Adipose tissue, the skeleton and cardiovascular disease

Peder Wiklund
People forget how fast you did a job - but they remember how well you did it.

Howard Newton
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Howard Newton
Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table of Contents</td>
<td>2</td>
</tr>
<tr>
<td>Abstract</td>
<td>4</td>
</tr>
<tr>
<td>Preface</td>
<td>6</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>7</td>
</tr>
<tr>
<td>Introduction</td>
<td>8</td>
</tr>
<tr>
<td>Obesity</td>
<td>9</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>10</td>
</tr>
<tr>
<td>Definitions of overweight and obesity</td>
<td>11</td>
</tr>
<tr>
<td>Pathogenesis of cardiovascular disease</td>
<td>12</td>
</tr>
<tr>
<td>Pathophysiology of obesity</td>
<td>14</td>
</tr>
<tr>
<td>Adipose tissue as an endocrine organ</td>
<td>15</td>
</tr>
<tr>
<td>Regulation and dysregulation of lipid metabolism</td>
<td>15</td>
</tr>
<tr>
<td>Obesity and CVD risk factor clustering</td>
<td>18</td>
</tr>
<tr>
<td>Measurement of fat mass</td>
<td>20</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>23</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>24</td>
</tr>
<tr>
<td>Definition of osteoporosis</td>
<td>25</td>
</tr>
<tr>
<td>Risk factors for osteoporosis</td>
<td>26</td>
</tr>
<tr>
<td>Bone structure</td>
<td>28</td>
</tr>
<tr>
<td>Bone modelling and remodelling</td>
<td>29</td>
</tr>
<tr>
<td>Measurement of bone mass</td>
<td>31</td>
</tr>
<tr>
<td>Adipose tissue, the skeleton and cardiovascular disease</td>
<td>33</td>
</tr>
<tr>
<td>Fat distribution and bone mineral density</td>
<td>34</td>
</tr>
<tr>
<td>Bone mineral density and cardiovascular disease</td>
<td>34</td>
</tr>
<tr>
<td>Obesity-associated CVD risk factors and osteoporosis</td>
<td>35</td>
</tr>
<tr>
<td>The skeleton and energy metabolism</td>
<td>37</td>
</tr>
<tr>
<td>Thesis aims and hypotheses</td>
<td>38</td>
</tr>
<tr>
<td>Materials and methods</td>
<td>39</td>
</tr>
<tr>
<td>DEXA</td>
<td>40</td>
</tr>
<tr>
<td>Regional fat mass measurements</td>
<td>40</td>
</tr>
<tr>
<td>Bone mineral density measurements</td>
<td>41</td>
</tr>
<tr>
<td>Study populations</td>
<td>41</td>
</tr>
<tr>
<td>The Bone density and fat mass database</td>
<td>42</td>
</tr>
<tr>
<td>The MONICA database</td>
<td>42</td>
</tr>
<tr>
<td>The VIP database</td>
<td>42</td>
</tr>
<tr>
<td>Statistical methods</td>
<td>44</td>
</tr>
<tr>
<td>Ethics</td>
<td>45</td>
</tr>
<tr>
<td>Results</td>
<td>46</td>
</tr>
<tr>
<td>Study I</td>
<td>46</td>
</tr>
<tr>
<td>Study II</td>
<td>48</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Study III</td>
<td>50</td>
</tr>
<tr>
<td>Study IV</td>
<td>52</td>
</tr>
<tr>
<td><strong>Discussion</strong></td>
<td>55</td>
</tr>
<tr>
<td>Regional obesity and cardiovascular disease</td>
<td>56</td>
</tr>
<tr>
<td>Osteoporosis and cardiovascular disease</td>
<td>59</td>
</tr>
<tr>
<td>The skeleton in energy metabolism</td>
<td>60</td>
</tr>
<tr>
<td>Adipose tissue, the skeleton and cardiovascular disease</td>
<td>61</td>
</tr>
<tr>
<td>Future research</td>
<td>64</td>
</tr>
<tr>
<td><strong>Summary and conclusions</strong></td>
<td>65</td>
</tr>
<tr>
<td><strong>Acknowledgements</strong></td>
<td>66</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>67</td>
</tr>
</tbody>
</table>
Abstract

Cardiovascular disease (CVD) is the leading cause of death in the Western World, although the incidence of myocardial infarction (MI) has declined over the last decades. However, obesity, which is one of the most important risk factors for CVD, is increasingly common. Osteoporosis is also on the rise because of an aging population. Based on considerable overlap in the prevalence of CVD and osteoporosis, a shared etiology has been proposed. Furthermore, the possibility of interplay between the skeleton and adipose tissue has received increasing attention the last few years with the discovery that leptin can influence bone metabolism and that osteocalcin can influence adipose tissue.

A main aim of this thesis was to investigate the effects of fat mass distribution and bone mineral density on the risk of MI. Using dual-energy x-ray absorptiometry (DEXA) we measured 592 men and women for regional fat mass in study I. In study II this was expanded to include 3258 men and women. In study III 6872 men and women had their bone mineral density measured in the total hip and femoral neck using DEXA. We found that a fat mass distribution with a higher proportion of abdominal fat mass was associated with both an adverse risk factor profile and an increased risk of MI. In contrast, a higher gynoid fat mass distribution was associated with a more favorable risk factor profile and a decreased risk of MI, highlighting the different properties of abdominal and gynoid fat depots (study I-II). In study III, we investigated the association of bone mineral density and risk factors shared between CVD and osteoporosis, and risk of MI. We found that lower bone mineral density was associated with hypertension, and also tended to be associated to other CVD risk factors. Low bone mineral density was associated with an increased risk of MI in both men and women, apparently independently of the risk factors studied (study III).

In study IV, we investigated 50 healthy, young men to determine if a high-impact loading intervention in the form of a series of jumps would lead to changes in glucose and lipid metabolism. We found that the intervention group had significantly lowered serum glucose levels compared to the control group. Changes in all metabolic parameters favored the intervention group with an increase in lipolysis from baseline and a decrease in cholesterol.

In summary, the proportion of abdominal and gynoid fat mass displayed contrasting associations to both CVD risk factors and MI risk. Abdominal fat mass was associated with a higher risk while a high proportion of gynoid fat...
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In summary, the proportion of abdominal and gynoid fat mass displayed contrasting associations to both CVD risk factors and MI risk. Abdominal fat mass was associated with a higher risk while a high proportion of gynoid fat mass was associated with a lower risk. Bone mineral density displayed an inverse association with MI risk, seemingly independently of CVD risk factors, suggesting other explanations to a shared pathogenesis. Finally, high impact loading on the skeleton in young, healthy men decreased serum glucose levels and tended to improve other metabolic parameters, suggesting that the skeleton can affect energy metabolism.
Preface


Abbreviations

ANOVA Analysis of variance
BMD Bone mineral density
BMI Body mass index
CI Confidence interval
CT Computed tomography
CV Coefficient of variation
CVD Cardiovascular disease
DEXA Dual energy x-ray absorptiometry
ECG Electrocardiogram
FFA Free fatty acids
HDL High density lipoprotein
HR Hazard ratio
LDL Low density lipoprotein
MI Myocardial infarction
MONICA Monitoring trends and determinants in cardiovascular disease
OC Osteocalcin
pDEXA Peripheral DEXA
pQCT Peripheral quantitative computed tomography
QCT Quantitative computed tomography
QUS Quantitative ultrasound
SAD Sagittal abdominal diameter
SAT Subcutaneous adipose tissue
SD Standard deviation
SNS Sympathetic nervous system
T2DM Type II diabetes
VAT Visceral adipose tissue
vBMD Volumetric BMD
VIP Västerbotten Intervention Programme
VLDL Very low density lipoprotein
WC Waist circumference
WHO World Health Organization
WHR Waist to hip ratio
Introduction

In the last century, Western countries have experienced numerous lifestyle changes. Life expectancy has never been higher, thanks to advances in healthcare. The abundance of food means that access to food is rarely an issue. The downside is that an increasingly sedentary lifestyle combined with an increased caloric intake has caused the prevalence of overweight and obesity to skyrocket in the last decades [1]. We are also seeing a shift in demographics; osteoporosis is increasing in prevalence as our population grows older.

The main comorbidities of obesity are insulin resistance [2] and cardiovascular disease (CVD) [3] and the main deleterious outcome of osteoporosis is fractures [4]. Obesity and osteoporosis have understandably been considered to be separate entities with different preventive strategies and treatments. Interestingly, the considerable overlap in the prevalence of CVD and osteoporosis is sufficient for a common etiology to be proposed. Additionally, several links between the skeleton and adipose tissue have recently been discovered expanding our knowledge of the interplay that occurs between these tissues.

Understanding how different body compositions, both relative to regional fat mass and bone mineral density (BMD), affects CVD risk is vital. Together with a deeper understanding of the links between the skeleton and adipose tissue, this could lead to improved preventive and therapeutical interventions, reducing the mortality and morbidity associated with obesity and osteoporosis.
Introduction

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We are also seeing a shift in demographics; osteoporosis is increasing in prevalence as our population grows older. The main comorbidities of obesity are insulin resistance\[2\] and cardiovascular disease (CVD)\[3\] and the main deleterious outcome of osteoporosis is fractures\[4\]. Obesity and osteoporosis have understandably been considered to be separate entities with different preventive strategies and treatments. Interestingly, the considerable overlap in the prevalence of CVD and osteoporosis is sufficient for a common etiology to be proposed. Additionally, several links between the skeleton and adipose tissue have recently been discovered expanding our knowledge of the interplay that occurs between these tissues.

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### Chapters

- Epidemiology
- Definitions of overweight and obesity
- Pathogenesis of cardiovascular disease
- Pathophysiology of obesity
- Measurement of fat mass
Obesity

Epidemiology

Obesity is a growing health concern. A combination of a sedentary lifestyle and excessive caloric intake are causing a rapid increase in the prevalence of overweight and obesity. This prompted the World Health Organization (WHO) to formally recognize obesity as a global epidemic in 1997 [5]. In Sweden about 33% of all adults are estimated to be overweight while an additional 12% are obese [6]. Furthermore, in young men, the prevalence of overweight and obesity have tripled and quadrupled over the last decades [1].

Obesity is associated with several adverse outcomes. It is known to reduce life span [7] and increase disability [8], and is associated with several serious chronic diseases. Increasing weight corresponds to increases in the incidence of CVD [3] and type II diabetes (T2DM) [2], as well as increased risk of osteoarthrosis [9,10], sleep apnoea [11] and certain cancers [12].

In Sweden, CVD is the leading cause of death [13], and is strongly influenced by obesity. However, even though the prevalence of obesity is increasing, neither CVD nor T2DM have shown corresponding increases. In fact, CVD is steadily declining in both men and women [14]. This discrepancy could be because the marked increase in overweight and obesity started in the early nineties, and the consequences have not yet surfaced. The proportions of overweight and obese in the Swedish population in 1999 were nearly identical to the US population in 1989, and the progression of the obesity epidemic is similar to what was observed in the US [15]. Since year 2000, the US has seen an increase in CVD mortality in certain age groups, after years of decline [16]. This suggests that if the development of obesity in Sweden continues to follow US trends, comorbidities of obesity might also increase in prevalence.
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**Definitions of overweight and obesity**

Overweight and obesity are defined as abnormal or excess fat accumulation that may impair health [5]. The most commonly used technique to classify overweight and obesity is body mass index (BMI). This is defined as weight in kilograms divided by the square of the height in meters (kg/m²) and emphasizes total weight relative to height. BMI is the criterion for overweight and obesity for WHO, which defines overweight as a BMI of between 25-29.9 and obesity defined as a BMI of 30 and above [5]. However, BMI might not be the best predictor of “dangerous” obesity. Instead, measures of central obesity have been advocated over the use of BMI. The central obesity criteria of waist circumference (WC) or waist to hip ratio (WHR) vary depending on population and country. However, the WHO criteria [17] are a WHR of >0.9 for men and >0.85 for women. The National Institutes of Health criteria [18] are a WC of >102 cm for men and >88 cm for women.
Pathogenesis of cardiovascular disease

Before discussing the pathophysiology of obesity, the underlying mechanism of CVD, namely atherosclerosis, must be considered. Many of the links between obesity and CVD that are discussed later directly concern atherosclerosis, so a brief summary of atherosclerosis is provided.

Atherosclerosis is a slow, complex disease that starts in childhood and progresses throughout life. It affects the walls of arteries causing a build-up of atheromatous plaques, narrowing the inner diameter, or lumen, of the blood vessel. The narrowing of the lumen can restrict blood flow to end organs, causing e.g. angina pectoris or claudicatio intermittens. However, advanced atheromatous plaques not only contribute to restriction of blood flow, but are often unstable and prone to rupture. In the worst-case scenario total obstruction of the vessel occurs due to thrombosis or emboli, causing permanent damage to the heart or brain from lack of oxygen [19].

The initiating factor that is considered to be a requisite for plaque formation is minimal injury of the endothelium, called endothelial dysfunction [20]. The first visible signs of the atherosclerotic process are small lipid deposits in the vessel wall called fatty streaks, seen as early as a few years of age [21]. The origin of fatty streaks is low density lipoprotein (LDL) cholesterol that becomes oxidized and entering the endothelium. The change in LDL structure causes macrophages to phagocytose the oxidized LDL, storing the lipids and cholesterol. Due to the lipid retention the macrophages change appearance and become foam cells. With increasing lipid deposition and higher LDL cholesterol concentrations in the blood overloaded foam cells die and form extracellular pools of lipids, stimulating adjacent smooth muscle cells to accumulate lipids [22], and turning the fatty streak into an atheromatous plaque. Over time, and exposure to various risk factors, the plaque builds. Extracellular pools of lipids and foam cells form the core of the plaque, and are separated from the lumen by a fibrous cap. The cells in the lipid core produce several pro-thrombotic factors, which can rapidly form a thrombus when in contact with the blood, either occluding the artery or detaching as an embolus that occludes a peripheral artery [20]. Atherosclerotic plaque progression is shown in Figure 1.
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Atherosclerotic plaque progression is shown in Figure 1.

**Figure 1.** Atherosclerotic plaque progression. The first visible sign of atherosclerosis is a fatty streak. After additional lipid accumulation and minor cell death, small extracellular lipid pools form, stimulating uptake into smooth muscle cells. Growth is mainly by lipid addition. Over time, the outer layer of the atherosclerotic plaque becomes fibrotic and prone to rupture, creating a risk of total occlusion from thrombosis.
Pathophysiology of obesity

Obesity is a heterogenous disease. Although total fat mass is consistently associated with increased CVD mortality and incident CVD, a subset of obese individuals is metabolically healthy, while a subset of normal weight individuals displays metabolic abnormalities [23,24].

Adipose tissue can be divided into subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). The SAT is located directly under the skin and is considered as healthy adipose tissue. VAT is located inside the peritoneal cavity, around the internal organs, and is suggested to be the driving force for the deleterious implications of obesity (Figure 2) [25].

Figure 2. Cross-sectional image of the abdomen of a normal weight women and an abdominally obese woman, demonstrating different proportions of SAT and VAT.
The normal function of the adipose tissue is to act as an energy reservoir, storing large amounts of triglycerides for release when the body is in need of energy [26]. To understand why obesity is associated with chronic diseases, it is important to consider the difference between the normal function of subcutaneous adipocytes, and the different properties of visceral adipocytes.

**Adipose tissue as an endocrine organ**

Long considered inert, the finding that adipocytes can produce and secrete a wide range of proteins and adipokines was a momentous discovery. Adipocytes are now recognized as active participants in energy regulation through a network of endocrine, paracrine, and autocrine signaling pathways. Adipocytes can influence energy regulation by releasing several adipose-tissue derived proteins, often referred to as adipokines or adipocytokines [26].

One of the many adipokines produced by both subcutaneous and visceral adipocytes is leptin. Leptin is secreted in direct proportion to the amount of fat mass, and functions as a metabolic signal of energy sufficiency [27]. This secretion is greater from subcutaneous than visceral adipocytes [28]. Adipokine secretion by visceral adipocytes is markedly different than their subcutaneous counterparts. IL-6, an adipokine associated with an increase in the systemic inflammation marker C-reactive protein, and TNF-α, an adipokine associated with insulin resistance, are synthesized and secreted more readily by VAT [29-32]. PAI-1, an adipokine involved in fibrinolysis regulation and thrombus formation, is also increased in visceral obesity [33]. Moreover, the synthesis of adiponectin, an adipokine suggested to be anti-inflammatory that is able to increase insulin sensitivity [34], is decreased in obesity [28].

**Regulation and dysregulation of lipid metabolism**

The main function of adipose tissue is the storage and release of triglycerides. Triglycerides contain three fatty acids, esterified to one glycerol molecule, with the fatty acids ultimately used as cellular energy. The main regulators of this process are insulin, which increases storage and inhibits lipolysis in periods of energy surplus, and catecholamines, which increase lipolysis and the release of free fatty acids (FFA) into the bloodstream in periods of energy shortage.

One difference between SAT and VAT is the anatomical location of the fat depots. Venous blood from SAT is returned to the heart via the systemic
veins, while venous blood from VAT is instead returned via the portal vein, creating a direct link between VAT and the liver [35].

Subcutaneous adipocytes are highly insulin sensitive. A high avidity for FFA and triglyceride storage allows the SAT to act as a metabolic sink, controlling the amount of FFA in the circulation [36]. VAT is less insulin sensitive than SAT [37]. Visceral adipocytes are, on the other hand, susceptible to cathecholamine-induced lipolysis. This altered inhibition/stimulation of lipolysis leads to increased FFA concentrations in the blood and a consequent increase of FFA influx to the liver. This causes an increase in FFA oxidation and increased very low density lipoprotein (VLDL) cholesterol secretion [38]. Additionally, glyconeogenesis is upregulated, which leads to hyperglycemia in addition to endogenous hyperlipidemia [39]. This dysregulation of lipid metabolism can cause ectopic fat accumulation in the liver, skeletal muscles and heart, inducing peripheral insulin resistance and aggravating the metabolic disturbances associated with visceral obesity [40].

In summary, because VAT drains via the portal vein and has a unique adipokine profile, it is thought to induce a prothrombotic and proinflammatory state that results in endothelial dysfunction [41]. This endothelial dysfunction precedes atherosclerotic plaques [42], and is now considered a pivotal component in the atherosclerotic process [20]. SAT, however, might confer protection against the metabolic deterioration associated with VAT. According to the post-prandial model of insulin resistance [43] chylomicrons derived from a meal are hydrolyzed by VAT. Because VAT is unable to effectively store the released FFA, increased flux to the liver ensues, ultimately causing ectopic fat accumulation. SAT and VAT compete to hydrolyze chylomicrons, and a higher amount of SAT might ameliorate the increased postprandial FFA flux attributable to VAT (Figure 3).
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Figure 3. Adverse properties of excess visceral fat accumulation. VAT has an altered adipokine profile and dysregulates lipid metabolism. This leads to adverse lipid levels, an increase in insulin resistance, and accumulation of ectopic fat, aggravating the atherosclerotic process. Subcutaneous fat accumulation works as a metabolic sink when in positive energy balance, providing protection from the metabolic disturbances of VAT accumulation.
Obesity and CVD risk factor clustering

The role of obesity in CVD, and in MI in particular, is part of a web of causation. The etiology of MI is multi factorial and influenced by several risk factors in addition to obesity. Hypertension, physical inactivity, smoking, age, heredity, sex, T2DM, and dyslipidemia are some of the strongest risk factors [44], and obesity interacts with several of these.

Obesity and hypertension

Hypertension and obesity are strongly associated, with a linear relationship between increasing BMI and systolic and diastolic blood pressure [45]. Obesity is suggested to influence blood pressure through several mechanisms, including alterations in the renin-angiotensin system [46], insulin resistance, increased sympathetic activity [47], and hyperlipidemia [48].

Essential hypertension is one of the most important risk factors for CVD. CVD risk increases linearly with increasing systolic and diastolic blood pressures [49]. The pathophysiology of hypertension in the development of CVD lies in both changes in hemodynamics and vascular physiology. With increasing blood pressure, end-organ damage occurs in the heart [50] and kidneys [51], among others. Hypertension is also associated with endothelial dysfunction [52], leading to an aggravated atherosclerotic process.

Obesity and smoking

The relationship between obesity and smoking is complex. Nicotine can increase energy expenditure [53] and lower appetite, thereby promoting weight loss, explaining the lower body weight found in smokers in some studies [54]. On the other hand, heavy smoking can increase the risk of obesity compared to light smoking, perhaps partly through the clustering of risk factors, such as physical inactivity, unhealthy diet, and high alcohol intake [55]. Smoking is also associated in a dose-dependent fashion with higher WHR, which is indicative of increased accumulation of VAT [56,57].

The risk for CVD among smokers is dose-dependent, with a doubled-to-tripled risk for CVD when smoking as few as 1-4 cigarettes a day [58]. After smoking cessation CVD risk quickly reverts to the level of non-smokers [59], suggesting that smoking-related changes are at least partly reversible. Smoking can affect CVD risk through several mechanisms. It can directly accelerate atherosclerosis by inducing a prothrombotic state and endothelial dysfunction [60]; it can also interact with other CVD risk factors. Smoking
has been shown to be unfavorably associated with lipid profile [61], and can contribute to insulin resistance [62] and abdominal obesity [56,57], factors that in turn can accelerate atherosclerosis.

**Obesity and type II diabetes**

The prevalence of obesity and T2DM are largely overlapping, enough that obesity is recognized as the most prominent cause of T2DM [63]. The risk of T2DM increases linearly with increasing body weight. Weight gain is associated with an increased risk, and even moderate weight loss can decrease risk [2,64]. Obesity may induce T2DM by several mechanisms. It can cause insulin resistance through the release of FFA into the liver, and by secreting pro-inflammatory cytokines from the VAT, and also by increasing cellular stress signaling [65,66]. T2DM is one of the main comorbidities of obesity as T2DM is associated with increased mortality and morbidity, especially in CVD [67].

Diabetes is associated with a doubled-to-tripled risk of MI [68]. Diabetes can affect CVD risk through different mechanisms. Hyperglycemia can cause increased glycosylation of almost any protein. Glycosylated proteins form advanced glycosylation end products that in turn can induce endothelial dysfunction, and accumulation and oxidation of LDL particles [69]. Aside from the effects of hyperglycemia, diabetes is also closely related to other CVD risk factors. Insulin resistance, together with a combination of central obesity, hypertension, and dyslipidemia, is called the metabolic syndrome [70], highlighting the strong relationships between obesity, T2DM, and other CVD risk factors.

**Obesity and dyslipidemia**

Obesity, and particularly abdominal obesity, is strongly associated with the presence of several lipid metabolism abnormalities. Increased triglycerides, LDL and VLDL cholesterol; and reduced high density lipoprotein (HDL) cholesterol are all associated with abdominal obesity [71,72]. Abdominal obesity can cause hypertriglyceridemia through the abnormal release of FFAs to the liver, inducing increased endogenous secretions of triglycerides in the form of VLDL [73]. Increased triglycerides are inversely associated with HDL cholesterol levels, and also inversely associated with LDL particle size and density, leading to a more atherogenic LDL particle [74,75]. The associations between triglyceride levels and nontraditional risk factors (increased insulin, apolipoprotein B levels and small, dense LDL particles) suggest that hypertriglyceridemia could be used with central obesity as a screening tool to identify persons at high risk of CVD [76].
Measurement of fat mass

Several methods for measuring fat mass have been developed. The most commonly used are BMI, WC and WHR. For measuring VAT computed tomography (CT) is the gold standard [77], although the high cost and low availability makes this method less commonly used. Alternatives for measuring fat mass include skinfold measurements, bioelectrical impedance, quantitative ultrasound (QUS), and dual energy x-ray absorptiometry (DEXA) [78,79].

Anthropometry

Body composition, especially fat mass, can be measured in many different ways. All have advantages and disadvantages. The most common methods are anthropometric and thus easy, quick and readily available. BMI is calculated as weight divided by the squared height and is a measure of general obesity. Although widely used in the clinical setting this method has several drawbacks [80]. Although predictive of the risk of MI, T2DM, and other adverse conditions, BMI gives only a crude measure of general obesity. Illustrated in Figure 4 are two individuals with the same weight and height, one of them lean and muscular and the other abdominally obese. They have the same BMI but different risks for CVD.

Figure 4. Comparison of two individuals with widely different body composition, but the same BMI.
Other easily obtained anthropometric measures are WC (measured as the horizontal circumference approximately at the umbilicus), WHR (ratio between WC and hip circumference, measured at the trochanters) and sagittal abdominal diameter (SAD, the distance from the back to the upper abdomen, measured in the supine position). Instead of general obesity, these methods all in some way assess the amount of VAT. As VAT is a stronger predictor of the adverse outcomes of obesity than total fat mass these measures are more predictive of CVD risk than BMI [81]. WC emphasizes the absolute amount of abdominal fat mass, similar to the SAD, while the WHR instead emphasizes the proportion of abdominal fat relative to hip size.

Whether any of these measures are superior to the others is inconclusive. When assessing CVD risk factors such as triglycerides, HDL cholesterol, insulin, and glucose levels, most studies find WC to be as good or better than WHR [82-84]. On the other hand, several large studies evaluating the risk of incident CVD found that WHR was more strongly associated [85], or tended to be more strongly associated [86] with the risk of incident CVD. In any case, the advantage of WHR, WC and SAD are attributed to the ability to estimate abdominal fat mass. However, these measures are still only anthropometric. A direct measure of fat mass requires other methods.

**Computed tomography**

CT is the gold standard for quantifying abdominal fat mass and VAT. VAT is usually measured by CT by taking a cross-sectional scan at the umbilicus. The contours of the SAT and the parietal peritoneum are manually traced and the intraperitoneal (visceral) fat volume calculated by subtracting the subcutaneous fat volume from the total fat volume of the scan. This has been shown to be highly correlated to total VAT in cadaver studies, and CT is thus used as the reference when investigating how well other methods can assess VAT [77].

**Dual energy x-ray absorptiometry**

DEXA is a low-dose radiation method that is gaining in popularity. It is based on a model that separates the body into three different compartments: bone mineral, lean mass and fat mass. It is primarily used to measure BMD, but also gives an accurate estimate of regional fat mass and lean mass. The advantage of DEXA is that since it is x-ray-based, it can accurately differentiate fat mass from other tissue, thereby circumventing many of the inherent drawbacks of anthropometry [87]. It can also accurately measure total body fat mass, abdominal fat mass, and any other regional fat depot using only a single measurement. The drawbacks of DEXA are that, while
strongly correlated to CT-measured total abdominal fat mass, it cannot
differentiate between SAT and VAT [88]. Also while a DEXA-measurement
involves less radiation and is more readily available than CT, it is still an
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More information on the accuracy and precision of DEXA is in Materials
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Osteoporosis

Epidemiology

Osteoporosis is generally a silent disease, characterized by reduced bone mass and deterioration of the bone tissue microstructure, leading to bone fragility and an increased risk of fractures (Figure 5). The development of osteoporosis often spans over several decades, gradually weakening the bone. Since the bone loss is asymptomatic, a low-energy fracture is often the first presenting sign [4].

Figure 5. Comparison of a bone with normal bone microstructure with an osteoporotic bone with decreased BMD and microstructural deterioration.

Osteopenia and osteoporosis currently are common, with an exponential increase in prevalence after 50 years of age [89]. Additionally, the risk of sustaining an osteoporotic fracture is 50% in women and 25% in men [90]. In Sweden, hip fractures alone occurs in 18,000 individuals each year [91], greatly affecting quality of life. Hip fractures are associated with a high morbidity and a considerable increase in mortality [92]. Hip fractures worldwide are predicted to increase fourfold by 2050 because of an increasingly elderly population [93].
Definition of osteoporosis

BMD is a continuous variable, with an inverse linear relationship to the risk of a fracture. The diagnosis of osteoporosis is therefore an arbitrary cut-off that represents a greatly increased risk of fracture compared to a normal bone [94].

The diagnosis of osteoporosis is based on measurements of BMD as specified by the WHO in 1994 [94], and later updated by the International Osteoporosis Foundation [89]. The preferred method for BMD measurements is DEXA. A BMD $\leq 2.5$ standard deviations (SDs) below that of young adult females from the same ethnic group is considered osteoporosis, while $\leq -1$ to $>-2.5$ SD is considered osteopenia. These reference values were defined in women, but the same values are used in men [89].

Figure 6. Schematic illustration of the accumulation and loss of bone mass during a lifetime for men and women. Peak bone mass occurs between the third and fourth decade of life, after which men have a continuous decline in bone mass. In women however, a faster decline in bone mass occurs the first years after menopause, after which the decline slows to be comparable to men.
Risk factors for osteoporosis

The risk of sustaining a fracture is inversely related to bone strength, estimated by BMD, and is directly related to the severity of trauma [95]. This thesis focuses on the association between BMD and CVD, and will be limited to risk factors for reduced BMD. Risk factors associated with obesity and osteoporosis, including smoking, hypertension, diabetes and hyperlipidemia are discussed in further detail in the chapter Adipose tissue, the skeleton and cardiovascular disease.

Risk factors can be divided into non-modifiable and modifiable risk factors (Table 1). Non-modifiable risk factors include genetic factors that are estimated to determine 50-80% of the variance in bone mass [96,97]. These are thus the strongest determinant of bone mass. Age is also an important risk factor, as with advancing age, the risk for osteoporosis increases linearly. Both men and women reach peak bone mass somewhere between the end of longitudinal growth and third or fourth decade, depending on bone site [98-100]. After peak bone mass is reached, a decrease in bone mass occurs throughout life. However, men and women do not have similar patterns of decrease. Men display a slow and continuous decrease while women have an accelerated bone loss in the years immediately after menopause, because of menopause-associated estrogen deficiency (Figure 6) [101-103].

Modifiable risk factors include physical inactivity, body weight, smoking, alcohol, malnutrition and several diseases and their treatments [104-107]. Physical inactivity is one of the most important risk factors. Physical activity is associated with increased BMD in children and adults [108,109] and also to amelioration of bone loss associated with aging [110]. Lifestyle factors such as smoking [111] and excess alcohol consumption [112] can also negatively influence BMD.
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<th>Non-modifiable risk factors</th>
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<td>Age</td>
<td>Physical inactivity</td>
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<td>Sex</td>
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Table 1. Risk factors for osteoporosis
**Bone structure**

The skeleton has several important functions in the body. It regulates calcium homeostasis in the blood, and is responsible for our ability to move, serving as levers for the skeletal muscles and protecting vital structures from trauma. To fulfill these roles, the skeleton must be able to withstand considerable force [113].

![Bone anatomy of the proximal femur](image)

**Figure 7.** Bone anatomy of the proximal femur.

All bones consist of two types of bone tissue, namely cortical and trabecular bone (Figure 7). Cortical bone comprises the outer surface of bone and is much denser than the trabecular bone, and provides most of the structural integrity of bone, contributing about 80% of the skeletal weight. Cortical bone consists of a system of cylindershaped units called osteons, which are running parallel to the length of the bone. In the center of each osteon is an Haversian canal, the blood vessel housing blood vessels and nerves. The osteons are in contact with the marrow cavity, the endosteum and other osteons through oblique and transverse canals. The structure of the cortical bone is, because of the dense organization of the osteons, very resilient to mechanical loading force [113].

Trabecular bone is much more porous than cortical bone, and houses blood vessels and hematopoietic stem cells. The osteons in the trabeculae are called packets, with the strongest and thickest trabeculae arranged in the direction of the greatest loading force on the bone. This allows for mechanical strength without unnecessary weight. Bone turnover is also higher in trabecular bone than cortical bone, signifying its important role in mineral metabolism [113].

Despite these differences, both bone types contain the same materials: organic bone matrix, bone mineral, and several types of bone cells. The organic bone matrix is the framework for bone mineral deposition. Adult bone is mainly composed of type I collagen, produced by osteoblasts (described below). The inorganic component of bone, the bone mineral, consists primarily of calcium and phosphorus, organized into hydroxyapatite crystals. The collagen gives the bone its resistance to torsional forces, while the bone mineral makes the bone stiff and provides resistance to compressional forces [113].

**Bone modelling and remodelling**

The skeleton is in a constant state of regeneration with two processes occurring simultaneously. Bone resorption is the process that breaks down bone. Bone formation is responsible for building up bone. Bone modelling is when bones change their overall shape in response to mechanical loading and other factors, while remodelling is the process by which bone is renewed to maintain bone strength [113]. Several factors influence bone turnover, most notably hormonal stimulation by testosterone and estrogen [114,115].

The cells responsible for constantly renewing bone tissue are osteoblasts, osteoclasts and osteocytes. Osteoblasts are bone-forming cells that synthesize and secrete type I collagen and enzymes that facilitate the mineralization process. Osteoclasts promote bone resorption by secreting acids and lysosomal enzymes onto the mineralized bone surface (Figure 8). The third type of cells is the osteocyte. Osteocytes are terminally differentiated, inactive osteoblasts. When osteoblasts secrete unmineralized bone matrix, some osteoblasts become trapped by the secreted collagen, and become incorporated into the matrix. These osteocytes develop long cytoplasmic processes, which connect them to other osteocytes via tiny tunnels.
Bone structure

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canals called canaliculi, forming a network of osteocytes [113]. The osteocytes sense changes in mechanical loading, and can influence osteoblastic and osteoclastic activity. This leads to altered bone turnover, in order to adapt to the current state of physical activity [116].

The renewal of bone tissue is continuous and allows the bone structure to adapt to the current loading state. Osteoclasts and osteoblasts move together as a single unit, called a basic multicellular unit, strongly coupling bone resorption and bone formation under most conditions. The resorptonal phase of remodelling takes about three weeks, while bone formation takes up to three months [117].

**Figure 8.** Osteoclast resorbing bone.
Measurement of bone mass

Several different techniques are used to measure bone mass. DEXA is the most common, but peripheral DEXA (pDEXA), QUS and peripheral quantitative computed tomography (pQCT) are also used. Other less commonly used techniques are quantitative computed tomography (QCT) and magnetic resonance imaging to investigate the microstructure of the skeleton [118].

Dual energy x-ray absorptiometry

DEXA is currently the most common bone densitometry technique, and the WHO criteria for osteoporosis are based on DEXA BMD measurements [89].

DEXA is based on a model that separates the body into three different compartments: bone mineral, lean mass and fat mass. It can measure total body BMD as well as BMD at specific skeletal sites, e.g. the spine, femoral neck or radius. The advantages of DEXA are high accuracy [119], short scan time, and a relatively low radiation dose. A limitation of DEXA is that all DEXA scans are two-dimensional so BMD measurements can be affected by bone size. When two different bones with the same volumetric BMD (vBMD, g/cm³) but different width and height are measured using DEXA, the larger bone will appear to have a higher BMD [118,120].

More information on the accuracy and precision of DEXA in Materials and methods.

Peripheral dual energy x-ray absorptiometry

pDEXA uses the same technique as DEXA. Because the device is smaller, the most notable difference is that pDEXA can measure BMD only at peripheral sites, such as the radius or calcaneus. The main advantage of pDEXA is that the equipment is portable, cheaper, and easier to use than a regular DEXA scanner [118]. Since hip fractures are the most serious complication of osteoporosis, BMD at the hip is of great interest. How well BMD measured peripherally correlates with BMD at the hip is unclear. Even though a low BMD at peripheral sites has been shown to increase the risk of future fractures, BMD of the hip is a better predictor of fracture risk [118].
Quantitative ultrasound

QUS uses sound waves instead of x-rays to assess bone strength. With sound, the properties measured are different than DEXA. QUS measures how fast sound travels through the bone and how the sound waves are absorbed. This is combined into a stiffness index that correlates to DEXA-derived BMD values [121]. Advantages of QUS include its low cost, the lack of ionizing radiation and the portability of the equipment [122]. The limitations are that different QUS devices can vary significantly when measuring the same parameter, as no universal standard exists for QUS [123]. Also, the WHO osteoporosis criteria are not applicable for QUS, and correlation coefficients between QUS measurements and DEXA measurements are in the range of 0.4-0.7 [124,125].

Computed tomography and peripheral computed tomography

QCT and pQCT are the most precise methods for measuring true vBMD. While the other methods rely on two-dimensional imaging, QCT scans several cross-sectional scans, measuring with high accuracy both the bone area and bone mineral content. It can also differentiate between the trabecular and cortical bone compartments [126]. The advantages of QCT lie in the high accuracy and ability to see not only BMD but also bone structure. The limitations are that it is expensive and results in higher radiation exposure than DEXA. Additionally, the WHO osteoporosis criteria are not applicable for QCT measurements [126,127].
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Adipose tissue, the skeleton and cardiovascular disease

Chapters

- Fat distribution and bone mineral density
- Bone mineral density and cardiovascular disease
- The skeleton and energy metabolism
Adipose tissue, the skeleton and cardiovascular disease

While traditionally regarded as unrelated diseases, new evidence suggests that the roles and functions of the skeleton and adipose tissue are more highly intertwined than previously thought. Leptin, a peptide hormone primarily manufactured by adipocytes, increases and decreases bone formation through actions in the hypothalamus, in addition to its appetite-regulating properties [128]. Additionally, osteocalcin (OC), a protein secreted solely by osteoblasts, has been shown to regulate glucose homeostasis through actions on the pancreas, as well as on adipose tissue, in animal models [129].

Fat distribution and bone mineral density

Low body weight is an important risk factor for hip fracture, since BMD is often correspondingly low. Obesity, on the other hand, is often associated with higher BMD. This has been attributed to the differences in load on the skeleton for different body weights, which results in differences in mechanical stimuli [130]. Several studies have found a correlation between total fat mass and BMD [131-133], and while the explanation of increased mechanical load on the skeleton is straightforward and logical, other explanations have been proposed. Adipocytes are an important source of estrogen production in post-menopausal women, and estrogen inhibits bone resorption [134]. Also, adipocytes secrete several adipokines, some of which may influence BMD [135]. But since neither adipose tissue nor the skeleton are inert organs, the ramifications of increasing body weight on bone are complicated. For example, an increased risk of fractures has been observed in obese children [136], and aging and menopause are both associated with increased fat mass, but a decline in BMD [101-103,137].

While total body weight might positively correlate to BMD, different distributions of body fat mass seem to differently influence BMD. Earlier studies investigating fat distribution and BMD found negative correlations between central obesity and BMD [138,139], suggesting that VAT might be a factor that cause lower BMD.

Bone mineral density and cardiovascular disease

BMD is usually described in the context of fracture risk. However, osteoporosis and CVD are often seen in the same individuals, suggesting that these two diseases might be linked. Prevalent CVD is related to low bone
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Bone mineral density and cardiovascular disease

BMD is usually described in the context of fracture risk. However, osteoporosis and CVD are often seen in the same individuals, suggesting that these two diseases might be linked. Prevalent CVD is related to low bone mass [140,141], and low bone mass has been shown to be associated with increased CVD mortality [142,143]. This relationship is partly regarded as a result of the normal aging process, as osteoporosis and CVD are very common diseases, but as noted above, a shared etiology has been proposed, independent of age. Suggested etiologies include hypertension [144], smoking [111], hyperlipidemia [145,146], and atherosclerosis [147,148]. Interestingly, these risk factors are, as discussed previously, also involved in a complex interplay with VAT (Figure 9).

**Obesity-associated CVD risk factors and osteoporosis**

**Hypertension**

Hypertension has, in several studies, been associated with lower BMD [149,144]. It has been postulated to decrease BMD by mobilizing calcium from the bone, leading to hypercalciuria and causing increased calcium losses [150]. In contrast, treatment with thiazide diuretics seems to improve BMD by reducing urinary calcium excretion [151,152].

**Hyperlipidemia**

Hyperlipidemia has been shown to be associated with BMD in several studies [153,154]. LDL cholesterol has been shown to reduce calcium uptake in bone and to promote vascular calcification [145], and is also associated with increased osteoclastic differentiation [146]. Furthermore, statins, which are widely used to treat hyperlipidemia, have been associated with increased BMD in some studies [155,156].

**Physical inactivity**

Physical activity has been shown in all ages to be beneficial for BMD, for both reaching a higher peak bone mass [157-159] and reducing post-menopausal bone loss [160,161]. Sedentary men and women have significantly lower BMD compared to active controls [162,163]. Additionally, performing light physical activity regularly has been shown to be associated with a 70% decreased risk of ischemic heart disease [164]. The level of physical activity throughout life is thus an important modifiable risk factor.

**Smoking**

Smoking is, in addition to CVD, also associated with lower BMD and greater rates of bone loss [165]. One factor is the lower body weight found in smokers [54], but nicotine might also have a direct effect on bone formation.
Smoking may influence hormone levels \[167,168\] and increase atherosclerotic burden \[60\].

**Diabetes**

The association between diabetes and BMD is not completely understood. In cases of diabetes type 1, BMD is decreased \[169\], while studies on individuals with T2DM have shown conflicting associations with BMD. Even though the fracture risk can be markedly increased in persons with T2DM \[170\], often average or even higher BMD is found in T2DM compared to healthy controls \[171\]. While T2DM is seemingly not associated with decreased BMD, other aspects of bone strength are influenced by this condition. Bone area is smaller, which can partially explain the increased BMD seen in some studies, as a specific amount of bone mineral within a smaller bone area would be interpreted as a higher BMD \[172\]. Nonetheless, diabetes is associated with changes in BMD and an increased risk of fractures, and is also associated with increased risk of CVD.

**Figure 9.** Schematic model of the association between BMD and CVD. CVD risk factors are associated with both obesity and BMD, and may contribute to the association between lower BMD and increased risk of CVD.
The skeleton and energy metabolism

Obesity-associated CVD risk factors appear to negatively influence BMD, and adipocyte-derived leptin can both up-regulate and down-regulate bone formation [128]. Adipose tissue might be able to influence skeletal metabolism in several ways, and the skeleton may be able to influence adipose tissue [129]. Only a few molecules produced in the skeleton are released into the systemic circulation, one of them OC.

In animals

OC, traditionally used as a bone formation marker [173], is able to influence glucose homeostasis by influencing adipose tissue in mice. OC knockout mice (OC-/-) have increased VAT, are insulin resistant, and have altered pancreatic β-cell function. Supplementing OC-/- mice with recombinant OC increases insulin sensitivity and lower fasting plasma glucose. The metabolic effects of OC are partly mediated through an increased expression of adiponectin in adipocytes [129].

In humans

The endocrine properties of the skeleton have also been investigated in humans. In mice, OC seems to mainly influence glucose metabolism, and whether this is similar in humans is of great interest. OC has in cross-sectional and longitudinal studies been shown to be associated with glucose metabolism. OC is a negative predictor of glucose levels [174,175], and persons with diabetes have lower OC levels than healthy controls [174,176]. When improvement occurs in individuals with poor glycemic control, an increase in OC is seen [177]. Studies investigating OC and lipid metabolism are inconclusive. While Fernández-Real et al. [178] showed that OC is inversely associated with triglycerides Zhou et al. [176] found a direct association between triglycerides and OC, and an inverse association between HDL cholesterol and OC. In addition to glucose and lipid metabolism, OC has also been shown to be associated with body composition. Inverse associations have been found for OC and BMI [174,179], total fat mass [174], and VAT [180], suggesting that the skeleton could be involved in fat accumulation.

The precise role of the skeleton, and particularly OC, in energy metabolism is not known. OC might as noted above be a marker of a beneficial metabolic phenotype, as it is associated with glycemic control, lower glucose levels, and body fat distribution. OC, however, is also associated with decreased femoral neck BMD [181], increased risk of MI [182] and increased risk of death [183].
Thesis aims and hypotheses

The overall aims of this thesis were to investigate the associations of body composition to CVD risk factors and incident MI, and to investigate whether the proposed interplay between the skeleton and energy metabolism might be influenced by high impact loading on the skeleton.

Study I-II. Regional fat mass is associated to CVD risk factors and incident CVD, but shows differential associations that depend on the location of the regional fat mass. By measuring regional fat mass with DEXA, the aims were to investigate how different regional fat depots are associated to CVD risk factors and the risk of MI.

Study III. BMD is suggested to be inversely associated with CVD risk. Several CVD risk factors also can negatively influence BMD, so a common etiology has been proposed between osteoporosis and CVD.

An aim was to investigate if BMD of the femoral neck and hip is associated with the risk of MI, and whether the association could be explained by associations between BMD and traditional CVD risk factors.

Study IV. The skeleton seems to be involved in various aspects of energy metabolism, with OC as the proposed link. We hypothesized that bone formation and skeletal metabolic signalling are coupled. The aim was to determine if a high impact exercise intervention would lead to changes in glucose and lipid metabolism in young men, and whether these changes could be explained by changes in OC levels.
Materials and methods, Results
Materials and methods

DEXA

In Study I-IV, a table-top DEXA (GE Lunar, Madison, WI, USA) was used for all bone mineral and regional fat mass investigations (Figure 10). Until 1998, a Lunar DPX-L was used. From 1998 a Lunar IQ was used.

Figure 10. Table-top DEXA machine.

Regional fat mass measurements

DEXA accurately measures total fat mass ($r^2=0.85-0.90$ compared to hydrostatic weighing) [184], abdominal fat mass ($r^2=0.76-0.92$ compared to CT) [88,185] and VAT ($r^2=0.44-0.62$ compared to CT) [88,186].

The coefficient of variation (CV, [mean/SD] x 100) is a measure of dispersion, with a low CV indicating a lower dispersion in repeated measurements. CVs were calculated from repeated scans and were 2% for total fat mass and 2% for abdominal fat mass.
**Bone mineral density measurements**

DEXA is the gold standard for measuring BMD, with the WHO basing the definitions of osteopenia and osteoporosis on DEXA BMD measurements [94]. DEXA accurately measures BMD in both total body and specific subregions, such as the spine and hip. Both femoral neck and total hip had a CV for BMD measurements of 1%.

**Study populations**

For Study I-III, the original cohort was gathered from the Bone density and fat mass database (described below). Study I, which investigated body composition and the association to CVD risk factors, included 592 men and women who had their total fat mass measured using DEXA. Data on blood pressure, lipids, and glucose were collected through the Västerbotten Intervention Programme (VIP, described below), and were available for all participants.

Study II investigated body composition and the risk of MI, and included 2336 women and 922 men who had their total fat mass measured. A subset of 1523 women and men also participated in the VIP. This subset was used to investigate the association of blood pressure, lipids, and glucose to regional fat mass and to investigate whether these factors would affect the association between regional fat mass and the risk of MI.

Study III investigated BMD and the risk of MI in 5490 women and 1382 men who had their BMD measured. A subset of 2730 women and 574 men had also participated in the VIP and thus had blood pressure, lipids, and glucose measured. This subset was used to investigate the association between the above background variables and BMD, and whether these variables affect the association between BMD and MI.

In Study IV, 50 healthy men aged 20-32 were enrolled. Participants were notified of the study through posters at sporting facilities and at Umeå University campus, Umeå, Sweden.
The Bone density and fat mass database

DEXA has been used since 1991 at the Sports Medicine Unit, Umeå University, Sweden, to measure BMD and total and regional fat mass. All individuals who have been measured by DEXA at the unit have been included in the Bone density and fat mass database. In early 2007, 12302 individuals had had BMD measured at some site. A subset of 4957 individuals had measured both total body BMD and regional fat mass. Reasons for BMD measurements were either a referral, most often from a general practitioner, or participation in a research project conducted at the Sports Medicine Unit. In 2005 and 2006, all individuals measured were categorised by the reason for DEXA scan. The most common causes were previous fracture (28.6%), fear of osteoporosis (23.1%), taking oral corticosteroid therapy (20.1%) or involvement in research projects (15.1%).

The MONICA database

The WHO MONICA project (MONitoring trends and determinants In CArdiovascular disease) was started by the WHO in the early 1980s with participating centres from all over the world. Its purpose was to gather information about the trends in MI incidence [187]. The project was discontinued in 1995, but continues as a regional project in Northern Sweden [188]. The original project included only patients between 25-65 years; however, since 2000 also patients aged 65-75 years have been included in the Northern Sweden MONICA project. To ensure high validity of MI diagnosis, strict criteria are used to validate MI. Originally, a MI diagnosis required either clear electrocardiogram (ECG) changes typical of MI according to the Minnesota code or cardiac enzyme elevation together with either typical chest pain or “probable” ECG changes and lesser symptoms. If the event was fatal it was considered to be a definite MI if they had findings visible to the naked eye of a fresh MI or coronary thrombosis [188]. In the late 1990s, troponins were introduced as biomarkers, and as of 2000 are used by all hospitals in northern Sweden. From 2000, all MI diagnoses are based on the presence of typical chest pain and troponin. If only one marker indicate a MI an ECG is analyzed before a diagnosis is made [189].

The VIP database

The VIP is an ongoing community intervention programme that annually invites all residents of Västerbotten county aged 40, 50 and 60 to a systematic risk factor screening and individual health counselling [190]. The VIP started in 1985 as the Norsjö Project. At that time, Västerbotten had one
of the highest incidences of CVD in Sweden and the project was initiated to reduce morbidity and mortality from CVD and diabetes. Residents 30 years of age were initially invited but participation after 1995 is restricted to those 40, 50 and 60 years of age. At screening, height and weight are measured in light clothing. Blood pressure is measured in the supine position using a mercury sphygmomanometer after 5 minutes of rest. An oral glucose tolerance test is performed with a 75 g oral glucose load according to WHO standards. Total cholesterol and triglycerides are analysed using a Reflotron bench-top analyser (Roche Diagnostics). Until 2004, this was also used to analyze plasma glucose. Since 2004, a Hemocue bench-top analyser (Quest Diagnostics) has been used. In addition to anthropometry and blood tests, all VIP participants are also invited to fill in a comprehensive questionnaire detailing factors such as social support, working environment, self-perceived health, previous illness, family history of disease, physical activity, tobacco, alcohol, and eating habits. In addition, the screening concludes with individual counselling in which the participants discuss the results of the screening. The results are stored in a database that is frequently used for research. All applications to extract data from the database are evaluated by a scientific board connected to the programme [190].
Statistical methods

Study I. Bivariate correlations between the different regional fat mass measures and continuous CVD risk factors were tested using Pearson’s coefficient of correlation. Differences between three or more groups were analysed using analysis of variance (ANOVA) and Bonferroni’s post hoc test. The relationships between different regional fat mass measures and categorical CVD risk factors were determined using logistic regression.

Study II. Differences between two groups were tested using Students t-test for independent samples. The relationships between different regional fat mass measures and MI events were determined using Cox proportional hazards models. The area under the receiver operator characteristic curve was compared for different regional fat mass measures in relation to the risk of MI. Logistic regression was used to investigate the relationships between quartiles of the different regional fat mass measures and categorical CVD risk factors.

Study III. Differences between two groups were estimated using the Student’s t-test for independent samples and the Mann-Whitney U test for the non-normally distributed variables. Correlations between BMD and risk factors were evaluated using Pearson’s correlation coefficient. Variables non-normally distributed were transformed using the natural logarithm. The relationships between BMD and vBMD, and MI risk were determined using Cox proportional hazards models. Logistic regression was used to investigate the relationship between different estimates of bone mass and different risk factors for MI, such as hypertension, diabetes, hypertriglyceridemia, and smoking.

Study IV. Differences between two groups were tested using the Student’s t-test for independent samples and the Mann-Whitney U-test for variables non-normally distributed. Variables were then transformed to normality using the square root of the variable in multivariate analyses. Changes in variables between baseline and study endpoint were tested using a paired samples t-test for the intervention and control group separately. To test for between-group differences between baseline and study endpoint, linear regression was used. Between-group differences in changes in metabolic variables were tested by several models: an unadjusted model, a model adjusted for baseline measurements of the dependent variable, a model adjusted for age, weight, and baseline measurements, and a model adjusted for baseline measurements and changes in accelerometer-measured physical activity.
A p-value of less than 0.05 was considered to be significant in all studies. SPSS for the PC (version 15 and 17, SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

**Ethics**

All studies (Study I-IV) were approved by the Regional Ethical Review Board in Umeå, Sweden.
Results

Study I

*Abdominal and gynoid fat mass are associated with cardiovascular risk factors in men and women.*

Aim

To explore the association between CVD risk factors and regional fat mass, with a focus on the contributions of abdominal fat mass and gynoid fat mass.

Methods

The study cohort consisted of 175 men and 417 women with a mean age of 45.3 years and 46.8 years, respectively. Information regarding CVD risk factors was collected through the VIP database (described above) and was available for all participants.

Results

In bivariate analyses, abdominal fat mass generally had the strongest correlation to CVD risk factors in men. In women the correlations of abdominal fat mass and the abdominal to gynoid fat mass ratio to CVD risk factors were similar. Gynoid fat mass *per se* and the gynoid to total fat mass ratio showed contrasting correlations with CVD risk factors. While gynoid fat mass showed a positive correlation, the gynoid to total fat mass ratio was negatively correlated to CVD risk factors.

To further investigate the associations of regional fat mass to CVD risk factors, a logistic regression was performed with the presence of impaired glucose tolerance (IGT), hypercholesterolemia, triglyceridemia or hypertension as dependent variables. After adjusting for age, follow-up time, physical activity, and smoking, the amount of abdominal fat mass was the strongest predictor for most CVD risk factors in men. In women, similar associations were found for abdominal fat mass and the abdominal to gynoid fat mass ratio for the CVD risk factors.

The gynoid to total fat mass ratio displayed significant negative associations for the risk of hypertriglyceridemia and hypertension for both men and women.
Conclusions

In both men and women, abdominal fat mass was strongly associated with the measured CVD risk factors. While gynoid fat mass *per se* was positively associated with CVD risk factors, the gynoid to total fat mass ratio was negatively associated with CVD risk factors.

These results suggest that abdominal fat mass is the strongest predictor of CVD risk factors, and that gynoid fat mass could have independent beneficial properties for the CVD risk factor profile.
Study II

Abdominal and gynoid adipose distribution and incident myocardial infarction in women and men

Aim

To explore the associations between the risk of MI and regional fat mass, with a focus on the contributions of abdominal fat mass and gynoid fat mass. A secondary objective was to determine if any association could be explained by the presence of CVD risk factors.

Methods

Total and regional fat mass was measured using DEXA in 2336 women and 922 men with a mean age of 56.2 years and 51.9 years, respectively. Information on MI incidences was from the MONICA database (described above). Altogether 104 first ever MIs were identified in the study population. Information regarding CVD risk factors was collected through the VIP database (described above) and the MONICA database (described above) and was available for 1523 subjects.

Results

In women, after adjusting for age and smoking, the abdominal to gynoid fat mass ratio was the strongest predictor of MI risk (hazard ratio [HR]= 2.44, 95% confidence interval [CI]: 1.79-3.32). In contrast, the gynoid to total fat mass ratio was negatively associated with MI risk (HR=0.57, 95% CI: 0.43-0.77). In men, no estimate of abdominal fat mass was associated with MI risk, but gynoid fat mass was negatively associated with MI risk (HR=0.69 95% CI: 0.48-0.98).

In women, after adjusting for the presence of confounding variables such as smoking, hypertension, hypertriglyceridemia or diabetes, the abdominal to gynoid fat mass ratio was still associated with an increased MI risk (p<0.05). In men, after adjustment for smoking, hypertension, hypertriglyceridemia, and diabetes, total fat mass and gynoid fat mass were both associated with a decreased MI risk (p<0.05).

Conclusion

In women the proportions of abdominal fat mass and gynoid fat mass displayed contrasting associations with MI risk. In men no association was
found for the different measures of abdominal fat mass and MI risk. However, gynoid fat mass was negatively associated with MI risk, seemingly independently of smoking, hypertension, hypertriglyceridemia or diabetes.
Study III

Low bone mineral density is associated with increased risk for myocardial infarction in men and women

Aim

To explore the association between BMD and MI risk. A secondary objective was to investigate whether shared risk factors for osteoporosis and CVD could explain any association.

Methods

Femoral neck and total hip BMD were measured using DEXA in 5490 women and 1382 men with a mean age of 53.1 and 58.0 years, respectively. Information on MI incidence was collected from the MONICA database. Altogether, 196 first-ever MIs were identified in the study population. Information on CVD risk factors was collected through the VIP database and the MONICA database, and was available for 2730 women and 574 men.

Results

After adjustment for age and BMI, lower BMD of the femoral neck and total hip was associated with increased MI risk for both women (HR= 1.33, 95% CI: 1.08-1.66 per SD decrease in femoral neck BMD) and men (HR= 1.74, 95% CI: 1.34-2.28 per SD decrease in total hip BMD).

In both women and men, lower BMD of the femoral neck was associated with hypertension. In women, BMD of the femoral neck was associated with diabetes (p<0.05), BMD of the total hip was associated with hypertriglyceridemia and BMD of the femoral neck and total hip was associated with smoking.

Adjusting for smoking, hypertension, diabetes, and hypertriglyceridemia in survival analysis did not materially change the results. After adjustment, the associations between BMD and MI risk were slightly attenuated in men (HR: 1.42-1.88 in the age and BMI-adjusted model versus 1.33-1.77 in the fully adjusted model) and similar attenuations were seen in women (HR: 1.06-1.25 versus 1.05-1.22).
Conclusion

Lower BMD was associated with an increase in MI risk for both men and women. Women had consistently lower HRs than men in all models. Adjusting for smoking, hypertension, diabetes, and hypertriglyceridemia only slightly attenuated these associations.
Study IV

*High impact loading of the skeleton is associated with decreased fasting glucose in young men*

**Aim**

To explore whether an intervention of high impact loading on the skeleton without cardiorespiratory demand would lead to changes in glucose and lipid metabolism. A secondary objective was to determine if changes in OC could explain any associations.

**Methods**

We investigated 50 men allocated to a control group or an intervention group. The intervention was an exercise protocol of six different jumps in sets of five, that subjected the lower extremities to high impact loading. In week 1 three training days were to be completed. For week 2 and 3 four training days were to be completed. For each subsequent week, an increase in the number of training days was made until every day was a training day.

The baseline characteristics of the control group were a mean age of 26.5 years and a BMI of 23.7. The intervention group had a mean age of 24.8 years and a BMI of 24.5. Blood samples were taken for OC, adrenalin, noradrenalin, glucose, HbA1c, insulin, and cholesterol. Microdialysis sampling was used to measure glycerol in the abdominal SAT as a marker of fasting lipolysis. Total physical activity was measured using an accelerometer.

**Results**

OC correlated to fat distribution by bivariate analysis, with a borderline significant association with abdominal fat mass (p=0.05) and significant correlation to the abdominal/gynoid fat mass ratio (p=0.03). Since OC also displayed a strong association with age (r=-0.59, p<0.01) a partial correlation was tested adjusting for age. In this model, OC showed no significant correlation with any of the anthropometric variables and was not correlated with any of the investigated metabolic parameters (glucose, HbA1c, insulin or cholesterol). OC in unadjusted models tended to correlate to high impact physical activity (p=0.05), but when adjusting for age, this correlation disappeared (p=0.20).

Within the intervention group, total cholesterol decreased (p=0.03), glycerol increased two-fold (p<0.01) while glucose and HbA1c tended to decrease (p=0.07). Between-group differences were tested thoroughly using several linear regression analysis models using the delta value as the dependent variable. In unadjusted analysis, the intervention group had significantly decreased glucose compared to the control group (p=0.02), with a tendency toward a difference for HbA1c (p=0.08) and noradrenalin (p=0.05). OC significantly increased in the control group (p=0.005) compared to the intervention group. When baseline values of the dependent variable were included in the model, the intervention showed borderline significant changes in glucose (p=0.07), and significant changes in adrenalin and noradrenalin (p=0.003 and 0.02, respectively). Additionally adjusting for age and weight did not change these associations. Adjusting for baseline values of the dependent variable and changes in accelerometer-measured physical activity, showed an association between the intervention and a decrease in glucose (p=0.046) and adrenalin (p=0.03), and tended to be associated with a decrease in noradrenalin (p=0.06). Changes in OC were significantly different between the intervention and control group in all models. Using the follow-up trait instead of the delta value as the dependent variable did not change the results.

**Conclusion**

The results suggested that high impact loading on the skeleton influences glucose metabolism, perhaps involving mechanisms other than only OC.
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Construction

Chapters

Regional obesity and cardiovascular disease

Osteoporosis and cardiovascular disease

The skeleton in energy metabolism

Adipose tissue, the skeleton and cardiovascular disease

Future research

RESULTS
Discussion

Chapters

Regional obesity and cardiovascular disease

Osteoporosis and cardiovascular disease

The skeleton in energy metabolism

Adipose tissue, the skeleton and cardiovascular disease

Future research
Discussion

Regional obesity and cardiovascular disease

Obesity is rapidly increasing in prevalence. Today more than half of all men and a third of all women in Sweden are considered overweight or obese [6]. Central obesity is recognized as the driving force behind the deleterious effects of obesity, as illustrated by definitions of metabolic syndrome. Central obesity is strongly associated with risk factor clustering, and is in the different criteria either a requisite for metabolic syndrome diagnosis, or a factor strongly indicative of metabolic syndrome [17,70,191]. The most common methods for measuring central fat distribution are anthropometric, such as WC or WHR. WHR not only measures abdominal obesity, but provides the context of hip circumference. An increased hip circumference is hypothesized to be protective against CVD [192,193]. However, since hip circumference is also affected by skeletal structure and lean mass, the effects of fat mass alone are difficult to discern.

In study I and II, we investigated the contributions of regional fat mass to traditional CVD risk factors, and to the risk of MI. We measured regional fat mass using DEXA. In study I, we showed that abdominal fat mass had the strongest association to CVD risk factors in men, and while in women the associations of the abdominal to gynoid fat mass ratio and abdominal fat mass per se was similar. Gynoid fat mass was positively associated with CVD risk factors. This was not surprising since gynoid fat mass was strongly correlated to total fat mass. When gynoid fat mass was expressed as the ratio between gynoid fat mass to total fat mass, associations to risk factors were inverted, with a high proportion of gynoid fat mass associated with lower risk.

In study II we investigated the association of the same fat mass depots to MI risk. The results showed that, in women, the proportion of abdominal fat mass expressed as the ratio of abdominal to gynoid fat mass was the strongest predictor of MI. The ratio of gynoid to total fat mass, on the other hand, was associated with a decreased MI risk. Surprisingly, in men abdominal fat mass was not significantly associated to the MI risk. Instead, gynoid fat mass per se was associated with a decreased MI risk. These observations remained after adjusting for hypertension, hypertriglyceridemia, smoking, impaired glucose tolerance, or diabetes.

When measuring fat mass to predict CVD risk factors, such as hypertension, hyperlipidemia or diabetes, WC is recommended over WHR because 1) WC is more strongly correlated to VAT than WHR [194]; 2) some studies found a
stronger association between WC and CVD risk factors than WHR [82-84]; and 3) the use of a ratio may compound measurement errors [195]. Additionally, changes in WC correlate better than WHR to changes in VAT. While WC is associated with the absolute amount of VAT, the WHR signifies the proportion of VAT. This reasoning is shown in Figure 11.

Figure 11. Accumulation of visceral and peripheral fat mass over several decades. Although there has been an absolute increase in VAT, reflected as an increased WC, WHR is unchanged.

In contrast, large studies investigating the association between body composition and incident CVD have found that WHR tends to be better than WC at predicting incident CVD risk [85,86,196-198].

The results of study I and II are partly in line with these results. In study I, abdominal fat mass showed the highest association to risk factor levels in men, while in women no measure tended to be clearly better. In study II, the
abdominal to gynoid fat mass ratio was the strongest predictor of MI risk in women, while in men no measure of fat mass was associated with increased risk. In women, the results of study I and II showed that the proportion of gynoid fat mass was inversely related to CVD risk factors, and was a negative predictor of MI risk. A higher proportion of gynoid fat is associated with decreased risk factor levels and thus hypothetically lower MI risk. However, our results from study II suggest that gynoid fat mass influence the risk of MI, and this is only partly mediated by association to traditional risk factors. Adjusting for hypertension, diabetes, hypertriglyceridemia and smoking did not substantially change the results.

Data on the association of MI to gynoid fat mass is scarce. Previous studies investigating the relationship of MI risk and gynoid fat mass almost exclusively used anthropometric measures such as WHR or hip circumference. Inherently, these methods are in ways measuring not only fat mass but also skeletal structure and muscle mass. The advantage of using DEXA instead of anthropometric methods is that DEXA accurately measures both total fat mass and regional fat mass, circumventing the confounding effects of skeletal structure and fat free mass.

Several hypotheses explain why gynoid fat mass is beneficial. First, higher leg fat mass is inversely associated with CVD risk factors, adjusting for trunk fat [199,200]. In part, this could be because gynoid adipocytes are different than visceral adipocytes in FFA metabolism [201]. Consequently, gynoid adipocytes might be able to act as a metabolic sink, buffering excess postprandial triglycerides, preventing ectopic lipid deposition and protecting against lipotoxicity in other organs [36]. Another hypothesis is that gynoid adipocytes have a different adipokine profile than visceral adipocytes. For example, higher leg fat mass is associated with adiponectin, an anti-inflammatory and insulin-sensitizing adipokine [34], after adjustment for trunk fat [202,203]. Expression of IL-6 and TNF-α also differs. These adipokines are thought to be involved in insulin resistance, since they are secreted in smaller quantities from SAT than from VAT [29-32].
Osteoporosis and cardiovascular disease

Osteoporosis is an insidious disease that progresses silently for decades until it manifests as a fragility fracture. The lifetime risk of an osteoporotic fracture is 50% for women and 25% for men [90].

The prevalence of osteoporosis and CVD overlap, and a shared etiology has been proposed. The risk of fractures after a CVD event has been studied, and seems to show that prevalent CVD increases the risk of a fracture [204,205,140]. Whether the association between CVD and fractures is because of decreased BMD is not fully understood. Falls are the most important risk factor for fractures, and risk of falling might be influenced by prevalent CVD. As stated above, several CVD risk factors, including hypertension, hyperlipidemia, smoking, and diabetes, not only increase the risk of incident CVD, but can also negatively affect BMD.

In study III we investigated whether low BMD increased MI risk, and whether traditional CVD risk factors explained any association. We investigated BMD in the femoral neck and total hip in a large group of men and women. We followed this cohort for a mean of 5.7 years. The most important finding was that an approximately 15% decrease in BMD of the femoral neck in women, and BMD of the total hip in men, increased the risk of MI by 33% in women and 76% in men. BMD was also associated with hypertension in both men and women, and with diabetes and smoking in women. In men, BMD tended to be associated with diabetes. Adjusting for these traditional CVD risk factors only marginally decreased the relationship between MI risk and low BMD. While low BMD is associated with a more adverse risk profile, the increase in MI risk because of low BMD may largely be mediated through other mechanisms.

Our results support the hypothesis that low BMD is a risk factor for CVD, and MI in particular. Several previous studies have investigated the association between BMD and CVD but their results were inconclusive. Farhat et al. [206] used a biracial population of black and white men and women and found that decreased BMD in black women and white men was associated with increased risk of CVD, while no associations were found for black men and white women. Samelson et al. [207] studied a cohort from the Framingham Study and found that being in the bottom quartile for BMD compared to the top quartile was associated with a 37% increased risk of any form of coronary heart disease in women. No associations were found in men. Szulc et al. [182] studied a cohort of men older than 50 years and found that the lowest quartiles of BMD of the spine, total body and radius were
associated with a doubled risk of a CVD event compared to the other three quartiles.

The disparate results of previous studies may be explained by the use of different measuring sites, and different composite CVD outcomes. The collective result of these studies suggest that BMD predicts risk of CVD, but the impact of CVD risk factors on the association of low BMD and risk of CVD is not fully elucidated.

**The skeleton in energy metabolism**

Several lines of evidence point not only to a link between osteoporosis and CVD, but between the skeleton and adipose tissue [129]. Total fat mass and VAT can have opposite associations with BMD [131,138]. This suggests a more intricate association than simply increased mechanical load on the skeleton. The answer might lie in adipokines secreted by the adipocytes. Leptin, a hormone responsible for regulating appetite, can influence bone formation by acting on the hypothalamus [128]. Adipose tissue influences the skeleton, and the skeleton may have have similar capabilities. One of the molecules released into systemic circulation by bone is OC. OC is exclusively produced by osteoblasts, and correlates with increases in bone gain. It is presumed to be a good marker of osteoblastic activity, and has been used as a bone formation marker. Recently, evidence has shown that it could also be an important player in glucose homeostasis by influencing the adipose tissue [129]. This suggests that bone formation and regulation of energy homeostasis might be coupled, and mediated through OC signalling.

In study IV we tested the hypothesis that high impact loading on the skeleton would lead to changes in glucose and lipid metabolism. Another objective was to investigate whether these putative changes could be explained by changes in OC levels.

Few studies have used high impact loading on the skeleton as an intervention, and also investigated metabolic variables. Kemmler et al. showed in menopausal women that two years of intense high impact exercise led to increased BMD, and decreased total cholesterol and triglycerides [208]. Similar results were found by Vainionpää et al., who found that in middle-aged, pre-menopausal women high impact exercise led to decreases in total cholesterol and LDL cholesterol [209]. Their interventions contained a large aerobic component, confounding the putative role of the high impact exercises in metabolic regulation. In our study we minimized the aerobic component to see whether high impact loading *per se* was associated with changes in glucose and lipid metabolism.
The intervention led to decreased glucose levels and decreased adrenalin, seemingly independent of aerobic exercise. All metabolic parameters favored the intervention group. Group-specific analyses showed that the intervention group had increased fasting lipolysis and decreased total cholesterol, although these changes were not significantly different from the control group. These results seem to support the hypothesis that the skeleton can influence glucose and lipid metabolism, but the proposed link, OC, was unchanged in the intervention group. Instead, adrenalin and noradrenalin significantly decreased in the intervention group, suggesting that the sympathetic nervous system (SNS) could be involved in skeletal metabolic signaling. Although no changes in OC were seen in the intervention group differences were still observed between the control and intervention groups. Speculatively, the intervention might have blunted a normal cyclical increase in OC or increased OC removal from the blood.

We did not find any significant associations between OC and body composition, baseline glucose, or lipid parameters. This is in contrast to previous studies that demonstrated an association between OC, body composition, and glucose levels [174,179,180]. The study populations in these studies were fundamentally different from our study population. Earlier studies used middle-aged men and women [179], the elderly [174] or individuals with overweight or obesity [180]. Bone metabolism is age-dependent, with altered levels of bone turnover markers in both men and women [210,211]. Additionally, acute aerobic and resistance exercise studies including only young, healthy individuals often fail to produce a change in OC [212-214]. Insulin resistance is associated with decreased levels of OC [215], and in studies including obese or diabetic individuals OC rises significantly after exercise [216,217]. This might mean that in an individual with tightly regulated metabolic parameters and normal bone turnover, the metabolic aspects of the skeleton plateaus and does not respond unless vigorously stressed. In contrast, an intervention in individuals with poor glycemic control or other adverse metabolic attributes could have a more profound effect than in healthy individuals. This could be because OC levels are depressed, suggesting impaired skeletal metabolic signaling.

**Adipose tissue, the skeleton and cardiovascular disease**

Obesity is a heterogenous disease. Although increased body weight is consistently associated with increased CVD mortality and incident CVD, a subset of obese individuals is metabolically healthy while a subset of normal weight individuals displays metabolic abnormalities. Studies on obese individuals show that about 1 in 5 does not have insulin resistance, dyslipidemia, or hypertension [23], a discrepancy that has been suggested to
be caused by a lower proportion of VAT relative to total fat mass. Similar explanations may explain the associations between obesity and BMD. While total fat mass is positively correlated with BMD, VAT is associated with increased bone loss, and inversely correlated with BMD [138,139]. VAT is thus a probable cause of both metabolic disturbances and lower BMD, perhaps partly explaining the association between low BMD and CVD.

As shown in study III, low BMD is associated with an increased risk of MI. The common pathophysiological link between CVD and lower BMD is suggested to be atherosclerosis [147,148]. The progression of atherosclerosis is influenced by several factors, e.g. hypertension, diabetes, smoking and dyslipidemia, which are also associated with visceral obesity as described above. Thus, excess VAT could influence BMD through metabolic disturbances. Lower BMD might, to some degree, be a marker of the metabolic burden caused by increased VAT. However, in this hypothesis, the skeleton is only a passive bystander, responding to changes in adipose tissue metabolism through one-way communication. This may not be the case, as illustrated by the proposed actions of OC [129]. Additionally, the results of study IV suggest that stressing the skeleton is associated with changes in glucose metabolism irrespective of aerobic exercise.

Physical activity could be a key factor in the association of VAT, lower BMD, and CVD. Physical activity is associated with VAT [218-220] and BMD in a dose-dependent fashion, and cessation of physical activity causes a decrease in BMD [221,159]. Physical activity also improves hypertension, insulin sensitivity and lipid profile [222,223], partly through a decrease in VAT and partly through improved metabolic and physical fitness [224-226]. The current literature is treating the beneficial effects of physical activity for metabolic health and for increased BMD as separate functions. The cardiorespiratory component is considered to be responsible for increased metabolic health, and the mechanical loading component of physical exercise is considered to be responsible for skeletal adaptment. However, parts of the beneficial metabolic effects of physical exercise may be mediated through efferent signaling pathways of the skeleton, as suggested by study IV (Figure 12). Bone turnover markers are used to assess the effect of physical activity on bone metabolism. Speculatively, skeletal metabolic signaling is coupled to bone turnover, as illustrated by OC and its role as a bone formation marker, and its newly proposed role in glucose and lipid metabolism [129]. Physical inactivity could therefore be associated with impaired skeletal metabolic signaling because of a decrease in mechanical loading, and subsequent alterations in bone turnover. Additionally, these changes might be more pronounced in older men and women as bone turnover is altered by age [210,211].
Speculatively, while VAT could increase bone loss, impaired skeletal metabolic signaling, perhaps presenting as increased bone loss, might induce VAT accumulation through decreased metabolic health.

**Figure 12.** Schematic model of how physical activity might affect the link between adipose tissue, the skeleton and CVD. Different activities confer different strains on the skeleton. Speculatively high-impact loading on the skeleton, independently of cardiorespiratory effects, might upregulate efferent skeletal metabolic signaling, increasing metabolic health and decreasing the risk of T2DM and CVD.
Future research

Low BMD increases the risk of CVD, an association that perhaps may be explained by the effects of atherosclerosis on both conditions. To investigate whether the association between BMD and CVD is dependent on atherosclerosis in longitudinal studies is of interest.

Another interesting hypothesis is that the skeleton may be involved in energy metabolism. If skeletal metabolic signaling affects metabolic health, these signaling pathways could perhaps partly explain the association between low BMD and CVD. Whether these pathways can be influenced by impact loading on the skeleton to improve metabolic health is inconclusive. Physical activity can be stratified according to skeletal strain, and investigating whether higher skeletal strain gives extra metabolic benefits, can expand our knowledge of the skeleton in energy metabolism. Moreover, if skeletal metabolic response is dependent on age and health status is unknown, and future studies should take this into consideration. Further research into the efferent signaling pathways of the skeleton is warranted.

The links between adipose tissue, the skeleton and CVD are of great interest, since identifying common mechanisms might improve risk stratification, and identify individuals at high risk for osteoporosis and CVD. Additionally, preventive and therapeutical interventions targeted at these common mechanisms might reduce the morbidity and mortality associated with these conditions.
Summary and conclusions

Different depots of regional fat mass seem to be associated with different risk profiles for CVD in both men and women. Abdominal fat mass is associated with higher CVD risk factor levels, while the proportion of gynoid fat mass is associated with lower CVD risk factor levels. In women, the same associations could also be found for the risk of MI, while in men, gynoid fat mass per se was associated with a decreased risk of MI.

A 15% decrease in BMD of the femoral neck for women, and 15% decrease of BMD of the total hip for men, increased the relative risk of MI with 33% in women and 74% in men. Lower BMD was associated with hypertension in both men and women, and lower BMD was in women also associated with diabetes and smoking. However, the association between BMD and MI was seemingly independent of these risk factors as adjusting for these conditions only slightly attenuated the association in both men and women. The MI risk associated with lower BMD was consistently higher in men than in women in all tested models.

Finally, a high-impact loading intervention consisting of a series of jumps was, after adjusting for physical activity, associated with a decrease in glucose levels compared to a control group. Favorable trends were found in other metabolic parameters as total cholesterol decreased and fasting lipolysis increased.

The results of this thesis corroborate the findings that abdominal obesity is associated with increases in CVD risk, and suggest that the gynoid fat mass depot is associated with a decreased CVD risk, partly mediated through associations with traditional CVD risk factors. Lower BMD is associated with an increased risk of MI, seemingly independently of traditional CVD risk factors. The finding that a high-impact loading intervention on the skeleton is associated with decreased glucose levels suggests that the role of the skeleton in energy metabolism can be stimulated through mechanical loading.
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85


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