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EFFECTS OF EXERCISE TRAINING ON BONE MARROW METABOLISM

Ronja Ojala

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“Believe you can and you’re halfway there.”
Theodore Roosevelt

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ABSTRACT

Bone marrow (BM) is the porous tissue found inside bones that consists of adipose tissue, hematopoietic cells, and other stem cells. BM adipose tissue has been shown to have high basal glucose uptake in humans, exceeding that of white adipose tissue. Obesity has been shown to suppress bone metabolism and increase the risk of insulin resistance and type 2 diabetes. Exercise training is known to improve bone mineral density and whole-body insulin sensitivity reducing the risk of type 2 diabetes. However, the effects of exercise training on BM metabolism remain unclear.

The aim of this thesis was to study the effects of short-term and long-term exercise training on BM metabolism using positron emission tomography (PET). We studied the effects of two weeks of exercise training on BM glucose and free fatty acid metabolism in healthy and insulin resistant participants. We also studied the effects of six months of regular exercise training on BM glucose metabolism in monozygotic twin pairs that were discordant for body weight. Lastly, to gain insight into the mechanisms in more detail, we studied the effects of high-fat diet and exercise training on BM glucose metabolism in rats.

We found that BM metabolism differs regarding anatomical location in both humans and rats, being higher in vertebrae and sternum and lower in long bones of the extremities. Already two weeks of exercise training improved BM glucose and free fatty acid metabolism regardless of glycemic status. Regular exercise training also improved femoral BM glucose metabolism. Interestingly lumbar vertebral BM glucose uptake was higher in participants with higher body weight and exercise training decreased lumbar vertebral BM metabolism even without reduction in weight. In rats, BM glucose metabolism was decreased by age but not significantly affected by the interventions.

In conclusion, this thesis suggests that BM is metabolically active and responds to exercise training. The response depends on its biological function and anatomical location.

KEYWORDS: Bone marrow, exercise training, insulin resistance, metabolism, obesity, positron emission tomography

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Sisätautioppi
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TIIVISTELMÄ

Luuydin on luiden sisällä olevaa huokoista kudosta, joka koostuu rasvasoluista, hematopoieettisista soluista, ja muista kantasoluista. Ihmisen luuytimen rasvasolut kuluttavat merkittäviä määriä sokeria, jopa enemmän kuin valkoisen rasvakudoksen solut. Ylipaino hidastaa luun aineenvaihduntaa ja lisää insuliiniresistenssin ja tyypin 2 diabeteksen kehittymisen riskiä. Liikuntaharjoittelun puolestaan tiedetään parantavan luun mineraalitiheyttä ja pienentävän tyypin 2 diabeteksen riskiä. Liikuntaharjoittelun vaikutuksista luuytimen aineenvaihduntaan tiedetään kuitenkin vasta vähän.

Tässä väitöskirjassa tutkittiin lyhyt- ja pitkäkestoisen liikuntaharjoittelun vaikutuksia luuytimen aineenvaihduntaan positroniemissiotomografialla (PET). Tutkimme kahden viikon liikuntaharjoittelun vaikutuksia luuytimen sokeri- ja rasvahappoaineenvaihduntaan keski-ikäisillä ja vain vähän liikuntaa harrastaneilla terveillä koehenkilöillä sekä koehenkilöillä, joiden paastosokeri oli koholla tai sokerinsieto heikentynyt. Tutkimme myös kuuden kuukauden säännöllisen liikuntaharjoittelun vaikutuksia luuytimen sokeriaineenvaihduntaan identtisillä kaksosilla, joilla oli lähtötilanteessa painoero. Lisäksi tutkimme korkearasvaisen ruokavalion ja liikunnan vaikutuksia rottien luuytimen sokeriaineenvaihduntaan.

Väitöskirjan tulokset osoittavat, että luuytimen aineenvaihdunta vaihtelee luuston eri osissa sekä ihmisillä että rotilla, ollen korkeampi selkärangan nikamissa sekä rintalastassa ja matalampi raajojen pitkissä luissa. Jo kahden viikon liikuntaharjoittelu paransi luuytimen sokeri- ja rasvahappoaineenvaihduntaa riippumatta lähtötilanteen sokeritasapainosta. Myös säännöllinen liikuntaharjoittelu paransi reisiluun luuytimen sokeriaineenvaihduntaa. Lannenikamien luuytimen sokerinkulutus oli korkeampaa koehenkilöillä, jotka olivat lähtötilanteessa painavampia, mutta sokerin kulutus väheni liikunnan vaikutuksesta ilman muutoksia tutkittavien painossa. Rotilla luuytimen aineenvaihdunta hidastui iän myötä, mutta interventioilla ei ollut vaikutusta luuytimen sokerin kulutukseen.

Yhteenvetona, luuydin on aineenvaihdunnallisesti aktiivista kudosta, joka reagoi fyysiseen aktiivisuuteen. Vaste riippuu luuytimen anatomisesta sijainnista ja biologisesta tehtävästä.

AVAINSANAT: Aineenvaihdunta, insuliiniresistenssi, liikuntaharjoittelu, luuydin, positroniemissiotomografia, ylipaino

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Abbreviations

[^{18}F]FDG	2-[^{18}F]fluoro-2-deoxy-D-glucose
[^{18}F]FTHA	14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid
BM	Bone marrow
BMAd	Bone marrow adipocyte
BMAT	Bone marrow adipose tissue
BMD	Bone mineral density
BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
cMAT	Constitutive marrow adipose tissue
CT	Computed tomography
CTX	C-terminal crosslinked telopeptides of type I collagen
DNA	Deoxyribonucleic acid
DZ	Dizygotic
ECG	Electrocardiogram
EDTA	Ethylenediamine tetra-acetate
FFA	Free fatty acid
FFAU	Free fatty acid uptake
FUR	Fractional uptake rate
GLUT	Glucose transporter
GU	Glucose uptake
HbA1c	Glycosylated hemoglobin
HDL	High-density lipoprotein
HFD	High-fat diet
HIIT	High-intensity interval training
HSC	Hematopoietic stem cell
hs-CRP	High-sensitivity C-reactive protein
HU	Hounsfield unit
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IR	Insulin resistant

LDL	Low-density lipoprotein
MICT	Moderate-intensity continuous training
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MSC	Mesenchymal stem cell
MZ	Monozygotic
OGTT	Oral glucose tolerance test
PET	Positron emission tomography
PINP	N-terminal propeptides of type 1 collagen
qCT	Quantitative computed tomography
RANKL	Receptor activator of nuclear factor kappa-B ligand
ROI	Region of interest
rMAT	Regulated marrow adipose tissue
SIT	Sprint-interval training
TAC	Time-activity curve
T2DM	Type 2 diabetes mellitus
TotalOC	Osteocalcin
ucOC	Uncarboxylated osteocalcin
VO_{2peak}	Aerobic capacity

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Ojala R, Motiani KK, Ivaska KK, Arponen M, Eskelinen JJ, Virtanen KA, Löyttyniemi E, Heiskanen MA, U-Din M, Nuutila P, Kalliokoski KK, Hannukainen JC. Bone Marrow Metabolism Is Impaired in Insulin Resistance and Improves After Exercise Training. *Journal of Clinical Endocrinology and Metabolism*. 2020, 105: e4290–303.

II Ojala R, Hentilä J, Lietzén MS, Arponen M, Heiskanen MA, Honkala SM, Virtanen H, Koskensalo K, Lautamäki R, Löyttyniemi E, Parkkola R, Heinonen OJ, Malm T, Lahti L, Rinne J, Eskola O, Rajander J, Pietiläinen KH, Kaprio J, Ivaska KK, Hannukainen JC. Bone marrow metabolism is affected by body weight and response to exercise training varies according to anatomical location. *Diabetes, Obesity and Metabolism*. 2024, 1:251-261.

III Ojala R*, Widjaja N*, Hentilä J, Jalo A, Helin J, Nissinen TA, Jalava N, Eskola O, Rajander J, Löyttyniemi E, Ivaska KK**, Hannukainen JC**. Evaluation of bone marrow glucose uptake and adiposity in male rats after diet and exercise interventions. *Manuscript*.

*These authors contributed equally to the manuscript.

1 Introduction

After its discovery, bone marrow adipose tissue (BMAT) was thought to be a non-active tissue filling the marrow cavities of bones. Since then, it has been recognized to be a metabolically active tissue that takes part in many physiological processes (Cawthorn *et al.*, 2014; Suchacki, Cawthorn and Rosen, 2016). Many different tissues fill the bone marrow (BM) cavity, including BMAT, hematopoietic tissue, and other stem cells.

Obesity and physical inactivity are major global public health concerns as they are becoming increasingly prevalent. Both of these can contribute to lifestyle-induced diseases such as type 2 diabetes mellitus (T2DM). Regulation of glucose homeostasis and the development of insulin resistance includes many different organs and tissues and is affected by genetics (Wilcox, 2005; Naukkarinen *et al.*, 2012; Willemsen *et al.*, 2015). BMAT comprises 8-10% of total adipose tissue mass and it has been shown to have high basal glucose uptake (GU) (Cawthorn *et al.*, 2014; Tencerova and Kassem, 2016; Suchacki *et al.*, 2020). However, the role of BM in developing insulin resistance and T2DM is not yet documented and needs to be studied further.

Exercise is a powerful tool to maintain glucose homeostasis and improve insulin sensitivity. Exercise training is easily accessible to all people regardless of financial status or place of residence. It is also a drug-free treatment option for the growing epidemic of lifestyle-induced diseases. However, the response to exercise is also affected by genetics and response to the same exercise training protocol can vary between individuals (Bouchard *et al.*, 2015). How exercise training affects BM metabolism in humans with insulin resistance and/or obesity is another question that needs to be studied further.

BM, especially BMAT, has been extensively studied to assess its unique characteristics, but human studies are scarce due to its location encased within the bone. Most previous studies on BMAT have been done in animals or *ex vivo* using samples collected during joint-replacement surgeries or *post mortem*. Positron emission tomography (PET), an imaging modality based on radioactivity, offers a novel and innovative method to study BM metabolism *in vivo* at the tissue level. PET imaging utilizing the radiotracers used in this study only requires cannulation of the

individual being studied and is less invasive as the surgeries that were previously needed for sample gathering.

Thus, the main aim of this thesis was to study the effects of exercise training on BM glucose and free fatty acid metabolism using PET imaging. It was hypothesized that both insulin resistance and higher body weight would suppress BM metabolism but that exercise training could counteract this effect.

2 Review of the Literature

2.1 Bone marrow and surrounding bone

2.1.1 Structure and composition

Bones support and protect other organs in the body as well as act as reserves of minerals and take part in calcium metabolism. They are initially formed during development through intramembranous or endochondral ossification process. Bones consist of living cells embedded in a mineralized organic matrix made primarily of type I collagen and hydroxyapatite. The hard outer layer of bones is cortical bone (Figure 1). Trabecular bone, also called spongy bone, can be found at the ends of long bones as well as vertebral bodies. BM is the porous tissue found inside bones. BM consists of adipose tissue (BMAT), hematopoietic tissue (blood cells and their precursors), and other stem cells in different combinations, sometimes interspersed with trabecular bone depending on anatomical location.

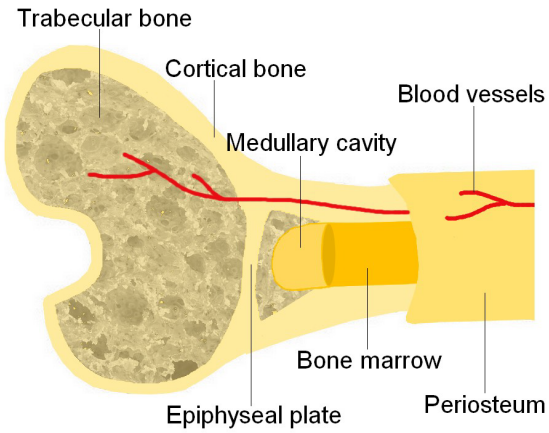

Figure 1 Schematic anatomy of a long bone and its bone marrow cavity, e.g. femur, tibia. Illustration by R Ojala 2024.

2.1.2 Bone marrow

BM has a different composition and different biological functions depending on its location. The ratio of the components of BM differ and change during growth and development. When a child is born, almost all their bone cavities are filled with red, hematopoietic BM tissue. During aging, this hematopoietic BM is slowly replaced by yellow, fatty BM tissue consisting mostly of adipose tissue from the periphery toward the axial skeleton (Kricun, 1985). In adults, hematopoietic BM persists in the axial skeleton and in the proximal ends of long bones, such as femur and humerus (Vogler and Murphy, 1988).

Hematopoietic BM, abundant in axial skeleton, produces blood cells while the BM in long bones in appendicular skeleton may serve as a specialized fat depot and consists in adults mainly of BMAT (Figure 2). Suchacki et al showed that BMAT is molecularly and functionally distinct from brown, beige, and white adipose tissue (Suchacki *et al.*, 2020). BMAT comprises 8-10% of total adipose tissue mass (Cawthorn *et al.*, 2014; Tencerova and Kassem, 2016) and has been shown to have high basal GU in humans, exceeding that of white adipose tissue in a fasted state as well as during the euglycemic-hyperinsulinemic clamp (Ojala *et al.*, 2020; Pham *et*

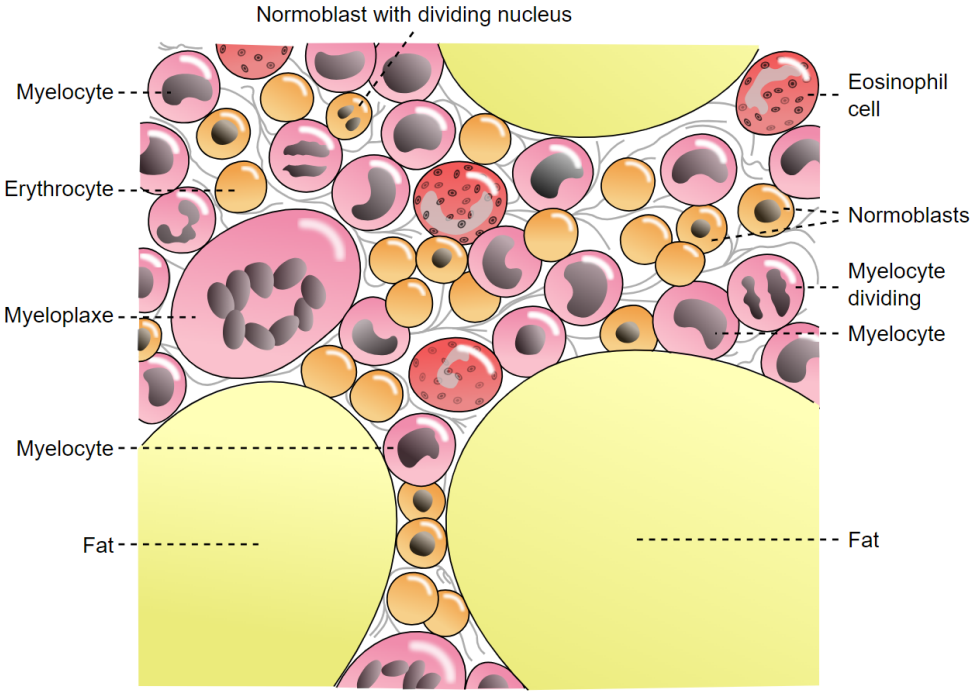

Figure 2 Simplified schematic anatomy of the bone marrow niche. Modified from Gray's Anatomy, plate 72 (1918). Vectorized by Mysid Inkscape (May 29th, 2010). Reprinted with permission from owner.

al., 2020; Suchacki *et al.*, 2020). Thus, improved BM metabolism may play a part in whole-body glucose metabolism.

BM adipocytes (BMAds) share similar morphological properties as extramedullary subcutaneous adipocytes consisting of a unilocular lipid droplet surrounded by a thin cytoplasm and with the nucleus situated at the cell periphery (Attané *et al.*, 2020). BMAds express glucose transporters (GLUT). Suchacki et al. speculated that the high basal GU of BMAT may be less insulin responsive (Suchacki *et al.*, 2020). They showed by qPCR that BMAT had increased GLUT1 and GLUT3 and decreased GLUT4 compared to white adipocytes. GLUT1 is mostly found in the central nervous system and at the surface of red blood cells. GLUT3 is present in neurons and has a high affinity for glucose. GLUT4 is insulin-regulated and found primarily in adipose tissue and striated muscle (Thorens and Mueckler, 2010).

BMAT may function in a manner similar to white adipose tissue, clearing and storing adipocytes (Lecka-Czernik, 2012). In addition to this, other roles have been proposed for BMAT in whole-body metabolism. BM adiposity has been linked to whole-body energy metabolism via adiponectin, a biomarker for insulin resistance that decreases in obesity and insulin-resistant individuals. Cawthorn et al. showed that secretion of adiponectin was higher in BMAT than white adipose tissue in humans during caloric restriction (Cawthorn *et al.*, 2014). Another cytokine released from BMAds that has potent local effects on skeletal remodeling is receptor activator of nuclear factor kappa-B ligand (RANKL), which drives osteoclastogenesis and ultimately bone resorption (Fan *et al.*, 2017). RANKL expression in BMAds is higher than in peripheral adipose depots suggesting that BMAds are unique adipocytes and reflect their site of origin (Fan *et al.*, 2017).

Scheller et al showed that BMAT can be subdivided into two distinct types: proximal, regulated BMAT (rMAT), which consists of single adipocytes interspersed with active hematopoiesis, and distal, constitutive BMAT (cMAT), which has a low number of hematopoietic cells, larger adipocyte size, develops earlier during development, and remains preserved upon systemic challenges. The lipid composition of white adipose tissue more closely mirrors that of rMAT, suggesting that lipid metabolism in white adipose tissue and rMAT adipocytes may be similar (Scheller *et al.*, 2015).

Red hematopoietic BM consists of differentiated hematopoietic stem cells (HSCs), hematopoietic progenitors and precursor cells, and non-differentiated HSCs. One of the main functions of these cells is to maintain a physiologically normal immune system by differentiating into a variety of cells, including red blood cells, different types of lymphoid and myeloid white blood cells, and osteoclasts. To maintain homeostasis of the hematopoietic system, these cells respond to pathologic environmental signals, such as infection (Singh *et al.*, 2008). Proliferation and

differentiation of HSCs into different blood cell types require a robust upregulation of energy metabolism (Hsu and Qu, 2013) which can affect BM GU measured by PET as more glucose is utilized in the BM niche. Devesa et al. found that lumbar vertebral BM activation (increased GU measured with [^{18}F]FDG-PET) was associated with increased immune system activation, increased hematopoiesis and with markers of systemic inflammation, such as high-sensitivity C-reactive protein (hs-CRP) (Devesa *et al.*, 2022).

In addition to HSCs, red BM consists of differentiated and non-differentiated mesenchymal stem cells (MSCs), which also constitute a minority of the BM cells (Pittenger *et al.*, 1999). MSCs are able to differentiate into specialized cells, such as osteoblasts and adipocytes, through complex cell processes regulated by chemical, physical and biochemical markers (Calvi and Link, 2014).

Femoral BM has been shown to be insulin sensitive in humans (Huovinen *et al.*, 2016; Pham *et al.*, 2020), but there are also studies with opposite findings (Suchacki *et al.*, 2020). Suchacki et al. compared insulin-stimulated Akt T308 phosphorylation in different tissues, including BMAT, after injection of vehicle and insulin in rats. In contrast, Pham et al. showed a significant increase in femoral BM GU between fasting state and insulin stimulation. Further, both Pham et al. and Huovinen et al. used state-of-the-art modalities including dynamic PET combined with computed tomography (CT) and euglycemic-hyperinsulinemic clamp to measure whole BM GU (Huovinen *et al.*, 2016; Pham *et al.*, 2020). More studies are needed to further investigate the effects of insulin on BM, and more specifically BMAT.

2.1.3 Bone modeling and remodeling

Bone is not an inert tissue but dynamic and constantly adapting its structure. Bone remodeling is a process where bone is renewed through sequential bone resorption and formation at the same location (Langdahl, Ferrari and Dempster, 2016). The main purpose of bone remodeling is to maintain bone mechanical strength by repairing bone microdamage and sustaining mineral homeostasis.

Bone remodeling persists throughout life. Approximately 10% of the skeleton is remodeled annually, most of which happens on the surface of trabecular bone. Remodeling is a process characterized by four phases: 1) the activation phase when the osteoclasts are recruited; 2) the resorption phase, when the osteoclasts resorb bone; 3) the reversal phase, where the osteoclasts undergo apoptosis and the osteoblasts are recruited; 4) the formation phase, where the osteoblasts lay down new organic bone matrix that subsequently mineralizes (Parfitt, 2002) (simplified in Figure 3). The bone formation phase lasts approximately 4-6 months. Eventually osteoblasts become trapped within the synthetized matrix and transform into osteocytes (Clarke, 2008). Osteocytes are connected to other osteocytes and

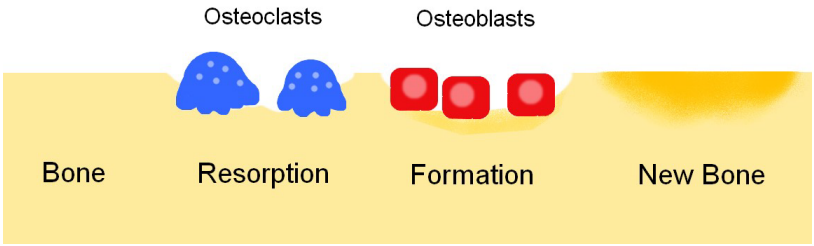

Figure 3 Simplified schematic of bone remodeling. Illustration by R Ojala 2024.

osteoblasts through an extensive tentacle-like network. Osteocytes are mechanosensory cells that can control the activity of surrounding osteoblasts and osteoclasts (Robling and Bonewald, 2020). They also have similar characteristics to both of these cell types as osteocytes can have the capacity to remove and replace their surrounding bone matrix (Robling and Bonewald, 2020).

After peak bone mass is attained, bone remodeling is balanced and bone mass is stable for 10–20 years until age-related bone loss begins. Bone loss related to aging is caused by increased resorptive activity and reduced bone formation (Dempster and Lindsay, 1993). The demand for energy caused by maintaining bone homeostasis is high, which is why the skeletal system is closely tied to whole-body energy metabolism. Fatty acids, glucose, and amino acids are the main fuel sources for bone cells recruited during bone remodeling (Rendina-Ruedy and Rosen, 2020).

Bone modeling happens when bone grows or changes shape due to a mechanical or physiological stimulus, such as physical activity (Clarke, 2008). In this process either bone formation or bone resorption occurs on a given bone surface.

It is not known whether BM metabolism affects bone turnover and homeostasis. Bone formation and resorption can be evaluated by measuring bone turnover markers from blood samples. N-terminal propeptide of type I collagen (PINP) is a bone formation marker produced by osteoblasts as the first part of bone matrix production and fibroblasts during fibroproliferative responses. However, the circulating concentrations of PINP seem to correspond to the biosynthetic events in bone, not soft tissues (Koivula, Risteli and Risteli, 2012). Bone resorption can be assessed by measuring C-terminal crosslinked telopeptide of type 1 collagen (CTX). CTX is released during bone resorption when collagen is being degraded by osteoclasts (Kuo and Chen, 2017). The International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommend one bone formation marker (PINP) and one bone resorption marker (CTX) to be used as reference markers when studying bone turnover (Vasikaran *et al.*, 2011).

Osteocalcin is an osteoblast-specific protein that is synthesized as a premolecule and secreted into circulation (Hauschka *et al.*, 1989). Osteocalcin has been used as a marker for bone remodeling (Schini *et al.*, 2023) but it is also thought to play a role in energy metabolism (Lee *et al.*, 2007). It is carboxylated within the osteoblast before being released into the bone matrix. However, both carboxylated and uncarboxylated forms of osteocalcin can be found in circulation (Poser *et al.*, 1980). It has been suggested, that only the uncarboxylated osteocalcin can act as a hormone and regulate insulin secretion (Lee *et al.*, 2007; Wei and Karsenty, 2015). Nevertheless, this suggests that bone can regulate glucose homeostasis via bone-derived osteocalcin.

2.2 Obesity, insulin resistance and type 2 diabetes mellitus

Obesity is defined by body mass index (BMI) (overweight BMI ≥ 25, obesity BMI ≥ 30), waist circumference (88 cm for women, 102 cm for men), or waist-to-hip ratio (0.85 or less for women, 0.9 or less for men) (Ness-Abramof and Apovian, 2008; Tutunchi *et al.*, 2020). Obesity and overweight are usually caused by an imbalance between energy intake and energy expenditure, although genetics, gut microbiota, chronic stress, and side effects from medications have been shown to affect weight gain (Tamashiro *et al.*, 2011; Naukkarinen *et al.*, 2012; Ali, 2013; Moran and Shanahan, 2014; Tilg *et al.*, 2020; Verhaegen and Van Gaal, 2021). Obesity and overweight are strongly associated with insulin resistance and T2DM (Haslam, 2010). In addition, higher weight can predispose the individual for other non-communicable diseases, such as cardiovascular disease and cancer. Obesity is not only associated with increased amount of adipose tissue, but also lipid accumulation in non-adipose tissues, such as liver and pancreas, known as ectopic lipid accumulation (VanHerpen and Schrauwen-Hinderling, 2008; Shulman, 2014).

Insulin is a peptide hormone secreted by β cells of islets of Langerhans in the pancreas. Insulin regulates the metabolism of carbohydrates, fats, and protein by stimulating the absorption of molecules like glucose from the blood to e.g. skeletal muscle, adipose tissue and liver (Wilcox, 2005). Insulin resistance is manifested by decreased insulin-stimulated glucose transport and metabolism as well as impaired suppression of hepatic glucose output (Reaven, 1995).

Initially during insulin resistance, β cells of the pancreas are able to compensate for the decreased insulin sensitivity by increasing insulin secretion. Once the condition progresses, the pancreas cannot cope with the increased insulin demand and impairment of glucose tolerance ensues. In the end, pancreatic failure occurs resulting in reduced insulin production. The proposed mechanisms of β cell demise include e.g. mitochondrial dysfunction, oxidative stress, glucotoxicity, and islet

inflammation resulting in severe β cell phenotypic alterations and loss of β cell mass by apoptosis (Prentki and Nolan, 2006). T2DM develops when insulin resistance is accompanied by pancreatic failure. Del Prato et al. suggested that hyperinsulinemia, *per se*, exacerbates insulin resistance (DelPrato *et al.*, 1994). Pancreatic lipid accumulation has also been associated with β cell dysfunction and reduced insulin production (VanHerpen and Schrauwen-Hinderling, 2008).

The diagnosis of T2DM is based on the understanding that when fasting plasma glucose, glycated hemoglobin (HbA1c), or glucose tolerance in the oral glucose tolerance test (OGTT) exceed certain threshold values, the state is called T2DM (American Diabetes Association, 2022) (Table 1). It should be noted that World Health Organization (WHO) and other diabetes organizations define the impaired fasting glucose (IFG) lower limit at 6.1 mmol/l.

Table 1. The diagnostic criteria for impaired fasting glucose, impaired glucose tolerance, and type 2 diabetes mellitus according to the American Diabetes Association.

	Fasting plasma glucose, mmol/l	**2h glucose in OGTT, mmol/l**	**HbA1c, mmol/l**
Impaired fasting glucose (IFG)	5.6–6.9	< 7.8	na
Impaired glucose tolerance (IGT)	< 7.0	7.8–11.0	na
Type 2 diabetes (T2DM)	≥ 7.0	≥ 11.1	≥ 48

OGTT, oral glucose tolerance test; HbA1c, glycated haemoglobin; na, not applicable.

Similar to white adipose tissue, BMAT stores significant quantities of fat and expresses insulin receptor (Lecka-Czernik, 2012). BMAT also responds to insulin-sensitizing anti-diabetic rosiglitazone, which increases cell sensitivity to insulin and increases lipid storage in adipocytes (Liu, Aronson and Lecka-Czernik, 2013). Rosiglitazone upregulates the expression of genes essential for fatty acid metabolism and insulin signaling in BMAT, but interestingly, there is no change in the expression of insulin-dependent glucose transporters, such as GLUT4. The response suggests BMAT is an insulin-sensitive tissue involved in fatty acid metabolism (Shockley *et al.*, 2009). This links BMAT with whole-body energy metabolism.

Obesity is associated with higher bone mineral density (BMD) (Qiao *et al.*, 2020) and increased BMAT (Bredella *et al.*, 2012; Lecka-Czernik *et al.*, 2015), but whether this contributes to bone fragility is unclear (Adler, Kaushansky and Rubin, 2014; Pagnotti and Styner, 2016). Obesity increases BMAT by adipose tissue hyperplasia and hypertrophy (Ambrosi *et al.*, 2017; Tencerova *et al.*, 2018). Increased BMAT is associated with states of impaired bone formation (Verma *et al.*, 2002; Yeung *et al.*,

2005). Studies have also found increased risk of bone fractures in T2DM patients, despite normal or increased BMD (Schwartz, 2016; Shanbhogue *et al.*, 2016; Napoli *et al.*, 2017). In support of this, increased BMAT has also been demonstrated in the setting of preserved or increased BMD in mice (Styner *et al.*, 2014; Doucette *et al.*, 2015; Lecka-Czernik *et al.*, 2015). Pagnotti and Styner suggest that the relationship between BMAT and bone volume is not reciprocal (Pagnotti and Styner, 2016).

In a pilot study, Huovinen et al. found that vertebral BM fat content was inversely associated with vertebral BM GU in healthy and diabetic pigs (Huovinen *et al.*, 2014). In their study, vertebral BM GU was approximately two-fold higher than muscle GU in a fasting state.

In morbidly obese participants bone remodeling was decreased (measured by e.g. CTX, PINP, and osteocalcin) when compared to participants with normal body weight (Ivaska *et al.*, 2017). Six months after bariatric surgery bone formation and resorption markers were significantly increased. The increase in bone turnover after surgery was affected by post-surgery remission of T2DM. Bone turnover markers appeared to increase more in those whose T2DM was in remission compared to those whose T2DM persisted.

2.3 Family and twin studies

Family and twin studies can be used to study whether genetics play a role in a physiological finding (Sahu and Prasuna, 2016). Monozygotic (MZ) twin pairs originate from the same zygote and thereby their DNA sequences are identical. Thus, phenotypic differences within a MZ twin pair can be attributed to acquired lifestyle factors and environmental exposures. Furthermore, MZ twins share similar exposures and environment during early life. Doing studies in MZ twin pairs makes it possible to study the other factors influencing weight gain and changes in glucose metabolism, outside of genetics. MZ twin pairs discordant for body weight at baseline are an excellent study model for acquired weight gain and obesity. The heavier co-twin acts as the altered metabolic state and the leaner co-twin as the clonal control for age, sex, genetic, and early environmental background.

According to data acquired from family and twin studies, obesity and metabolic diseases, such as T2DM, are strongly influenced by genetic factors (Naukkarinen *et al.*, 2012; Ali, 2013; Willemsen *et al.*, 2015). Genetics also have an important role in the variation of exercise capacity and response to the same exercise training protocol (Bouchard *et al.*, 2015).

In a summary review of findings from MZ twin pairs discordant for physical activity (assessed by interviews and questionnaires) Kujala et al. found that more active co-twins had higher physical fitness, reduced body fat, reduced visceral fat, reduced liver fat, increased lumen diameter of conduit arteries, increased BMD in

loaded bone areas, and an increased number of large high-density lipoprotein particles (Kujala *et al.*, 2022). In a study by Ma et al. using MZ and dizygotic (DZ) twin pairs discordant for long-term leisure time physical activity, they found that active members of MZ twin pairs had better markers for bone strength and health compared to their less active co-twins (Ma *et al.*, 2009). The trends were similar, but less consistently so in DZ pairs as in MZ pairs. Ma et al. suggest that leisure time physical activity, independent of genes, could improve bone quality and, thus, prevent osteoporosis and osteoporotic fractures (Ma *et al.*, 2009).

A 30-year follow-up study of MZ and DZ twin pairs discordant for physical activity showed that independent of familial background, physical activity protects from weight gain, especially of that in the abdominal region, consequently also protecting from diseases associated with overweight and obesity (Waller, Kaprio and Kujala, 2007). Mustelin et al. had similar results in younger MZ and DZ co-twins. They found that inherited factors significantly influence body weight and adiposity in sedentary individuals, but that in physically active individuals the effect of genes is diminished (Mustelin *et al.*, 2008).

Thus, epigenetics also play a role in the development of metabolic diseases. Duncan et al. found after an epigenome-wide association study on buccal cells from MZ twin pairs discordant for physical activity that the more physically active co-twins had lower signs of metabolic disease measured by waist circumference and BMI (Duncan *et al.*, 2022). The more active co-twins also had epigenetic markers linked to lower metabolic syndrome. They identified several DNA methylation sites associated with the physiological traits of interest as well as with over fifty genes previously found to be specific to vigorous physical activity and metabolic risk factors. The study suggests that markers of metabolic disease are also affected by environmental factors as well as just their inherited genetics (Duncan *et al.*, 2022).

To our knowledge there are no previous family or twin studies on the effects of exercise training on bone marrow metabolism.

2.4 Exercise training

2.4.1 Type 2 diabetes mellitus

Regular exercise training is a powerful tool to maintain glucose homeostasis and improve insulin sensitivity. Exercise training in T2DM patients improved management of blood glucose levels, body weight, lipids, blood pressure, cardiovascular disease, mortality, and overall quality of life (Kelley *et al.*, 2002; Wing *et al.*, 2011; Gregg *et al.*, 2012; The Look AHEAD Research Group, 2013; Espeland *et al.*, 2014; Rejeski *et al.*, 2015).

Most of the positive effects of exercise training on whole-body glucose metabolism are attributed to the exercise-induced adaptations on the skeletal muscle GU (Stanford and Goodyear, 2014). Both insulin and exercise acutely increase skeletal muscle GU by translocation of GLUT4 to the plasma membrane from an intracellular location. Regular exercise increases skeletal muscle GU by increasing GLUT4 protein expression and the number of mitochondria (Stanford and Goodyear, 2014).

These exercise-induced improvements in the skeletal muscle play an important role in the prevention and treatment of T2DM, because skeletal muscle makes up a big part of the systemic insulin resistance that starts the pathogenesis of T2DM (DeFronzo and Tripathy, 2009; Kahn, Cooper and Del Prato, 2014).

BMAds express GLUT4 (Suchacki *et al.*, 2020). This speaks to the possibility of the same pathway being utilized in improving BMAT GU than in skeletal muscle after exercise and insulin stimulation, but this needs to be studied further. Also, it is unclear how exercise training affects BM in insulin resistant/T2DM patients.

2.4.2 Bone health

The human skeleton responds to mechanical stimulus by increasing or decreasing bone modeling and remodeling (Clarke, 2008; Langdahl, Ferrari and Dempster, 2016). The beneficial effect of exercise on musculoskeletal strength is known: exercise induces bone formation, encourages growth of skeletally supportive tissues, inhibits bone resorption, and alters skeletal architecture through effects on cells involved in skeletal adaptation, such as osteocytes (Judex, Gross and Zernicke, 1997; Pagnotti and Styner, 2016).

Exercise may induce an osteogenic effect via mechanosensor activation (Clarke, 2008). Mechanical stimuli induce bone cellular activity in osteocytes and osteoblasts, which are able to communicate with each other (Robling and Bonewald, 2020). The most effective exercise load in regard to bone strength in rats has been shown to be when separating loading into short bouts followed by periods of rest (Robling *et al.*, 2002). To improve skeletal status, exercise like soccer, volleyball, tennis, and squash are most effective as these sports include the type of load most effective for skeletal strength (Karlsson and Rosengren, 2012).

Exercise is associated with higher BMD and lower fracture incidence than expected by age and sex (Karlsson and Rosengren, 2012). Exercise has been found to have a different effect on BMD regarding age, sex, underlying pathological conditions, type of exercise (e.g. low-impact and high-impact resistance training or endurance training), site of the BMD measurement and method of the BMD measurement (Karlsson and Rosengren, 2012). The strongest response to mechanical stimuli occur during growth, especially in the pre-pubertal or early peri-pubertal

period (Kannus *et al.*, 1995; Iuliano-Burns *et al.*, 2005). In elderly, physical activity seems to reduce age-related bone loss or marginally improve BMD (Heinonen *et al.*, 1996; Bérard, Bravo and Gauthier, 1997).

However, BMD alone is not a sufficient marker for bone health. How obesity influences the effect of exercise on bone has not been widely studied (Zouhal *et al.*, 2022). The effects of weight-bearing exercise on bone have been well established (Beck *et al.*, 2016; Weaver *et al.*, 2016), but in obese participants Zouhal et al. found that the results can mostly be explained by already high, obesity-induced baseline BMD levels (Zouhal *et al.*, 2022). The review by Zouhal et al. included 10 studies (889 initial records including obese men and women) where the training duration was at least eight weeks with 2-3 sessions/week of aerobic, resistance, and combined training.

2.4.3 Bone marrow

Exercise-induced bone formation correlates with suppression of BMAT in mice (Styner *et al.*, 2014). In their study, the exercise group had voluntary access to running wheels for six weeks. The mentioned association might be due to calorie expenditure from this depot or from mechanical biasing of MSC lineage away from fat and toward bone, or a combination thereof (Pagnotti and Styner, 2016).

In mice treated with the anti-diabetes drug, rosiglitazone (a PPARγ-agonist known to increase BMAT and fracture risk) for six weeks, femur BMAT volume was higher than in non-treated controls (Styner *et al.*, 2015). However, exercise intervention significantly lowered BMAT accumulation in rosiglitazone-treated mice and prevented BMAT accumulation in control mice (Styner *et al.*, 2015). Also in this study, the exercise group had voluntary access to running wheels for six weeks. Noteworthy also is, that exercise induction of trabecular bone volume is unhindered by rosiglitazone (Case *et al.*, 2013; Styner *et al.*, 2015). Thus, exercise is a powerful regulator of bone remodeling, pushing marrow stem cells from adipogenic towards osteogenic lineage in mice.

In humans, Huovinen et al. studied the effects of four months of resistance training on BM insulin sensitivity and GU (Huovinen *et al.*, 2016). They found that training increased femoral BM GU in frail offspring of obese mothers but not in frail offspring of lean mothers. Vertebral BM GU was not affected by resistance training. Femoral BM consists mostly of BMAT. They suggested that maternal obesity altered BMAT metabolism and that glucose metabolism of these two anatomical locations is regulated differently. Also, Heinonen et al. showed an acute increase in femoral BM GU with increasing exercise intensity during cycling in healthy participants (Heinonen *et al.*, 2019).

However, in addition to these two studies, very little is known about BM metabolism *in vivo* in humans and how it is affected by exercise, body weight, and glycemic status. This thesis aims to answer these questions.

3 Aims of the study

Obesity suppresses bone metabolism and increases the risk of insulin resistance and T2DM. In turn, exercise training improves whole-body insulin sensitivity and bone health. However, the metabolic activity of bone marrow under these conditions remains elusive.

The aim of this thesis was to apply dynamic PET/CT imaging to get insight into glucose and fatty acid metabolism in bone marrow in humans and rats *in vivo*, and to evaluate the effect of exercise interventions, body weight, and insulin resistance on bone marrow metabolism.

The specific aims of this thesis were:

1. To assess two different anatomical locations and investigate the short-term effects of two training methods, sprint-interval training (SIT) and moderate-intensity continuous training (MICT), on BM glucose and free fatty acid metabolism in two different anatomical locations as well as bone turnover markers at baseline and after a two-week exercise training intervention between healthy and insulin resistant men as well as insulin resistant men and women (Study I).
2. To study the long-term effects of six months of regular exercise training on BM glucose metabolism and adiposity as well as bone mineral density and bone turnover markers in MZ co-twins discordant for body weight (Study II).
3. To examine age-related changes in BM glucose metabolism and whether dietary and/or exercise intervention can reverse high-fat diet induced changes in BM glucose metabolism and adiposity in rats (Study III).

4 Materials and Methods

4.1 Human studies

4.1.1 Participants and study designs

4.1.1.1 Study I (HITPET)

This study was part of a larger study entitled "The Effects of Short-Time High-Intensity Interval Training on Tissue Glucose and Fat Metabolism in Healthy Subjects and in Patients with Type 2 Diabetes (HITPET)" (NCT01344928). The study was conducted at Turku PET Centre (University of Turku, Turku, Finland), Turku University Hospital (Turku, Finland), and the Paavo Nurmi Centre (Turku, Finland) between March 2011 and September 2015 in compliance with the Declaration of Helsinki. The study protocol was approved by the ethical committee of the Hospital District of Southwest Finland (decision 95/180/2010 §228). Before any measurements were performed the purpose and potential risks of the study were explained and written informed consent was obtained.

Middle-aged, sedentary, healthy subjects and subjects with insulin resistance (IR) were recruited for the study via newspaper advertisements, personal contacts, and traditional and electronic bulletin boards. The inclusion criteria for healthy subjects (n = 28, aged 40-55 years, all male) were:

- male sex
- age 40–55 years
- body mass index (BMI) of 18.5–30 $kg \cdot m^{-2}$
- VO_{2peak} <40 $mL \cdot kg^{-1} \cdot min^{-1}$
- normal glycemic control

Inclusion criteria for IR subjects (n = 26, aged 43-55 years, male/female = 16/10) were the same as for healthy subjects except for a BMI of 18.5–35 $kg \cdot m^{-2}$ and an impaired glucose tolerance or T2DM according to the criteria of the American Diabetes Association (American Diabetes Association, 2015), an HbA1c of less than

7.5 mmol·L^{-1}, and no insulin treatment in case of T2DM. Exclusion criteria for the study were:

- any chronic disease (other than diabetes for IR participants), a medical defect, or injury that interfered with everyday life
- high blood pressure (>140/90 mmHg in healthy participants and >160/100mmHg for IR participants)
- a history of eating disorders
- a history of asthma
- use of tobacco products
- use of anabolic steroids, additives, or any other substrates
- significant use of alcohol
- current or a history on regular and systematic exercise training
- presence of ferromagnetic objects in the body
- any other condition that in the opinion of the investigator could create a hazard to the participant's safety, endanger the study procedures, or interfere with the interpretation of the study results

Of the 26 IR subjects 17 (11 men) met the criteria for T2DM and 9 (5 men) met the criteria for prediabetes having impaired fasting glucose concentrations and/or impaired glucose tolerance as defined by American Diabetes Association guidelines (American Diabetes Association, 2015). Of the 17 subjects with T2DM, 13 were treated with at least 1 type of oral hypoglycemic medication. The median diabetes duration was 4.2 years. Four subjects (1 man) met the criteria for T2DM at screening and had no previous medication. In addition, 7 IR subjects were taking statins. In total, 7 subjects dropped out during the intervention, 1 due to exercise-induced hip pain, 1 due to training induced migraine, 1 due to claustrophobic feeling within the magnetic resonance imaging (MRI) scanner, and 4 due to personal reasons. All participants were asked not to change their habitual dietary intake during the study period.

Study design is shown in Figure 4. Initial screening consisted of a physical examination, an oral glucose tolerance test (OGTT), and a VO_{2peak} test to assess the participant's glycemic status and aerobic capacity. At least 1 week after the screening day [^{18}F]FTHA-PET study was performed to measure free fatty acid uptake (FFAU) in thoracic vertebral, lumbar vertebral, and femoral BM. The following day, [^{18}F]FDG-PET study was performed during euglycemic-hyperinsulinemic clamp to measure whole-body insulin sensitivity (M-value) and GU in BM in the same anatomical regions. Subjects were asked to avoid exhausting exercise and

caffeinated and alcoholic beverages and to stop all antidiabetic medications 48 h prior to any measurements.

After the pre-training measurements the subjects were randomized into two training groups for the 2-week exercise intervention, SIT and MICT. The final group sizes for healthy subjects were n = 14 for SIT and n = 14 for MICT, and for IR subjects, n = 13 for SIT and n = 13 for MICT.

After the training intervention, all measurements were repeated ~48 h after the last training session. [^{18}F]FTHA-PET study was performed first and on the following day, ~72 h after the last training session, [^{18}F]FDG-PET study was performed. Finally, OGTT and VO_{2peak} test were repeated ~96 h after the last training session.

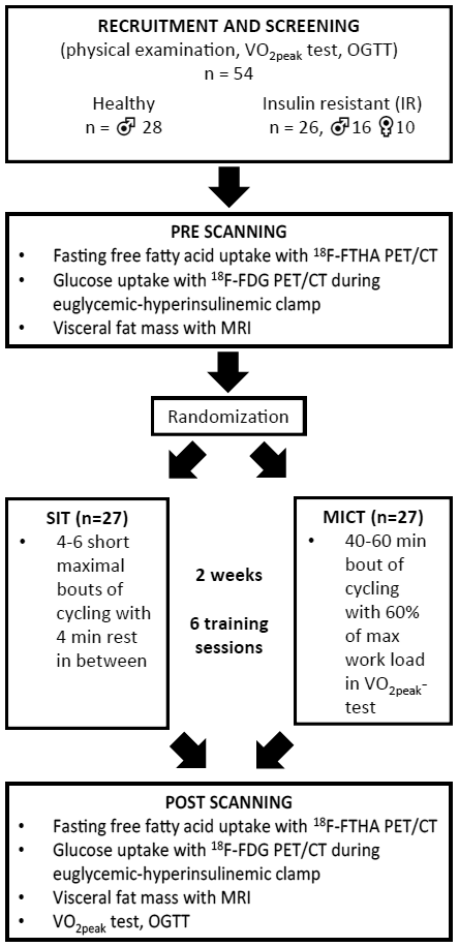

Figure 4 Study design for Study I (HITPET). Abbreviations: ^{18}F-FDG, 2-[^{18}F]fluoro-2-deoxy-D-glucose; ^{18}F-FTHA, 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid; MICT, moderate-intensity continuous training; MRI, magnetic resonance imaging; OGTT, oral glucose tolerance test; SIT, sprint-interval training; VO2peak test, aerobic capacity. Reprinted with permission from Study I.

4.1.1.2 Study II (CROSSYS)

This study is part of a larger study entitled 'Systemic cross-talk between brain, gut, and peripheral tissues in glucose homeostasis: effects of exercise training (CROSSYS)' (NCT03730610). The study was conducted at Turku PET Centre (University of Turku, Turku, Finland), Turku University Hospital (Turku, Finland), University of Turku (Turku, Finland) and Paavo Nurmi Centre (Turku, Finland) between January 2019 and October 2021 according to Good Clinical Practice and in compliance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland (decision 100/1801/2018 §438 and §548). Before any measurements were performed, the purpose and possible risks and benefits of the study were explained and written informed consent was obtained for all participants.

The participants were MZ twin pairs that were recruited from three unique population-based longitudinal twin studies in collaboration with University of Helsinki (Kaidesoja *et al.*, 2019; Kaprio *et al.*, 2019; Rose *et al.*, 2019). The participants were all born in Finland and were of European descent. Inclusion criteria for CROSSYS were:

- MZ twins
- BMI within-pair difference $\geq 2\ kg \cdot m^{-2}$ and/or insulin resistance
- at least one of the co-twins is overweight ($BMI > 25\ kg \cdot m^{-2}$)

Exclusion criteria were:

- $BMI > 60\ kg \cdot m - 2$
- body mass > 170 kg
- waist circumference > 150 cm (due to the gantry limitations of PET and MRI equipment)
- mental disorder or poor compliance
- eating disorder or excess use of alcohol
- active ulcus disease
- diabetes requiring insulin treatment or fasting glucose $> 10\ mmol \cdot l^{-1}$
- pregnancy
- past dose of radiation
- claustrophobia
- presence of ferromagnetic objects in the body

- physical disability that would prevent exercising, or any other condition which could potentially endanger participant's health during the study or interfere with the interpretation of the results

Twelve MZ twin pairs, discordant for BMI (eight female, four male pairs; 40.4 ± 4.5 years; mean BMI 32.9 ± 7.6 kg/m2, mean difference between co-twins 7.6 kg/m2 (min 2.2 kg/m2, max 18.4 kg/m2)), participated in our study. Of the leaner co-twins, five met the criteria for impaired fasting glucose (IFG) and two for impaired glucose tolerance (IGT) as defined by American Diabetes Association guidelines (American Diabetes Association, 2019). Of the heavier co-twins, seven met the criteria for IFG and two for IGT. Two co-twins in the heavier group were treated for hypertension. No participants were treated for diabetes, hyperlipidemia, or used medication that affects bone metabolism. Use of medication did not change during the study. All female participants were premenopausal, none used oral contraceptive medications but eight women had hormonal intrauterine devices. Participants were asked not to change their habitual dietary intake or physical activity outside of the intervention during the study. There were no differences in the reported amounts of total energy, carbohydrates, protein, and fat between the leaner and heavier co-twins at baseline or after the intervention. In total, three co-twins did not receive the intended intervention, one co-twin because of pregnancy midway through the study and one twin pair because of claustrophobic feeling of one co-twin.

Study design is shown in Figure 5. The eligibility of the participants was first evaluated in a phone interview, followed by a screening visit to ensure that the inclusion criteria for the twin pair were fulfilled. Within 1–4 weeks after the screening visit, the twin pairs arrived to baseline measurements, which are described in detail below. Then, both co-twins exercised four times a week for 6 months in their place of residence, and received guidance from a personal trainer once a week. After 6 months, the same measurements were repeated as at baseline. Participants were asked to avoid strenuous exercise as well as caffeinated drinks and alcohol 48 h before each study visit. The usage of nicotine-products was not restricted prior to the measurements. Participants were asked to fast overnight (at least 10 h) before all the study visits.

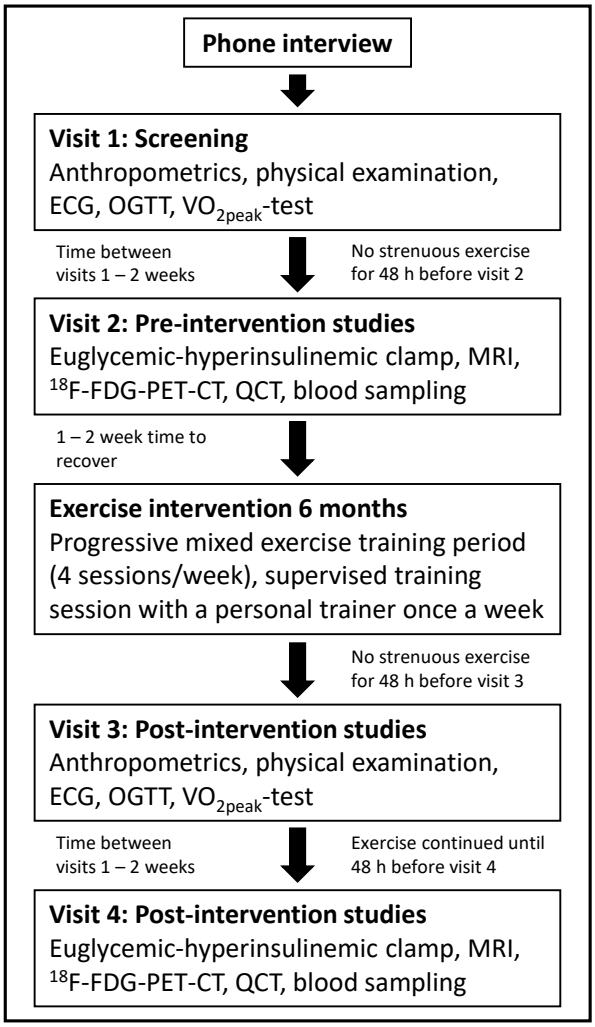

Figure 5 Study design for Study II (CROSSYS). Abbreviations: ^{18}F-FDG, 2-[^{18}F]fluoro-2-deoxy-d-glucose; CT, computed tomography; ECG, electrocardiogram; MRI, magnetic resonance imaging; MZ, monozygotic; OGTT, oral glucose tolerance test; PET, positron emission tomography; QCT, quantitative computed tomography; VO_{2peak} test, aerobic capacity. Reprinted with permission from Study II.

4.1.2 Exercise interventions

4.1.2.1 Two-week SIT and MICT exercise intervention (Study I)

For HITPET, study participants were randomized into SIT and MICT groups. Interval training refers to series of short bursts of exercise followed by recovery periods or low-intensity exercise. The term sprint-interval training (SIT) is used

when the interval training protocol consists of exercise bouts lasting ≤30 seconds at maximal intensity (all-out) and close to VO_{2max} (Buchheit and Laursen, 2013; Weston, Wisløff and Coombes, 2014). MICT refers to traditional endurance training performed at a constant intensity without rest intervals, such as brisk walking, jogging or cycling.

Both training groups had in total six supervised training sessions within two weeks in controlled laboratory conditions at Turku PET Centre with at least one recovery day between sessions. Given the nature of the intervention, no blinding was used. The total duration of exercise training during the six sessions for the SIT group was only 15 minutes whereas for the MICT group the total time was 300 minutes.

The SIT sessions consisted of 4–6 maximal all-out cycling bouts (Monark Ergomedic 894E; MONARK, Vnasbro, Sweden) of 30 s with a 4-min recovery period in between (Wingate protocol). During the recovery period, the subjects could either remain still or do unloaded cycling. The amount of cycling bouts started at 4 and was increased by 1 bout after every other training session. The study participants were familiarized with the SIT protocol ~1 week before the intervention by doing two 30-second bouts.

The MICT sessions consisted of 40 to 60 min of cycling at a moderate intensity with a load of 60% of their individual VO_{2peak} intensity (Tunturi E85; Tunturi Fitness, Almere, Netherlands). The cycling time started at 40 min and was increased by 10 min every other training session until 60 min was reached.

4.1.2.2 Six-month regular exercise training intervention (Study II)

For CROSSYS, the exercise training intervention consisted of 27 ± 2 weeks of progressive exercise training. The training period included one supervised (local personal trainer) and three unsupervised home-based exercise training sessions per week. The intensity of the training was progressive, and the individual training loads were determined with the help of a personal trainer to meet the intensities of the protocol (Heiskanen *et al.*, 2021).

The training program included aerobic endurance, resistance and high-intensity interval training (HIIT). Endurance training was performed twice a week. Endurance training could be implemented in many ways and the participant was allowed to choose the most suitable form of exercise and free to switch between different forms of training, such as running, cycling, swimming, or rowing.

Resistance training was performed once a week and the loads corresponded to approximately 75% of the one repetition maximum throughout the training period. Each session included exercises for both upper and lower body. The training loads were determined three times during the intervention. The exercises became more challenging as the intervention progressed.

HIIT was performed once a week in short exercise bouts with very high intensity and recovery periods between bouts, consisting of circuit type training, cross training, and uphill/stair running. Generally the term HIIT is used for interval training protocols consisting of 1-4 minute exercise bouts performed at 80-100% of maximal heart rate with recovery periods in between (Buchheit and Laursen, 2013; Weston, Wisløff and Coombes, 2014).

The training program included two deload weeks in order to avoid overtraining and injuries. The training adherence based on heart-rate monitor data (PolarA370; Polar) was 88% without a difference between co-twins.

4.1.3 Euglycemic-hyperinsulinemic clamp and whole-body insulin sensitivity (M-value) (Studies I and II)

[^{18}F]FDG-PET imaging was performed during a euglycemic-hyperinsulinemic clamp, which is a method to measure whole-body insulin-stimulated glucose uptake (M-value). This value is considered to be the most definitive measure of insulin sensitivity in humans (DeFronzo, Tobin and Andres, 1979).

The study was performed after an overnight fast (≥10h) as originally described by DeFronzo et al (DeFronzo, Tobin and Andres, 1979). Antidiabetic treatment was withheld for 72 h, and the participants were asked to avoid exhausting exercise 48 h before the clamp.

A primed-constant insulin (Actrapid 100 U·ml^{-1}, NovoNordisk, Bagsvaerd, Denmark) infusion was started at 40 mU·m^{-2}·min^{-1} for the first four minutes of the study. For the next three minutes the infusion rate was decreased to 20 mU·m^{-2}·min^{-1}. After seven minutes, the infusion rate was further reduced to 10 mU·m^{-2}·min^{-1} for the remainder of the study. Exogenous glucose infusion was started 4-10 minutes after the start of the insulin infusion and further adjusted according to blood glucose concentration aiming at the steady level of 5 mmol/l. Arterialized venous blood samples were collected before and every five minutes during the clamp.

Whole-body insulin sensitivity (M-value) was calculated from the glucose values obtained in the steady state of 5 mmol/l lasting at least for 20 min (DeFronzo, Tobin and Andres, 1979).

4.1.4 Imaging and image analysis

4.1.4.1 Positron emission tomography (Studies I and II)

The PET/CT images were acquired using GE Discovery TM ST System (General Electric Medical Systems, US) for Study I and Discovery MI ((DMI), GE

Healthcare, US) for Study II. CT images were acquired for anatomical reference and radiodensity extraction. The participants fasted for ≥10 h before the PET studies. Antecubital veins of both arms were cannulated for the PET studies. One catheter was used to inject the radiotracers, whereas the other one was for blood sampling. To arterialize venous blood for the length of the study, an electrically powered heating cushion was placed under the arm where the blood samples were taken from.

For HITPET the [^{18}F]FDG-PET study (157 [SEM 0.9] MBq) started 91 min (SD 2) after the start of the clamp and lumbar vertebral (vertebrae Th12-L3), femoral (mid-diaphysis), and thoracic vertebral (vertebrae Th1-4) regions were scanned starting ~47, ~67, and ~93 min after tracer injection, respectively. In the IR group, thoracic region was not scanned. BM FFAU was measured using the radiotracer [^{18}F]FTHA in a fasting state. Lumbar (vertebrae Th12-L3), femoral (mid-diaphysis), and thoracic regions (vertebrae Th1-Th4) were then scanned starting at ~46, ~65 and ~86 min after tracer injection (156 [SEM 1.1] MBq), respectively.

For CROSSYS, the [^{18}F]FDG (155 ± 8 MBq) tracer injection was given and PET imaging started ~84 min after the start of the clamp and lumbar vertebral (vertebrae L2-L4) and femoral (mid-diaphysis) regions were imaged ~56 and ~68 min after the injection, respectively.

All obtained imaging data were corrected for attenuation, dead time, and decay and then reconstructed. Carimas software (http://turkupetcentre.fi) was used to manually draw 3-dimensional regions of interest (ROIs) in the marrow cavities of femurs and thoracic and lumbar vertebrae. CT images were used as anatomical reference. The ROIs were carefully drawn to only include the marrow cavity and to leave out cortical bone and surrounding tissue. A representative example of the shape and positioning of the ROIs can be seen in Figure 6. From these ROIs, time activity curves (TACs) were extracted. TAC data was analyzed using FUR model (Thie, 1995). A lumped constant of one was used for BM.

4.1.4.2 Computed tomography (Studies I and II)

CT images were acquired during PET imaging as anatomical references and for PET image attenuation. We measured the radiodensity of BM to study its adiposity. The radiodensity of tissue is expressed in Hounsfield units (HU), obtained from a linear transformation of attenuation coefficients based on the arbitrary definitions of air (−1000 HU) and water (0 HU). On this scale, fat has a density of −60 to −120 HU (Goldman, 2007). In BM, the amount of fat cannot be precisely quantified by this method, but fat content can be estimated with the help of HUs. Thus, the lower the HU, the higher the fat content.

For radiodensity analysis, ROIs were drawn onto the CT images using Carimas software. In the thigh area, a ROI covering the entire mid-shaft of the femur was

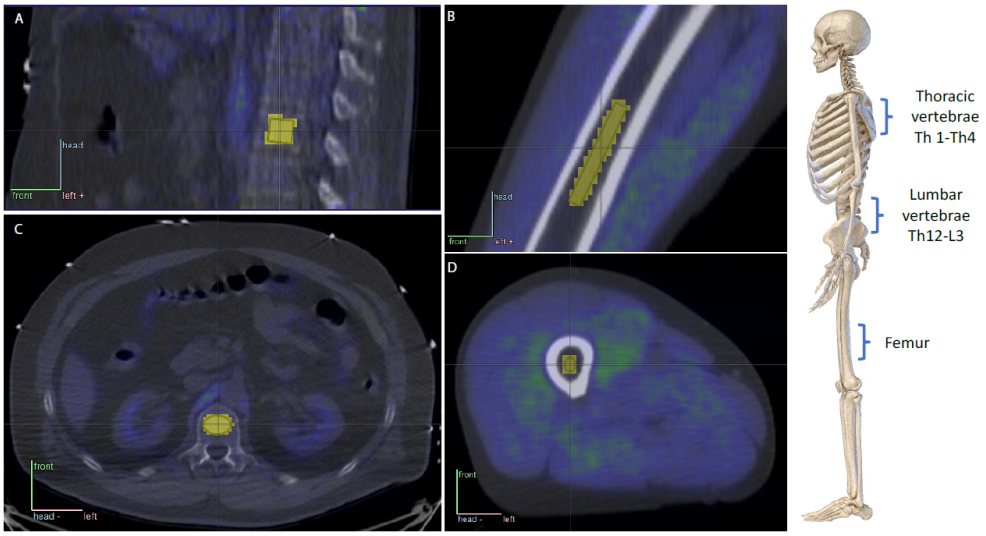

Figure 6 An example from of the shape and positioning of the region of interest (ROI), shown in yellow, from which time activity curves were extracted. Sagittal PET/CT image of lumbar vertebral (A) and femoral (B) regions. Transaxial PET/CT image of lumbar vertebral (C) and femoral (D) regions. CT scans were used as anatomical reference. Reprinted with permission from Study I.

drawn. The CT voxels within this ROI were then thresholded to separate cortical bone from the BM tissue. The HU threshold level for differentiating cortical bone from BM tissue was considered to be 400 HU based on visual evaluation as well as previously documented HU range of cortical bone and BM (Singhal and Bredella, 2019). In lumbar area, ROIs were drawn onto the CT images carefully avoiding cortical bone as in ROIs for PET image analysis. No thresholding was necessary, as only trabecular bone area was included.

In Study II, quantitative computed tomography (qCT) was applied to measure volumetric BMD using Discovery MI computed tomography (CT) scanner (GE Healthcare, Waukesha, WI, US) with a solid Mindways QCT Phantom (Mindways Software Inc., Austin, TX, US, model QCT Pro) immediately after the [^{18}F]FDG-PET scan. Vertebrae L2 to L4 were scanned in the supine position. Calibration phantom was used to convert HU into bone mineral equivalents in mg·cm^{-3} using QCT Pro software (Mindways Software Inc., Austin, TX, USA), with which the CT images were analyzed.

4.1.4.3 Magnetic resonance imaging (Studies I and II)

In Study I, MRI scans were performed using Philips Gyroscan Intera 1.5T CV Nova Dual scanner (Philips Medical Systems). Abdominal area axial T1 weighted dual fast field echo images (echo time 2.3 and 4.7 ms, repetition time 120 ms, slice thickness

10 mm without gap) were obtained. To measure different adipose tissue masses, the images were analyzed using SliceOmatic software version 4.3 (http://www.tomovision.com/products/sliceomatic.html). To obtain the visceral adipose tissue mass, the pixel surface area was multiplied with the slice thickness and the density of adipose tissue, 0.9196 kg/l.

In Study II, visceral adipose tissue was measured using magnetic resonance imaging with Siemens Magnetom Skyra fit 3 T MRI system (Siemens Healthcare, Erlangen, Germany). Carimas software was used to create fat fraction maps in which fat image of the T1 VIBE Dixon scan is divided by the sum of fat and water images. Visceral fat was segmented from fat fraction maps by drawing two-dimensional ROIs in every 5-10 slices starting from the ends of the heads of the femurs and ending to the xiphoid process, and creating a three-dimensional volume of interest from them using the interpolation feature of the Carimas software. Then, all voxels with an intensity value <0.5 (i.e. fat fraction over 50%) were excluded and the remaining volume of interest was considered as visceral adipose tissue.

4.1.5 Bone turnover markers (Studies I and II)

For Study I, blood samples were collected on the morning of the [^{18}F]FTHA-PET study and for Study II, on the morning of the PET study day between 8 and 10 a.m. after an overnight fast (≥10 h). EDTA plasma and serum samples were stored as aliquots at −80°C.

Bone formation was assessed by measuring intact N-terminal propeptides of type I collagen (PINP) using IDS-iSYS Intact PINP assay (IDS Ltd, UK). Bone resorption was assessed by measuring C-terminal crosslinked telopeptides of type I collagen (CTX) with IDS-iSYS CTX-I (CrossLaps) assay (IDS Ltd, UK).

Bone-specific osteocalcin, a marker of bone remodeling, was measured with a 2-site immunoassay (Paldánius *et al.*, 2012). The assay detects total osteocalcin (TotalOC) and is based on monoclonal antibodies 2H9 and 6F9. We also measured uncarboxylated form of osteocalcin (ucOC), which has been suggested to regulate glucose metabolism (Wei and Karsenty, 2015). We used an immunoassay based on ucOC-specific recombinant antibody Fab-AP1324 and expressed the results as the ratio of uncarboxylated to total osteocalcin (ucOC/TotalOC) (Arponen *et al.*, 2020).

4.1.6 Other measurements

4.1.6.1 Body composition and oral glucose tolerance test (Studies I and II)

Body composition was measured with a bioimpedance monitor (InBody 720, Mega Electronics, Kuopio, Finland). Weight and height of the participants were measured by standard procedures.

A 2-h, 75-g OGTT was performed after the participants had fasted for ≥10 h. After ingestion of glucose, blood samples were collected at 0, 15, 30, 60, 90, and 120 min to determine glucose and insulin concentrations in the blood.

4.1.6.2 Aerobic capacity (Studies I and II)

Aerobic capacity was determined by an incremental bicycle ergometer test (Ergoline 800s; VIASYS Healthcare) to indicate the baseline aerobic performance as well as the effectiveness of the exercise training intervention. The tests were performed at Paavo Nurmi Centre, University of Turku, Turku, Finland and supervised by a trained exercise physiologist.

For participants in Study I and men in Study II, the test started with a workload of 50 W which was then increased by 30 W every 2 min until volitional exhaustion. For women in Study II, the test started with a workload of 40 W which was then increased by 20 W every 2 min until volitional exhaustion. Ventilation and gas exchange were measured (Jaeger Oxycon Pro; VIASYS Healthcare or Vyntus CPX, Vyaire Medical Gmbh, Leibnizstrasse, Hoechberg, Germany) during the test. Tests before and after the intervention were conducted using the same device for each participant. Lactate concentration was measured from capillary blood samples immediately and 1 min after exhaustion (YSI 2300 Stat Plus; YSI Incorporated Life Sciences, Yellow Springs, OH). The heart rate of the participants was followed continuously with an ECG-machine (CardioSoft GE, CardioSoft V6.51; GE Medical Systems Information Technologies, USA). The highest 1 minute mean value of oxygen consumption (VO_{2peak}) related to body weight was used for both studies as the measure for aerobic capacity.

4.2 Animal studies

4.2.1 Study design (Study III)

This study is part of a larger study entitled CROSRAT (Jalo *et al.*, 2024). The study was approved by the State Provincial Office of Southern Finland (permission number

ESAVI/4080/2019). Male Sprague-Dawley rats were housed in standard cages of two to four rats in an environmentally controlled facility (12/12h light-dark cycle, 21°C, 55% humidity) at the Central Animal Laboratory of the University of Turku (Turku, Finland). Food and water were provided *ad libitum*.

The study design is shown in Figure 7. Eight-week old rats were divided into five intervention groups (n=16-24 per group) for 24-weeks as follows: (I) age-control group fed with normal chow diet for 24 weeks (^{24}Chow); (II) high-fat diet group fed with high-fat diet for 24 weeks (^{24}HFD); (III) exercise intervention group fed with HFD for 24 weeks with the possibility of voluntary running exercise for the last 12 weeks of the intervention (^{12}HFD+^{12}(HFD+E)); (IV) diet intervention group fed with an initial 12 weeks of HFD then switched to normal chow diet for another 12 weeks (^{12}HFD+^{12}(Chow)); and (V) combined intervention group of an initial 12 weeks of HFD then a switch to normal chow diet with a possibility of voluntary running exercise for the last 12 weeks of the intervention (^{12}HFD+^{12}(Chow+E)). Additionally, eight-week-old rats without intervention (baseline, age 8 wks, ^{0}Chow, n=9) or fed with normal chow diet for 12 weeks (^{12}Chow, age 20 wks, n=6) were imaged with PET/CT to elucidate the effects of aging.

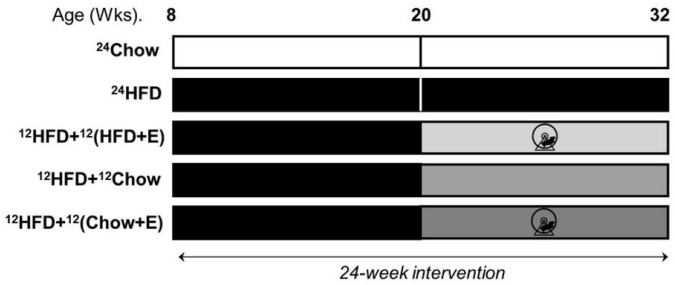

Figure 7 Study design for Study III (CROSRAT). Abbreviations: HFD, high-fat diet; E, exercise. Reprinted with permission from Study III.

The rats were fed either standard chow (control diet, RM3 (E) Soya free, Special Diets Services, United Kingdom; 15.43 MJ/kg: 11.5 % from fat, 27.0 % from protein, 61.5% from carbohydrates), or high-fat diet (Western diet, 1.5 % cholesterol, ssniff Spezialdiäten GmbH, Soest, Germany; 21.8 MJ/kg: 42.0 % from fat, 15.0 % from protein, 43.0 % from carbohydrates) for 12 or 24 weeks according to the study design.

For the exercise groups, rats were housed individually in cages with free access to running wheels (Intellibio, Seichamps, France) from 4 pm to 8 am for four consecutive days a week followed by three days of rest. Individual running distance was recorded daily with the activity wheel system and ActiWheel software (Intellibio, Seichamps, France).

After the intervention, animals were weighed and body composition was measured with EchoMRI™ 1100 Analyzer (EchoMRI LLC, Houston, TX, USA). Four days before the imaging studies, fasting (4h) blood glucose was measured from the lateral tail vein with a glucometer (Contour XT, Bayer, Germany). Blood samples were collected into lithium-heparin plasma collection tubes, and plasma was stored at -80 °C. Fasting plasma insulin was measured according to the manufacturer protocol (Mercodia, Uppsala, Sweden). HOMA-IR index was calculated using fasting blood glucose and insulin values (fasting glucose (mmol/l) × fasting insulin (μU/ml)/22.5)).

4.2.2 Euglycemic-hyperinsulinemic clamp (Study III)

At the end of the intervention, 8-13 rats from each group underwent PET imaging with [^{18}F]FDG combined with euglycemic-hyperinsulinemic clamp. The rat was weighed and administered 0.05 mg/kg buprenorphine for analgesia, after which the rat was anesthetized with isoflurane/oxygen mixture (4% isoflurane for induction, 2.5% for maintenance). Surgical femoral artery and vein catheterization was performed for a 4-hour fasted, anaesthetized rat. The animal was then transported to the scanner (Inveon Multimodality PET/CT device (Siemens Medical Solutions, Knoxville, TN, USA)) and after a CT scan the catheters were combined with a peristaltic pump and Swisstrace Twilite II coincidence detector.

Insulin clamp was initiated with a 120 mU/kg/min infusion for 3 minutes, followed by 60 mU/kg/min infusion until the blood glucose level dropped to 6.0 mmol/L. Then the insulin infusion was lowered to 18 mU/kg/min for the rest of the clamp protocol. Glucose infusion (20% v/v) was started at the same time. Blood glucose was measured (Contour XT, Bayer) before the initiation of the clamp, every 3 minutes for the first 25 minutes and every 5 minutes onwards. Whole-body insulin sensitivity (M-value) was calculated from at least a 20-minute time-period with a steady blood glucose level (~5 mmol/L) (DeFronzo, Tobin and Andres, 1979).

4.2.3 Imaging and image analysis (Study III)

After a steady blood glucose level of approximately 5 mmol/L was reached, a 45-min PET scan, with 30 x 10 s, 15 x 60 s and 5 x 300 s framing, was started simultaneously with an intravenous injection of [^{18}F]FDG (20 MBq). Arterial blood coincidences were recorded with Swisstrace Twilite II coincidence detector and PMOD v. 4.1 (Psample, PMOD, Zurich, Switzerland), which enabled the measurement of the whole blood arterial input function with a temporal resolution of one second without blood loss during the PET scan. Cross-calibration between Swisstrace Twilite II and Inveon Multimodality PET/CT scanner was performed

prior to every experiment with the same tracer batch used for the injection. The arterial whole blood coincidences were corrected for the background signal counts, the time of the injection, radioactive decay, and the premeasured calibration factor between coincidence detector and Inveon Multimodality PET/CT scanner.

For the analysis of insulin-stimulated BM GU, animals with higher than intended insulin infusion rate or those with incomplete PET-CT image sets were excluded. Insulin-stimulated BM GU was measured from successful PET imaging data (n = 3-8 animals/group) using Carimas software (https://carimas.fi). Three-dimensional ROIs were manually drawn into the BM cavities of the humeri (mid-diaphysis) and the sternum similarly to Studies I and II. BM TACs were computed from the dynamic PET images. FUR was calculated as a ratio of average BM radioactivity concentration between 30 and 45 min post injection and integral of blood TAC. The lumped constant, representing the difference in transport and phosphorylation rates of FDG and glucose was assumed 1.

After the scan, rats were sacrificed under terminal anesthesia. Subsequently, tibiae from the rats were collected and prepared for further analysis.

4.2.4 Bone samples (Study III)

Tibiae (n = 4-10 per group) were fixed in 10% neutral buffered formalin overnight and decalcified in a decalcifying solution (10% EDTA, 1 M NaH_2PO_4, 0.5 M Na_2HPO_4) for 16 days. Decalcified tibiae were oriented longitudinally for paraffin embedding and trimmed approximately 40% deep from the surface and sectioned as 4 µm thick sections.

Histological bone sections were then stained with 2 µg/mL rabbit polyclonal anti-perilipin1 (Thermofisher, Massachusetts, USA; PA1–1051, AB_2167579) and 2 µg/mL Alexa Fluor® 594-conjugated goat polyclonal anti-rabbit IgG (Abcam, Cambridge, UK; ab150080, AB_2650602) to visualize BMAds. The slides were then imaged with Pannoramic Midi Slide scanner (3DHISTECH, Budapest, Hungary). Two 1 mm × 1 mm regions of interest (ROI) were drawn from each section in the center of BM cavity at 9 mm distal from the growth plate and non-specifically at the adipocyte-rich area of distal region of the tibia representing analysis at the proximal and distal marrow of the tibia, respectively. BMAd density (N.Ad/mm2) and individual area (Ad.Ar) were analyzed using the immunofluorescent-based script (Script 2) (Widjaja *et al.*, 2023) in ImageJ version 1.53c (Abramoff, Magalhães and Ram, 2003).

4.3 Statistics

The normal distribution of variables was evaluated visually and tested using Shapiro-Wilk test. Logarithmic (log10) or square root transformations were performed to fulfil normal distribution. The statistical tests were performed as 2-sided and the level of statistical significance was set at 0.05. The analyses were performed using SAS System, version 9.4 for Windows (SAS Institute, Cary, NC, US) (Study I and II) or Prism 9 (GraphPad, San Diego, USA) (Study III).

4.3.1 Study I (HITPET)

Sample size was calculated for the whole study based on its primary outcome (skeletal muscle GU) (Eskelinen *et al.*, 2015). For healthy participants, a total of 24 participants (12 in each group) were calculated to give > 90% power of detecting a 20% unit difference in insulin-stimulated GU in quadriceps femoris (estimated increase in SIT 40% vs. estimated increase in MICT 20%, SD 15) with a level of significance at 5%. For IR participants, a total of 20 participants (10 in each group) were calculated to give corresponding statistical power (estimated increase in SIT 60% vs. estimated increase in MICT 30%, SD 20). No sample size calculation was performed on the outcome measures of this study.

Statistical analyses were performed using hierarchical mixed linear models with compound symmetry covariance structure. First, the differences between healthy and IR men were studied with the model, which included 1 within-factor term (time; indicating the overall mean change between baseline and measurement after the intervention), 2 between-factor terms (glycemic status: healthy and IR men; training: SIT and MICT), and 2 interaction terms (time × glycemic status: indicating whether mean change during the study was different between healthy and IR men; time × training: indicating whether mean change during the study was different between SIT and MICT). IR women were only included in comparisons within the IR group to avoid mixing the effects of sex and glucose intolerance. Second, differences between SIT and MICT in IR participants, including both men and women, were studied using a model that included within-factor time (time; indicating the overall mean change between baseline and measurement after the intervention), 2 between-factor terms (training: SIT and MICT; sex: male and female), and 2 interaction terms (time × sex: indicating whether mean change during the study was different between IR men and IR women; time × training: indicating whether mean change during the study was different between SIT and MICT). The analyses were carried out using the intention-to-treat principle and included all the randomized participants. Due to the chosen analysis method, also participants with missing data could be included into statistical modelling. Furthermore, model-based means (SAS least square means) and 95% confidence intervals are reported for all the parameters.

Correlations were calculated using Pearson's correlation (Spearman's rank correlation for non-normally distributed data).

To study which variables affect the GU and FFAU of BM, we used the multivariate regression analysis, which is a technique that estimates a single regression model with multiple outcome variables and 1 or more predictor variables.

4.3.2 Study II (CROSSYS)

The sample size calculations were based on liver fat content and VO_{2max} results from an earlier cross-sectional study in twins discordant for physical activity and fitness (Hannukainen *et al.*, 2005, 2011), and on M-value (Kaye *et al.*, 2012). No sample size calculation was performed on the outcome measures of this study.

Statistical analyses were performed using a linear mixed model for repeated time points using compound symmetry covariance structure. The differences between the co-twins were studied with the model, which included one or two within factors; twin effect, i.e. group defined as within-factor (group: leaner and heavier co-twins) and time as within-factor if outcome was measured several times (time; indicating the overall mean change between baseline and measurement after the intervention), and one interaction term (time × group: indicating whether mean change during the study was different between the leaner and heavier co-twins). The statistical unit was defined to be twin.

The analyses were carried out using the intention-to-treat principle and included all the participants. Because of the chosen analysis method, also participants with missing data were included into statistical modelling. Furthermore, model-based means (SAS least square means) and 95% confidence intervals (CI) are reported for all the parameters. Correlations were calculated using Pearson's correlation (Spearman's rank correlation for non-normally distributed data).

4.3.3 Study III (CROSRAT)

Pearson's correlation analysis was performed to determine the correlation between two continuous variables. Mean differences among the groups were analyzed with one-way ANOVA with Dunnett's correction for multiple comparison. To test the effect of interventions, group (II) served as the high-fat control group (^{24}HFD). To test the effect of anatomical BM depot and age, or the difference in BMAd size distribution, covariance analysis with Tukey's or Dunnett's correction for multiple comparisons was performed. Difference in running distance between groups was tested using Student's t-test.

5 Results

The following section will present the main results of this thesis. More detailed results can be found in the original articles (Study I-III).

5.1 Exercise interventions improved aerobic capacity, whole-body insulin sensitivity and/or body composition

5.1.1 Two-week SIT and MICT exercise intervention (Study I)

Before the two-week intervention, IR men had impaired aerobic capacity ($p < 0.001$), higher body mass, higher body mass index (BMI) and higher whole-body fat percentage, and their lipid and glucose profiles were impaired (all $p < 0.001$) compared to healthy men (Table 2). Training improved aerobic capacity similarly in both groups (time $p = 0.003$, time*IR $p = 0.23$) (Table 2). When divided by training mode, only SIT improved aerobic capacity in IR subjects with no differences between men and women (data not shown). IR men had significantly lower whole-body insulin sensitivity (M-value) at baseline ($p < 0.001$), but it improved after training with no differences between the groups or training modes (time $p < 0.001$) (Table 2). After the training intervention whole-body fat percentage decreased in the whole group and there were significant improvements in the lipid and glucose profiles (time all $p < 0.05$) (Table 2) and no difference was found in the training response between the groups. Except for the higher increase in aerobic capacity after SIT, we did not observe any other differences between the training modes in the measured parameters.

Table 2. Subject characteristics of Study I (HITPET) between healthy and IR men before and after exercise intervention.

Parameter	Healthy men		IR men		P-value		
	Pre	Post	Pre	Post	Baseline	Time	Time*IR
Anthropometrics							
Weight (kg)	83.6 [79.7;87.5]	83.3 [79.4;87.2]	96.3 [91.2;101.3]	96.2 [91.0;101.3]	**<0.001**	0.22	0.80
BMI (kg/m^2)	26.1 [25.1;27.1]	26.0 [25.0;27.0]	30.4 [29.1;31.8]	30.4 [29.0;31.7]	**<0.001**	0.17	0.70
Whole body fat& (%)	22.6 [20.9;24.3]	21.7 [20.0;23.3]	28.8 [26.5;31.2]	28.1 [25.7;30.4]	**<0.001**	**<0.001**	0.78
Visceral fat† (kg)	2.5 [2.0;3.2]	2.4 [1.9;3.08]	4.3 [5.4;3.4]	4.1 [3.1;5.1]	**0.002**	**0.002**	0.48
VO_{2peak} (mL/kg/min)	34.2 [32.7;35.7]	35.7 [34.2;37.2]	29.3 [27.2;31.4]	30.0 [27.9;32.1]	**<0.001**	**0.003**	0.23
Glucose profile							
$Glucose_{fasting}$& (mmol/L)	5.5 [5.4;5.7]	5.7 [5.5;6.0]	7.2 [6.9;7.6]	7.1 [6.8;7.5]	**<0.001**	0.26	0.086
M-value& (µmol/min/kg)	35.3 [30.0;40.6]	38.7 [33.3;44.1]	17.5 [10.3;24.8]	21.6 [14.2;29.0]	**<0.001**	**<0.001**	0.11
HbA_1c (mmol/mol)	36.9 [35.2;38.6]	34.8 [33.0;36.5]	39.6 [37.3;41.8]	37.5 [35.2;39.9]	0.071	**<0.001**	0.87
HbA1c (%)	5.5 [5.4;5.7]	5.3 [5.2;5.5]	5.8 [5.6;6.0]	5.6 [5.4;5.8]	0.080	**<0.001**	0.90
Lipid profile							
$FFA_{fasting}$ (mmol/L)	0.70 [0.62;0.77]	0.62 [0.54;0.69]	0.69 [0.60;0.78]	0.68 [0.59;0.78]	0.86	**0.04**	0.11
Cholesterol (mmol/L)	4.9 [4.6;5.3]	4.4 [4.1;4.7]	4.7 [4.3;5.2]	4.3 [3.9;4.8]	0.44	**<0.001**	0.52
HDL& (mmol/L)	1.4 [1.3;1.5]	1.3 [1.2;1.4]	1.2 [1.1;1.4]	1.1 [1.0;1.2]	0.08	**<0.001**	0.66
LDL (mmol/L)	3.1 [2.9;3.4]	2.8 [2.5;3.1]	2.7 [2.3;3.1]	2.6 [2.2;3.0]	0.09	**<0.001**	0.16
Triglycerides& (mmol/L)	0.94 [0.81;1.11]	0.83 [0.70;0.98]	1.70 [1.38;2.10]	1.50 [1.19;1.90]	**<0.001**	0.08	0.96
Bone markers							
Osteocalcin (ng/ml)	7.97 [7.28;8.65]	7.64 [6.92;8.36]	6.64 [5.72;7.55]	6.76 [5.78;7.74]	**0.02**	0.66	0.32
PINP&	51.3 [45.7;57.4]	48.1 [42.6;54.3]	38.0 [32.4;44.5]	38.9 [32.9;46.0]	**0.003**	0.68	0.37

All values are model based means [95% confidence intervals]. Log transformation & or square root transformation † was performed to fulfill normal distribution assumption. P-value for Baseline indicates the differences between healthy and IR men. The p-value for Time indicates the change between pre and post measurements in the whole study group. The p-value for Time*IR interaction indicates if the change in the parameter was different between healthy and IR men. IR, insulin resistant; BMI, body mass index; VO2peak, aerobic capacity; M-value, whole-body insulin sensitivity; HbA1c, glycosylated hemoglobin; FFA, free fatty acids; HDL, high density lipoprotein; LDL, low density lipoprotein; PINP, procollagen type 1 N-terminal propeptide. Reprinted with permission from Study I.

5.1.2 Six-month regular exercise training intervention (Study II)

Before the six-month intervention, heavier co-twins had lower aerobic capacity relative to body weight (VO_{2peak}, $p = 0.002$) and more visceral adipose tissue ($p = 0.002$) compared with leaner co-twins (Table 3). At baseline, heavier co-twins also had a worse glucose profile manifested as higher fasting insulin and HbA1c, and lower whole-body insulin sensitivity (M-value) than leaner co-twins (all $p < .05$). Training improved aerobic capacity similarly in both groups ($p = 0.001$, Table 3). However, training had no statistically significant effect on body weight or body composition. Systolic and diastolic blood pressure decreased after training similarly in leaner and heavier co-twins (both $p < 0.05$). Whole-body insulin sensitivity increased in both groups similarly ($p = 0.022$) (Table 3).

5.1.3 12-week diet and/or exercise intervention in rats (Study III)

High-fat feeding (^{24}HFD) significantly increased body weight (11.6%, $p = 0.002$) and whole-body fat percentage (25.6% higher, $p = 0.004$) when compared to the age-matched chow-fed control group (^{24}Chow; Figure 8). On average, diet (^{12}HFD+^{12}Chow), exercise (^{12}HFD+^{12}(HFD+E)), and combined intervention (^{12}HFD+^{12}(Chow+E)) decreased body weight and whole-body body fat percentage compared to ^{24}HFD group. Rats with combined intervention had on average the lowest whole-body fat percentage (11.50%) despite being heavier in body weight than the baseline ^{24}Chow group. Further, HFD increased the average levels of fasting blood glucose and plasma insulin compared to the ^{24}Chow group (Figure 8). Dietary intervention with exercise had the most pronounced effect in reducing plasma insulin ($p = 0.005$). However, dietary intervention alone significantly reduced fasting blood glucose ($p = 0.03$) compared to the ^{24}HFD. Whole-body fat percentage correlated positively with weight ($p < 0.001$, $r = 0.48$), fasting insulin ($p < 0.001$, $r = 0.66$) and HOMA-IR index ($p < 0.001$, $r = 0.64$). On average, ^{24}HFD group had the highest HOMA-IR index and both diet (^{12}HFD+^{12}Chow) and combined groups (^{12}HFD+^{12}(Chow+E)) significantly reduced HOMA-IR index ($p = 0.03$ and $p = 0.001$, respectively) (Figure 8). Whole-body insulin sensitivity (M-value) did not significantly differ between the intervention groups. The running distance (m/day) during intervention was similar in both exercising groups (^{12}HFD+^{12}(HFD+E) and ^{12}HFD+^{12}(Chow+E)) ($p = 0.16$; Figure 8). Running distance (m/day) correlated negatively with weight ($p = 0.005$, $r = -0.47$) and whole-body fat percentage ($p = 0.002$, $r = -0.52$).

Table 3. Subject characteristics of Study II (CROSSYS) between leaner and heavier co-twins before (pre) and after (post) exercise training intervention.

	Leaner co-twins		Heavier co-twins		P-value		
	Pre	**Post**	**Pre**	**Post**	**Baseline**	**Time**	**Time*group**
Anthropometrics	n = 12	n = 10	n = 12	n = 11			
Age (years)	40.4 [37.5;43.4]		40.4 [37.5;43.4]				
Weight (kg)	86.4 [72.4;100.4]	86.9 [72.6;101.2]	108.7 [94.2;123.3]	108.0 [93.1;122.9]	**0.001**	0.95	0.37
BMI (kg/m^2)	29.1 [25.2;33.0]	29.3 [25.3;33.2]	36.7 [32.7;40.7]	36.4 [32.4;40.4]	**<0.001**	0.92	0.41
Whole body fat (%)	30.4 [21.3;39.6]	29.5 [20.3;38.7]	40.6 [36.5;44.7]	40.0 [35.9;44.1]	**<0.001**	0.37	0.72
Visceral adipose tissue (kg)	3.38 [2.13;4.64]	3.22 [2.03;4.42]	5.83 [4.74;6.93]	5.46 [4.42;6.50]	**0.002**	0.067	0.28
Lean mass (kg)	33.1 [30.0;36.3]	33.9 [30.6;37.2]	35.9 [31.9;39.8]	36.2 [32.1;40.3]	**0.003**[a]	0.14	0.10
Systolic BP (mmHg)	131.4 [120.4;143.4]	122.3 [114.1;131.1]	136.1 [128.4;144.4]	126.8 [121.2;132.7]	0.38	**0.011**[b]	0.98[a]
Diastolic BP (mmHg)	80.1 [73.3;86.9]	77.1 [71.2;83.0]	86.9 [80.2;93.5]	78.3 [72.5;84.0]	0.074	**0.017**	0.091
VO_{2peak} (ml/kg/min)	32.4 [26.9;37.8]	35.1 [29.9;40.2]	25.6 [23.2;28.0]	28.3 [26.1;30.6]	**0.003**	**0.001**	0.94
hs-CRP (mg/l)	0.81 [0.41;1,61]	0.56 [0.21;1.47]	1.42 [0.75;2.70]	1.14 [0.46;2.85]	**0.005**[a]	0.30[a]	0.45[a]
Glucose profile							
Fasting glucose (mmol/l)	5.5 [5.2;5.7]	5.5 [5.2;5.7]	5.7 [5.4;5.9]	5.8 [5.6;6.1]	0.39	0.37	0.42
Fasting insulin (mU/l)	6.6 [5.1;8.7]	6.3 [4.3;9.3]	11.1 [8.7;14.2]	9.9 [6.9;14.1]	**0.006**[a]	0.50[a]	0.71[a]
HbA_{1c} (mmol/mol)	34.9 [32.9;36.9]	34.7 [32.2;37.1]	36.5 [35.0;38.0]	36.0 [34.2;37.7]	**0.047**[a]	0.58	0.68
HbA_{1c} (%)	5.3 [5.2;5.5]	5.3 [5.1;5.5]	5.5 [5.4;5.6]	5.4 [5.3;5.6]	**0.049**[a]	0.59	0.67
M-value (µmol/min/kg)	37.5 [28.0;47.0]	46.9 [31.7;62.1]	23.0 [16.1;29.9]	31.4 [20.4;42.3]	**0.007**	**0.022**	0.82

All values are model based means [95% confidence intervals]. P-value for Baseline indicates the differences between leaner and heavier co-twins. P-value for Time indicates the change between pre and post measurements in the whole study group. P-value for Time*group interaction indicates if the mean change in the parameter was different between leaner and heavier co-twins. Statistically significant p-values (p<0.05) are highlighted in **bold**. Eight female and four male twin pairs. For M-value leaner co-twins PRE n = 11, POST n = 10, heavier co-twins PRE n = 11, POST n = 9. For visceral adipose tissue leaner co-twins PRE n = 10, POST n = 10, heavier co-twins PRE n = 8, POST n = 9.

Abbreviations: VO_{2peak}, aerobic capacity; hs-CRP, high-sensitivity C-reactive protein; M-value, whole-body insulin sensitivity. [a]Logarithmic or [b]Square root transformation was performed to fulfill normal distribution assumption. Reprinted with permission from Study II.

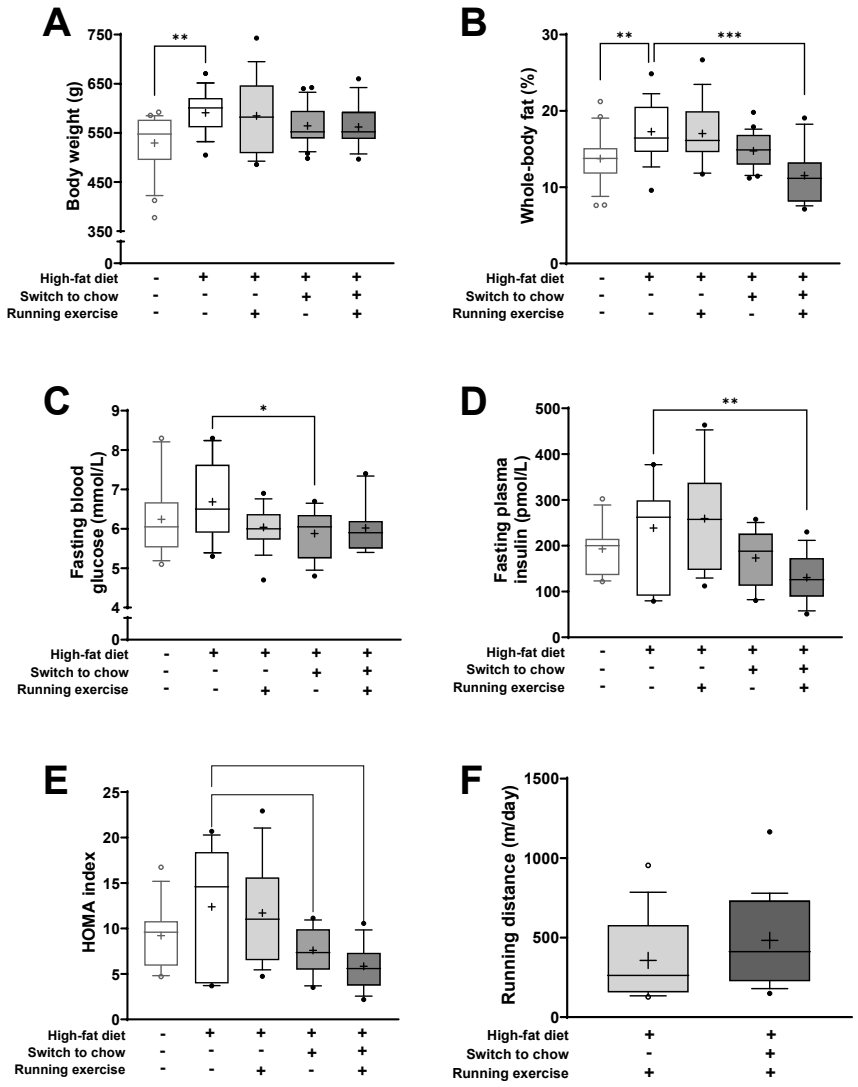

Figure 8 Study III (CROSRAT) **(A)** Body weight and **(B)** whole-body fat-percentage at the end of study (n = 16-24/group). Fasting plasma levels of **(C)** glucose (n = 12-18/group) and **(D)** insulin (n = 11-16/group). **(E)** Homeostatic model assessment for insulin resistance (HOMA-index; n = 11–16/group). **(F)** Mean daily running distance of exercising rats (^{12}HFD+^{12}(HFD+E), n = 16; and ^{12}HFD+(^{12}Chow+E) groups, n = 19). Data is presented as boxes representing quartiles and whiskers representing the 10th and 90th percentile. Median is marked with a single line and mean value is marked with a plus in each box. One-way ANOVA with Dunnet's correction for multiple comparison was performed in **A – E** to compare mean values between groups. The ^{24}HFD group served as the control group. Student's t-test was performed to compare mean difference between groups in **F**. *p< 0.05, **p< 0.01, ***p< 0.001. Reprinted with permission from Study III.

5.2 Bone marrow metabolism differs between anatomical locations (Study I-III)

Both BM insulin-stimulated GU and fasting FFAU differed regarding the anatomical region in healthy subjects at baseline of Study I (HITPET) (Figure 9). Insulin-stimulated GU was significantly higher in lumbar vertebral BM than in thoracic vertebral BM ($p < 0.0001$). Further, GU in femoral BM was significantly lower than GU in lumbar vertebral or thoracic vertebral BM ($p < 0.0001$ for both). Fasting FFAU was higher in lumbar vertebral and thoracic vertebral than in femoral BM ($p < 0.0001$ for both). Similar regional differences in GU and FFAU were observed in the IR group with lumbar vertebral and femoral BM (Figure 9). Thoracic vertebrae were not scanned in the IR group.

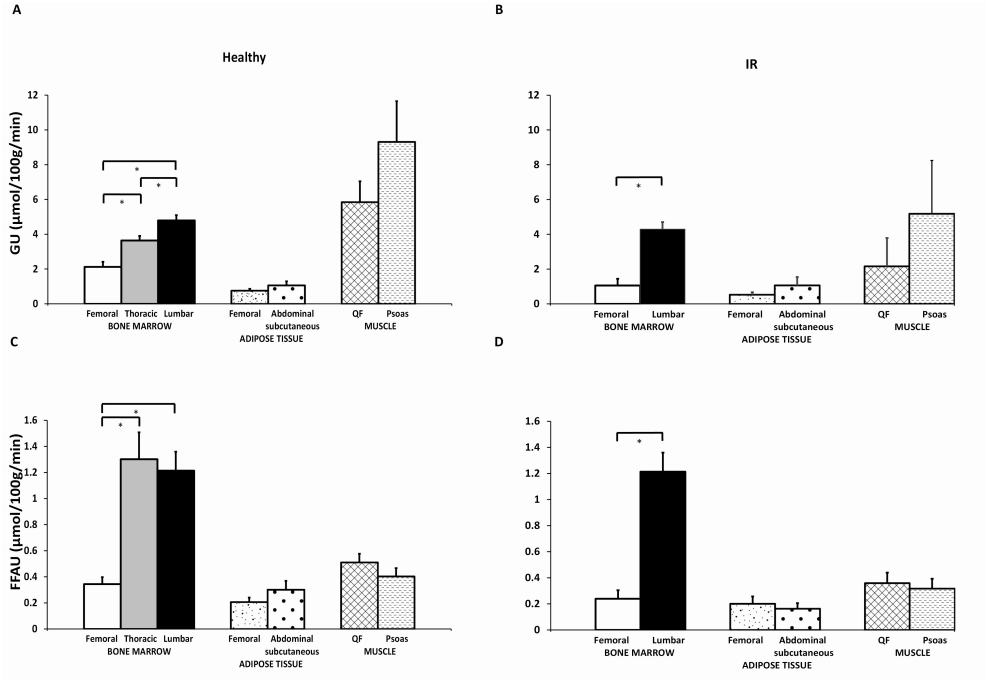

Figure 9 Bone marrow substrate uptake differs according to anatomic region in Study I (HITPET). Statistical analyses were done only between femoral, thoracic vertebral and lumbar vertebral bone marrow results. *P < 0.0001. Data are model-based means with 95% confidence intervals. Abbreviations: FFAU, free fatty acid uptake; GU, glucose uptake; IR, insulin resistant; QF, quadratus femoris muscle. Reprinted with permission from Study I.

Similar regional differences in BM GU were also observed in MZ twin pairs in Study II, as lumbar vertebral BM GU was significantly higher than femoral BM GU ($p < 0.001$) (Figure 12). In the rat study (Study III), we evaluated different skeletal sites (humerus and sternum) than in human studies. However, similar differences in

BM GU between anatomical sites were also observed in rats: BM GU in the axial skeleton (sternum) was significantly higher than in appendicular skeleton (humerus) ($p < 0.001$) (Figure 15).

5.3 Two weeks of exercise training improves bone marrow metabolism (Study I)

Insulin-stimulated GU in femoral BM was impaired in IR men compared to healthy men ($p < 0.0001$) (Figure 10). Training improved GU similarly in both groups. When femoral muscle GU was included as a covariate, the change in femoral BM GU was no longer significant (data not shown). In lumbar vertebral BM GU, no training induced changes were observed (Figure 10).

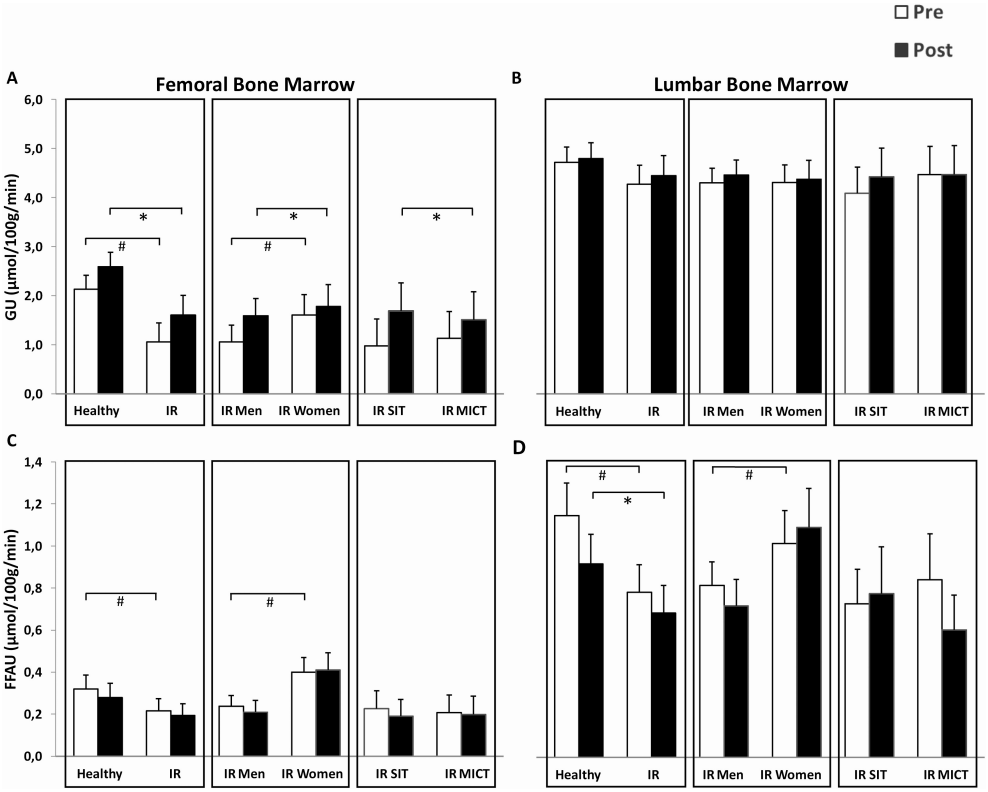

Figure 10 Study I (HITPET) (A-B) Bone marrow insulin-stimulated GU is impaired in IR group and improves after training. (C-D) Bone marrow fasting FFAU is higher in healthy subjects and IR women but improves after training in lumbar vertebrae. # difference at baseline, $p < 0.05$. *difference between pre- and post-measurements. $p < 0.05$. Data are model-based means with 95% confidence intervals. Abbreviations: FFAU, free fatty acid uptake; GU, glucose uptake; IR, insulin resistant; MICT, moderate-intensity continuous training; SIT, sprint-interval training. Reprinted with permission from Study I.

Fasting FFAU in femoral BM was impaired in IR men compared to healthy men ($p = 0.016$) and higher in women compared to men ($p < 0.001$) (Figure 10). This same phenomenon was seen in lumbar vertebral BM ($p = 0.002$ and $p = 0.023$, respectively) (Figure 10). Training decreased lumbar vertebral BM FFAU similarly in healthy and IR men. However, no change was seen in comparisons between sexes or training modes.

Femoral BM GU correlated positively with whole-body insulin sensitivity ($p < 0.0001$, $r = 0.76$) and lumbar BM GU ($p = 0.0004$, $r = 0.40$) and negatively with BMI ($p = 0.0008$, $r = -0.51$) (Figure 11). Both femoral and lumbar vertebral BM GU correlated positively with aerobic capacity (femoral BM $p = 0.014$, $r = 0.39$, and lumbar BM $p = 0.017$, $r = 0.38$). Femoral BM GU correlated positively with lumbar vertebral BM GU ($p = 0.0004$, $r = 0.40$) but did not correlate with femoral or lumbar BM FFAU (Figure 11).

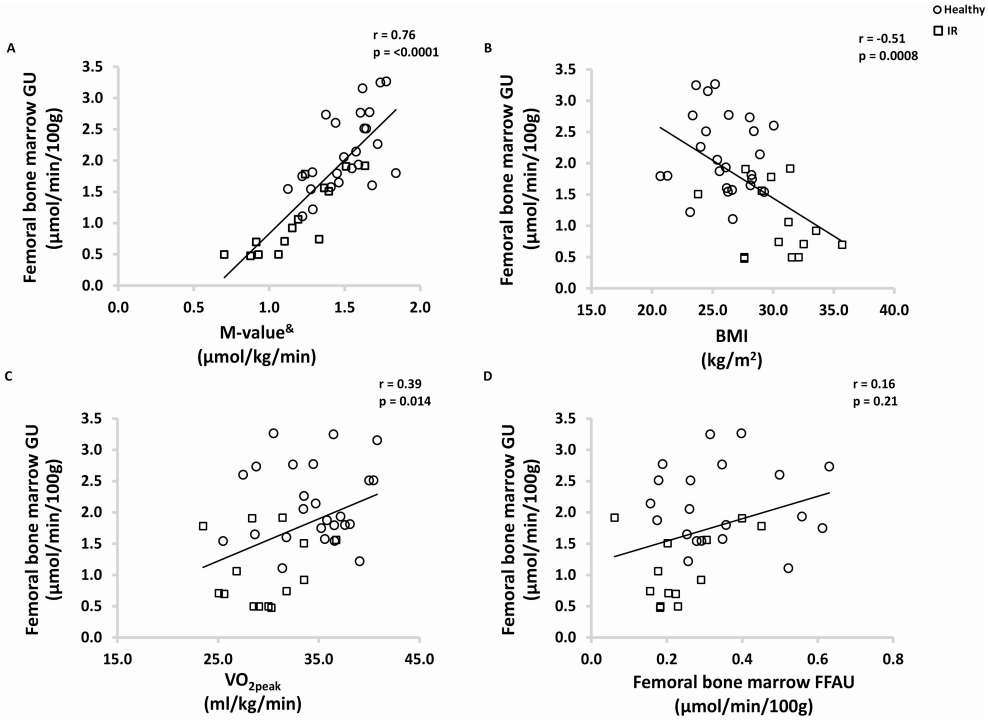

Figure 11 Baseline correlations for Study I (HITPET). Healthy subjects have been marked with a circle and IR subjects with a square. Femoral bone marrow GU correlates positively with whole body insulin sensitivity, aerobic capacity, and negatively with BMI. There was no correlation between femoral bone marrow GU and FFAU. Abbreviations: BMI, body mass index; FFAU, free fatty acid uptake; GU, glucose uptake; IR, insulin resistant; M-value, whole-body insulin sensitivity; VO_{2peak}, aerobic capacity. &Logarithmic transformation was performed to fulfill normal distribution assumption. Reprinted with permission from Study I.

Radiodensity, used to assess BM adiposity, was higher in lumbar vertebral bone marrow (186.1 HU) than in femoral bone marrow (81.0 HU) in men ($p < 0.0001$). Femoral bone marrow radiodensity was significantly lower in healthy men (74.5 HU) compared to IR men (87.5 HU, $p = 0.035$). There was no difference in lumbar vertebral bone marrow radiodensity between healthy (191.6 HU) compared to IR subjects (180.5 HU, $p = 0.35$). There were no exercise induced changes in any of the groups. There was no significant difference between femoral or lumbar vertebral bone marrow radiodensity between IR men and women. Femoral bone marrow radiodensity correlated positively with weight ($p = 0.009$, $r = 0.29$), BMI ($p = 0.016$, $r = 0.27$), fasting glucose ($p = 0.023$, $r = 0.27$), and fasting FFA ($p = 0.025$, $r = 0.26$) and correlated inversely with femoral bone marrow insulin stimulated GU ($p = 0.035$, $r = -0.25$). Lumbar vertebral bone marrow radiodensity correlated negatively with age ($p = 0.031$, $r = -0.25$) and whole body fat percentage ($p = 0.025$, $r = -0.25$) and positively with VO_{2peak} ($p = 0.002$, $r = 0.34$).

A multivariate regression analysis was conducted to study the predictors of femoral and lumbar BM GU and FFAU using the key variables (glycemic status, weight, visceral adipose tissue volume, M-value, VO2peak, fasting glucose, osteocalcin, PINP, and BM radiodensity). At baseline, the only statistically significant finding was the association between femoral BM GU and M-value ($R2 = 0.78$, $P < 0.0001$). Also, when we analyzed the change in response to exercise training, M-value was the only statistically significant predictor for femoral BM GU ($R2 = 0.59$, $P = 0.0004$). None of the aforementioned key variables was a statistically significant predictor for BM FFAU.

5.4 Regular exercise training improves bone marrow metabolism even without reduction in weight (Study II)

Heavier co-twins had higher lumbar vertebral BM insulin-stimulated GU ($p < 0.001$) compared with their leaner co-twins at baseline (Figure 12). No significant difference was observed between the groups in femoral BM GU, BMD, or lumbar vertebral or femoral BM radiodensity.

In all participants at baseline, lumbar vertebral BM GU correlated negatively with whole-body insulin sensitivity (M-value, $r = -0.52$, $p = 0.013$) and positively with body composition (BMI ($r = 0.54$, $p = 0.009$), fat mass ($r = 0.61$, $p = 0.003$) and whole-body fat percentage ($r = 0.52$, $p = 0.014$)), HbA1c ($r = 0.53$, $p = 0.011$) and hs-CRP ($r = 0.77$, $p < 0.001$). Lumbar vertebral BM radiodensity correlated positively with BMD ($r = 0.61$, $p = 0.003$), femoral BM radiodensity ($r = 0.59$, $p = 0.004$), aerobic capacity ($r = 0.68$, $p < 0.001$), bone turnover markers (serum TotalOC ($r = 0.54$, $p = 0.012$), PINP ($r = 0.56$, $p = 0.008$) and CTX ($r = 0.51$, $p = 0.019$)), and

negatively with fat mass ($r = -0.52$, $p = 0.013$), whole-body fat percentage ($r = -0.50$, $p = 0.019$), visceral adipose tissue mass ($r = -0.50$, $p = 0.035$) and hs-CRP ($r = -0.58$, $p = 0.010$) at baseline. BMD correlated positively with BMI at baseline ($r = 0.45$, $p = 0.033$).

The 6-month-long training intervention significantly increased femoral BM insulin-stimulated GU in both groups similarly (time $p = 0.008$) (Figure 12). Interestingly, training response in lumbar vertebral BM GU was different between the groups (time × group $p = 0.023$), as lumbar vertebral BM GU tended to decrease in the heavier co-twins ($p = 0.061$), while there was no significant change in the leaner co-twins ($p = 0.90$) (Figure 12). The training intervention had no effect on vertebral BMD, or lumbar vertebral or femoral BM radiodensity. Change in lumbar vertebral BM GU correlated positively with the change in hs-CRP after exercise training ($r = 0.61$, $p = 0.006$).

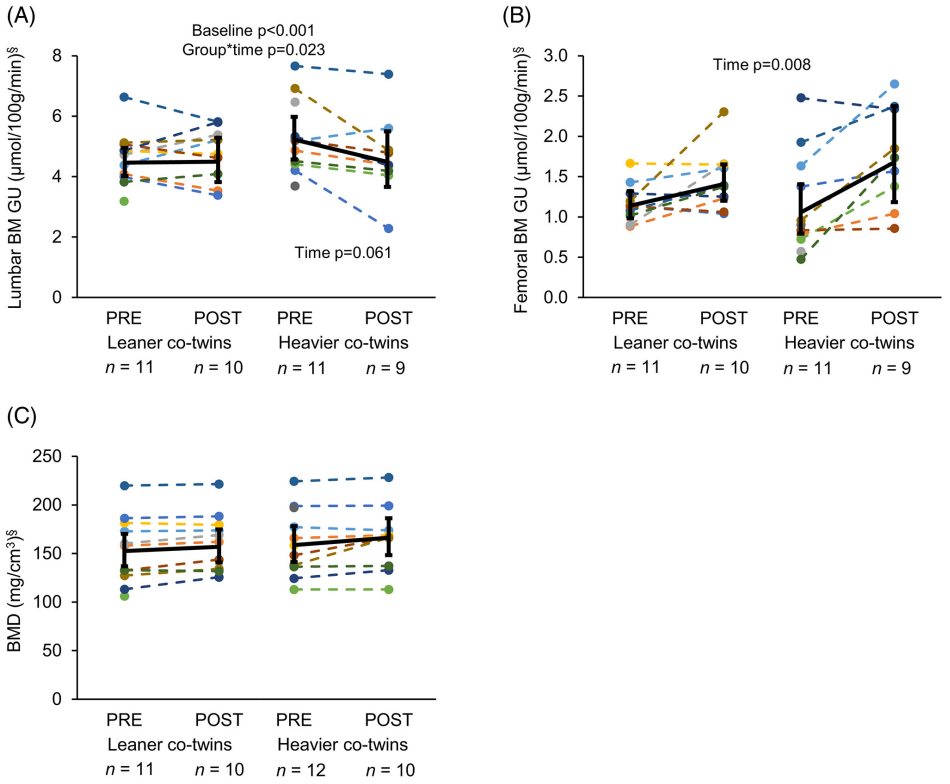

Figure 12 Study II (CROSSYS) (A) Lumbar BM GU is higher in heavier co-twins compared with leaner co-twins at baseline and decreases after training intervention in heavier co-twins. (B) There is no significant difference in femoral BM GU between leaner and heavier co-twins at baseline, and GU increases in both groups after training intervention. (C) There is no difference in BMD between leaner and heavier co-twins before and after training intervention. Data are model-based means with 95% confidence intervals. P-value for baseline indicates the differences between leaner and heavier co-twins at baseline. P-value for time indicates the change between pre- and post-measurements in the whole study group. P-value for group × time interaction indicates if the mean change in the parameter was different between leaner and heavier co-twins. BM, bone marrow; BMD, bone mineral density; GU, insulin-stimulated glucose uptake. §Logarithmic transformation was performed to fulfil normal distribution assumption. Reprinted with permission from Study II.

5.5 Bone turnover markers were lower in insulin resistance and in participants with higher body weight but not affected by exercise training intervention (Studies I and II)

In Study I at baseline, both markers of bone formation, osteocalcin and PINP, were lower in IR than healthy men (Table 2), and correlated positively with whole-body insulin sensitivity and femoral BM GU (Figure 13). PINP also correlated positively with femoral BM FFAU ($p = 0.027$, $r = 0.37$), and lumbar vertebral BM FFAU ($p = 0.026$, $r = 0.37$), and negatively with BMI ($p = 0.033$, $r = -0.34$) and blood triglycerides ($p = 0.017$, $r = -0.37$). However, two weeks of exercise training had no effect on osteocalcin or PINP concentrations in either group (Table 2).

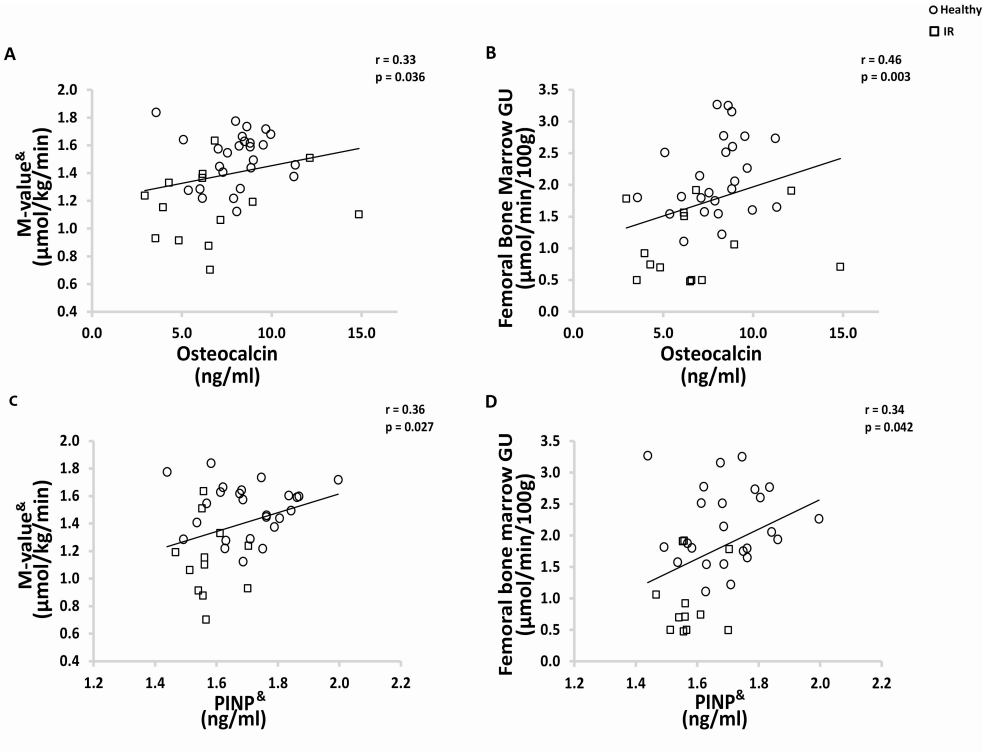

Figure 13 Bone turnover marker correlations in Study I (HITPET) at baseline. Healthy subjects have been marked with a circle and IR subjects with a square. **(A-B)** Osteocalcin correlates positively with whole-body insulin sensitivity and femoral bone marrow GU. **(C-D)** Also PINP correlates positively with whole-body insulin sensitivity and femoral bone marrow GU. Abbreviations: GU, glucose uptake; IR, insulin resistant; M-value, whole-body insulin sensitivity; PINP, procollagen type 1 N-terminal propeptide. &Logarithmic transformation was performed to fulfill normal distribution assumption. Reprinted with permission from Study I.

In Study II, bone turnover markers TotalOC, PINP and CTX were all significantly lower (all $p < 0.01$) in heavier co-twins than in leaner co-twins at baseline (Figure 14). There was no significant difference in plasma ucOC or plasma ucOC/TotalOC ratio between the groups. Osteocalcin, PINP, CTX and plasma ucOC correlated positively with each other at baseline ($p < 0.001$ for all). PINP and CTX correlated negatively with visceral adipose tissue mass ($r = -0.68$, $p = 0.002$ and $r = -0.52$, $p = 0.026$, respectively), and similar association was observed for TotalOC ($r = -0.45$, $p = 0.06$) at baseline. Six months of regular exercise training had no effect on the levels of the analyzed bone turnover markers (Figure 14).

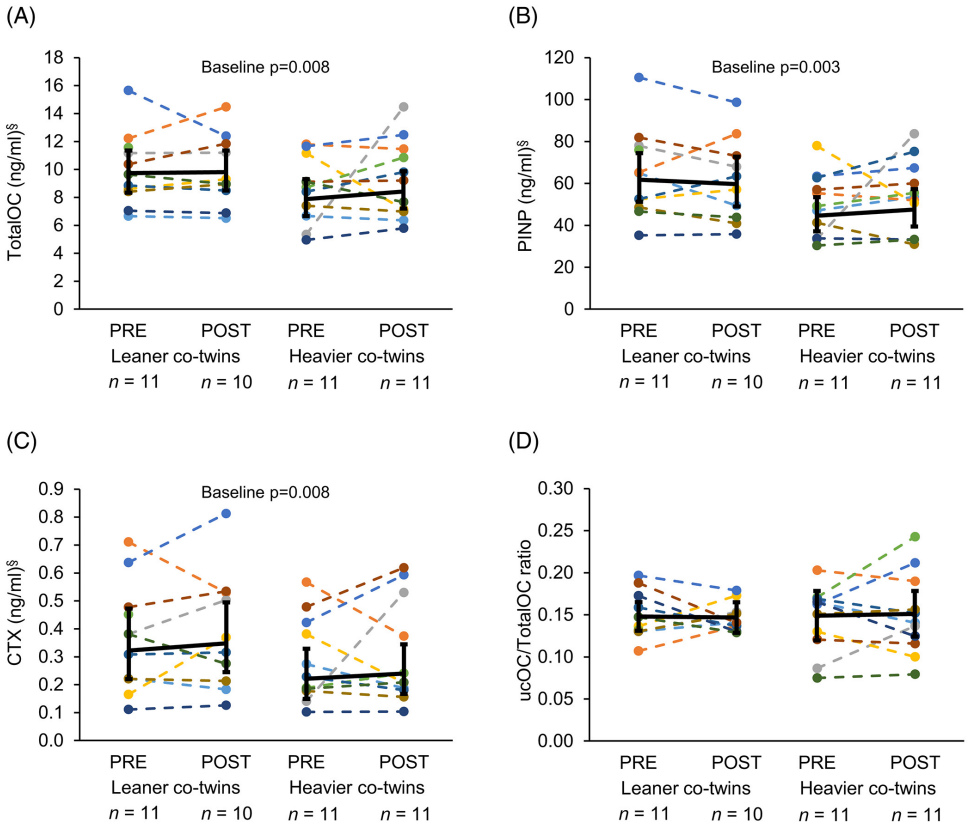

Figure 14 In Study II (CROSSYS), bone turnover markers (TotalOC, PINP and CTX) were lower in heavier co-twins compared with leaner co-twins at baseline. There was no difference between the groups in the plasma ucOC/TotalOC ratio. No exercise effect was seen in bone turnover markers. Data are model-based means with 95% confidence intervals. P-value for baseline indicates the differences between leaner and heavier co-twins at baseline. Abbreviations: CTX, C-terminal crosslinked telopeptide of type I collagen; PINP, N-terminal propeptide of type 1 collagen; TotalOC, total osteocalcin; ucOC, uncarboxylated osteocalcin. §Logarithmic transformation was performed to fulfil normal distribution assumption. Reprinted with permission from Study II.

5.6 Bone marrow glucose uptake is age and site dependent in rats (Study III)

The level of BM GU was age- and site-dependent in the chow group (Figure 15). Sternal and humeral BM GU was significantly higher in 8-week-old rats (^{0}Chow) ($p < 0.001$) than in 20 (^{12}Chow) and 32-week-old (^{24}Chow). At 32 weeks, there was no statistically significant difference in BM GU between any of the intervention groups. However, when we compared the two anatomical locations, sternal BM GU was significantly higher than humeral BM GU when all animals were included in the analysis at 32 weeks.

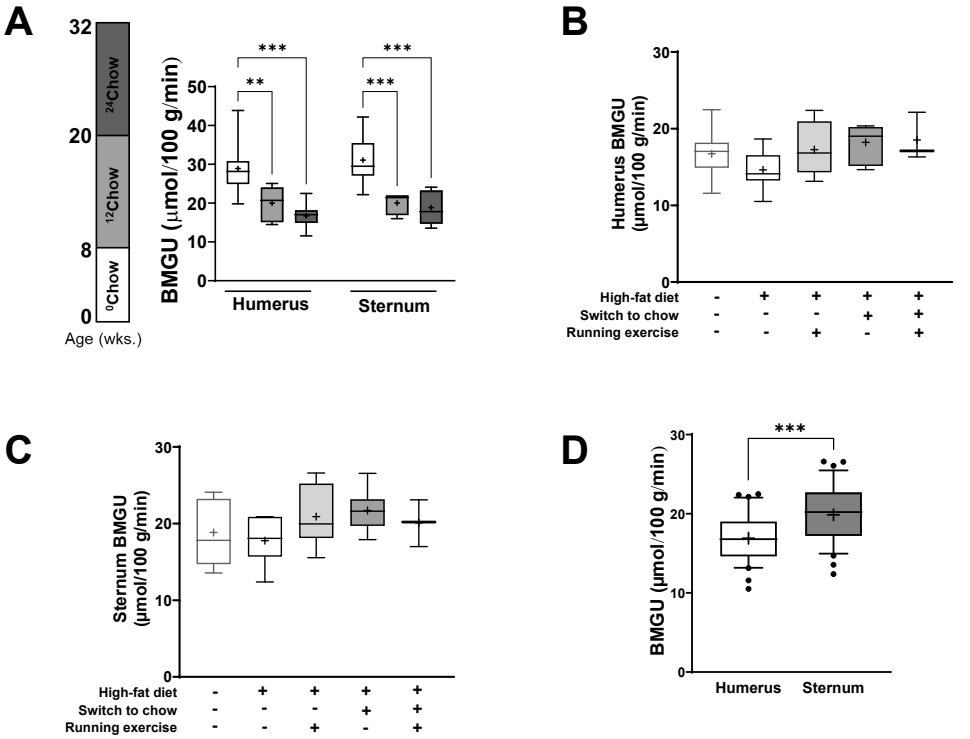

Figure 15 BM insulin-stimulated GU is site- and age-dependent. **(A)** Sternal and humeral BM GU was higher at 8 than at 20 or 32 weeks of age in the control chow group (n = 6-9/group). There was no difference at 32 weeks in **(B)** humeral or **(C)** sternal BM GU (n = 3-8/group). **(D)** Sternal BM GU was significantly higher than humeral at 32 weeks when all intervention groups were combined (n = 32). Data is presented as boxes representing quartiles and whiskers representing the 10th to 90th percentiles. Median is marked with a single line and mean value is marked with a plus sign in each box. Covariance analysis with Tukey's correction for multiple comparison was performed in A and one-way ANOVA with Dunnett's correction for multiple comparison was performed in B and C to compare mean values between groups. The ^{24}HFD group served as the control group for Dunnett's comparison. Student's t-test was performed to compare mean difference between groups in D. *p<0.05, **p<0.01, ***p<0.001. BM, bone marrow; GU, insulin-stimulated glucose uptake. Reprinted with permission from Study III.

5.7 Bone marrow adipocytes resist diet-induced hypertrophy in response to a dietary and/or exercise intervention in rats (Study III)

We evaluated BM adiposity in histological tibia sections (Figure 16). The median size of BMAds in the proximal tibia was significantly larger in ^{24}HFD group than in ^{24}Chow group (815 μm^2 versus 592 μm^2, p = 0.03), indicating a hypertrophic response of BMAds with high-fat diet. However, the hypertrophy of BMAds was not evident in the distal region of the tibia. On average, diet, exercise, and combined intervention slightly decreased the median size of BMAds in the tibia, although this was not statistically significant and the size of BMAds was still larger than in ^{24}Chow control group at 32 weeks. The median size of BMAds in the distal tibia was significantly larger than that in the proximal tibia (822 μm^2 versus 716 μm^2, p = 0.003, data not shown) at 32 weeks. The frequency of small BMAds (200 – 600 μm^2) in the proximal tibia was lower in the ^{24}HFD group compared to ^{24}Chow group (p < 0.001). In line with this, the frequency of large BMAds (1200 – 1600 μm^2) was higher in the ^{24}HFD group compared to the ^{24}Chow group. The distribution of BMAds in the distal tibia was similar across all groups. Histologically, BMAds were more interspersed with BM cells in the proximal tibia than in distal tibia. Regardless, the density of BMAds in the proximal and distal tibia remained similar after interventions.

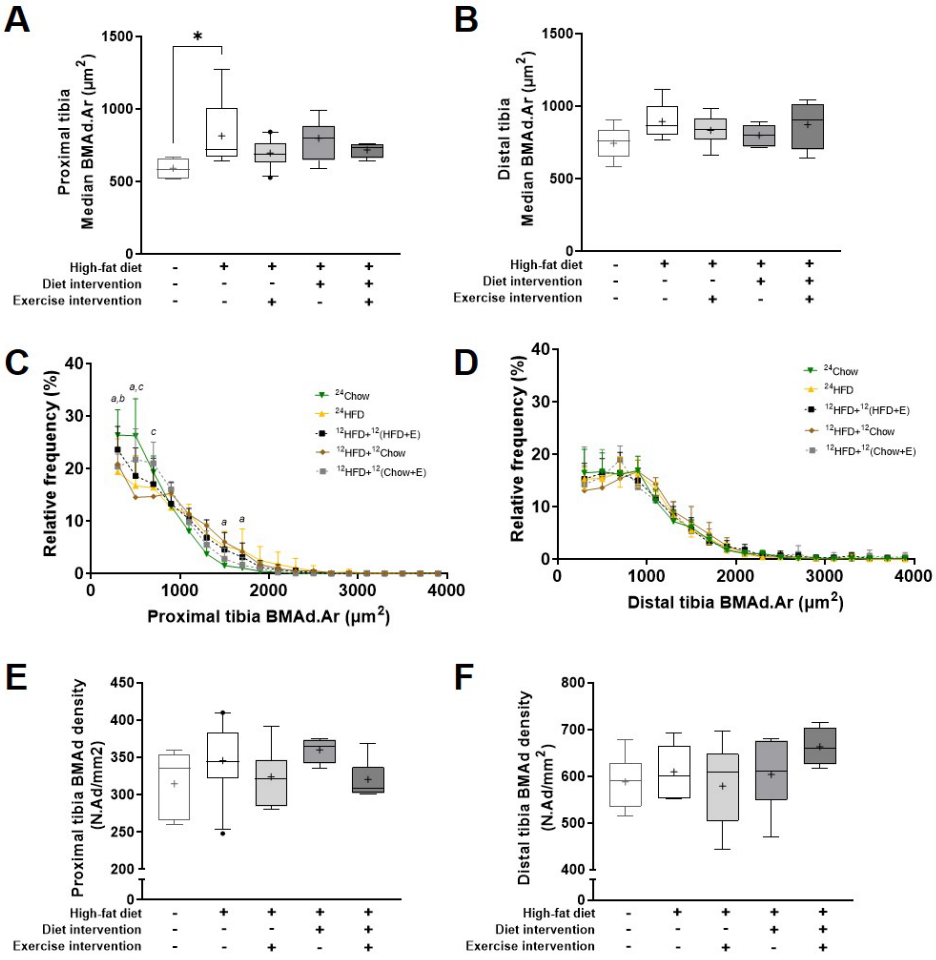

Figure 16 BMAds resist diet-induced hypertrophy in response to a dietary and/or exercise intervention. (A) High-fat diet increased the median size of BMAds in the proximal tibia, but not in (B) the distal tibia. Frequency distribution of the size of BMAds in (C) proximal and (D) distal tibia. A bin width of 200 µm^2 applied from a lower and upper bin centre of 300 µm^2 and 3900 µm^2, respectively. Small letters indicate a significant difference in the mean frequency distribution between the high-fat control group (^{24}HFD) and age-matched control (^{24}Chow, *a*) or the exercising group (^{12}HFD+^{12}(HFD+E), *b*) or the combination group (^{12}HFD+^{12}(Chow+E), *c*) (E and F). The density of BMAds were not affected by study intervention. One-way ANOVA with Dunnett's correction for multiple comparison was performed in A, B, E and F to compare mean values between groups. Data is presented as boxes representing quartiles and whiskers representing the 10th to 90th percentiles. Median is marked with a single line and mean value is marked with a plus sign in each box. Covariance analysis with Dunnett's correction for multiple comparison was performed in C and D to compare group wise mean frequency distribution in each bin. The ^{24}HFD group served as the control group. n = 4-10/group. *p< 0.05. BMAd, bone marrow adipocyte. Reprinted with permission from Study III.

6 Discussion

6.1 Bone marrow metabolism differs regarding anatomical location

One of the major findings in this thesis is that BM metabolism differs regarding anatomical location in humans and rats. GU was higher in BM of axial skeleton (lumbar vertebrae or sternum) and lower in BM of long bones (femur or humerus). The same phenomenon can be seen regardless of glycemic status and also in lipid metabolism (FFAU) in humans.

This finding can be explained by the differences in the composition and function of BM. In adult humans, the marrow cavity of femur is mostly filled with yellow, fatty BM, whereas the lumbar vertebral region still actively produces blood cells (Kricun, 1985; Vogler and Murphy, 1988). This proliferation and differentiation of hematopoietic cells may consume more energy than serving as a specialized fat depot (Hsu and Qu, 2013).

Our data also agrees with Suchacki et al, who found that BMAT had high basal GU compared to white adipose tissue and GU in axial skeleton was even higher than in skeletal muscle. However, because of the imaging modalities used in our study, we cannot differentiate between the different tissue types in the BM cavity and can only present our findings for the whole BM niche. In our study, BM insulin-stimulated GU was also higher than white adipose tissue GU in all evaluated anatomical locations and regardless of glycemic status. Suchacki et al suggested that the higher GU in BMAT was required for normal metabolic function and the ability for BMAT to act as a local energy source to support hematopoiesis, but this should be investigated further (Suchacki *et al.*, 2020).

Nevertheless, the high basal and insulin-stimulated GU suggests that BM metabolism may play a part in whole-body insulin sensitivity and influence systemic glucose homeostasis.

6.2 Short-term and regular exercise training improve femoral bone marrow metabolism

Femoral BM GU increased already after two weeks of exercise training in Study I and the same phenomenon was observed after six months of regular exercise training in Study II.

In Study I at baseline, healthy men had higher femoral BM GU than IR men. IR women also had higher BM GU than IR men. Females are known to be more insulin sensitive than men (Yki-Järvinen, 1984; Kautzky-Willer *et al.*, 2012), and our results show this also at BM level. However, there were no differences in lumbar vertebral BM GU between the groups. The need for energy of lumbar vertebral BM, e.g. containing hematopoietic tissue, seems to be constant and not affected by glycemic status or sex. However, femoral BM appears to serve mainly as a fat depot, which has been shown to be insulin sensitive and respond to exercise (Huovinen *et al.*, 2016; Pham *et al.*, 2020).

When we further studied the difference between the two anatomical locations using CT-derived HU, the radiodensity was lower in femoral than lumbar vertebral BM indicating higher fat content in femoral BM. However, the BM cavity includes adipose tissue, hematopoietic tissue, and trabecular bone. Therefore, attenuation measurements of BM cavity include multiple tissues and adipose tissue alone cannot be quantified. Nevertheless, the lower the HU, the higher the fat content (Singhal and Bredella, 2019). In this context, we found that lumbar vertebral BM radiodensity was in inverse relationship with age and whole-body fat percentage suggesting either an increase in adiposity or decrease in trabecular bone in lumbar BM cavity with aging and obesity. In Study I, in femoral diaphyseal BM cavity, where the influence of trabecular bone on BM radiodensity is negligible, we found lower femoral BM radiodensity (high-fat content) in healthy compared to IR subjects. Further, we found that there is a direct correlation of femoral BM radiodensity with body weight and BMI and an inverse relationship with insulin-stimulated femoral BM GU. These findings are in line with Ermetici et al (Ermetici *et al.*, 2018), who measured BM fat content using proton magnetic resonance spectroscopy, and it was found that BM fat content (%) was inversely related to the index of whole-body IR. It is possible that a higher fat content (more adipocytes) in BM in healthy compared to IR subjects drives an increase in insulin-stimulated GU since marrow adipocytes express insulin receptors (Lecka-Czernik, 2012), and insulin stimulates GU in BM adipose tissue (Pham *et al.*, 2020). In line with these findings femoral BM GU, but not lumbar vertebral BM GU, correlated negatively with BMI and positively with whole-body insulin sensitivity, and IR subjects had lower insulin-stimulated BM GU than healthy subjects at baseline.

After the training intervention in Study I, we found an increase in femoral BM insulin-stimulated GU but not in lumbar vertebral and thoracic vertebral BM GU. In

femoral BM, training increased GU in all groups. This finding agrees with previous data from the same study protocol regarding the changes in skeletal muscle GU where Eskelinen et al. showed that GU improved only in the working muscles of the lower extremities and not in the upper body muscles (Eskelinen *et al.*, 2015). The training intervention consisted of bicycle ergometer training, which mainly strains the lower extremities, likely explaining why GU improved only in femoral BM. Our data agree also with the findings of Huovinen and colleagues, who investigated the effects of whole-body resistance training on insulin-stimulated BM in elderly subjects and found that GU improved in femoral BM but not in vertebral BM after training (Huovinen *et al.*, 2016). The difference in training response between vertebral and femoral BM GU can also be explained by the amount of fat and the role of BM. Hematopoietic tissue in lumbar vertebral BM may not be as easily affected by external stimuli, such as exercise training, compared to femoral BM.

Increase in the femoral BM insulin-stimulated GU was no longer significant when we corrected the statistical analysis for muscle GU. This suggests that the increase in femoral BM GU was not independent of the increase of GU in the surrounding muscle tissue. Indeed, in the multivariate analysis M-value showed to be the only statistically significant predictor of BM insulin-stimulated GU. Our finding of increased femoral BM GU may be also partially due to the PET methodology related spillover effect — that is, spilling of activity from the neighboring high activity tissues (muscles) to the less active areas (bone tissue). However, cortical bone between BM cavity and skeletal muscle tissue should minimize the spillover effect in our analyses (Figure 6). Our study suggests that BM metabolism is improved by exercise training, however, further studies are needed to clarify the proportion of independent and muscle metabolism-induced changes.

In Study I at baseline, FFAU was higher in healthy compared to IR men, as well as IR women compared to IR men. However, high FFAU in other fat depots is generally associated with higher weight and/or worse metabolic profile. In this study it may be explained by higher adiposity as described earlier. At baseline, females had higher amount of circulating FFAs than males, which may explain the difference in FFAU between sexes. Exercise training had no effect on the amount of circulating FFAs. FFAU was not affected by exercise training in the IR group. The sample size for IR men and women was small so this response should be investigated further.

In Study II, femoral BM GU did not differ between leaner and heavier co-twins at baseline. However, femoral GU at baseline was lower compared with healthy lean participants and in line with the values we published in participants with insulin resistance in Study I. Femoral BM GU was also lower compared with the results in an elderly cohort of frail and control study participants (Huovinen *et al.*, 2016). In Study II, participants were allocated to leaner and heavier groups within each twin pair based on BMI. Although the mean difference in BMI between the groups was

7.6 kg/m^2, both groups can be classified as overweight on average (BMI >25) and there were subjects with IFG and/or IGT in both leaner and heavier twin groups. Thus, our data collectively suggest, that femoral BM GU is more strongly associated with glycemic status than body weight alone.

After training, femoral BM GU increased in leaner and heavier co-twins similarly regardless of the significant weight difference at baseline and without training-induced weight loss. This is in line with the results of Study I.

Together this data suggests that metabolism in femoral BM can be improved by exercise training independent of glycemic status, body weight at baseline, and training-induced weight loss. The data suggests as well that not one exercise modality is better than the other (regarding BM metabolism), as long as you move your body. Thus, improved femoral BM metabolism due to exercise training may be beneficial for patients in risk of developing diseases associated with insulin resistance.

6.3 Effects of exercise training on lumbar bone marrow are tied to its biological function

Lumbar vertebral BM has not been shown to be insulin-sensitive (Pham *et al.*, 2020; Suchacki *et al.*, 2020). Intriguingly, in Study II, heavier co-twins had higher lumbar vertebral BM GU than their leaner co-twins at baseline and lumbar vertebral BM GU correlated positively with body weight at baseline. Obesity induces low-grade inflammation (Pereira and Alvarez-Leite, 2014) and at baseline, heavier co-twins had higher levels of hs-CRP, an inflammation marker, compared with leaner co-twins. Thus, our results suggest that in the heavier co-twins increased low-grade inflammation may activate the cells of the immune system and hematopoiesis in lumbar vertebral BM resulting in a higher need for energy in BM at baseline. We also found a positive correlation between lumbar vertebral BM GU and hs-CRP at baseline. This suggested link between lumbar vertebral BM GU and low-grade inflammation is supported by the findings of Devesa et al. in a large cohort of more than 700 participants as they showed that lumbar vertebral BM activation was associated with increased immune system activation, increased hematopoiesis and with markers of systemic inflammation, such as hs-CRP (Devesa *et al.*, 2022).

In Study II, study groups responded differently to training with respect to lumbar vertebral BM GU. In heavier co-twins, lumbar vertebral BM GU decreased close to the level of leaner co-twins, while there was no change in leaner co-twins. Decrease in lumbar vertebral BM GU correlated positively with the decrease in hs-CRP, suggesting reduced systemic inflammation and lower GU in lumbar vertebral BM after exercise training. Regular exercise training decreases systemic low-grade inflammation (Gonzalo-Encabo *et al.*, 2021). Our data suggest that the decrease in

lumbar vertebral BM GU in heavier co-twins may be explained by reduced need for energy because of the decrease in inflammation-induced hematopoiesis. To our knowledge, this has not been studied before in a preclinical setting and is an interesting topic for future research.

In Study I, we found no effect of exercise on lumbar vertebral BM GU when we analyzed the effects of short-term exercise training on BM metabolism. We speculate the 2-week intervention to be too short to induce changes in low-grade inflammation.

In Study II, we measured BM radiodensity to assess BM fat content. There was no difference in femoral or lumbar vertebral BM radiodensity between co-twins at baseline, and femoral or lumbar vertebral BM radiodensity did not change in either group regardless of 6 months of regular exercise training. With the used imaging modalities, we cannot differentiate between different tissue types in the BM cavity. However, the lower the HU, the higher the fat content (Singhal and Bredella, 2019). There was also no significant change in body weight or body composition in either group. In Study I, femoral BM radiodensity was lower in healthy men compared with men with insulin resistance. Furthermore, 2 weeks of exercise training did not induce changes in any of the groups. This suggests that exercise training without weight loss has no effect on BM adiposity, and that the changes we see in BM metabolism after exercise training are caused by changes in tissue metabolism rather than changes in fat infiltration into BM.

6.4 Bone turnover markers are more closely tied to changes in weight than affected by exercise training

Osteocalcin is a biochemical bone formation marker that is produced by osteoblasts. In Study I, osteocalcin concentrations were lower in IR than in healthy group at baseline, which is in line with previous observations (Ivaska *et al.*, 2017). It has also been shown that circulating osteocalcin is negatively associated with IR, obesity, and diabetes (Addai, Zarkos and Tolekova, 2019). Our results support these findings, as osteocalcin correlated positively with whole-body insulin sensitivity. In addition, to our knowledge, we showed for the first time that osteocalcin correlated positively with femoral BM GU at baseline. Another bone formation marker evaluated in this study, PINP, is synthesized by osteoblasts as part of Type I collagen formation, and it has been recommended to be used as a reference analyte for bone turnover markers in observational and intervention studies (Vasikaran *et al.*, 2011). It has not been clearly established yet how IR or diabetes affects PINP concentration (Costantini and Conte, 2019). Similarly to osteocalcin, PINP concentration was lower in the IR group than in the healthy group. PINP also correlated positively with BM GU and FFAU, whole-body insulin sensitivity, and negatively with BMI. However, there

were no significant changes in either of the bone markers after the exercise training intervention. A 2-week training intervention may be too short to induce significant changes in circulating osteocalcin or PINP concentrations. Also, training consisted of cycling, which may not stimulate bone turnover as much as, for example, running or other high-impact exercise (Heinonen *et al.*, 1996; Beck *et al.*, 2016).

In Study II we also measured BMD using qCT. Obesity is associated with higher BMD (Qiao *et al.*, 2020). In Study II, there was no difference in BMD between co-twins at baseline, and both groups had mean BMD within normal reference interval, that is above the upper limit for osteopenia 120 mg/cm^3 (The American College of Radiology, 2023). BMD correlated positively with BMI at baseline. However, how the effect of exercise on bone is influenced by obesity has not been widely studied (Zouhal *et al.*, 2022). While the benefits of weight-bearing exercise on bone are well established (Beck *et al.*, 2016; Weaver *et al.*, 2016), the effects of exercise are mostly explained by baseline BMD level. We found no training effect in BMD in either group. In accordance to our study, Zouhal et al. suggest that in people with overweight or obesity, BMD is not expected to increase, as they already have high BMD values (Zouhal *et al.*, 2022). Six months may also be too short a time to detect significant changes in BMD (Brunnquell *et al.*, 2021). Interestingly, when we analyzed BMD individually, not taking into account the study participants' twin pair status, there was a statistically significant increase of 4.1% ($p = 0.005$, 95% CI 1.5; 6.8) in BMD (data not shown). It appears that with our current statistical model and relatively small number of participants we were not able to detect a statistically significant change in BMD in response to exercise training.

In Study II we also measured bone turnover markers to assess the effect of exercise training on bone metabolism at the molecular level. Being more dynamic than BMD, bone turnover markers are routinely employed for rapid monitoring of both anti-resorptive and anabolic treatments (Eastell *et al.*, 2018). At baseline, bone turnover was suppressed in heavier co-twins and in all participants bone turnover markers associated negatively with lumbar vertebral BM radiodensity. These outcomes agree with previous findings (Addai, Zarkos and Tolekova, 2019). However, exercise training had no effect on bone turnover markers. We previously showed, that bone turnover markers, suppressed by obesity, increased and reached levels beyond normal-weight control subjects 6 months after bariatric surgery, suggesting a high bone turnover rate postoperatively after significant reduction in body weight (Ivaska *et al.*, 2017). It seems that bone turnover markers are more closely associated with changes in weight and body adiposity and not as drastically affected by exercise training *per se*.

6.5 Bone marrow glucose uptake is age- and site-dependent but not significantly affected by exercise in rats

A long-term (24 wks) exposure to HFD markedly increased body weight, whole-body adiposity and hypertrophy of BMAds in the proximal tibia in rats, consistent with previous findings in mice (Tencerova *et al.*, 2018). Animals with both diet and exercise intervention had the most pronounced reduction in whole-body fat percentage when compared to ^{24}HFD and ^{24}Chow groups. HOMA-IR index, a measure of insulin resistance, was significantly lower in both groups that switched to chow diet compared to ^{24}HFD group suggesting improved glucose homeostasis. Interestingly, running exercise during HFD did not decrease the whole-body fat percentage when compared to the sedentary ^{24}HFD group.

BM GU was higher in 8-week-old rats compared to older rats (aged 20 or 32 weeks) in both humeral and sternal BM. This phenomenon may be explained by the function of BM and the physiological "red-to-yellow" transition (Kricun, 1985). The BM cavity of young rodents, as well as humans, consists predominantly of hematopoietic tissue, which, during aging, is replaced by adipocyte-rich yellow BM. As in humans, the proliferation and differentiation of hematopoietic stem cells into different blood cell types requires a robust upregulation of energy metabolism compared to less metabolically active BMAT (Hsu and Qu, 2013). Also, the need for energy caused by growing may be reflected in the BM GU. Taken together, high BM GU in younger rats may represent more active hematopoiesis and bone formation than low BM GU in skeletally mature and BMAT-enriched marrow of older rats.

At 32 weeks, sternal BM GU was significantly higher than humeral BM GU when all intervention groups were combined. Cline et al showed in rats that cellular composition of humeral and sternal BM is different as sternal BM contains more hematopoietic tissue (Cline and Maronpot, 1985). Our data agrees with this finding, which is reflected by higher BM GU in the sternum than in humerus. The same phenomenon can be seen in humans when comparing vertebral and femoral BM GU as previously stated (Huovinen *et al.*, 2016; Ojala *et al.*, 2020, 2024). Additionally, we found that humeral, but not sternal, BM GU correlated inversely with whole-body fat percentage in rats that were fed HFD. It seems that higher BM GU tied to hematopoiesis in the axial skeleton is more independently regulated than BM GU in BM of long bones. Our data suggests that even small differences in BM cellularity can result in significant differences in BM metabolism and GU.

Despite differences in body weight, whole-body fat percentage, and glycemic status, we did not observe any statistically significant differences in BM GU between intervention groups. However, on average it seems that both switching to standard chow and starting exercise training increase BM GU compared to ^{24}HFD group. This

agrees with our previous findings from human Studies I and II. We have also shown that changes in BM metabolism can occur even without change in BM adiposity measured using radiodensity in CT images (Ojala *et al.*, 2020, 2024). It seems that this same phenomenon can be seen in rats and changes in BM GU and adiposity can occur independent of each other. However, this requires further studies.

One reason that could explain the finding of no differences between groups in BM GU and whole-body insulin sensitivity (M-value), unlike our previous findings in studies in humans, is that in rats [^{18}F]FDG PET/CT imaging was performed during isoflurane anesthesia unlike humans that were awake (Huovinen *et al.*, 2016; Ojala *et al.*, 2020, 2024; Jalo *et al.*, 2024). Isoflurane anesthesia has been shown to cause hyperglycemia in humans and in rats (Lattermann *et al.*, 2001; Tanaka *et al.*, 2009, 2011). The increased levels of plasma glucose are a consequence of impaired insulin secretion, impaired glucose clearance and increased glucose production (Lattermann *et al.*, 2001; Tanaka *et al.*, 2009). To achieve successful imaging of rodents, they need to be anesthetized for the duration of the study. This may affect the reliability of the results of the euglycemic-hypersinsulinemic clamp and BM GU in this study. We believe that BM metabolism can be affected by exercise training. However, this study design was unsuccessful in showing this in rats. Also, the effect of aging in decreasing insulin stimulated BM GU may concomitantly mask the effect of our intervention. To elucidate the effect of anesthesia and aging on insulin sensitivity measurements further studies are needed.

Although interventions reduced whole-body fat percentage, neither diet nor exercise intervention was able to reduce BM adiposity by total adipocyte area close to the age-matched control (^{24}Chow). Indeed, BMAT has been reported to be distinct from extramedullary adipose depots (Cawthorn *et al.*, 2014; Scheller *et al.*, 2019; Attané *et al.*, 2020; Pham *et al.*, 2020), which may explain why BMAT was relatively resistant to interventions in our experimental model. For instance, the limited lipolytic capacity of BMAds compared to subcutaneous white adipocytes may partly explain the resistance of BMAT to remodel under external stimuli (Scheller *et al.*, 2019; Attané *et al.*, 2020). Further, BMAT is presented with higher expression of inflammation-related genes compared to other extramedullary adipose tissues under basal condition (Tencerova *et al.*, 2018); this may prime adipogenesis upon early exposure to the HFD. In accordance with this, BM global gene expression showed a pro-adipogenic and pro-inflammatory profile with HFD (Sontam *et al.*, 2017). In their study, rats with exercise intervention, which is similar to our ^{12}HFD+^{12}(HFD+E) group, remained pro-adipogenic but not pro-inflammatory after exercise training when compared to the sedentary HFD rats; this may explain the relatively stable morphology of BMAds observed in our study. However, specific changes in BMAds remain to be investigated.

The hypertrophy of adipocytes in the BM cavity of the proximal tibia was most pronounced in the ^{24}HFD group. Interestingly, this hypertrophic response to HFD was site-specific as we did not observe similar changes in the morphology of BMAds in the distal tibia. This may suggest a heterogeneity in the population of BMAds in the tibia similar to the work by Scheller et al. (Scheller *et al.*, 2015). They showed that BM of the tibia houses two distinct subpopulations of adipose tissue, rMAT and cMAT. rMAT resides in the proximal region with BMAds surrounded by BM cells and is more responsive to external stimuli whereas cMAT in the distal region comprises of tightly clustered BMAds that are more resistant to external stimuli. This established heterogeneity may partly explain the differences observed in the response of BMAds in the proximal and distal regions in our model.

In Study III, we show that HFD increased the size of BMAds in the proximal tibia which correlated inversely with insulin-stimulated BM GU suggesting that BMAT is not protected from obesity. Although more studies are needed to mechanistically link the size of BMAds and BM GU, research on white adipocytes show the association between adipocyte hypertrophy and insulin resistance (Kim *et al.*, 2015; McLaughlin *et al.*, 2016; Verboven *et al.*, 2018). The analyzed regions for BM adiposity (proximal and distal tibia) and BM insulin-stimulated GU (humerus and sternum) were different in Study III. However, BM of the tibia and humerus are similar in that marrow cellularity, BMAT volume and BM GU are comparable (Cline and Maronpot, 1985; Cawthorn *et al.*, 2016; Suchacki *et al.*, 2020).

While many studies have shown marked reduction in BM adiposity with diet and exercise intervention, our study model showed a rather moderate effect. This may have been the result of the use of less energy-dense diet and intermittent voluntary running intensity compared to others (Styner *et al.*, 2014, 2017; Scheller *et al.*, 2016; McCabe *et al.*, 2019). Further, the difference in the duration of the interventions and the age of animals may affect the response of BMAT to the interventions. For instance, ageing-related expansion of BMAT (Scheller *et al.*, 2015; Ambrosi *et al.*, 2017; Tratwal *et al.*, 2020) may have masked the effect of intervention in older animals. It might be that the effect of the interventions on basic anthropometrics as well as BMAT and BM metabolism could have been more pronounced after a longer or more rigorous training or with a diet containing more fat. Our study intervention was designed to reflect the feasibility in clinical practice and provide mechanistic insights that parallels to our previous human studies (Ojala *et al.*, 2020, 2024).

Overall, the results of Study III suggest that BMAT is not protected from the effects of obesity on tissue morphology while BM GU is unaffected. To reverse BMAd hypertrophy and induce favorable changes in BM GU, a more intense intervention may be required.

6.6 Strengths, limitations and future aspects

A major strength of this thesis is that it comprehensively covers effects of both short-term (Study I) and long-term (Study II) exercise training on BM metabolism as well as provides insights into the mechanisms behind these effects through the rat *in vivo* study (Study III). First, we showed that exercise training can affect BM metabolism, and then we showed that these were not only acute effects of exercise but adaptations in BM after regular exercise training.

Another strength of this thesis is the use of state-of-the-art imaging modality, dynamic PET, to measure substrate uptake into tissues. Also, the PET imaging was conducted during insulin-stimulated conditions to assess whole-body insulin sensitivity (M-value) in both humans and rats. This value is considered to be the most definitive measure of insulin sensitivity in humans (DeFronzo, Tobin and Andres, 1979).

An additional strength of Study II is the unique study design which allows us to study the effects of acquired weight and exercise on bone and BM as the effects of genetics and shared environmental factors can be excluded in the analysis for causal factors.

A strength of Study III is the use of both *in vivo* and *ex vivo* methods to study the connection between morphology of BMAds and BM GU. To our knowledge, this is the first study to combine dynamic PET imaging and morphological histology to assess the effects of high-fat diet induced obesity and exercise on BMAds and BM GU.

This thesis is not without limitations. One of the limitations of this thesis is the low number of participants included in the studies. The number of study subjects for Study I was relatively small but typical for exercise training trials using demanding molecular imaging modalities. In Study II we sent letters to all twin pairs (n = 53) in the cohorts but the study was performed during the COVID-19 pandemic, and a number of possible study participants perhaps decided not to participate because of this. In Study III, 8-13 rats in each group underwent PET imaging. However, because of problems during the imaging after the intervention, a smaller number of rats finally completed imaging successfully. It was not possible to redo the experiment because of time and money constraints.

In Study I, running or other high-impact loading exercise would probably have been the best type of exercise to stimulate BM metabolism and bone turnover optimally. However, to standardize the SIT and MICT protocols in laboratory settings in sedentary subjects, we preferred cycling in the study. In Study II the intervention was personalized to suit the participants' day to day life, and included loading exercise two to four times a week.

In this thesis, with the used PET/CT modalities, it was not possible to differentiate between the different tissues inside the BM cavity so the reported

uptake values are for the whole BM niche, not for certain tissue types. With the emergence of whole-body PET scanners, the dose of the radiotracer can be decreased as the whole body can be imaged at one time, and subsequently the resolution of CT could be increased to better study the contents of the BM niche.

Another modality to consider in future BM studies is magnetic resonance imaging (MRI) as well as magnetic resonance spectroscopy (MRS). MRI has been proven as a safe and non-ionizing imaging method in contrast to PET/CT (Formica and Silvestri, 2004). MRI has become the preferred imaging method to evaluate diseases of BM (Porter, Shields and Olson, 1986). MRI and MRS could be used to assess BM adiposity and the lipid composition of BM adipocytes to better understand BM fatty acid metabolism (Beekman *et al.*, 2023). BM fat fraction can be studied using several MRI techniques, e.g. short T1 inversion recovery, chemical shift imaging (Dixon method), and MRS. However, contrary to PET/CT, none of these methods can assess metabolism *in vivo*.

Finally, further translational research is needed to better understand the role and dynamics of the different tissue types in BM in response to exercise training, and the role of BM in systemic glucose homeostasis.

7 Conclusion

In summary, this thesis shows that

1. Bone marrow metabolism differs regarding anatomical location in humans and in rats. Short-term exercise training improves bone marrow glucose and free fatty acid metabolism similarly in healthy and insulin resistant men, similarly in men and women and regardless of training method. Bone turnover markers osteocalcin and PINP are associated with insulin sensitivity.
2. When genetic variability is controlled, regular exercise training increases femoral bone marrow glucose metabolism independent of body weight while there is no effect on bone marrow adiposity, bone mineral density or bone turnover markers. Interestingly, lumbar vertebral bone marrow glucose metabolism is higher in participants with higher body weight and regular exercise training counteracts this effect even without reduction in weight. Bone marrow seems to respond differently to increased body weight and physical activity depending on its biological function and anatomical location.
3. In rats, bone marrow insulin-stimulated glucose uptake decreases during aging. High-fat diet increases the size of bone marrow adipocytes in the proximal but not distal region of the tibia. These phenomena are not significantly affected by exercise and/or diet intervention.

To conclude, exercise training improves over-all health and bone marrow metabolism even without reduction in weight. This thesis shows that bone marrow is metabolically active and responds to physical activity depending on its cellular composition and anatomical location. More studies are warranted to establish the role of bone marrow in whole-body glucose metabolism.

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References

Abramoff, M., Magalhães, P. and Ram, S. J. (2003) 'Image Processing with ImageJ', Biophotonics International, 11, pp. 36–42.

Addai, D., Zarkos, J. and Tolekova, A. (2019) 'The bone hormones and their potential effects on glucose and energy metabolism', Endocrine regulations, 53(4), pp. 268–273.

Adler, B. J., Kaushansky, K. and Rubin, C. T. (2014) 'Obesity-driven disruption of haematopoiesis and the bone marrow niche', Nature Reviews Endocrinology, 10(12), pp. 737–748.

Ali, O. (2013) 'Genetics of type 2 diabetes.', World journal of diabetes, 4(4), pp. 114–123.

Ambrosi, T. H. et al. (2017) 'Adipocyte Accumulation in the Bone Marrow during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration', Cell Stem Cell, 20(6), pp. 771-784.e6.

American Diabetes Association (2015) 'Classification and diagnosis of diabetes', Diabetes Care, 38(Supplement 1), pp. S8–S16.

American Diabetes Association (2019) '2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2019', Diabetes Care, 42(Supplement_1), pp. S13–S28.

American Diabetes Association (2022) '2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2022', Diabetes Care, 45(Supplement_1), pp. S17–S38.

Arponen, M. et al. (2020) 'Recombinant Antibodies with Unique Specificities Allow for Sensitive and Specific Detection of Uncarboxylated Osteocalcin in Human Circulation', Calcified Tissue International, 107(6), pp. 529–542.

Attané, C. et al. (2020) 'Human Bone Marrow Is Comprised of Adipocytes with Specific Lipid Metabolism', Cell Reports, 30(4), pp. 949-958.e6.

Beck, B. R. et al. (2016) 'Exercise and Sports Science Australia (ESSA) position statement on exercise prescription for the prevention and management of osteoporosis', Journal of Science and Medicine in Sport, (20), pp. 438–445.

Beekman, K. M. et al. (2023) 'Osteoporosis and Bone Marrow Adipose Tissue', Current osteoporosis reports, 21(1), pp. 45–55.

Bérard, A., Bravo, G. and Gauthier, P. (1997) 'Meta-analysis of the effectiveness of physical activity for the prevention of bone loss in postmenopausal women', Osteoporosis International, 7(4), pp. 331–337.

Bouchard, C. et al. (2015) 'Personalized Preventive Medicine: Genetics and the Response to Regular Exercise in Preventive Interventions', Progress in Cardiovascular Diseases, 57(4), pp. 337–346.

Bredella, M. A. et al. (2012) 'Vertebral Bone Marrow Fat Is Positively Associated With Visceral Fat and Inversely Associated With IGF-1 in Obese Women', Obesity, 19(1), pp. 49–53.

Brunnquell, C. L. et al. (2021) 'Sources of error in bone mineral density estimates from quantitative CT', European Journal of Radiology, 144, p. 110001.

Buchheit, M. and Laursen, P. B. (2013) 'High-Intensity Interval Training, Solutions to the Programming Puzzle', Sports Medicine, 43(5), pp. 313–338.

Calvi, L. M. and Link, D. C. (2014) 'Cellular complexity of the bone marrow hematopoietic stem cell niche', Calcified Tissue International, 94(1), pp. 112–124.

Case, N. et al. (2013) ‘Mechanical input restrains PPARγ2 expression and action to preserve mesenchymal stem cell multipotentiality’, Bone, 52(1), pp. 454–464.

Cawthorn, W. P. et al. (2014) ‘Bone Marrow Adipose Tissue Is an Endocrine Organ that Contributes to Increased Circulating Adiponectin during Caloric Restriction’, Cell Metabolism, 20(2).

Cawthorn, W. P. et al. (2016) ‘Expansion of Bone Marrow Adipose Tissue During Caloric Restriction Is Associated With Increased Circulating Glucocorticoids and Not With Hypoleptinemia’, Endocrinology, 157(2), pp. 508–521.

Clarke, B. (2008) ‘Normal Bone Anatomy and Physiology’, Clinical journal of the American Society of Nephrology, 3 Suppl 3.

Cline, J. M. and Maronpot, R. R. (1985) ‘Variations in the Histologic Distribution of Rat Bone Marrow Cells with Respect to Age and Anatomic Site’, Toxicologic Pathology, 13(4), pp. 349–355.

Costantini, S. and Conte, C. (2019) ‘Bone Health in Diabetes and Prediabetes’, World Journal of Diabetes, 10(8), pp. 421–445.

DeFronzo, R. A., Tobin, J. D. and Andres, R. (1979) ‘Glucose clamp technique: a method for quantifying insulin secretion and resistance.’, The American journal of physiology, 237(3), pp. E214-23.

DeFronzo, R. A. and Tripathy, D. (2009) ‘Skeletal Muscle Insulin Resistance Is the Primary Defect in Type 2 Diabetes’, Diabetes Care, 32(suppl_2), pp. S157–S163.

DelPrato, S. et al. (1994) ‘Effect of sustained physiologic hyperinsulinaemia and hyperglycaemia on insulin secretion and insulin sensitivity in man’, Diabetologia, 37(10), pp. 1025–1035.

Dempster, D. W. and Lindsay, R. (1993) ‘Pathogenesis of osteoporosis’, The Lancet, 341(8848), pp. 797–801.

Devesa, A. et al. (2022) ‘Bone marrow activation in response to metabolic syndrome and early atherosclerosis’, European Heart Journal, 43(19), pp. 1809–1828.

Doucette, C. R. et al. (2015) ‘A High Fat Diet Increases Bone Marrow Adipose Tissue (MAT) But Does Not Alter Trabecular or Cortical Bone Mass in C57BL/6J Mice’, Journal of Cellular Physiology, 230(9), pp. 2032–2037.

Duncan, G. E. et al. (2022) ‘Epigenome-wide association study of physical activity and physiological parameters in discordant monozygotic twins’, Scientific Reports 2022 12:1, 12(1), pp. 1–17.

Eastell, R. et al. (2018) ‘DIAGNOSIS OF ENDOCRINE DISEASE: Bone turnover markers: are they clinically useful?’, European Journal of Endocrinology, 178(1), pp. R19–R31.

Ermetici, F. et al. (2018) ‘Bone marrow fat contributes to insulin sensitivity and adiponectin secretion in premenopausal women’, Endocrine, 59(2), pp. 410–418.

Eskelinen, J.-J. et al. (2015) ‘Muscle-specific glucose and free fatty acid uptake after sprint interval and moderate-intensity training in healthy middle-aged men’, Journal of Applied Physiology, 118(9), pp. 1172–1180.

Espeland, M. A. et al. (2014) ‘Impact of an Intensive Lifestyle Intervention on Use and Cost of Medical Services Among Overweight and Obese Adults With Type 2 Diabetes: The Action for Health in Diabetes’, Diabetes Care, 37(9), pp. 2548–2556.

Fan, Y. et al. (2017) ‘Parathyroid Hormone Directs Bone Marrow Mesenchymal Cell Fate’, Cell Metabolism, 25(3), pp. 661–672.

Formica, D. and Silvestri, S. (2004) ‘Biological effects of exposure to magnetic resonance imaging: An overview’, BioMedical Engineering Online, 3(1), pp. 1–12.

Goldman, L. W. (2007) ‘Principles of CT and CT technology’, Journal of Nuclear Medicine Technology, pp. 115–128.

Gonzalo-Encabo, P. et al. (2021) ‘The Role of Exercise Training on Low-Grade Systemic Inflammation in Adults with Overweight and Obesity: A Systematic Review’, International Journal of Environmental Research and Public Health, 18(24), p. 13258.

Gregg, E. W. et al. (2012) ‘Association of an Intensive Lifestyle Intervention With Remission of Type 2 Diabetes’, JAMA, 308(23), pp. 2489–2496.

Hannukainen, J. C. et al. (2005) ‘Cardiac structure and function in monozygotic twin pairs discordant for physical fitness’, Journal of Applied Physiology, 99(2), pp. 535–541.
Hannukainen, J. C. et al. (2011) ‘Liver and pancreatic fat content and metabolism in healthy monozygotic twins with discordant physical activity’, Journal of Hepatology, 54(3), pp. 545–552.
Haslam, D. (2010) ‘Obesity and diabetes: the links and common approaches’, Primary Care Diabetes, 4(2), pp. 105–112.
Hauschka, P. V. et al. (1989) ‘Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone’, Physiological Reviews, 69(3), pp. 990–1047.
Heinonen, A. et al. (1996) ‘Randomised controlled trial of effect of high-impact exercise on selected risk factors for osteoporotic fractures’, Lancet, 348(9038), pp. 1343–1347.
Heinonen, I. et al. (2019) ‘Increase of Glucose Uptake in Human Bone Marrow With Increasing Exercise Intensity’, International Journal of Sport Nutrition and Exercise Metabolism, 29(3), pp. 254–258.
Heiskanen, M. A. et al. (2021) ‘Systemic cross-talk between brain, gut, and peripheral tissues in glucose homeostasis: effects of exercise training (CROSSYS). Exercise training intervention in monozygotic twins discordant for body weight’, BMC Sports Science, Medicine and Rehabilitation, 13(1), pp. 1–19.
Hsu, P. and Qu, C. K. (2013) ‘Metabolic plasticity and hematopoietic stem cell biology’, Current Opinion in Hematology, 20(4), pp. 289–294.
Huovinen, V. et al. (2014) ‘Vertebral bone marrow glucose uptake is inversely associated with bone marrow fat in diabetic and healthy pigs: [18F]FDG-PET and MRI study’, Bone, 61, pp. 33–38.
Huovinen, V. et al. (2016) ‘Femoral Bone Marrow Insulin Sensitivity Is Increased by Resistance Training in Elderly Female Offspring of Overweight and Obese Mothers’, PLOS ONE, 11(9).
Iuliano-Burns, S. et al. (2005) ‘Diet and exercise during growth have site-specific skeletal effects: A co-twin control study’, Osteoporosis International, 16(10), pp. 1225–1232.
Ivaska, K. K. et al. (2017) ‘Changes in bone metabolism after bariatric surgery by gastric bypass or sleeve gastrectomy’, Bone, 95, pp. 47–54.
Jalo, A. et al. (2024) ‘Mechanisms Leading to Increased Insulin-Stimulated Cerebral Glucose Uptake in Obesity and Insulin Resistance: A High-Fat Diet and Exercise Training Intervention PET Study with Rats (CROSRAT)’, Journal of Functional Morphology and Kinesiology, 9(2), p. 58.
Judex, S., Gross, T. S. and Zernicke, R. F. (1997) ‘Strain Gradients Correlate with Sites of Exercise-Induced Bone-Forming Surfaces in the Adult Skeleton’, Journal of Bone and Mineral Research, 12(10), pp. 1737–1745.
Kahn, S. E., Cooper, M. E. and Del Prato, S. (2014) ‘Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future’, The Lancet, 383(9922), pp. 1068–1083.
Kaidesoja, M. et al. (2019) ‘FinnTwin16: A Longitudinal Study from Age 16 of a Population-Based Finnish Twin Cohort.’, Twin Research and Human Genetics, 22(6), pp. 530–539.
Kannus, P. et al. (1995) ‘Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players’, Annals of Internal Medicine, 123(1), pp. 27–31.
Kaprio, J. et al. (2019) ‘The Older Finnish Twin Cohort — 45 Years of Follow-up’, Twin Research and Human Genetics, 22(4), pp. 240–254.
Karlsson, M. K. and Rosengren, B. E. (2012) ‘Training and bone – from health to injury’, Scandinavian Journal of Medicine & Science in Sports, 22(4), pp. e15–e23.
Kautzky-Willer, A. et al. (2012) ‘Influence of Increasing BMI on Insulin Sensitivity and Secretion in Normotolerant Men and Women of a Wide Age Span’, Obesity, 20(10), pp. 1966–1973.
Kaye, S. M. et al. (2012) ‘Obesity-related derangements of coagulation and fibrinolysis a study of obesity-discordant monozygotic twin pairs’, Obesity, 20(1), pp. 88–94.
Kelley, D. E. et al. (2002) ‘Dysfunction of Mitochondria in Human Skeletal Muscle in Type 2 Diabetes’, Diabetes, 51(10), pp. 2944–2950.
Kim, J. I. et al. (2015) ‘Lipid-Overloaded Enlarged Adipocytes Provoke Insulin Resistance Independent of Inflammation’, Molecular and Cellular Biology, 35(10), pp. 1686–1699.

Koivula, M. K., Risteli, L. and Risteli, J. (2012) ‘Measurement of aminoterminal propeptide of type I procollagen (PINP) in serum’, Clinical Biochemistry, 45(12), pp. 920–927.

Kricun, M. E. (1985) ‘Red-yellow marrow conversion: Its effect on the location of some solitary bone lesions’, Skeletal Radiology, 14(1), pp. 10–19.

Kujala, U. M. et al. (2022) ‘Physical activity and health: Findings from Finnish monozygotic twin pairs discordant for physical activity’, Scandinavian Journal of Medicine & Science in Sports, 32(9), pp. 1316–1323.

Kuo, T. R. and Chen, C. H. (2017) ‘Bone biomarker for the clinical assessment of osteoporosis: Recent developments and future perspectives’, Biomarker Research, 5(1), pp. 1–9.

Langdahl, B., Ferrari, S. and Dempster, D. W. (2016) ‘Bone modeling and remodeling: potential as therapeutic targets for the treatment of osteoporosis’, Therapeutic Advances in Musculoskeletal Disease, 8(6), pp. 225–235.

Lattermann, R. et al. (2001) ‘Understanding the mechanisms by which isoflurane modifies the hyperglycemic response to surgery’, Anesthesia and Analgesia, 93(1), pp. 121–127.

Lecka-Czernik, B. (2012) ‘Marrow fat metabolism is linked to the systemic energy metabolism’, Bone, 50(2), pp. 534–539.

Lecka-Czernik, B. et al. (2015) ‘High bone mass in adult mice with diet-induced obesity results from a combination of initial increase in bone mass followed by attenuation in bone formation; implications for high bone mass and decreased bone quality in obesity’, Molecular and Cellular Endocrinology, 410, pp. 35–41.

Lee, N. K. et al. (2007) ‘Endocrine Regulation of Energy Metabolism by the Skeleton’, Cell, 130(3), pp. 456–469.

Liu, L., Aronson, J. and Lecka-Czernik, B. (2013) ‘Rosiglitazone disrupts endosteal bone formation during distraction osteogenesis by local adipocytic infiltration’, Bone, 52(1), pp. 247–258.

Ma, H. et al. (2009) ‘Long-Term Leisure Time Physical Activity and Properties of Bone: A Twin Study’, Journal of Bone and Mineral Research, 24(8), pp. 1427–1433.

McCabe, L. R. et al. (2019) ‘Exercise prevents high fat diet-induced bone loss, marrow adiposity and dysbiosis in male mice’, Bone, 118, pp. 20–31.

McLaughlin, T. et al. (2016) ‘Adipose Cell Size and Regional Fat Deposition as Predictors of Metabolic Response to Overfeeding in Insulin-Resistant and Insulin-Sensitive Humans’, Diabetes, 65(5), pp. 1245–1254.

Moran, C. P. and Shanahan, F. (2014) ‘Gut microbiota and obesity: Role in aetiology and potential therapeutic target’, Best Practice & Research Clinical Gastroenterology, 28(4), pp. 585–597.

Mustelin, L. et al. (2008) ‘Physical activity reduces the influence of genetic effects on BMI and waist circumference: a study in young adult twins’, International Journal of Obesity, 33(1), pp. 29–36.

Napoli, N. et al. (2017) ‘Mechanisms of diabetes mellitus-induced bone fragility’, Nature Reviews Endocrinology, 13(4), pp. 208–219.

Naukkarinen, J. et al. (2012) ‘Causes and consequences of obesity: the contribution of recent twin studies’, International Journal of Obesity, 36(8), pp. 1017–1024. doi: 10.1038/ijo.2011.192.

Ness-Abramof, R. and Apovian, C. M. (2008) ‘Waist Circumference Measurement in Clinical Practice’, Nutrition in Clinical Practice, 23(4), pp. 397–404.

Ojala, R. et al. (2020) ‘Bone marrow metabolism is impaired in insulin resistance and improves after exercise training’, Journal of Clinical Endocrinology and Metabolism, 105(12), pp. e4290–e4303.

Ojala, R. et al. (2024) ‘Bone marrow metabolism is affected by body weight and response to exercise training varies according to anatomical location’, Diabetes, Obesity and Metabolism, 26(1), pp. 251–261.

Pagnotti, G. M. and Styner, M. (2016) ‘Exercise regulation of marrow adipose tissue’, Frontiers in Endocrinology, 7, p. 94.

Paldánius, P. M. et al. (2012) ‘The Effect of Oral Glucose Tolerance Test on Serum Osteocalcin and Bone Turnover Markers in Young Adults’, Calcified Tissue International, 90(2), pp. 90–95.

Parfitt, A. M. (2002) 'Targeted and nontargeted bone remodeling: relationship to basic multicellular unit origination and progression', Bone, 30(1), pp. 5–7.
Pereira, S. S. and Alvarez-Leite, J. I. (2014) 'Low-Grade Inflammation, Obesity, and Diabetes', Current Obesity Reports, 3(4), pp. 422–431.
Pham, T. T. et al. (2020) 'Human Bone Marrow Adipose Tissue is a Metabolically Active and Insulin-Sensitive Distinct Fat Depot', J Clin Endocrinol Metab, 105(7), pp. 1–11.
Pittenger, M. F. et al. (1999) 'Multilineage potential of adult human mesenchymal stem cells', Science, 284(5411), pp. 143–147.
Porter, B. A., Shields, A. F. and Olson, D. O. (1986) 'Magnetic Resonance Imaging of Bone Marrow Disorders', Radiologic Clinics of North America, 24(2), pp. 269–289.
Poser, J. W. et al. (1980) 'Isolation and sequence of the vitamin K-dependent protein from human bone. Undercarboxylation of the first glutamic acid residue', Journal of Biological Chemistry, 255(18), pp. 8685–8691.
Prentki, M. and Nolan, C. J. (2006) 'Islet β cell failure in type 2 diabetes', Journal of Clinical Investigation, 116(7), p. 1802.
Qiao, D. et al. (2020) 'Association of obesity with bone mineral density and osteoporosis in adults: a systematic review and meta-analysis', Public Health, 180, pp. 22–28.
Reaven, G. M. (1995) 'Pathophysiology of insulin resistance in human disease', Physiological Reviews, 75(3), pp. 473–486.
Rejeski, W. J. et al. (2015) 'Aging and Physical Function in Type 2 Diabetes: 8 Years of an Intensive Lifestyle Intervention', The Journals of Gerontology: Series A, 70(3), pp. 345–353.
Rendina-Ruedy, E. and Rosen, C. J. (2020) 'Lipids in the Bone Marrow: An Evolving Perspective', Cell Metabolism, 31(2), pp. 219–231.
Robling, A. G. et al. (2002) 'Improved Bone Structure and Strength After Long-Term Mechanical Loading Is Greatest if Loading Is Separated Into Short Bouts', Journal of Bone and Mineral Research, 17(8), pp. 1545–1554.
Robling, A. G. and Bonewald, L. F. (2020) 'The Osteocyte: New Insights', Annual Review of Physiology, 82, pp. 485–506.
Rose, R. J. et al. (2019) 'FinnTwin12 Cohort: An Updated Review', Twin Research and Human Genetics, 22(5), pp. 302–311.
Sahu, M. and Prasuna, J. G. (2016) 'Twin Studies: A Unique Epidemiological Tool', Indian journal of community medicine, 41(3), pp. 177–182.
Scheller, E. L. et al. (2015) 'Region-specific variation in the properties of skeletal adipocytes reveals regulated and constitutive marrow adipose tissues', Nature Communications, 6(6), p. 7808.
Scheller, E. L. et al. (2016) 'Changes in skeletal integrity and marrow adiposity during high-fat diet and after weight loss', Frontiers in Endocrinology, 7, p. 102.
Scheller, E. L. et al. (2019) 'Bone marrow adipocytes resist lipolysis and remodeling in response to β-adrenergic stimulation', Bone, 118, pp. 32–41.
Schini, M. et al. (2023) 'Bone Turnover Markers: Basic Biology to Clinical Applications', Endocrine Reviews, 44(3), pp. 417–473.
Schwartz, A. V. (2016) 'Epidemiology of fractures in type 2 diabetes', Bone, 82, pp. 2–8.
Shanbhogue, V. V et al. (2016) 'Type 2 diabetes and the skeleton: new insights into sweet bones', The Lancet Diabetes & Endocrinology, 4(2), pp. 159–173.
Shockley, K. R. et al. (2009) 'PPARγ2 nuclear receptor controls multiple regulatory pathways of osteoblast differentiation from marrow mesenchymal stem cells', Journal of Cellular Biochemistry, 106(2), pp. 232–246.
Shulman, G. I. (2014) 'Ectopic Fat in Insulin Resistance, Dyslipidemia, and Cardiometabolic Disease', New England Journal of Medicine, 371(12), pp. 1131–1141.
Singh, P. et al. (2008) 'Vaccinia Virus Infection Modulates the Hematopoietic Cell Compartments in the Bone Marrow', Stem Cells, 26(4), pp. 1009–1016.

Singhal, V. and Bredella, M. A. (2019) 'Marrow adipose tissue imaging in humans', Bone, 118, pp. 69–76.

Sontam, D. M. et al. (2017) 'A memory of early life physical activity is retained in bone marrow of male rats fed a high-fat diet', Frontiers in Physiology, 8, p. 476.

Stanford, K. I. and Goodyear, L. J. (2014) 'Exercise and type 2 diabetes: Molecular mechanisms regulating glucose uptake in skeletal muscle', Advances in Physiology Education, 38(4), pp. 308–314.

Styner, M. et al. (2014) 'Bone marrow fat accumulation accelerated by high fat diet is suppressed by exercise', Bone, 64, pp. 39–46.

Styner, M. et al. (2015) 'Exercise Regulation of Marrow Fat in the Setting of PPARγ Agonist Treatment in Female C57BL/6 Mice', Endocrinology, 156(8), pp. 2753–2761.

Styner, M. et al. (2017) 'Exercise Decreases Marrow Adipose Tissue Through ß-Oxidation in Obese Running Mice', Journal of Bone and Mineral Research, 32(8), pp. 1692–1702.

Suchacki, K. J. et al. (2020) 'Bone marrow adipose tissue is a unique adipose subtype with distinct roles in glucose homeostasis', Nature Communications, 11(1), pp. 1–18.

Suchacki, K. J., Cawthorn, W. P. and Rosen, C. J. (2016) 'Bone marrow adipose tissue: Formation, function and regulation', Current Opinion in Pharmacology, 28, pp. 50–56.

Tamashiro, K. L. et al. (2011) 'Chronic stress, metabolism, and metabolic syndrome', Stress, 14(5), pp. 468–474.

Tanaka, K. et al. (2009) 'Mechanisms of Impaired Glucose Tolerance and Insulin Secretion during Isoflurane Anesthesia', Anesthesiology, 111(5), pp. 1044–1051.

Tanaka, K. et al. (2011) 'Differential effects of propofol and isoflurane on glucose utilization and insulin secretion', Life Sciences, 88(1–2), pp. 96–103.

Tencerova, M. et al. (2018) 'High-Fat Diet–Induced Obesity Promotes Expansion of Bone Marrow Adipose Tissue and Impairs Skeletal Stem Cell Functions in Mice', Journal of Bone and Mineral Research, 33(6), pp. 1154–1165.

Tencerova, M. and Kassem, M. (2016) 'The bone marrow-derived stromal cells: Commitment and regulation of adipogenesis', Frontiers in Endocrinology, 7, p. 127.

The American College of Radiology (2023) ACR-SPR-SSR practice parameter for the performance of musculoskeletal quantitative computed tomography (QCT). Available at: https://www.acr.org/-/media/ACR/Files/Practice-Parameters/QCT.pdf?la=en.

The Look AHEAD Research Group (2013) 'Cardiovascular Effects of Intensive Lifestyle Intervention in Type 2 Diabetes', New England Journal of Medicine, 369(2), pp. 145–154.

Thie, J. A. (1995) 'Clarification of a Fractional Uptake Concept', Journal of Nuclear Medicine, 36(4), pp. 711–712.

Thorens, B. and Mueckler, M. (2010) 'Glucose transporters in the 21st Century', American Journal of Physiology. Endocrinology and metabolism, 298(2), pp. 141–145.

Tilg, H. et al. (2020) 'The intestinal microbiota fuelling metabolic inflammation', Nature Reviews Immunology, 20(1), pp. 40–54.

Tratwal, J. et al. (2020) 'MarrowQuant Across Aging and Aplasia: A Digital Pathology Workflow for Quantification of Bone Marrow Compartments in Histological Sections', Frontiers in Endocrinology, 11, p. 480.

Tutunchi, H. et al. (2020) 'What are the optimal cut-off points of anthropometric indices for prediction of overweight and obesity? Predictive validity of waist circumference, waist-to-hip and waist-to-height ratios', Health Promotion Perspectives, 10(2), pp. 142–147.

VanHerpen, N. A. and Schrauwen-Hinderling, V. B. (2008) 'Lipid accumulation in non-adipose tissue and lipotoxicity', Physiology and Behavior, 94(2), pp. 231–241.

Vasikaran, S. et al. (2011) 'International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine Position on bone marker standards in osteoporosis', Clinical Chemistry and Laboratory Medicine, 49(8), pp. 1271–1274.

Verboven, K. et al. (2018) ‘Abdominal subcutaneous and visceral adipocyte size, lipolysis and inflammation relate to insulin resistance in male obese humans’, Scientific Reports, 8(1), pp. 1–8.
Verhaegen, A. A. and Van Gaal, L. F. (2021) ‘Drugs Affecting Body Weight, Body Fat Distribution, and Metabolic Function—Mechanisms and Possible Therapeutic or Preventive Measures: an Update’, Current Obesity Reports, 10(1), pp. 1–13.
Verma, S. et al. (2002) ‘Adipocytic proportion of bone marrow is inversely related to bone formation in osteoporosis’, Journal of Clinical Pathology, 55(9), pp. 693–698.
Vogler, J. B. and Murphy, W. A. (1988) ‘Bone marrow imaging’, Radiology, 168(3), pp. 679–93.
Waller, K., Kaprio, J. and Kujala, U. M. (2007) ‘Associations between long-term physical activity, waist circumference and weight gain: a 30-year longitudinal twin study’, International Journal of Obesity, 32(2), pp. 353–361.
Weaver, C. M. et al. (2016) ‘The National Osteoporosis Foundation’s position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations’, Osteoporosis International, 27(4), pp. 1281–1386.
Wei, J. and Karsenty, G. (2015) ‘An overview of the metabolic functions of osteocalcin’, Reviews in Endocrine and Metabolic Disorders, 16(2), pp. 93–98.
Weston, K. S., Wisløff, U. and Coombes, J. S. (2014) ‘High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis’, British Journal of Sports Medicine, 48(16), pp. 1227–1234.
Widjaja, N. et al. (2023) ‘Perilipin-1 immunostaining improves semi-automated digital quantitation of bone marrow adipocytes in histological bone sections’, Adipocyte, 12(1).
Wilcox, G. (2005) ‘Insulin and insulin resistance’, The Clinical biochemist. Reviews, 26(2), pp. 19–39.
Willemsen, G. et al. (2015) ‘The Concordance and Heritability of Type 2 Diabetes in 34,166 Twin Pairs From International Twin Registers: The Discordant Twin (DISCOTWIN) Consortium.’, Twin Research and Human Genetics, 18(6), pp. 762–771.
Wing, R. R. et al. (2011) ‘Benefits of Modest Weight Loss in Improving Cardiovascular Risk Factors in Overweight and Obese Individuals With Type 2 Diabetes’, Diabetes Care, 34(7), pp. 1481–1486.
Yeung, D. K. W. et al. (2005) ‘Osteoporosis is associated with increased marrow fat content and decreased marrow fat unsaturation: A proton MR spectroscopy study’, Journal of Magnetic Resonance Imaging, 22(2), pp. 279–285.
Yki-Järvinen, H. (1984) ‘Sex and Insulin Sensitivity’, Metabolism, 33(11), pp. 1011–1015.
Zouhal, H. et al. (2022) ‘Effects of Exercise Training on Bone Health Parameters in Individuals With Obesity: A Systematic Review and Meta-Analysis’, Frontiers in Physiology, 12, p. 807110.