism of vascular calcification - a systematic review. Med Sci Monit 18:RA1-RA11.

63. Cappuccio FP, Kalaitzidis R, Duneclift S, Eastwood JB (2000) Unravelling the links between calcium excretion, salt intake, hypertension, kidney stones and bone metabolism. J Nephrol 13:169-177.

64. Park JS, Choi SB, Rhee Y, et al. (2015) Parathyroid hormone, calcium, and sodium bridging between osteoporosis and hypertension in postmenopausal Korean women. Calcif Tissue Int 96:417-429.

65. Chai H, Ge J, Li L, Li J, Ye Y (2021) Hypertension is associated with osteoporosis: a case-control study in Chinese postmenopausal women. BMC Musculoskelet Disord 22:253-253.

66. Cappuccio FP, Meilahn E, Zmuda JM, Cauley JA (1999) High blood pressure and bone-mineral loss in elderly white women: a prospective study. Study of Osteoporotic Fractures Research Group. Lancet 354:971-975.

67. Xu B, Han L, Liu H, et al. (2013) Cardiovascular disease and hip fracture among older inpatients in Beijing, China. BioMed Res Int 2013: 493696

68. Gerber Y, Melton LJ, 3rd, McNallan SM, et al. (2013) Cardiovascular and noncardiovascular disease associations with hip fractures. Am J Med 126:169.e119-126.

69. Vestergaard P, Rejnmark L, Mosekilde L (2009) Hypertension is a risk factor for fractures. Calcif Tissue Int 84:103-111.

70. Sennerby U, Melhus H, Gedeborg R, et al. (2009) Cardiovascular diseases and risk of hip fracture. JAMA 302:1666-1673.

71. Dominic E, Brozek W, Peter RS, et al. (2020) Metabolic factors and hip fracture risk in a large Austrian cohort study. Bone Reports 12:100244

72. Yang S, Nguyen ND, Center JR, Eisman JA, Nguyen TV (2014) Association between hypertension and fragility fracture: A longitudinal study. Osteoporos Int 25:97-103.

73. Pérez-Castrillón JL, Martín-Escudero JC, Alvarez Manzanares P, et al. (2005) Hypertension as a risk factor for hip fracture [2]. Am J Hypertens 18:146-147.

74. den Uyl D, Nurmohamed MT, van Tuyl LHD, Raterman HG, Lems WF (2011) (Sub)clinical cardiovascular disease is associated with increased bone loss and fracture risk; A systematic review of the association between cardiovascular disease and osteoporosis. Arthritis Res Ther 13:R5

75. Anagnostis P, Karagiannis A, Kakafika AI, et al. (2009) Atherosclerosis and osteoporosis: Age-dependent degenerative processes or related entities? Osteoporos Int 20:197-207.

76. McFarlane SI, Muniyappa R, Shin JJ, Bahtiyar G, Sowers JR (2004) Osteoporosis and cardiovascular disease: Brittle bones and boned arteries, is there a link? Endocrine 23:1-10.

77. Ye C, Xu M, Wang S, et al. (2016) Decreased Bone Mineral Density Is an Independent Predictor for the Development of Atherosclerosis: A Systematic Review and Meta-Analysis. PLoS One 11:e0154740.

78. Veronese N, Stubbs B, Crepaldi G, et al. (2017) Relationship Between Low Bone Mineral Density and Fractures With Incident Cardiovascular Disease: A Systematic Review and Meta-Analysis. J Bone Miner Res 32:1126-1135.

79. Szulc P (2012) Association between cardiovascular diseases and osteoporosis-reappraisal. Bonekey Rep 1:144.

80. Luan L, Li R, Wang Z, et al. (2016) Stroke increases the risk of hip fracture: a systematic review and meta-analysis. Osteoporos Int 27:3149-3154.

81. Barzilay JI, Buzkova P, Cauley JA, et al. (2018) The associations of subclinical atherosclerotic cardiovascular disease with hip fracture risk and bone mineral density in elderly adults. Osteoporos Int 29:2219-2230.

82. Ge G, Li J, Wang Q (2019) Heart failure and fracture risk: a meta-analysis. Osteoporos Int 30:1903-1909.

83. Collins TC, Ewing SK, Diem SJ, et al. (2009) Peripheral arterial disease is associated with higher rates of hip bone loss and increased fracture risk in older men. Circulation 119:2305-2312.

84. Peláez VC, Ausín L, Mambrilla MR, Gonzalez-Sagrado M, Castrillón JLP (2015) Ankle-brachial index, risk of clinical fractures, mortality and low bone mass in nursing home residents. Eur Rev Med Pharmacol Sci 19:1577-1582.

85. Liu FL, Lin CS, Yeh CC, et al. (2017) Risk and outcomes of fracture in peripheral arterial disease patients: two nationwide cohort studies. Osteoporos Int 28:3123-3133.

86. von Muhlen D, Allison M, Jassal SK, Barrett-Connor E (2009) Peripheral arterial disease and osteoporosis in older adults: the Rancho Bernardo Study. Osteoporos Int 20:2071-2078.

87. Hyde Z, Mylankal KJ, Hankey GJ, Flicker L, Norman PE (2013) Peripheral arterial disease increases the risk of subsequent hip fracture in older men: The Health in Men Study. Osteoporos Int 24:1683-1688.

88. Gamba G (2009) The thiazide-sensitive Na+-Cl- cotransporter: Molecular biology, functional properties, and regulation by WNKs. Am J Physiol Renal Physiol 297:F838-F848.

89. Fontenelle LF, Sarti TD (2019) Kidney Stones: Treatment and Prevention. Am Fam Physician 99:490-496.

90. Hsu YJ, Yang SS, Cheng CJ, et al. (2015) Thiazide-Sensitive Na(+) -Cl(-) Cotransporter (NCC) Gene Inactivation Results in Increased Duodenal Ca(2+) Absorption, Enhanced Osteoblast Differentiation and Elevated Bone Mineral Density. J Bone Miner Res 30:116-127.

91. Dvorak MM, De Joussineau C, Carter DH, et al. (2007) Thiazide diuretics directly induce osteoblast differentiation and mineralized nodule formation by interacting with a sodium chloride co-transporter in bone. Am J Physiol Renal Physiol 18:2509-2516.

92. Wasnich RD, Benfante RJ, Yano K, Heilbrun L, Vogel JM (1983) Thiazide effect on the mineral content of bone. N Engl J Med 309:344-347.

93. Sigurdsson G, Franzson L (2001) Increased bone mineral density in a population-based group of 70-year-old women on thiazide diuretics, independent of parathyroid hormone levels. J Intern Med 250:51-56.

94. Morton DJ, Barrett-Connor EL, Edelstein SL (1994) Thiazides and bone mineral density in elderly men and women. Am J Epidemiol 139:1107-1115.

95. Sowers MR, Clark MK, Jannausch ML, Wallace RB (1993) Body size, estrogen use and thiazide diuretic use affect 5-year radial bone loss in postmenopausal women. Osteoporos Int 3:314-321.

96. Cauley JA, Cummings SR, Seeley DG, et al. (1993) Effects of thiazide diuretic therapy on bone mass, fractures, and falls. The Study of Osteoporotic Fractures Research Group. Ann Intern Med 118:666-673.

97. Wasnich R, Davis J, Ross P, Vogel J (1990) Effect of thiazide on rates of bone mineral loss: a longitudinal study. BMJ 301:1303-1305.

98. Transbøl I, Christensen MS, Jensen GF, Christiansen C, McNair P (1982) Thiazide for the postponement of postmenopausal bone loss. Metabolism 31:383-386.

99. Lim LS, Fink HA, Kuskowski MA, Cauley JA, Ensrud KE (2005) Diuretic use and bone mineral density in older USA men: the osteoporotic fractures in men (MrOS) study. Age Ageing 34:504-507.

100. Adland-Davenport P, McKenzie MW, Notelovitz M, Pendergast JF (1985) Thiazide diuretics and bone mineral content in postmenopausal women. Am J Obstet Gynecol 152:630-634.

101. Bolland MJ, Ames RW, Horne AM, et al. (2007) The effect of treatment with a thiazide diuretic for 4 years on bone density in normal postmenopausal women. Osteoporos Int 18:479-486.

102. Reid IR, Ames RW, Orr-Walker BJ, et al. (2000) Hydrochlorothiazide reduces loss of cortical bone in normal postmenopausal women: a randomized controlled trial. Am J Med 109:362-370.

103. LaCroix AZ, Ott SM, Ichikawa L, Scholes D, Barlow WE (2000) Low-dose hydrochlorothiazide and preservation of bone mineral density in older adults: A randomized, double-blind, placebo-controlled trial. Ann Intern Med 133:516-526.

104. Wasnich RD, Davis JW, He YF, Petrovich H, Ross PD (1995) A randomized, double-masked, placebo-controlled trial of chlorthalidone and bone loss in elderly women. Osteoporos Int 5:247-251.

105. Renneboog B, Musch W, Vandemergel X, Manto MU, Decaux G (2006) Mild chronic hyponatremia is associated with falls, unsteadiness, and attention deficits. Am J Med 119:71.e71-71.e78.

106. Kinsella S, Moran S, Sullivan MO, Molloy MGM, Eustace JA (2010) Hyponatremia independent of osteoporosis is associated with fracture occurrence. Clin J Am Soc Nephrol 5:275-280.

107. Adams AL, Li BH, Bhandari S, et al. (2019) Chronic hyponatremia and association with osteoporosis among a large racially/ethnically diverse population. Osteoporos Int 30:853-861.

108. Burst V, Grundmann F, Kubacki T, et al. (2017) Thiazide-Associated Hyponatremia, Report of the Hyponatremia Registry: An Observational Multicenter International Study. Am J Nephrol 45:420-430.

109. Yang LJ, Wu PH, Huang TH, Lin MY, Tsai JC (2018) Thiazide-associated hyponatremia attenuates the fracture-protective effect of thiazide: A population-based study. PLoS One 13:e0208712.

110. Berry SD, Mittleman MA, Zhang Y, et al. (2012) New loop diuretic prescriptions may be an acute risk factor for falls in the nursing home. Pharmacoepidemiol Drug Saf 21:560-563.

111. Tinetti ME, Han L, Lee DH, et al. (2014) ANtihypertensive medications and serious fall injuries in a nationally representative sample of older adults. JAMA Internal Medicine 174:588-595.

112. Ang HT, Lim KK, Kwan YH, et al. (2018) A Systematic Review and Meta-Analyses of the Association Between Anti-Hypertensive Classes and the Risk of Falls Among Older Adults. Drugs and Aging 35:625-635.

113. Kahlaee HR, Latt MD, Schneider CR (2017) Association Between Chronic or Acute Use of Antihypertensive Class of Medications and Falls in Older Adults. A Systematic Review and Meta-Analysis. Am J Hypertens 31:467-479.

114. Zang G (2013) Antihypertensive drugs and the risk of fall injuries: A systematic review and meta-analysis. J Int Med Res 41:1408-1417.

115. Albasri A, Hattle M, Koshiaris C, et al. (2021) Association between antihypertensive treatment and adverse events: systematic review and meta-analysis. The BMJ, 372, n189.

116. Aung K, Htay T (2011) Thiazide diuretics and the risk of hip fracture. Cochrane Database Syst Rev Cd005185.

117. Jones G, Nguyen T, Sambrook PN, Eisman JA (1995) Thiazide diuretics and fractures: Can meta-analysis help? J Bone Miner Res 10:106-111.

118. Wiens M, Etminan M, Gill SS, Takkouche B (2006) Effects of antihypertensive drug treatments on fracture outcomes: a meta-analysis of observational studies. J Intern Med 260:350-362.

119. Donmez BO, Ozdemir S, Sarikanat M, et al. (2012) Effect of angiotensin II type 1 receptor blocker on osteoporotic rat femurs. Pharmacol Rep 64:878-888.

120. Zhao X, Wang JX, Feng YF, et al. (2014) Systemic treatment with telmisartan improves femur fracture healing in Mice. PLoS ONE 9: e92085.

121. Garcia P, Schwenzer S, Slotta J, et al. (2010) Inhibition of angiotensin-converting enzyme stimulates fracture healing and periosteal callus formation - Role of a local renin-angiotensin system. Br J Pharmacol 159:1672-1680.

122. Lamparter S, Kling L, Schrader M, Ziegler R, Pfeilschifter J (1998) Effects of angiotensin II on bone cells in vitro. J Cell Physiol 175:89-98.

123. Corrao G, Mazzola P, Monzio Compagnoni M, et al. (2015) Antihypertensive Medications, Loop Diuretics, and Risk of Hip Fracture in the Elderly: A Population-Based Cohort Study of 81,617 Italian Patients Newly Treated Between 2005 and 2009. Drugs Aging 32:927-936.

124. Kwok T, Leung J, Zhang YF, et al. (2012) Does the use of ACE inhibitors or angiotensin receptor blockers affect bone loss in older men? Osteoporos Int 23:2159-2167.

125. Chen HY, Ma KY, Hsieh PL, Liou YS, Jong GP (2016) Long-term Effects of Antihypertensive Drug Use and New-onset Osteoporotic Fracture in Elderly Patients: A Population-based Longitudinal Cohort Study. Chin Med J (Engl) 129:2907-2912.

126. Thorell K, Ranstad K, Midlov P, Borgquist L, Halling A (2014) Is use of fall risk-increasing drugs in an elderly population associated with an increased risk of hip fracture, after adjustment for multimorbidity level: a cohort study. BMC Geriatr 14:131.

127. Kunutsor SK, Blom AW, Whitehouse MR, Kehoe PG, Laukkanen JA (2017) Renin-angiotensin system inhibitors and risk of fractures: a prospective cohort study and meta-analysis of published observational cohort studies. Eur J Epidemiol 32:947-959.

128. Ruths S, Bakken MS, Ranhoff AH, et al. (2015) Risk of hip fracture among older people using antihypertensive drugs: a nationwide cohort study. BMC Geriatr 15:153.

129. Solomon DH, Mogun H, Garneau K, Fischer MA (2011) Risk of fractures in older adults using antihypertensive medications. J Bone Miner Res 26:1561-1567.

130. Cheng YZ, Huang ZZ, Shen ZF, et al. (2017) ACE inhibitors and the risk of fractures: a meta-analysis of observational studies. Endocrine 55:732-740.

131. Salcuni AS, Carnevale V, Battista C, et al. (2017) Primary aldosteronism as a cause of secondary osteoporosis. Eur J Endocrinol 177:431-437.

132. Notsu M, Yamauchi M, Yamamoto M, Nawata K, Sugimoto T (2017) Primary Aldosteronism as a Risk Factor for Vertebral Fracture. J Clin Endocrinol Metab 102:1237-1243.

133. Wu VC, Chang CH, Wang CY, et al. (2017) Risk of Fracture in Primary Aldosteronism: A Population-Based Cohort Study. J Bone Miner Res 32:743-752.

134. Moore RE, Smith CK, 2nd, Bailey CS, Voelkel EF, Tashjian AH, Jr. (1993) Characterization of beta-adrenergic receptors on rat and human osteoblast-like cells and

demonstration that beta-receptor agonists can stimulate bone resorption in organ culture. Bone Miner 23:301-315.

135. Khosla S, Drake MT, Volkman TL, et al. (2018) Sympathetic β1-adrenergic signaling contributes to regulation of human bone metabolism. J Clin Invest 128:4832-4842.

136. Takeda S, Elefteriou F, Levasseur R, et al. (2002) Leptin regulates bone formation via the sympathetic nervous system. Cell 111:305-317.

137. Bonnet N, Benhamou CL, Malaval L, et al. (2008) Low dose beta-blocker prevents ovariectomy-induced bone loss in rats without affecting heart functions. J Cell Physiol 217:819-827.

138. Reid IR, Lucas J, Wattie D, et al. (2005) Effects of a beta-blocker on bone turnover in normal postmenopausal women: a randomized controlled trial. J Clin Endocrinol Metab 90:5212-5216.

139. Rejnmark L, Vestergaard P, Mosekilde L (2006) Treatment with beta-blockers, ACE inhibitors, and calcium-channel blockers is associated with a reduced fracture risk: a nationwide case-control study. J Hypertens 24:581-589.

140. Yang S, Nguyen ND, Eisman JA, Nguyen TV (2012) Association between beta-blockers and fracture risk: a Bayesian meta-analysis. Bone 51:969-974.

141. Torstensson M, Hansen AH, Leth-Moller K, et al. (2015) Danish register-based study on the association between specific cardiovascular drugs and fragility fractures. BMJ Open 5:e009522.

142. Toulis KA, Hemming K, Stergianos S, Nirantharakumar K, Bilezikian JP (2014) beta-Adrenergic receptor antagonists and fracture risk: a meta-analysis of selectivity, gender, and site-specific effects. Osteoporos Int 25:121-129.

143. Halici Z, Borekci B, Ozdemir Y, Cadirci E, Suleyman H (2008) Protective effects of amlodipine and lacidipine on ovariectomy-induced bone loss in rats. Eur J Pharmacol 579:241-245.

144. Boesgaard S, Hyldstrup L, Feldstedt M (1991) Changes in calcium homoeostasis and bone formation in patients recovering from acute myocardial infarction: effect of verapamil treatment. Danish Study Group on Verapamil in Myocardial Infarction. Eur J Clin Pharmacol 41:521-523.

145. Albers MM, Johnson W, Vivian V, Jackson RD (1991) Chronic use of the calcium channel blocker nifedipine has no significant effect on bone metabolism in men. Bone 12:39-42.

146. Rejnmark L, Vestergaard P, Mosekilde L (2006) Treatment with beta-blockers, ACE inhibitors, and calcium-channel blockers is associated with a reduced fracture risk: A nationwide case-control study. J Hypertens 24:581-589.

147. Lim LS, Fink HA, Kuskowski MA, et al. (2008) Loop diuretic use and increased rates of hip bone loss in older men: the Osteoporotic Fractures in Men Study. Arch Intern Med 168:735-740.

148. Lim LS, Fink HA, Blackwell T, Taylor BC, Ensrud KE (2009) Loop diuretic use and rates of hip bone loss and risk of falls and fractures in older women. J Am Geriatr Soc 57:855-862.

149. Rejnmark L, Vestergaard P, Heickendorff L, Andreasen F, Mosekilde L (2006) Loop diuretics increase bone turnover and decrease BMD in osteopenic postmenopausal women: Results from a randomized controlled study with bumetanide. J Bone Miner Res 21:163-170.

150. Wallerstedt SM, Hoffmann M (2019) Evidence synthesis based on non-randomised studies—a critical review of studies leading to conclusions on fall risk properties of loop diuretics/beta-blockers. Eur J Clin Pharmacol 75:1731-1738.

151. Shimbo D, Bowling CB, Levitan EB, et al. (2016) Short-term risk of serious fall injuries in older adults initiating and intensifying treatment with antihypertensive medication. Circ Cardiovasc Qual Outcomes 9:222-229.

152. Berry SD, Zhu Y, Choi H, Kiel DP, Zhang Y (2013) Diuretic initiation and the acute risk of hip fracture. Osteoporos Int 24:689-695.

153. de Vries M, Seppala LJ, Daams JG, et al. (2018) Fall-Risk-Increasing Drugs: A Systematic Review and Meta-Analysis: I. Cardiovascular Drugs. J Am Med Dir Assoc 19:371.e371-371.e379.

154. Xiao F, Qu X, Zhai Z, et al. (2015) Association between loop diuretic use and fracture risk. Osteoporos Int 26:775-784.

155. Lai SW, Cheng KC, Lin CL, Liao KF (2017) Furosemide use and acute risk of hip fracture in older people: A nationwide case–control study in Taiwan. Geriatr Gerontol Int 17:2552-2558.

156. Paik JM, Rosen HN, Gordon CM, Curhan GC (2016) Diuretic Use and Risk of Vertebral Fracture in Women. Am J Med 129:1299-1306.

157. Stoltzfus JC (2011) Logistic regression: a brief primer. Acad Emerg Med 18:1099-1104.

158. Bradburn MJ, Clark TG, Love SB, Altman DG (2003) Survival analysis part II: multivariate data analysis--an introduction to concepts and methods. Br J Cancer 89:431-436.

159. Stel VS, Dekker FW, Tripepi G, Zoccali C, Jager KJ (2011) Survival analysis II: Cox regression. Nephron Clin Pract 119:c255-260.

160. Cioci AC, Cioci AL, Mantero AMA, et al. (2021) Advanced Statistics: Multiple Logistic Regression, Cox Proportional Hazards, and Propensity Scores. Surg Infect (Larchmt) 22:604-610.

161. Leslie WD, Lix LM, Wu X (2013) Competing mortality and fracture risk assessment. Osteoporos Int 24:681-688.

162. Cauley JA, Cawthon PM, Peters KE, et al. (2016) Risk Factors for Hip Fracture in Older Men: The Osteoporotic Fractures in Men Study (MrOS). J Bone Miner Res 31:1810-1819.

163. Buzkova P (2021) Competing risk of mortality in association studies of non-fatal events. PLoS ONE 16:e0255313

164. Austin PC, Lee DS, Fine JP (2016) Introduction to the Analysis of Survival Data in the Presence of Competing Risks. Circulation 133:601-609.

165. Buzkova P, Barzilay JI, Mukamal KJ (2019) Assessing risk factors of non-fatal outcomes amid a competing risk of mortality: the example of hip fracture. Osteoporos Int 30:2073-2078.

166. Hjerpe P, Merlo J, Ohlsson H, Bengtsson Boström K, Lindblad U (2010) Validity of registration of ICD codes and prescriptions in a research database in Swedish primary care: A cross-sectional study in Skaraborg primary care database. BMC Med Inform Decis Mak 10:23-23.

167. Hasselstrom J, Zarrinkoub R, Holmquist C, et al. (2013) The Swedish Primary Care Cardiovascular Database (SPCCD): 74 751 hypertensive primary care patients. Blood Press 23:116-125.

168. Qvarnstrom M, Kahan T, Kieler H, et al. (2013) Persistence to antihypertensive drug treatment in Swedish primary healthcare. Eur J Clin Pharmacol 69:1955-1964.

169. Bubien JK (2010) Epithelial Na+ channel (ENaC), hormones, and hypertension. J Biol Chem 285:23527-23531.

170. Lewerin C, Ljungman S, Nilsson-Ehle H (2007) Glomerular filtration rate as measured by serum cystatin C is an important determinant of plasma homocysteine and serum methylmalonic acid in the elderly. J Intern Med 261:65-73.

171. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. (2019) Sarcopenia: Revised European consensus on definition and diagnosis. Age Ageing 48:16-31.

172. Kindblom JM, Ohlsson C, Ljunggren O, et al. (2009) Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men. J Bone Miner Res 24:785-791.

173. Lewerin C, Nilsson-Ehle H, Jacobsson S, et al. (2014) Serum estradiol associates with blood hemoglobin in elderly men: the MrOS Sweden study. J Clin Endocrinol Metab 99:2549-2556.

174. Kristjansdottir HL, Lewerin C, Lerner UH, et al. (2020) High Plasma Erythropoietin Predicts Incident Fractures in Elderly Men with Normal Renal Function: The MrOS Sweden Cohort. J Bone Miner Res 35:298-305.

175. Kristjansdottir HL, Lewerin C, Lerner UH, et al. (2018) High Serum Serotonin Predicts Increased Risk for Hip Fracture and Nonvertebral Osteoporotic Fractures: The MrOS Sweden Study. J Bone Miner Res 33:1560-1567.

176. Erben RG (2018) Physiological Actions of Fibroblast Growth Factor-23. Frontiers in Endocrinology 9: 267-267.

177. Feskanich D, Willett WC, Stampfer MJ, Colditz GA (1997) A prospective study of thiazide use and fractures in women. Osteoporos Int 7:79-84.

178. Lacroix AZ (1991) Thiazide diuretic agents and prevention of hip fracture. Comprehensive Therapy 17:30-39.

179. Puttnam R, Davis BR, Pressel SL, et al. (2017) Association of 3 Different Antihypertensive Medications With Hip and Pelvic Fracture Risk in Older Adults: Secondary Analysis of a Randomized Clinical Trial. JAMA Intern Med 177:67-76.

180. Rejnmark L, Vestergaard P, Mosekilde L (2005) Reduced fracture risk in users of thiazide diuretics. Calcif Tissue Int 76:167-175.

181. Schoofs MWCJ, Van Der Klift M, Hofman A, et al. (2003) Thiazide Diuretics and the Risk for Hip Fracture. Ann Intern Med 139:476-482+I436.

182. Heidrich FE, Stergachis A, Gross KM (1991) Diuretic drug use and the risk for hip fracture. Ann Intern Med 115:1-6.

183. Juraschek SP, Simpson LM, Davis BR, et al. (2019) Effects of Antihypertensive Class on Falls, Syncope, and Orthostatic Hypotension in Older Adults: The ALLHAT Trial. Hypertension 74:1033-1040.

184. Xiao X, Xu Y, Wu Q (2018) Thiazide diuretic usage and risk of fracture: a meta-analysis of cohort studies. Osteoporos Int 29:1515-1524.

185. Charkos TG, Liu Y, Yang S (2020) Thiazide diuretics and risk of hip fracture: A Bayesian meta-analysis of cohort studies. Global Epidemiology 2:100025.

186. Kruse C, Eiken P, Vestergaard P (2016) Continuous and long-term treatment is more important than dosage for the protective effect of thiazide use on bone metabolism and fracture risk. J Intern Med 279:110-222.

187. Herings RMC, Stricker BHC, De Boer A, et al. (1996) Current use of thiazide diuretics and prevention of femur fractures. J Clin Epidemiol 49:115-119.

188. Ray WA, Downey W, Griffin MR, Melton Iii LJ (1989) Long-term use of thiazide diuretics and risk of hip fracture. Lancet 1:687-690.

189. Schlienger RG, Kraenzlin ME, Jick SS, Meier CR (2004) Use of beta-blockers and risk of fractures. JAMA 292:1326-1332.

190. Rodenburg EM, Hoorn EJ, Ruiter R, et al. (2013) Thiazide-associated hyponatremia: a population-based study. Am J Kidney Dis 62:67-72.

191. Kao YT, Huang CY, Fang YA, Liu JC (2020) The association between renin angiotensin aldosterone system blockers and future osteoporotic fractures in a hypertensive population – A population-based cohort study in Taiwan. Int J Cardiol 305:147-153.

192. Chen CI, Yeh JS, Tsao NW, et al. (2017) Association between renin-angiotensin-aldosterone system blockade and future osteoporotic fracture risk in hypertensive population: A population-based cohort study in Taiwan. Medicine (Baltimore) 96:e8331.

193. Reid IR, Gamble GD, Grey AB, et al. (2005) beta-Blocker use, BMD, and fractures in the study of osteoporotic fractures. J Bone Miner Res 20:613-618.

194. Song HJ, Lee J, Kim YJ, et al. (2012) beta1 selectivity of beta-blockers and reduced risk of fractures in elderly hypertension patients. Bone 51:1008-1015.

195. Yang S, Nguyen ND, Center JR, Eisman JA, Nguyen TV (2011) Association between beta-blocker use and fracture risk: the Dubbo Osteoporosis Epidemiology Study. Bone 48:451-455.

196. Majumdar SR, Ezekowitz JA, Lix LM, Leslie WD (2012) Heart failure is a clinically and densitometrically independent risk factor for osteoporotic fractures: Population-based cohort study of 45,509 subjects. J Clin Endocrinol Metab 97:1179-1186.

197. Mortensen SJ, Mohamadi A, Wright CL, et al. (2020) Medications as a Risk Factor for Fragility Hip Fractures: A Systematic Review and Meta-analysis. Calcif Tissue Int 107

198. Reyes C, Estrada P, Nogués X, et al. (2014) The impact of common co-morbidities (as measured using the Charlson index) on hip fracture risk in elderly men: A population-based cohort study. Osteoporos Int 25:1751-1758.

199. Ungprasert P, Wijarnpreecha K, Thongprayoon C, Cheungpasitporn W (2018) Peripheral arterial disease and risk of hip fracture: A systematic review and meta-analysis of cohort studies. J Postgrad Med 64:220-225.

200. Criqui MH, Aboyans V (2015) Epidemiology of Peripheral Artery Disease. Circ Res 116:1509-1526.

201. Goto NA, Weststrate ACG, Oosterlaan FM, et al. (2020) The association between chronic kidney disease, falls, and fractures: a systematic review and meta-analysis. Osteoporos Int 31:13-29.

202. Cauley JA, Kassem AM, Lane NE, Thorson S (2016) Prevalent peripheral arterial disease and inflammatory burden. BMC Geriatr 16:213.

203. Pasqualini L, Ministrini S, Macura A, et al. (2016) Increased Bone Resorption: A Possible Pathophysiological Link Between Hypovitaminosis D and Peripheral Arterial Disease. Eur J Vasc Endovasc Surg 52:352-359.

204. Eriksson AL, Moverare-Skrtic S, Ljunggren O, et al. (2014) High-sensitivity CRP is an independent risk factor for all fractures and vertebral fractures in elderly men: the MrOS Sweden study. J Bone Miner Res 29:418-423.

205. Kanis JA, Johnell O, Oden A, et al. (2005) Smoking and fracture risk: A meta-analysis. Osteoporos Int 16:155-162.

206. Banks E, Joshy G, Korda RJ, et al. (2019) Tobacco smoking and risk of 36 cardiovascular disease subtypes: Fatal and non-fatal outcomes in a large prospective Australian study. BMC Medicine 17:128-128.

207. Navas-Acien A, Selvin E, Sharrett AR, et al. (2004) Lead, cadmium, smoking, and increased risk of peripheral arterial disease. Circulation 109:3196-3201.

208. Tellez-Plaza M, Guallar E, Fabsitz RR, et al. (2013) Cadmium exposure and incident peripheral arterial disease. Circ Cardiovasc Qual Outcomes 6:626-633.

209. Wallin M, Barregard L, Sallsten G, et al. (2016) Low-Level Cadmium Exposure Is Associated with Decreased Bone Mineral Density and Increased Risk of Incident Fractures in Elderly Men: The MrOS Sweden Study. J Bone Miner Res 31:732-741.

210. Moayeri A, Mohamadpour M, Mousavi SF, et al. (2017) Fracture risk in patients with type 2 diabetes mellitus and possible risk factors: A systematic review and meta-analysis. Ther Clin Risk Manag 13:455-468.

211. Schwartz AV (2016) Epidemiology of fractures in type 2 diabetes. Bone 82:2-8.

212. Shanbhogue VV, Hansen S, Frost M, Brixen K, Hermann AP (2017) Bone disease in diabetes: another manifestation of microvascular disease? Lancet Diabetes Endocrinol 5:827-838.

213. Sennerby U, Farahmand B, Ahlbom A, Ljunghall S, Michaëlsson K (2007) Cardiovascular diseases and future risk of hip fracture in women. Osteoporos Int 18:1355-1362.

214. Yang Q, Li Z, Li W, et al. (2019) Association of total testosterone, free testosterone, bioavailable testosterone, sex hormone-binding globulin, and hypertension. Medicine (Baltimore) 98:e15628.

215. Wei D, Hou J, Liu X, et al. (2021) Interaction between testosterone and obesity on hypertension: A population-based cross-sectional study. Atherosclerosis 330:14-21.

216. Mellstrom D, Johnell O, Ljunggren O, et al. (2006) Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MrOS Sweden. J Bone Miner Res 21:529-535.

217. Perakakis N, Farr OM, Mantzoros CS (2021) Leptin in Leanness and Obesity: JACC State-of-the-Art Review. J Am Coll Cardiol 77:745-760.

218. Meng XH, Tan LJ, Xiao HM, Tang BS, Deng HW (2019) Examining the causal role of leptin in bone mineral density: A Mendelian randomization study. Bone 125:25-29.

219. Ghaedian MM, Nazari Jaz A, Momeni M, Ghaedian T, Samiei N (2020) Plasma leptin level is positively associated with blood pressure measures independent of gender and BMI. Clin Exp Hypertens 42:31-35.

220. Galletti F, D'Elia L, Barba G, et al. (2008) High-circulating leptin levels are associated with greater risk of hypertension in men independently of body mass and insulin resistance: results of an eight-year follow-up study. J Clin Endocrinol Metab 93:3922-3926.

221. Barba G, Russo O, Siani A, et al. (2003) Plasma leptin and blood pressure in men: graded association independent of body mass and fat pattern. Obes Res 11:160-166.

222. Zhang Y, Zhang DZ (2018) Circulating parathyroid hormone and risk of hypertension: A meta-analysis. Clinica Chimica Acta 482:40-45.

223. Oinonen L, Tikkakoski A, Koskela J, et al. (2021) Parathyroid hormone may play a role in the pathophysiology of primary hypertension. Endocrine Connections 10:54-65.

224. Qu Z, Yang F, Hong J, Wang W, Yan S (2020) Parathyroid Hormone and Bone Mineral Density: A Mendelian Randomization Study. J Clin Endocrinol Metab 105:1-8.