Epidemiology of Physical Activity and Fragility Fractures

KARL STATTIN
Abstract

Fragility fractures mainly affect elderly individuals and often cause long term pain, loss of function and higher mortality rates. Physical activity improves balance, increases muscle strength and bone mineral density, and may reduce the risk of fragility fractures. The aim of this thesis is to investigate the association between physical activity and fragility fractures.

In Paper I the risk of hip and any fracture was investigated across levels of habitual walking/bicycling and exercise in participants from the population-based Cohort of Swedish Men (COSM) and Swedish Mammography Cohort (SMC). Individuals walking/bicycling a maximum of 20 minutes per day had a lower risk of hip and any fracture than individuals who did not walk or bicycle. The risk of hip and any fracture was gradually lower with increasing levels of exercise. In Paper II participants in the cross-country skiing race Vasaloppet were compared to non-participants from the general population, and were found to have a higher risk of any and forearm fracture but a lower risk of hip, proximal humerus and lower leg fracture. There was no difference in the risk of vertebral fracture. In Paper III, the association between physical activity and cardiovascular candidate plasma protein concentrations were analyzed in participants from the EpiHealth cohort and the Swedish Mammography Cohort Clinical. Of 184 assayed proteins, 75 associations with physical activity were discovered and 28 subsequently replicated in multivariable adjusted models. In Paper IV the COSM, SMC and the Vasaloppet cohort were combined to achieve as wide a range of physical activity as possible and a common measure of physical activity was created using generalized structural equation modeling (GSEM). Low levels of physical activity were associated with higher risk of any and hip fracture but lower risk of wrist fracture. Individuals with physical activity close to the median of the combined cohort had the lowest risk of fracture, and higher levels of physical activity was associated with a higher risk of any fracture.

In conclusion, physical activity is associated with a lower risk of major fractures such as hip fractures, but may in large quantities increase the risk of wrist and any fracture. Physical activity is associated with more beneficial concentrations of 28 cardiovascular plasma proteins.

Keywords: physical activity, fracture, fragility fracture, epidemiology, cohort study, proteomic, walking, exercise, cross-country skiing, skiing, Vasaloppet.

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To Anna Pia
List of Papers

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


IV  **Stattin K**, Höijer J, Hållmarker U, Larsson SC, Wolk A, Michaëlsson K, Byberg L. (2020) The risk of different fracture types across a wide range of physical activity levels, from sedentary individuals to elite athletes. *(manuscript)*

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### Abbreviations

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<tr>
<td>AHA</td>
<td>American Heart Association</td>
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<td>ACSM</td>
<td>American Heart Association</td>
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<td>BMD</td>
<td>bone mineral density</td>
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<td>BMI</td>
<td>body mass index</td>
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<td>CI</td>
<td>confidence interval</td>
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<td>COSM</td>
<td>Cohort of Swedish Men</td>
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<td>DAG</td>
<td>directed acyclic graph</td>
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<td>DLW</td>
<td>doubly-labeled water</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>DXA</td>
<td>dual energy x-ray absorptiometry</td>
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<td>GSEM</td>
<td>generalized structural equation modeling</td>
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<td>HK</td>
<td>hasardkvot</td>
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<tr>
<td>HR</td>
<td>hazard ratio</td>
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<tr>
<td>ICD</td>
<td>International Statistical Classification of Diseases and Related Health Problems</td>
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<tr>
<td>KI</td>
<td>konfidensintervall</td>
</tr>
<tr>
<td>LISA</td>
<td>Longitudinell integrationsdatabas för sjukförsäkrings- och arbetsmarknadsstudier (Longitudinal Integration Database for Health Insurance and Labor Market Studies)</td>
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<td>METs</td>
<td>metabolic equivalents of task</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>RCT</td>
<td>randomized controlled trial</td>
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<td>SEM</td>
<td>structural equation modeling</td>
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<td>SMC</td>
<td>Swedish Mammography Cohort</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<td>SMCC</td>
<td>Swedish Mammography Cohort Clinical</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Introduction

Fragility fractures mainly affect elderly individuals\(^1\) and the most common fragility fractures are fractures of the wrist, hip and vertebrae.\(^2\) Every year 70,000 individuals in Sweden suffer a fragility fracture, and the life time risk in 50-year-old women and men is 50% and 25%, respectively.\(^2\) The most common mechanism is a fall from standing.\(^1\) Fragility fractures are associated with considerable morbidity, loss of function and mortality. After a hip fracture, the most severe fragility fracture, many patients suffer long-term pain,\(^3\) loss of independence,\(^3, 4\) reduced quality of life,\(^5, 6\) and 20% die within one year.\(^7\) Other fragility fractures, such as wrist fractures, also often lead to long-term suffering due to for example neuropathy, malunion or secondary osteoarthritis.\(^8\) It is hence of great importance to find prophylactic treatments to reduce the risk of fragility fractures. Physical activity reduces the risk of falling,\(^9\) increases bone mineral density to a modest degree\(^10\) and has been associated with a lower risk of hip fractures.\(^11-13\)

Most, but not all,\(^14, 15\) previous observational studies have shown an association between physical activity and lower risk of hip fractures.\(^11-13, 16-18\) Other fracture sites have been less studied, but physical activity may decrease the risk of humerus fracture\(^19, 20\) while increasing the risk of wrist fracture.\(^21-24\) Walking is the most commonly performed modality of physical activity,\(^25\) and is also the most studied modality concerning hip fracture risk.\(^11, 12, 18\) Fewer studies have been able to show an association between more strenuous exercise and hip fracture.\(^13, 16\) Most studies investigate mainly postmenopausal women\(^12, 16, 18, 26\) and individuals performing low-to moderate amounts of physical activity.\(^27-32\) A high number of participants and long follow-up is generally required to study fragility fractures, and hence few interventional studies have successfully studied the association. Meta-analyses of randomized controlled trials (RCTs) have however shown a decreased risk of overall fracture risk with physical activity interventions,\(^9, 32-34\) but have been unable to differentiate by fracture site, type of physical activity or examine dose-response patterns. Reduced risk of falling and improved bone density has been posited as underlying mechanisms, but neither fully explains the observed associations.

Thus, it is not fully known if the association between physical activity and fragility fractures is similar for all fracture sites, how modality, frequency, intensity or duration of physical activity affect the risk of fracture, how a potential dose-response relation is shaped, or if the association is similar in men.
and women or across all ages. Neither is it fully known how the association is mediated.
Background

Fragility Fractures

Definition
There is no uncontroversial definition of fragility fractures, possibly due to their multifactorial etiology. One approach is to define fragility fractures as low-energy fractures,\(^{35}\) such as fractures caused by a fall from standing height or less.\(^{36}\) The World Health Organization (WHO) has opted to define fragility fractures as “a fracture caused by injury that would be insufficient to fracture normal bone.”\(^{37}\) However, basing the definition on bone strength is problematic as although all fractures except facial fractures are associated with bone strength, the risk attributable to bone strength is low.\(^{38}\) Further, bone strength affects the risk of both high- and low-energy fractures.\(^{39}\) Due to the lack of a standard definition, which fractures constitute fragility fractures may vary across cited articles. In the Papers included in the present thesis, all fractures except fractures of the skull irrespective of mechanism are considered fragility fractures.

Epidemiology
The most common fragility fractures are wrist, hip and vertebral fractures.\(^{2}\) Sweden has a high incidence of fragility fractures compared to other countries\(^{40}\) with a national annual incidence of 70,000.\(^{2}\) The risk of most fractures increase linearly with age, whereas the risk of hip fracture increases near-exponentially.\(^{1}\) Women have a higher risk of fragility fracture than men\(^{1}\) and 50-year old women and men in Sweden have a 50% and 25% lifetime risk of suffering a fragility fracture, respectively.\(^{2}\) The mean age is 65 years at first wrist fracture, 67 years at first clinical vertebral fracture and 79 at first hip fracture.\(^{3}\) Vertebral fractures differ from other fragility fractures in that they are often subclinical, and only a minority of radiographic fractures are clinically diagnosed.\(^{41}\)
Etiology

A fracture occurs when forces applied to a bone exceed its’ strength, which most commonly happens due to a fall, frequently from standing height. Most such falls occur indoors. Among community-dwelling individuals above 65 years of age, 30% fall every year and 15% fall two or more times per year. About every other fall result in a minor injury and 3-4% of falls lead to fracture. The orientation of a fall and protective responses influence the risk of fracture and which site may be affected. Breaking the fall decreases the risk of fracture. Falls straight down or sideways increase the risk of hip fracture, as do falls striking the hip. Landing on a hand decreases the risk of hip fracture, but increases the risk of wrist fracture. Finally, soft tissue at the site of impact may pad the underlying bone, decreasing the force of impact.

Bone is built up of central porous trabecular bone surrounded by dense cortical bone, which is the main determinant of bone strength. Extracellular matrix makes up 90% of bone and consists primarily of collagen type I, providing shape and tensile strength, and hydroxyapatite, providing stiffness. Bone contains three main cell types: osteoblasts that synthesize and mineralize bone matrix, osteocytes that maintain bone matrix and communicate with other cells, and osteoclasts that resorb bone. Bone strength is clinically assessed by measuring bone mineral density (BMD) using dual-energy X-ray absorptiometry (DXA) and comparing the individuals’ BMD to a reference population of young healthy women. Osteoporosis, the medical condition of having weak bones, has been operationally defined by the WHO in Caucasian women as a BMD of lower than 2.5 standard deviations compared to the mean among young adult women, whereas cutoffs for other groups are less certain.

Risk Factors

Theoretically, any factor influencing the risk of falling, orientation of a fall, protective responses, force of impact or bone strength may affect the risk of fracture. Several factors have been observed to increase the risk of fracture, but the underlying mechanism is not always known.

The risk of hip fracture increases near-exponentially with increasing age, whereas the risk of fractures of the proximal humerus and vertebrae increases linearly. Women have a higher risk of fragility fracture than men due to several factors, including longer life span, higher prevalence of impaired balance, lower bone mass, differences in bone geometry and accelerated bone loss after menopause. Body size and composition affects the risk of fracture. Being tall increases the risk of clavicle, proximal humerus, hip, forearm, wrist, patella and ankle fractures. Low body mass index (BMI) is a strong risk factor for fracture and high BMI is a weak protective factor.
Both lean\textsuperscript{(61, 62)} and fat\textsuperscript{(63, 64)} mass is positively associated with BMD. Adiposity increases the risk of falls\textsuperscript{(62)} but serves as padding that reduces the force of impact.\textsuperscript{(50)} High BMI is associated with an elevated risk of ankle\textsuperscript{(16, 57)}, lower leg and humerus fractures,\textsuperscript{(60)} but a lower risk of hip,\textsuperscript{(16, 57)} wrist,\textsuperscript{(16, 57)} and vertebral fractures.\textsuperscript{(57)} Several medical conditions, for example cardiovascular disease\textsuperscript{(65)} and diabetes\textsuperscript{(66)} increase the risk of fracture. Glucocorticoid treatment leads to loss of bone mass,\textsuperscript{(67)} and medications that increase the risk of falls, such as benzodiazepines, increase the risk of fracture.\textsuperscript{(68)} A number of lifestyle factors influence the risk of fragility fractures, including smoking\textsuperscript{(18)} and diet.\textsuperscript{(69)} Poor protective responses contribute to risk of falling\textsuperscript{(70)} and characteristics of falls. Several measures of poor neuromuscular function,\textsuperscript{(27)} including weak triceps,\textsuperscript{(48)} poor balance,\textsuperscript{(54)} poor mobility,\textsuperscript{(49)} lower limb dysfunction\textsuperscript{(71)} and poor walking ability\textsuperscript{(72)} increase the risk of fracture. In a twin study, 40\% of hip fractures were attributable to self-reported poor balance.\textsuperscript{(54)} Low BMD is a strong risk factor for fragility fractures\textsuperscript{(73)} and increases the risk of both low- and high-energy fractures.\textsuperscript{(39)} BMD has different effects across different fracture sites.\textsuperscript{(38)} However, the risk of fractures attributable to BMD is rather low (the population attributable risk of osteoporosis to hip fracture was 28\% in a cohort study of women 65 years or older)\textsuperscript{(38)} and 80\% of individuals suffering a fragility fracture do not have osteoporosis.\textsuperscript{(74)}

Consequences

Fragility fractures negatively affect health-related quality of life,\textsuperscript{(6)} physical functioning and independence.\textsuperscript{(4, 5)} Mortality is increased after hip,\textsuperscript{(7)} proximal humerus\textsuperscript{(7)} and vertebral fractures,\textsuperscript{(7, 75)} but is contested for other fractures.\textsuperscript{(7, 75)} Men have a higher mortality than women following hip fracture.\textsuperscript{(76-79)} Mortality is higher the first six months after fracture and subsequently decreases,\textsuperscript{(77)} but it is controversial whether it fully attenuates over the following years.\textsuperscript{(76-79)} It is not certain to what extent the excess mortality is due to the fracture itself and what is due to preexisting frailty and health status.\textsuperscript{(77, 79)}

Physical Activity

Definition

Caspersen et al defined physical activity as “any bodily movement produced by skeletal muscles that results in energy expenditure”.\textsuperscript{(80)} Physical activity may be characterized according to modality (type of physical activity, e.g. running or bicycling), frequency (how often it is performed), duration (how long the activity is sustained) and intensity (how straining the activity is) or to
which domain of life it belongs to (e.g. leisure or occupational physical activity). They further defined exercise as a subcategory of physical activity that is “planned, structured, repetitive and has as a final or intermediate objective the improvement or maintenance of physical fitness”.(80) Physical fitness is in turn defined as a set of health- or skill-related attributes, and should be distinguished from physical activity.(80)

Measuring Physical Activity

To fully characterize physical activity it is hence necessary to describe modality, frequency, duration and intensity. Several methods are used to measure physical activity, but no method provides ideal measurements of all dimensions of physical activity and all methods have strengths and weaknesses. Although it is possible to combine complementary methods to improve characterization, few studies have reliable and valid measures of all parameters. Which characteristic of physical activity is most important depends on study question, and choice of measurement method should hence be guided by study hypothesis. Methods used to measure physical activity can be divided into objective methods and subjective methods.(81)

Objective techniques of measuring physical activity includes calorimetry, accelerometers and heart rate monitors. The gold standard for measuring physical activity is direct calorimetry (measurement of heat production), but is due to practical difficulties seldom used.(82) Indirect calorimetry methods, such as doubly labeled water (DLW),(82) measure energy expenditure through oxygen consumption or carbon dioxide production. DLW is very accurate but only provides information on energy expenditure.(81, 82) Cost and practical considerations preclude its use on a large scale, but it may be used to validate other methods.(82) More commonly applied objective methods include accelerometers, heart rate monitors and devices combining the two.(81, 82) Accelerometers record frequency and amplitude of acceleration in one or several directions. Strong correlations between energy expenditure and accelerometer data has been shown for many activities, including walking and running, but accelerometers may poorly capture other activities, such as bicycling.(81) Intensity is typically assessed using cutoff values calibrated for the specific accelerometer model.(83) Heart rate may be monitored to measure the cardiorespiratory demands of an activity. Heart rate is linearly associated with energy expenditure in moderate to vigorous activity,(82) whereas at lower intensities, this association is modified by for example ambient temperature, body weight, age, sex and fitness level. Consequently, cutoff points in heart rate where the person is likely to participate in moderate or vigorous physical activity are frequently used.(81, 82) Common strengths of objective methods are accurate measurements of frequency, duration and intensity of physical activity free from recall or social desirability bias, i.e. that individuals are prone to over-report
desirable habits such as physical activity. Common weaknesses include inability to differentiate activity by modality and domain of life, and relatively high cost and nontrivial inconvenience to study participants, which limits study size and duration of measurement.

Subjective methods use various self-report approaches, such as self- or interviewer-administered questionnaires or diaries/logs. Due to low cost and little burden placed on respondents, subjective methods enable accumulation of data on sufficiently large sample sizes to be used in multivariable analyses of rare events and are the most common methods of ascertaining physical activity in epidemiological research. Subjective methods allow for classification of all dimensions of physical activity (including modality and domain of life), but the information is affected by interpretation of the question, cognitive demands of recall and accurate reporting, difficulties in estimating characteristics of physical activity, recall bias, and social desirability bias. Measurement of intensity presents unique difficulties in self-reported data and several methods are used, including numeric rating scales (e.g. Borg scale of perceived exertion) and scales using physiologic cues (such as “breathing lightly/heavily”, “causing small/large increases in heart rate” or “sweating lightly/heavily”). Although not valid for assessing energy expenditure at the individual level, self-report questionnaires can categorize and rank study participants according to level of activity. Several physical activity questionnaires have been validated against different criterion methods. Simple methods tend to have greater validity and reproducibility than more complex ones. In a brief questionnaire simply asking whether the individual spends most time in sedentary activity, walks for pleasure, engages in recreational sport or partakes in hard physical exercise or competitive sport, increased physical activity has been shown to be associated with higher capillary density of the vastus lateralis muscle, higher maximal oxygen uptake and higher maximal workload. The questionnaire used in the Cohort of Swedish Men (COSM) and Swedish Mammography Cohort (SMC) for the present thesis has been shown to have a concordance correlation of 0.65 for reproducibility, 0.56 to 0.64 for validity against 7-day activity records and 0.38 for validity against accelerometer data.

Metabolic Equivalents of Task (METs) may be used to compare intensities between different activities or compare amounts of physical activity across individuals or studies. One MET is equal to the energy cost of sitting quietly, i.e. 1 kcal per kilogram bodyweight per hour. The intensity of activities is expressed as multiples of METs, e.g. walking at 5 kilometers per hour corresponds to 3.5 METs, while running at 20 kilometers per hour corresponds to 19.8 METs. Sedentary activities are in the span of 1-1.5 METs, light activities 1.6-2.9 METs, moderate activities 3-5.9 and vigorous activities ≥6 METs. MET scores can be calculated by multiplying intensity with time spent in each activity and then adding METs from all different activities. MET scores may be calculated from one or several domains of life, i.e. leisure activities only...
(yielding “leisure METs”), occupational activities only, or total activity. Although useful in comparing physical activity across individuals or studies, METs only gives a summary measure of activity and does not permit comparison of specific characteristics of the physical activities performed.

Epidemiology of Physical Activity

Adult Swedish men and women perform physical activity of moderate or vigorous intensity for a median of 31 minutes per day (interquartile range 18-47 minutes) measured with accelerometers. In a self-administered questionnaire, 66% of Swedish men and women aged 18-64 years and 54% of men and women aged ≥65 years met the WHO physical activity guidelines (see below). In a telephone interview survey, 45% of adult Americans met physical activity recommendations, defined as 30 minutes of moderate activity five or more days per week or 20 minutes of vigorous activity three or more days per week, whereas 16% performed no bout of moderate or vigorous activity of at least 10 minutes per week. Physical activity is negatively associated with age, body mass index, and smoking, but is positively associated with education. Women walk more whereas men perform more strenuous activities.

Walking is the most common modality of outdoors physical activity in Sweden, and 86% of respondents to a national survey reported walked the past month. Walking, followed by running, are the most common modalities of physical activity in the United States, performed by 47% and 13% of adults each month, respectively. Popularity of other modalities of physical activity vary by sex.

The WHO and the American Heart Association (AHA) recommend at least 150 minutes of moderate or 75 minutes of vigorous endurance physical activity and 2 sessions of muscle-strengthening exercise per week. The American College of Sports Medicine (ACSM) previously recommended 30 minutes of physical activity at least five days per week, but now has similar recommendations as the WHO and the AHA.

Physical Activity and Health

Since the seminal works by Morris and Paffenbarger studying, among other groups, bus drivers and conductors and college alumni, physical activity has been associated with a range of health benefits such as a lower risk of several cancers, cardiovascular disease, depression, fragility fractures, and all-cause mortality. Physical inactivity has also emerged as an important part in the frailty syndrome, which has gained recent interest as a major determinant for ill health in the elderly. Several
mechanisms potentially mediating these associations have been found, including beneficial effects on blood pressure,\textsuperscript{(115, 116)} blood lipids,\textsuperscript{(117, 118)} and cardiorespiratory fitness,\textsuperscript{(119)} lower risk of type 2 diabetes,\textsuperscript{(120, 121)} and lower levels of chronic inflammation.\textsuperscript{(122-124)} Unfortunately, most individuals are not sufficiently active,\textsuperscript{(93, 95)} and the World Health Organization ranks inactivity as the fourth leading risk factor for mortality globally.\textsuperscript{(125)}

Physical Activity and Fragility Fractures

As fractures are relatively uncommon events within the timeframe of most studies, previous studies exploring the association between physical activity and fracture risk have either investigated the association with proxy markers believed to lie on the causal path to fracture (e.g. risk of falling or bone strength) or the risk of fracture itself.

Physical Activity and Falls

Most fragility fractures are caused by a fall,\textsuperscript{(43)} and the association between physical activity, falling and fracture is multifaceted. Meta-analyses of randomized controlled trials have shown that exercise interventions reduce the risk of falling\textsuperscript{(9, 32-34, 126)} and the number of falls.\textsuperscript{(9, 33)} Multicomponent exercise programs, involving for example balance and strength training, appear most effective.\textsuperscript{(9, 33)} The risk of falling and fracture are increased during activity,\textsuperscript{(127)} but physical activity seems to improve balance. Inactive individuals, especially elderly, have poorer ability to handle challenges to their balance, thus falling more often,\textsuperscript{(31)} falling in low-challenge environments such as home,\textsuperscript{(43)} and falling poorly.\textsuperscript{(27)} When active individuals do suffer a fall, they are more likely to catch the fall with an outstretched hand, decreasing the risk of hip fractures\textsuperscript{(48)} but increasing the risk of wrist fractures.\textsuperscript{(27, 48)} Conversely, inactive individuals are less likely to parry falls and more likely to fall sideways or onto the hip, increasing the risk of hip fractures.\textsuperscript{(48, 49, 71)} Hence, physical activity seems not only to be associated with the risk of falling but also with the characteristics of falls and protective responses.

Physical Activity and Bone

Physical activity improves bone strength by increasing BMD and altering bone geometry. Leisure physical activity has been positively associated with hip BMD in observational studies.\textsuperscript{(21, 128)} Meta-analyses of randomized controlled trials have shown that exercise increases BMD, and that high-force exercise yields the greatest increase in hip BMD (circa 1%) and combination exercise the greatest increase in spine BMD (circa 3%).\textsuperscript{(10)} Cross-sectional studies of athletes have indicated that physical activity is also associated with improvements in bone geometry, such as increasing cortical thickness, larger cross-sectional area and more dense trabeculae.\textsuperscript{(129-131)} These improvements were associated with the magnitude of the forces applied to the specific site
during activity, and high-impact or odd-impact exercise were most effective. (130, 131)

**Physical Activity and Fragility Fractures**

Fractures, especially hip fractures, require large study sizes and long follow-up in order to accumulate sufficient number of fractures to permit analysis. Meta-analyses of randomized controlled trials have generally shown that exercise interventions reduce the risk of fracture, (9, 32-34, 132) but have been unable to differentiate on fracture site or investigate the effect of different characteristics of physical activity. Previous observational studies have mainly investigated the risk of hip fracture, and most, but not all, (14) have shown an association between physical activity and a lower risk of hip fracture. (11-13, 16-18) The association with other fracture sites are less well-known, but physical activity may be associated with a lower risk of humerus fracture (19, 20) but a greater risk of wrist fractures (21-24) and fractures of any site. (15, 22) Women in the early postmenopausal period have been most frequently studied, (12, 16, 18, 26) but similar estimates have been found in elderly men. (11, 13, 31) Most studies investigate elderly with low to moderate levels of physical activity, (27-32) and the association for younger individuals or individuals with high levels of physical activity is not as well known. (22, 133) The shape of the association between physical activity and the risk of fracture is also poorly known. Some, but not all, (14, 134) previous studies have indicated a possible dose-response decrease in the risk of hip fracture with increasing levels of light activity (11, 12, 17, 18, 135) or strenuous physical activity. (13, 16, 26, 136-138) However, previous studies frequently suffer from low power, (11, 12, 18, 136, 138) narrow range of physical activity (11, 12, 18, 26, 135) or broad categories of physical activity. (13, 18, 26, 134-138)

In summary, previous studies are frequently underpowered, collect data on only one or a few fracture sites, often include mostly postmenopausal women and have limited range of exposure. Comparisons between studies are hampered by differences in levels of physical activity, population studied and baseline fracture risk. Thus, it is not fully known how modality, frequency, duration or intensity of physical activity affects the risk of fracture, how high levels of physical activity is associated with the risk of fracture, what the shape of a possible dose-response relation is, or if the effects are similar for both sexes and all ages.

**Proteomics**

Proteomics, a portmanteau of protein and genome coined in 1995, is classically defined as the large-scale quantification of all proteins (i.e. the “proteome”) in an organism, tissue or cell. Currently, proteomics more widely refers to the study of the structure, modifications, expression or function of proteins or interactions between proteins. The proteome is even more complex to study
than the genome, as the former varies across organs and physiologic states and is subject to post-translational modifications (such as phosphorylation and methylation) whereas the latter is the same throughout the body and is relatively stable over time.\textsuperscript{(139, 140)} Previous epidemiological studies have shown an association between cardiovascular disease and fracture\textsuperscript{65} and suggested common pathophysiological mechanisms, such as inflammation.\textsuperscript{(141, 142)} Investigating the association between physical activity and the plasma proteome may thus discover both mechanisms underlying the association between physical activity and cardiovascular disease and fracture and identify pathways where their pathogeneses intersect.

Associations between plasma protein concentration and cardiovascular disease has been studied to improve understanding of pathophysiological mechanisms and evaluate candidate protein biomarkers that could improve prediction models or become potential drug targets. Large-scale proteomic studies have found protein patterns associated with increased risk of atrial fibrillation,\textsuperscript{(143)} congestive heart failure,\textsuperscript{(144)} aortic stenosis\textsuperscript{(145)} and ischemic stroke,\textsuperscript{(146)} and proteins involved in inflammation\textsuperscript{(142)} and fat metabolism\textsuperscript{(147)} have been shown to affect the pathogenesis of cardiovascular disease.

To date, no large-scale study of how physical activity affects the plasma proteome has been conducted, but several studies have investigated one or a few proteins. Although a bout of physical exertion is followed by a transient peak in inflammatory and pro-coagulant proteins,\textsuperscript{(148-150)} physical activity is associated with a reduction of chronic systemic inflammation and prothrombotic activity.\textsuperscript{(150)} The underlying mechanism is not fully understood,\textsuperscript{(150)} but observational studies have found associations between physical activity and lower levels of C-reactive protein,\textsuperscript{(123, 124, 151-156)} white blood cell count,\textsuperscript{(151, 153-155)} interleukin-6,\textsuperscript{(123, 124, 152, 155, 156)} amyloid-A and tumor necrosis factor alpha,\textsuperscript{(155, 156)} often indicating dose-response relations.\textsuperscript{(151, 153, 154)} Physical activity is associated with reduced levels of several factors involved in hemostasis, including fibrinogen,\textsuperscript{(151, 153-155)} coagulation factors VIII\textsuperscript{(151, 153)} and IX, platelet count, von Willebrand factor, blood viscosity,\textsuperscript{(152)} fibrin D-dimer and tissue plasminogen activator antigen.\textsuperscript{(152, 153)} Physical activity is also associated with lower levels of leptin,\textsuperscript{(157)} a pro-inflammatory adipokine involved in energy balance and associated with high fat mass and insulin resistance.\textsuperscript{(158)} Meta-analyses of physical activity trials have corroborated some of these findings, showing lower levels of C-reactive protein,\textsuperscript{(156, 159, 160)} tumor necrosis factor alpha, interleukin-6,\textsuperscript{(156, 159)} fibrinogen,\textsuperscript{(119, 160)} leptin and angiotensin II but higher interleukin-18 in the intervention groups.\textsuperscript{(119)}
Aims

Overall Aim

The overall aim of this thesis was to investigate how different quantities and intensities of physical activity affects the risk of a range of fragility fractures and to investigate how physical activity is associated with cardiovascular candidate plasma protein concentrations.

Paper I

The aim of the first paper was to investigate the association between time spent walking or bicycling and time spent doing more strenuous exercise and the rate of hip and any fracture in the population-based Cohort of Swedish Men (COSM) and Swedish Mammography Cohort (SMC).

Paper II

The aim of the second paper was to investigate the rate of any, hip, proximal humerus, lower leg, forearm and vertebral fractures in a cohort of participants in Vasaloppet, a long-distance cross-country skiing race, and non-participants from the general population, using race participation, race distance, number of races run and finishing time as proxies of high levels of physical activity.

Paper III

The aim of the third paper was to investigate how leisure physical activity is associated with plasma concentrations of 184 cardiovascular candidate proteins in a cross-sectional discovery and replication study using the population-based EpiHealth and Swedish Mammography Cohort Clinical (SMCC).
Paper IV

The aim of the fourth paper was to explore the shape of the association between physical activity and the rate of any, wrist, proximal humerus, spine and hip fracture, using a combined dataset of participants from the COSM, the SMC and Vasaloppet skiers, to study individuals with as wide a range of physical activity as possible.
Methods

Paper I

In the first paper, participants from the COSM and the SMC were included. The COSM recruited participants in 1997 by mailing a lifestyle questionnaire to all men residing in Västmanland and Örebro counties born 1918-1952. Of all eligible men, 49% (n=48,850) answered. The SMC invited women living in Uppsala county born 1914-1948 and women living in Västmanland county born 1917-1948 to answer a lifestyle questionnaire and attend a mammography screening program in 1987. Of the invited women, 74% answered. A second more extensive questionnaire was administered in 1997 to respondents of the first questionnaire still residing in the study area with a response rate of 70% (n=39,277). The COSM and SMC are considered comparable to the Swedish population considering age distribution, BMI and education. The 1997 lifestyle questionnaires were similar in COSM and SMC and contained amongst other things information on walking/bicycling and exercise habits, height, weight, education, cohabitation status, smoking status, alcohol consumption, a food frequency questionnaire, use of vitamin D and/or calcium-containing supplements and ever use of systemic cortisone. Energy-adjusted vitamin D and calcium intake have been calculated from reported food intake. Leisure physical activity was inquired in two multiple choice questions: daily walking/bicycling (with the options “hardly ever”, “<20 minutes”, “20-40 minutes”, “40-60 minutes”, “60-90 minutes” and “>90 minutes”) and weekly exercise (“<1 hour”, “1 hour”, “2-3 hours”, “4-5 hours”, “>5 hours”). The National Patient Register was used to ascertain prevalent comorbidities and incident hip fractures and fractures of any site from 1998 to 2014 using a valid method. The National Patient Register has near-complete coverage of inpatient and specialist care in Sweden and has high validity. Prevalent comorbid conditions were used to calculate weighted Charlson comorbidity index.

Cox proportional hazards regression was used to calculate hazard ratios (HR) and 95% confidence intervals (CI) of hip and any fracture across levels of walking/bicycling and exercise separately and jointly adjusted for above noted covariates. Men and women with incorrect personal identification number or who died or were diagnosed with cancer (except non-melanoma skin cancer) before 1st January 1998 were excluded, leaving 66,940 individuals
who had all information necessary for inclusion in the analysis of walking/bicycling and fracture, and 65,622 individuals for inclusion in the analysis of exercise and fracture.

Paper II

Paper II included participants from the Vasaloppet cohort. Vasaloppet is a Swedish long-distance cross-country skiing race that started in 1922. Women were not allowed to participate 1924-1980, and are still underrepresented. Vasaloppet currently attracts 50,000 participants annually,\(^{(166)}\) from recreational exercisers to world-class skiers. The Vasaloppet cohort was created by collecting race data from all participants 18 years or older having participated in a Vasaloppet race 1991-2009. The unique personal identification number issued to all Swedish residents was used to link this information to the National Patient Register to ascertain prevalent comorbidities and incident fractures and to the Longitudinal Integration Database for Health Insurance and Labor Market Studies (LISA) to garner age, sex, cohabitation status, income and education. Statistics Sweden then supplied comparison individuals, with the same covariate information, that have not participated in Vasaloppet, frequency-matched on sex, county of residence and age in 5-year spans. Participation in a Vasaloppet race (yes/no), race distance (non-participant, 30/45 km or 90 km), number of races participated in (0, 1, 2-3, \(\geq 4\)) and finishing time (in percentage of the winner’s time to account for differences in skiing conditions between years) were used as proxies for physical activity. Outcomes of interest were any, hip, proximal humerus, lower leg, forearm and vertebral fractures. Vasaloppet race data and covariate information were time updated on a yearly basis. After exclusion of individuals not living in Sweden, 118,204 men and 71,757 women having raced 501,129 Vasaloppet races and 505,194 non-participants were included in the study.

Cox proportional hazards regression was used to calculate hazard ratios in the analyses of Vasaloppet participation, race distance and number of races using Vasaloppet non-participants as reference. Restricted cubic splines were used to model the hazard ratio of each fracture according to finishing time among Vasaloppet participants. All analyses were adjusted for age (as time scale), sex, county of residence, weighted Charlson comorbidity index, cohabitation status, income quintile by 5-year spans and education. Sensitivity analyses adjusting the main analysis for relevant lifestyle covariates were performed using Vasaloppet participants and non-participants that have answered the SMC and COSM questionnaires.
The third paper included participants from the EpiHealth and Swedish Mammography Cohort Clinical (SMCC). The EpiHealth cohort aims to collect data on 300,000 individuals randomly chosen from the Swedish population between 45 and 75 years of age to investigate interactions between genes and lifestyle factors in the development of common degenerative disorders. Information has been collected through a web-based lifestyle questionnaire, physical examination, bioimpedance measurement and blood sampling. Leisure physical activity was ascertained using a five-point Likert scale, labeled: 1 “Mostly sitting”, 2 (blank), 3 “Walking 30 minutes/day”, 4 (blank), 5 “Strenuous activity 60 minutes/day”. Blood from 2,500 individuals have been analyzed for protein concentration, and 2,239 individuals had all information necessary for inclusion in Paper III. The SMCC is a subcohort of 5,022 women from the SMC that have answered additional questionnaires, undergone clinical examination and dual-energy x-ray absorptiometry (DXA) and have had blood samples collected. The examination took place in 2003-2009 in five phases with similar, but not identical, questionnaires. Physical activity in the SMCC is assessed as in the SMC. In the analysis of walking/bicycling, 4,320 women from SMCC had all the prerequisite information for inclusion, and in the analysis of exercise 4,289 women from SMCC had all the necessary information for inclusion. Blood from both cohorts have been analyzed using the Olink Proseek Multiplex Cardiovascular 2 and 3 panels. The Proseek Multiplex method uses a proximity extension assay, where antibodies with attached oligonucleotides link pairwise to proteins in the sample. This causes the oligonucleotides to be brought into proximity and dimerize. A deoxyribonucleic acid (DNA) polymerase is added, which creates an extension to the joined oligonucleotides, creating a polymerase chain reaction (PCR) template. Next, all oligonucleotides are amplified using universal primers. Uracil-DNA glycosylase removes unbound primers and partly digest the templates, allowing detection and quantification using quantitative PCR. This method yields relative protein concentrations which are log2-transformed and standardized per analysis plate, setting the mean to zero and the standard deviation to one, before statistical analysis.

Associations between protein concentrations and leisure physical activity were discovered in EpiHealth and subsequently replicated in SMCC. Discovery was performed by fitting one multivariable linear regression model for each protein with protein concentration as dependent variable and cross-sectionally collected leisure physical activity, sex, age, occupational physical activity, daily servings of fruit and vegetables, daily servings of meat, smoking status, alcohol consumption, education, cohabitation status and analysis chip number as dependent variables. The False Discovery rate described by Benjamini and Hochberg was used to control for multiple testing. Associations
found statistically significant in the discovery phase were then assessed for replication in SMCC using cross-sectionally (from 2003-2009) and longitudinally (from 1997) collected walking/bicycling and exercise as leisure physical activity and similar covariates as in the discovery phase plus analysis phase and glomerular filtration rate and alanine aminotransferase, to adjust for kidney and liver function respectively. In the replication phase associations with a p-value of <0.05 were considered statistically significant. Adiposity was considered a potential mediator, and the association between leisure physical activity and protein concentration independent of adiposity was investigated by adjusting the discovery and replication for body fat percentage, assessed with bioimpedance in EpiHealth and DXA in SMCC.

Paper IV

In order to assess the shape of the association between physical activity and the rate of fracture, participants from the previously described COSM, SMC and Vasaloppet cohorts were combined to create a dataset with individuals with a wide range of physical activity, from sedentary individuals to world-class skiers. Participants from COSM and SMC were combined with individuals from the Vasaloppet cohort born 1930-1949 having participated in a Vasaloppet race 1997-1999, yielding a study population of 63,989 individuals, including 28,749 men from COSM (of which 508 had participated in Vasaloppet), 22,256 women from SMC (of which 159 had participated in Vasaloppet) and 12,984 individuals from the Vasaloppet cohort.

To permit comparison between cohorts, the overlap between the cohorts (i.e. the 667 COSM and SMC respondents that have participated in Vasaloppet) was used to create a common measure of physical activity through generalized structural equation modeling (GSEM). First, a GSEM model was used to construct a latent continuous physical activity variable using values from observed variables, such as socioeconomic information (education, cohabitation status and age), race data (Vasaloppet participation, race distance, number of years raced and finishing time) and COSM/SMC questionnaire answers (walking/bicycling, exercise, and physical activity at work). Next, a physical activity value was calculated for participants in all cohorts using information available for that specific individual. The rate of any, wrist, proximal humerus, spine and hip fracture was then assessed with the latent physical activity variable as the exposure in proportional hazards models using restricted cubic splines adjusted for age, sex, cohabitation status and Charlson comorbidity index.
Figure 1. Assumed interrelations between physical activity and observed variables used in construction of the latent physical activity variable and subsequent allocation of physical activity values to study participants in Paper IV. Boxes with solid outlines are variables available in all cohorts, boxes with dashed outlines are available in Vasaloppet and boxes with dotted outlines are available in COSM and SMC. Arrows represent the direction of causality: boxes with arrows pointing to the physical activity variable are thought to influence the activity level, while boxes with arrows from the physical activity variable are thought to be influenced by physical activity.
Results

Paper I

The mean age at baseline was 61 years and 42% of participants were women. Individuals in the higher strata of walking/bicycling and exercise tended to have lower BMI, fewer comorbidities, and were less frequently current smokers. During a maximal follow-up of 17 years, 5,153 hip and 15,043 any fractures occurred in the analysis of walking/bicycling, 5,011 hip fractures 14,626 any fractures occurred in the analysis of exercise and 4,824 hip fractures and 14,178 any fractures occurred in the joint analysis of both walking/bicycling and exercise. In the analysis of walking/bicycling, the greatest reduction in rate of fracture was seen between participants walking/bicycling <20 minutes per day and participants hardly ever walking/bicycling, both for hip (HR 0.77; 95% CI 0.70-0.85) and any (HR 0.87; 95% CI 0.82-0.92) fracture, with minor differences in higher strata of walking/bicycling. The rate of fracture decreased linearly with increasing levels of exercise. Participants exercising one hour per week had lower rate of hip (HR 0.87; 95% CI 0.80-0.96) and any (HR 0.94; 95% CI 0.89-0.99) fracture compared to participants exercising less than one hour per week (Figure 2).
Figure 2: Adjusted hazard ratio with 95% confidence intervals (CI) of hip fracture and any fracture by levels of walking/bicycling and exercise, with the lowest level as reference. Adjusted for age (as time scale), sex, height, body mass index, education, cohabitation status, smoking status, alcohol consumption, systemic cortisone use, vitamin D and/or calcium supplement use, energy-adjusted vitamin D and calcium intake and weighted Charlson comorbidity index.\(^{165}\)

In the combined analysis of both walking/bicycling and exercise using a common reference group, walking/bicycling and exercise showed similar reductions in rates of hip and any fracture (Figure 3).
Figure 3. Adjusted hazard ratio with 95% confidence interval of hip (panel A) and any fracture (panel B) for stratified analysis of walking/bicycling and exercise, using the group with lowest level of physical activity (walking/bicycling and exercise) as reference. Adjusted for age (as time scale), sex, height, body mass index, education, cohabitation status, smoking status, alcohol consumption, systemic cortisone use, vitamin D and/or calcium supplement use, energy-adjusted vitamin D and calcium intake and weighted Charlson comorbidity index.\(^{165}\)
Paper II

Mean age at baseline for the 1991 race was 41 years and 7% were women. During the study period the number of participants per race increased, as did the proportion of women. Vasaloppet participants had higher education and less comorbidities than non-participants. During a mean follow-up of 9.6 years, 53,177 any fractures, 2,929 hip, 3,107 proximal humerus, 11,875 lower leg, 11,733 forearm and 2,391 vertebral fractures occurred. In Kaplan-Meier failure functions, Vasaloppet participants had a higher proportion of forearm fractures and a lower proportion of hip, proximal humerus, lower leg and vertebral fractures. No difference was visible for any fracture. The functions of forearm, lower leg and vertebral fractures separated early, whereas the functions for hip and proximal humerus fractures diverged only in older age (Figure 4).

Figure 4: Unadjusted Kaplan-Meier failure functions of any, hip, proximal humerus, lower leg, forearm and vertebral fractures in Vasaloppet participants (solid line) and non-participants (dotted line) with age as time scale.

In adjusted Cox proportional hazard analyses, Vasaloppet participants had a higher rate of any (HR 1.03; 95 % CI 1.01- 1.05) and forearm fractures (HR 1.11; 95% CI 1.06-1.15), a lower rate of hip (HR 0.75; 95 % CI 0.67-0.83), proximal humerus (HR 0.89; 95 % CI 0.82-0.98), and lower leg fracture (HR
0.93; 95 % CI 0.89-0.97), and a similar rate of vertebral fracture (HR 0.97; 95 % CI 0.88-1.07) (Figure 5) compared to non-participants. The hazard ratios were similar among Vasaloppet participants irrespective of race distance or number of races run compared to non-participants. Sensitivity analyses of Vasaloppet participants and non-participants from the SMC and COSM adjusting for lifestyle covariates changed the estimates with less than 1 %.

**Figure 5:** Hazard ratio and 95 % confidence interval (CI) of any, hip, proximal humerus, lower leg, forearm and vertebral fractures in Vasaloppet participants compared to non-participants adjusted for age (as time scale), sex, county of residence, weighted Charlson comorbidity index, cohabitation status, income quintile in 5-year age spans and education.

In the analysis comparing participants according to finishing time, 13,949 fractures of any type, 412 hip, 676 proximal humerus, 2,849 lower leg, 3,436 forearm and 571 vertebral fractures occurred. Participants close to the median finishing time (195 % of the winners’ time) had the lowest rate of fracture. Participants with slower finishing times had an increased rate of any and proximal humerus fracture, while participants with a faster finishing time had a higher rate of any and forearm fractures (Figure 6).
Figure 6: Hazard ratio (HR) and 95% confidence interval (CI) of any, hip, proximal humerus, lower leg, forearm and vertebral fractures among Vasaloppet participants by finishing time adjusted for age (as time scale), sex, county of residence, weighted Charlson comorbidity index, cohabitation status, income quintile by 5-year age spans and education. Finishing time modeled as a restricted cubic spline with three knots and the median as reference.

Paper III

The mean age at baseline was 61 years in EpiHealth and 67 years in SMCC. EpiHealth consisted of 50% women whereas SMCC included only women. Individuals in the highest level of physical activity had lower body fat percentage, were less likely to smoke and consumed more fruit and vegetables. In the discovery phase 75 proteins were associated with leisure physical activity in the EpiHealth cohort. In the replication phase, 49 associations were replicated using walking/bicycling and 31 using exercise as measure of physical activity in SMCC. β-coefficients were similar in models using walking/bicycling and exercise, and 28 associations were replicated in both models (Figure 7). Seven associations have not been previously described in human: paraoxonase 3, cystatin B, cathepsin Z, alpha-L-iduronidase, prostasin, growth differentiation factor 2 and tumor necrosis factor receptor superfamily member 11A.
Figure 7: Proteins significant in both replication models with β-coefficients (corresponding to number of standard deviations change in protein concentration per one-step change in leisure physical activity (LPA)) and 95% confidence intervals from each different model. All models adjusted for age, occupational physical activity, daily servings of fruit and vegetables, daily servings of meat, smoking status, alcohol consumption, education, cohabitation status, analysis chip number, and, in SMCC; phase of investigation, glomerular filtration rate and alanine aminotransferase.
The associations were approximately linear for most proteins, but tended to level out at higher levels of physical activity. Estimates were similar across tertiles of body fat. In the analysis adjusting both the discovery and replication phases for adiposity, seven proteins were discovered to be associated with physical activity independently of body fat percentage and four of those were replicated in both the models using walking/bicycling and exercise: cystatin B, fatty acid binding protein 4, interleukin-1 receptor antagonist and paraoxonase 3.

Paper IV

The mean age at baseline was 57 years, and 41% of participants were women. Individuals with higher physical activity scores were slightly older, had less comorbidities, reported higher levels of walking/bicycling and exercise, were more likely to have participated in Vasaloppet, have skied more races and have faster finishing times. During a maximal follow-up of 13 years, 8,506 fractures of any site, 2,164 wrist fractures, 779 proximal humerus fractures, 908 hip fractures, and 346 spine fractures occurred. Individuals close to the median physical activity level in the combined cohort generally had the lowest rate of fracture. Individuals with physical activity lower than the median had a lower rate of wrist fracture, but a higher rate of any and hip fracture. The rate of hip fracture was gradually lower with higher activity until the median level of activity, above which the rate was similar. Individuals with physical activity higher than the median had a higher rate of any fracture, but had otherwise similar rate of fracture as individuals close to the median. Proximal humerus fracture was not associated with physical activity, whereas spine fracture had a U-shaped association with wide confidence intervals (Figure 8).
Figure 8: associations between physical activity and any, wrist, proximal humerus, spine and hip fracture adjusted for age, sex, education, cohabitation status and Charlson comorbidity index. Physical activity modeled as restricted cubic splines with three knots and the median as reference. Associations presented as hazard ratios (HR, black line) and 95% confidence intervals (CI, gray area).
Discussion

Main Findings

In the papers included in this thesis physical activity was associated with a lower risk of fracture at most sites. In Paper I, both walking/bicycling and exercise were associated with a lower rate of hip and any fracture. Previous studies have reported a lower rate of hip fracture with frequent walking, but the results for exercise has been discordant and only large studies have been able to show an association, which is consistent with a smaller effect estimate. We also demonstrated that walking/bicycling and exercise were associated with similar reductions in rate of fracture compared to a common reference group, which no previous study has done. In Paper II, participation in Vasaloppet was associated with a higher rate of any and forearm fracture but a lower rate of hip, proximal humerus and lower leg fractures compared to non-participants. No previous study has investigated individuals with as high levels of physical activity or studied as many fracture sites. Previous studies of less active individuals have shown an association between physical activity and a higher risk of wrist fracture but a lower risk of hip and humerus fracture. Physical activity has previously been associated with a higher risk of any fracture in younger and more active individuals and a lower risk in elderly, congruent with our results. In Paper III, associations between physical activity and concentrations of 28 cardiovascular plasma proteins were discovered and replicated, of which seven have not previously been described in humans. Four of the associations were independent of body fat. The proteins found to be associated with physical activity are involved in for example inflammation and fat metabolism, and further research may yield insights into how physical activity benefits health. Paper IV included participants with a wider range of physical activity than previously studied, from sedentary individuals to elite athletes. The lower half of the exposure range showed a gradually lower rate of hip and any fracture but a progressively higher rate of wrist fracture with increasing physical activity. This is consistent with previous studies investigating hip and wrist fractures among individuals with low to moderate levels of physical activity. The non-linearities found may in part explain previous conflicting results for any fracture. The associations for wrist
and hip fracture leveled off at higher physical activity levels, which may indicate possible threshold values above which further physical activity may not contribute to additional changes in fracture risk.

Methodological Considerations

Study Populations
Papers I, II and IV have the common strengths of having prospectively followed a large cohort of men and women with a wide age range for a long time, during which a high number of fractures have occurred. Fractures were ascertained through national registers of high completeness and validity, mitigating loss to follow-up and misclassification. Participants in COSM and SMC were recruited from the general population and are considered representative thereof and have similar age span and range of exposure as previous population-based studies of physical activity and fracture, aiding comparison and external validity. The EpiHealth cohort is also randomly drawn from the population. Although healthy volunteer bias may affect who joins the COSM, SMC and EpiHealth, this is non-differential across exposure categories. This is however not the case in the Vasaloppet cohort. Vasaloppet participants are likely to be more health-conscious and healthy than non-participant comparison individuals, which may affect results. A directed acyclic graph-based (DAG) approach was used to attempt to adjust for this (see below).

Measurement of Physical Activity
Self-administered questionnaires were used to gather information on physical activity in Papers I and III (and COSM/SMC participants in Paper IV). The COSM/SMC questionnaire inquires about walking/bicycling and exercise, which are wide categories designed to capture most leisure physical activities performed by study participants. The items capture the quantity of physical activity rather than the frequency or duration and poorly specifies intensity, as although walking implies light to moderate intensity (fast walking eventually becomes running), bicycling may be performed with great effort and exercise may involve gentle as well as strenuous exercise. However, the questionnaire has been validated against activity records and accelerometer data with acceptable results. The EpiHealth questionnaire item does not inquire about specific characteristics of physical activity, but rather places the individual on a continuum of activity through a simple grading of dose and intensity (point 3 on the Likert scale, walking, implies light to moderate intensity; and point 5
specifically states that the activity is strenuous). Both methods however allow ranking individuals according to physical activity level.

Using self-administered questionnaires (together with follow-up in national registers) allowed large study sizes, permitting analysis of several fracture sites, investigation of dose-response associations and comparison of walking/bicycling and exercise in Paper I. However, limitations of this design include misclassification of exposure due to difficulties in accurate recall or social desirability bias, which would lead to an attenuation of the results. The accuracy of the measurement decays over time as participants may change their physical activity habits, and repeat measurements would have led to more accurate exposure ascertainment. More questions characterizing modality, frequency, duration and intensity of physical activity would have enabled further analyses, but may on the other hand have led to fewer individuals completing the questionnaire, reducing sample size or causing more missing data.

In Paper II, objective results from Vasaloppet participation were used as a proxy for physical activity, thus avoiding potential misclassification introduced by subjective self-report. Vasaloppet participants have higher levels of physical activity than the general population, but unfortunately no information on physical activity on the individual level was available for the Vasaloppet non-participants. Non-participants were assumed to have low to moderate levels of physical activity, but if they were more active than anticipated it would lead to an attenuation of estimates. Exposure and covariate information were time updated to improve classification and reduce immortal time bias. Using race data makes translation into habitual levels of physical activity, and comparison with other studies, difficult.

In Paper III, physical activity was assessed in different ways in EpiHealth and SMCC. The questionnaires have unfortunately not been validated against one another. EpiHealth has the strength of simplicity whereas COSM/SMC allows for greater detail. It is possible that the walking/bicycling item in COSM/SMC captures the lower range of the EpiHealth item (points 1 through 3) and the exercise item captures the higher range (points 4 and 5). However, the aim of the study was to explore overall associations between physical activity and protein concentration and not to compare associations between different characteristics of physical activity.

Covariates

In Papers I and III (and among COSM/SMC participants in Paper IV) covariates were ascertained in self-administered questionnaires, allowing collection of a wide range of covariates with a high level of detail (e.g. weekly servings of a series of dietary products), but suffering from aforementioned limitations such as difficulty of accurate recall and social desirability bias.
In Vasaloppet participants in Papers II and IV socioeconomic covariates and comorbidities were acquired from national Swedish registers. Using a directed acyclic graph\(^{(173)}\) based method, adjusting for these factors was determined to close confounding pathways through healthy lifestyle habits (e.g. smoking, diet), but unfortunately information on such covariates themselves were not available for the entire cohort. Although residual confounding may thus persist, adjustment for confounders in a sensitivity analysis of Vasaloppet participants who have also answered the COSM or SMC questionnaires did not alter estimates materially in Paper II.

A common weakness in all papers is possible confounding by functional status, i.e. frailty or fitness, as frail individuals are less likely to engage in physical activity and more likely to fracture.\(^{(174)}\) However, both frailty and fitness may conversely be viewed as mediators between physical activity and fracture, as inactivity leads to functional decline\(^{(175)}\) and activity leads to increased fitness\(^{(119)}\) which could subsequently affect the risk of fracture. If frailty or fitness are seen as confounders statistical adjustment would be desirable, but if they are considered mediators adjustment would lead to an underestimation of the total association. Unfortunately no measure of frailty or fitness were available in our cohorts. However, in an attempt to control for confounding by frailty in Paper I, we adjusted for risk factors for frailty such as age, sex, smoking and comorbidity. We further performed sensitivity analyses to exclude potentially frail individuals, such as restricting the analysis to only participants with weighted Charlson comorbidity index of zero or to only men with good self-reported health, which both showed similar results as the main analysis. To attempt to control for frailty in Paper II, we performed a sensitivity analysis adjusting for lifestyle factors using Vasaloppet participants having answered the SMC or COSM questionnaires, which showed similar results as the main analysis. Paper II may be especially susceptible to confounding by fitness as physical activity is not measured in itself but is proxied by ski race performance, which is affected by both physical activity and fitness. Fully separating the effects of fitness and physical activity in Paper II would be difficult, but Vasaloppet participants reported higher levels of leisure exercise than non-participants and amount of preparation increased with increasing race distance and faster finishing time, indicating an association between physical activity and our proxy measures.

Proteomics

Paper III stands out from the other papers in several aspects of its method. Instead of longitudinal data, it relied mainly on cross-sectional information. As both exposure and outcome are measured at the same time it is impossible to determine the causal direction. The physical activity variables are ordinal variables, but were used as continuous variables in the linear regression mod-
els to yield a single measure of association (a single beta-coefficient and corresponding p-value). This assumes a linear association, which was subsequently visually assessed in validated proteins. Confounders of interest were determined using a DAG-based method, where the same DAG model was assumed to describe potential confounding pathways between physical activity and all proteins. This is not necessarily accurate, as the association between physical activity and different proteins may be confounded by different covariates (e.g. the association between physical activity and proteins involved in inflammatory pathways may be confounded by smoking, whereas proteins involved in lipid metabolism may not). However, there is not enough knowledge about the analyzed proteins and what factors influence their expression to permit construction of individual DAGs for each protein. Further, the construction of 184 individual DAGs (and corresponding statistical models) would be unmanageable. The results should be considered mainly as exploratory and hypothesis-generating.

GSEM

Paper IV relies on the construction of a latent physical activity variable using GSEM. GSEM is an extension of structural equation modeling (SEM), allowing for generalized responses (binary, ordinal, etc. instead of only continuous), multilevel modeling and different missing patterns. Although this method allows creation of a common latent physical activity variable, thus permitting comparison of individuals from different cohorts with different measures of physical activity, interpretation of the results warrants caution. The process of creating the latent variable is partly subjective, as the analyst chooses which observed variables are to be included in the model and names the latent variable. If important variables (available or not) are omitted, the model may be misspecified. The decision to exclude or include observed variables are influenced by how they affect the latent variable, which may improve validity. GSEM does not aid in naming the variable, and although we labeled it “physical activity”, it may also describe other healthy behaviors. The latent variable was constructed from the overlap between cohorts, and if later extrapolation of the variable to the entirety of the cohorts is to be valid, the overlap must be representative of the rest of the cohorts. The latent variable is unitless and depends on the physical activity in the studied cohorts, making interpretation and comparison with other studies difficult. However, when physical activity was plotted against the observed variables, physical activity levels close to the median corresponded roughly to 20–40 minutes of daily walking/bicycling or 2–3 hours of weekly exercise.
Clinical implications

Fragility fractures are a major public health concern as they are common, frequently cause substantial morbidity, declines in quality of life, incur considerable healthcare costs and may increase mortality. As fragility fractures predominantly affect older people an ageing population will lead to more individuals at risk. Pharmacological treatment may be used in select high-risk patient groups, whereas physical activity may be a possible prophylactic treatment available and effective for all individuals at risk.

Several findings in Paper I may have clinical implications: similar associations with lower fracture risk were observed for both walking/bicycling (which is generally not strenuous and requires little equipment or supervision) and for exercise. For walking/bicycling, the largest difference was observed between sedentary and minimally active individuals, whereas exercise showed a dose-response pattern. As a significant proportion of the population are not sufficiently active, they may thus reduce their risk of fracture substantially if they assumed limited physical activity habits. In Paper I, the type of physical activity mattered less than performing any physical activity regularly, and although small amounts of physical activity was beneficial, additional activity was even better. The associations were similar in both sexes, across a range of age groups and in healthy individuals as well as those with comorbidities, indicating generalizability to a large part of the population.

In Paper II, high levels of physical activity was associated with a higher rate of minor fractures (such as wrist fractures) but a lower rate of major fractures (such as hip and proximal humerus fractures). This should allay fears that strenuous exercise increases the risk of major fractures. On the contrary, it appears beneficial for fracture risk. Vasaloppet participants were on average younger and more active than participants in previous studies, thus belonging to a group to whom previous results may not be generalizable to.

In Paper III, physical activity was associated with beneficial concentrations of 28 plasma proteins involved in inflammatory and atherosclerotic pathways. As Paper III was exploratory in design and aimed to discover new associations, the results need to be replicated in future studies before clinical implications may be drawn. However, the results could indicate which biological pathways are positively affected by physical activity, and thus which medical conditions that may be amenable to physical activity as therapy or prophylaxis. The associations often had dose-response patterns, indicating that although even small amounts of physical activity have beneficial effects on the plasma proteome, further activity may yield additional benefits.

Paper IV indicated that the association between physical activity and fracture may be non-linear, and that there may be a level above which further physical activity is not associated with changes in fracture risk. If these levels could be found and validated in future studies, these threshold values might
potentially be used to find optimal physical activity levels for public health recommendations for fracture prevention.

In Papers I, II and IV, physical activity was not specifically impact-oriented (as walking, bicycling and skiing is conversely rather gentle), yet was associated with lower risk of fracture, which may imply that specific impact-oriented bone-strengthening physical activity is not necessary for a reduction in the risk of fracture.

Implications for Future Research

The papers included in this thesis has expanded the knowledge concerning the association between different quantities, modalities and intensities of physical activity and fracture as well as the shape of the association between physical activity and fracture. Future research could use this information to find optimal quantities, modalities and intensities of physical activity for fracture prevention and to investigate the mechanisms underlying the associations.

In Paper I, II and IV, low to moderate levels of physical activity was associated with a lower risk of most fractures, whereas high levels of physical activity in Papers II and IV was associated with a higher risk of some fractures, indicating that the association is differential on fracture site and may be non-linear with possible threshold levels. This has several implications for future research: first, as the association is different across fracture sites, it may be inappropriate to study the association between physical activity and overall risk of fracture, as opposing associations may hide important findings in a summary estimate. Second, if the association is not linear, physical activity should not be used as a continuous variable, estimates should be given for each level of physical activity and thought should be given to the range of physical activity represented in a study when interpreting results. This may in part explain previous ostensibly contradicting results, where physical activity increases the risk of any fracture some studies but decreases the risk in others, as they may be studying different ranges of the association. Future research should seek to explain the non-linearities and find the possible threshold values. It is possible that physical activity at some point no longer substantially reduces the risk of falls or improves BMD, while the risk of fracture remains high during activity or even that individuals undertake gradually more challenging activities with increasing fitness, thus further increasing their risk during activity. Future research should investigate how specific modalities at different physical activity levels affect the risk of fracture to find optimal modalities and quantities for fracture prevention.

Previously shown associations between walking, bicycling and cross-country skiing and BMD are not proportionate to the decrement in risk of fracture observed in Papers I, II, IV and previous studies,
indicating that other mechanisms may be more important. Possible candidate mechanisms may include decreased risk of falling through improved muscle strength, balance or proprioception, or “better falling”, i.e. parrying falls, decreasing the force of impact or protecting vulnerable structures when falling due to for example better protective responses. If future research could discern through which mechanisms the association between physical activity and fracture are mediated, physical activity interventions could be tailored to improve those specific skills or traits.

In Paper III, leisure physical activity was associated with 28 cardiovascular proteins. We found several pathways where the pathogenesis of cardiovascular disease and fracture intersect: growth-differentiation factor 2 (also known as bone morphogenic protein 9), which is involved in glucose and lipid metabolism and angiogenesis and is one of the strongest inducers of bone synthesis; tumor necrosis factor receptor superfamily member 11A, which is involved in atherosclerosis and osteoclast differentiation and activation; and several markers of inflammation, such as interleukin-6 and tumor necrosis factor alpha 1 and 2. Future research into pathways highlighted in this study may yield new insights into how physical activity affects health and how cardiovascular disease and fracture are related. Deeper knowledge of specific pathways through which physical activity affects health may permit design of agonists or antagonists that increase or decrease the effect of said pathways, thus creating an “exercise pill” conferring some of the benefits of physical activity. An oral small-molecule inhibitor of fatty acid-binding protein 4, which was associated with physical activity in Paper III, has been developed and tested in mice, in whom it protected against atherosclerosis and type 2 diabetes.
Summary and conclusion

In Paper I, both daily walking/bicycling and weekly exercise was associated with similarly lower risk of hip and any fracture, and the largest difference was seen between the lowest and second-lowest levels of physical activity. In Paper II, high levels of physical activity, as proxied by Vasaloppet participation, was associated with a higher risk of any and forearm fracture, a lower risk of hip, proximal humerus and lower leg fracture, but not associated with vertebral fracture. In Paper III, leisure physical activity was associated with 28 proteins involved in for example inflammation, fat metabolism and atherosclerosis. In Paper IV, the association between physical activity and fracture was non-linear, where study participants with physical activity close to the median of the cohort had the lowest risk of fracture, less active individuals had a higher risk of any and hip fracture but lower risk of wrist fracture, and more active individuals had higher risk of any fracture. Physical activity had no association with proximal humerus fracture and a weak U-shaped association with spine fracture.

In conclusion, physical activity is associated with a lower risk of fragility fracture but the association is non-linear. The greatest difference in risk is seen comparing sedentary to moderately active individuals. Although high levels of physical activity is associated with a higher risk of minor fractures, all levels of activity is associated with a lower risk of severe fractures such as hip fractures. These results could be used in future studies to design interventions for fracture prevention and indicate that sedentary individuals could reduce their risk of fracture if they adopted limited physical activity habits. Physical activity is associated with more beneficial levels of 28 plasma proteins involved in atherosclerosis. Future studies investing these pathways may advance our understanding how physical activity affects health.
Bakgrund

Fragilitetsfrakturer sker framförallt hos äldre, ofta till följd av ett fall från stående. Varje år sker ca 70 000 fragilitetsfrakturer i Sverige, och de vanligaste typerna är brott på handleden, höften och ryggens kotor. Fragilitetsfrakturer leder ofta till långvarig smärta och nedsatt funktion och kan i värsta fall leda till döden. Av de som drabbas av höftbrott dör 20 % inom ett år. Fysisk aktivitet minskar risken för fall och ökar bentätheten, vilket skulle kunna minska risken för fragilitetsfrakturer. Syftet med avhandlingen var att utreda hur fysisk aktivitet är kopplat till risken för fragilitetsfrakturer.

Delarbete I

I det första delarbetet inkluderades deltagare från befolkningsstudierna Cohort of Swedish Men (COSM) och Swedish Mammography Cohort (SMC). COSM består av män boende i Västmanland och Örebro län födda 1918-1952. SMC består av kvinnor boende i Uppsala län födda 1914-1948 och kvinnor boende i Västmanland län födda 1917-1948. Båda kohorterna har svarat på livsstilsformulär 1997 (med svarsfrekvens på 49 % respektive 70 %) med information om promenad/cykling, motion, längd, vikt, utbildning, civilstånd, rökning, alkoholkonsumtion, användande av calcium- eller vitamin D-tillskott, kortisonmedicinering samt energijusterat calcium- och vitamin D-intag. Information om komorbiditet och incidenta frakturer inhämtades från Patientregistret. Cox-regression användes för att beräkna hasardkvoter (HK) och konfidensintervall (KI) för höftfraktur och alla frakturer över nivåer av promenad/cykling och motion. I analysen av promenad/cykling inkluderades 66 940 individer, och i analysen av motion inkluderades 65 622 individer. Den största riskskillnaden i analysen av promenad/cykling sågs mellan individer som promenerade/cyklande <20 minuter per dag, jämfört med de som nästan aldrig promenerade/cyklande, både för höft (HK 0.77; 95 % KI 0.70-0.85) och för alla (HK 0.87; 95 % KI 0.82-0.92) frakturer, med endast mindre skillnader mellan högre frekvenser av promenad/cykling. Frakturrisken minskade linjärt med ökande nivå av motion. Individer som motionerade en timme per vecka hade lägre risk för höft (HK 0.87; 95 % KI 0.80-0.96) och alla (HK 0.94; 95 % CI 0.89-0.99) frakturer jämfört med de som motionerade mindre än en timme per
vecka. I en kombinerad analys av promenad/cykling och motion var båda typerna av fysisk aktivitet kopplade till liknande lägre risk för fraktur.

Delarbete II

I denna studie inkluderades alla individer 18 år eller äldre som deltagit i Vasaloppet mellan 1991 och 2009 samt kontrollindivider från befolkningen som inte åkt Vasaloppet matchade på kön, ålder i 5-årsintervall och län. Totalt inkluderades 118 204 manliga och 71 757 kvinnliga Vasaloppsdeltagare och 505 194 kontrollindivider. Med hjälp av deltagarnas personnummer kopplades tävlingsdata till Patientregistret och Longitudinella integrationsdatabasen för sjukförsäkrings- och arbetsmarknadsstuder (LISA) för att samla information om kön, ålder, sjukdomar, civilstånd, inkomst och utbildning. Vasaloppsdeltagande, tävlingsdistans, antal lopp och målgångstid (i procent av vinners tid för att ta skillnad i skidföre i beaktan) användes som proxy för fysisk aktivitet. Cox-regression användes för att beräkna hasardkvoter för alla frakturer samt höft-, proximal humerus-, underbens-, underarms- och kotfraktur för Vasaloppsdeltagare jämfört med icke-deltagare samt deltagare uppdelade på tävlingsdistans och antal lopp. Restricted cubic splines användes för att jämföra frakturrisken över målgångstid. Vasaloppsdeltagare hade högre risk för alla frakturer (HK 1.03; 95 % KI 1.01- 1.05) och underarmsfrakturer (HK 1.11; 95% KI 1.06-1.15), men lägre risk för höft- (HK 0.75; 95 % KI 0.67-0.83), proximal humerus- (HK 0.89; 95 % KI 0.82-0.98), och underbensfraktur (HK 0.93; 95 % KI 0.89-0.97), och liknande risk för kotfraktur (HK 0.97; 95 % KI 0.88-1.07) jämfört med icke-deltagare. Tävlingsdistans eller antal lopp påverkade inte frakturrisken, men deltagare nära median-målgångstiden hade längst frakturrisken bland Vasaloppssåkare. Deltagare med långsammare målgångstid hade högre risk för alla frakturer och proximal humerusfraktur och deltagare med snabbare målgångstid hade högre risk för alla frakturer och underarmsfrakturer.

Delarbete III

antikroppsbaserad metod för att beräkna relativa proteinkoncentrationer. Associationer mellan fysisk aktivitet och proteinkoncentration upptäcktes först i EpiHealth genom linjär regression med proteinkoncentration som beroende variabel och fysisk aktivitet som oberoende, justerat för ålder, kön, aktivitet på arbetet, daglig konsumtion av frukt och grönt, daglig konsumtion av kött, rökning, alkoholkonsumtion, utbildning och civilstånd. False Discovery Rate användes för att justera för multipla signifikantester. Associationer signifikanta i detta första steg togs sedan vidare till SMCC där en liknande analys gjordes för att bekräfta fynden. I EpiHealth upptäcktes 75 möjliga samband. I SMCC bekräftades därefter 49 samband i en analys med promenad/cykling som mått på fysisk aktivitet och 31 i en analys med motion. Tjugoåtta samband kunde bekräftas i båda analyserna, varav sju samband inte har tidigare beskrivits hos människa och fyra samband var oberoende av kroppsfett.

Delarbete IV


Konklusion

Sammanfattningsvis är fysisk aktivitet kopplat till lägre risk för fragilitetsfrakturer, framförallt mer allvarliga frakturer såsom höftfrakturer. Jämfört med en stillasittande livsstil är även mindre mängder fysisk aktivitet kopplad till lägre frakturrisk. Högta nivåer av fysisk aktivitet minskar också risken för allvarliga frakturer, men kan öka risken för mindre allvarliga frakturer såsom handledsbrott. Fysisk aktivitet är också kopplat till mer fördelaktiga koncentrationer av
28 plasmaproteiner förekommande i bland annat inflammatoriska processer och fettmetabolism.
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)