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EXERCISE TRAINING- INDUCED EFFECTS ON BROWN AND WHITE ADIPOSE TISSUE METABOLISM IN HUMANS

Positron emission tomography studies
in health and insulin-resistance

Piryanka Motiani



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To my brother Kumail

ABSTRACT

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Exercise training-induced effects on brown and white adipose tissue metabolism in humans: Positron emission tomography studies in health and insulin resistance

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White adipose tissue (WAT) not only serves as a passive energy storage but also has an endocrine role releasing hormones that play a major role in the regulation of whole-body glucose homeostasis and insulin sensitivity. Active brown adipose tissue (BAT) is able to consume lipids and glucose to generate heat. The dysregulation of BAT and WAT may predispose a person to become obese and insulin resistant. Exercise training is established to reduce fat mass and insulin resistance. Some of the exercise-induced benefits may be dose-specific. However, only a few studies exist examining the effects of training on BAT metabolism in humans that are cross-sectional in nature and the results are contradictory. There are no controlled prospective intervention studies that have investigated exercise-induced effects on BAT metabolism directly in humans. Furthermore, there is no clear evidence that exercise improves WAT metabolism.

The aim of this thesis was to investigate the effects of short-term (2wks) exercise training with either, sprint interval training (SIT) or moderate intensity continuous training (MICT) on BAT and WAT metabolism in middle-aged sedentary healthy (BMI 26.1 ± 2.4 ; age 48 ± 5) and insulin resistant (IR) subjects (BMI 30.1 ± 2.5 ; age 49 ± 4). Further, the effects of longer term (6wks) progressive endurance and resistance exercise training on cold-induced BAT metabolism in healthy men (BMI 23 ± 0.9 ; age 31 ± 7) were studied. BAT and WAT glucose and free fatty acid was determined using positron emission tomography (PET).

The results show that modifications after exercise training are not only adipose tissue depot-specific but also the type of exercise (SIT vs MICT) induces different responses. Training decreased insulin stimulated BAT glucose uptake but had no effect on cold stimulated BAT glucose uptake in healthy subjects. At baseline IR had impaired WAT GU compared to healthy subjects which normalized after training. SIT improves WAT insulin resistance while MICT decreases WAT free fatty acid metabolism in IR. This suggests that different adipose tissue depots respond differently to the metabolic demands of exercise training. Moreover, intensity affects different substrate uptake from WAT. This data suggests that changes in adipose tissue metabolism may help whole body insulin action. Overall, exercise-induced BAT and WAT adaptations provide potential therapeutic targets for obesity and type 2 diabetes.

Keywords: brown adipose tissue, exercise training, sprint interval training, insulin resistance, metabolism, positron emission tomography.

TIIVISTELMÄ

Piryanka Motiani

Liikuntaharjoittelu aikaansaamat vasteet ruskean ja valkoisen rasvakudoksen aineenvaihduntaan ihmisillä:

Positroniemissiotomografia tutkimuksia terveillä ja insuliiniresistenteillä henkilöillä.

Turun yliopisto, Lääketieteellinen tiedekunta, Kliininen fysiologia ja isotoppilääketiede, Turun kliininen tohtoriohjelma, PET-keskus, Turku, Suomi. Annales Universitatis Turkuensis, Turku, 2019

Valkoinen rasvakudos ei toimi ainoastaan elimistön passiivisena energiavarastona, vaan se on myös aktiivinen endokriininen elin, joka vapauttaa muun muassa koko kehon sokeriaineenvaihduntaan ja insuliiniherkkyyteen vaikuttavia hormoneja. Aktiivinen ruskea rasvakudos pystyy hyödyntämään elimistön rasvahappoja ja sokeria lämmön tuottamiseksi. Ruskean ja valkoisen rasvan toiminnan häiriintyminen saattaa johtaa lihavuuteen ja insuliiniresistenssin kehittymiseen. Liikuntaharjoittelun on osoitettu vähentävän rasvakudoksen määrää ja insuliiniresistenssiä. Osa liikunnan aikaansaamista vaikutuksista saattaa olla sidottuja liikunnan volyyymiin. Vain harvat aiemmat tutkimukset käsittelevät liikuntaharjoittelun vaikutuksia ihmisen ruskean rasvakudoksen aineenvaihduntaan, ja tulokset ovat ristiriitaisia. Tämä on ensimmäinen kontrolloitu interventiotutkimus, joissa tutkitaan liikunnan aikaansaamia suoria vasteita ruskean rasvan aineenvaihduntaan ihmisillä. Ei myöskään ole aiempaa selkeää näyttöä siitä, että liikuntaharjoittelu parantaa valkoisen rasvan aineenvaihduntaa.

Tämän väitöskirjatyön tavoitteena oli selvittää lyhytaikaisen (2 viikkoa) sprintti-intervalliharjoittelun (SIT) ja kohtalaiskuormitteisen tasasykkeisen harjoittelun (MICT) vaikutuksia valkoisen ja ruskean rasvakudoksen aineenvaihduntaan keski-ikäisillä terveillä (BMI 26.1 ± 2.4 ; ikä 48 ± 5) ja insuliiniresistenteillä tutkittavilla (BMI 30.1 ± 2.5 ; ikä 49 ± 4). Lisäksi tutkittiin pitkäkestoisemman (6 viikon) progressiivisen yhdistetyn kestävyys- ja voimaharjoittelun vaikutuksia kylmän indusoimaan ruskean rasvakudoksen aineenvaihduntaan terveillä miehillä (BMI 23.0 ± 0.9 ; ikä 31 ± 7). Ruskean ja valkoisen rasvakudoksen sokeri- ja rasvahappoaineenvaihduntaa tutkittiin positroniemissiotomografialla.

Tutkimuksen tulokset osoittavat, että liikuntaharjoittelun aikaansaamat vasteet rasvakudoksen aineenvaihdunnassa riippuvat sekä rasvavarastosta, että myös liikuntaharjoittelun tyypistä (SIT vs. MICT). Liikuntaharjoittelu alensi ruskean rasvakudoksen insuliinistimuloitua sokerin ottoa, mutta sillä ei ollut vaikutusta kylmästimuloitun ruskean rasvakudoksen sokerin ottoon terveillä tutkittavilla. Jo kahden viikon harjoittelu paransi lähtötilanteessa terveisiin verrattuna heikentynyttä valkoisen rasvakudoksen sokeriaineenvaihduntaa tutkittavilla, joilla oli insuliiniresistenssi. SIT paransi valkoisen rasvakudoksen insuliiniherkkyyttä tehokkaammin kuin MICT, kun taas MICT vaikutti enemmän valkoisen rasvakudoksen rasvahappoaineenvaihduntaan. Näiden tulosten perusteella näyttäisikin siltä, että liikuntaharjoittelun vasteet rasvakudoksen aineenvaihdunnassa ovat riippuvaisia rasvavarastosta sekä liikunnan intensiteetistä. Tämä tutkimus ehdottaa myös, että muutokset rasvakudoksen aineenvaihdunnassa voivat parantaa koko kehon insuliiniherkkyyttä. Yhteenvetona, liikuntaharjoittelun vasteet ruskeassa ja valkoisessa rasvassa voivat tarjota terapeuttisen kohteen lihavuudessa ja tyypin 2 diabeteksessa.

Avainsanat: Ruskea rasva, liikuntaharjoittelu, sprintti-intervalliharjoittelu, insuliiniresistenssi, aineenvaihdunta, positroniemissiotomografia.

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ABBREVIATIONS

[¹¹ O]O ₂	Oxygen-15-labelled oxygen
[¹⁵ O]H ₂ O	Oxygen-15- labelled water
[¹⁸ F]FDG	[¹⁸ F]2-fluoro-2-deoxy-D-glucose
[¹⁸ F]FTHA	14(R,S)-[¹⁸ F]fluoro-6-thia-heptadecanoic acid
AC	Adenylyl cyclase
ACC	Acetyl CoA carboxylase
AMPK	5' adenosine monophosphate-activated protein kinase
Acetyl CoA	Acetyl coenzyme A
ANGPTL	Angiopietin-like proteins
ACSM	American college of sports medicine
ANP	Atrial natriuretic peptide
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ATGL	Adipose triglyceride lipase
ASAT	Abdominal subcutaneous adipose tissue
BAIBA	β-aminoisobutyric acid
BAT	Brown adipose tissue
BMI	Body mass index
BNP	B-type natriuretic peptide
cAMP	3'-5'-cyclic adenosine monophosphate
CD36	Cluster of differentiation 36
CREB	cAMP response-element binding protein
CT	X-ray computed tomography
CIDEA	Cell death activator
CRP	C-reactive protein
diHOME	Dihydroxy-9Z-octadecenoic acid
DIO2	Type 2 deiodinase
DNL	De novo lipogenesis
EE	Energy expenditure
FAS	Fatty acid synthase
FFAs	Free fatty acids
FAB4	Fatty acid binding protein-4
FGF-21	Fibroblast growth factor 21
FATPs	Fatty acid transporter proteins
FNDC5	Fibronectin type III domain containing 5
FSAT	Femoral subcutaneous adipose tissue
FFAU	Free fatty acid uptake

FASN	Fatty acid synthase
GLUT4	Glucose transporter-4
GLUTs	Glucose transporters
G _s protein	Stimulatory G protein
GU	Glucose uptake
GDP	Guanosine diphosphate
hMADS	Human multipotent adipose-derived stem cells
HSL	Hormone sensitive lipase
HIIT	High intensity interval training
HDL	High density lipoprotein
HBA1C	Glycated Hemoglobin
HGF	Hepatocyte growth factor
HR _{max}	Maximum heart rate
HU	Hounsfield units
IDF	International diabetes federation
IL-6	Interleukin-6
IL-8	Interleukin-8
IR	Insulin resistance
IRS	Insulin receptor substrate
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IDL	Intermediate density lipoprotein
K _i	Influx rate constant
LPL	Lipoprotein lipase
LDL	Low density lipoproteins
LC	Lumped constant
MCP-1	Monocyte chemoattractant protein 1
MCT	Monocarboxylate transporter
MRI	Magnetic resonance imaging
MAGL	Monoacylglycerol lipase
mRNA	Messenger ribonucleic acid
MRS	Magnetic resonance spectroscopy
mTOR	Mechanistic target of rapamycin
MICT	Moderate intensity continuous training
MG	Monoacylglycerol
MYF5	Myogenic factor 5
M-value	Whole body insulin sensitivity
NE	Nor-epinephrine
NST	Non-shivering thermogenesis
NP	Natriuretic peptides

NMR	Nuclear magnetic resonance
NGF	Nerve growth factor
OGTT	Oral glucose tolerance test
p38 MAPK	p38 mitogen-activated protein kinase
PAX7	Paired box protein 7
PET	Positron emission tomography
PGC1- α	Peroxisome proliferator-activated receptor-gamma coactivator-1 alpha
PKA	Protein kinase A
PkB	Protein kinase B
PPAR γ	Peroxisome proliferator-activated receptor gamma
PRDM16	PR domain containing 16
PI3K	Phosphoinositide-3 kinase
PDK-4	Pyruvate dehydrogenase lipoamide kinase isozyme 4
QF	Quadriiceps femoris
ROIS	Region of interest
SAT	Subcutaneous adipose tissue
SCD1	Microsomal stearyl-CoA desaturase 1
SNS	Sympathetic nervous system
SIT	Sprint interval training
SFF	Signal fat fraction images
SUV	Standard uptake values
T2DM	Type 2 diabetes mellitus
T3	Triiodothyronine
T4	Thyroxine
TAG	Triacylglycerol
TCA	Tricarboxylic acid
THR s	Thyroid hormone receptors
TRL	Triglyceride-rich lipoproteins
TNF- α	Tumor necrosis factor- α
UCP-1	Uncoupling protein -1
VAT	Visceral adipose tissue
VEGFA	Vascular endothelial growth factor A
VEGFD	Vascular endothelial growth factor D
VLDL	Very low density lipoprotein
VO $_{2max}$	Maximal oxygen consumption
VO $_{2peak}$	Peak oxygen uptake
WAT	White adipose tissue
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications, which are referred in the text by the corresponding Roman numerals, I-III.

Motiani P, Virtanen KA, Motiani KK, Eskelinen JJ, Middelbeek RJ, Goodyear LJ, Savolainen AM, Kemppainen J, Jensen J, Din MU, Saunavaara V, Parkkola R, Löyttyniemi E, Knuuti J, Nuutila P, Kalliokoski KK, Hannukainen JC. 2017. Decreased insulin-stimulated brown adipose tissue glucose uptake after short-term exercise training in healthy middle-aged men. *Diabetes, Obesity and Metabolism*. 19 (10):1379-1388.

Motiani P, Teuvo J, Saari T, Virtanen KA, Honkala SM, Middelbeek RJ, Goodyear LJ, Eskola O, Löyttyniemi E, Hannukainen JC*, Nuutila P*. 2018. Exercise training alters lipoprotein particles independent of brown adipose tissue metabolic activity. *Obesity Science & Practice*. 10.1002/osp4.330.

Motiani P*, Honkala SM*, Motiani K, Eskelinen JJ, Virtanen KA, Löyttyniemi E, Nuutila P, Kalliokoski KK, Hannukainen JC. 2018. Exercise training improves depot specific adipose tissue metabolism regardless of baseline glucose tolerance and sex. Manuscript.

*These authors shared equal contribution.

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1. INTRODUCTION

The number of patients worldwide characterized with obese, and type 2 diabetes mellitus (T2DM) has increased radically over the past 50 years (Hruby and Hu, 2015). Obesity is serious health risk and an economic burden on society. According to world health organization (WHO) more than 1.9 billion adults worldwide are overweight and nearly one-third of the population is obese (World Health Organization, 2018). In obesity, there is an altered energy balance in which energy intake exceeds the energy expenditure (Hill et al., 2012). This lack of balance leads to excessive accumulation of adipose tissue. Obesity is strongly associated with several comorbidities including T2DM, cardiovascular disease and cancers and an increased risk of mortality (Yu et al., 2017). Therefore, there is an urgent need for new therapeutic options to prevent and treat obesity and its associated comorbidities (Poher et al., 2015).

White adipose tissue (WAT) and brown adipose tissue (BAT) are the two major types of adipose tissues critically implicated in energy storage and dissipation (Cannon and Nedergaard, 2012; Gesta et al., 2007). Energy imbalance and disturbances in WAT and BAT metabolism are the underlying factors in the obesity epidemic and are linked with the rise of metabolic disorders including T2DM (Gaggini et al., 2015). The previous notion that only infants have BAT changed after the presence of BAT was verified in adults (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). BAT activation increases energy expenditure and improves whole-body glucose and lipid metabolism (Ouellet *et al.*, 2012; Chen *et al.*, 2013; Stanford *et al.*, 2013). Cold exposure is established means of study for BAT activation as the primary role of BAT is to maintain non-shivering thermogenesis (Cannon and Nedergaard, 2004).

Exercise can effectively combat obesity and T2DM (Coyle et al., 2012; Crandall et al., 2008). There is compelling evidence of exercise induced benefits to skeletal muscle metabolism and cardiovascular fitness (Goodyear et al., 1998; Holloszy and Coyle, 1984; Myers, 2003). However, the mechanisms by which exercise confers health benefits remain unclear.

In obesity there are several dysfunctions of WAT including WAT mass expansion, and increased storage of fat (ectopic fat) in metabolically active non-adipose tissues like the liver and skeletal muscle (Chouchani and Kajimura, 2019; Shulman, 2014) as well as the development of insulin resistance (IR). However, there is no clear consensus at the moment of whether decreased BAT mass or activity might contribute to changes in IR; consequently whether these disruptions in adipose tissue metabolism can be prevented by exercise training is still unexplored. One of the proposed mechanisms for exercise induced benefits that has gained considerable attention is the browning of WAT. The exact mechanism of the browning of WAT are not clearly understood, it could occur due to

increased sympathetic innervation of WAT (Bartness and Ryu, 2015) and exercise-induced release of hormone and cytokines such as catecholamines, interleukin-6, irisin and fibroblast growth factor 21 (Pedersen and Febbraio, 2012)

Altogether, while the numerous positive outcomes of exercise training on metabolic health are obvious in humans, the contribution of adipose tissue in playing a role in the underlying improvements remains elusive. The aims of this doctoral work were to investigate the effects of exercise training and the training mode, SIT vs MICT on BAT metabolism on healthy middle-aged men (Study I), and WAT metabolism in middle-aged men and women with prediabetes and T2DM (Study III). In addition, the effects of combined endurance and resistance training on BAT metabolism in young healthy men were also studied (Study II). The results of these studies provide knowledge on adipose tissue adaptations to exercise training.

2. REVIEW OF LITERATURE

2.1 Types of adipose tissue

2.1.1 *White adipose tissue*

White adipose tissue (WAT) is a loose connective tissue consisting of adipocytes and stromal-vascular fraction cells including vascular endothelial cells, fibroblasts, macrophages, pre-adipocytes and immune cells (Otto and Lane, 2005). White adipocytes contain one large fat lipid droplet abundant in triglycerides and some mitochondria. Typically, in lean humans, 20-28% of total body mass is WAT. However, this may vary with age, sex and metabolic health status. In fact, in obesity it can account for approximately 80% of body weight (Thompson et al., 2012). Adipose tissue has the ability to store triglycerides as a form of energy. It also functions in an endocrine manner by secreting numerous hormones, cytokines/adipokines influencing whole-body glucose and lipid metabolism and energy homeostasis (Jazet et al., 2003). WAT can be categorized into, subcutaneous adipose tissue (SAT) situated underneath the skin and visceral adipose tissue (VAT) surrounding the abdominal organs. VAT is located around the kidneys, intestines and heart. VAT is in close vicinity to the digestive tract and drains directly into the portal circulation. VAT is important as it plays a role in hepatic insulin resistance and liver steatosis because liver is directly exposed to releasing free fatty acids from VAT via portal vein (Choe et al., 2016). SAT is the largest adipose tissue depot. The abdominal SAT acts as a short term energy store that takes up free fatty acids after a meal and has an immediate response to lipolytic stimuli, while the femoral SAT provides safe, long-term energy storage that takes up fatty acids between the meals and displays reduced lipolytic responses (Karpe and Pinnick, 2015).

In obesity both SAT and VAT masses are increased and this expansion could be due to hypertrophy (enlarged adipocytes) and hyperplasia (increased number of adipocytes) or both (Wajchenberg, 2000). The increase in WAT mass because of adipocyte hypertrophy results in impaired adipocyte function and is linked to metabolic disorders in adult humans (Hirsch and Knittle). On the other hand, hyperplastic WAT expansion is beneficial as it increases adiponectin and formation of new adipocytes decreases basal fatty acids release, hypoxia, immune cell activation and improves insulin sensitivity (Choe et al., 2016).

Women have greater whole-body adiposity than men. Despite men having lower fat mass men are predisposed to have greater incidence of T2DM and early abnormalities of glucose metabolism than women (Kuhl et al., 2005). Men are predisposed to fat accumulation in the VAT and abdominal SAT (upper body region), whereas women accumulate more fat as femoral SAT (lower body region) (Bhatt, Dhillo et al. 2017a, Geer, Shen 2009, Golbidi, Laher 2014). SAT does not grow uniformly. In the state of excessive calorie consumption,

femoral SAT undergoes hyperplasia, whereas abdominal SAT undergoes hypertrophy (Tchkonina et al., 2010). Generally distribution of fat in the lower body region is related with positive effects on metabolic health including increased insulin sensitivity (Balakrishnan et al., 2012). Women have better insulin-sensitivity than men (Mittendorfer, 2005). This idea is supported by evidence showing women having higher skeletal muscle GU during insulin stimulation, increased whole-body glucose disposal and better insulin suppression of endogenous glucose production (EGP) than men (Lundsgaard and Kiens, 2014; Nuutila et al., 1995). There are limited studies about sex differences in human adipose tissue GU. There are many factors that could contribute to the observed differences in insulin sensitivity which could be due to different patterns of fat distribution, fat cell size, cortisol metabolism, levels of intrahepatic fat, adiponectin and other inflammatory markers (Bloomgarden, 2003; Geer and Shen, 2009). Interestingly, it has been shown that femoral SAT in women is more efficient at taking up fatty acids from meals via LPL and free fatty acids directly from circulation (Raitakari et al., 1996). As a consequence, adipocytes in femoral SAT of pre-menopausal women are larger, yet remain insulin sensitive (Johnson et al., 2001).

2.1.2 Brown and beige adipose tissue

A brown adipocyte consists of tiny fat droplets, granular cytoplasm and numerous mitochondria, thereby contributing to the brown color (Figure 1) (Cinti, 2005). Brown adipose tissue (BAT) produces excess heat instead of ATP generation due to the mitochondrial uncoupling protein 1 (UCP-1) (Nicholls, Bernson et al. 1978). UCP-1 is activated by free fatty acids (FFAs) and permits protons to leak across the membrane, bypassing ATP synthase, and dissipating chemical energy in the form of heat (Cannon and Nedergaard, 2004). Furthermore, it is a highly vascularized and a densely innervated tissue.

BAT regulates normal body temperature in small animals and protect newborns from hypothermia (Cannon and Nedergaard, 2004). Although the presence of BAT in human infants is established, it was assumed for many years to have no clinical or physiologic significance in adults. However, radiologists while studying metabolically active tumors with [¹⁸F]FDG PET/CT, detected high rates of glucose uptake in the neck region that were symmetrical in nature (Cohade et al., 2003; Engel et al., 1996; Hany et al., 2002). In 2007, the presence of active BAT and its metabolic importance to adults was initially claimed (Nedergaard et al., 2007) and finally verified in 2009 by three prominent research groups (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). Interestingly, the BAT activity in humans has shown to differ in relation to body mass index, gender, age and outdoor temperature (Ouellet et al., 2011). This suggests BAT may play important metabolic roles in health.

In rodents and newborns BAT is located predominately in the interscapular region referred to as the classic BAT depot. The other locations of BAT in newborns are in the neck surrounded by blood vessels, muscles, supraclavicular, mediastinum and around the aorta, pancreas, spleen, kidneys and supra renal glands (Heaton 1972). In adult humans, BAT is located in the supraclavicular region (Hany et al., 2002), paraaortic, pericardial, paraspinal, perisplenic and supra-renal regions (Nedergaard and Cannon, 2013). As a heat generating tissue in humans, the dense capillary network of BAT ensures blood warming as it flows through the activated BAT while its central location in the body makes integral core regions warm during cold exposure (Cinti, 2009).

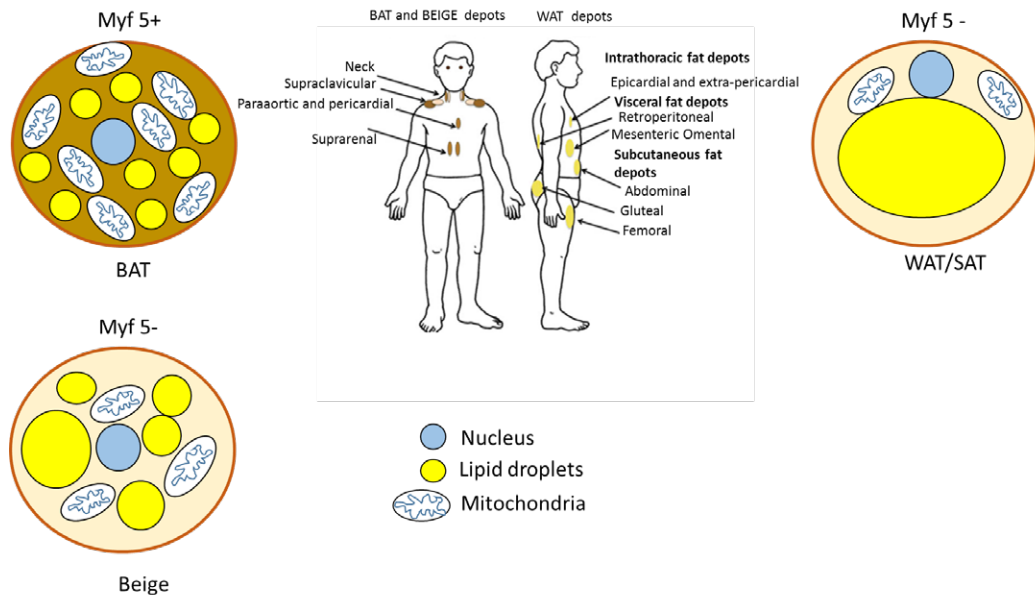


Figure 1. Anatomical locations of adipose tissues. Modified from (Gaggini et al., 2015)

Previously, the assumption was that all adipocytes that are positive for UCP-1 expression could be classified as brown adipocytes. However, later studies documented the occurrence of brown-like cells intermixed in the white fat depots, known as the ‘beige’, ‘recruitable brown’ or ‘brite’ adipocytes in adult humans were also UCP-1 positive (Sharp et al., 2012; Wu et al., 2012). Beige adipocytes have comparable characteristics to classic brown adipocytes but distinct origins. For instance, beige adipocytes have high mitochondrial numbers and several tiny lipid droplets. Although beige adipocytes express lower UCP-1 levels than classical brown adipocytes, they are capable of inducing thermogenesis at an almost similar level to classic brown adipocytes when activated (Boström et al., 2012; Giralt and Villarroya, 2013). In recruitable adipocytes the expression of UCP1 is low but it can be upregulated after proper stimulation such as cold exposure or exercise (Kajimura and Saito, 2014; Moonen et al., 2018; Wu et al., 2013).

Classic BAT originates from myogenic factor 5 (*MYF5*⁺) and paired boxed proteins 7 (*PAX7*⁺) progenitors similar to skeletal muscle (Seale, Sabourin et al. 2000). Another transcription factor, the PR domain zinc finger protein 16 (*PRDM16*) is an important determinant in the differentiation of brown adipocytes. In the presence of *PRDM16*, the precursor cells of brown adipocytes differentiate into ‘classical brown adipocyte’ but if this transcription factor is knocked out, the cells will be redirected to differentiate into myocytes (Seale et al., 2008). In adulthood, muscle satellite cells can also produce brown adipocytes (Lee et al., 2013; Lepper and Fan, 2010; Yin et al., 2013). Although the origin of beige adipocytes is still unclear they are thought to be derived from white adipocytes precursors from a different lineage called myogenic factor (*MYF5*⁻) (Seale et al., 2000; Timmons et al., 2007; Walden et al., 2009). It is noteworthy that interconversion of white adipocyte into beige adipocyte and vice versa can occur by trans differentiation (Harms et al., 2014; Young et al., 1984). Since the discovery of beige adipose tissue in rodents, it has not been absolutely clear as to what type of BAT adult humans have. However, it was reported in some studies that human brown fat depots, such as supraclavicular and periaxillary fat, are composed predominately of beige adipocytes (Sharp et al., 2012; Wu et al., 2012). Nonetheless, some preliminary data suggest that human BAT includes both classical and beige adipocytes (unpublished data).

2.1.3 Thermogenesis and substrate utilization by BAT

Body temperature is regulated by the central nervous system. In cold conditions, thermoreceptors on the skin sense the drop in ambient temperature. The afferent peripheral nerves transmit this information to the spinal cord and brain. This information is processed mainly by the ventromedial hypothalamus (Contreras et al., 2017). The efferent sympathetic nerves send a response to the periphery and BAT. Postganglionic SNS efferent nerves release nor-epinephrine (NE) (Cinti, 2009), which in turn, binds to β_3 adrenergic receptors (and activate a chain of intracellular reactions that subsequently activate thermogenesis (Morrison et al., 2012). Adenylyl cyclase (AC) is then activated by β_3 adrenergic receptors (Bourová et al., 2000). AC converts adenosine triphosphate (ATP) to 3'-5'-cyclic adenosine monophosphate (cAMP) (Patel et al., 2001). The cAMP activates the enzyme, protein kinase A (PKA). Various proteins are phosphorylated by PKA such as the peroxisome proliferator-activated receptor co-activator 1 α (PGC1 α), the cAMP response-element binding protein (CREB) (Thonberg et al., 2002), perilipin (Chaudhry and Granneman, 1999) and hormone sensitive lipase (HSL) (Krintel et al., 2009). Under normal conditions, the FFAs released from hydrolysis of triglycerides, are consequently oxidized to form acetyl coenzyme A, which produces adenosine triphosphate molecules (ATP) after full oxidization via Krebs Cycle and electron transport chain activity. However, brown adipocytes have elevated UCP-1 protein activity that increases leakage of protons, thereby releasing heat instead of producing ATP. This is termed non-shivering/facultative thermogenesis (Cannon and Nedergaard, 2004). Heat generation in

BAT can also take place from other pathways like creatinine cycling (Kazak et al., 2015) and the glycerol-3-phosphate shuttle (Anunciado-Koza et al., 2008; Lardy and Shrago, 1990).

When thermogenesis is triggered, circulatory glucose and free fatty acids are used by brown adipocytes. The transport of glucose is facilitated by the glucose transporter proteins 1 (GLUT1) and 4 (GLUT4). Insulin upregulates both GLUT1 and GLUT4 (Aldiss et al., 2017). The fate of glucose after the uptake is not clearly known. BAT eliminates roughly half of the glucose molecules as lactate (Reshef et al., 2003). The residual glucose is probably diverted to glycerol production (Reshef et al., 2003), intracellular triglyceride synthesis or fatty acid synthesis (*de novo* lipogenesis). The majority of glucose taken by BAT during cold exposure is not used to fuel mitochondrial oxidative metabolism, thus BAT GU can be disconnected from thermogenesis (Carpentier et al., 2018).

In brown adipocytes, fatty acid metabolism is a topic of active investigation. BAT may utilize fatty acids from two different pools of circulation namely plasma FFAs and triglyceride-rich lipoproteins (TRL) (Berbée et al., 2015; Blondin et al., 2017a; Carpentier et al., 2011; Khedoe et al., 2015; Waurisch et al., 2011) (Figure 2). BAT may also take up FFAs from *de novo* synthesis (Schreiber et al., 2017). FFAs are released by the lipolysis of intracellular lipid droplets in BAT. Additionally, during fasting WAT releases FFA via intracellular triglyceride lipolysis. FFAs activate UCP-1 and are the mainly utilized for BAT thermogenesis (Cannon and Nedergaard, 2004). A recent study in rats and humans showed that during cold exposure intracellular triglycerides lipolysis is required to generate BAT thermogenesis (Blondin et al., 2017b). While other studies have shown that BAT thermogenesis can be activated through alternative pathways rather than intracellular triglyceride lipolysis (Schreiber et al., 2017; Shin et al., 2017).

When TRL reach the blood stream lipoprotein lipase (LPL) hydrolyzes them into FFA and monoacylglycerol (MG) for uptake in BAT (Figure 2). The enzyme LPL is bound at the endothelial cell surface and is an important source of FFA in BAT (Bartelt et al., 2012). The fatty acid transporters, cluster of differentiation (CD-36) and fatty acid transport proteins (FATPs) receive the FFA released by LPL. Importantly, there are six isoforms of FATPs, and FATP1 and 4 are found exclusively in BAT (Gimeno, 2007). These proteins translocate FFA into cells (Hagberg et al., 2010). They have active long-chain acyl CoA synthetase and when over-expressed increase FFA uptake (Bartelt et al., 2012; Stienstra et al., 2014). In addition to glucose, BAT may also use acylcarnitines as a substrate for BAT thermogenesis (Simcox et al., 2017).

2.2 Brown adipose tissue activity

2.2.1 Cold-induced BAT activation

BAT function is dormant at room temperature and at resting state (Orava et al., 2011; Virtanen et al., 2009). Cold exposure is by far the most robust natural and physiological inducer for activation of BAT. Skin temperature is reduced in response to acute cold exposure (van Marken Lichtenbelt et al., 2009; Yoneshiro et al., 2011). However, in subjects with an active functional BAT, particularly the supraclavicular region, temperature on the skin surface is not reduced. This suggests that BAT produces heat (Yoneshiro et al., 2011). Furthermore, resting energy expenditure is increased by cold (van Marken Lichtenbelt et al., 2009; Orava et al., 2011; Ouellet et al., 2012; Saito et al., 2009) in those subjects with greater BAT metabolic activity in cold conditions (Yoneshiro et al., 2011).

In humans, BAT activation can be measured *in vivo* using non-invasive combined PET/CT imaging. Different tracers are used to precisely measure several metabolic and physiological functions. In BAT studies quantitative tissue-specific glucose uptake rate or semi-quantitative FDG (18F-fluro-D-deoxyglucose uptake) are most often used as an indirect marker of thermogenesis.

There is a markedly increased FDG uptake in the supraclavicular and neck region during the winter season compared to summer (Ouellet et al., 2011). Besides variability in seasons, exposure to acute cold may extensively raise the metabolic activity of BAT. Acute cold exposure increases BAT GU by ten-fold compared to room temperature (Orava et al., 2011). Importantly, only a few tissues such as skeletal muscle have the ability to increase the metabolic rate so considerably during short stimulation. Cold acclimation by repeated cold exposures can increase BAT volume (Ouellet et al., 2012) and metabolic BAT GU (Blondin et al., 2014; van der Lans et al., 2013; Yoneshiro et al., 2013). Additionally, cold exposure also significantly increases human BAT oxidative metabolism (Blondin et al., 2015a; Din et al., 2016; Muzik et al., 2013), blood flow (Din et al., 2016; Muzik et al., 2013), and fatty acid uptake (Blondin et al., 2015a, 2017b).

2.2.2 Insulin-stimulated glucose uptake in BAT

While cold is a robust activator and recruiter of the BAT function, the use of long-term cold exposure by people in the modern world remains impractical. BAT activation has also been linked to eating (Rothwell and Stock, 1997). In post prandial condition, insulin plays a prominent role. Insulin concentrations are slowly increased after the initial period of eating. In the post prandial period, the plasma insulin level increases up to 70-100 m U/l from approximately 3-10 mU/l at fasting. Insulin stimulation created by euglycemic hyperinsulinemic clamp technique, analogous to postprandial levels, is targeted to reach

insulin concentrations of 70-100 mU/l (Virtanen, 2018). In this type of experimental set-up, substrate uptake from tissues can be quantified with PET during insulin stimulation (Nuutila et al., 1995).

Insulin and cold may activate BAT thermogenesis via SNS activation by 5 and 12-fold, respectively (Orava et al., 2011; Virtanen, 2018). Conversely, during steady state of hyperinsulinemia, BAT perfusion is not increased similar to cold conditions; this implies that BAT thermogenesis may not be directly affected by insulin (Orava et al., 2011). Nevertheless, insulin may stimulate thermogenesis by inhibiting warm-sensitive neurons in the hypothalamus (Labbé et al., 2015; Sanchez-Alavez et al., 2010). Vosselman et al. showed that a meal containing predominately carbohydrate increases BAT FDG uptake comparable to that after cold stimulus (Vosselman et al., 2013). Recently, BAT postprandial metabolism was studied in comparison to cold exposure by using [^{15}O]O $_2$, [^{15}O]H $_2\text{O}$ and [^{18}F]FTHA (U Din et al., 2018). BAT oxygen consumption and blood flow were enhanced after eating a meal rich in carbohydrates in a similar manner to cold stimulus, thus suggesting the hypermetabolism of BAT after a meal (U Din et al., 2018).

Insulin stimulation increases BAT GU at a level similar to skeletal muscle GU (Orava et al., 2011). Therefore, BAT can be considered an insulin sensitive tissue (Nuutila et al., 1995; Orava et al., 2011). Although the amount of BAT in the body is minor, and BAT GU plays a small role in contributing to whole-body insulin sensitivity, insulin stimulated GU in BAT correlates with whole body insulin sensitivity (M-value) (Orava et al., 2013). The increase in insulin-stimulated GU in BAT is considered to be mediated GLUT4 (Figure 2) (Omatsu-Kanbe et al., 1996), whose translocation is facilitated by IRS-2 (Fasshauer et al., 2000) (Figure 2).

Both cold and insulin stimulation increase BAT GU and whole body energy expenditure (Orava et al., 2011). However, cold and insulin have relatively diverse effect on BAT metabolism as the metabolic milieu is not the same. During cold SNS activation causes high plasma FFA concentration whereas, during insulin stimulation, there is low concentration of plasma FFA. Therefore, during cold, lipolysis from WAT is prominent, but during insulin stimulation, it is inhibited by elevated insulin levels (Virtanen, 2018). This phenomenon could be explained by increased plasma NE levels during cold, but such changes cannot be identified during insulin stimulation.

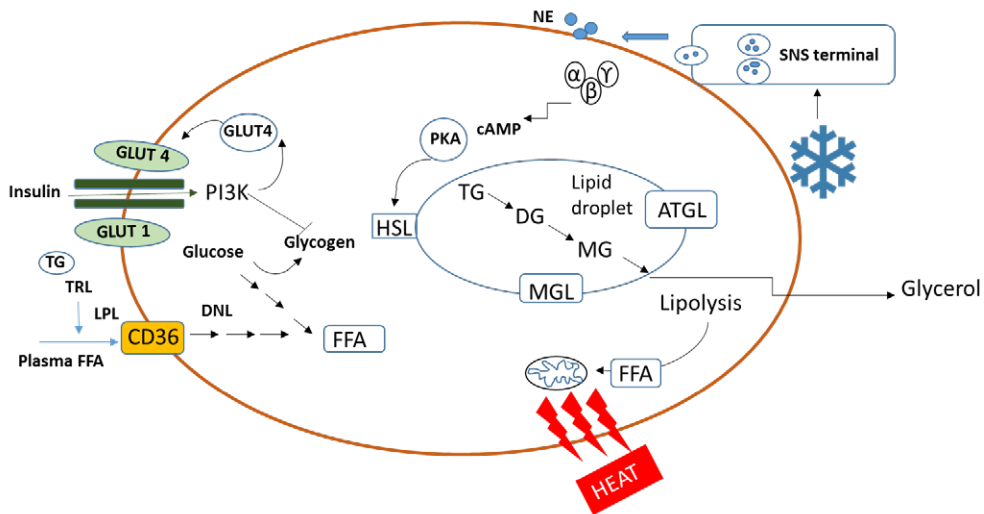


Figure 2. Key metabolic signaling pathways in brown adipocyte during cold and insulin stimulation. Modified from (Peng et al., 2015).

2.2.3 BAT and insulin resistance

Currently, there is much interest in understanding how activating BAT can prevent and/or treat obesity as well as T2DM. BAT GU is reduced in obesity and T2DM, which may be related to genetic variants of IR (Latva-Rasku et al., 2018; Lee et al., 2010; Orava et al., 2013). In either case, in healthy subjects, whole-body insulin sensitivity is negatively associated with BAT lipid content (Raiko et al., 2015) and reduced BAT GU is associated with increased fat content (Blondin et al., 2015b; Holstila et al., 2017; Koskensalo et al., 2017). This indicates that the higher the BAT lipid content the reduced ability of BAT to clear FFA's from the circulation. In times of energy excess such as in obesity, the lipid pool is increased considerably possibly also affecting mitochondrial function and oxidation. One of the functions of BAT is to buffer the lipids from the circulation in order to protect from systemic hyperlipidemia (Berbée et al., 2015; Chondronikola et al., 2016; Waurisch et al., 2011). Therefore, if BAT fails to function or has reduced activity as a consequence of obesity, it might start to take resemblance to white adipocyte and increase the lipid pool inside the cell. It is not very clear whether activating BAT will realistically be a cure for obesity or whether decrease in BAT activity predisposes to obesity particular people to obesity.

Interestingly, cold-induced BAT GU positively associates with insulin-stimulated BAT GU (Orava et al., 2013). IR impairs BAT function as found in obesity. In lean subjects, insulin-stimulated GU and blood flow has been shown to be twice as high as that in obese subjects (Orava et al., 2013). Additionally, the cold-induced BAT GU correlated positively with cold-induced blood flow in the same study indicating that the impairment in BAT

GU is associated with reduced blood flow in obesity (Orava et al., 2013). In line with this, according to recent report in rodents, a hypoxic state in BAT leads to dysfunction in mitochondria and whitening of adipocyte (Shimizu et al., 2014).

BMI and BAT activity have inverse associations (van Marken Lichtenbelt et al., 2009; Ouellet et al., 2011; Saito et al., 2009). However, the human data regarding the effects of weight loss on BAT GU in morbidly obese subjects with or without T2DM after bariatric surgery is controversial (Rodvalho et al., 2017; Vijgen et al., 2011).

AMPK is key component for the preservation of mitochondrial function in BAT (Desjardins and Steinberg, 2018), thus improvements in BAT insulin sensitivity after weight loss could be explained with an increase in AMPK activity. Obese and IR subjects have reduced SAT AMPK activity (Xu et al., 2012). However, BAT AMPK activity in T2DM has not been explored so far. It will be interesting to investigate whether BAT AMPK activity in obese and IR subjects is also altered similar to WAT. In rodents, BAT transplantation improves SAT and myocardial insulin sensitivity by releasing BAT-derived hormonal factors indicating an endocrine role of BAT (Stanford et al., 2013). However, the molecular mechanisms of insulin resistance and hormonal functions of BAT are not widely explored in humans and remain to be determined.

2.3 WAT metabolism

2.3.1 Glucose and free fatty acid metabolism in WAT

At rest, the quantitative role of WAT for whole body glucose disposal in lean subjects averages about 11% and in obese subjects around 21% (Horowitz et al., 2001). In a fasting state, glucose uptake in WAT is regulated by the GLUT1 via facilitated diffusion (Figure 3A) (Kahn, 1992a). Under insulin stimulation, glucose uptake in white adipocytes increases 20 to 30 fold, indicating marked insulin sensitivity (Petersen and Shulman, 2018). In addition to the increased glucose uptake, insulin suppresses lipolysis and thus lowers plasma FFA concentrations (Perry et al., 2015; Petersen and Shulman, 2018; Reaven, 1988). Some studies have shown that in humans quantitatively WAT plays a minor role and only accounts for <5% of an oral glucose load (Jackson et al., 1988; Kowalski and Bruce, 2014). Insulin activates glucose transport by two mechanisms, either by GLUT translocation (Cushman and Wardzala, 1980) or by increasing the amount of GLUT proteins (Cusin et al., 1990). In WAT under insulin stimulation, glucose is taken up predominately via GLUT 4 (Figure 3). Similar to skeletal muscle cells in the insulin signaling pathway, the activation of phosphoinositide-3 kinase (PI3K) and insulin receptor substrate (IRS) is crucial in adipocytes to increase glucose transport (Shepherd and Kahn, 1999).

Once glucose enters the cell, hexokinase immediately phosphorylates glucose (Katzen et al., 1970). However, the fate of this glucose is not clearly known. Either glucose undergoes oxidation in Krebs/TCA cycle (Cannon and Nedergaard, 1979), it is stored as glycogen, or it undergoes conversion to lactate. Alternatively, *de novo* lipogenesis and triglyceride synthesis can occur (Figure 3). WAT may function this way as a glucose sink (Virtanen et al., 2005). To further elaborate this point as adipocyte GU generates glycerol-3-phosphate for fatty acid esterification, it mediates the lipid storage in WAT and reducing lipid flow to the liver and muscle (Petersen and Shulman, 2018). Furthermore during periods of excessive energy intake WAT stores excessive free fatty acids and glycerol as triglycerides in their adipocytes with limited energy expenditure (Seale and Lazar, 2009). In WAT of T2DM and obese subjects binding of insulin to its receptor is not altered but instead the insulin receptor substrate has reduced phosphorylation and diminished tyrosine kinase activity (Bolinder et al., 1986; Freidenberg et al., 1987, 1988). Interestingly, weight loss improves both adipose tissue IR and defective adipocyte insulin receptor kinase activity (Freidenberg et al., 1988). However, mechanisms for these underlying adipocyte receptor defects are unknown.

In the WAT of lean adults about 80,000 kcal are stored as triglycerides (Horowitz, 2003). Triglycerides are released as fatty acids from WAT through lipogenesis after eating and lipolysis after fasting (Hankir and Klingenspor, 2018). Circulating fatty acids are released either from dietary fats or from endogenous *de novo* lipogenesis (DNL), mediated majorly by acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS). After consumption of a carbohydrate meal there is activation of FAS in WAT that converts glucose to fatty acids (Hillgartner et al., 1995). Dietary fatty acid uptake in adipocytes occur sequentially. First, lipoprotein lipase (LPL) situated in capillary endothelium hydrolyzes circulating triglycerides and then tissues take up the released free fatty acids. In WAT, fatty acids are stored as triglycerides to provide energy when required. Triglycerides are carried from circulating chylomicrons or very low density lipoproteins (VLDLs). During fasting or after exercise, WAT releases fatty acids through triglyceride lipolysis. The lipolysis that began with adipose triglyceride lipase (ATGL) is thereafter continued by hormone sensitive lipase (HSL) and mono-acylglycerol lipase (MAGL); this release FFA and glycerol, for energy production in other tissues like the liver, and skeletal and cardiac muscles (Huijsman et al., 2009). Fatty acids are taken up by passive and facilitated diffusion, which relies on the concentration gradient between the interstitial and intracellular fluid (Corpeleijn et al., 2009). The diffusion is carried out by specific transport proteins that change the fractional free fatty acid uptake (the percentage of fatty acids available from plasma that are extracted by the organ (Glatz et al., 2002). A principal fatty acid transporter of WAT is CD36 (Coburn et al., 2000) (Figure 4A). Additionally, Angiopoietin-like proteins (specifically, ANGPTL3 and ANGPTL4) have an important role in regulating lipid metabolism by inhibiting lipoprotein lipase (Shan et al., 2009).

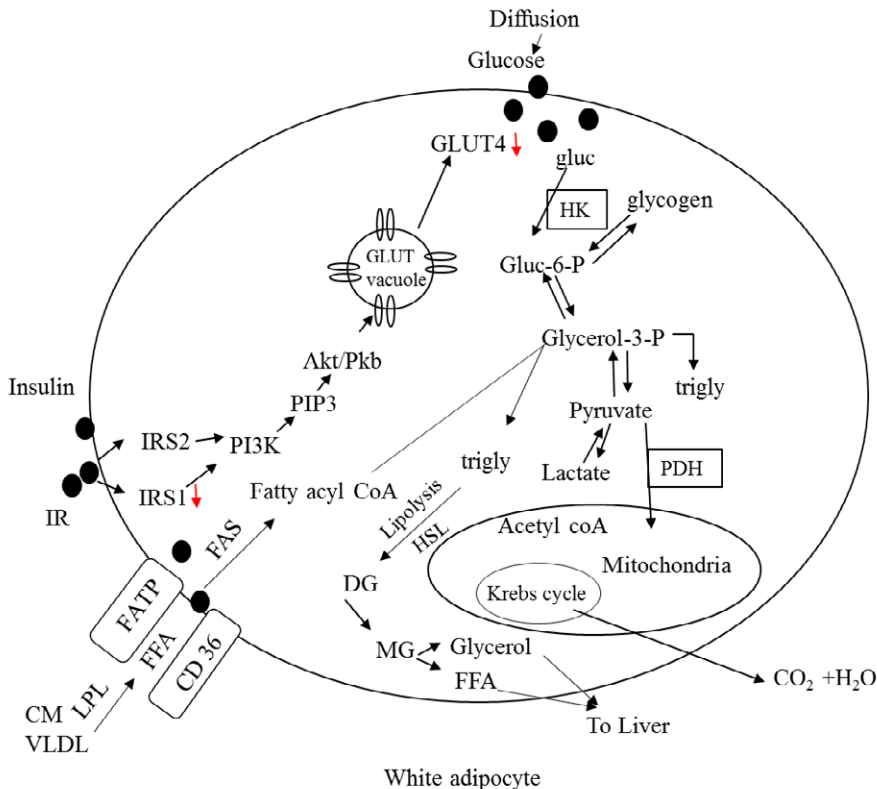


Figure 3. Illustration of the regulation of glucose and free fatty acid uptake in white adipocyte. Red arrows: impaired possible mechanisms observed in insulin resistance and obesity in white adipocyte. GLUT 4, glucose transporter 4; IRS, insulin receptor substrate; PI3K, phosphoinositide-3-kinase; PIP3, phosphatidylinositol-3,4,5-triphosphate; Pkb, protein kinase B; Gluc-6-P, glucose 6 phosphate; trigly, triglycerides; HK, hexokinase and PDH, pyruvate dehydrogenase; hormone sensitive lipase (HSL); TRL, triglyceride rich lipoproteins; FATP, fatty acid transporter protein; cluster of differentiation 36 CD36; FAS, fatty acid synthetase. References are provided in the main text.

WAT has a thermogenic response to chronic cold exposure. This chronic cold exposure induces sympathetic activation. The interaction of nor-epinephrine with β_3 adrenergic receptors situated on the membrane of white adipocytes triggers a cascade that leads to increased UCP-1 expression leading to browning of WAT (Montanari et al., 2017). Recent animal studies have shown that cold exposure can cause browning of WAT through interleukin-6, fibroblast growth factor FGF21 and irisin (Fisher et al., 2012; Villarroya et al., 2017). However, browning in response to cold exposure is not clear in humans.

2.3.2 WAT and insulin resistance/T2DM

Insulin resistance (IR) plays a major role in the pathophysiology of T2DM. IR means decreased insulin action on its targets on whole-body level, tissue level or cell level compared to normal physiological response (Petersen and Shulman, 2018). IR manifests as reduced stimulation of GU in skeletal muscle, the liver, WAT and BAT, intestine,

cardiac muscles well decreased suppression of lipolysis in WAT and impaired endogenous glucose production (EGP) suppression from liver and kidneys (Iozzo, 2010; Mäkinen et al., 2015; Orava et al., 2013; Petersen and Shulman, 2018). It is evident that defective insulin action in both insulin target tissues and β -cells is required for the development of fasting hyperglycemia and T2D (Kahn, 2003).

WAT together with skeletal muscle, is a site of peripheral IR in prediabetes and T2DM (Ciaraldi et al., 1982). IR in obesity and T2DM is manifested by reduced glucose transport and metabolism during insulin stimulation in adipocytes (Ciaraldi et al., 1982; Garvey et al., 1991; Kashiwagi et al., 1983; Reaven, 1995). Accumulation of both abdominal SAT (Goodpaster et al., 1997) and VAT have been associated with IR and T2DM (Després et al., 1989; Goodpaster et al., 1997; Karpe and Pinnick, 2015). In comparison to SAT, VAT has a higher lipolytic activity due to a higher expression of β -adrenergic receptors (Goedecke and Micklesfield, 2014; Nielsen et al., 1991). The positive association between VAT and metabolic complications of obesity are due to high rates of VAT FFA release into the portal vein (Björntorp, 1990; Kissebah and Peiris, 1989). This results in increased hepatic FFA delivery which is considered to increase very low density lipoprotein (VLDL) production (Kissebah and Peiris, 1989; Lewis et al., 1995) and IR with regard to hepatic glucose production (Ferrannini et al., 1983). Furthermore, insulin-stimulated GU in abdominal SAT and VAT is reduced in obesity and T2DM when measure per litre of tissue implying IR. However, a larger fat mass counteracts this impairment so that the GU when expressed per whole depot is in a true sense similar to or higher than that of a normal-weight or lean subject (Dadson et al., 2015; Koskensalo et al., 2017; Mitrou et al., 2009; Virtanen et al., 2002).

Previous studies indicate defective antilipolytic effect of insulin in obesity, IR and T2DM (McGarry, 1992, 2002; Nellesmann et al., 2012), there are also higher FFA levels postprandially in obesity and in people who are on a high fat diet. However, some studies suggest that increased lipolysis in obese subjects is because of higher fat mass and not due to reduced antilipolytic effect of insulin (Arner et al., 1990; Robinson et al., 1998).

In obesity, the most prominent dysfunctions are WAT inflammation (Boutens and Stienstra, 2016; Johnson and Olefsky, 2013; Osborn and Olefsky, 2012), IR (Abdul-Ghani et al., 2008), hypoxia (Trayhurn, 2013), reduced adipogenesis and mitochondrial dysfunction (Jokinen et al., 2017; Yin et al., 2014). Adipose tissue function is strongly associated with adipocyte cell size suggesting that adipocyte size contributes towards the metabolic dysfunction associated with obesity (Heinonen et al., 2014b; Tchernof and Després, 2013; Weyer et al., 2000). When the storage capacity of WAT (SAT) is exceeded adipocytes become hypertrophic. Hypertrophic adipocytes have more IR as they promote low-grade inflammation and decrease the ability of WAT to buffer lipids, leading to accelerated lipolysis and increased concentration of FFA (Farnier et al., 2003; Varlamov

et al., 2010). Expansion of the adipose tissue in human obesity is considered to be homeostatic stress and is linked with increased adipocyte cell death (Murano et al., 2008). Stressed adipocytes recruit bone marrow derived macrophages through chemotactic signals. These adipose tissue macrophages deposit in “crown-like structures” around dead adipocytes and secrete cytokines with autocrine, paracrine and endocrine effects (Murano et al., 2008; Olefsky and Glass, 2010). In obese and inflamed adipose tissue predominately M1 macrophages secrete pro-inflammatory markers like IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1) and TNF- α . Furthermore, serum concentrations of pro-inflammatory markers like C-reactive protein, TNF- α , IL-1 β , IL-6 and MCP-1 have been shown to correlate with IR events leading to T2D (Jager et al., 2007; Spranger et al., 2003). C-reactive protein is positively associated with abdominal adiposity (Lemieux et al., 2001) and IL-6, IL-1 β and TNF- α can directly inhibit insulin signaling (Jager et al., 2007; Rotter et al., 2003). Notably, the main insulin-stimulated glucose transporter, GLUT4, is reduced in SAT in subjects with IR and T2DM (Kahn, 1992b; Rondinone et al., 1997) and has an inverse association with adipocyte hypertrophy (Carvalho et al., 2001). For an enlarged adipose tissue, there is increased demand for vascular supply to maintain oxygen delivery. Therefore, adipogenesis and angiogenesis are considered to be tightly regulated simultaneous processes to ensure adipose tissue expansion. It is not completely clear whether expansion of WAT due to inadequate vascularization leads to hypoxia, as some studies support the idea (Kabon et al., 2004; Pasarica et al., 2009a) while others do not (Hodson et al., 2013; Trayhurn, 2013). Indeed it has been shown that lower adipose tissue blood flow is major factor in decreased insulin-stimulated GU in case of IR (Ferrannini et al., 2018; Raitakari et al., 1996).

2.4 Effects of exercise training on health

Sedentary behavior is a major behavioral factor in metabolic diseases. Generally, the term physical activity indicates all movements that increase energy expenditure. However, exercise training is planned controlled physical activity designed to improve physical fitness. Regular exercise training leads to improvements in body composition (i.e. decrease in abdominal fat masses and body weight) (Smith, 2018; Thomas et al., 2013), better lipid profile as reflected by lower triglyceride and low density lipoproteins (LDL) and elevated high density lipoprotein (HDL) levels (Mitsui et al., 2012; Warburton et al., 2006), improved glucose tolerance and insulin sensitivity (Tan et al., 2018), decreased systemic inflammation (Batista Jr. et al., 2010; Lira et al., 2009; Sharman et al., 2015), increased cardiac function (Barauna et al., 2005; Batista et al., 2008; Laterza et al., 2007), reduced blood pressure (Imazu et al., 2017; Sharman et al., 2015) and increased endothelial function (Heinonen et al., 2014a). The main physiological adaptations involve increased blood flow for delivery of oxygen in all tissues particularly in the heart and skeletal muscles (Figure 4) (Heinonen et al., 2014a). The structural adaptations mainly in skeletal muscle include increase in skeletal muscle mass, change in fiber composition and an

increase in mitochondrial content (Holloszy, 1967) that enable the utilization of these nutrients for energy production.

Exercise causes a number of hormonal responses with a rise in catecholamines that increase lipolysis. One of the key mechanisms post-exercise is the increase in the activity of the 5'-adenosine monophosphate-activated protein kinase (AMPK), an enzyme that senses the energy state of the cells. The main energy of the cell comes from oxidizing fat and carbohydrate. When there is need for extra energy, AMP and ADP levels rise whereas ATP levels are low in the cells. This causes an increase in AMPK activity that then in turn stimulates oxidation of FFAs, uptake of glucose and mitochondrial biogenesis (Carling et al., 2012; Day et al., 2017). AMPK may also regulate cellular glycogen content, but the underlying mechanisms are not clear (Musi et al., 2003). Exercise also increases the expression of peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1- α), a master regulator of mitochondrial biogenesis (formation of new mitochondria) and oxidative metabolism (Knutti and Kralli, 2001). Recently, activation of BAT and WAT browning have been proposed to be the potential part of an unknown underlying mechanism by which exercise exerts a beneficial effect on human health (Lehnig and Stanford, 2018; Sanchez-Delgado et al., 2015; Tarantola et al., 2015). However, the cellular mechanism by which exercise acts on BAT and WAT remains to be elucidated.

Physical inactivity is an independent cause of ectopic fat accumulation (Pedersen, 2009). Reduction of ectopic stores by enhancing mitochondrial uncoupling and increasing cellular expenditure that could reduce insulin resistance (Schrauwen and van Marken Lichtenbelt, 2016). Increased energy turnover by exercise may enhance insulin sensitivity by increasing the turnover rate of fat in ectopic fat sites, and hence protect against the harmful effects of excessive fat storage. However, this needs further testing (Schrauwen and van Marken Lichtenbelt, 2016), particularly as it relates to different exercise prescriptions.

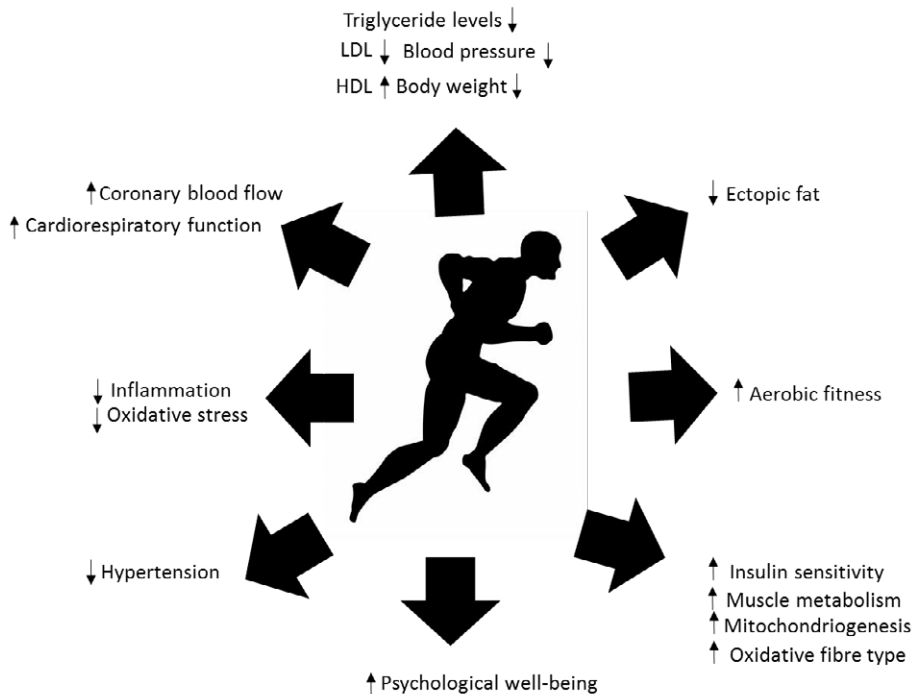


Figure 4. Effects of exercise. References found in the main text.

2.4.1 Modes of exercise training

Exercise is generally categorized as endurance and/or resistance exercise training, both of which promote substantial health benefits. However, the effects of exercise vary depending on the intensity, type, frequency, prior training status and duration of exercise (Wisloff et al., 2009).

Moderate intensity continuous training (MICT) is defined as training of an endurance-type carried out with persistent intensity without a rest-period in between. For example, brisk walking, cycling and jogging. Typically, MICT protocol of one session comprises running or cycling at 60-70% of VO_{2max} for 45-60 minutes. Conventionally, MICT has been the most common category of exercise endorsed to increase physical fitness and over-all health-related parameters. MICT is relatively easy to start with but considering the fact that it consumes considerable amount of time, it may limit adherence to this type of exercise regularly.

High intensity interval training (HIIT) is popular particularly among athletes because it challenges the aerobic energy system and rapidly improves physical performance (Billat, 2001). During the last decade, high intensity interval training (HIIT) has gained attention among the general public, as it is an alternative option for busy people since HIIT is effective and less time-consuming. Low-volume HIIT is defined as relatively short

vigorous bouts of exercise lasting for 1-4 min that elicit 80-100% of maximal heart rate, interspersed by episodes of rest or exercise with minimum intensity. Intense HIIT protocols performed with exceptionally high, > 100% of $\text{VO}_{2\text{max}}$, each bout lasting for about 30 secs, are categorized in term SIT (sprint interval training). There is growing consensus that various forms of HIIT despite the lower total exercise volume and training duration can be as beneficial as traditional high-volume moderate intensity endurance training in inducing positive whole-body health promoting adaptations (Martin et al., 1995; Rehn et al., 2013; Weston et al., 2014).

2.5 Effects of exercise training on BAT activity

Exercise training may influence BAT metabolism (Ruiz et al., 2015). Exercise training increases sympathetic activity and the release of catecholamines, which promotes lipolysis in BAT via β_3 adrenergic receptors. Acute exercise training increases BAT lipolysis and thermogenesis mainly through activation of UCP-1 (Ruiz et al., 2015). The chronic effects of exercise on BAT include increase in mitochondrial biogenesis, BAT hyperplasia, browning or inducing *de novo* differentiation giving rise to the classical interscapular and perirenal brown adipocyte (Giordano et al., 2016). Stimulation of β_3 adrenergic receptors promotes UCP-1, PGC1 α expression and mitochondrial biogenesis, thermogenic capability and conversion of white to brown adipocytes (Carpentier et al., 2018).

2.5.1 Direct influence of exercise on BAT activity

In rodents, BAT activity in response to exercise training has been widely studied although the results have been inconsistent, suggesting decreased, unchanged or increased activity (Table 1). Interestingly, increases in BAT activity was mostly seen with swimming intervention studies. The deductions made by swimming intervention studies can be biased as it is highly possible that it is the cold water and not the training that induces BAT adaptations. The inconsistent outcomes in the aforementioned studies may also be partly explained by the different methods used with rodents.

In humans, there are few studies that have investigated the effects of exercise on BAT metabolism. Vosselman et al. investigated the impact of chronic exercise on BAT [^{18}F]FDG uptake using static imaging and comparing 12 healthy lean young sedentary men to 12 endurance-trained athletes in a cross-sectional study. The subjects were exposed to cold conditions for 2 h and BAT activity was measured using [^{18}F]FDG-PET/CT (Vosselman et al., 2015). Cold-induced BAT [^{18}F]FDG uptake was lower in endurance-trained athletes compared to healthy lean sedentary men, suggesting regular endurance training is linked with decreased cold-stimulated BAT FDG uptake (Vosselman et al., 2015). A similar trend was also found when comparing female athletes (lower BAT volume and activity) to non-athletes (Singhal et al., 2016). These interesting results highlight that similar to obese subjects, trained athletes also show reduced BAT activity

(van Marken Lichtenbelt et al., 2009). Habitual physical activity associated positively with increased BAT activity (Dinas et al., 2015), indicating that light exercise may increase BAT activity in untrained sedentary people. In contrast, another trial showed that objectively measured physical activity levels do not change either BAT volume or activity (Acosta et al., 2018).

There are no exercise intervention studies investigating BAT free fatty acid (FFA) uptake directly in humans. Recently, it was shown that moderate intensity exercise training significantly increases a novel circulating lipid 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME) in all human subjects regardless of sex, age or physical activity (Stanford et al., 2018). Previously, 12,13-diHOME has been shown to be elevated after short-term (1 hour) and chronic (7-11 days) cold exposure in humans (Lynes et al., 2017). Although having the same outcome, cold and exercise have opposite mechanism of action on 12, 13-diHOME. It has been proposed that during cold conditions 12,13-diHOME acts in an autocrine mode, whereas after exercise BAT releases 12,13-diHOME stimulating the release of fatty acids into the active skeletal muscle and performs in an endocrine manner (Stanford et al., 2018).

Table 1. Studies on exercise training and BAT.

REFERENCE	METHOD	EXPERIMENTAL GROUPS	EXERCISE INTERVENTION PROTOCOL	FINDINGS
INCREASE IN BAT ACTIVITY				
(HIRATA AND NAGASAKA, 1981)	Assessment of calorogenic response to cold immersion (in water at 18°C up to neck) and Infusion of nor-epinephrine	Physically fit, control and cold-acclimated rats	3 hr swimming in water at 36 °C for 6-8 weeks	Cold immersion increased oxygen consumption in cold-acclimated and physically fit rats compared to control rats. Infusion of nor-epinephrine increased oxygen consumption significantly more in cold-acclimated and physically trained rats compared to control rats.
(HIRATA, 1982)	Blood flow measurement by gamma-labelled plastic microspheres during 30-min infusion of nor epinephrine	Physically trained and control rats	2 hr swimming in water at 32°C 36°C and 38 °C for 10-11 weeks	Increased blood flow to BAT
(OH-ISHI ET AL., 1996)	Western blotting of UCP, Northern blot analysis of UCP mRNA	Young and old male mice	Swimming 1 h/day, 5 days/week at 35-36°C for 6 weeks	2-fold increases in BAT mass, increase in UCP content in BAT, no effect in UCP-1 mRNA expression
(XU ET AL., 2011)	UCP-1 expression (immunohistochemical staining for UCP-1) adipocyte progenitor cells population (Flow cytometry)	Sedentary and, exercise trained rats with normal diets	Motorized treadmill, 40 min/day, 5 days/week for 8 weeks	Increase in UCP-1 in BAT, increase in the number of progenitor cells in BAT
DECREASE IN BAT ACTIVITY				
(WU ET AL., 2014)	Western blot UCP-1, PGC1 α , AMPK, Phospho-AMPK, ATGL, FNDC3, GADPH and β -actin	Sedentary & exercise-trained rats fed ad libitum either low fat or high fat diets	Treadmill, 1h/day, 5days/week for 8 weeks	Sedentary HF diet: BAT mass, PGC1- α and UCP-1 content and palmitate oxidation increased.

					Exercise LF diet: PGC1- α and UCP-1 content and palmitate oxidation reduced. BAT UCP-1, UCP-2 and UCP-3 mRNA expression did not change
(BOSS ET AL., 1998)	UCP-1, UCP-2, UCP-3 mRNA	Trained rats	Endurance training	Swimming 30 min/day for 21 days with resting interval of 3 min at 25°C	Trained animals had lower serum T3 and deiodinating activity (around 25%) than untrained rats in basal state and after cold exposure. 5'-D activity decreased in IBAT after training.
(SULLO ET AL., 2004)	5'D enzyme assay from interscapular BAT measured at basal state and after 30 min of cold exposure. Serum concentration of T3 and T4 determined by radioimmunoassay.	Untrained rats, swimming controls, swimming trained controls			
LE BLANC ET AL., 1982	Plasma T3 levels, BAT size, nor-epinephrine levels	Rats fed with laboratory chow and cafeteria diet		Swimming 2 h/day for 10 weeks	Cafeteria diet induced thermogenesis and increase in T3 levels increased BAT size. Exercise training did not increase nor-epinephrine induced thermogenesis in laboratory chow fat rats or in rats on cafeteria diet.
NO EFFECT IN BAT ACTIVITY					
(RICHARD ET AL., 1986)	Mitochondrial GDP binding	Sedentary and exercise trained rats		Motorized treadmill at two different environments at either 24°C or 4°C Progressive time increase starting with 10 min, 2 times a day with 4 h rest between the bouts and after 10 days, 1h 2 times a day and 7 days/week for 45 days	Exercise training led to significant decreases in both BAT weight and protein content. BAT thermogenesis (GDP binding) was not significantly modified by exercise training, regardless of the temperature at which the rats were trained.
(STERN ET AL., 1987)	Oxygen consumption in response to infusion of nor-epinephrine. Nor-epinephrine stimulated blood	Sedentary and exercise trained rats		Treadmill running 90 min/day 5 days/week for 6 weeks	No significant differences in resting oxygen consumption, norepinephrine induced oxygen consumption, BAT mass or blood flow

	flow and the BAT mass by microsphere technique.	Untrained and trained rats		Forced running on treadmill (30 min, twice a day, 5days/week for 5 weeks	Exercise increased heat production in BAT in untrained rather than in trained rats. Interscapular BAT weight did not change, no difference of nor-epinephrine induced thermogenesis between the groups
(SHIBATA AND NAGASAKA, 1987)	Heat production measured by recording temperature of colon and interscapular BAT by thermistor probe and constantan thermocouple. Nor-epinephrine infused through a catheter in right jugular vein.				
(SEGAWA ET AL., 1998)	Mitochondrial GDP binding, UCP-1 content	Sedentary and trained rats		Treadmill running 5 days/wk for 9 weeks	No definite effect of training was noted on either in UCP-1 content or GDP binding
HUMAN STUDIES					
(VOSSELMAN ET AL., 2015)	BAT FDG uptake measured after 2-hr mild cold exposure by [18F]FDG-PET/CT scan. Subcutaneous WAT biopsies (gene expression assays for UCP-1, CIDEA, CD137 AND TMEM26)	Endurance trained athletes and lean healthy sedentary men		Endurance exercise 3 times/week for 2 years	Cold induced BAT FDG uptake significantly lower in athletes vs. sedentary men. No differences in gene expression of classical brown and beige adipocyte markers in subcutaneous WAT between the groups.
(SINGHAL ET AL., 2016)	BAT GU and volume measured after 2 hrs of cooling vest by [18F]FDG-PET/CT scan	Female athletes & non-athletes		Athletes: Weight bearing aerobic exercises for ≥ 4 hrs/week or running ≥ 20 miles/week for at least 6 months in the year preceding the study. Non-athletes: < 2 hrs per week of weight bearing exercise activity	A trend in lower cold-induced BAT FDG uptake and volume in athletes.
(ACOSTA ET AL., 2018)	BAT FDG uptake and volume measured after 2 hrs of personalized cold exposure by [18F]FDG-PET/CT scan	Young healthy sedentary males and females		Physical activity light, moderate, vigorous and moderate vigorous for 7 days (24hrs/day)	Objectively measured free living physical activity levels have no effect on BAT volume and activity

2.5.2 Indirect influence of exercise on BAT activity

Independent of SNS activation, BAT can be activated by a group of novel myokines/cytokines released into the circulation by muscles and peptides released by the heart during exercise (Figure 5). Skeletal muscle and BAT share some features and characteristics (Table 2). Notably, both are insulin-sensitive tissues (Orava et al., 2011). Exercise training improves skeletal muscle insulin-sensitivity. However, it is unclear whether training also improves BAT insulin sensitivity. The factors that may cause browning of WAT via increasing UCP-1 or enhance mitochondrial biogenesis in BAT are released during exercise include, but are not limited to irisin, interleukin-6 (IL-6), β -aminoisobutyric acid (BAIBA), fibroblast growth factor-21 (FGF-21) and cardiac natriuretic peptides (Figure 4).

Irisin

Irisin is a myokine discovered in 2012 (Boström et al., 2012). It is secreted mainly by skeletal muscle in response to exercise (Boström et al., 2012) and is produced by the cleavage from the precursor protein, fibronectin type III domain containing 5 (FNDC5), which recruits beige cells in WAT (Boström et al., 2012; Lee et al., 2014). Lee et al. showed a two-fold increase in irisin after 10 weeks of endurance training. In contrast, many studies showed that neither acute nor chronic exercise training alter circulating irisin levels (Aydin et al., 2013; Hecksteden et al., 2013; Kurdiova et al., 2014; Moraes et al., 2013; Norheim et al., 2014; Pekkala et al., 2013). Hence, the potential role and function of irisin particularly as a browning agent in humans is still unresolved (Elsen et al., 2014).

Interleukin- 6

The release of IL-6 from skeletal muscle and WAT have multiple functions (Ma et al., 2015). In skeletal muscle, IL-6 stimulates glycolysis and improves responses to insulin (Pedersen and Febbraio, 2008). Although it is known to be a pro-inflammatory cytokine during times of fat mass accretion, it also has anti-inflammatory effects post-exercise (Pedersen and Fischer, 2007a). These dual effect of IL-6 depends on the environment, in muscle cells it acts as a pro-inflammatory and in immune cell it can act as an anti-inflammatory factor (Pedersen and Febbraio, 2008). Exercise significantly increases serum IL-6 as much as 100-fold (Pedersen and Fischer, 2007b). Transplantation of BAT from IL-6 knock out mice into WAT depots blunts metabolic responses including reduced insulin sensitivity and a decreased amount of weight loss (Stanford et al., 2013). This indicates that IL-6 is essential to sustain the metabolic changes caused by BAT transplantations (Stanford et al., 2013). IL-6 seems to be a crucial mediator of BAT metabolism in animal models, however, whether the effects persist in humans remain unclear.

β -aminoisobutyric acid (BAIBA)

BAIBA was recognized in early 2014 (Roberts et al., 2014) and it was reported that BAIBA levels in muscle cells are controlled by PGC1- α and increases the expression of genes specific to brown-adipocyte. In mice, 3 wks of voluntary wheel cage running and in humans (HERITAGE Family Study) (Bouchard et al., 1995) after 20 weeks of supervised submaximal aerobic exercise training caused a significant increase in circulating BAIBA (Roberts et al., 2014). Additionally, BAIBA increased expression of the beiging markers UCP1, CIDEA and PRDM16 in human-induced pluripotent stem cells. These data together highlight that exercise induces a significant increase in circulating BAIBA in relation to browning of WAT in mice, stem cells, and human subjects.

Table 2. Common characteristics of brown adipose tissue and skeletal muscle.

	Brown/Beige adipose tissue	Skeletal muscle
Mitochondria	High (Cannon and Nedergaard, 2004)	High (Larsen et al., 2012)
Oxidative capacity	High (Blondin et al., 2014)	High (Holloszy and Coyle, 1984)
Vasculature	Dense (Hausberger and Widelitz, 1963)	Dense (Segal, 2005)
Innervation	SNS (Bartness et al., 2010)	Motor (Barker et al., 1974)
Function	Burn energy (Cannon and Nedergaard, 2004)	Burn energy (Block, 1994)
Energy storage	Triglycerides (Cannon and Nedergaard, 2004; Scheja and Heeren, 2016)	Glycogen (Bergström et al., 1967)
Insulin sensitivity	High (Orava et al., 2011)	High (Ferrannini et al., 1988; Thiebaud et al., 1982)

Fibroblast growth factor 21

FGF21 is produced by the liver, skeletal muscle, BAT, WAT and pancreatic β cells (Muise et al., 2008; Nishimura et al., 2000; Schulz et al., 2011). In response to chronic cold exposure in mice, FGF21 expression was shown to increase in BAT and WAT along with an increase in UCP-1 expression indicating browning of WAT (Fisher et al., 2012). FGF21 can penetrate through the blood brain barrier and increase SNS activity, thereby promoting an increased expression of genes of thermogenesis and higher energy expenditure (Wu et al., 2012). It was speculated that some of the metabolic enhancements after exercise could be related to an increase in FGF21. However, studies have shown mixed results. In one study after 1h of leg cycling, there was an increase in FGF21 muscle expression (Catoire et al., 2014). Another study showed no effect on serum FGF21 after acute exercise, but after 2 weeks of daily treadmill exercise (Bruce's protocol) training FGF21 concentration was observed to increase (Cuevas-Ramos et al., 2012). In contrast, 3 weeks of SIT was shown to decrease FGF21 expression in skeletal muscle (Scalzo et al., 2014). Furthermore, cardiorespiratory fitness is inversely linked to serum FGF21 levels (Pedersen et al., 2015).

Thus, whether FGF21 expression in BAT is associated with alterations in human BAT mass, activity and function to be established following training in obese adults.

Cardiac natriuretic peptides

The heart releases hormones called cardiac natriuretic peptides, atrial NP (ANP) and the B-type natriuretic peptide (BNP), in response to stretching of the cardiac wall or under pressure and volume overload (Brenner et al., 1990; Gardner et al., 2007). Traditionally, natriuretic peptides counteract excessive cardiac wall stress through its known actions such as natriuresis, diuresis and vasodilation. Cardiac natriuretic peptides (NPs) are one of the mediators to activate UCP-1 expression, mitochondrial biogenesis and thermogenesis in BAT (Bordicchia et al., 2012). Exercise seems to increase these peptides concomitantly with catecholamines and insulin. Systemically, NPs increase the lipolytic effect (Sengenès et al., 2000) and increase fatty acids that could be supplied to the myocardium and muscle during aerobic exercise. Furthermore, an increase in circulating NPs have been linked to improved postprandial fat oxidation in humans (Birkenfeld et al., 2005, 2008) and weight loss (Chainani-Wu et al., 2010; Chen-Tournoux et al., 2010). There are also other factors that improve metabolic function secreted by BAT termed as 'batokines' (Villarroya et al., 2017). One such example is the nerve growth factor (NGF). NGF plays a role in angiogenesis and is considered to increase sympathetic innervation and number of pre-adipocytes in BAT promoting thermogenesis (Né Chad et al., 1994; Nisoli et al., 1996)

As WAT has a higher mass than BAT, and with BAT having great metabolic potential, increasing the amount of beige/brown adipocytes in WAT may be a viable option to combat obesity related disease. However, whether exercise-induced browning is physiologically relevant is unknown. Several theories exist to explain the reason for the browning that occurs after exercise in rodents. Indeed, browning may be induced by an improvement in the redox regulatory capacity (Sepa-Kishi and Ceddia, 2016). Lactate is one of the examples of redox metabolites. Lactate is formed after anaerobic metabolism and enhances browning through its transport by the monocarboxylate transporters (Carriere et al., 2014). Others have disputed that browning after exercise is implausible though because skeletal muscle is already producing heat and this would leave little or no room to promote browning of white adipocyte (Saugen et al., 1995). Others, nonetheless, suggest that after exercise there is a decrease in adipocyte size and reduction in WAT lipid content, thus lowering whole-body insulation and stimulating the release of more heat therefore cause browning (Lehnig and Stanford, 2018).

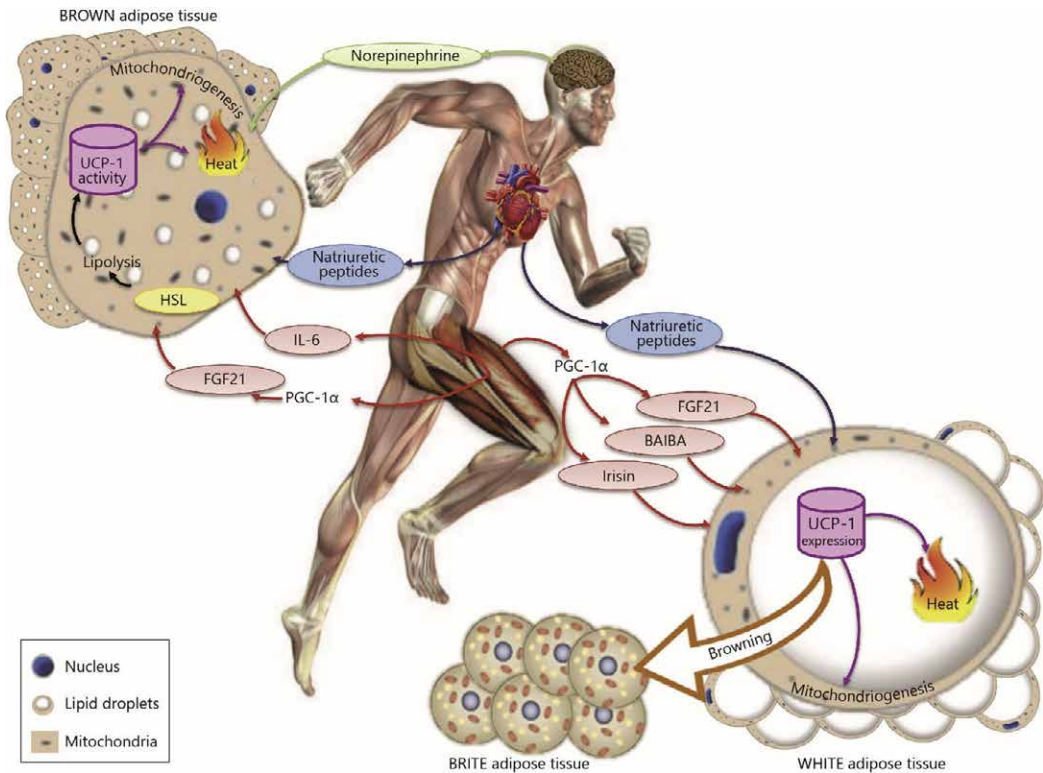


Figure 5. The underlying mechanisms of BAT activation with exercise through activation of the sympathetic nervous system, heart and skeletal muscle. IL-6, interleukin 6; HSL, hormone sensitive lipase; FGF21, fibroblast growth factor 21; BAIBA, β -aminoisobutyric acid Reprinted with permission from (Sanchez-Delgado et al., 2015). Copyright © 2015 S. Karger AG, Basel.

2.6 Effects of exercise on WAT

As mentioned earlier numerous exercise-induced changes are secondary to weight loss (decreased fat mass). Exercise training reduces the amount of VAT more than over all fat (Borel et al., 2015; Giannopoulou et al., 2005). Exercise-induced reduction of VAT, independent of weight loss, may reverse negative consequences such as ectopic fat storage in skeletal muscle, liver, heart and pancreas. Although different types of exercise training seem to decrease fat from all depots, exercise-induced reduction in SAT mass may be region and sex-specific. While men seem to have greater reductions in abdominal SAT mass in response to exercise training, women show substantial reductions from femoral SAT mass. VAT or SAT mass reduction in response to exercise training could be elucidated either by decreases in adipocyte size or adipocyte number. Physical intervention studies have shown decreases in abdominal adipocyte cell size (Björntorp, 1976; Després et al., 1984, 1985) but no changes in abdominal adipocyte number (Björntorp, 1976; Després et al., 1984). Additionally, increased physical activity can also maintain weight through increased catabolism of lipids (Mika et al., 2019).

Previously, endurance trained athletes have been shown to have increased abdominal SAT insulin sensitivity compared to sedentary subjects using the microdialysis technique (Stallknecht et al., 2000). Additionally, abdominal SAT GLUT4 protein expression has been demonstrated to increase in T2DM subjects after 4 weeks of bicycle training (Hussey et al., 2011). Studies on the effects of exercise training on VAT, abdominal and femoral SAT glucose metabolism in humans using imaging are sparse. In a randomized controlled study, Reichkender et al. investigated the effect of 11 week of moderate or high dose aerobic physical exercise in sedentary and moderately overweight young men on insulin stimulated GU in VAT, abdominal and femoral SAT using FDG PET/CT. Interestingly, training decreased insulin-stimulated GU in abdominal SAT based on SUV_{max} but did not change GU in VAT or femoral SAT independent of training doses (Reichkender et al., 2013). It is noteworthy that in the same study they also found higher GU in VAT than in SAT at baseline (Reichkender et al., 2013) which is consistent with previous studies (Christen et al., 2010; Ng et al., 2012; Viljanen et al., 2009; Virtanen et al., 2005). Higher GU in VAT is due to its increased cellularity (more adipocytes per gram than in subcutaneous fat) (Rebuffé-Scrive et al., 1989) and more effective lipid mobilization (Mårin et al., 1992), indicating a more active metabolic role of VAT compared to abdominal SAT. The discrepant results in endurance trained athletes and Reichkender et al. study might be due to different methods used. Microdialysis is a technique to evaluate biochemical processes inside various tissues. The limitation of this technique is limited access to organs deeper inside the body as the recovery period to prevent inflammation caused by insertion of catheter may have impacted the findings. In contrast the limitation of microdialysis technique to measure insulin sensitivity can be overcome from PET imaging. There drawback of using semi-quantitative standard uptake values (SUV) is that the changes of the biodistribution of FDG in the body are neglected. Therefore, when only relative SUV values are reported misinterpretation of physiology of tissue can occur.

Interestingly, transplantation of abdominal SAT from trained mice into sedentary recipient mice have remarkable changes with better glucose tolerance, improved insulin sensitivity and metabolic improvements in other tissues including skeletal muscle and BAT (Stanford et al., 2015). The findings of this study indicated a probable endocrine role of SAT in releasing adipokines that mediate communication between the tissues and contribute to the improved metabolic homeostasis with exercise. The mechanism for this effect may involve adipo cytokines influencing the GU in other tissues, liver function and “browning” of SAT. It has been shown that changes in lifestyle significantly regulates adipokines such as leptin, adiponectin and resistin because of weight reduction by increasing adiponectin and decrease in leptin and resistin (Esposito et al., 2003; García de la Torre et al., 2008; Jones et al., 2009; Jung et al., 2008). It has been proposed that these adipocytokines mediate the cross-talk of tissues with other tissues. For instance, brain can indirectly sense exercise via adiponectin released by adipose tissue or FGF21 released by liver (Pedersen, 2019). VAT releases higher amounts of IL-8 than SAT (Li et al., 2017). In humans, there

has been conflicting data on the effects of exercise training and regulation of adipokines (Christiansen et al., 2013; Højbjerg et al., 2007; Klimcakova et al., 2006; Kohut et al., 2006; Rokling-Andersen et al., 2007). This is possibly due to the type and intensity of different exercise training program used in these interventions. Anaerobic type of exercises may cause an increase in adipocytokines (Boström et al., 2012; Li et al., 2017). It has been shown that short dynamic high-intensity exercises induce great biochemical, hormonal and immune changes. Single anaerobic exercises and series of other anaerobic exercises induce oxidative stress (Bogdanis et al., 2013; Meyer et al., 2001; Wiecek et al., 2015, 2018b, 2018a). It seems that HIIT type of exercises increase IL-6, adiponectin and lowers leptin concentrations (Avazpor et al., 2016; Guerra et al., 2011). Nonetheless, further research is required on the influence of exercise of varying intensities on the secretion of adipocytokines, exercise training improves SAT inflammation (Haczeyni et al., 2015). It has been proposed that the long-lasting anti-inflammatory effects of exercise may be facilitated by reduction in VAT mass (Pedersen, 2009) and subsequently by less lipotoxicity and secretion of adipokines. In humans, increased systemic levels of IL-6 are associated with obesity which most likely originate from the immune cells in adipose tissue (Mohamed-Ali et al., 1997; Wedell-Neergaard et al., 2018). On the contrary, the reduction of inflammation after exercise training could be due to increased production of IL-6 that decreased production of TNF- α (Starkie et al., 2003). The release of epinephrine and nor-epinephrine after exercise training can also inhibit pro-inflammatory cytokine production (Ignatowski et al., 1996). In obese subjects, macrophage infiltration has been shown to be a strong predictor of IR (Klötting et al., 2010), although whether exercise training decreases macrophage infiltration leading to insulin sensitivity remains to be explored.

In rodents exercise training increases mitochondrial activity in both SAT and VAT (Boström et al., 2012; Stallknecht et al., 1991; Stanford et al., 2015; Vernochet et al., 2012; Wu et al., 2014) by enhancing the tissue's oxidative capability. There seems to be only two reports in humans studying exercise-induced changes in abdominal SAT mitochondrial activity. In a 6-month exercise intervention, increased oxidative phosphorylation in SAT was reported in healthy sedentary males (Rönn et al., 2014). These results are similar to a 4 week exercise intervention showing an increased PGC1- α mRNA expression in the SAT irrespective of sex or baseline glucose tolerance status, highlighting that exercise training favored enhanced mitochondrial biogenesis (Ruschke et al., 2010).

Exercise training activates lipolysis and depletes the adipose tissue lipid stores which are associated with many alterations in adipose tissue fatty acid metabolism (Mika et al., 2019). Liver free fatty acid uptake (FFAU) and VAT fat mass has been shown to be 30% and 20% lower in the more active healthy twin brother compared to the less active twin (Hannukainen et al., 2007). This suggests that physical activity decreases liver FFAU via

decreased VAT mass thereby reducing the rate of lipolysis and the FFA flux to liver (Hannukainen et al., 2007). IR is a complex mechanism with impaired FFA uptake (FFAU) metabolism in several tissues such as skeletal muscle, liver, pancreas and brain (Blaak et al., 2000; Heiskanen et al., 2018; Honkala et al., 2018; Turpeinen et al., 1999). In contrast, in young healthy monozygotic twin-brothers discordant for physical activity and fitness their resting skeletal muscle FFAU did not differ between the more active (having 18% higher VO_{2max}) or less active twin-pair (Hannukainen et al., 2007). Exercise training increases FFAU in working skeletal muscles of subjects with pre-diabetes and T2DM (Sjöros et al., 2018) suggesting that exercise ameliorates the impaired FFA metabolism in skeletal muscle, whereas it remains unchanged in healthy subjects (Eskelinen et al., 2015). Whether exercise intensity impacts this lipid turnover, however, remains unclear.

2.7 Summary of the literature review

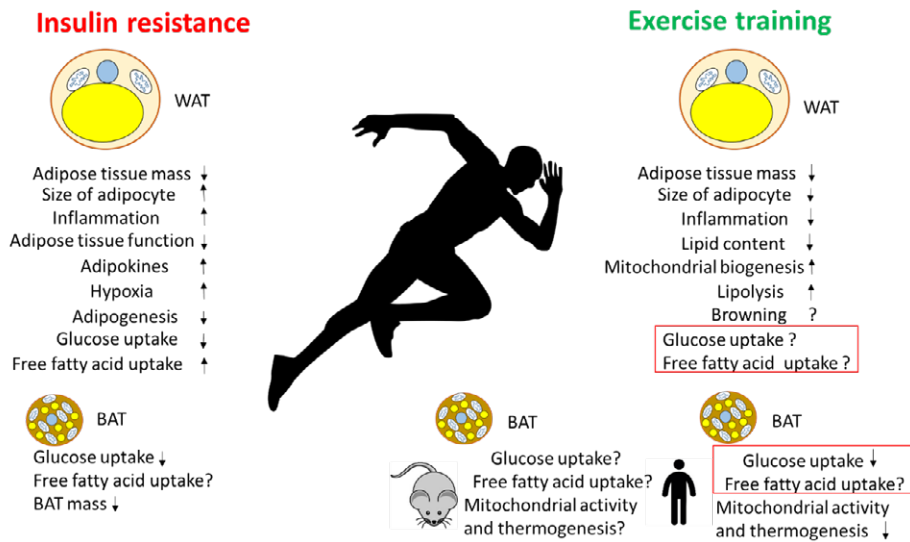


Figure 6. Adipose tissue changes (WAT and BAT) with insulin resistance (left). Exercise-induced changes in adipose tissue (WAT and BAT) (right) (references are provided in main text). The red boxes indicates the focus of this thesis.

3. AIMS OF THE STUDY

The aims of the thesis were:

- 1) To investigate the effects of a short-term 2-week sprint interval training (SIT) versus moderate intensity continuous training (MICT) on BAT substrate metabolism (insulin-stimulated GU and fasting free fatty acid uptake) in sedentary middle-aged men (I).

Study hypothesis: SIT would activate BAT by increasing insulin-stimulated GU. In comparison, SIT would activate BAT GU more than MICT. SIT and MICT would improve fasting BAT free fatty acid uptake (FFAU).

- 2) To study how six-weeks of training intervention (combination of SIT, MICT and resistance exercise training influences cold-induced BAT GU in healthy men and the role of serum metabolites in relation to BAT GU (II).

Study hypothesis: A six-week training intervention would increase cold-induced BAT GU.

- 3) To compare the effects exercise-induced adaptations after a short-term 2-weeks of SIT or MICT in abdominal and femoral subcutaneous, and visceral adipose tissue in three different groups:
 - a. healthy and IR men (pre diabetic or T2DM subjects) (III),
 - b. in IR men and women (III) and
 - c. SIT versus MICT in IR subjects (III)

Study hypothesis:

- a. Exercise training would improve adipose tissue metabolism (both insulin-stimulated GU and fasting FFAU) similarly in healthy and IR men.
- b. Exercise training has superior effects on adipose tissue metabolism (both insulin-stimulated GU and fasting FFAU in women than in men.
- c. SIT is superior to MICT in improving adipose tissue insulin-stimulated GU and MICT would decrease fasting FFAU in IR subjects.

4. MATERIALS AND METHODS

4.1 Study Subjects

The subjects were recruited by researchers at the Turku PET Centre. All of the subjects were recruited by traditional bulletin boards, personal contacts, newspaper and electronic advertisements. The study protocols were permitted by the Ethical committee of the Hospital District of Southwest Finland (Turku, Finland, decision 95/180/2010 §228 (I and III) and decision 28/1801/2013 §228 (II)). These studies were carried out according to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

Studies I and III are sub studies of the HITPET (NCT01344928) where a total of 28 healthy subjects and 26 insulin resistant subjects were studied. Study I subjects consisted of 18 healthy males for whom insulin stimulated BAT FDG PET/CT studies were successfully performed. The inclusion criteria for healthy subjects in Study I and III were male, 40 – 55 years of age, BMI 18.5–30 kg/m², no exercise on a regular basis (< 2 days/week or less), VO_{2peak} < 40 ml/kg/min and normal glycaemic control. Study II EXEBAT (NCT03359824) included 11 healthy males with the inclusion criteria, age 18-45 years, BMI 20-25 kg/m², normal glycaemic control and no history of exercise on a regular basis (< 3 days/week), and VO_{2max} < 40ml/min/kg min.

Study III included 28 healthy men and 26 IR subjects (n = 26; 10F/16M), the inclusion criteria were the same as in the healthy subjects (see above) except for a BMI of 18.5–35 kg/m² and an impaired glucose tolerance according to the criteria of the American Diabetes Association (American Diabetes Association, 2017), an HbA1c of < 7.5% and no insulin treatment in the case of T2DM. Of the 26 IR subjects, 16 had T2DM and 10 had impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). At the screening, 5 subjects were newly diagnosed with T2DM and had no previous medication. Of the 11 T2DM subjects, the median diabetes duration was 4.2 years and they were treated with oral hypoglycaemic agents (11 with metformin; 5 with sitagliptin and 1 with glimepiride).

Screening was done for all subjects and included a medical history, physical examination, routine clinical blood tests, a two-hour oral glucose tolerance test (2h-OGTT), BP and anthropometric measurements. The characteristics of the study subjects are shown in Table 3. The exclusion criteria used for the study subjects in all studies (I-III) included:

- 1) Any significant chronic disease or medical defect requiring medical treatment other than prediabetic or T2DM subjects
- 2) High blood pressure (>140/90 mmHg in healthy subjects and >160/100 mmHg in prediabetic or T2DM subjects)

- 3) Smoking, heavy use of alcohol or use of nicotine products
- 4) Current or history of regular and systematic exercise training
- 5) Previous participation in PET studies
- 6) Presence of ferromagnetic objects in the body (contraindicating MRI)
- 7) Any other condition that in 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.

Table 3. Study subjects.

IR, insulin resistant; BMI, body mass index.

	Study I	Study II	Study III	
Study population	Healthy	Healthy	Healthy	IR
Number of subjects	18	11	28	26
Gender (men/women)	18/0	11/0	28/0	16/10
Pre-T2DM/T2DM	-	-	-	13/13
Age (years)	47 ± 5	31 ± 7	48 ± 5	49 ± 4
Body mass (kg)	83 ± 8	73 ± 7	84 ± 9	92 ± 13
BMI (kg/m²)	25.9 ± 2.4	22.8 ± 0.9	26.1 ± 2.4	30.7 ± 2.8
Body fat (%)	22.4 ± 4.4	17.1 ± 1.6	22.6 ± 4.2	33.4 ± 7.5
Aerobic capacity (mL/kg⁻¹/min⁻¹)	34.3 ± 4.6	38.7 ± 7.6	34.2 ± 4.1	27.2 ± 4.6

4.2 Study designs

4.2.1 Study I and III (HITPET)

Studies I and III were a part of a larger research effort entitled ‘The Effects of Short-Term High-Intensity Interval Training on Tissue Glucose and Fat Metabolism in Healthy Subjects and in Patients with Type 2 diabetes’ (NCT01344928). There are previous reports published from this study (Eskelinen et al., 2015; Heiskanen et al., 2016; Honkala et al., 2018; Motiani et al., 2017). This study project was performed in two stages. The first stage of the measurements included middle-aged healthy sedentary men and was carried out between March 2011 and February 2013. In the second phase, subjects were middle-aged men and women with pre-diabetes or pre-diabetes or T2DM, and the measurements were performed between February 2013 and October 2015. The VO_{2peak} test and analysis of body composition, was done at the Paavo Nurmi Centre, University of Turku, Finland. The rest of the measurements and training interventions were carried out in the Turku PET Centre, Turku, Finland.

To assess the level of physical fitness, subjects performed a VO_{2peak} test on a bicycle ergometer under guidance of an exercise physiologist on the day of screening or the day after described in detail in chapter 4.3.4. Baseline scans were done, at least one week after the screening on two subsequent days. On the first study day, magnetic resonance imaging (MRI) and fasting [^{18}F] FTHA PET/CT scanning were performed. The second measurement day started with [^{18}F] FDG-PET/CT scanning during a euglycemic-hyperinsulinemic clamp. Subsequent to all the pre-training measurements, the subjects were randomized into SIT and MICT training groups within both the healthy and IR groups. The training interventions are described in detail in Chapter 4.3. All the measurements were repeated after the 2-week exercise training intervention starting with the MRI and the [^{18}F]-FTHA-PET/ CT scans $\sim 48h$, [^{18}F]FDG-PET study $\sim 72h$, and OGTT and VO_{2peak} test $\sim 96h$ after the last training session.

Subjects fasted for at least ten hours prior to the screening day. Anti-diabetic drugs were with-drawn for 72 hours and the subjects were instructed to refrain from caffeinated drinks and to avoid exhausting exercise 48 hours prior to the measurements. Diet was not taken into account prior to these metabolic tests and in the entire study. The study design is shown in Figure 7

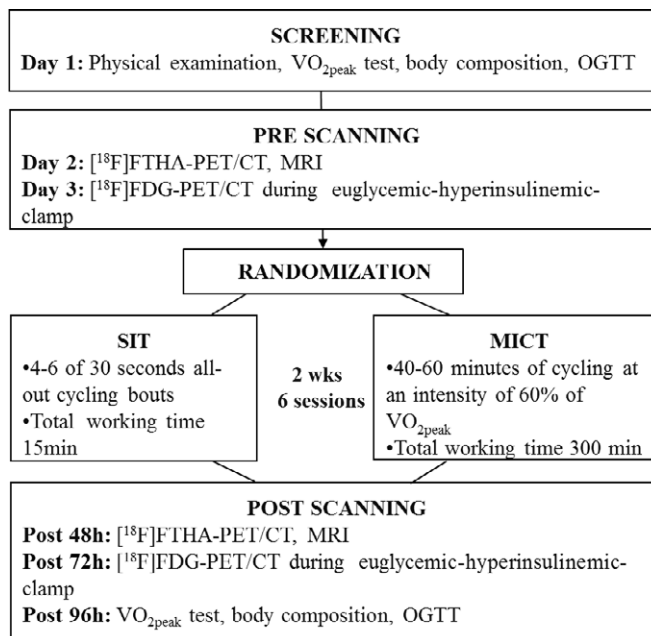


Figure 7. Schematic of Studies I and III (HITPET). The study was carried out in two phases with the same protocol. VO_{2peak} , (aerobic capacity); [^{18}F]FDG (2-[^{18}F]fluoro-2-deoxy-D-glucose); [^{18}F]FTHA (14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid); PET (positron emission tomography); OGTT (oral glucose tolerance test); CT (computed tomography); MRI (magnetic resonance imaging); SIT (sprint interval training); MICT (moderate intensity continuous training).

4.2.2 Study II (EXEBAT)

Study II entitled ‘Brown adipose tissue activation: Effect of exercise training and irisin’ (NCT03359824) was performed at the Turku PET Centre, the University of Turku, and Turku University Hospital (Turku, Finland) between August 2013 and May 2015.

In Study II, at the initial screening visit, a medical history, physical examination, electrocardiography (ECG), indirect calorimetry at room temperature for resting metabolism, blood sampling, and a 2h 75g OGTT were performed. This was followed by a sub-maximal bicycle ergometer test on another day. Each subject underwent three [¹⁸F] FDG-PET/MRI scans after overnight fasting conditions. The first scan was performed before exercise training at room temperature, the second scan before exercise training under cold exposure, and the third scan after exercise training during cold exposure. The first and second scans were performed in a random order on two separate days, with the minimum duration between the two scanning sessions being one week. Cold-induced fasting glucose uptake in BAT, skeletal muscles and various WAT depots were measured under normal room temperature and during cold stimulation in order to identify active BAT. Subsequently, the subjects performed six weeks of exercise training. After the training intervention, and 48 hrs after the last training session, a sub-maximal bicycle ergometer test was performed. The third PET/MRI scan was performed 48-72 hrs after the sub maximal test (except in one subject post 7 days). Indirect calorimetry, OGTT and PET/MRI studies were performed after at least a 10 h fast. Caffeinated drinks and alcohol were prohibited ~48h before the studies. All the screening visit measurements were repeated about 48-72 hrs after the third PET/MRI scan. The complete study flow is shown in Figure 8.

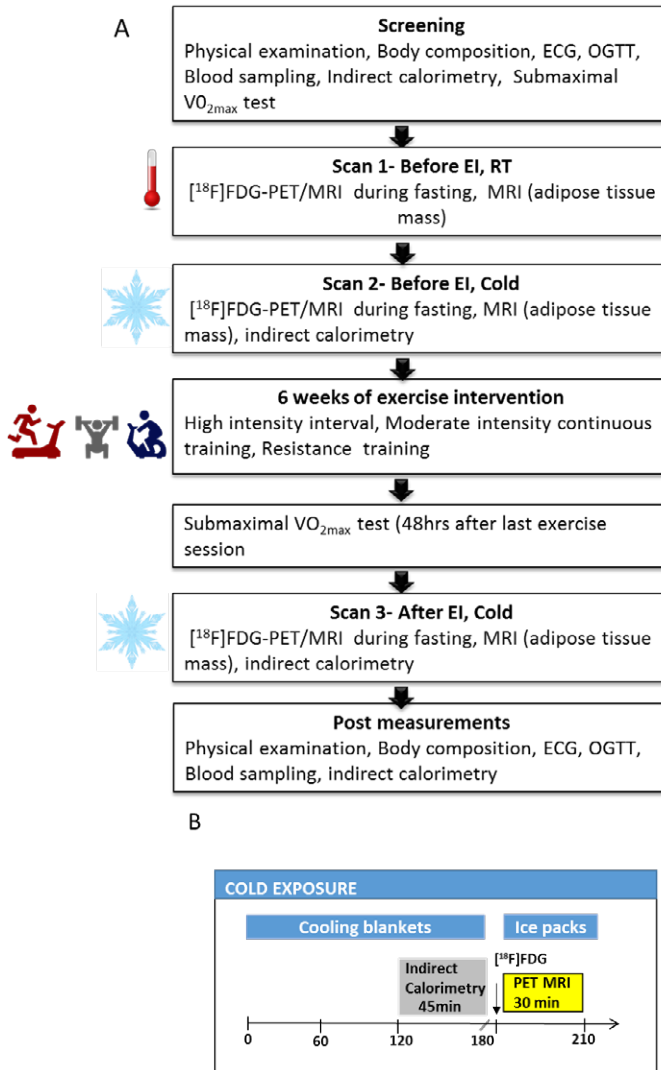


Figure 8. Schematic of the design of Study II. A. The scanning protocol to measure fasting BAT GU at room temperature, before and after exercise intervention during cold stimulus. B Cold exposure scans in detail From Motiani et al. 2019 (II).

4.2.3 Cold Exposure (II)

Personalized cold exposure was started 2 hrs before the PET scan using a cooling blanket (Blanketrol III, Cincinnati Sub-Zero, Cincinnati, OH, USA). The cooling was started with 4 to 6 °C water circulating in the cooling blanket. The temperature was gradually increased if shivering was reported by the subjects or visually observed by the investigator. During PET/MR scanning, icepacks were used to maintain the cooling. Room temperature (RT) was maintained at ~ 22°C.

4.2.4 Skin temperature measurements (II)

The skin temperature was measured during scanning using a digital thermometer (Art.183, Termometerfabriken Viking AB, Eskilstuna, Sweden). The temperature sensing probe was attached to the lateral abdominal skin surface.

4.3 Exercise training intervention (I, III)

In studies I and III, both training interventions (SIT and MICT) consisted of 6 training sessions over a period of 2 weeks with at least one day of recovery between subsequent sessions. The time duration for the SIT group was only 15 minutes whereas in the MICT group the total time was 300 minutes.

4.3.1 Sprint interval training (SIT) protocol

The SIT sessions consisted of 4-6 x 30s maximal all-out cycling bouts (Monark Ergomedic 894E, MONARK, Vnasbro, Sweden) with a 4 minute recovery between each bout. Heart rate was determined by (RS800CX; Polar Electro Ltd., Kempele, Finland) and rating of perceived exertion was recorded throughout each session to gauge appropriate intensity was performed. The number of bouts increased progressively starting with 4 bouts and increasing by one every other session. Each bout started with 5-s acceleration to maximal cadence without any resistance, followed by an immediate increase of the load (for healthy subjects 7.5% of whole body weight in kg, for IR 10% of lean body mass in kg) for 30s.

4.3.2 Moderate-intensity continuous training (MICT) protocol

A session of MICT consisted of continuous aerobic cycling at an intensity of 60% of VO_{2peak} (Tunturi E85, Tunturi Fitness, Almere, The Netherlands). The duration of cycling was 40 mins for session 1 and 2, 50 min for session 3 and 4, and 60 min for sessions 5 and 6. Sessions were carried out 3 days in a week.

4.3.3 Endurance and resistance training protocol (II)

The training-intervention was six weeks and consisted of a combination of moderate-intensity continuous training (MICT, 75-85% of HR_{max}), sprint-interval training (SIT, >90% of HR_{max}) and resistance training. The MICT included mainly running, but also indoor cycling and cross-trainer exercise was allowed. SIT was performed either by cycle-ergometer (Monark Ergomedic 828E, MONARK, Vnasbro, Sweden) or stair-run. Cycle-ergometer SIT training sessions consisted of 5 x 30s maximal all-out cycling bouts with 4-minutes recovery between the bouts according to the original protocol as described previously (Burgomaster et al., 2005). Stair-run SIT-training sessions consisted of 6 x 30-45s maximal stair-run bouts with 4 minutes recovery between the bouts. The resistance training was divided into two different programs, leg- or upper body-oriented (see detailed

in Table 4). Training loads were individually determined in the first training session by the participant's ability to complete 10 repetitions for a given exercise. Each week, three of the training sessions were guided by a personal trainer. All training sessions were monitored via a heart-rate monitor (Suunto, Finland).

4.3.4 Aerobic fitness tests (I-III)

Aerobic fitness tests in Studies I and III were calculated with a peak oxygen uptake (VO_{2peak}) cycling ergometer test (Ergoline 800s; VIASYS Healthcare, Germany). The test was performed approximately one week before the first training session and 96 hours after the last training session. The subjects fasted for two hours before testing. The initial workload was 50 W and was increased by 30 W every two minutes until exhaustion. Ventilation and gas exchange (Jaeger Oxycon Pro, VIASYS Healthcare Germany) was measured and reported as the mean value per minute. The peak respiratory exchange ratio was more than 1.15 and peak blood lactate measured from the fingertip capillary samples was greater than 8.0 mmol/l for all tests obtained immediately and 1 minute after exhaustion. The highest 1 minute mean value of oxygen consumption (VO_{2peak}) related to body weight (ml/kg/min) was used in all cases. Peak workload was calculated as an average workload during the last two minutes of the test and used as a measurement of maximal performance.

In Study II, the indirect bicycle test was conducted as tripartite sub-maximal test according to WHO protocol (Coleman DC, 1971). The test started with a four-minute light warm up and was followed by three four-minute work periods with an increasing work load. The workloads were determined individually based on the age, BMI, sex and physical activity level. The cycle frequency was kept at 60 rpm. The cycling intensity was increased so that at the end of the test subject reached approximately 80% of the theoretical maximal heart rate and rating of perceived exertion no more than 17 (Borg scale 6-20). Blood pressure and heart rate were monitored throughout the test. To calculate theoretical VO_{2max} , following formula was used.

$$VO_{2max} = \frac{12.35 \times P(w)}{BM (kg)} + 3.5$$

Where P = maximal power output based on the workloads and heart rate during submaximal VO_{2max} test, BM= body mass and 3.5= resting oxygen consumption.

Table 4. Training protocol. Subjects underwent a six week exercise training intervention. Three of the session each week were supervised and during the weeks 4-6 two sessions were performed individually. SIT, sprint interval training; MICT, moderate intensity continuous training. From original publication (II)

	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Week 1	MICT 30 min		MICT 30 min + resistance training 2		MICT 45 min + resistance training 1		
Week 2	SIT (Wingate- test) + stretching		MICT 30 min + resistance training 2		MICT 45 min + resistance training 1	MICT (independent) 30 min	
Week 3	SIT (Wingate- test) + resistance training 1		MICT 45 min + resistance training 2		SIT (stair-run) + stretching	MICT (independent) 45 min	
Week 4	SIT (Wingate- test) + resistance training 1	MICT (independent) 45 min	MICT 45 min + resistance training 2		SIT (stair-run) + stretching	MICT (independent) 45 min	
Week 5	SIT (Wingate- test) + resistance training 1	MICT (independent) 45 min	MICT 45 min + resistance training 2		SIT (stair-run) + stretching	MICT (independent) 60 min	
Week 6	SIT (Wingate- test) + resistance training 1	MICT (independent) 45 min	MICT 45 min + resistance training 2		SIT (stair-run)	MICT (independent) 60 min	

<u>Resistance training 1 - upper body</u> <u>(Turku PET Centre)</u>	<u>Resistance training 2 - lower body,</u> <u>(Kupittaa sports hall)</u>
Burpee + Kettlebell swing 3x10	Leg press 3x10
Bicep curl 3x10	Deadlift 3x10
Dip push-up 3x10	Leg extension 3x10
Push-up 3x10	Leg curl 3x10
Plank (max effort) x3	Sit-up 3x12

4.4 Image acquisition (I-III)

PET/CT (I and III). Participants underwent four PET sessions: one [^{18}F] FTHA PET and [^{18}F] FDG PET before and after the training intervention. The PET/CT images were

acquired using GE Discovery TM ST System (General Electric Medical Systems, Milwaukee, WI, USA). Both antecubital veins were cannulated for the PET studies; one was used for the injection of radiotracers [^{18}F] FTHA and [^{18}F] FDG, and the other for continuous arterialized blood sampling. The arm that was used for blood sampling was heated with an electrically powered cushion to arterialize the venous blood throughout the scan. Scanning was performed in supine position. On the first day, an [^{18}F]FTHA-bolus (155 [SD 9] MBq) was injected and dynamic imaging of the abdominal region (frames 3x300s) was acquired starting ~46 minutes after the tracer injection, followed by the femoral (frames 3x300s) region starting at ~65 minutes and neck starting at ~85 minutes (3x300s) regions.

On the second PET study day, ~91 minutes after the start of the euglycemic-hyperinsulinemic clamp, [^{18}F] FDG (157 [SD 10] MBq) was injected, and dynamic scanning of the abdominal area started ~46 minutes after the injection. Thereafter, scanning of femoral starting at ~65 minutes and neck regions followed at ~87 minutes. To measure the plasma radioactivity of the tracer input function, arterialized venous blood samples were collected repeatedly during [^{18}F] FTHA and [^{18}F] FDG scanning. Anatomical references were obtained from the acquired CT images.

PET/MRI (II). The [^{18}F] FDG-PET and MRI scans were performed with an integrated 3T Philips Ingenuity TF PET/MR scanner. Both antecubital veins were cannulated for the PET studies; one was used for [^{18}F]-FDG injection and the other one for blood sampling. For the scans performed at room temperature T1 (flip angle 10° , TE 2.3 ms, TR 4.1 ms) weighted MR images were used and for cold scans a modified 2-point Dixon sequence (mDixon, flip angle 10° , TE 1.08 ms, TR 6.5 ms, $\Delta\text{TE} = 0.9$ ms) was used to provide an anatomical reference. At the same time as the injection of [^{18}F]FDG [Scan 1: 153 (SD 9.0), Scan 2: 155 (SD 7.9), Scan 3, the: 154 (SD 10.1) MBq] dynamic PET scan was started (frames 7x60 s, 4x300 s and 1x180 s) and the scanning of the neck area was continued for 30 minutes. PET images were reconstructed in a 144 x 144 matrix with an isotropic voxel size of 4 mm, using a fully 3-D row action maximum likelihood algorithm (3D-RAMLA) with 2 iterations and 4 subsets (Browne and de Pierro, 1996). PET data was corrected for photon attenuation, physical decay, dead time, scatter and random coincidences. Plasma radioactivity was measured with an automatic gamma counter (I-III) (Wizard 1480 3", Wallac, Turku, Finland).

4.5 Magnetic resonance imaging (I-III)

A 1.5-T MRI scanner (Gyrosan Intera CV Nova Dual scanner (Philips, Amsterdam, Netherlands) with an internal body coil was used to obtain whole body (from head to knee) axial T1- weighted dual fast field echo images (TE 2.3 and 4.7 ms, TR 120ms, slice thickness 10mm without gap) (Study I, III). In Study II, 3T Philips Ingenuity TF PET/MR scanner was used to obtain modified 2-point Dixon images.

Adipose tissue masses in the abdominal region were measured at the level of intervertebral disc L2/L3, as previously described (Abate et al., 1994). MRI scans were performed both before and after exercise intervention. Abdominal SAT and VAT masses were analysed using SliceOmatic software v.4.3 (<http://www.tomovision.com/products/sliceomatic.htm>). To obtain the tissue mass, the pixel surface area was multiplied by the slice thickness and the density of adipose tissue 0.9196 kg/L (Study I-III).

4.6 Regions of interest: adipose tissue and skeletal muscle (I-III)

Carimas 2.9 (www.pet.fi/carimas) was used for image analysis. Three-dimensional regions of interest (3-D ROIs) were drawn on fused dynamic PET and CT or MRI images. 3-D ROIs were drawn on supraclavicular BAT, neck white adipose tissue from the posterior cervical region SAT (n), visceral adipose tissue (VAT) at the level of umbilicus, abdominal subcutaneous adipose tissue (ASAT) on planes superior to the umbilicus, femoral subcutaneous adipose tissue (FSAT), quadriceps femoris muscle (QF) at the mid-region of the thigh and skeletal muscles close to BAT (sternocleidomastoid and levator scapulae) and distant from BAT (deltoid and trapezius muscles). The regions of interest (ROI) were manually outlined from C1 to T4 vertebrae for the neck muscles within the field of view.

4.7 Quantification of glucose and free fatty acid uptake rate (I-III)

Fractional uptake rate (FUR) was used to calculate glucose and free fatty acid uptake from supraclavicular BAT, ASAT, FSAT, VAT and quadriceps femoris muscle. For glucose uptake, the value obtained was further divided by lumped constant (LC), a factor used to correct the uptake rate of a PET tracer to that of the substrate of interest (I,III).

The influx rate constant (K_i) of [^{18}F]FDG for the supraclavicular BAT, WAT, sternocleidomastoid, levator scapulae, deltoid and trapezius muscles were determined using the model of Gjedde-Patlak and graphical analysis (II).

Glucose uptake and free fatty acid uptake (FFAU) rates were calculated as follows: plasma glucose/FFA concentration $\times K_i/LC$. The LC for [^{18}F]-FDG in BAT, WAT, and skeletal muscle was set to 1.14, 1.14, and 1.2 respectively (I-III).

4.8 BAT mass (I-II)

PET/CT (I). BAT mass was analyzed by thresholding the voxels from all the potential sites of BAT (cervical, supraclavicular, and axillary adipose depots) based on Hounsfield units (HU) in CT image (-250 and -50 HU). All voxels above 2.9 $\mu\text{mol}/100\text{g}/\text{min}$ glucose uptake on parametric PET images were included. Lastly, the volume of all these voxels (cm^3) was converted into mass by using BAT density of 0.92 g/cm^3 .

PET/MRI (II). BAT mass, in the cervico-upper thoracic region was estimated from fused cold PET-MRI images before and after exercise intervention using Carimas 2.9 software (www.pet.fi/carimas). MRI signal fat fraction (SFF) images were created from water component images (W) and fat component images (F) obtained from a mDixon sequence using the formula (Franz et al., 2015).

$$SFF = F / (F + W)$$

The SFF images were calculated using in-house software developed in MATLAB2015b (Mathworks Inc, Natick, MA) and SPM8 (Wellcome Trust, UCL). A mask was drawn on the SFF maps at all potential cervico-upper thoracic BAT sites (cervical, supraclavicular, and axillary depots), using a fat fraction threshold of 40% to identify and measure BAT while excluding blood vessels, muscle and bones (Holstila et al., 2017; Lundström et al., 2015; Trexler et al., 2017) the mask was then transferred to the parametric [¹⁸F]FDG PET image to exclude any voxels that had $GU < 3.0 \mu\text{mol}/100\text{g}/\text{min}$ from the mask. The voxels in the mask then underwent a 2nd thresholding step with $GU > 3.0 \mu\text{mol}/100\text{g}/\text{min}$ used to include voxels representing active BAT. Previously, it has been shown that during cold exposure, $GU > 3.0 \mu\text{mol}/100\text{g}/\text{min}$ represents active BAT (Orava et al., 2011). Finally, the volume of all these voxels (cm^3) was converted into mass by assuming the density of BAT to be $0.92\text{g}/\text{cm}^3$.

4.9 Other measurements

4.9.1 Indirect calorimetry (I-III)

Energy expenditure (EE) and whole body substrate utilization was measured with indirect calorimetry. Metabolic rate measurements (VO_2 , CO_2 and EE) were determined using an open-system indirect calorimeter Deltrac (Deltatrac II; Datex-Ohmeda). The procedure involves the placement of the subject's head under a plastic transparent hood (canopy) connected to the analyzer. The indirect calorimetry was done on the [¹⁸F] FDG-PET day and it started with subjects who had an overnight fast and indirect calorimetry was performed first for about 30 mins before the start of the clamp to stimulate the fasting conditions and then after the start of the clamp they underwent another round of calorimetry for about 30 mins to stimulate the post prandial situation (I,III). Measurements were performed while fasting during screening at room temperature, during cold exposure for 45 mins before the PET imaging and once with the post measurements at room temperature (II). Whole body energy expenditure (EE) and substrate utilization were calculated according to Weir equation (Weir, 1949) The protein oxidation rate was accounted for in the equations by considering urinary nitrogen production to be $13\text{g}/24\text{h}$ (I-III).

4.9.2 Adipocytokines(I)

Serum fasting adipokine concentration of NGF, IL-6, IL-8, leptin, HGF, MCP-1, and TNF- α were analyzed using the Adipokine Magnetic Bead Panel 2 (Cat#HADK2MAG-61K, Millipore, Billerica, MA) on the Luminex-Multiplex analyzer (Millipore, Billerica, MA). The samples were taken 48 hrs after the last training session.

4.9.3 Plasma Catecholamines (I-II)

Plasma samples were collected on 1ml plastic tubes in ice containing EGTA and reduced glutathione as a preservative. Plasma concentrations of catecholamines were analyzed using a chromsystems reagent kit for HPLC analysis (Chromsystems Instruments and Chemicals GmbH, Munich, Germany) with the Agilent ChemStation chromatography program.

4.9.4 Blood measurements (I-III)

Plasma total and HDL-cholesterol, triglycerides, and glucose were measured from the venous blood samples with an automatized enzymatic assay and insulin using automatized electro-chemiluminescence immunoassay (Cobas 8000, Roche Diagnostics GmbH, Mannheim, Germany). LDL-cholesterol concentration was calculated using the Friedewald formula.

4.9.5 Oral glucose tolerance test (OGTT) (I-III)

A 2-hour OGTT was done after subjects had fasted for at least 10 hours. The subjects drank a 330-ml solution containing 75 g of glucose (Nutrical, Nutricia Medical, Turku, Finland). Blood samples were collected before drinking the solution and at 15, 30, 60, 90, and 120 min after drinking for glucose and insulin analysis (I-III). To estimate whole-body insulin sensitivity, the matsuda index was calculated using the following formula (II):

$$\text{Matsuda index} = \frac{100}{\text{sqrt}} \left(G_0 \left(\frac{\text{mg}}{\text{dL}} \right) \times \text{Ins}_0 \left(\frac{\text{mU}}{\text{L}} \right) \times \left(\text{Gavg} \left(\frac{\text{mg}}{\text{dL}} \right) \times \text{Insavg} \left(\frac{\text{mU}}{\text{L}} \right) \right) \right)$$

Where G_0 = Glucose measurement taken at time point 0, Ins_0 = Insulin measurement taken at time point 0, Gavg = Average of glucose, and Insavg = Average of insulin

4.9.6 Body composition (I-III)

A Bioimpedance monitor (In Body 720, Mega Electronics Ltd., Kuopio, Finland) was used for the measurement of body composition at the Paavo Nurmi Centre, University of Turku. The measurements were performed at the same time of day before and 96 hours after the training intervention. The weight, height and waist cm of the subjects were measured by standard procedures given by the American college of sports medicine (Pescatello et al., 2013).

4.9.7 Whole-body insulin stimulated glucose uptake (M-value) (I and III)

A euglycemic hyperinsulinemic clamp was conducted after a 10-hour fast (DeFronzo et al., 1979). The euglycemic hyperinsulinemic clamp was started approximately 90 minutes before the injection of the glucose tracer [¹⁸F] FDG on the PET study day. During the first 4 minutes, a primed-constant insulin (Actrapid, 100 U/ml, Novo Nordisk, Bagsvaerd, Denmark) infusion was initiated at a rate of 480 ml/h of body surface area. After the first 4 minutes the infusion rate was reduced to 240ml/h for three minutes, and then further reduced to 120 ml/h for the remaining duration of the clamp. An exogenous glucose infusion was started at 4 minutes after the start of the insulin infusion at a rate of the subjects weight (kg)/0.1g/h. The glucose infusion rate was doubled after 10 minutes and then adjusted based on the blood glucose concentration to maintain it as close to 5mmol/l as possible. Blood samples were collected every 5 minutes to during the first 30 minutes to adjust the glucose infusion rate. After 30 minutes, the samples were collected every 5 to 10 minutes to check the glucose levels. The whole body glucose uptake (M-value) was calculated from the glucose infusion rate and measured glucose values collected during the PET scan that was started 91 min (SD 20) after the start of clamp.

4.9.8 NMR spectroscopy (II)

Metabolic biomarkers were quantified from plasma of eleven individuals using high-throughput proton NMR metabolomics (Nightingale Health Ltd, Helsinki, Finland). EDTA plasma samples were obtained at five different time points on three different scan days. The first sample was obtained on scan day 1 at baseline, the second and third samples on scan day 2 at baseline and 2 hours after cold exposure (before exercise intervention), and the fourth and fifth samples were taken at baseline and 2 hours after cold exposure (after exercise intervention) on scan day 3. This method provides simultaneous quantification of lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and various low-molecular metabolites, including amino acids, ketone bodies and gluconeogenesis-related metabolites in molar concentration units. Details of the experimentation and applications of the NMR metabolomics platform have been described previously (Soininen et al, *Circ Cardiovasc Genet* 2015; 8: 192-206). Glycerol and glycine were unquantifiable from EDTA plasma samples and were not measured in this study.

The 14 lipoprotein subclass sizes were defined as follows: extremely large VLDL with particle diameters from 75 nm upwards and a possible contribution of chylomicrons, five VLDL subclasses (average particle diameters of 64.0 nm, 53.6 nm, 44.5 nm, 36.8 nm, and 31.3 nm), IDL (28.6 nm), three LDL subclasses (25.5 nm, 23.0 nm, and 18.7 nm), and four HDL subclasses (14.3 nm, 12.1 nm, 10.9 nm, and 8.7 nm). The following components of the lipoprotein subclasses were quantified: phospholipids, triglycerides, cholesterol, free cholesterol, and cholesteryl esters. The mean size for VLDL, LDL and HDL particles was

calculated by weighting the corresponding subclass diameters with their particle concentrations.

4.9.9 Adipose tissue biopsies, gene expression and plasma biomarkers (III)

The abdominal SAT biopsies were taken 6-8cm lateral from the umbilicus under local anesthesia after all PET scans were performed. To exclude the acute effect on gene expression, post-exercise biopsies were collected 72 hours after the last training session. The adipose tissue samples were snap frozen in liquid nitrogen immediately after excision and stored in -80°C for the gene expression. Deep-frozen tissues were homogenized with 700 µl TRIsure (Bioline) using bead-based tissue homogenizer (MO BIO Laboratories, USA). RNA was further purified by spin columns using RNeasy Plus Micro Kit (Qiagen) according to manufacturer's instructions. The RNA concentration was determined photometrically using Nanodrop (Thermo Fisher Scientific). Gene expression analysis of ANGPTL4, CD36, GLUT4, FABP4, FASN, PPARY and PDK4 was performed with quantitative real-time PCR (BioRad) using SYBR green gene expression assays (FastStart Universal SYBR Green Master Mix, Roche). The Cq values of the technical triplicates were averaged for each sample and normalized to 36B4 and YWHAZ expression. Primer pairs are listed in Table 5.

Plasma concentrations of the tumor necrosis factor (TNF- α), Vascular endothelial growth factor D (VEGFD) and vascular endothelial growth factor A (VEGFA) and also C-reactive protein (CRP) were measured by multiplex bead assay analysis (Luminex® Performance Assay Multiplex Kit; Procarta® Immunoassay Affymetrix, Santa Clara US) according to the manufacturer's instructions. Samples were analyzed with Luminex® 200™ using the Luminex xPonent software (Luminex, USA).

Table 5. Primer sequences used for quantitative real time PCR analysis.

Gene	Forward (5'-3')	Reverse (5'-3')
ANGPTL4	AGGCAGAGTGGACTATTTG	CCTCCATCTGAGGTCATC
CD36	AGCTTTCCAATGATTAGACG	GTTTCTACAAGCTCTGGTTC
GLUT4	TCCTTCCTCATTGGTATCATC	CCAAGGATGAGCATTTCATAG
FABP4	CAAGAGCACCATAACCTTAG	CTCGTTTTCTCTTTATGGTGG
FASN	AACTCCAAGGACACAGTCACCAT	CAGCTGCTCCACGAACTCAA
PPAR γ	GGGCTCCAATTTTTTCATTGTGA C	CCACACAGGTAACAAAATACATTC TAAGG
PKD4	GGATAGATCCCAGGTCGCTAGG	GGCTACTGTAAAAGTCCCGCTCTG
YWHAZ	ACTTTTGGTACATTGTGGCTTCA A	CCGCCAGGACAAACCAGTAT
36B4	CGACCTGGAAGTCCAACACTAC	ATCTGCTGCATCTGCTTG

5.0 Statistical analyses (I-III)

Studies I and III. The sample size of Study I and III were based on the primary outcome (skeletal muscle) of a larger research project (HITPET NCT01344928). For the healthy subjects, a total of 24 subjects (12/training group) and for the IR subjects a total of 20 subjects (10/training group) were calculated to give > 90% power of detecting a 20% difference in insulin-stimulated GU in the quadriceps femoris muscle. In the healthy subjects, the estimated increase in SIT was 40% and in MICT 20% (SD 15) and in the IR subjects in SIT 60% and MICT 30% (SD 20) with a level of significance at 5%. The sample size calculated for the entire study was used as there are no previous studies regarding the effects of SIT and MICT in adipose tissue metabolism. The final number of subjects recruited was 28 and 26 for the healthy and IR groups, respectively.

Altogether there were 7 drop-outs in the study, of which 2 were from the healthy group and 5 from the IR group. In the healthy group, both subjects dropped out during the training intervention, 1 from MICT group because of hip pain and 1 from the SIT group because of personal reasons. In the IR group, there were 2 drop-outs in the SIT groups; of which 1 discontinued before the intervention because of claustrophobic feelings in the PET scanner and 1 during the intervention, and 3 dropped out of the MICT group; all for personal reasons.

In all studies, normal distribution of the variables was tested by the Shapiro-Wilk test and evaluated visually. Logarithmic or square root transformations were performed when variables were not normally distributed. The level of statistical significance for all tests

was set at $p < 0.05$. All correlation analyses were performed between the variables on a whole group level using Pearson's correlation.

A statistical program package, version 9.3 for Windows (SAS Institute, Cary, NC, USA) was used for statistical calculations. Statistical analyses were performed with hierarchical linear mixed models compound symmetry covariance structure suitable for repeated measurements (PROX MIXED procedure). The model included one within-factor (training; before and after intervention in whole group) interaction term (training * group; the SIT and MICT groups behaved differently for the change in parameter with significant differences between the training modes) and a further interaction term (training * BAT; the high BAT and low BAT groups behaved differently for the change in parameter with significant difference between them) (I). The baseline characteristics of the groups were compared by a two-way analysis of variance including the main effect of health status (healthy vs. IR, a) or training mode (IR SIT vs. MICT, b) or sex (IR women vs. men, c) (III). Missing data points were accounted for by a restricted maximum likelihood estimation within the linear mixed models. All values are reported as model-based mean (SAS least square means) values [95% CI] from all the parameters measured before and after the training.

Study II. The sample size of Study II was based on the observed 55 and 34% improvements in skeletal muscle and myocardial glucose uptake after 5-months of moderate intensity exercise training in patients with dilated cardiomyopathy (Kempainen et al., 2003; Stolen et al., 2003). Assuming that the change in tissue glucose uptake would change by 15% after 2 weeks training, a total of 10 subjects would be needed to show the difference at an alpha level of 0.05. Cold has been shown to increase the BAT activation 10-fold compared to room temperature situation (Orava et al., 2011). With alpha level of 0.05, a total of 9 subjects would be needed to find a similar increase in BAT activation.

All analyses were performed using IBM SPSS software (version 22). The results are expressed as model-based means and 95% confidence intervals (CI) unless shown otherwise. A paired t-test was used to compare mean values of all end points of interest between measurements before and after exercise intervention. A linear mixed model for repeated measurements was used to study mean changes over time. The model included (time; indicating overall mean change between all different time points) and outdoor temperature was taken as a covariate in the model. A compound symmetry covariance structure was used in the analyses

6. RESULTS

This chapter presents the main results of the thesis. More details can be found in the original research papers (I-III).

6.1 Anthropometry and lipid profile in response to a 2 weeks and 6 weeks of exercise intervention (I-III)

We examined the responses of an exercise intervention in physically untrained healthy men after 2 weeks of SIT and MICT (I and III) and 6 weeks of a combination of SIT, MICT and resistance training (II). The physiological training adaptations are summarized in Table 6. Visceral fat mass was reduced and aerobic capacity increased in all studies. In addition, 2 weeks of exercise training induced a reduction in whole body fat percentage, subcutaneous fat mass, fasting free fatty acids, total cholesterol and increased HDL cholesterol independent of intensity. However, these changes were not seen after the 6 weeks of exercise intervention. When observing the results of the NMR spectroscopy, 6 weeks of training decreased plasma triglycerides, total cholines, and in particular phosphatidylcholines and fatty acids. Six weeks of training increased docosahexaenoic acid and citrate concentration. Training also reduced VLDL particle size, whereas there was no change in LDL and HDL particle size. Exercise training induced the most changes in chylomicron composition and in VLDL and HDL. Total lipids and cholesterol within chylomicrons and medium VLDL decreased after exercise training. Triglycerides decreased within chylomicrons, medium and small VLDL: small LDL and all classes of HDL, except very large and large HDL (data not shown).

Table 6. Characteristics of healthy subjects (Studies I-III) and their training responses.

HITPET (Studies I and III, Healthy subjects) 2 weeks of exercise intervention							<i>p</i> -values	
	Pre	Post	SIT Pre	SIT Post	MICT Pre	MICT Post	Time	Time* training
<i>n</i>	28	26						
<i>Anthropometrics</i>								
Age (years)	48 [46;49]							
BMI (kg/m ²)	26.1 [25.1;27.1]	26.0 [25.0;27.0]	25.9 [24.2;27.3]	25.7 [24.3;27.1]	26.4 [25.0;27.8]	26.4 [24.9;27.8]	0.14	0.19
Weight (kg)	83.6 [80.2;87.1]	83.3 [79.9;86.8]	83.1 [78.2;88.0]	82.6 [77.7;87.4]	84.1 [79.3;89.0]	84.1 [79.2;89.0]	0.21	0.30
Fat percentage (%)	22.1 [20.5;23.9]	21.1 [19.5;22.7]	22.2 [19.8;24.7]	22.1 [18.7;23.6]	22.9 [20.4;25.3]	22.1 [19.7;24.6]	<0.001	0.56
Fat free mass (kg)	64.3 [62.3;66.3]	64.8 [62.8;66.7]	64.0 [61.2;66.8]	64.4 [61.5;67.2]	64.6 [61.8;67.3]	65.1 [62.4;67.9]	0.02	0.56
Subcutaneous fat mass (kg)	4.1 [3.5;4.7]	4.0 [3.4;4.6]	4.0 [3.3;4.8]	3.9 [3.2;4.7]	4.4 [3.7;5.2]	4.3 [3.4;5.2]	0.04	0.51
Visceral fat mass (kg)	2.5 [2.0;3.2]	2.4 [1.9;3.0]	2.9 [2.1;3.8]	2.8 [1.8;3.5]	2.6 [1.7;3.5]	2.5 [1.8;3.4]	0.04	0.73
VO _{2peak} (ml/kg/min)	34.2 [32.5;35.9]	35.7 [34.0;37.4]	34.7 [32.4;37.1]	36.7 [34.3;39.1]	33.7 [31.3, 36]	34.7 [32.4;37.1]	0.001	0.27
<i>Glucose profile</i>								
Glucose _{fasting} (mmol/L)	5.5 [5.3;5.7]	5.3 [5.1;5.5]	5.4 [5.1;5.7]	5.2 [4.9;5.5]	5.6 [5.4;5.9]	5.5 [5.2;5.8]	0.26	0.77
Insulin _{fasting} (mmol/L)	4.7 [3.7;5.9]	5.8 [4.6;7.4]	5.2 [3.8;7.2]	4.8 [3.4;6.6]	5.8 [4.1;8.1]	6.0 [4.3;8.4]	0.80	0.46
HOMA-IR	1.3 [1.0;1.6]	1.8 [1.4;2.1]	1.3 [0.8;1.7]	1.7 [1.2;2.2]	1.4 [0.9;1.8]	1.9 [1.4;2.4]	0.02	0.91
M value (μmol/kg/min)	35.1 [29.1;41.1]	38.5 [32.5;44.6]	38.2 [30.1;46.4]	42.8 [34.5;51.0]	31.9 [23.1;40.7]	34.2 [25.4;43.1]	0.03	0.45
<i>Lipid profile</i>								
FFA _{fasting} (mmol/L)	0.70 [0.6;0.7]	0.62 [0.5;0.7]	0.61 [0.5;0.7]	0.59 [0.4;0.7]	0.78 [0.6;0.9]	0.67 [0.5;0.8]	0.052	0.14
Triglycerides _{NP} (mmol/L)	0.94 [0.8;1.1]	0.83 [0.7;0.9]	1.02 [0.8;1.2]	0.97 [0.7;1.2]	0.96 [0.7;1.1]	0.80 [0.6;0.9]	0.07	0.37
LDL-cholesterol (mmol/L)	3.1 (2.8;3.4)	2.7 [2.4;3.9]	3.4 [3.0;3.8]	2.8 [2.4;3.3]	2.9 [2.5;3.3]	2.7 [2.3;3.1]	0.001	0.03
HDL-cholesterol (mmol/L)	1.3 (1.2;1.5)	1.2 [1.1;1.4]	1.4 [1.2;1.6]	1.2 [1.1;1.4]	1.4 [1.2;1.6]	1.3 [1.1;1.5]	<0.001	0.28
Total cholesterol (mmol/L)	4.9 [4.5;5.2]	4.4 [4.0;4.7]	5.3 [4.8;5.7]	4.6 [4.1;5.0]	4.7 [4.3;5.2]	4.4 [3.9;4.9]	<0.001	0.06

EXEBAT (Study II, Healthy subjects) 6 weeks of exercise intervention			
	Pre	Post	p-value
<i>n</i>	11	10	
<i>Anthropometrics</i>			
Age (years)	31.1 ± 7.5		
BMI (kg/m ²)	22.8 ± 1.0	23.1 ± 1.0	0.53
Weight (kg)	73.3 ± 7.7	73.6 ± 8.1	0.51
Fat percentage (%)	17.1 ± 1.7	17.0 ± 1.5	0.20
Fat free mass (kg)	60.6 ± 6.2	61.1 ± 6.4	0.15
Subcutaneous fat mass (kg)	2.2 ± 0.7	2.1 ± 0.4	0.37
Visceral fat mass (kg)	1.1 ± 0.4	0.9 ± 0.5	0.02
VO _{2max} (ml/kg/min)	38 ± 9.3	47 ± 8.2	0.01
<i>Glucose profile</i>			
Glucose _{fasting} (mmol/L)	5.2 ± 0.3	5.3 ± 0.4	0.41
Insulin _{fasting} (mmol/L)	5.5 ± 2.4	6.1 ± 2.3	0.62
Matsuda index	8.8 ± 4.6	7.4 ± 2.6	0.24
HOMA-IR	1.3 ± 0.6	1.4 ± 0.5	0.58
<i>Lipid profile</i>			
FFA _{fasting} (mmol/L)	0.39 ± 0.18	0.44 ± 0.19	0.56
Triglycerides _{fp} (mmol/L)	0.91 ± 0.30	1.03 ± 0.39	0.23
LDL-cholesterol (mmol/L)	2.63 ± 1.14	2.36 ± 0.89	0.18
HDL-cholesterol (mmol/L)	1.61 ± 0.32	1.62 ± 0.33	0.97
Total cholesterol (mmol/L)	4.7 ± 1.2	4.5 ± 1.1	0.28

Values in HITPET are model based means (95% CI). Values in EXEBAT are means ± SD. BMI, body mass index; VO_{2peak}, peak oxygen uptake; VO_{2max}, maximal oxygen uptake; fp, fasting plasma.

6.2 Comparisons of baseline characteristics based on different groups (III)

Healthy vs IR. In a 2-weeks of exercise intervention, compared to IR men, healthy men had lower body adiposity, a better blood lipid profile and glucose homeostasis at baseline (Table 7). There were similar reductions in total cholesterol levels and whole body-fat percentage in healthy and IR subjects after exercise training. At baseline, IR subjects had a 16% lower VO_{2peak} compared to healthy men ($p<0.001$, Table 7). Aerobic capacity in the whole study population (men, $n=44$) increased by 3% with no significant differences in the training response between the healthy and IR groups. After intervention, the aerobic capacity remained decreased in IR subjects compared to healthy subjects. Whole body insulin sensitivity was impaired in IR subjects with a 54% reduced M-value compared to the healthy men ($p<0.001$, Table 7). Insulin sensitivity improved in both healthy and IR groups (10% and 23%, respectively, time $p=0.01$, time*IR, $p=0.12$), although insulin sensitivity remained low in the IR subjects compared to the healthy subjects ($p=0.004$) after the intervention.

Men vs Women. Compared to IR men, IR women at baseline were older than the men having increased whole-body adiposity, higher abdominal SAT mass and plasma FFAs (Table 8). Exercise reduced body mass, abdominal SAT and VAT masses, cholesterol, LDL, HDL and HbA1c without any differences between the groups (Table 8). However, when these variables were assessed with age as the covariate body mass, BMI and body fat percentage reduction was no longer significant ($p=0.06$, $p=0.052$, $p=0.051$).

IR SIT vs MICT. There were no significant differences in training induced responses between the two training modes in IR subjects. Training reduced visceral fat mass, HDL, LDL, total cholesterol, HbA1c and increased insulin sensitivity without any significant differences between SIT and MICT (Table 9). As a whole group, whole body mass and abdominal SAT mass tended to decrease. Aerobic capacity was increased only by SIT (SIT +5% vs. MICT 0.0%, time*training $p=0.048$) (Table 9).

Table 7. Baseline characteristics of healthy and insulin resistant (IR) men. The p-value for 'Baseline' denotes the baseline differences between healthy (n=28) and IR (n=16) groups and 'Time' shows all healthy and IR males after 2 weeks of training. 'Time*IR' demonstrates whether there is an interaction between the pre-post change and health status. All the data are presented as model-based means [95% confidence interval, CI]. Logarithmic transformation has been done to the variables with * and square transformation to the variables with □ to achieve a normal distribution. The values are LS means translated into the original unit; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free fatty acid; HbA1c, glycated hemoglobin; M-value, insulin sensitivity; VO₂peak, aerobic capacity. Modified from Motiani P et al (11)

	Healthy men		Insulin resistant (IR) men		Baseline	Time	Time* IR
	Pre	Post	Pre	Post			
N	28	26	16	13			
Age (y)	48		49				
BP systolic (mmHg)	125.2 [122.1;128.3]	128.7 [125.4;131.9]	138.6 [134.5;142.7]	132.1 [127.6;136.6]	<0.001	0.31	0.002
BP diastole (mmHg)	77.6 [75.4;79.9]	78.7 [76.2;81.1]	87.6 [84.6;90.6]	81.8 [78.5;85.2]	<0.001	0.06	0.01
Body mass (kg)	83.6 [79.7;87.5]	83.3 [79.4;87.2]	96.3 [91.1;101.5]	96.1 [90.9;101.3]	<0.001	0.20	0.80
BMI (kg/m ²)	26.1 [25.1;27.1]	26.0 [25.0;27.0]	30.4 [29.0;31.8]	30.3 [29.0;31.7]	<0.001	0.17	0.72
Body fat (%) ^{&}	22.1 [20.5;23.9]	21.1 [19.5;22.7]	28.4 [25.6;31.5]	27.6 [24.9;30.7]	<0.001	<0.001	0.31
Waist (cm)	95.5 [92.4; 98.6]	94.8 [91.7;98.0]	105.3 [101.0;109.6]	104.7 [100.4;109.0]	0.0006	0.02	0.84
Subcutaneous fat (kg) [^]	4.0 [3.6;4.5]	4.0 [3.6;4.0]	5.5 [4.8;6.4]	5.5 [4.8;6.4]	<0.001	0.03	0.93
Visceral fat (kg) [^]	3.0 [2.7;3.4]	2.9 [2.6;3.3]	4.2 [4.9;3.5]	4.0 [4.8;3.4]	0.002	0.002	0.54
Cholesterol (mmol/L) ^{&}	4.9 [4.5;5.2]	4.4 [4.0;4.7]	4.6 [4.2;5.1]	4.2 [3.8;4.7]	0.44	<0.0001	0.59
HDL cholesterol (mmol/L) ^{&}	1.3 [1.2;1.5]	1.2 [1.1;1.3]	1.2 [1.0;1.3]	1.0 [0.9;1.2]	0.08	<0.001	0.63
LDL cholesterol (mmol/L)	3.1 [2.8;3.4]	2.7 [2.4;3.0]	2.7 [2.3;3.1]	2.5 [2.1;2.9]	0.09	0.002	0.16
Triglycerides (mmol/L) ^{&}	0.9 [0.8;1.1]	0.8 [0.7;0.9]	1.6 [1.3;2.0]	1.5 [1.1;1.8]	<0.001	0.08	0.94
Fasting FFA (mmol/L)	0.70 [0.62;0.77]	0.62 [0.54;0.70]	0.69 [0.60;0.78]	0.68 [0.58;0.78]	0.86	0.07	0.15
HbA1c (mmol/L) ^{&}	36.7 [35.0;38.4]	34.5 [32.9;36.2]	39.1 [36.8;41.6]	37.1 [34.9;39.5]	0.09	<0.001	0.75
HbA1c(%)	5.5 [5.4;5.7]	5.3 [5.2;5.5]	5.7 [5.6;6.0]	5.5 [5.4;5.8]	0.07	<0.001	0.87
Fasting glucose (mmol/L) ^{&}	5.5 [5.3;5.8]	5.5 [5.2;5.7]	6.7 [6.3;7.0]	6.6 [6.3;7.0]	<0.001	0.57	0.79
Fasting insulin (mmol/L) ^{&}	4.7 [3.7;5.8]	5.8 [4.6;7.4]	14.4 [10.8;19.3]	13.6 [10.0;18.5]	<0.001	0.34	0.10
M-value (μmol/kg/min)	32.3 [26.7;39.0]	35.7 [29.4;43.3]	14.8 [11.4;19.2]	19.3 [14.8;25.1]	<0.001	0.001	0.12
Aerobic capacity (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

Table 8. Baseline characteristics of men and women in insulin resistant subjects (IR). The p-value for 'Baseline' indicates the baseline differences between men and women. 'Time' means all IR subjects after 2 weeks of training. 'Time*sex' demonstrates if there is an interaction between the pre-post change and sex. All the data are presented as model-based means [95% confidence interval, CI]. Logarithmic transformation has been done to the variables with * and square transformation to the variables with □ to achieve a normal distribution. The values are LS means translated into the original unit.; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free fatty acid; HbA1c, glycated hemoglobin; TAT, total adipose tissue M-value, insulin sensitivity, VO₂peak, aerobic capacity. Modified from Motiani P et al (11).

	IR Men			IR Women			Baseline	Time	Sex* training
	Pre	Post	13	Pre	Post	8			
N	16	13		10	8				
Age (y)	47 [45;49]			52 [20;55]			0.002	-	-
BP systole (mmHg)	138.6 [133;144]	132.3 [126;138]		142.4 [135;149]	139.8 [132;147]		0.39	0.02	0.32
BP diastole (mmHg)	87.6 [84;91.1]	81.9 [78.0;85.8]		86.9 [82.4;91.3]	81.1 [76.2;86.1]		0.75	0.004	0.99
Height (m)	1.8 [1.7;1.8]			1.7 [1.6;1.7]			< 0.001		
Body mass (kg)	96.4 [90.0;102.7]	96.2 [89.8;102.5]		84.0 [75.8;92.1]	83.0 [74.8;91.1]		0.02	0.04	0.12
BMI (kg/m ²)	30.5 [29.0;32.0]	30.4 [28.9;31.9]		30.4 [28.5;31.9]	30.1 [28.1;32.0]		0.97	0.03	0.09
Whole body fat (%) ^{&}	28.5 [26.3;30.8]	27.7 [25.5;30.0]		40.7 [36.7;45.2]	39.3 [35.4;43.6]		< 0.001	0.02	0.77
Waist (cm)	105.3 [100.7;109.8]	104.7 [100.2;109.3]		102.2 [96.4;108.1]	101.8 [95.9;107.7]		0.40	0.08	0.98
Subcutaneous fat (kg) [^]	6.0 [5.0;7.1]	5.9 [5.0;7.0]		9.1 [7.3;11.3]	8.8 [7.1;10.9]		0.003	0.03	0.25
Adj.SAT (TAT&height)	5.7 [5.1;6.5]	5.8 [5.1;6.6]		8.6 [7.2;10.4]	8.6 [7.3;10.3]		0.01	0.78	0.76
Visceral fat (kg) [^]	4.2 [3.4;5.1]	4.0 [3.2;4.9]		2.3 [1.8;3.0]	2.2 [1.7;2.8]		0.002	0.01	0.75
Adj.VAT (TAT&height)	4.5 [3.7;5.4]	4.4 [3.6;5.3]		1.8 [1.4;2.4]	1.8 [1.4;2.4]		< 0.001	0.42	0.90
Cholesterol (mmol/L) ^{&}	4.7 [4.3;5.2]	4.3 [3.9;4.8]		5.0 [4.4;5.8]	4.4 [3.9;5.1]		0.35	0.01	0.52
HDL cholesterol (mmol/L) ^{&}	1.2 [1.1;1.4]	1.1 [0.9;1.3]		1.5 [1.3;1.7]	1.5 [1.2;1.7]		0.048	0.03	0.22
LDL cholesterol (mmol/L)	2.7 [2.2;3.1]	2.6 [2.1;3.0]		2.9 [2.4;3.5]	2.4 [1.9;3.0]		0.43	0.01	0.15
Triglycerides (mmol/L) ^{&}	1.7 [1.3;2.2]	1.5 [1.1;2.0]		1.2 [0.9;1.8]	1.2 [0.8;1.7]		0.12	0.56	0.70
Fasting FFA (mmol/L)	0.69 [0.61;0.78]	0.68 [0.59;0.77]		0.96 [0.84;1.1]	0.90 [0.78;1.0]		< 0.001	0.25	0.40
HbA1c (mmol/L) ^{&}	39.6 [37.1;42.2]	37.7 [35.1;40.3]		39.7 [36.5;43.0]	37.9 [34.6;41.2]		0.99	0.003	0.91
HbA1c (%)	5.8 [5.5;6.0]	5.6 [5.4;5.8]		5.8 [5.5;6.1]	5.6 [5.3;5.9]		0.99	0.002	0.89
Fasting glucose (mmol/L) ^{&}	6.7 [6.2;7.2]	6.7 [6.2;7.2]		6.6 [6.1;7.2]	6.4 [5.8;7.0]		0.80	0.38	0.47
Fasting insulin (mmol/L) ^{&}	13.1 [9.2;18.6]	12.1 [8.5;17.4]		8.3 [5.4;12.6]	8.2 [5.3;12.7]		0.09	0.56	0.65
M-value (μmol/kg/min)	14.8 [10.8;20.3]	19.4 [14.0;27.0]		17.4 [11.8;25.5]	20.3 [13.4;30.6]		0.69	0.04	0.54
Aerobic capacity (ml/kg/min)	29.4 [27.6;31.2]	30.0 [28.1;31.8]		23.9 [21.6;26.3]	24.6 [22.2;27.0]		0.002	0.10	0.89

Table 9. Baseline characteristics of SIT and MICT training groups in insulin resistant subjects (IR). The p-value for 'Baseline' describes the baseline differences between SIT (n=13) and MICT (n=13) groups and 'Time' shows all IR subjects after 2 weeks of training 'Time*training' demonstrates whether there is an interaction between the pre-post change and training mode. All the data are presented as model-based means [95% confidence interval, CI]. Logarithmic transformation has been done to the variables with * and square transformation to the variables with □ to achieve a normal distribution. The values are LS means translated into the original unit.; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free fatty acid; HbA1c, glycated hemoglobin; M-value, insulin sensitivity; V_{O2}peak, aerobic capacity. Modified from Motiani P et al (11).

	IR SIT		IR MICT		Baseline	Time p-value	Time* training
	Pre	Post	Pre	Post			
N (M/F)	13 (9/4)	11(7/4)	13 (7/6)	10(6/4)			
Age (y)	48 [46;49]		47[44;49]				
BP systole (mmHg)	133.8 [128.2;139.4]	131.2 [125.4;137.1]	146.2 [140.7;151.8]	139.0 [133.0;145.0]	0.001	0.008	0.18
BP diastolic (mmHg)	85.9 [82.0;89.7]	81.5 [77.4;85.7]	88.8 [83.0;92.7]	81.6 [77.3;86.0]	0.17	0.003	0.40
Body mass (kg)	91.6 [84.0;99.2]	91.2 [83.6;98.8]	92.0 [84.4;99.6]	91.5 [83.9;99.1]	0.95	0.09	0.96
BMI (kg/m ²)	29.9 [28.3;31.5]	29.8 [28.2;31.4]	31.0 [29.4;32.6]	30.8 [29.2;32.4]	0.35	0.08	0.84
Whole body fat (%) ^{&}	32.2 [28.1;36.9]	31.2 [27.2;35.8]	33.0 [28.8;37.8]	32.0 [27.9;36.8]	0.79	0.01	0.92
Waist (cm)	103.7 [98.5;108.8]	103.5 [98.3;108.6]	104.6 [99.4;109.8]	103.7 [98.5;108.9]	0.80	0.07	0.24
Subcutaneous fat (kg) [^]	7.1 [5.7;8.8]	6.9 [5.5;8.6]	7.1 [5.7;8.7]	6.9 [5.6;8.6]	0.97	0.046	0.90
Visceral fat (kg) [^]	3.1 [2.3;4.1]	3.0 [2.2;3.9]	3.6 [2.7;4.7]	3.4 [2.6;4.4]	0.42	0.01	0.43
Cholesterol (mmol/L) ^{&}	4.7 [4.2;5.2]	4.0 [3.6;4.5]	5.0 [4.4;5.6]	4.7 [4.2;5.3]	0.41	0.006	0.15
HDL cholesterol (mmol/L) ^{&}	1.3 [1.1;1.5]	1.2 [1.0;1.4]	1.3 [1.1;1.6]	1.3 [1.1;1.5]	0.83	0.01	0.81
LDL cholesterol (mmol/L)	2.6 [2.2;3.0]	2.3 [1.8;2.7]	3.0 [2.5;3.5]	2.8 [2.2;3.2]	0.19	0.02	0.62
Triglycerides (mmol/L) ^{&}	1.5 [1.1;2.0]	1.3 [1.0;1.8]	1.5 [1.1;2.1]	1.5 [1.1;2.0]	0.90	0.44	0.70
Fasting FFA (mmol/L)	0.74 [0.61;0.85]	0.75 [0.62;0.88]	0.83 [0.72;0.95]	0.77 [0.65;0.89]	0.22	0.43	0.17
HbA1c (mmol/L) ^{&}	39.6 [36.8;42.3]	37.8 [35.0;40.6]	39.5 [36.8;42.3]	37.5 [34.7;40.3]	0.99	0.001	0.79
HbA1c (%)	5.8 [5.5;6.0]	5.6 [5.4;5.9]	5.8 [5.5;6.0]	5.6 [5.3;5.8]	0.99	0.001	0.79
Fasting glucose (mmol/L) ^{&}	6.9 [6.4;7.4]	6.9 [6.4;7.4]	6.5 [6.0;6.9]	6.3 [5.8;6.8]	0.22	0.45	0.43
Fasting insulin (mmol/L) ^{&}	12.6 [8.4;18.8]	11.0 [7.3;16.5]	9.5 [6.6;13.7]	9.8 [6.7;14.2]	0.30	0.43	0.23
M-value (μmol/kg/min)	16.8 [11.9;23.9]	22.2 [15.4;32.0]	14.4 [10.4;19.8]	17.6 [12.5;24.8]	0.54	0.02	0.71
Aerobic capacity (ml/kg/min)	27.0 [24.4;29.7]	28.4 [25.7;31.1]	27.4 [24.7;30.1]	27.2 [24.5;29.9]	0.85	0.14	0.048

Cold exposure increased the whole-body energy expenditure. However, exercise did not have any effect on whole-body energy expenditure (Figure 9A). During the cold exposure compared to room temperature before the exercise intervention, whole-body fat oxidation was increased (233%, $p < 0.001$). Similarly, during cold exposure after exercise training whole-body fat oxidation increased (108% $p = 0.001$). (Figure 9C).

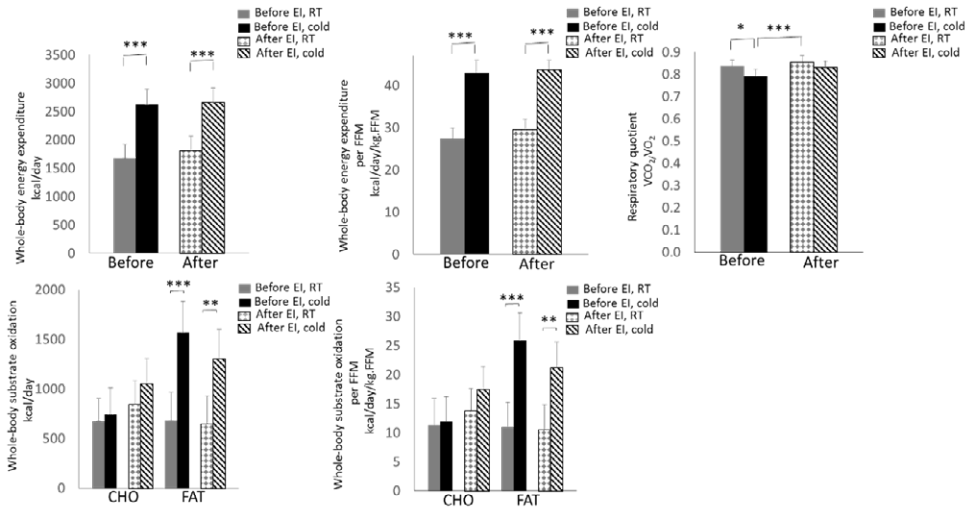


Figure 9. Whole-body energy expenditure measurements at room temperature, during cold stimulation before and after exercise intervention. (A) Whole-body energy expenditure. (B) Rate of whole body energy expenditure per kilogram of fat free mass. (C) Whole-body substrate oxidation. (D) Rate of whole body substrate oxidation per kilogram of fat free mass. Values are means [95%, CI]. *** $p < 0.001$, ** $p = 0.001$.

6.4 Effects of cold and exercise on circulatory hormones and catecholamines (II)

Nor-epinephrine increased during cold stimulation before and after exercise. (Figure 10)

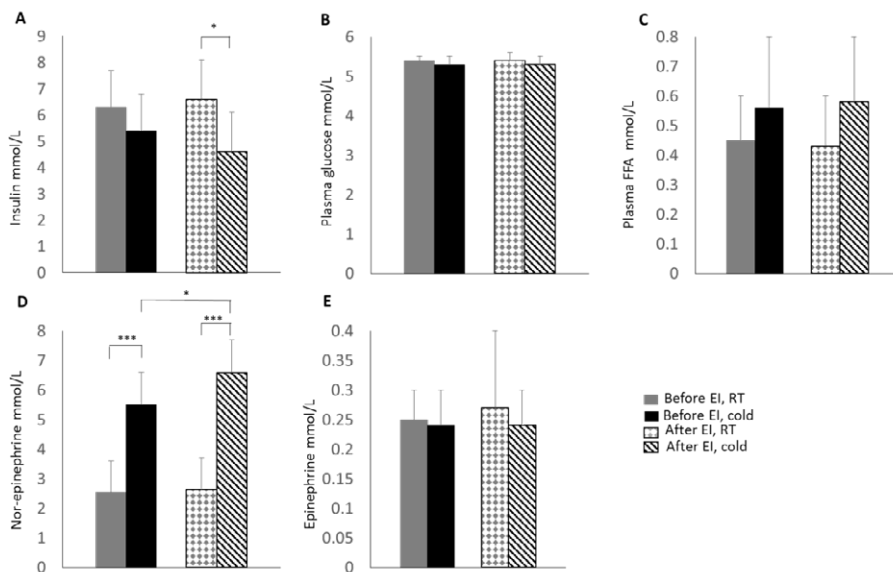


Figure 10. Concentration of circulatory hormones and metabolites at room temperature (RT), during cold stimulation before exercise intervention (EI) and after exercise intervention (EI). (A) Insulin, (B) Plasma glucose, (C) Plasma free fatty acids, (D) Nor-epinephrine, (E) Epinephrine. Values are means [95% CI]. *** $p < 0.001$, ** $p = 0.001$, * $p = 0.04$.

6.5 Effects of SIT and MICT on brown adipose tissue glucose metabolism (I)

No differences in either BAT GU (time $p = 0.08$, time*training $p = 0.95$) (Figure 11A) or BAT FFAU were observed between the two training intensities (time $p = 0.21$, time*training $p = 0.70$). Due to the high variability in brown adipose tissue activity it has been proposed previously that glucose uptake $>2.9 \mu\text{mol}/100\text{g}/\text{min}$ represents active BAT during euglycaemic-hyperinsulinaemic clamp. Based on pre-intervention insulin-stimulated BAT GU the subjects were divided into high BAT ($>2.9 \mu\text{mol}/100\text{g}/\text{min}$; $n = 6$) and low BAT ($<2.9 \mu\text{mol}/100\text{g}/\text{min}$; $n = 12$) groups (Figure 11B).

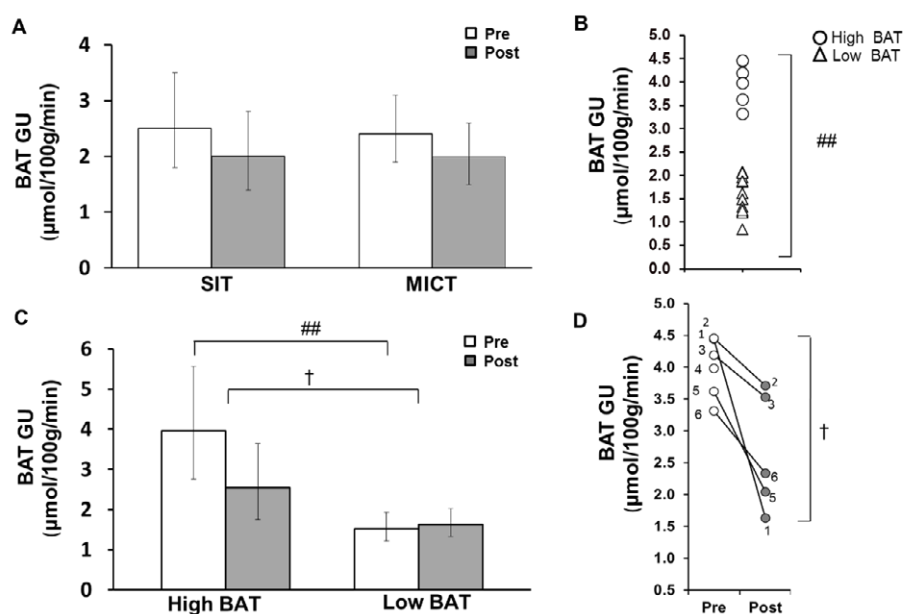


Figure 11. (A) Randomization into SIT and MICT (B) Division of subjects into high BAT (circles) and low BAT groups (triangles) with a cut-off point of ($2.9 \mu\text{mol}/100\text{g}/\text{min}$) at baseline. (C) BAT insulin stimulated glucose uptake (GU) before (white bars) and after the exercise intervention (grey bars). (D) Insulin stimulated BAT glucose uptake in each individual in high BAT group before and after the exercise intervention. ## $p < 0.0001$ # $p = 0.03$ difference between the groups at baseline, †† $p = 0.01$, † $p = 0.02$ indicates whether the high BAT and low BAT groups behaved differently for the change in parameter with significant difference between them (training*BAT). Modified from Motiani P et al. (I).

Division and characteristics of high and low BAT activity groups. The characteristics of subjects with high BAT and low BAT activity are shown in Table 10. Interestingly, at baseline subjects with high BAT activity had lower body adiposity and leptin concentrations, as well as higher whole-body insulin sensitivity and HDL cholesterol compared to subjects with low BAT activity. These data suggest high vs. low BAT individuals have a better metabolic phenotype. Subjects with high BAT activity also showed reduced IL-6 levels, increased circulating MCP-1 levels, carbohydrate oxidation (during insulin stimulation) compared to low BAT activity group (Table 11).

BAT, WAT and muscle metabolism in high and low BAT activity groups. The main finding was that 2 weeks of exercise training decreased insulin-stimulated BAT GU in high BAT group, whereas there was no change in the low BAT group (training*BAT $p = 0.02$, Figure 11C). In contrast, training decreased BAT FFAU in the low BAT activity group, but not in the high BAT activity group (training*BAT, $p = 0.01$, Figure 12B). Exercise training led to a higher quadriceps muscle glucose uptake in both groups similarly (training $p = 0.0009$, training*BAT $p = 0.25$). In contrast, glucose uptake in femoral WAT tended to be higher in the high BAT compared with low BAT activity group (training $p = 0.02$,

training*BAT $p = 0.07$). Training did not change muscle FFAU in either group. At baseline and after training, there were no changes in BAT mass or radiodensity. Interestingly, training increased IL-6 concentrations in the high BAT group, but decreased it in the low BAT activity group (training*BAT $p = 0.02$).

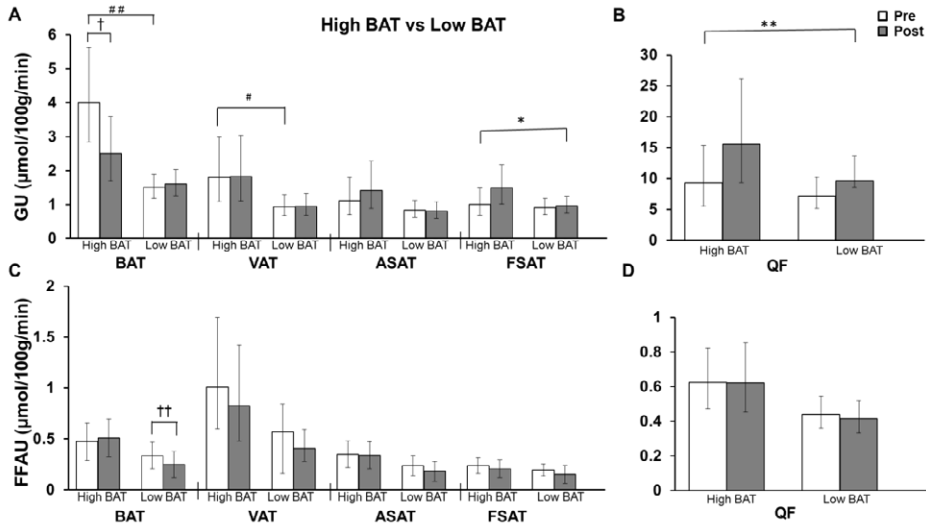


Figure 12 Insulin-stimulated glucose uptake (GU) and free fatty acid uptake (FFAU) A and B: Insulin-stimulated GU. C and D: Free fatty acid uptake.

Results are presented as mean and (95% CI)

$p < 0.0001$ differences between the groups at baseline

* $p = 0.02$, ** $p = 0.0009$ mean changes in pre- and post- measurements in whole group (training)

† $p = 0.02$, †† $p = 0.01$ indicates whether high BAT and low BAT activity groups behaved differently for the change in variable with significant difference between them (training*BAT) ASAT, abdominal subcutaneous adipose tissue; FSATF, femoral subcutaneous adipose tissue; VAT; visceral adipose tissue, QF, quadriceps femoris muscle. Modified from Motiani P et al. 2017 (1).

Table 10. The characteristics of high BAT and low BAT study subjects before (Pre) and after 2-weeks of exercise training (Post)

	Groups based on BAT activity				<i>p</i> - value	
	High BAT (n=6) PRE	High BAT (n=6) POST	Low BAT (n=12) PRE	Low BAT (n=12) POST	Training	Training* BAT
<i>Anthropometrics</i>						
Body weight (kg)	74.7 (82,91) [#]	74.8 (68,81)	86.5 (82,91)	86.4 (82,91) [#]	0.95	0.71
BMI(kg/m ²)	23.6 (22,25) [#]	23.7 (22,26) [#]	26.9 (26,28)	26.8 (26,28)	0.93	0.75
Fat percentage (%)	18.7 (15, 23) [#]	17.6 (14, 22)	24.1 (21, 26)	23.7 (21, 26)	0.04	0.37
Free fat mass	59.7 (57, 63).	60.5 (57,64)	65.5 (63, 68)	65.8 (64, 68),	0.03	0.28
Waist (cm)	87.1 (81, 93) [#]	86.3 (80, 93) [#]	98.7 (94, 103)	98.1 (94,102)	0.08	0.68
VO _{2peak} (ml/kg/min)	36.1(32,41)	39.1(35,44)	33.4 (31, 36)	34.9 (32,38)	0.0005	0.16
Systolic BP (mm Hg)	120 (113, 127)	122 (116, 129) [#]	127 (122, 133)	131 (127,135)	0.13	0.61
Diastolic BP (mmHg)	77 (71, 83)	76 (69, 81)	77 (73,81)	78 (74, 82)	0.80	0.53
Heart rate (bpm)	60.9 (55,67)	58.0 (52, 64)	61.0 (57, 65)	59.8 (56, 64)	0.17	0.57
Subcutaneous fat mass (kg)	3.3 (1.9, 4.6) ^(#)	3.2 (1.9, 4.5)	4.8 (3.9, 5.7)	4.7 (3.8, 5.6)	0.15	0.85
Visceral fat mass (kg)	&1.2 (0.7, 2.1) [#]	1.1 (0.6, 1.1)	3.0 (2.0, 4.3)	2.8 (1.9 ,4.1)	0.009	0.30
<i>Glucose Profile</i>						
M value (μmol/kg/min)	48.9 (36, 62) [#]	49.3 (36, 62)	31.1 (22, 40)	36.8 (28, 46)	0.13	0.18
Glucose _{fasting} (mmol/L)	5.3 (5.1,5.6)	5.8 (5.4,6.2)	5.5 (5.3,5.7)	5.7 (5.5,5.9)	0.05	0.34
Glucose _{clamp} (mmol/L)	5.1 (4.5,5.7)	5.3 (4.8,5.8)	5.5 (5.3,5.8)	5.5 (5.2,5.7)	0.47	0.35
Insulin _{fasting} (mmol/L)	&3.6 (2.5, 5.4) [#]	3.9 (2.7, 5.8)	5.7 (4.4,7.4)	5.7 (4.4,7.5)	0.54	0.62
Insulin _{clamp} (mmol/L)	79.5 (64,95)	81.7(66,98)	72.3 (62,83)	74.3 (64,85)	0.59	0.99
HbA1c (%)	5.3 (5.0, 5.6)	5.1 (4.8, 5.4)	5.7 (5.5, 5.9)	5.4 (5.2, 5.6)	0.003	0.70
<i>Lipid Profile</i>						
FFA _{fasting}	0.82(0.5,1.1)	0.91 (0.6,1.2)	0.65 (0.4,0.9)	0.61 (0.4,0.8)	0.80	0.47
FFA _{clamp}	0.46 (0.3,0.6)	0.51 (0.4,0.7)	0.41 (0.3,0.5)	0.37 (0.3,0.5)	0.93	0.38
Cholesterol (mmol/L)	4.85 (4.0, 5.7)	4.24 (3.4, 5.1)	5.09 (4.5, 5.7)	4.61 (4.0, 5.2)	0.002	0.66
LDL (mmol/L)	2.90 (2.2, 3.6)	2.46 (1.7, 3.2)	3.40 (2.9, 3.9)	3.03 (2.5, 3.5)	0.004	0.77
HDL (mmol/L)	&1.57(1.4,1.8) [#]	1.50 (1.3, 1.7) [#]	1.17 (1.1, 1.3)	1.12 (1.0, 1.2)	0.27	0.99
Triglycerides (mmol/L)	0.80 (0.5, 1.1)	0.59 (0.3, 0.9) [#]	1.10 (0.9, 1.3)	1.00 (0.8, 1.2)	0.08	0.51

Abbreviation: HbA1c, glycated haemoglobin.

Values are means and 95% CIs.

&Log transformation was done to achieve normal distribution

#*p* <0.05, ##*p* <0.01 and (#)*p* = 0.052 significant difference between the groups in corresponding time point. #*p* <0.05 difference between groups in corresponding time point. *p* value (training) indicates the mean changes in pre and post measurements in the whole group. *p* value(training*BAT) indicates whether high BAT and low BAT groups behaved differently for the change in variable with significant difference between them. Bolded values are statistically significant. Modified from Motiani P et al. 2017 (II).

Table 11. Circulatory hormones, metabolites and energy oxidation of the high BAT and low BAT groups after 2 weeks of exercise training

	Groups based on BAT activity				p-value	
	High BAT PRE	High BAT POST	Low BAT PRE	Low BAT POST	Training BAT	Training *
<i>Circulatory hormones and metabolites</i>						
Epinephrine	&0.14 (0.09,0.23)	0.17 (0.11, 0.27)	0.14 (0.10,0.19)	0.25 (0.18,0.34)	0.02	0.19
Norepinephrine	&1.67 (1.2, 2.2)	2.09 (1.6, 2.8)	1.95 (1.6, 2.4)	2.17 (1.8, 2.6)	0.13	0.57
Irisin	66.1 (50, 82)	64.3 (47, 82)	73.0 (62, 84)	64.3 (47, 82)	0.33	0.62
NGF (pg/ml)	&0.64 (0.3, 1.5)	0.50 (0.2, 1.4)	1.13 (0.7, 1.9)	1.39 (0.8, 2.4)	0.95	0.37
IL-6 (pg/ml)	&0.19 (0.04, 0.9) [#]	0.28 (0.05,1.5)	1.33 (0.5,0.3)	0.41 (0.2,1.0)	0.27	0.04
IL-8 (pg/ml)	4.86 (2.5,7.2)	4.42 (1.9,6.9)	6.42 (5.1,7.8)	6.15 (4.8,7.5)	0.48	0.87
Leptin (pg/ml)	1625(1723,432) [#]	1769 (1129,4688)	5030 (3499,661)	4373 (2792,5953)	0.64	0.47
HGF (pg/ml)	348 (128, 568)	312 (77.9, 547)	441 (313, 570)	354 (225, 483)	0.17	0.55
MCP-1 (pg/ml)	285 (204, 366) [#]	300 (217, 384)	200 (153, 248)	215 (168, 263)	0.20	0.99
TNF- α (pg/ml)	&2.79 (1.7, 4.6)	2.67 (1.6, 4.5)	4.99 (3.7, 6.7)	4.29 (3.2, 5.8)	0.27	0.53
<i>Energy oxidation</i>						
Adj REE _{fasting} (MJ/day)	&6.62 (6.1, 7.2)	6.62 (6.1, 7.2) [#]	6.81 (6.4, 7.2)	6.86 (6.5, 7.3)	0.88	0.88
AdjREE _{clamp} (MJ/day)	&7.09 (6.6, 7.6)	6.99 (6.5, 7.5) [#]	7.19 (6.8, 7.6)	7.15 (6.8, 7.5)	0.58	0.85
Carbohydrate _{fasting} (g/min)	2.27 (1.6, 3.0)	1.80 (1.1, 2.5)	2.18 (1.7, 2.7)	2.32 (1.8, 2.9)	0.58	0.32
Carbohydrate _{clamp} (g/min)	3.66 (2.9, 4.4) [#]	3.08 (2.3, 3.8)	2.86 (2.3, 3.4)	2.91 (2.3, 3.4)	0.34	0.26
Fat _{fasting} (g/min)	2.33 (1.6, 3.0)	2.82 (2.1, 3.5)	2.65 (2.1, 3.2)	2.82 (2.0, 3.1)	0.54	0.34
Fat _{clamp} (g/min)	0.98 (0.2, 1.7) [#]	1.51 (0.8, 2.2)	2.36 (1.8, 2.9)	2.22 (1.7, 2.7)	0.47	0.27
Protein _{fasting} (g/min)	1.13 (1.1, 1.2)	1.18 (1.1, 1.2)	1.15 (1.1, 1.2)	1.14 (1.1, 1.2)	0.44	0.25
<i>Others</i>						
BAT mass (g)	&80.1 (43,148)	81.5 (42,157)	64.9(43,98)	61.8 (41,94)	0.95	0.89
BAT radiodensity (CT HU)	-87.5 (-93,-82)	-88.4 (-94,-83)	-91.6 (-95,-88)	-91.0 (-95,-87)	0.93	0.58

Abbreviation: REE, resting energy expenditure
Values are means and 95% CIs.

&Log transformation was done to achieve normal distribution.

[#] $p < 0.05$ difference between groups in corresponding time point. p value (training) indicates the mean changes in pre and post measurements in the whole group. p value(training*BAT) indicates whether high BAT and low BAT groups behaved differently for the change in variable with significant difference between them. Bolded values are statistically significant. Modified from Motiani P et al. 2017 (II).

6.6 Effects of exercise training on BAT metabolism in healthy men (II)

Cold-exposure stimulated BAT GU \sim 4-fold compared to room temperature in all subjects (Figure 13). In addition, cold increased GU in skeletal muscles located close to BAT +56% (sternocleidomastoid and levator scapulae muscles), but not in muscles distant to BAT (deltoid, trapezius and pectoralis major muscles) and subcutaneous WAT (Figure 13 A). There was no significant change in cold-induced GU in BAT, WAT or skeletal muscles after 6 weeks of exercise training during cold exposure (Figure 13 A). There was high variation in the cervico-upper thoracic region BAT mass with a mean of 171 ± 31 grams (range 135-225 grams). Interestingly, BAT mass decreased by -42 % after training (Figure 13 c).

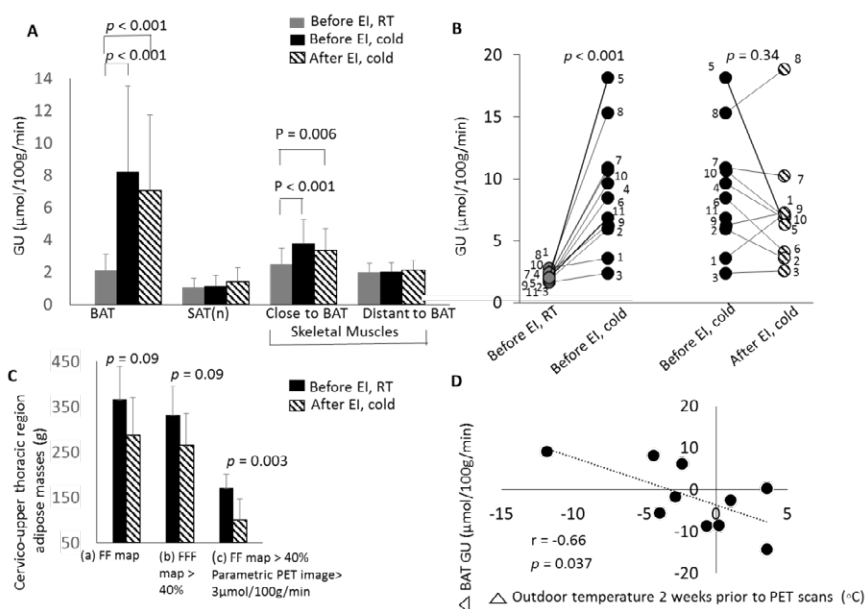


Figure 13. Glucose uptake (GU) was increased during cold exposure both before and after exercise intervention (EI) compared to the room temperature (RT) in BAT, and less but also significantly in muscles close to the BAT area. B. Individual BAT glucose uptakes during cold exposure before and after exercise intervention. C. (a) BAT mass ($n=7$) was studied starting with MRI signal fat fraction (SFF) images of potential cervico-upper thoracic BAT sites (b) Thresholding $> 40\%$ cleaned non-adipose structures off. (c) When BAT masses were estimated thereafter by thresholding only areas with concomitant parametric BAT GU $> 3 \mu\text{mol}/100\text{g}/\text{min}$, exercise seems to decrease the mass of BAT $*p = 0.03$, paired sample Student's t -test \pm SD. Modified from Motiani et al. (II).

6.7 Effects of exercise training on WAT glucose and FFAU metabolism (III)

Healthy vs Insulin resistant (IR)

At baseline, insulin-stimulated glucose uptake expressed per 100 g of tissue in VAT, abdominal SAT, and femoral SAT was significantly lower in IR subjects than in healthy subjects (-29%, -32%, and -50% respectively, all $p < 0.001$, Figure 14A). However, these

baseline differences in VAT and abdominal SAT were no longer significant when adipose tissue glucose uptake per mass was calculated as a whole depot (Figure 14B). As a whole group at baseline, GU in VAT, abdominal and femoral SAT correlated positively with whole body insulin sensitivity and aerobic capacity but negatively with, body adiposity, VAT and abdominal SAT masses, fasting glucose, fasting insulin, and triglycerides (Figure 16).

At baseline, the free fatty acid uptake per 100g of tissue was significantly lower in VAT and abdominal SAT in IR subjects than in healthy subjects (both-35% respectively, both $p < 0.001$, Figure 14A) but no difference in femoral SAT FFAU was observed ($p = 0.97$). However, when free fatty acid uptake was measured per whole depot, the baseline differences were no longer significant (Figure 14B).

Training led to a similar higher VAT GU (time $p < 0.001$, time*IR $p = 0.10$) and femoral SAT GU (time $p < 0.001$, time*IR $p = 0.27$) in both healthy and IR subjects. After 2-weeks of training, femoral SAT GU stayed lower in the IR group when compared with the healthy controls (74%, $p < 0.001$). Abdominal SAT GU did not change after training either in the whole group or when divided according to groups. The change in GU in all measured adipose tissues was positively associated with the change in whole body insulin sensitivity (VAT, $r = 0.38$, $p = 0.02$ abdominal SAT $r = 0.35$, $p = 0.35$ and femoral SAT $r = 0.37$, $p = 0.04$, data not shown).

Exercise training reduced VAT FFAU (time $p = 0.01$, time*IR $p = 0.58$) and had a tendency to similarly reduce FFAU in abdominal and femoral SAT in healthy and IR subjects (time $p = 0.06$, time*IR $p = 0.84$ and time $p = 0.07$, time*IR $p = 0.97$, respectively). Compared to the healthy subjects, the FFAU remained at a lower level in abdominal SAT of the IR group (54%, $p < 0.001$) after the training intervention. VAT and abdominal SAT FFAU exhibited no correlation with aerobic capacity, whole body-insulin sensitivity or parameters of body adiposity (Figure 16).

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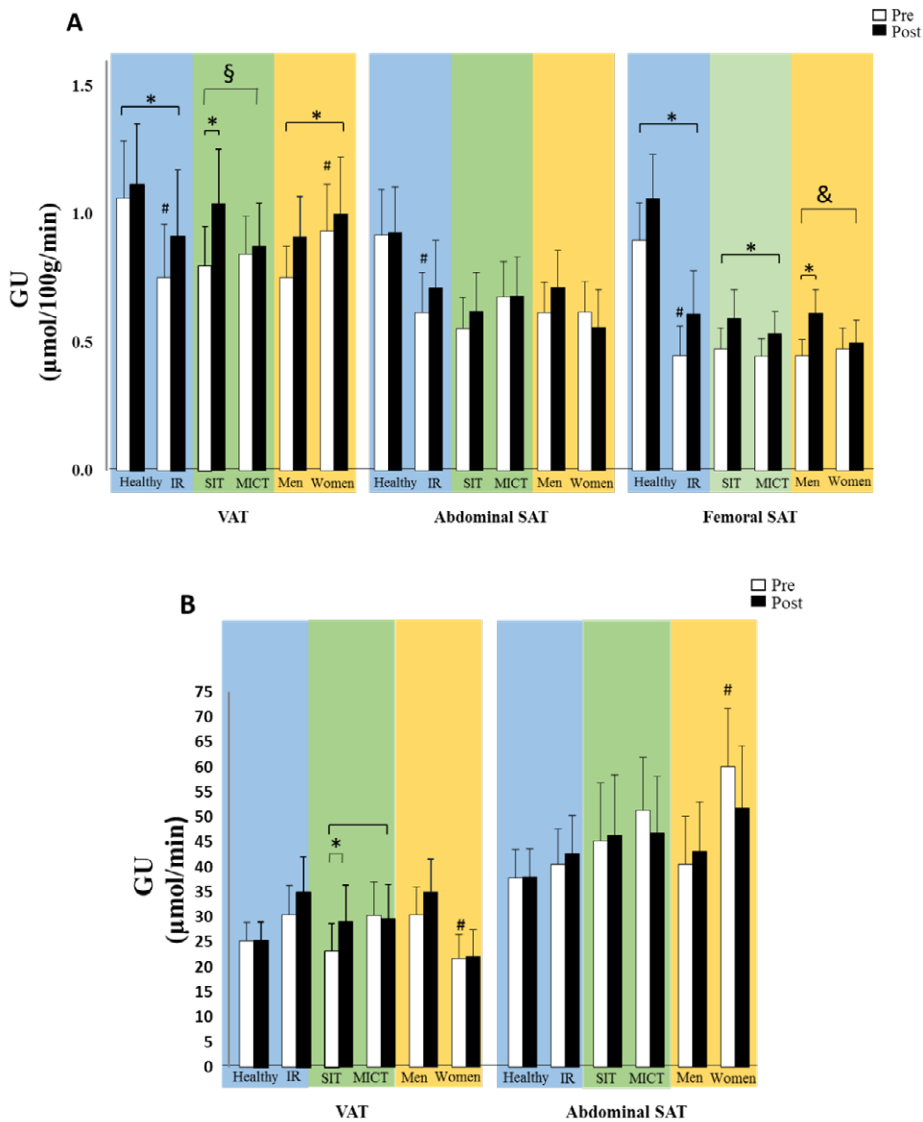


Figure 14. Insulin stimulated glucose uptake (GU) before (white bars) and after (black bars) the training intervention in visceral adipose tissue (VAT), abdominal subcutaneous tissue (SAT) and femoral SAT. A: GU values are expressed in per 100 grams of adipose tissue B: GU expressed per whole fat depot. GU is compared in three different comparisons: healthy vs insulin resistant (IR) men (blue), insulin resistant men vs insulin resistant women (green) and sprint interval training (SIT) vs moderate intensity continuous training (MICT) in IR subjects (yellow). All data is expressed as means and (95% CI). # $p < 0.001$; baseline differences between insulin resistant and healthy men and between men and women. * $p < 0.05$; the effect of exercise training over time (pre-post) as a whole group § $p < 0.05$ different response after SIT and MICT, & $p < 0.05$; different response after training between men and women.

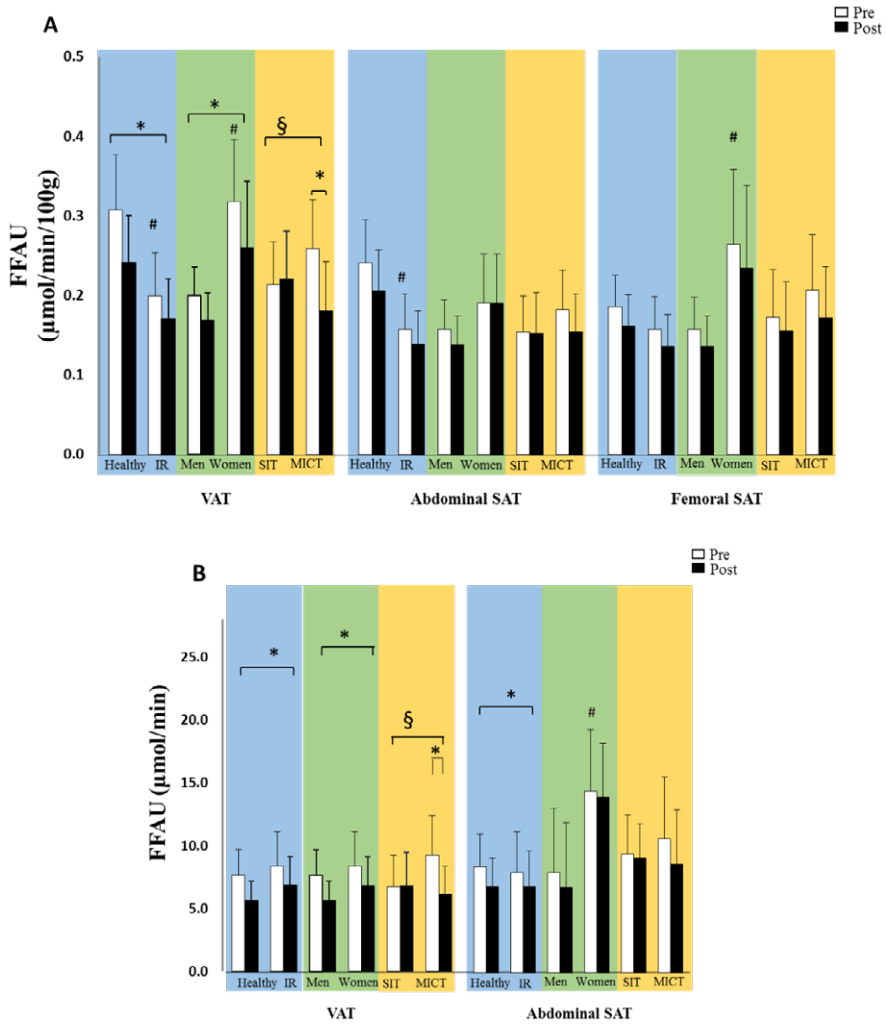


Figure 15. Free fatty acid uptake (FFAU) before (white bars) and after (black bars) the training intervention in visceral adipose tissue (VAT), abdominal subcutaneous tissue (SAT) and femoral SAT. A FFAU values are expressed in per 100 grams of adipose tissue B: FFAU expressed per whole fat depot. FFAU is compared in three different comparisons: healthy vs insulin resistant (IR) men (blue), insulin resistant men vs insulin resistant women (green) and sprint interval training (SIT) vs moderate intensity continuous training (MICT) in IR subjects (yellow) All data is expressed as means and (95% CI). # $p < 0.001$; baseline differences. * $p < 0.05$; the effect of exercise training over time (pre-post) as a whole group, § $p < 0.05$. different response after SIT and MICT, & $p < 0.05$; different response after training between men and women.

IR men vs women

At baseline, in contrast to IR males, VAT GU and FFAU (19%, $p=0.04$ and 37%, $p=0.002$, respectively) and femoral SAT FFAU (18%, $p=0.02$) were significantly higher in females with no differences between abdominal SAT GU (Figure 14A and 15A). The training response in VAT was not different between men and women as both had raised GU (21% and 8%, respectively, time $p=0.04$, time*sex $p=0.32$) and reduced FFAU (-15% and -17%, respectively, time $p=0.03$, time*sex $p=0.84$) but the response did differ as regard femoral SAT, with only men showing improved GU after exercise training (37% vs.7%, time*sex $p=0.02$, Fig.12A).

SIT vs MICT

When IR subjects were randomized into two training modes, both SIT and MICT increased femoral SAT GU (25% and 20%, respectively, time $p=0.004$, time*training $p=0.74$). However, SIT raised VAT GU (30% vs. 4%, time*training, $p=0.03$, Figure 14A), whereas MICT decreased the VAT FFAU (-30% vs. 3%, $p=0.01$, Figure 15A). Interestingly, the differential responses between the two training intensities were still present when the VAT GU and FFAU were calculated per depot (Figure. 14B and 15B). Abdominal SAT GU or FFAU did not change.

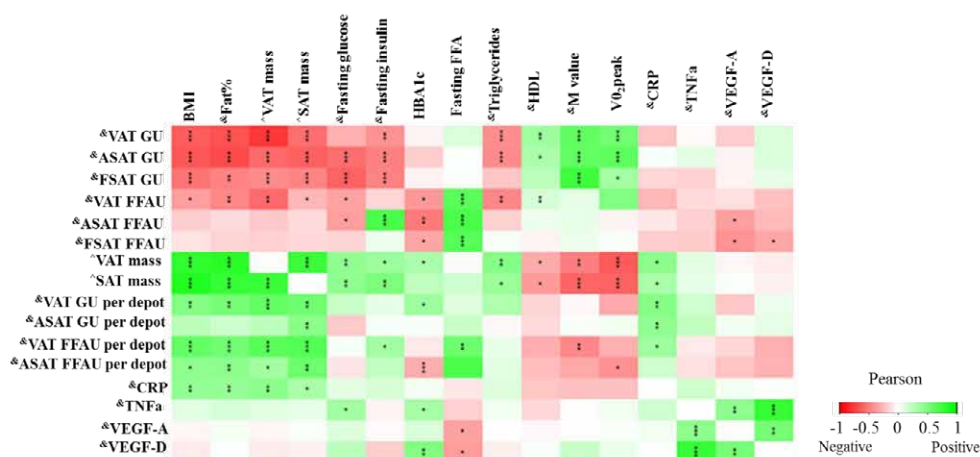


Figure 16. Pearson correlations for the measured variables at baseline in the male subjects of the study population ($n=44$). Statistically significant correlations $*p<0.05$, $**p<0.01$ are highlighted with green (positive correlations) or red (negative correlations). &Log transformation and ^Square root transformation was done to achieve normal distribution. VAT GU: visceral glucose uptake, ASAT GU: abdominal subcutaneous adipose tissue glucose uptake, FSAT GU: femoral subcutaneous glucose uptake, VAT FFAU: visceral free fatty acid uptake, ASAT FFAU: abdominal subcutaneous adipose tissue free fatty acid uptake, FSAT FFAU: femoral subcutaneous free fatty acid uptake, VAT: visceral adipose tissue and ASAT: abdominal subcutaneous adipose tissue. Modified from Motiani et al. (III).

6.8 Exercise training downregulates genes of free fatty acid metabolism (III)

Abdominal SAT revealed no differences in gene expression of CD36, ANGPTL4, CD68, GLUT4, FAB4, FASN, PPARG and PDK-4 in healthy and IR subjects either at baseline or after exercise training (data not shown). This gene selection was based on the PET results demonstrating an increase in GU and decrease in FFAU overall with similar trends in VAT, abdominal and femoral SAT. However, when gene expression was analyzed only in IR subjects there was a decrease in CD36 ($p = 0.04$) and ANGPTL4 gene expression ($p = 0.04$) without any differences between SIT and MICT (Figure 17). Regarding, plasma inflammatory markers and markers of vascularization, in healthy and insulin resistant subjects as a whole group, there was a decrease in TNF- α ($p = 0.02$), VEGFA ($p = 0.01$) and a tendency to decrease VEGFD ($p = 0.12$). However, when analyzed according to training modes or sex in IR group, there were no differences in TNF- α , CRP, VEGFA and VEGFD (Figure 18).

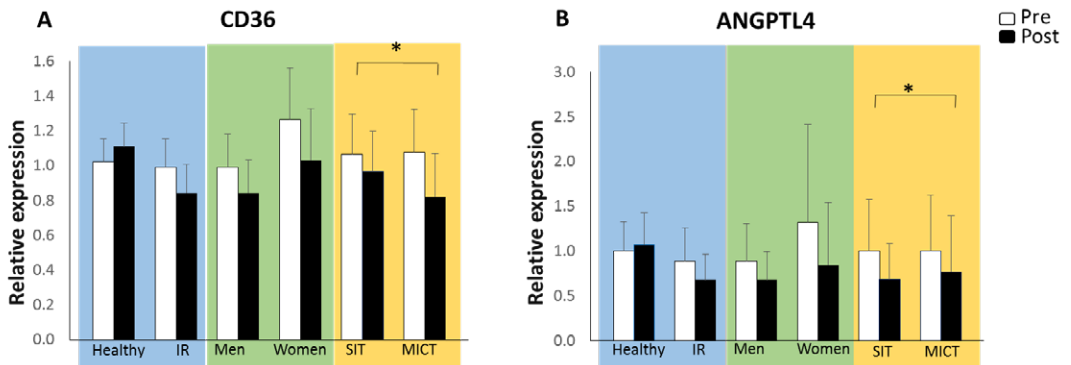


Figure 17. Gene expression from abdominal subcutaneous tissue (SAT) before (white bars) and after (black bars) the training intervention. Gene expression is compared in three different comparisons: healthy vs insulin resistant (IR) men (blue), insulin resistant men vs insulin resistant women (green), sprint interval training (SIT) vs moderate intensity continuous training (MICT) in IR subjects (yellow). All data is expressed as means and (95% CI). the effect of exercise training over time (pre-post) as a whole group, * $p < 0.05$.

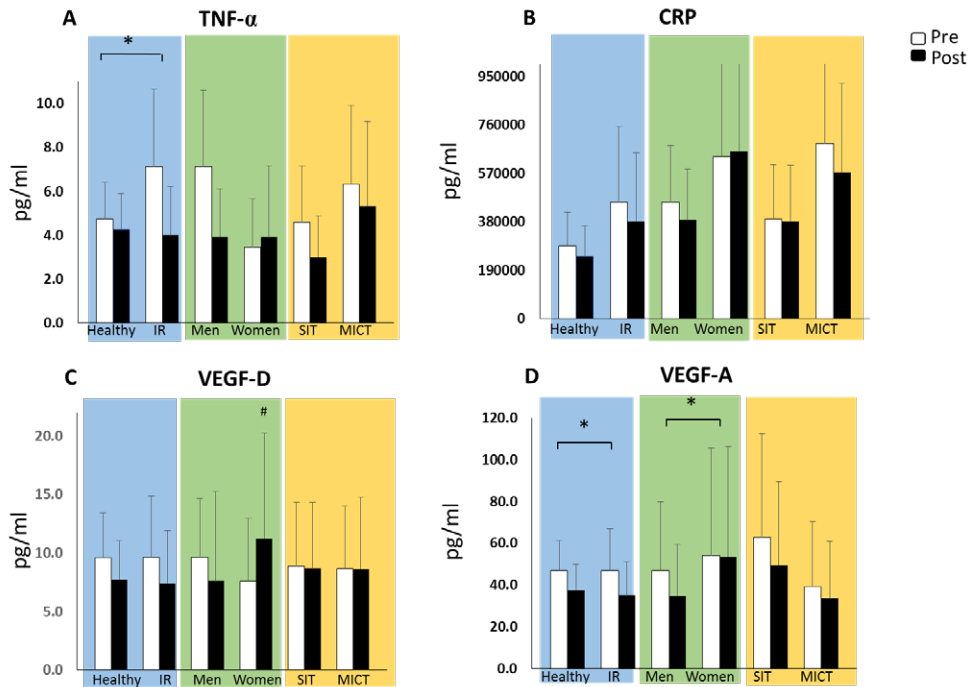


Figure 18. Plasma markers before (white bars) and after (black bars) the training intervention. Plasma markers are compared in three different comparisons: healthy vs insulin resistant (IR) men (blue), insulin resistant men vs insulin resistant women (green) and sprint interval training (SIT) vs moderate intensity continuous training (MICT) in IR subjects (yellow). All data is expressed as means and (95% CI). the effect of exercise training over time (pre-post) as a whole group, * $p < 0.05$.

7 DISCUSSION

7.1 Does exercise effect human BAT GU? (I-II)

For a decade, numerous studies have investigated the role of BAT as a part of normal physiology related to weight regulation. Exercise was suggested as one of the possible interventions for stimulating BAT activity. Subsequent to the first convincing hypothesis regarding irisin as a potential skeletal muscle derived mediator in browning of WAT after exercise training, this thesis has attempted to advance the understanding of exercise-induced BAT regulation by testing the role of exercise intensity on BAT glucose and lipid metabolism in relation to whole-body insulin sensitivity, energy metabolism and systemic inflammation in at risk individuals for chronic disease.

Previous human imaging studies related to the potential role of exercise on BAT metabolism were either cross-sectional in nature or retrospective studies, thereby limiting understandings of the influence exercise training has on BAT metabolism. It is also not clear whether exercise intensity or volume matters on human BAT adaptation. Thus in this study, we expected SIT training to be more effective than MICT training in stimulating BAT in overweight healthy adults for two reasons. Firstly, with SIT exercise there is greater SNS activity compared to MICT as reflected by elevated heart rate. For BAT activation and recruitment increase in SNS activity is crucial, as it causes the release of nor-epinephrine from sympathetic nerves which activates β_3 -adrenergic receptor and rapidly stimulates intracellular lipolysis. This in turn result in increased fuel uptake and oxidation for heat production by UCP1. Secondly, it has also been proposed that BAT recruitment is mediated by the change in intracellular oxidation-reduction (redox) state (Jeanson et al., 2015). This oxidation-reduction imbalance due to excess reactive oxygen species (ROS) is also called oxidative stress (Parker et al., 2018). SIT elicits greater systemic oxidative stress compared to MICT (Fisher-Wellman and Bloomer, 2009; Parker et al., 2014). However, BAT GU did not increase following 2 weeks of SIT or MICT in our study population. In fact, contrary to our hypothesis 2 weeks of exercise training decreased BAT insulin-stimulated GU, high active BAT at baseline (Figure 11, Study I). While difficult to directly compare our work to the literature given differences in techniques, exercise intervention, and population studied, the results presented here concur with a cross-sectional study showing decreased cold induced BAT GU in athletes compared to sedentary subjects (Vosselman et al., 2015). It is noteworthy that all the subjects in this later work appeared to have high BAT activity when 18-FDG uptake values were quantified by semi-quantitative standard uptake values (SUVs) (Vosselman et al., 2015b). In the current study, BAT GU was measured using quantitative dynamic PET data instead of SUVs. Although previous studies have used cold exposure to measure fasting BAT GU, we expanded upon this work in this study as BAT GU was studied during

insulin-stimulation. Interestingly, insulin-stimulated BAT GU has been shown to correlate with cold-stimulated BAT GU (Orava et al., 2013). BAT GU has also been shown to increase by 5-fold by insulin-stimulation compared to a fasting state at room temperature in subjects with active BAT (Orava et al., 2011). Although the training period was short, it significantly increased aerobic capacity and QF muscle GU during insulin stimulation. The increase in aerobic capacity suggests that exercise training was effective and fitness adaptation occurred. Increased aerobic capacity correlates with increased insulin-sensitivity. Similar to skeletal muscle, BAT is also an insulin-sensitive tissue. The decreased BAT GU after training in subjects who had better BAT insulin sensitivity at baseline could be a compensatory response. An interesting question that remains is the reason for the decrease in BAT GU after exercise training since BAT is most likely not a source of energy during exercise. There was no change in whole-body energy consumption and whole-body insulin sensitivity, which only tended to improve (Table 11, Study I). One explanation could be that body reduces BAT GU to supply substrate to increased skeletal muscle GU and preserve whole body homeostasis in insulin-stimulated state (Pedersen et al., 2007; Reichkender et al., 2013). However, when skeletal muscle GU was tested as a covariate this explanation was ruled out. In rodents, there is evidence of more glycogen in the BAT of exercise-trained rats compared to non-trained rats (Jessen and Goodyear, unpublished data). Thus, if this is similar in humans, the found reduction in BAT GU in the present study could be described by the increased glycogen after training impeding the insulin-stimulated BAT GU.

Strikingly, after dividing the subjects into high BAT and low BAT activity based on previously defined criteria, the two groups differed in baseline characteristics before the training intervention. When compared to subjects in the low BAT group, high BAT subjects had reduced body adiposity and visceral fat mass along with higher insulin sensitivity and HDL cholesterol concentration (Table 10, Study I), all of which have been shown to associate with BAT GU (Chondronikola et al., 2014; Cypess et al., 2009; Matsushita et al., 2014; Saito et al., 2009; Wang et al., 2015). Previously, it has been shown that BAT positive subjects have improved insulin-stimulated glucose disposal compared to BAT negative subjects (Chondronikola et al., 2014). In line with this, the subjects with high BAT activity also had greater whole-body and muscle insulin sensitivity, along with higher carbohydrate and reduced fat oxidation during insulin stimulation. These data promote the notion that high baseline activity may be beneficial for obesity and insulin resistance. There were lower levels of IL-6 and leptin and higher levels of monocyte chemoattractant protein-1 (MCP-1) in high BAT subjects. The lower levels of IL-6 and leptin could probably be explained by reduced body adiposity and higher macrophages that secrete IL-6 and MCP-1, which have pro- and anti-inflammatory effects. IR is associated positively with high levels of these cytokines and are also elevated in obesity and T2DM (Dela and Stallknecht, 2010; Wellen and Hotamisligil, 2003). Consistent with previous studies, there were lower levels of leptin in high BAT subjects compared to low

BAT subjects (Zhang et al., 2013). Indeed, it is difficult to draw concrete conclusion whether high BAT activity contributes to a positive profile of adipokines or the reverse. However, the present study suggests that BAT can be important for metabolic regulation and a potential pharmacological target for insulin resistance.

In rodents, BAT has been demonstrated to secrete IL-6, and exercise induced IL-6 activates SAT browning (Knudsen et al., 2014). Stanford et al has shown that BAT transplantation into the VAT in mice increased circulating IL-6 levels, accompanied by improved glucose homeostasis, greater energy consumption and decreased adiposity in the recipient wild type but not in IL-6 knock out mice implying that these effects were dependent on IL-6 (Stanford et al., 2013). It has been suggested that the effects reliant on IL-6 may enhance lipolysis and increase insulin sensitivity in SAT and heart via an increase in GLUT-1 protein expression (Stanford et al., 2013). Contrary to our findings, Vosselman et al. reported no correlation between IL-6 and cold-induced BAT GU (Vosselman et al., 2015b). Therefore, whether IL-6 contributes to BAT metabolism and functions in humans warrants additional studies.

Six weeks of exercise training did not affect cold-stimulated BAT GU in sedentary normal weight healthy males (Figure 13, Study II). This in line with a randomized controlled trial that showed no effect of 6 months of combined strength and endurance exercise training on BAT volume and BAT FDG uptake after a personalized cold-exposure in young adults (Sánchez-Delgado, 2018). Interestingly, in the same study there was a marginal stimulatory effect on BAT SUV peak only with moderate-intensity training and not with vigorous-intensity training (Sánchez-Delgado, 2018). The results in the present study are contrary with those of Vosselman et al. who showed in a cross-sectional study design that endurance trained athletes have lower cold-stimulated BAT GU compared to lean sedentary controls (Vosselman et al., 2015). This discrepancy could be explained by the different methods used to measure BAT GU. In the Vosselman et al. study static PET acquisition was used to provide BAT GU based on semiquantitative SUV (Vosselman et al., 2015). The disadvantage of using SUV is that the alterations of the biodistribution of FDG in the body are more likely to be misinterpreted. For example, if cooling stimulates shivering in the muscles leading to marginal increases in GU, it reduces the FDG plasma concentration and its retention in BAT, which is not considered when using SUV. In this study a controlled exercise intervention was carried out and dynamic PET imaging was used. Multiple images are acquired in dynamic imaging at various time points to form a time-activity curve of the rate of ^{18}F -FDG in BAT (Chen et al., 2016; Muzi et al., 2012; Ong et al., 2018). In comparison to static imaging in which only one single frame is chosen to represent the whole tracer metabolism, dynamic PET imaging allows better determination of tracer uptake and retention (Muzi et al., 2012). These data suggest that in humans, exercise training either decreases or has no change in BAT GU. The beneficial effects of exercise on BAT itself may be independent of BAT glucose metabolism.

Since fat oxidation increased after exercise training in the present study, glucose may not be the fuel of choice for BAT after training. However, this is mere speculation and further studies are required. A recent study demonstrated that exercise leads to the release of a BAT derived lipid species 12, 13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME) that increases the release of fatty acids into active skeletal muscles, implying the crucial role of BAT in facilitating lipid rather than glucose metabolism after exercise (Stanford et al., 2018). Unfortunately, we did not perform scans using [¹⁸F] FTHA tracer in this study so we cannot confirm whether circulating fatty acid are utilized more than glucose as a substrate by BAT after exercise training. It would have been an optimal situation if both BAT GU and BAT FFAU could be studied simultaneously in the same experimental setting. The measurement of these two substrates could possibly allow a better understanding of the substrate metabolism of BAT after exercise training.

Cold exposure increased BAT GU in BAT and skeletal muscles that were located close to BAT, but not in skeletal muscles located further away from BAT (Din et al., 2016; Orava et al., 2011; Virtanen et al., 2009). One possible explanation for this is that cold exposure might have caused shivering in those muscles that were located in close proximity to BAT. Despite the protocol being designed to study BAT during non-shivering state, it is challenging to neglect shivering due to greater mass of skeletal muscles (42%) compared to the BAT (1%) of the whole body-weight in adult humans.

Outdoor temperature influences BAT GU (Cohade et al., 2003; Saito et al., 2009). In a study by Saito et al. it was demonstrated that healthy volunteers have a higher increase in cold-induced BAT GU during winter, in contrast to summer (Saito et al., 2009). These results suggested a cold-induced BAT hyperplasia may eventually lead to increase in BAT GU (Saito et al., 2009). In the present study, an inverse correlation was seen between the absolute change in the outdoor temperature and absolute change on the cold-induced BAT GU before and after exercise intervention. These results indicated that a high outdoor temperature is related to a decrease in BAT GU particularly in subjects that were scanned in the beginning of summer (May to June). Change in outdoor temperature is a potential confounding factor, we used outdoor temperature as a co-variate in the linear mixed model. After running an ANCOVA we identified no change in the results.

This study extended the observations from previous studies showing an increase in aerobic fitness and decrease in VAT mass after training (Ghahramanloo et al., 2009; Keller et al., 2001). Exercise altered the lipid profile and fatty acids measured by nuclear magnetic resonance spectroscopy. Lipoproteins are heterogeneous combination of lipids composed mainly of cholesterol and triglycerides (Badeau et al., 2014). Disturbances in the metabolism of lipoproteins may contribute to onset of T2DM (Badeau et al., 2014). Exercise training decreased the particle size of very large density lipoprotein (VLDL). The higher the VLDL size the lower the HDL concentrations, partly because cholesterol ester from triglyceride rich lipoproteins are transferred to HDL. Moreover, when these

triglyceride rich lipoproteins are further undergoing lipolysis, they lead to the formation of LDL cholesterol. There was a reduction in medium VLDL after exercise training that could not be clarified by BAT GU but rather appeared to be a general response to training.

BAT has a potential to treat obesity by increasing energy expenditure and T2D as its activity increases glucose uptake. These studies evaluated the impact of different intensities on BAT activation. The results from both short-term and long-term studies indicate that exercise is not a potent stimulus for stimulating BAT GU regardless of fat loss or increases in fitness. Although these results differ from the exercise training studies in rodents showing positive adaptations to BAT (De Matteis et al., 2013; Xu et al., 2011). It is noteworthy that humans are not exactly similar to rodents, having larger body size which is associated with a lower ratio of body surface area to mass. This results in better insulation for humans but less dissipation of heat than mice. It is most likely for these reasons that BAT constitute such lower percentage of the whole body mass in humans (Hui and Rabinowitz, 2018).

It is known that the metabolic and cardiovascular benefits of body mass reduction depends on various factors like improved insulin secretion and action, increased adiponectin production, increased in GLP-1 production, changes in gut microbiota and reduction of subclinical inflammation (de Carvalho et al., 2009; Henry and Gumbiner, 1991; Marfella et al., 2004; Tremaroli et al., 2015). Recent studies also suggest that BAT GU is another factor that could play a role and is affected by body mass reduction (Vijgen et al., 2011). In these studies we saw reduction in whole body adiposity but did not observe any major weight changes in healthy middle-aged overweight men or young healthy men. Furthermore, we did not implement any diet modifications to restrict energy intake. The subjects in these studies maintained their weight. As exercise “heats” the body, BAT activity could decline as compensatory response overall (Study I). It is probable that with major weight loss we could have found more changes in BAT GU. In future, it would be interesting to perform an exercise intervention study in obese and T2DM subjects. These findings contribute to an initial understanding of how exercise can influence BAT metabolism in humans. A more detailed investigation is necessary to clarify the effects of other substrates and metabolites. For instance, latest study suggests that succinate can activate BAT thermogenesis (Mills et al., 2018). From a clinical standpoint, the results suggest that BAT, regardless of not being activated by exercise, is an important component of metabolic health.

7.1.2 Exercise induced changes in BAT radiodensity and BAT mass (I-II)

BAT radiodensity indicates indirectly the intracellular triglyceride content of BAT (Ouellet et al., 2012). BAT radiodensity has been shown to be inversely associated with obesity parameters (BMI, hip circumference and waist circumference) (U Din et al., 2017). A higher BAT radiodensity reflects a metabolically healthy lipid profile (U Din et al.,

2017). In T2DM, obese and overweight subjects BAT radiodensity is reduced compared to healthy controls indicating a shift towards BAT lipid storage or a lipolytic dysfunction of BAT (Blondin et al., 2015b; Dadson et al., 2018; Pasarica et al., 2009b). Previous studies have shown increases in BAT radiodensity after acute cold exposure and bariatric surgery suggesting a decrease in BAT triglyceride content (Baba et al., 2010; Dadson et al., 2018; Ouellet et al., 2012; U Din et al., 2018). Two-weeks of exercise training did not change BAT radiodensity implying no exercise-induced effect in BAT lipid content (Table 11, Study I).

Previous rodent studies have shown inconsistent results regarding BAT mass. A 6-weeks of swimming intervention in rats (1h/day, 5 days/wk at 35-36°C) showed a 2-fold increase in BAT mass (Oh-ishi et al., 1996). In another study, 8-weeks of endurance training decreased BAT mass in rats, suggesting a decreased need for BAT heat production as the working skeletal muscles produced excess heat during exercise (Wu et al., 2014). It has been previously shown that in obese subjects weight loss tended to increase BAT GU but did not change BAT mass (Orava et al., 2013). It is noteworthy that in this training a 5-month weight loss program was used with a combination of a very low calorie diet and physical activity, obese subjects lost about 12.5% of body weight and had decreased whole body energy expenditure after weight loss (Orava et al., 2013). Therefore, it is a probability that the tendency towards increased BAT GU helps with the regulation of whole-body thermogenesis given the decline in whole-body energy expenditure. It is important that in morbidly obese subjects cooling may not be optimal to induce BAT thermogenesis or morbidly obese subjects may lack active BAT altogether (Vijgen et al., 2011). Interestingly, in the same study no association was found between 12.5% weight loss and cold induced BAT GU even though some of the obese subjects had high BAT GU values (Orava et al., 2013). In contrast to a study in morbidly obese subjects, body mass reduction of around 28% was more effective in inducing BAT activation in non-diabetic than in diabetic subjects (Rodvalho et al., 2017). There was no change in BAT mass after 2 weeks of exercise training (Table 11, Study I). However, 6 weeks of exercise induced a significant decrease in BAT mass suggesting that BAT activation decreases after exercise intervention; most likely in an adaptive effort to conserve energy (Figure 13, Study II). However, this is mere speculation and it is difficult to draw conclusions regarding the underlying mechanisms of decreased BAT mass in humans after exercise training.

7.2 WAT specific responses to exercise training in different groups (III)

Exercise training improves insulin sensitivity and reduces ectopic fat stored in tissues like the muscles and heart (Eskelinen et al., 2015; Honkala et al., 2017) Studies investigating the effects of exercise on adipose tissue have shown that blood flow and lipolysis are increased in adipose tissue during exercise (Frayn, 1999; Thompson et al., 2012; Tsiloulis and Watt, 2015). This study is novel in showing different training responses within three different comparisons in WAT glucose metabolism during insulin stimulation and lipid

metabolism during fasting combined with plasma biomarkers and abdominal SAT biopsies.

Healthy vs IR. Our first aim was to compare the effects of 2 weeks of exercise between healthy and IR men. Before training, as expected IR subjects had higher VAT and SAT masses compared to healthy men. At baseline, IR men had decreased insulin-stimulated GU per 100g in VAT, abdominal, and femoral SAT compared to healthy subjects as shown previously (Dadson et al., 2015; Virtanen et al., 2002). An inverse correlation between adipose tissue mass and adipose tissue GU was found in all measured fat depots implying a decreased insulin sensitivity in expanding fat depots. Previously, similar associations have also been found in obese and T2DM subjects (Oliveira et al., 2015). Obese subjects have been shown to have 60% lower GU per mass compared to lean subjects both in VAT and abdominal SAT (Virtanen et al., 2002). In the present study, compared to healthy men, IR men had lower GU per 100g in VAT (-29%), abdominal SAT (-32%) and femoral SAT (-50%). Interestingly, both in the present study and in the previous study by Virtanen et al., when GU was calculated per tissue depot, there was no difference between the IR/obese and healthy group. This was seen in both the VAT and abdominal SAT depot because of the higher mass of fat depots in the IR group. This indicates that fat mass likely contributes to the differences in GU.

We have discrepant results regarding adipose tissue FFAU compared to the previous study (Bucci et al., 2015). In the present study fasting FFAU per 100g was decreased in IR subjects compared to healthy subjects both in VAT and abdominal SAT. In contrast, Bucci et al. showed 100% higher VAT FFAU per 100g and no difference in abdominal SAT FFAU in obese subjects with metabolic syndrome compared to non-obese subjects (Bucci et al., 2015). In the current study, this impairment in adipose tissue lipid uptake in the presence of IR and T2D may be partly attributed to decreased facilitated FFAU via CD36 in adipocytes (Stinkens et al., 2015). However, previous studies have reported higher CD36 protein expression in abdominal SAT and VAT of obese, overweight and T2D compared with lean subjects (Bonen et al., 2006). There was no difference in abdominal SAT CD36 between healthy and IR subjects, It also might be that these subjects did not have long standing history of T2DM and this IR group comprised a combination of prediabetes and T2DM men therefore the higher VAT FFAU might develop in later stages of T2DM and this impairment is still early to manifest.

Exercise training induced a similar reduction in VAT and abdominal SAT mass after the training intervention in both groups. In both healthy and IR men, exercise training led to a significant increase in VAT and femoral SAT GU, a decrease in VAT FFAU, and a tendency towards decreased FFAU in both studied SAT depots. However, exercise training did not change abdominal SAT GU. This is in contrast with the study by Reichkendler and colleagues where they found that 11-weeks of endurance training (>70%

VO_{2max}) did not change the insulin-stimulated GU rate in VAT or femoral SAT but reduced abdominal SAT GU rate when estimated by SUV_{mean} in healthy sedentary moderately over-weight men. These differences could be explained by different training method as in the study by Reichkender et al. exercise intervention consisted of mixed endurance training (rowing, running, bicycling, elliptical training) and in our study only bicycling (Reichkender et al., 2013). Exercise training stimulates adrenergic receptors which induces lipolysis.

We also found a significant increase in femoral SAT GU. As such femoral SAT is not involved in muscle contraction, but is located close to exercising skeletal muscles. Therefore, the rapid improvement in muscle GU could play a role in the improvement in femoral SAT. However, we tested this phenomenon by using femoral muscle GU as a covariant, and the increase in femoral SAT then became no longer significant ($p=0.82$). Thus, it could be possible that myokines are released into local adipose tissue depots by the working skeletal muscles which may regulate local adipose tissue GU (Pedersen, 2011). Abdominal SAT and VAT GU in the IR group was not found to be different from the healthy subjects after the intervention. However, femoral SAT GU in the IR group remained lower post training intervention

Previously, the majority of studies on adipose tissue metabolism have aimed at investigating diet-induced weight loss. Viljanen et al. measured the effect of diet-induced weight loss using PET imaging (Viljanen et al., 2009). They did not find any improvement in fasting VAT and abdominal SAT GU even though significant weight loss was seen after a 6-week diet (Viljanen et al., 2009). Similarly another study by Bucci et al. did not show any change in VAT and abdominal FFAU despite significant weight loss after 6-week of diet (Bucci et al., 2015). Dadson et al. found no change in fractional FFAU in abdominal SAT and VAT after bariatric surgery despite reduction in VAT and SAT mass. Thus, it seems that exercise training affects adipose tissue lipid metabolism differently from diet or surgery induced weight loss (Dadson et al., 2017). Additionally, gene expression (CD36, ANGPTL4, CD68, GLUT4, FAB4, FASN, PPARY and PDK-4) from abdominal SAT samples showed no change. It has been previously shown that GLUT4 expression is downregulated in abdominal SAT in subjects with impaired insulin sensitivity and T2D are associated with adipocyte hypertrophy (Kahn, 1992b; Rondinone et al., 1997). However, in the present study we did not observe baseline difference in abdominal SAT GLUT4 between healthy subjects and IR subjects. It is well known that regular exercise training improves adipose tissue function by increased lipolysis, FFA mobilization and decreased adiposity. In animals, several studies have shown increases in the expression of several metabolic proteins including GLUT4 both in SAT and VAT (Gollisch et al., 2009; Stanford and Goodyear, 2016). However, the data on the human studies on mechanistic responses in adipose tissue after exercise training is sparse and more conflicting. Previous studies have showed that abdominal SAT GLUT4 protein expression increases in T2DM

subjects after 4-weeks of bicycle training (Hussey et al., 2011; Stallknecht et al., 2000). However, a recent study by Florez-Opazo et al. showed no change in SAT GLUT4 expression after 10-days exercise intervention in healthy males (Flores-Opazo et al., 2018). This finding is in line with our present study as we did not observe significant increase in GLUT4 expression nor the GU in abdominal SAT after 2-weeks bicycle training in healthy and IR subjects. Thus, these results suggest that longer training periods are needed to achieve beneficial changes in GLUT4 expression. Unfortunately, GLUT4 was only measured from abdominal SAT, in which glucose uptake did not improve. Thus, improvements in VAT and femoral SAT GLUT4 expression may have occurred as their glucose uptake increased after exercise training.

When adipose tissue expands in size it becomes hypoxic and has less vascularization and more inflammation which leads to IR (Sun et al., 2012). VEGF-A is an endothelial growth cell factor that is accountable for majority of pro-angiogenic activity in adipose tissue (Hausman and Richardson, 2004; Zhang et al., 1997). There was a decrease in plasma VEGFA which coincides with reduced adipose tissue masses and lower angiogenesis. As such, the decrease of VEGFA after exercise training could be due to decreased SAT mass requiring less need for angiogenesis. Interestingly, exercise training decreased plasma TNF- α indicating a decrease in inflammation. However, we found no effects of exercise training on abdominal SAT gene expression of CD68, which is a marker of AT macrophage infiltration. This suggests that 2 weeks is too short intervention to show reduction in inflammation in abdominal SAT itself but overall inflammation in the body is reduced consistent with previous studies indicating attenuation of systemic inflammation after exercise training (Görgens et al., 2015).

IR men vs women. Our second aim was to compare IR men with IR women in response to 2 weeks of exercise intervention. Women favor adipose tissue storage, while in men mobilization of fat is more efficient. Gender differences are important to an understanding of metabolism in order to enhance prevention, diagnosis and design therapeutic intervention for both sexes (Link and Reue, 2017).

In humans, men have more VAT deposition compared to women. However, women store more SAT and have a higher percentage of body fat than men. In the present study men had 45% higher VAT mass compared to women and these results are consistent with previous studies (Kuk and Ross, 2009). Women had 42% higher body fat, and a 51% higher amount of abdominal SAT compared to men. Despite higher levels of body fat, women often have greater insulin sensitivity than men (Macotela et al., 2009; Mittendorfer, 2005; Nuutila et al., 1995). At baseline women had both higher GU and FFAU in VAT and higher femoral SAT FFAU compared to men. Similar to our baseline findings in adipose tissue, it has been shown that women have increased FFAU in skeletal muscle accompanied by higher intramyocellular lipid content and higher capacity for β -oxidation of long chain fatty acids (Maher et al., 2010). The explanation for these

differences is not exactly known, but could be sex steroids. Sex steroid hormones, in particular estrogen, are known to play a role in the regulation of adipose tissue development and function, as well as whole body insulin sensitivity (Newell-Fugate, 2017). However, both VAT GU increased and FFAU decreased after exercise training in both groups irrespective of sex. The difference in training response was seen in femoral SAT GU, which only improved in men. This could be explained by a higher muscle mass in men requiring more glucose as a substrate after training. These findings are against our hypothesis where we expected a greater response to exercise training in women but instead we observe an increase in femoral SAT GU of men indicating that there might be differential responses to training in different sexes.

IR SIT vs MICT. Our third aim was to compare the effects of high-volume MICT and short-term SIT. Because the IR group consisted of men and women, the effects of SIT and MICT were compared in the IR subjects only (n=26). Both SIT and MICT led to similar improvements in body adiposity, whole body insulin sensitivity and femoral SAT GU but SIT was superior to induce greater aerobic capacity. Previous reports have also suggested similar or higher improvements in aerobic capacity and insulin sensitivity with SIT (Burgomaster et al., 2005; Weston et al., 2014). To date, the mechanisms of the greater improvements seen in SIT or aerobic capacity and glucose homeostasis are poorly understood. It has been suggested that the rapid depletion of glycogen stores of the working skeletal muscle during SIT could be one of the mechanisms underlying the greater effectiveness in improving insulin sensitivity by SIT (Metcalfé et al., 2012; Romijn et al., 1993).

In the present study, only SIT increased VAT GU and MICT decreased FFAU. SIT has been proposed to increase mitochondrial content thereby increasing fat oxidation which would lead to an enhancement in insulin sensitivity (Astorino and Schubert, 2018). Increases in VAT GU by SIT in the present study after exercise training could be due to higher β -adrenergic receptor density observed in VAT. It has also been proposed that exercise training with higher intensities activates β -adrenergic receptors and causes higher secretion of lipolytic hormones that are associated with fat oxidation and post-exercise energy expenditure (Horowitz, 2003). It could also be that loss of VAT mass after exercise training caused lower inflammation, hence improving VAT GU. Moreover, VAT has more β -adrenergic receptors than SAT which could explain the higher exercise-induced improvement in VAT GU (Mauriege et al., 1987). One more reason for SIT being effective in increasing VAT GU could be that the energy during SIT is supplied by glycogen stores. Interestingly, the findings remained the same when VAT GU was calculated per depot, thus highlighting the clinical significance of exercise. The improvement in GU was also observed in femoral SAT with no differences between SIT and MICT. These findings emphasize the effectiveness especially SIT, on VAT glucose metabolism in IR subjects.

Only MICT reduced VAT FFAU. It has been shown that an increase in adipose tissue fatty acid mobilization occurs following low-intensity exercise. It has been suggested that alternative fat stores, such as intramuscular fat, are used as intensity increases (Thompson et al., 2012). The energy utilized during the MICT training is produced mainly from lipids via lipolysis (Thompson et al., 2012). Since [^{18}F]FTHA is a trapping palmitate analogue, tissue specific FAU is product of arterial plasma FFA concentration and K_i , which represents the amount of accumulated tracer in the tissue compared to the amount of tracer available in the plasma. Therefore, the decrease in FFAU could be also explained by differences in plasma FFA concentration. However, there were no differences in plasma FFA concentration after training and yet reduction in K_i was seen only after MICT ($p=0.003$).

Interestingly, another report from the same data set showed that working skeletal muscle (QF) FFAU increased without any differences between SIT and MICT (Sjöros et al., 2018). This suggests that adipose tissue has a different response which is also intensity specific to skeletal muscle after exercise training (Sjöros et al., 2018). Although there were no changes in abdominal SAT FFAU, we found marked decreases in CD36 expression and ANGPTL4 in abdominal SAT after two weeks of exercise with no differences between SIT or MICT. Both CD36 and ANGPTL4 are regulators of fatty acid metabolism. The down-regulation of these genes could therefore provide an insight into mechanisms behind decreased FFAU in adipose tissue after exercise training.

7.3 Strengths, limitations and future aspects

This thesis work contributes towards an understanding of BAT and WAT regulation after exercise training. In this thesis, dynamic PET imaging was used, which allowed a quantitative measurement of the tissues of interest. Furthermore, the imaging data was combined with WAT biopsies, energy expenditure measurements and systemic blood values to elucidate the physiological events.

However, the thesis also has its limitations. The number of study subjects in Studies I and II was relatively small with the inclusion of only males. Ideally in Study I, the supraclavicular neck scans should have been performed in all healthy subjects but the idea to investigate BAT metabolism was initiated later on in the study resulting in small number of scans from the neck region. In future studies, females should also be included. In Study II cold exposure was used to measure BAT GU using personalized cooling protocol. This particular personalized protocol was chosen to maximize non-shivering thermogenesis in all subjects but it was a subjective approach to the method used for perceiving shivering. It is possible that these results are biased because of individual variations to the cold stimulus used in this study for each subject. The skin temperature was recorded from one area (lateral abdominal skin surface) which could limit the thermogenic precision. A room temperature scan after training intervention would be ideal but was not performed due to

the hazards of radiation. When planning for future studies detection of muscle shivering by using electromyography and more comprehensive measurements of core and skin temperature could be used to confirm proper stimulation of BAT. In the future, liquid perfused suits could be considered as an option in the cooling experiments (Blondin et al., 2017a). Further, the verification of increased metabolic activity in imaging by means of BAT biopsies was not possible. Age was also a limitation in these studies in future it would be of great interest if an older population could be studied. The strengths of Study III is the demonstration of the training effects on adipose tissue metabolism combined with WAT adipose tissue biopsies in healthy and IR subjects. However, a major limitation was small number of subjects. The PET measurements were limited by the unsuccessful measurements due to technical difficulties and problems in tracer production. There was a high drop-out rate because of the extensive training. The study subjects in the IR group were combination of both pre-diabetic and T2DM subjects. Excluding subjects with pre-diabetes (IFG, IGT) from the analysis or performing analysis grouping according to diabetes status (pre-diabetes/T2DM) did not alter the results. Medication was used as a covariate in the statistical analysis, but it did not confound our results. The IR group included both men and women. As gender has a major impact on body adiposity and may also cause huge variation in adipose tissue metabolism, the females (n=10) were excluded from the IR group. As the healthy group consisted of only males the analysis comparing healthy and IR subjects was done only for the males. Ideally, these groups should have been gender matched. The initial idea of the study was to recruit only men in both groups, but due to challenges encountered in recruiting male T2DM subjects, women were included in order to complete the study in a reasonable time frame.

Adipose tissue is a complex organ with much variation in regards to the type of fat, the depot in which it is stored in, the size of the adipocytes, and the capability to transdifferentiate into other types of fat cells in response to different stimuli. Further research to explore the physiology of adipose tissue will provide more knowledge about the pathophysiology of diabetes. It will also offer exciting opportunities to investigate possible pharmaceutical targets for metabolic diseases. The findings from this thesis highlight the underused potential of exercise in rapidly restoring the impaired VAT glucose metabolism in IR subjects. In the future it would be interesting to explore whether exercise could prevent or delay adipose tissue dysfunction in IR and T2DM subjects.

8. CONCLUSIONS

- Short-term SIT or MICT did not have any effect on insulin-stimulated BAT GU or fasting BAT FFAU in healthy sedentary middle-aged men. However, when subjects were stratified based on active BAT GU $\geq 3\mu\text{mol}/100\text{g}/\text{min}$, subjects with high BAT GU displayed a metabolically more favorable phenotype compared to subjects with low BAT GU. In summary, BAT seems to have a different response to short-term exercise training than skeletal muscle, i.e. it decreases insulin-stimulated BAT GU. Therefore, BAT glucose metabolism in human subjects may be downregulated after exercise training.
- Six weeks of training intervention (combination of SIT, MICT and resistance training) did not affect cold-induced BAT GU in sedentary healthy males. The changes in lipid particles and metabolites may regulate some of the favorable effects of exercise on whole-body metabolism and may offer therapeutic potential for obesity and metabolic diseases independent of BAT activation.
- At the baseline, IR subjects had reduced GU in VAT, ASAT and FSAT and increased VAT and abdominal SAT masses. Adipose tissue GU in all measured fat depots correlated positively with aerobic capacity suggesting that improving aerobic fitness might enhance adipose tissue glucose metabolism.
- Short-term exercise training improves adipose tissue metabolism (both insulin-stimulated GU and fasting FFAU) similarly in healthy and IR men. There was a differential response to training in IR men to women. In fact, men showed greater increases in femoral SAT insulin-stimulated GU than women. However, in IR subjects SIT seems to be more effective in improving VAT insulin resistance and MICT decreased VAT lipid uptake. This suggests that exercise intensity plays a role in substrate selection and thus may ameliorate IR in tissue-specific manner.

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