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University of Turku

ADIPOSE TISSUE AND BRAIN METABOLIC ADAPTATIONS TO SPRINT INTERVAL  
TRAINING AND MODERATE-INTENSITY CONTINUOUS TRAINING;  
Studies in healthy and insulin resistant subjects

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*What you get by achieving your goals is not as important as what you become by achieving your goals.*

## **ABSTRACT**

Sanna Maria Honkala

### **Adipose tissue and brain metabolic adaptations to sprint interval training and moderate-intensity continuous training; studies in healthy and insulin resistant subjects**

University of Turku, Faculty of Medicine, Department of Clinical Physiology and Nuclear Medicine, Doctoral Programme in Clinical Research, Turku PET Centre

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**Background:** Insulin resistance (IR) is associated with metabolic disturbances in several tissues, including increased insulin-stimulated glucose uptake in brain, decreased glucose uptake in adipose tissue and accumulation of fat in and around the myocardium. Whether these disturbances can be normalized by exercise is still uninvestigated. Thus, the aims of this study were to determine the myocardial adiposity in and around myocardium, to elucidate exercise training-induced metabolic adaptations in brain and adipose tissue and to compare the training responses between, sprint interval training (SIT) vs. moderate-intensity continuous training (MICT) in subjects with IR.

**Methods:** Middle-aged, sedentary healthy subjects (n=28) and subjects with IR (n=26) were randomized into SIT and MICT interventions for two weeks. Brain and adipose tissue metabolism in terms of glucose uptake and free fatty acid uptake and myocardial adiposity were determined non-invasively using positron emission tomography (PET), computed tomography (CT), magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS).

**Results:** Two-week of exercise training, both SIT and MICT, decreased epi- and pericardial fat volumes and adipose tissue glucose and fatty acid metabolism similarly in healthy subjects and subjects with IR despite the baseline differences. In short-term, only SIT showed improvements in aerobic capacity, visceral adipose tissue glucose uptake and a decrease in insulin-stimulated brain glucose uptake in IR subjects.

**Conclusions:** Exercise training has beneficial effects on adipose tissue volume and metabolism despite the baseline glucose tolerance. This study also showed for the first time, that exercise training can decrease insulin-stimulated brain glucose uptake. These findings further support the therapeutic potential of exercise training in subjects with IR.

**Keywords:** exercise training, high-intensity interval training, sprint interval training, insulin resistance, type 2 diabetes mellitus, metabolism, positron emission tomography

# TIIVISTELMÄ

Sanna Maria Honkala

**Rasvakudoksen ja aivojen aineenvaihdunnan vasteet kovatehoiseen intervalliharjoitteluun ja kohtalaiskuormitteiseen tasasykkeiseen harjoitteluun; tutkimuksia terveillä ja insuliiniresistenteillä henkilöillä.**

Turun yliopisto, Lääketieteellinen tiedekunta, Kliininen fysiologia ja isotooppilääketiede, Turun kliininen tohtoriohjelma, Valtakunnallinen PET -keskus

Turun yliopiston julkaisuja, Painosalama Oy, Turku, Suomi, 2018

**Tausta:** Insuliiniresistanssin on havaittu olevan yhteydessä aineenvaihdunnan häiriöihin useissa kudoksissa. Näitä häiriöitä ovat muun muassa aivojen lisääntynyt glukoosinotto, rasvakudoksen alentunut insuliiniherkkyys sekä rasvan kertyminen sydänlihaksen sisään ja ympärille. Vielä on monilta osin tuntematta, voidaanko näitä muutoksi normalisoida liikuntaharjoittelun avulla. Täten, tämän tutkimuksen tarkoituksena oli selvittää kahden intensiteetiltään eroavan liikuntaharjoittelumenetelmän vaikutuksia aivojen ja eri rasvakudosten rasva- ja sokeriaineenvaihdunnassa terveillä ja insuliiniresistenteillä henkilöillä.

**Menetelmät:** Tutkimukseen osallistuneet keski-ikäiset, inaktiiviset terveet miehet (n=28) sekä tyypin 2 diabetesta sairastavat naiset ja miehet (n=26) satunnaistettiin kohtalaiskuormitteiseen (MICT) tai korkeatehoiseen intervalliharjoitteluryhmään (SIT) kahden viikon ajaksi. Aivojen ja rasvakudoksen sokeri- ja rasvahappoaineenvaihduntaa sekä sydänlihaksen rasvoittumista tutkittiin positroniemissiotomografialla (PET), tietokonetomografialla (TT), magneettiresonanssikuvantamisella (MRI) sekä magneettiresonanssispektroskopiolla (MRS).

**Tulokset:** Kahden viikon liikuntaharjoittelu, sekä SIT että MICT, alensi sydäntä ympäröivien epi- ja perikardiaali rasvamassojen määrää sekä paransi rasvakudoksen glukoosi- ja rasvahappoaineenvaihduntaa ilman eroja terveiden ja insuliiniresistenttien välillä huolimatta erosta lähtötasossa. Ainoastaan SIT harjoittelu paransi kestävyyskuntoa, viskeraalirasvan insuliiniherkkyttä sekä alensi aivojen insuliini-stimuloitua glukoosinottoa insuliiniresistenteillä henkilöillä.

**Johtopäätökset:** Liikuntaharjoittelulla on suotuisia vaikutuksia rasvakudosten määrään sekä aineenvaihduntaan insuliiniherkkyden lähtötasosta riippumatta. Tutkimuksessa osoitettiin myös ensimmäistä kertaa, että liikuntaharjoittelu alentaa aivojen sokeriaineenvaihduntaa. Nämä löydökset vahvistavat liikuntaharjoittelun terapeuttista potentiaalia henkilöillä, joilla on insuliiniresistenssi.

**Avainsanat:** liikuntaharjoittelu, korkeatehoisen intervalliharjoittelu, sprintti intervalliharjoittelu, insuliiniresistanssi, tyypin 2 diabetes, aineenvaihdunta, positroni emissio tomografia kuvantaminen



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## **ABBREVIATIONS**

BMI	Body mass index
EE	Energy expenditure
FDG	2-deoxy-2-[ <sup>18</sup> F]fluoro-D-glucose
FFA	Free fatty acid
FAU	Free fatty acid uptake
FTHA	14( <i>R,S</i> )-[ <sup>18</sup> F]fluoro-6-thia-heptadecanoic acid
GLUT	Glucose transporter
GU	Glucose uptake
HIIT	High-intensity interval training
IRS-1	Insulin receptor substrate 1
MICT	Moderat intensity continuous training
MTC	Myocardial triglyceride content
M-value	Whole-body insulin-stimulated glucose uptake
OGTT	Oral glucose tolerance test
ROI	Region of interest
SAT	Subcutaneous adipose tissue
SIT	Sprint interval training
T2DM	Type 2 diabetes mellitus
TG	Triglyceride
IR	Insulin resistance
VAT	Visceral adipose tissue
VO <sub>2max</sub>	Maximal oxygen uptake
VO <sub>2peak</sub>	Peak oxygen uptake
VOI	Volume of Interest

## **LIST OF ORIGINAL PUBLICATIONS**

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

- I. Honkala Sanna M.\*, Motiani Priyanka\*, Motiani Kumail K., Eskelinen Jari-Joonas, Virtanen Kirsi A., Löyttyniemi Eliisa, Nuutila Pirjo, Kalliokoski Kari K., Hannukainen Jarna C. Exercise training improves depot specific adipose tissue metabolism regardless of baseline glucose tolerance and sex. *Submitted*.
- II. Honkala Sanna M., Motiani Kumail K, Eskelinen Jari-Joonas, Savolainen Anna, Saunavaara Virva, Virtanen Kirsi A., Löyttyniemi Eliisa, Kapanen Jukka, Knuuti Juhani, Kalliokoski Kari K., Hannukainen Jarna C. 2017. Exercise training reduces intrathoracic fat in healthy subjects and subjects with defective glucose tolerance. *Medicine & Science in Sport & Exercise* 49(7), 1313-1322.
- III. Honkala Sanna M., Johansson Jarkko, Motiani Kumail K., Eskelinen Jari-Joonas, Virtanen Kirsi A., Löyttyniemi Eliisa, Knuuti Juhani, Nuutila Pirjo, Kalliokoski Kari K, Hannukainen Jarna C. 2017. Short-term interval training alters brain glucose metabolism in subjects with insulin resistance. *Journal of Cerebral Blood Flow and Metabolism*. Doi: 10.1177/0271678X17734998. [Epub ahead of print]

\* These authors shared equal contribution.

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# 1 INTRODUCTION

The prevalence of obesity, insulin resistance (IR), and type 2 diabetes mellitus (T2DM) has increased dramatically over the past 50 years. In modern, developed countries, the population-based prevalence of insulin resistance is approaching 20% and the incidence of T2DM in adults is 5-10%, making it the most common endocrine disorder in westernized populations. Globally, approximately 8.3% of the population suffer from diagnosed or undiagnosed T2DM (Reunanen, 2006; Koski, 2015; International Diabetes Federation, 2017), and 42% of Finnish men and 33% of Finnish women have some type of defect in glucose regulation (Peltonen *et al.*, 2006). Globally, it is predicted that the number of people with T2DM will increase by 50% in next 20 years, leading to huge effects on public health (Danaei *et al.*, 2011; Whiting *et al.*, 2011). While there are several factors influencing the development of insulin resistance, it has been shown that the most powerful factors are the behaviors that are related to urbanization and modern lifestyles (Malik, Willett and Hu, 2013).

T2DM is a chronic condition, which occurs when there is a raised level of glucose in the blood. Insulin is an important hormone, which is produced by pancreatic cells, and facilitates the transport of glucose to tissue cells where the glucose is utilized as energy. If the target tissue is unable to respond to insulin, the glucose concentration in the blood increases leading to hyperglycemia (International Diabetes Federation, 2017). Prolonged hyperglycemia can have an effect on several tissues and lead to serious complications, such as cardiovascular diseases, neuropathy, nephropathy and eye diseases, such as retinopathy and blindness. By the time individuals are diagnosed with T2DM, around 50% already show signs of complications. Thus, T2DM is the fifth most common cause of death in the world. (National Task Force on the Prevention and Treatment of Obesity, 2000)

T2DM results from the interaction between a genetic background (non-modifiable risk factors) and behavioral and environmental risk factors (modifiable risk factors) (Hu *et al.*, 2001; Stanford and Goodyear, 2014). Obesity and weight gain are the most prominent modifiable risk factors for the development of pre-diabetes and T2DM, together with its complications, with 80% of patients with T2DM being classified as obese (Venables and Jeukendrup, 2009). Although the exact mechanism through which obesity increases the risk of T2DM are not fully understood, obesity has been shown to have a strong correlation with IR and T2DM (Reaven, 1988; International Diabetes Federation, 2017). When compared to normal weight subjects (BMI of 18.5-24.9 kg·m<sup>-3</sup>), overweight subjects (25.0-29.9 kg·m<sup>-3</sup>) have 3-5 times the risk, class I obese subjects (30.0-34.9 kg·m<sup>-3</sup>) 5-10 times the risk and

class II and III ( $\geq 35.0$ - $39.9$  and  $\geq 40.0$   $\text{kg}\cdot\text{m}^{-3}$ ) more than 10 times the risk of developing T2DM (Ford, Williamson and Liu, 1997). Other lifestyle related factors, acting either through obesity or independently of obesity, may play role in the diabetes risk. Physical inactivity elevates the risk of T2DM in individuals with normal weight and the degree of aerobic fitness is directly linked to the level of IR. Physical inactivity is regarded as an individual risk factor for T2DM as such and many observational studies have clearly shown that a dose-response relationship between increased physical activity and decreased risk of T2DM is independent of body weight and weight gain (Janssen *et al.*, 2002; Ross *et al.*, 2004).

In obesity, excess triglycerides (TGs) accumulate not only in the peripheral fat depots, but also into the abdominal cavity (visceral adipose tissue, VAT) and in and around the internal organs such as the liver, muscle, and heart. These fat depots are commonly called ectopic fats and in normal conditions their role is thought to serve as fuel during exercise and act as a lipid storage after dietary fat intake (shown in skeletal muscle and myocardium) (Coen and Goodpaster, 2012; Bucher *et al.*, 2014). An increased volume of ectopic fats lead to local lipotoxicity and the metabolites that are secreted from VAT and ectopic fat may be involved in mediating insulin resistance (Coen and Goodpaster, 2012). Thus, it has been suggested that regional fat distribution is independent and as important a risk factor for metabolic and cardiovascular diseases as the whole body fat quantity as such (Shimabukuro *et al.*, 2013). Although subcutaneous adipose tissue (SAT), VAT and ectopic fat depots are closely associated and play a role in the development of metabolic implications, accumulation of VAT and ectopic adipose tissue in the liver, heart, pancreas and other tissues have been showed to increase the risk for IR and T2DM more than abdominal and peripheral SAT. (Gaggini, Saponaro and Gastaldelli, 2015) The increased adiposity of the heart has also been associated with an increased risk for cardiomyopathy and heart failure. (Kankaanpaa *et al.*, 2006; Iacobellis and Willens, 2009; Talman *et al.*, 2014; Gaggini, Saponaro and Gastaldelli, 2015)

In addition to fat distribution, insulin resistance has been linked with several other metabolic disturbances, such as altered brain glucose metabolism. Previous studies have shown that obese and insulin resistant subjects have higher insulin-stimulated glucose uptake in the brain than healthy controls (Hirvonen *et al.*, 2011). Another study has shown that weight loss is associated with decreased insulin-stimulated brain glucose uptake in morbidly obese subjects (Tuulari *et al.*, 2013). In addition, brain free fatty acid (FFA) uptake is shown to reduce substantially after rapid weight loss in subjects with metabolic syndrome (Karmi *et al.*, 2010). However, the effect of exercise training on brain glucose and FFA uptake has not yet been investigated.

The general public and the science community have shown great interest in high-intensity interval training (HIIT) during the last decade, as it could serve as a time-saving health-enhancing exercise method for urbanized people with a busy lifestyle. Low-volume HIIT is characterized by short exercise bouts with high intensity, which are repeated after short recovery periods. HIIT protocols, that are performed with extremely high intensities ( $>100\%$  of  $VO_{2max}$ ), are specified in the term ‘sprint interval training’, SIT, as they differ from protocols with lower intensities (80-100% of  $VO_{2max}$ ). A growing body of evidence suggests that different forms of HIIT induce numerous physiological health-enhancing adaptations that are similar to traditional high-volume moderate-intensity endurance training despite the lower total exercise volume and training time commitment (Martin J Gibala and McGee, 2008; Rehn *et al.*, 2013; Weston *et al.*, 2014). However, it is unclear whether exercise training, either SIT or more traditional moderate-intensity continuous training (MICT,  $>60\%$  of  $VO_{2max}$ ), is an effective method to improve glucose and FFA uptake in the brain and adipose tissue and reduce myocardial ectopic fat accumulation along with improvements in general metabolic health. Thus, the aims of this study were to elucidate the effects of exercise training and the training mode, SIT vs. MICT, on myocardial ectopic fat in healthy men and men with IR and further, on the glucose and FFA metabolism in the brain and adipose tissue of subjects with IR.

## 2 REVIEW OF LITERATURE

### 2.1 Prevention and management of insulin resistance and type 2 diabetes mellitus (T2DM) through exercise training

#### 2.1.1 *Insulin resistance and T2DM*

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder that usually develops during adulthood. Patients with T2DM form a heterogeneous group and pathogenesis T2DM is extremely complex. T2DM can be described as a cluster of interrelated metabolic disturbances, including: (1) insulin resistance in muscle, liver, and adipocytes; (2) impaired insulin secretion in the pancreas; and (3) abnormalities in glucose and FFA uptake of internal organs (Goldstein, 2002; DeFronzo, 2004). The mechanisms by which T2DM leads to complications are not fully understood, but they involve the direct effects of lipotoxicity along with inflammation, oxidative stress and endothelial dysfunction (DeFronzo, 1988; van den Oever *et al.*, 2010). T2DM is typically developed through prediabetes, a stage that is assessed as impaired fasting plasma glucose concentration (IFG) or impaired glucose tolerance (IGT). According to the WHO, IFG is defined as a fasting plasma glucose value of 6.1 to 6.9 mmol·L<sup>-1</sup>. IGT can be identified by an oral glucose tolerance test (OGTT) and is defined as a plasma glucose value of 7.8 to 11.0 mmol·L<sup>-1</sup> after two hours of OGTT. IGT is an intermediate state between normal glucose tolerance and T2DM. T2DM is defined as a fasting blood glucose value >7.0 mmol·L<sup>-1</sup> and/or 2-h OGTT blood glucose value >11.1 mmol·L<sup>-1</sup>. (World Health Organization, 2006; American Diabetes Association, 2017a). Individuals with prediabetes have a 10-times higher risk of developing T2DM compared to healthy individuals and therefore form an important target group for interventions aimed at preventing diabetes (*Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. The DECODE study group. European Diabetes Epidemiology Group. Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe.*, 1999).

Both prediabetes and T2DM are characterized by insulin resistance. Insulin resistance is defined as a reduced ability to respond to insulin in the insulin-sensitive tissues, such as skeletal muscle, the liver or adipose tissue. Insulin resistance affects glucose disposal from the blood and insulin suppression of hepatic glucose output leading to increased blood glucose levels. Insulin resistance in skeletal muscle and other target tissues, such as liver and adipose tissue, lead to increased levels of glucose in the circulating blood, a stage that is called as hyperglycemia. The

pathophysiology of hyperglycemia in established T2DM relates to both hepatic and muscle insulin resistance. (Taylor, 2012) Skeletal muscle insulin resistance is widely considered to be one of the primary defects in the development of T2DM (DeFronzo and Tripathy, 2009; Kahn, Cooper and Prato, 2014). Skeletal muscle (and liver) insulin resistance may be caused by the defects in fatty acid oxidation and mitochondrial function seen in IR subjects. Increased FFA levels lead to accumulation of active lipid intermediates, such as long chain fatty acid CoAs, ceramides and diacylglycerols inside the cell. These intermediates may lead to impaired insulin-signaling. (Kraegen and Cooney, 2008) Obesity and T2DM lead to liver fat accumulation, which is closely associated with hepatic insulin resistance. In normal conditions, the increased secretion of insulin suppresses endogenous glucose production (EGP) (for example after meal). During the state of T2DM, the suppressing effect of insulin in EGP declines leading to increased plasma glucose levels. (Samuel and Shulman, 2012) The development of T2DM is heterogeneous, and previous studies have reported different results of the primary defects in T2DM. For example, in a previous calorie restriction study with T2DM subjects reported a decrease in liver fat, normalization of hepatic insulin sensitivity and fasting plasma glucose, but no change in muscle insulin resistance after weight loss. (Petersen *et al.*, 2005) Insulin resistance is strongly linked to obesity and physical inactivity and it is also associated with other disturbances, such as dyslipidemia and hypertension. (DeFronzo, 1988, 2004; Goldstein, 2002). Insulin resistance related dyslipidemias include increased triglyceride levels, decreased high-density lipoprotein (HDL) cholesterol, and changes in the composition of low-density lipoprotein (LDL) cholesterol. (Howard, 1999)

Glucose is the most important fast energy source for the human body, and its uptake by tissues is assured by multiple mechanisms. Glucose uptake is regulated by insulin in all insulin-sensitive tissues, such as skeletal muscle, the heart and adipose tissue (Nolan, Damm and Prentki, 2011). The major site for insulin-stimulated glucose uptake in humans is skeletal muscle, but it is also utilized in several other tissues (DeFronzo and Tripathy, 2009). Glucose is important especially for the brain, as the neurons mainly use glucose as an energy source. Moreover, neurons are not able to store glucose, which makes the constant availability of glucose in the brain essential for the whole organism (Mergenthaler *et al.*, 2013). Other organs, such as the liver, skeletal muscle, and heart, are more adaptive to the changes in glucose levels and glucose metabolism in these tissues is adjusted according to prevalent insulin and glucagon levels, physical activity, and availability of energy sources (glucose, FFAs, amino acids and lactate). Insulin-signaling is a complex process and disturbance in any step of the signaling may block the signaling to the cell. The initial step in insulin mediated glucose uptake is the activation of the glucose transport system, which leads to an influx of glucose into the target cells. In skeletal muscle and adipose tissue, insulin promotes glucose uptake

into the cells by activating a complex cascade of phosphorylation-dephosphorylation reactions. For example, in skeletal muscle the binding of insulin to the insulin receptor activates IRS-1/PI-3 kinase/Akt pathway, which leads to the translocation of GLUT4 to the sarcolemma, followed by entry of glucose into the cell. The integrity of this pathway is essential for normal insulin-mediated glucose uptake in target tissues. (Taniguchi, Emanuelli and Kahn, 2006; Huang and Czech, 2007). The balance between insulin and glucose is mediated by the crosstalk between  $\beta$  cells and insulin-sensitive tissues. The stimulation of  $\beta$ -cells releases insulin and thereby mediates glucose, amino acid and FFA uptake in insulin-sensitive tissues. As a result, insulin-sensitive organs give feedback to  $\beta$ -cells of their insulin need (Kahn, Cooper and Prato, 2014). This process is mediated by multiple mechanisms, which are still partly unknown, but it has been suggested that the co-work between the brain and humoral system could have a central role in regulation of glucose metabolism (Nolan, Damm and Prentki, 2011; Kahn, Cooper and Prato, 2014). Insulin-signaling may be disturbed for example by plasma FFAs, cytokines, and inflammatory markers, all of which are related to the accumulation of VAT and ectopic fat.

In addition to glucose, FFA is an important energy source in most human tissues and it is a primary energy source for the liver, resting skeletal muscles, and myocardium. When demand for energy rises, for example, during starvation and exercise, FFAs are released from fat depots, such as subcutaneous adipose tissue (SAT), to serve as an energy for the skeletal muscles, liver, and myocardium in order to spare glucose for the brain. Thus, especially SAT works as a buffer for energy: releasing and storing it according to the dominant physiological state of the body. Defects in buffering capacity lead to increased plasma FFA concentrations and accumulation of TGs into tissues. Both fatty acid uptake and fatty acid oxidation are dysregulated in obesity and T2DM, resulting in the accumulation of lipids as triglycerides in non-adipose tissues such as those of the skeletal muscles, liver, pancreas and heart (Blaak, 2003; van Herpen and Schrauwen-Hinderling, 2008; Coen and Goodpaster, 2012). Previous studies have shown, that skeletal muscle fatty acid uptake (FAU) is decreased in subjects with IR (Turpeinen *et al.*, 1999) and T2DM (Blaak, 2003) compared to healthy subjects; however, in the adipose tissue and liver FAU is shown to be increased in obese subjects compared to lean (Bucci *et al.*, 2015; Immonen *et al.*, 2018). The decreased fatty acid oxidation along with the increased FFA concentrations in the blood lead to the accumulation of the TGs in and around internal organs. These fat depots are called ectopic fat. Untypical ectopic fat accumulation in non-adipose tissues is associated with cardiometabolic complications (Shimabukuro *et al.*, 2013; Lim and Meigs, 2014). Ectopic fat accumulation is discussed in detail in Chapter 2.2.2. Elevated plasma FFA levels may be predictive of the transition of patients from prediabetes to T2DM (Charles *et al.*, 1997).

A number of proteins can stimulate fatty acid transport. Important fatty acid transporters are fatty acid translocase (FAT/CD36), plasma membrane fatty acid binding protein (FABPpm) and a family of integral plasma membrane fatty acid transport proteins (FATP) (Ibrahimi *et al.*, 1999). Some studies have suggested that FAT/CD36 could play a central role in insulin resistant states such as obesity and T2DM. Increased TG content in insulin-sensitive organs (for example muscle, heart, and liver) can interrupt insulin action by interfering with post-receptor insulin signaling. Lipids release FFAs that can directly block insulin-signaling pathways and thus promote insulin resistance (Luiken *et al.*, 2002; Chabowski *et al.*, 2006).

High FFA levels may also interrupt endogenous glucose production in liver (Boden and Shulman, 2002). The effect is emphasized in VAT which shows high lipolytic activity and has a direct access to the portal circulation. In fact, an increased flux in FFA via the portal vein from the VAT to the liver is suggested to be one of the mechanisms causing the accumulation of ectopic fat in the liver (Björntorp, 1992; Zierath *et al.*, 1998). Because increased levels of glucose, FFAs and cytokines in circulating blood all simultaneously exist in insulin resistance, it is difficult to separate the contribution of each metabolic defect in the pathogenesis of T2DM (DeFronzo and Tripathy, 2009).

During a state of hyperglycemia,  $\beta$ -cells attempt to overcome the underlying defect of insulin resistance by increasing insulin secretion, leading to a state that is called hyperinsulinemia. In the early stages of IR, the increased insulin secretion compensates for the IR and prevents hyperglycemia. However, the compensatory mechanisms fail during a chronic increase of IR and eventually lead to the development of T2DM. Thus, when T2DM is diagnosed, IR is accompanied with  $\beta$ -cell dysfunction. During the development of T2DM,  $\beta$ -cell function is progressively lost. However, recent studies have suggested that  $\beta$ -cell dysfunction is already present in the early stages of T2DM progression (Kahn, Cooper and Prato, 2014). At the time of T2DM diagnosis, as much as 50 % of  $\beta$ -cell function may be lost (DeFronzo, Eldor and Abdul-Ghani, 2013).  $\beta$ -cells are damaged due to the chronic hyperglycemia and increased amount of FFAs (DeFronzo, 2004; Kahn, Cooper and Prato, 2014). The metabolism of glucose in  $\beta$ -cells produces a cell toxic reactive oxygen species (ROS) that are normally neutralized by catalase and superoxide. However, the amount of ROS increases with increasing blood glucose levels and the neutralization system is not able to compensate for the increased amount of ROS during hyperglycemia. At this point, ROS is able to damage components of  $\beta$ -cells and they may also induce apoptosis. TGs also accumulate inside the pancreas. Ectopic fat accumulation in the pancreas has shown to decrease insulin secretion in  $\beta$ -cells and hence promote the development of  $\beta$ -cell failure (Kelpe, Johnson and Poirout, 2002).

### 2.1.2 Exercise recommendations for insulin resistant and T2DM subjects

Exercise is a well-established tool to prevent and manage metabolic and cardiovascular diseases. Indeed, daily exercise training decreases cardiovascular, metabolic and all-cause mortality by 40–70%, regardless of age, sex and presence of disease (Wen *et al.*, 2011). For patients with IR or T2DM, exercise improves blood glucose control, contributes weight loss, improves general well-being, and reduces the risk of comorbidities including hyperlipidemia, hypertension, and ischemic heart disease. Exercise training has both acute and persistent effects on insulin sensitivity and glucose disposal and therefore exercise training is recommended on a regular basis to prevent and manage T2DM. The effects of exercise can be either direct enhancements in target-tissue metabolism and function or secondary effects after exercise induced weight management and/or weight loss. The effects of exercise training are depended on prevalent physical fitness, exercise intensity, exercise frequency and duration of each exercise session. In addition, all individuals do not respond similarly to a given training stimulus. Therefore, optimal training benefits occur only when exercise programs focus on individual needs and capacities. (Buford, Roberts and Church, 2013)

Traditional exercise guidelines have focused on increasing low- to moderate- intensity physical activity in sedentary individuals. The current guidelines set by American College of Sports Medicine (ACSM) for health-enhancing physical activity recommend at least 150 minutes of moderate-intensity continuous training (50-70% HR<sub>max</sub>) or 75 minutes on vigorous-intensity training (70-85% HR<sub>max</sub>) per week. This includes activities such as walking, jogging, and cycling, or anything that causes a continuous increase in heart rate. In addition, resistance training and stretching is recommended 2-3 times per week. In resistance training, ACSM recommends performing eight to 10 repetitions per set and two to three sets total, at a sufficiently challenging weight (defined as 75% to 80% of the one repetition maximum) (Garber *et al.*, 2011). The same recommendations are applied in the Finnish version of health-enhancing physical activity recommendations (UKK-instituutti, 2017). Recently, it has been suggested that high-intensity interval training should be added as an option in exercise guidelines (O'Hagan, De Vito and Boreham, 2013; Buresh and Berg, 2017). The exercise recommendation for insulin resistant and T2DM subjects are similar to the general exercise recommendations (American Diabetes Association, 2017b).

There is no consensus about the optimal amount and intensity of exercise required for improvement in whole body and tissue-specific metabolism. It has also been claimed that some individuals are non-responders according to current dose recommendations (Bouchard and Rankinen, 2001). Montero and Lundby (2017) demonstrated recently with 78 healthy individuals that by doing longer and more

intensive training sessions, the number of non-responders can be reduced to zero (Montero and Lundby, 2017). Many studies have shown that exercise intensity plays a central role when determining the physiological responses to exercise training, showing greater benefits with higher intensity (Wisloff *et al.*, 2007; Garber *et al.*, 2011; Rehn *et al.*, 2013). It is likely that moderate intensity exercise is emphasized in exercise recommendations as activities, such as walking and cycling, are easy and safe to execute. However, especially low- to moderate-intensity training may not be able to provide an appropriate stimulus to increase cardiorespiratory fitness. This is specially highlighted in people with T2DM, who have a poor fitness level to start with and the improvements in health outcomes are rarely achieved only by moderate-intensity walking (Johnson *et al.*, 2008). Thus, individual supervised exercise training involving higher intensity exercise may be the most effective method to improve cardiorespiratory fitness and reduce hyperglycemia in T2DM.

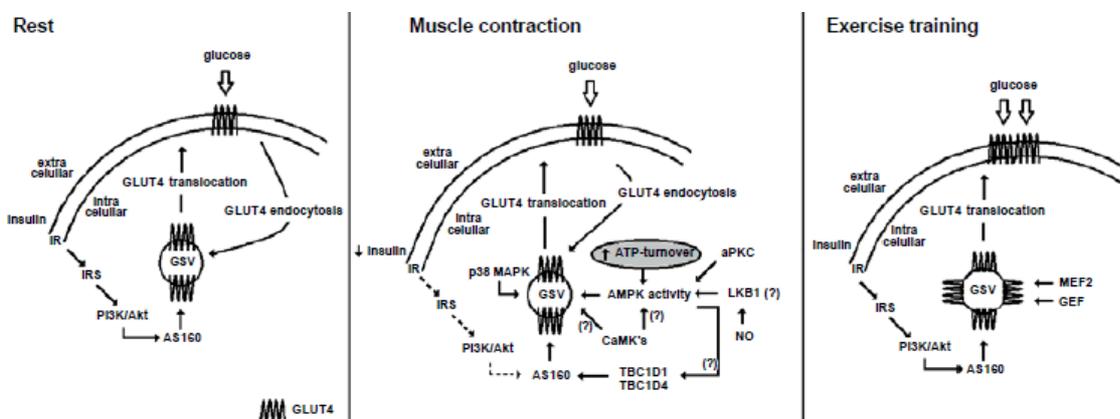
Despite the clear evidence showing the beneficial effect of exercise training in the prevention and treatment of IR and T2DM, subjects are still not engaging in sufficient physical activity. For example, approximately only 10% of Finnish adults meet the physical activity recommendations (Husu *et al.*, 2014). Most of the inactive people report lack of time as the main barrier. Therefore, there is an urgent need for new time-saving low-volume exercise methods, which could motivate busy people to exercise more. High-intensity interval training has shown a huge potential as a time-saving training mode with similar health-enhancing effects to traditional aerobic training with lower intensities. However, there are still unanswered questions regarding high-intensity training.

Physical activity is a general term that includes all movement that increases energy expenditure (EE). Exercise training is a more specific form of physical activity that is structured and designed to improve physical fitness. Both physical activity and exercise are important to prevent and manage IR and T2DM. Exercise training includes both resistance and endurance training, which both have a beneficial effect on health. However, this thesis will focus on endurance training of different exercise intensities.

### ***2.1.3 The mechanisms to improve insulin sensitivity by endurance exercise training***

There are many possible mechanisms by which increased physical activity leads to improved metabolic health in subjects with IR and T2DM. Remarkably, single bout of aerobic exercise can already significantly increase whole-body glucose transposal and muscle insulin sensitivity acutely (Pruett and Oseid, 1970; Richter

*et al.*, 1989; Goodyear and Kahn, 1998; Richter and Hargreaves, 2013; Stanford and Goodyear, 2014). Skeletal muscle is a highly flexible organ and it adapts to changes in use. The adaptation depends on the nature and quantity of the exercise bouts. Thus, one of the most important mechanisms through which exercise improves metabolic health of IR and T2DM subjects is the adaptation in skeletal muscle insulin sensitivity. Exercise can stimulate skeletal muscle glucose uptake also independently of insulin. Hence, exercise has two distinct mechanisms to promote glucose uptake. In fact, studies have shown that exercise-stimulated glucose uptake is normal in individuals with T2DM (Martin, Katz and Wahren, 1995). Both muscle contraction and insulin increase skeletal muscle glucose uptake by translocation of GLUT4; however, previous animal studies have demonstrated that the translocation of GLUT4 occurs by a distinct signaling mechanism (Figure 1). As described earlier (in paragraph 2.1.1) in insulin-stimulated glucose uptake to skeletal muscles the insulin binds to the insulin receptor, leading to activation of the IRS-1/PI-3 kinase/Akt pathway, which leads on to the translocation of GLUT4 (Folli *et al.*, 1992; Goodyear *et al.*, 1995). Nevertheless, exercise (without the insulin effect) does not have an effect on the insulin receptor nor activate IRS-1/PI-3 kinase/Akt pathway (Goodyear *et al.*, 1995). An acute exercise bout activates several signaling pathways, but the pathways necessary for GLUT4 translocation are not well understood. During muscle contraction there are several changes, such as an increase in intracellular  $Ca^{2+}$  concentration, changes in energy status (increased AMP/ATP) and increased ROS (reactive oxygen species) and activation of the 5'-adenosine monophosphate-activated protein kinase (AMPK). These changes stimulate several signaling pathways, which eventually lead to the translocation of GLUT4 (Fig 1)(Goodyear and Kahn, 1998). The acute effects of exercise on glucose control are seen for up to 72h. Thus, the acute effects of exercise are rather short-lived if they are not followed by another exercise session within 2-3 days (Thompson *et al.*, 2001).



**Fig 1. Metabolic pathways of insulin-stimulated glucose uptake at rest and pathways leading to changes in GLUT4 expression in the skeletal muscle following acute exercise and exercise training.** Both muscle contraction and insulin increase skeletal muscle glucose uptake by translocation of GLUT4

but the activation occurs by means of a distinct signaling mechanism. During insulin-stimulated glucose uptake insulin binds to the insulin receptor, leading to activation of the IRS-1/PI-3 kinase/Akt pathway, which leads on to the translocation of GLUT4. During exercise-stimulated glucose uptake several signaling pathways are activated, but the pathways necessary for GLUT4 translocation are not well understood. Regular exercise training leads to improvements in insulin-stimulated glucose uptake. Reprinted with permission from (Lehnen, 2013).

There is substantial evidence both in animals and humans, that regular exercise training increases the expression of GLUT4 in skeletal muscle and thus improves insulin-stimulated glucose uptake (Goodyear and Kahn, 1998; Richter and Hargreaves, 2013; Stanford and Goodyear, 2014). Several cross-sectional studies comparing athletes and sedentary subjects have shown that athletes have higher GLUT4 expression in skeletal muscle (Andersen *et al.*, 1993). Intervention studies with healthy and insulin resistant physically inactive subjects have shown increase in GLUT4 expression after exercise training. One potential pathway leading to increased insulin sensitivity could be activation of AMPK after exercise training. AMPK is a metabolic master switch regulating several intracellular systems. AMPK is activated by phosphorylation by kinases such as liver kinase B1 (LKB1) and is regulated by cellular energy demand. Insulin resistant subjects show reduced exercise-induced activation of AMPK, but can reach full potential in training with higher intensities (Sriwijitkamol *et al.*, 2007). Both acute and chronic exercise interventions have shown that exercise training with moderate- and high-intensities induces AMPK activation and thereby leads to changes in gene expression favoring GLUT4 translocation (Figure 1) (Gibala *et al.*, 2009; Röhling *et al.*, 2016). Chronic exercise can also lead to higher rates of tyrosine phosphorylation of key molecules (Akt) in the insulin signaling cascade in the muscles of healthy as well as insulin-resistant individuals (Frøsig *et al.*, 2007).

Regular exercise training increases muscle mass and thus leads to higher energy consumption in muscle. Previous studies have shown that relative muscle mass is inversely associated with insulin resistance, which means that the higher muscle mass is associated with better insulin sensitivity and lower risk for T2DM. (Srikanthan and Karlamangla, 2011) The increase in skeletal muscle mass is highlighted after resistance training, which is recommended at least once a week by the national exercise guidelines.

As mentioned earlier, obesity is the leading cause of several diseases, such as IR and T2DM. Aerobic exercise training increases energy expenditure and thereby can lead to weight loss. However, exercise-induced weight loss is rather slow, and therefore regular exercise combined with healthy nutrition is the most efficient method for reducing the risk for T2DM. A Finnish study by Tuomilehto and colleagues showed that lifestyle intervention can reduce the risk of T2DM. The study

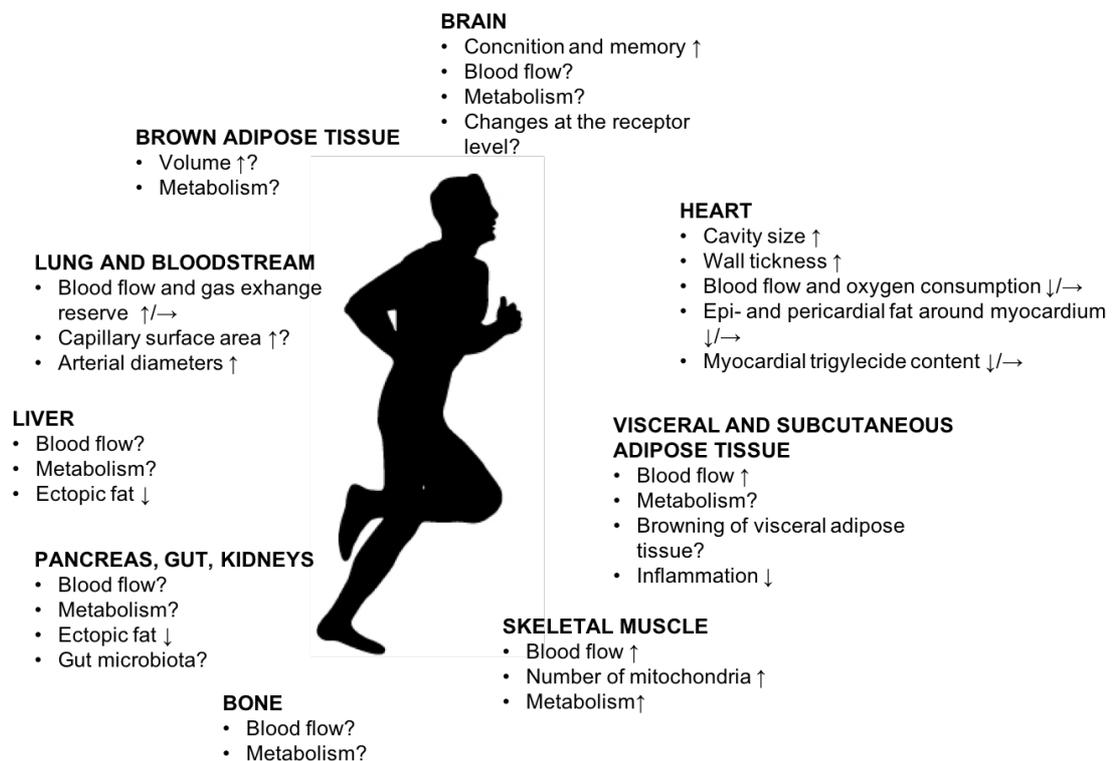
consisted of 522 overweight subjects with pathological glucose tolerance. The study subjects were instructed to increase physical activity and reduce caloric and fat intake and subjects were monitored for 3.2 years. Impressively, the risk for developing T2DM was reduced by 58% (Tuomilehto *et al.*, 2001). An exactly similar finding was seen in a study by Knowler and colleagues after a 2.8-year diet and exercise intervention study with 3234 subjects with pathological glucose tolerance (Knowler *et al.*, 2002). The Cochrane Review from 2017, comprising 5238 overweight or obese individuals studied 12 randomized controlled trials and concluded that if physical activity is combined with a restricted diet and dietary counseling, the effects on weight are more significant than physical activity without a restricted diet (Hemmingsen *et al.*, 2017).

Obesity and IR are also associated with changes in immunological and hormonal cross talk involving interleukin 6 (IL-6), tumor necrosis factor alpha (TNF- $\alpha$ ), or adiponectin. These cytokines and adipokines are part of inflammatory processes and can also affect molecular signaling pathways modulating glucose uptake. Exercise training has been shown to reduce inflammatory markers, which is accompanied by improvements in glucose uptake. Low-grade inflammation is emphasized in VAT and ectopic fat depots and indeed, it has been shown that exercise can reduce the amount of VAT and ectopic fat individually from diet and weight loss (Passos and Gonçalves, 2014). It is of note that these fat depots are extremely important risk factors for T2DM and reduction in ectopic and VAT may be even more important than general weight loss. These fat depots are discussed in detail in Chapter 2.2.1.

Low cardiorespiratory fitness is a well-known risk factor for chronic diseases such as cardiovascular disease, T2DM, and obesity. Aerobic training and high-intensity exercise especially have been shown to improve aerobic fitness in a dose-response related manner. Aerobic fitness can be measured as a maximal oxygen consumption ( $VO_{2max}$ ), which correlates with cardiorespiratory fitness. Aerobic fitness is improved by exercise-induced enhancements in cardiac function, blood flow, myoglobin levels, mitochondrial function, oxidative enzyme levels and muscle fiber structure. Previous studies have suggested that the improvement in  $VO_{2max}$  seems to be more important factor than the BMI in reducing all-cause mortality (Rehn *et al.*, 2013; Weston *et al.*, 2014). Exercise training has been shown to increase the amount and size of mitochondria in skeletal muscle, which leads to improvement in oxidative capacity. Increase in oxidative capacity and oxidative enzymes induces increased fatty acid oxidation in mitochondria both in healthy and T2DM subjects (Hey-Mogensen *et al.*, 2010). This process is mainly regulated by PGC- $1\alpha$ , which has been shown to increase especially after high-intensity training

(Gibala *et al.*, 2012). Mitochondrial impairment has been linked to IR, and consequently, improvement of mitochondrial function may restore skeletal muscle insulin signaling (Bruce *et al.*, 2006).

Most of the previous exercise studies on IR/T2DM subjects have concentrated on the skeletal muscle and whole-body IR. However, IR and T2DM may affect the metabolism, blood flow, and function of several other tissues too, such as the gut, adipose tissue, liver, pancreas, heart and bone. These metabolic changes might be possible to reverse by exercise training (Fig 2). However, the exercise-induced physiological adaptations and mechanisms in these organs remain largely unknown. (Heinonen *et al.*, 2014).



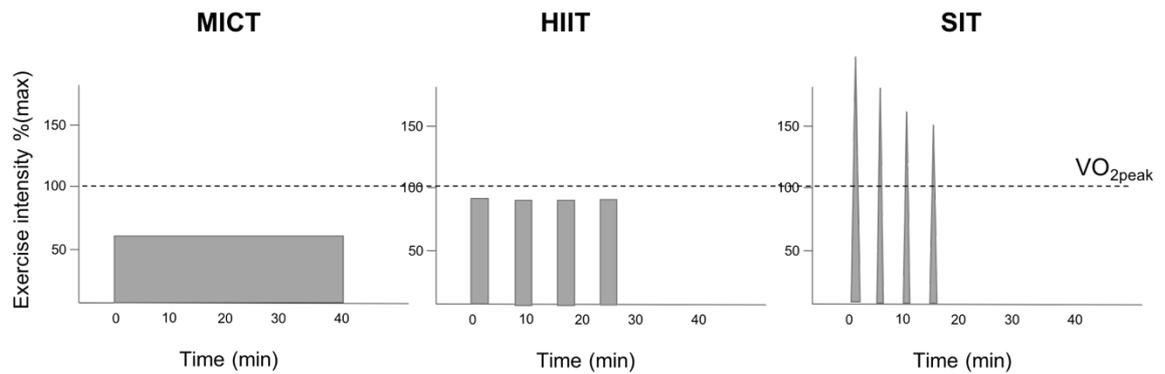
**Figure 2. The tissue-specific effects of regular long-term endurance exercise training.** The main physiological and structural adaptations include increased blood flow and oxygen consumption especially in the heart and skeletal muscle. However, metabolic physiological adaptations and driving-force mechanisms especially in bone, inner organs and brain remain largely unknown and there still remains many questions unsolved. ↑ increases; → no change in response or may increase or decrease; ↓ decreases. Modified with a permission from (Heinonen *et al.*, 2014).

#### 2.1.4 Interval training and management of insulin resistance and T2DM

High-intensity interval training (HIIT) was introduced by Woldemar Gerscher 70 years ago and was followed by world record performances. Ever since, HIIT has

been a popular exercise mode in athletes' training programs since it effectively challenges the aerobic energy system and leads to rapid aerobic improvements even in highly trained people (Coyle, 2005; Koral *et al.*, 2017). However, during the last decade, HIIT has gained considerable interest from the general public, as it could serve as a time-efficient and alternative approach to traditional endurance training for westernized, busy populations. HIIT has also been the focus of scientific research, and various studies and meta-analyses have elucidated the effect of different HIIT protocols in healthy subjects and in subjects with lifestyle induced diseases, such as IR and T2DM. A growing body of evidence suggests that several modes of HIIT can lead to similar improvements in fitness and health as seen after moderate-intensity endurance training (Gist *et al.*, 2014).

The term HIIT refers to short exercise bouts performed with high intensity with recovery periods in between. These bouts are commonly performed with an "all-out" effort, which is close or above the work load at individual  $VO_{2max}$ . In the previous studies, there are numerous different HIIT protocols, which might vary in many different aspects, such as the exercise intensity and duration, intensity and duration of recovery between bouts, the number of bouts, exercise modality and resting time between the training sessions (Buchheit and Laursen, 2013). This makes comparison between HIIT studies difficult. It has been approved by the science community that the terminology of high intensity interval training protocols would be specified by two terms; the term "HIIT" standing for the intensities of aerobic interval training (80-100% of  $VO_{2max}$ ) and the term "sprint interval training" (SIT) for supra-maximal efforts (>100% of  $VO_{2max}$ ) (Fig 3) (Gibala, Gillen and Percival, 2014). The common SIT protocol used in research is so called the "Gibala method" and involves repeated maximal-intensity 30 second cycling bouts on a specialized cycling-ergometer (i.e., repeated Wingate's test). The resting time between the bouts is 4 minutes (Burgomaster *et al.*, 2005; Gibala *et al.*, 2006). However, this type of training requires a high level of motivation and a specialized ergometer. Furthermore, the extremely intense nature of training may induce feelings of fatigue and pain and therefore may not be suitable for some individuals. Hence, there has been a request for the development of alternative HIIT/SIT protocols, which may be more suitable for specific populations, taking into account for example different age and disease groups (Coyle, 2005). One suitable option could be another protocol developed by Gibala's group, consisting of 60-second bouts at an intensity of 90% of  $HR_{max}$  (Gibala *et al.*, 2012). However, the optimal protocol still remains unknown and is probably highly individual (Weston *et al.*, 2014).



**Figure 3. Visual demonstration of moderate-intensity continuous training (MICT), high intensity interval training (HIIT) and sprint interval training (SIT) protocols.** MICT training consist of continuous exercise with constant work effort of about 60% of  $VO_{2max}$ . HIIT consists of exercise bouts of a few minutes with a work effort of 80-100% of  $VO_{2max}$  with short resting periods between. SIT consists of tens of seconds exercise bouts with supra-maximal work effort of more than 100% of  $VO_{2max}$ . Edited with permission from (Nightingale *et al.*, 2017).

SIT has shown to be an efficient method to reverse metabolic defects in skeletal muscle in sedentary populations. Earlier studies have demonstrated that low-volume SIT is an efficient stimulus to achieve adaptations that are usually associated with high-volume moderate intensity endurance training despite the remarkable reduction in the total time commitment and exercise volume (Eskelinen *et al.*, 2015, Burgomaster *et al.*, 2005; Gibala *et al.*, 2006). Some studies have suggested that SIT improves exercise capacity, cardiovascular fitness and insulin sensitivity and reduces VAT similarly or even more effectively compared to traditional moderate-intensity endurance training (Burgomaster *et al.*, 2008; Milanovic, Sporis and Weston, 2015). Other added benefits of SIT are improvements in endothelial function and blood pressure (Weston, Wisløff and Coombes, 2014). The efficiency of high intensity training is likely a result of the high level of motor unit activation during exercise bouts and rapid depletion of muscle glycogen levels, thereby promoting a greater increase in post-exercise muscle insulin sensitivity. SIT training specifically stresses recruitment and adaptation of type II muscle fibers, which are also known as fast twitch fibers. Enzyme activity in type II fibers is not effectively increased during low-intensity endurance training. (Coyle, 2005).

Several studies exist comparing traditional moderate-intensity continuous training (MICT) and different HIIT/SIT protocols showing that both training protocols lead to a similar increase in muscle mitochondrial content and improvements in oxidative capacity (Gibala *et al.*, 2006; Burgomaster *et al.*, 2008). The literature contains a number of reports that 6-8 weeks of HIIT leads to improved mitochondrial function in skeletal muscle (Henriksson and Reitman, 1976; Dudley, Abraham and Terjung, 1982). HIIT and SIT has been shown to rapidly increase GLUT4-expression and improve insulin sensitivity and  $\beta$ -cell function (Little *et al.*, 2011; Madsen

*et al.*, 2015). The studies comparing SIT and MICT have shown partly controversial results, suggesting that the differences between SIT and MICT are mild (Burgomaster *et al.*, 2005; Martin J Gibala and McGee, 2008; Little *et al.*, 2010). Regarding the glucose homeostasis, also Iellamo and colleagues showed similar improvement in fasting glucose after 12 weeks of either HIIT or MICT intervention in subjects with cardiac dysfunction (Iellamo *et al.*, 2014).

Numerous studies comparing different HIIT/SIT protocols to MICT have shown significantly faster improvement in cardiorespiratory fitness after HIIT/SIT. A recent meta-analysis of studies in participants with lifestyle-related metabolic disease reported that 9 of the 10 studies showed greater improvement after HIIT compared to MICT (Weston, Wisløff and Coombes, 2014). In these studies, the duration and used exercise intensity of these supervised interventions were 4 to 16 weeks, and the intensity of training 75-120% of maximal output, respectively. The increase in cardiorespiratory fitness after HIIT was approximately double the increase after moderate-intensity continuous training (19% vs 10%, respectively) (Weston, Wisløff and Coombes, 2014). This finding is important as cardiovascular fitness is an independent predictor of mortality. Another meta-analysis also concluded similar finding and, in addition, showed that the improvements seen after HIIT are greater in less fit subjects (Milanovic, Sporis and Weston, 2015). In the meta-analysis that included 13 short-term SIT intervention studies (all-out effort, duration of 2 to 8 weeks), SIT improved aerobic fitness in 11 studies (Sloth *et al.*, 2013).

Although the EE during the low-volume HIIT/SIT session is remarkably lower than that in the high-volume MICT, SIT increases post-training EE and fat oxidation more than MICT. Skelly and colleagues demonstrated that HIIT (1 minute bouts at intensity 90% of  $VO_{2max}$  and 1 minute rest) and MICT (53 minutes at intensity of  $VO_{2max}$ ) induced similar 24-h EE after a single exercise session despite the fact that during the exercise session EE was significantly higher in the MICT group than in HIIT group. Thus, during hours of recovery after the training session EE elevated higher in the SIT group. Hence, HIIT increases exercise-induced post oxygen consumption (EPOC) more than MICT, which leads to higher post EE (Skelly *et al.*, 2014). In addition, higher intensity exercise results in secretion of lipolytic hormones, which are associated with greater post-exercise EE and fat oxidation (Horowitz, 2003). Irving and colleagues performed a 4-month intervention study in which subjects performed exercises with different intensities but matched EE during each training session. Higher intensity training resulted in a reduction in total and abdominal SAT, whereas no change was seen in the lower intensity training group, which could be explained by higher post exercise EE after training with higher intensity (Irving *et al.*, 2008). Hence, this may explain the comparable

or greater changes in body composition reported despite lower total training volume and time commitment after HIIT/SIT (Yoshioka *et al.*, 2001; Skelly *et al.*, 2014).

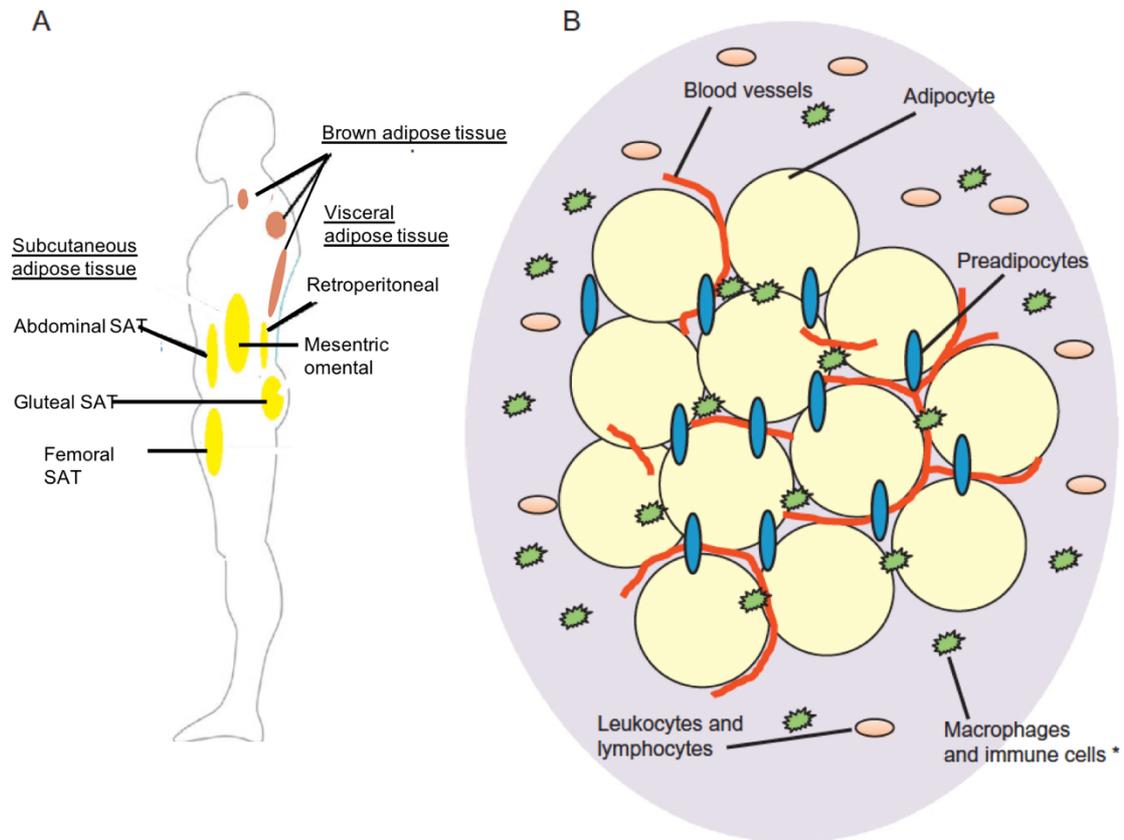
Studies directly comparing HIIT to traditional, moderate-intensity exercise in people with IR and T2DM are less common. Few recent studies have examined whether the effects of different interval training protocols could be applied to subjects with IR and T2DM and if HIIT/SIT is safe for subjects with T2DM. Study by Little and colleagues first showed that two weeks of HIIT (60 second bouts of cycling at 90% maximal effort with 60 second recovery) already increased skeletal muscle GLUT4 expression and markers of mitochondrial activity, and decreased blood glucose concentration in subjects with T2DM (Little *et al.*, 2011). The beneficial effects were also later observed in the study by Shaban and colleagues in which nine T2DM subjects performed two weeks of SIT training with 30 seconds maximal bouts with 4 minutes rest between. They observed that blood glucose was reduced immediately after each session and that IR, assessed by fasting homeostasis model assessment scores was reduced after a two-week intervention. However, they did not observe difference in fasting insulin or glucose levels (Shaban, Kenno and Milne, 2014).

Because high intensity exercise has been associated with increased risk of acute cardiovascular events, there is concern regarding the safety of implementing SIT in any clinical population. However, review by Wisloff and colleagues concluded that high-intensity protocols could be particularly effective for improving cardiac function (Wisloff *et al.*, 2007). Interestingly, recent study also showed that healthy subjects as well as subjects with prediabetes can adhere to interval training more than to MICT, because of its structural protocol (Jung *et al.*, 2015). In addition, similar adherence between HIIT and MICT was seen in obese subjects in 6-week intervention study by Vella and Colleagues (Vella, Taylor and Drummer, 2017). All these finding support the huge potential of SIT as a time-saving and motivating method for subjects at risk for T2DM. Certainly, more work is needed to examine the feasibility, impact, and safety of SIT among different subgroups of the T2DM population.

## **2.2 Tissue-specific adaptations after exercise training**

### ***2.2.1 Subcutaneous and visceral adipose tissue***

Adipose tissue accounts for 5-60% of body weight, depending on gender and level of obesity (Walker *et al.*, 2007). Adipose tissue consists mainly of white adipocytes and vascular-stromal fraction cells such as vascular endothelial cells, fibroblasts and immune cells (Otto and Lane, 2005). Different types of adipocytes with different metabolic characteristics exist and they can roughly be divided into white and brown adipose tissue. White adipose tissue can further be divided into subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and intrathoracic fat according to the location (Figure 4). The role of white adipose tissue is to store energy in the form of triglycerides, but it also has a central role in glucose and fat metabolism and is an active endocrine organ (Walker *et al.*, 2014). In contrast, the function of brown adipose tissue is to transfer energy from food into heat through the presence of the uncoupling protein 1 (UCP1). Recent studies have suggested that there could be a browning effect of the white adipose tissue after exercise training or medication, producing beige adipocytes that are functionally between white and brown adipose tissue (Sacks *et al.*, 2009; Virtanen *et al.*, 2009; Gaggini and Gastaldelli, 2015). Browning could provide a mechanism for weight management in the future (Kim and Plutzky, 2016). Brown adipose tissue is not studied in this thesis and therefore is not discussed further.



**Figure 4. Adipose tissue localization and composition in humans.** A. Human adipose tissue depots can be roughly divided into brown and white adipose tissue. Brown adipose tissue (BAT) is high-energy consuming, thermogenic fat depot, which is located in the neck area. White adipose tissue depots can be divided into visceral, subcutaneous, and intrathoracic depots. Visceral adipose tissue can be further divided into retroperitoneal and mesenteric omental depots and is located in the abdominal cavity. Subcutaneous adipose tissue can be divided into abdominal, gluteal, and femoral depots according to their anatomical locations. Subcutaneous adipose tissue is located directly below the skin. B. Adipose tissue consists mainly of white adipocytes and vascular-stromal fraction cells such as vascular endothelial cells, fibroblasts, and immune cells. Modified with permission from (Tsiloulis and Watt, 2015).

A study by Walker and colleagues in 1954, first showed that android and truncal (upper body) adipose tissue distribution has greater association with metabolic disturbances than gynoid (lower body) adipose tissue (Vague, 1956). This study was the first to suggest, that obesity-related diseases are not associated only with the fat per se but also with the distribution of fat. This suggestion has been further supported by several studies (Ohlson *et al.*, 1985; Carey *et al.*, 1997; Goedecke and Micklesfield, 2014). The phenomenon has also been confirmed in metabolically obese normal weight (MONW) and metabolically healthy obese (MHO) phenotypes. MONW are characterized by having a normal BMI but having IR or metabolic syndrome. In contrast, MHO subjects are obese, but are insulin sensitive and have lower VAT, ectopic fat and cardiorespiratory fitness than MONW subjects (Karelis *et al.*, 2004; Conus, Rabasa-Lhoret and Péronnet, 2007). Thus, it is

now well recognized, that the quality of the adipose tissue is more important than the quantity as such. Numerous studies have also confirmed that AT is not a single homogeneous tissue and that different regional depots have individual function and metabolic diversity (Kelley *et al.*, 2000; Gillian E Walker *et al.*, 2007). The distribution of VAT and SAT is individual and is dependent on various factors, such as age, nutrition, and the energy homeostasis in the adipose tissue. For example, women have a higher proportional amount of total body fat than men, however, men tend to have a higher central adiposity and women tend to accumulate fat on the hips and limbs and therefore are more protected from metabolic disturbances (Ohlson *et al.*, 1985; Macotela *et al.*, 2009).

VAT is located in the abdominal cavity and can be divided into mesenteric omental and retroperitoneal depots (Fig 4). Although the total volume of adipose tissue is a risk factor for development of IR and T2DM, it is believed that the volume of VAT is more linked to metabolic disturbances. In fact, previous studies have shown a correlation between VAT volume and IR, while no correlation is seen individually with SAT (Hoffstedt *et al.*, 1997; Wajchenberg, 2000; Neeland *et al.*, 2012; Borel *et al.*, 2015). It is already well recognized that adipocytes in VAT are lipolytically more active than adipocytes in SAT and therefore, VAT contributes substantially to the hepatic FFA delivery in subjects with excess visceral fat (Votruba and Jensen, 2007; Hajer, van Haeften and Visseren, 2008; Bjørndal *et al.*, 2011). However, abdominal subcutaneous fat is the major source of systemic free fatty acids taking into account the higher volume of SAT. The metabolic activity of the cell is dependent on its mitochondrial content. VAT adipocytes have a higher number of mitochondria compared to SAT adipocytes (Deveaud *et al.*, 2004) and have hence higher metabolic activity. In addition, it has been found that VAT has a higher expression of  $\beta$ -adrenergic receptors compared to SAT, which can contribute to the higher lipolytic activity. VAT releases free fatty acid (FFA) directly into the portal vein making the liver the first target of unsuppressed lipolysis and promoting hepatic TG synthesis and accumulation and thus may impair the hepatic insulin response (Bjørndal *et al.*, 2011). Other mechanism explaining the causative role of VAT in metabolic disturbances might be caused by accumulation of inflammatory cells, an alteration in the production of adiponectin and defect in PPAR- $\gamma$  signaling, a lower angiotensin capability or hypoxia (Hocking *et al.*, 2013).

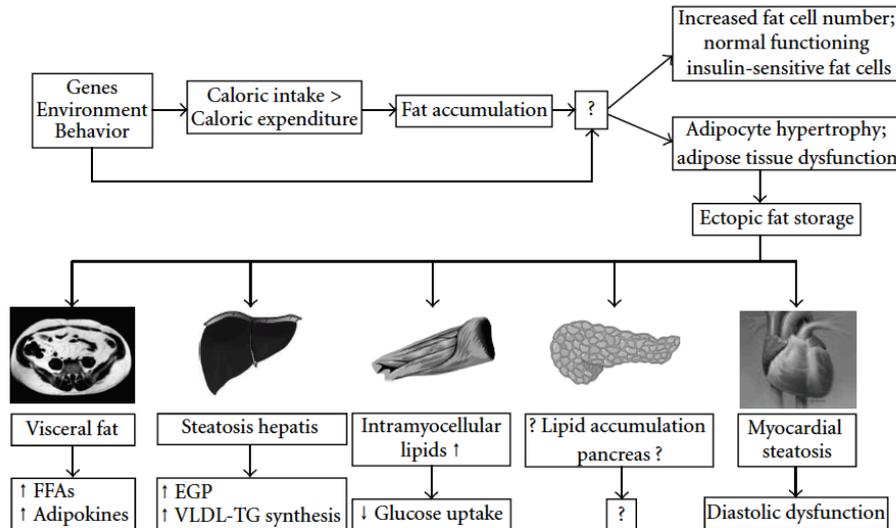
SAT accounts for more than 80% of total body fat and is located both in the lower and upper parts of the body directly below the skin. SAT in the abdominal, gluteal and femoral areas share some characteristics but differ in various manners. Meanwhile the excess accumulation of abdominal SAT is linked to metabolic disturbances and accumulation of ectopic fats; gluteofemoral SAT might have a protective role against IR and cardiovascular diseases (Snijder *et al.*, 2003;

Manolopoulos, Karpe and Frayn, 2010). Abdominal SAT can be anatomically divided into superficial and deep SAT and are separated by Scarpa's fascia. Deep abdominal SAT has been suggested to have similar characteristics to VAT and therefore it associates with IR. However, this heterogeneity has not been seen in all studies. Recent study have shown that superficial and deep abdominal SAT are homogenous at least in terms of gene expression and FFA composition (Petrus *et al.*, 2017). As previously discussed, SAT is metabolically less active than VAT and therefore may have better storage capacity. Thus, SAT is thought to act as a buffer for energy, by storing the TGs in periods of high energy intake and releasing FFAs during the periods of fasting or exercise (Frayn, 2002). In addition to its storage function, both VAT and SAT are active endocrine organs, which produce multiple hormones and hundreds of different adipokines, such as leptin, adiponectin, angiotensinogen, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), and plasminogen activator inhibitor-1 (PAI-1) (Hajer, van Haeften and Visseren, 2008).

Obesity is demonstrated to lead to several dysfunctions in adipose tissue. The most evident dysfunctions that are seen during weight gain are adipose tissue inflammation, hypoxia, insulin resistance, mitochondrial dysfunction and impaired adipogenesis. Obesity has been shown to increase adipose tissue inflammation and increase the recruitment of immune cells in adipose tissue. Eventually, a positive feedback cycle is formed in which macrophages recruit more immune cells, and finally, a state of chronic inflammation is induced. Studies have shown that the number of macrophages correlates with IR (Otto and Lane, 2005). Two types of macrophages (M1 and M2) secrete many inflammation-related adipokines and cytokines, such as TNF- $\alpha$ , interleukins and MCP-1 (Hajer, van Haeften and Visseren, 2008). Increased secretion of TNF- $\alpha$  activates adipocytes and consequently promotes lipolysis and FFA in the plasma. In obesity, the secretion of around 200 different adipokines is altered, which may also have systemic effects. These adipokines and cytokines can interfere with the immune system and have effects on hepatocytes, skeletal muscle and other tissues that contribute to the metabolic dysfunction that is associated with obesity and T2DM.

The possibility of an adipocyte to expand is limited, leading to hypertrophy of the adipocytes (Figure 5). A recent study showed that obese subjects had both hyperplasia and hypertrophy compared to the healthy controls, which was reversed by weight loss (Dadson *et al.*, 2016). To ensure a sufficient energy and oxygen supply and FFA and adipocytokine transport, an extended microvasculature is needed in enlarged adipose tissues. Therefore, adipogenesis and angiogenesis are closely associated processes during adipose tissue enlargement. Human adipocytes can grow up to ~20 fold in diameter and several thousand-fold in volume (Jernås *et al.*, 2006). When adipocytes reach their maximal size, stress signals are released. For

example, hypoxia can occur when vascularization is inadequate for the expanded adipose tissue. Further on, hypoxia can be attributed to the expression of angiogenic factors, such as VEGF and an unfolded protein response. Hypertrophic and stressed adipocytes have increased capacity to take up and release free fatty acids (FFAs). This induces an overflow of lipids towards non-adipose tissues such as skeletal muscle, liver, pancreas, and heart (Snel *et al.*, 2012). Accumulation of fat into non-adipose tissues (ectopic fat) are discussed in more detailed in the next chapter (2.2.2)



**Figure 5. Ectopic fat depositions and tissue-specific consequences of accumulation of adipose tissue.**

The accumulation of fat is induced by a combination of genetic background, environmental factors, and behavioral actions (physical inactivity and increased energy intake). Fat accumulation leads to an increased number and size of adipocytes. Adipocyte hypertrophy leads to an inflammatory response which ultimately leads to ectopic fat deposition and local and peripheral consequences. FFA (free fatty acid); EGP (endogenous glucose production); VLDL-TG (very low-density lipoprotein-triglyceride). Reprinted with permission from (Snel *et al.*, 2012).

Adipose tissue plays a major role in the regulation of glucose homeostasis and insulin sensitivity. Adipose tissue glucose uptake (GU) and fatty acid uptake (FAU) might reflect adipose tissue metabolic activity and give insights into relationship between adipose tissue and IR. Previous studies have shown that obese subjects have increased GU in VAT and SAT depots compared to lean subjects during an insulin clamp (Virtanen *et al.*, 2002). Previous intervention studies have been concentrated on diet-induced weight-loss. Surprisingly, weight reduction did not change abdominal VAT or SAT insulin-stimulated GU or fasting FAU in a 6-week very low-calorie diet intervention study despite significant reduction in VAT and abdominal SAT mass. However, abdominal SAT and VAT masses correlated inversely with insulin-stimulated GU in the same tissue and positively with whole-body insulin sensitivity (Viljanen *et al.*, 2009).

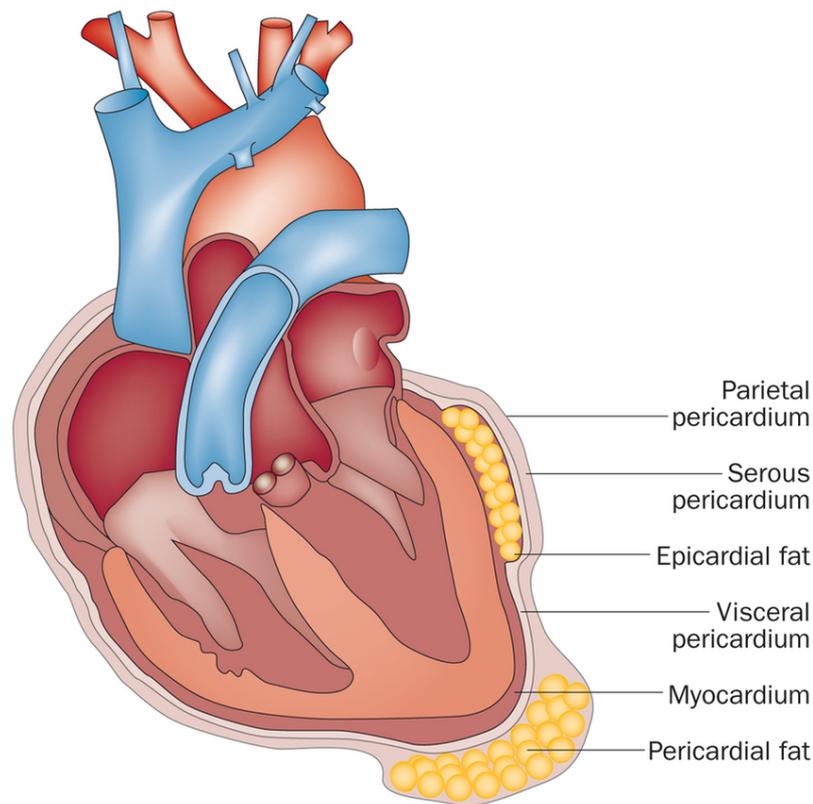
Exercise training has shown to be an effective method to decrease the volume of VAT and SAT, even without significant weight loss. Previous studies have observed that a higher amount of VAT rather than overall fat may be decreased in response to the exercise training (Giannopoulou *et al.*, 2005; Borel *et al.*, 2012). This phenomenon is most likely explained by the higher level of adrenergic receptors in VAT than SAT. It has also been suggested that the higher intensity exercise is more efficient in reducing the volume of VAT and SAT as it results in the secretion of lipolytic hormones, which are associated with post-exercise energy expenditure and fat oxidation (Horowitz, 2003). The reduction in adipose tissue mass leads to improvement in adipose tissue function. Several studies of bariatric surgery induced weight loss have shown that abnormalities, such as inflammation, can be reversed by weight loss (Sams *et al.*, 2016; Labrecque *et al.*, 2017). However, the effect of exercise training of different intensities on adipose tissue metabolism are still poorly investigated.

### **2.2.2 Ectopic fat in and around myocardium**

In addition to SAT and VAT, adipose tissue accumulates in and around internal organs, such as the liver, pancreas, skeletal muscle, and heart. These fat depots are believed to accumulate as the impaired clearance and storage of TGs in SAT lead to a 'leak' of TGs into non-adipose tissues and VAT. If in these tissues, the lipid supply exceeds the oxidative capacity intracellular fat accumulation occurs and organ function might be impaired (Després and Lemieux, 2006; Despres *et al.*, 2008; Shimabukuro *et al.*, 2013). Ectopic fat accumulation in and around tissues such as the liver, pancreas, adipose tissues, heart and VAT are linked to cardiometabolic complications commonly experienced in T2DM. The harmfulness of ectopic fat accumulation depends on the specific organ, fat content, composition and localization. For example, an increase in accumulation of intramuscular lipids (IMCL) is found in type 2 diabetes (van Loon and Goodpaster, 2006). Moreover, strong positive correlations between insulin resistance and IMCL have been reported (Krssak *et al.*, 1999). Strikingly, IMCL is also increased in highly-trained healthy athletes, despite being insulin sensitive (Goodpaster *et al.*, 2001). The finding in athletes suggest that intramuscular fats can serve as local energy source during muscle contraction, and in athletes is not thought to be harmful, a phenomenon known as the "athletes paradox" (Coen and Goodpaster, 2012). Previous studies in obese and highly-trained athletes have shown different composition of intramuscular fats in athletes and in obese subjects. In obese subjects, increase in fatty acid metabolites, such as fatty acyl-CoA, ceramides and diacylglycerol has been observed, of which the last two are likely to induce defects in the insulin signaling cascade causing skeletal muscle insulin resistance (Goodpaster *et al.*, 2001;

Addison *et al.*, 2014). It has been suggested that the differences in the relationship between IMCL and insulin resistance in obese/T2DM individuals and highly-trained individuals could be partly explained by the differences in muscle fiber type composition (Goodpaster *et al.*, 2001). Previous studies have shown that different ectopic fat depots can be reduced by weight loss and physical activity. This thesis will focus on ectopic fat accumulation in the heart.

The untypical accumulation of adipose tissue in and around the heart has gained increasing interest as it has been found to be a risk factor for IR, cardiomyopathy, coronary artery disease and heart failure. The volume of ectopic fat in the heart associates with body adiposity, circulation of FFAs and TG concentrations, fasting plasma glucose and insulin levels and blood pressure (Kankaanpaa, Lehto, Parkka, Komu, Viljanen, Ferrannini, Knuuti, Nuutila, Parkkola and Iozzo, 2006; Sacks and Fain, 2007; Rijzewijk *et al.*, 2008; Lim and Meigs, 2014; Talman *et al.*, 2014; Nagy *et al.*, 2017; Nerlekar *et al.*, 2017). In the heart, TGs can accumulate either inside the myocardium (myocardial triglyceride content, MTC) or outside the myocardium (intrathoracic fat) located between the myocardium and pericardium (epicardial fat), or between the pericardium and the chest wall (pericardial fat) (Fig 6). Throughout this thesis, the term “myocardial ectopic fat” refers to all fat depots in and around the myocardium (MTC, epicardial and pericardial fat), and the term “intrathoracic fat” refers to fat outside the myocardium (epicardial and pericardial fat). These myocardial fat depots have been proposed to have a cardioprotective role by acting as a buffer to protect the myocardium from a high triglyceride load and, in contrast, to act as a rapid energy source for example during starvation and exercise training (Guzzardi and Iozzo, 2011; Gaborit, Abdesselam and Dutour, 2013; Talman *et al.*, 2014).



**Figure 6. An anatomical demonstration of the locations of different fat depots in and around the myocardium.** Epicardial adipose tissue is defined as the fat located between the myocardium and visceral pericardium. Pericardial adipose tissue is the fat depot located between the visceral pericardium and the chest wall. MTC refers to the triglyceride content inside the myocardium. Reprinted with permission from (Iacobellis, 2015).

Epicardial and pericardial fat depots share some characteristics, but are also different in various manners, such as their anatomy, biochemistry, embryonic cell differentiation and function (Iacobellis, 2009). In contrast to epicardial fat, derived from the splanchnopleuric mesoderm, pericardial fat originates from the primitive thoracic mesenchyme (Iacobellis, 2009). Epicardial fat is not separated from the myocardium by any fascia and shares the microcirculation from the coronary arteries with the myocardium, while pericardial fat blood is supplied by the branches of the internal mammary artery. Compared to other fat deposits, epicardial fat has a smaller cell size, a distinct fatty acid composition, and a higher fatty acid and lower glucose metabolism (Iacobellis and Willens, 2009). High FFA metabolism supports the role of epicardial fat as an energy source for the myocardium. Epicardial fat also secretes defensive cytokines and adipokines (e.g. adiponectin and IL-10) and attenuate vascular torsion and hence, is not considered harmful under normal physiological conditions (Iacobellis and Willens, 2009; Talman *et al.*, 2014). Epicardial fat has also been found to have a 5-times higher UCP-1 expression than other adipose tissue depots in the body, which suggest that epicardial

fat shares some characteristics with brown adipose tissue and thus could protect the myocardium from hypothermia (Sacks *et al.*, 2009). The metabolic role of pericardial fat is still unclear, but increased pericardial fat may evolve into a higher risk for metabolic diseases compared to epicardial fat (Sicari *et al.*, 2011).

The mechanisms relating epi- and pericardial fat to increased cardiovascular and metabolic risk factors are complex and incompletely studied. Under pathological circumstances, such as obesity and T2DM, epi- and pericardial fat have been suggested to release adipokines and pro-inflammatory cytokines, which may lead to chronic low-grade inflammation and thus potentially contributing further to the development and progression of atherosclerotic lesions and coronary artery disease (Guzzardi and Iozzo, 2011; Bucher *et al.*, 2014). Some studies have also observed decreased adiponectin and increased leptin production in epicardial fat in subjects with metabolic and cardiovascular disturbances, which could enhance the development of coronary artery disease (Iacobellis *et al.*, 2005; Eiras *et al.*, 2008). Decreased adiponectin expression leads to increased TNF- $\alpha$  production, which further promotes the inflammation and oxidative stress (Iacobellis, Malavazos and Corsi, 2011).

Myocardial triglyceride content describes the percentage of TGs of the whole mass of the myocardium. MTC has been studied mainly in animal studies but an increasing number of human studies have been conducted in the recent years. Increased MTC has been shown to have a positive association with mitochondrial dysfunction and increased oxidative stress, which especially increases the risk of cardiac dysfunctions (McGavock *et al.*, 2007; Lim and Meigs, 2014). The increased MTC is associated with aging, left ventricle systolic and diastolic dysfunction, left ventricle hypertrophy, myocardial fibrosis and apoptosis, and even with premature death. Increased MTC could also be associated with the changes in energy metabolism of the myocardium. Previous studies have also shown correlation with MTC and IR. For example, the study by McGavock and colleagues showed a 2-fold MTC in type 2 diabetic subjects compared to healthy controls. The same study also showed positive correlation between MTC and VAT (McGavock *et al.*, 2007). MTC has been shown to be a rather active TG store, as two days of fasting increases MTC by 3-fold (Reingold *et al.*, 2005).

Exercise training has been shown to reduce MTC and epicardial fat in non-diabetic obese individuals (Table 1). However, the few studies performed with T2DM patients have not revealed promising results. In the study by Schrauwen-Hinderling and colleagues (Schrauwen-Hinderling *et al.*, 2011), the 3-month combined endurance and strength training improved whole-body insulin sensitivity and the LV ejection fraction but not MTC. Similarly, in the study by Jonker and colleagues, no response was observed in MTC or epicardial fat after six months of endurance

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training in T2DM subjects, although hepatic TG content, VAT, and pericardial fat decreased (Jonker *et al.*, 2013). Overall, the data on the effects of exercise training on MTC is limited. It is also unclear whether exercise training could increase MTC as it occurs in skeletal muscle, which is the phenomenon known as the “athlete’s paradox”. No previous studies have studied the effect of training with higher intensities on myocardial adiposity.

**Table 1. Review of the studies on exercise training and myocardial adiposity in humans.** All of the previous studies have been conducted with moderate-intensity or combination of endurance and resistance training. NM, not measured; EAT, epicardial adipose tissue; PAT, pericardial adipose tissue; MTC, myocardial triglyceride content.

Authors	Study subjects	Intervention	BMI	Whole body fat %	Aerobic fitness	EAT	PAT	MTC
Kim et al 2008	24 obese men (BMI 30.7±3.3 kg•m <sup>-3</sup> )	12-wk moderate-intensity (60-70% of VO <sub>2max</sub> )	Decreased (p<0.001)	Decreased (p<0.001)	Increased (p<0.001)	Decreased (p<0.001)	NM	NM
Jonker et al 2013	12 T2DM subjects (BMI 28.7±1.2 kg•m <sup>-3</sup> )	6 months moderate intensity training and high-altitude trekking	Decreased (p<0.05)	NM	NM	No change (p=0.9)	Decreased (p=0.02)	No change (p=0.9)
Wu et al 2016	39 obese subjects (BMI 32.6±4.5 kg•m <sup>-3</sup> )	12-wk moderate intensity	Decreased (p<0.001)	NM	NM	Decreased (p<0.001)	Decreased (p<0.001)	NM
Schrauwen-Hinderling et al 2010	14 overweight men (BMI 29.9±0.01 kg•m <sup>-3</sup> )	12-wk moderate-intensity/resistance	No change (p=0.8)	No change (p=0.1)	Increased (p=0.001)	NM	NM	Decreased (p=0.02)
Schrauwen-Hinderling et al 2011	11 T2DM subjects (BMI 29.9±0.01 kg•m <sup>-3</sup> )	12-wk moderate-intensity/resistance	No change (p=0.8)	No change (p=0.11)	Increased (p=0.001)	NM	NM	No change (p=0.15)
Sai et al 2013	15 lean healthy subjects and 10 male athletes	Cross-sectional	-	-	-	NM	NM	Lower in the athletes (p=0.045)
Bucher et al 2014	10 lean men (BMI 22.75±1.4 kg•m <sup>-3</sup> )	Single 2h bout (50-60% of VO <sub>2max</sub> )	-	-	-	NM	NM	Decrease (p=0.002)
Billet et al 2010	11 lean men (23.6±0.8 kg•m <sup>-3</sup> )	Single 2h bout (50% of VO <sub>2max</sub> )	-	-	-	NM	NM	Decrease (p=0.003)

### 2.2.3 Brain metabolism

The brain is a highly glucose dependent organ and its main fuel is glucose. Once in the brain, glucose is used for ATP synthesis, to maintain the neuronal and non-neuronal stability and for the generation of neurotransmitters. Glucose cannot completely be replaced as an energy source in the brain but, for example, during starvation or exercise it can be supplemented with lactate or ketone bodies (van Hall *et al.*, 2009; Mergenthaler *et al.*, 2013). The brain has a limited capacity to store energy and therefore a constant glucose supply to the brain is pivotal and ensured by several mechanisms (Peters *et al.*, 2004). Disturbed brain glucose metabolism may lead to several diseases affecting both the brain as well the whole body (Mergenthaler *et al.*, 2013).

Glucose enters the central nervous system (CNS) across the endothelial membranes via saturable GLUT1 glucose transporters, which are mediated through insulin signaling by brain insulin receptors (InsRb). InsRb are widely expressed in CNS, but especially in the hypothalamus, cerebellum, and cortex. Impaired InsRb action has been hypothesized to be involved with dysfunctions in CNS (Schulingkamp *et al.*, 2000; Bingham *et al.*, 2002; Mergenthaler *et al.*, 2013). Neuronal glucose uptake from extracellular matrix occurs via GLUT3, which has a much higher transport rate than GLUT1 (Mergenthaler *et al.*, 2013). In addition to neurons, glucose is taken up by a particular type of glial cells, astrocytes. Astrocytes play a central role in brain energy metabolism via astrocyte-lactate neuron-shuttle. Glucose enters astrocytes via GLUT1 glucose transporters (Bélanger, Allaman and Magistretti, 2011).

The brain and especially the hypothalamus are key regulators of whole-body glucose homeostasis. The brain regulates food intake and hepatic glucose production based on the feedback from adiposity related signals (insulin and leptin) (Pagotto, 2009; Schwartz *et al.*, 2013). In addition, although the brain does not use FFAs as an energy source, it has been suggested that the brain obtains nutrition related feedback via FFAs and therefore the FFAs may have a role in CNS control of whole-body energy homeostasis and food intake. Hence, the role of brain FFA uptake (FAU) and whole-body energy metabolism remains unclear (Pocai *et al.*, 2006; López, Lelliott and Vidal-Puig, 2007; Pagotto, 2009; Schwartz *et al.*, 2013). Central glucose sensing of hormones and nutrients, such as glucose, insulin, leptin and FFAs, and peripheral regulation of glucose homeostasis are closely linked. Studies with neuron-specific insulin receptor disturbed (NIRKO) mice have suggested that neuronal insulin signaling regulates peripheral glucose homeostasis (Brüning *et al.*, 2000) and that there exists a direct link between central glucose sensing and the peripheral glucose level. Studies have shown that a sudden increase in CNS

glucose level results in a decrease in blood glucose and insulin levels and suppression of hepatic endogenous glucose production (EGP) (Lam, Chari and Lam, 2009).

Earlier, it was thought that brain is not insulin sensitive organ, but recent studies have shown opposite (Bingham *et al.*, 2002; Mergenthaler *et al.*, 2013). It has also been shown that insulin stimulation increases brain GU in subjects with IR, but not in healthy subjects (Hirvonen *et al.*, 2011). This finding suggests that during insulin stimulation, brain GU behaves contrary to peripheral tissues, such as skeletal muscle. However, subjects with IR have shown to have increased FAU in the brain similar to other tissues, such as liver (Karmi *et al.*, 2010). Interestingly, one recent study showed that bariatric surgery induced weight loss decreases insulin stimulated brain GU (Tuulari *et al.*, 2013). In addition, brain FAU was seen to be substantially reduced after rapid weight loss in subjects with metabolic syndrome (Karmi *et al.*, 2010).

Long-term physiological adaptations to exercise training in the human brain have been poorly studied. However, exercise training studies on the brain have shown that exercise has beneficial effects on cognition, depression and memory in humans (Loprinzi *et al.*, 2013; Erickson and Hillman, 2015). Animal studies have also found that exercise training can increase mitochondrial biogenesis and induce neuroprotective molecular changes (Bayod *et al.*, 2011; Steiner *et al.*, 2011). However, exercise training studies on brain glucose and free fatty acid metabolism in humans are extremely scarce. In a previous cross-sectional study, brain GU in contrast was shown to decrease with increasing exercise intensity when studied acutely after one 35-minute bicycle session. In this study it was suggested that brain GU decrease was possibly due to an increased use of lactate as an energy source (Kemppainen *et al.*, 2005). However, a recent study by Robinson and colleagues showed a decreased brain GU after a 12-weeks combined high-intensity training intervention in healthy adults (Robinson, Lowe and Nair, 2017). There are no prior studies that have investigated training-induced responses in brain GU during insulin stimulation and brain FAU during fasting in IR subjects.

### **2.3 Summary of the literature review**

Physical activity, as a cornerstone in the prevention and treatment of IR and T2DM, has marked acute and chronic effects on the regulation of GU and on inflammatory processes. A single bout of exercise acutely activates pathways that lead to exercise-stimulated GU in the skeletal muscle. However, the acute effects of a single exercise bout are transient and resting GU will return to the same level

if the exercise is not repeated after 2-3 days. Hence, exercise training is recommended on a regular basis. Numerous exercise training studies have shown increased skeletal muscle mitochondria and GLUT4 expression, which are associated with improved skeletal muscle insulin sensitivity and general metabolic health both in healthy and IR subjects.

Unfavorable regional adiposity can be considered a major risk factor for IR and T2DM. Subjects with IR have shown to have increased amount of SAT, VAT and other ectopic fats, of which the VAT and ectopic fat in and around internal organs seem to contribute more to the development of IR. VAT and SAT have been shown to be reduced in response to exercise training, but the effect on epi- and pericardial fat and MTC are less investigated. In addition, the metabolic adaptations after exercise training in VAT and SAT are not fully known. Insulin resistant subjects have also shown increased insulin stimulated GU and FAU in the brain. Weight-loss and acute exercise have been shown to reduce insulin-stimulated brain GU, but the effects of exercise training remain completely unstudied.

Given that a lack of time is the number-one reported barrier to regular exercise participation, it is possible that low-volume SIT may be an attractive option for increasing physical activity levels. SIT involves a substantially lower total exercise volume and time commitment and has therefore been praised as a time-efficient exercise option. It is already well recognized that SIT can elicit rapid improvements in cardiovascular and metabolic health. However, it remains to be studied whether all the benefits of traditional aerobic exercise can be achieved with low-volume SIT and whether it is effective for individuals with T2DM.

### 3 AIMS OF THE STUDY

Physical activity is a cornerstone in the prevention and treatment of IR and T2DM and has been shown to induce marked acute and chronic effects on the regulation of glucose uptake and on inflammatory processes. Recent studies have shown that the beneficial adaptations that are usually seen after MICT, can be achieved by a significantly smaller volume of SIT (Martin J Gibala and McGee, 2008; Rehn *et al.*, 2013; Weston *et al.*, 2014). Earlier studies have shown that short-term SIT induces at least similar improvements in aerobic capacity and insulin sensitivity compared to MICT in healthy middle-aged men (Eskelinen *et al.*, 2015, Gibala *et al.*, 2006). However, it is unclear whether exercise training, either SIT or MICT, is an effective method to improve glucose and FFA uptake in brain and adipose tissue and reduce myocardial ectopic fat accumulation along with improvements in general metabolic health.

The aims of the current thesis were to compare the effects after two weeks of short-term low-volume SIT or high-volume MICT on:

1. Visceral and subcutaneous fat glucose and FFA uptake in healthy men and men with IR.
2. Fat content in and around the myocardium in healthy men and in men with IR.
3. Brain glucose and FFA uptake in subjects with IR.

## 4 SUBJECTS AND STUDY DESIGN

This thesis was a part of a larger study 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). From that study, several reports have been published earlier. All the experiments were performed at the Turku PET Centre, the University of Turku, and Turku University Hospital (Turku, Finland) and the Paavo Nurmi Centre (Turku, Finland) between March 2011 and September 2015. The study was approved by the ethical committee of the Hospital District of Southwest Finland (Turku, Finland, decision 95/180/2010 §228) and was carried out according to the Declaration of Helsinki. All participants gave written informed consent.

### 4.1 Subjects

Middle-aged physically inactive healthy subjects and subjects with pre-diabetes (impaired fasting glucose or impaired glucose tolerance) or T2DM were recruited for the study via newspaper advertisements, personal contacts, and electronic and traditional bulletin boards. The inclusion criteria for healthy subjects ( $n=28$ ) were male sex, age 40 – 55 years, BMI 18.5–30  $\text{kg}\cdot\text{m}^{-2}$ ,  $\text{VO}_{2\text{peak}} < 40 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  and normal glycemic control. The exclusion criteria were: any chronic disease, a medical defect or injury that interfered with everyday life; a history of eating disorders; a history of asthma; use of tobacco products; use of anabolic steroids, additives or any other substrates; significant use of alcohol; current or a history of regular and systematic exercise training of any other condition that in the opinion of the investigator could create a hazard to the participant’s safety, endanger the study procedures or interfere with the interpretation of the study results. For the insulin resistant subjects ( $n=26$ ; 10 females and 16 males), the inclusion criteria were the same as in healthy subjects except for a BMI of 30–35  $\text{kg}\cdot\text{m}^{-2}$  and an impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) according to the criteria of the American Diabetes Association (American Diabetes Association, 2017a), an HbA1c of less than 7.5 % and no insulin treatment in cases of T2DM. Of the 26 IR subjects, 16 had T2DM and 10 IFG and/or IGT. At the screening, five subjects were newly diagnosed with T2DM and had no previous medication. In the other eleven T2DM subjects, the median diabetes duration was 4.2 years and they were treated by oral hypoglycaemic agents (11 with metformin; 5 with sitagliptin and 1 with glimepiride). The study population in different sub-studies varied according to the study questions and data limitations (Table 2).

Randomization of the healthy subjects was performed in two phases. First, a random permuted block of 24 subjects with a 1:1 allocation ratio was generated. Because of technical problems in the PET studies, another random permuted block of 4 subjects was also generated. Therefore, the final group sizes were n=14 for the SIT and n=14 for the MICT in the healthy subjects (totally n=28). Randomization of the IR subjects (n=26) was performed in blocks of four subjects with a 1:1 ratio.

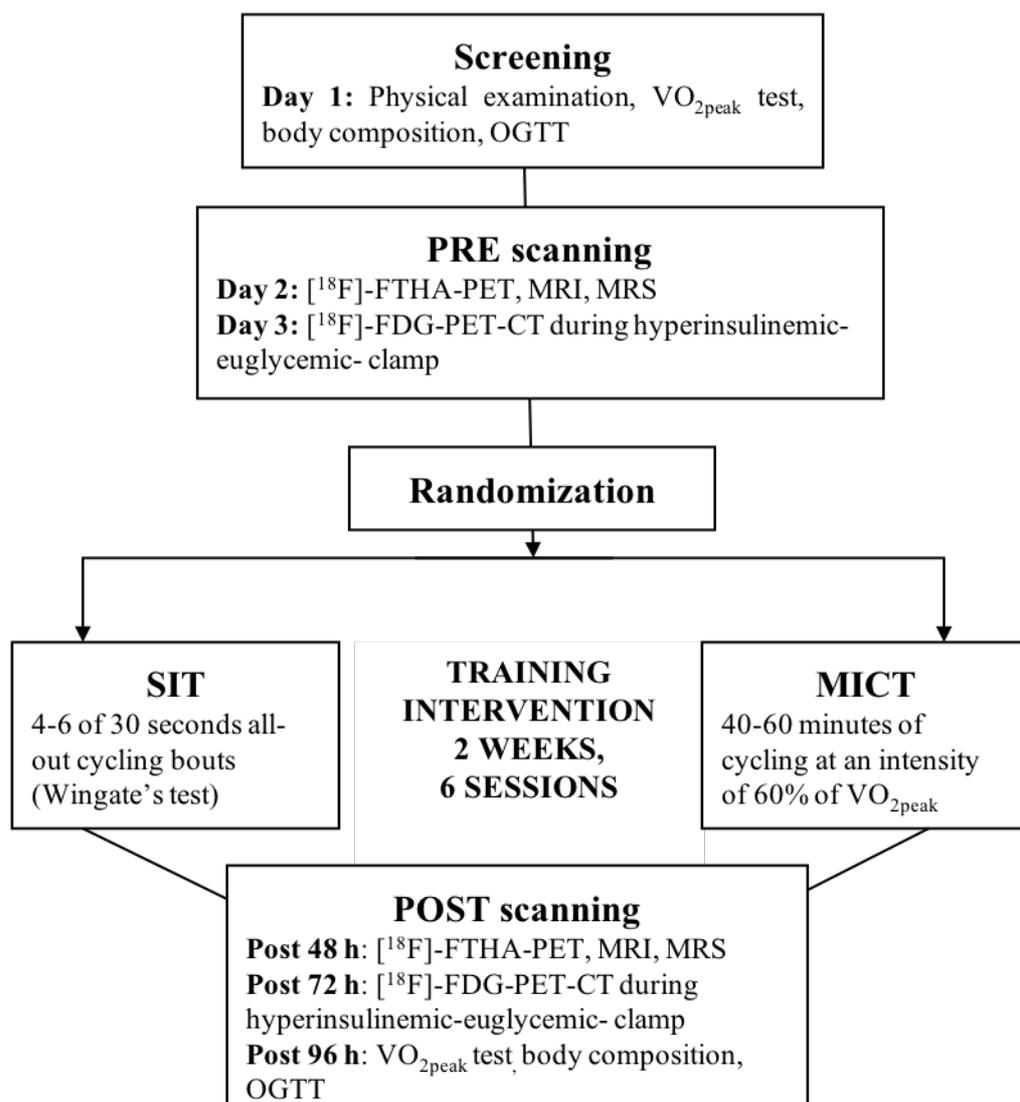
**Table 2.** Study subjects in different sub-studies. Paper I and II compared exercise training induced responses in adipose tissue metabolism in healthy men (n=28) and men with IR (pre-T2DM or T2DM, n=16). Paper I also compared responses in SIT and MICT in IR men and women (n=26). Paper III compared the effects of SIT and MICT in brain metabolism IR men and women (n=21). Five of the IR subjects lacked the scan from the brain area and thus were not included in the study.

	<b>Paper I and II</b> Healthy vs IR SIT vs MICT		<b>Paper I</b> SIT vs MICT Men vs Women	<b>Paper III</b> SIT vs MICT
<b>Group</b>	<b>Healthy</b>	<b>IR</b>	<b>IR</b>	<b>IR</b>
<b>N</b>	28	16	26	21
<b>Sex (men/women)</b>	28/0	16/0	16/10	12/9
<b>pre-T2DM/T2DM</b>	-	3/13	13/13	8/13
<b>Age (years)</b>	48 ± 5	49 ± 4	47 ± 3	49 ± 4
<b>Weight (kg)</b>	83.6 ± 8.7	89.5 ± 10.9	93.5 ± 9.8	91.0 ± 13.5
<b>BMI (kg•m<sup>-2</sup>)</b>	26.1 ± 2.4	30.2 ± 2.4	30.1 ± 2.6	30.5 ± 2.7
<b>Body fat (%)</b>	22.6 ± 4.1	33.3 ± 7.5	28.5 ± 4.5	33.6 ± 7.5
<b>VO<sub>2peak</sub> (mL•kg<sup>-1</sup>•min<sup>-1</sup>)</b>	34.2 ± 4.0	27.3 ± 4.6	29.6 ± 3.5	27.0 ± 4.7

IR (insulin resistance); SIT (sprint interval training); MICT (moderate intensity continuous training); T2DM (type 2 diabetes mellitus); BMI (body mass index), VO<sub>2peak</sub> (aerobic capacity).

## 4.2 Study Design

The measurements and the training interventions were carried out in the Turku PET Centre, Turku, Finland, except for the  $\text{VO}_{2\text{peak}}$  test and body composition analysis, which were performed at the Paavo Nurmi Center, University of Turku, Finland. The complete study flow is shown in Figure 7. Before the study, subjects were interviewed and physically examined by a medical doctor, including basic laboratory samples and a 2-hour oral glucose tolerance test (OGTT). On the same day or the day after, subjects performed a  $\text{VO}_{2\text{peak}}$  test with the supervision of an exercise physiologist to determine the level of physical fitness. At least one week after, a pre-training scanning was performed at the Turku PET Centre on two subsequent days. Myocardial fat content was measured by magnetic resonance spectroscopy (MRS) and VAT and SAT volumes by magnetic resonance imaging (MRI) during the first measurement day. MRI/MRS was followed by [ $^{18}\text{F}$ ]FTHA PET to measure the free fatty acid uptake of the brain and fat tissues in a fasted state. The second measurement day started with a measurement of the whole body insulin sensitivity using a euglycemic hyperinsulinemic clamp and was followed by [ $^{18}\text{F}$ ]FDG PET-CT during a steady state euglycemic hyperinsulinemic clamp to measure the glucose uptake of the brain and fat tissues at rest and also computed tomography (CT) to measure epi- and pericardial fat volume.



**Figure 7. Study design. Subjects underwent similar measurements before and after a two-week training intervention of either SIT or MICT.**  $VO_{2peak}$  (aerobic capacity); OGTT (oral glucose tolerance test);  $[^{18}F]$ FTHA (14(R,S)- $[^{18}F]$ fluoro-6-thia-heptadecanoic acid); PET (positron emission tomography); MRI (magnetic resonance imaging); MRS (magnetic resonance spectroscopy);  $[^{18}F]$ FDG (2- $[^{18}F]$ fluoro-2-deoxy-D-glucose); CT (computed tomography); SIT (sprint interval training); MICT (moderate-intensity continuous training).

After all the pre-training measurements, the subjects were randomly divided 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 two-weeks training intervention starting with  $[^{18}F]$ FTHA PET, MRS, MRI and CT scans at 48 hours after the last training session,  $[^{18}F]$ FDG PET-CT during a steady state euglycemic hyperinsulinemic clamp at 72 hours and an aerobic fitness and blood sampling at 96 hours after the last training session.

Subjects were asked to fast for at least ten hours prior to the screening day and all scanning days. Fasting was controlled by interview of the subjects. Anti-diabetic

treatment was withheld for 72 hours and the subjects were asked to avoid exhaustive exercise 48 hours prior to the measurements.

### 4.3 Training intervention

Both training interventions took two weeks and included six supervised training sessions. The duration of the intervention was based on previous studies showing improvements in aerobic fitness and insulin sensitivity after only six training sessions (Burgomaster *et al.*, 2005, 2008) and also considering the intense nature of the SIT intervention. It is noteworthy that the time spent during the training intervention (SIT 15 minutes vs MICT 300 minutes) was much less in SIT than MICT. Each session was performed in laboratory conditions at the Turku PET Centre with the supervision of a member of the study group.

#### 4.3.1 Moderate-intensity continuous training (MICT) protocol

The MICT training protocol was planned to meet the current health-enhancing exercise recommendation of 150 minutes of moderate-intensity exercise per week. Each MICT session consisted of 40-60 minutes of cycling at a moderate intensity, which was determined as 60% of the measured  $\text{VO}_{2\text{peak}}$  workload (Tunturi E85, Tunturi Fitness, Almere, Netherlands) based on previous MICT protocols (Gibala *et al.*, 2006). The duration of the MICT increased progressively starting with 40 minutes and increasing by ten minutes every other session up to 60 minutes.

#### 4.3.2 Sprint-interval training (SIT) protocol

The SIT protocol was based on the study originally described by Burgomaster and colleagues showing that only 15 minutes and two weeks of SIT improves exercise capacity and muscle insulin sensitivity (Burgomaster *et al.*, 2005).

Subjects were familiarized with SIT during the screening days, as each of the subjects performed two 30 seconds all-out exercise bouts. During the intervention, each SIT sessions consisted of 15 minutes warm-up, 4-6  $\times$  30 seconds all-out cycling bouts with a 4 minutes recovery between each bout and 10 minutes cool down. The number of bouts increased progressively starting with four bouts and increasing by one every other session up to six bouts. The training load was individually determined (for healthy subjects 7.5% of whole body weight in kg, for IR subjects 10% of lean body mass in kg). Each bout started with prompt acceleration

to maximum frequency without resistance. As the maximum frequency was accomplished, a sudden increase of load was applied with a special cycling ergometer (Monark Ergonomic 894E, MONARK, Vnasbro, Sweden), and cycling was carried out for 30 seconds at the maximum speed. The subjects were motivated to keep up the maximum pace by cheering them during each bout, using the slogans such as 'Keep on going, you can do it!' and 'Go, go, go'. Subjects cycled without resistance during a 4 minutes recovery period.

## 5 METHODS

### 5.1 Measuring glucose and FFA metabolism - positron emission tomography

Positron emission tomography (PET) is a non-invasive nuclear imaging technique that provides information about physiological function of the human tissues. There exist several radioisotopes, which of [ $^{18}\text{F}$ ]FDG is the most commonly used. In this thesis, [ $^{18}\text{F}$ ]FDG and [ $^{18}\text{F}$ ]FTHA tracers were used to measure glucose and FFA uptakes. [ $^{18}\text{F}$ ]FDG is a radiotracer for glucose metabolism. After injection, it enters the cell in the same manner as the normal glucose. Once FDG has entered the cell, it is phosphorylated, which traps FDG into the cell (Rudroff, Kindred and Kalliokoski, 2015). [ $^{18}\text{F}$ ]FTHA is a palmitate analogue for fatty acid metabolism. [ $^{18}\text{F}$ ]FTHA is injected into circulation from where it enters tissues. In the tissue, it subsequently enters either the mitochondria or is incorporated into complex lipids, mostly triglycerides. In the mitochondria [ $^{18}\text{F}$ ]FTHA undergoes initial steps of  $\beta$ -oxidation and is thereafter trapped as further  $\beta$ -oxidation is blocked by its sulfur heteroatom. The half-life of [ $^{18}\text{F}$ ]FTHA and [ $^{18}\text{F}$ ]FDG is 110 minutes. (Lederer *et al.*, 1967; DeGrado, Coenen and Stocklin, 1991)

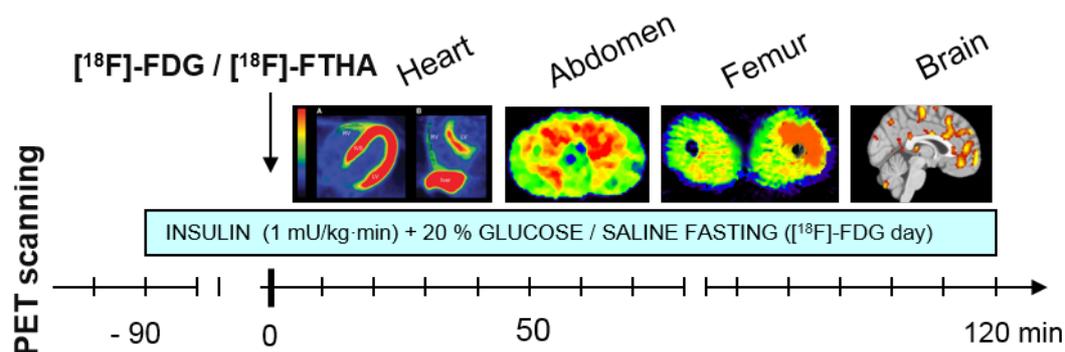
#### 5.1.1 PET image acquisition

Participants underwent four PET sessions: one [ $^{18}\text{F}$ ]FTHA PET and one [ $^{18}\text{F}$ ]FDG PET before and after the training intervention. Antecubital veins of both arms were cannulated for the PET studies. One catheter was used to inject the radiotracers [ $^{18}\text{F}$ ]FTHA and [ $^{18}\text{F}$ ]FDG, whereas the second one was for blood sampling. The arm was heated using an electrically powered cushion to arterialize the venous blood samples. The subjects were placed in a supine position in a PET scanner (GE Discovery TM ST System, Milwaukee, WI, USA).

On the first scanning day, an [ $^{18}\text{F}$ ]FTHA-bolus (155 [SD 9] MBq) was injected. Once the tracer was injected into the vein, the scanning of the chest area was started immediately and continued for 40 minutes (data not used in the present thesis). Dynamic imaging of the abdominal region (frames  $3 \times 300$  seconds) was acquired starting  $\sim 46$  minutes after the tracer injection, followed by the femoral (frames  $3 \times 300$  seconds) region at  $\sim 65$  minutes, and finally  $\sim 80$  minutes after injection the head region (figure 8). Plasma radioactivity was measured with an automatic gamma counter (Wizard 1480 3", Wallac, Turku, Finland) and blood samples for calculation of input function were collected at 4.5, 7.5, 10, 20, 30 and 40 minutes

after the injection. The production of [ $^{18}\text{F}$ ]FTHA was performed as previously described. (DeGrado, Coenen and Stocklin, 1991)

On the second PET study day, approximately 90 minutes after the start of the euglycemic-hyperinsulinemic clamp, [ $^{18}\text{F}$ ]FDG (157 [SD 10] MBq) was injected, and dynamic scanning of the heart started immediately and abdominal area started ~48 minutes after the injection and was followed by femoral and head area similarly to the [ $^{18}\text{F}$ ]FTHA PET. The production of [ $^{18}\text{F}$ ]FDG was performed as previously described. (Hamacher, Coenen and Stöcklin, 1986) To measure the plasma radioactivity for tracer input function, arterialized venous blood samples were collected repeatedly during the scanning. Arterialized venous plasma glucose was determined in duplicate by the glucose oxidase method and the mean of these values was taken to represent the plasma glucose. (Analox GM9 Analyzer; Analox Instruments LTD, London, United Kingdom)



**Figure 8.** The timeline of positron emission tomography (PET) imaging. During the [ $^{18}\text{F}$ ]FDG scanning day, a euglycemic-hyperinsulinemic clamp was started 90 minutes before the injection of the radiotracer. Both in [ $^{18}\text{F}$ ]FTHA and [ $^{18}\text{F}$ ]FDG scanning, the chest area was scanned immediately after tracer injection, the abdomen area was scanned ~50 minutes (48 minutes and 46 minutes, respectively) after the tracer injection, the femoral area ~65 minutes after the injection and the head area ~80 minutes after the injection. [ $^{18}\text{F}$ ]FDG (2- $^{18}\text{F}$ fluoro-2-deoxy-D-glucose); [ $^{18}\text{F}$ ]FTHA (14(R,S)- $^{18}\text{F}$ fluoro-6-thia-heptadecanoic acid); PET (positron emission tomography).

A euglycemic-hyperinsulinemic clamp was performed after a 10-hour fast based on the original protocol by DeFronzo and colleagues (DeFronzo, Tobin and Andres, 1979). The clamp was started 90 minutes before injection of the [ $^{18}\text{F}$ ]FDG PET radiotracer (Fig 8). During the first 4 minutes, a primed-constant insulin (Actrapid, 100 U·ml $^{-1}$ , Novo Nordisk, Bagsvaerd, Denmark) infusion was started at a rate of 480 ml·h $^{-1}$  of body surface area. After the first 4 minutes, the infusion rate was decreased to 240 ml·h $^{-1}$  for three minutes, and then further decreased to 120 ml·h $^{-1}$  for the rest of the clamp. An exogenous glucose infusion was started at 4 minutes after the beginning of the insulin infusion at a rate of the subject's weight

(kg)·0.1g<sup>-1</sup>·h<sup>-1</sup>. The glucose infusion rate was doubled after 10 minutes and then adjusted based on the blood glucose concentration to maintain it as close to 5 mmol·l<sup>-1</sup> as possible. The glucose infusion rate was doubled after 10 minutes and then adjusted based on the blood glucose concentration to maintain it as close to 5 mmol·l<sup>-1</sup> as possible. Blood samples were collected before the clamp and every 5 minutes during the first 30 minutes to adjust the glucose infusion rate. After 30 minutes, the samples were collected every 5 to 10 minutes to determine the glucose concentration for adjusting the glucose infusion rate. The whole-body glucose uptake (M-value) was calculated from the glucose infusion rate and the glucose values obtained in the steady state. For these calculations, it is assumed that endogenous glucose production is completely suppressed by hyperinsulinemia so that the M-value is equal to the amount of glucose infused. M-value was calculated in 20 minutes time spans starting from time point 20 minutes. The first 20 minutes are not included to increase the reliability.

### 5.1.2 PET image analysis

*Adipose tissue.* All the VAT and SAT imaging data was corrected for dead time, decay, and measured photon attenuation, and then reconstructed by scanner software using 3D-OSEM. A Carimas (version 2.9, Turku PET Centre, Finland) was used to analyze all acquired PET-CT images. The regions of interest (ROIs) were drawn manually on the abdominal SAT, on planes superior to the umbilicus, VAT at the level of the umbilicus and in the femoral SAT at the mid-region of the thigh using CT as an anatomical reference. The rate constant (K<sub>i</sub>) for the uptake of radiotracer ([<sup>18</sup>F]FTHA, [<sup>18</sup>F]FDG) into the cells was calculated using tissue time activity curves obtained from the VAT and abdominal and femoral SAT, using a fractional uptake method. Regional GU and FAU were calculated by multiplying regional specific K<sub>i</sub> by corresponding plasma glucose or free fatty acid concentration respectively. A Lumped constant (LC) value 1.0, which is a correction factor accounting for difference in transport and phosphorylation between [<sup>18</sup>F]FDG and glucose, was used for the adipose tissue GU (Nuutila *et al.*, 2000).

*Brain.* All imaging data was preprocessed using SPM8 (Wellcome institute, London, UK). Firstly, all DICOM data was converted into Nifti-format using SPM-DICOM import. Secondly, within each PET session the frame-to-frame misalignments were compensated for by using a mutual information (MI) based rigid registration. A visual inspection was deemed adequate for the alignment of the PET- and CT- data and thus no re-alignment was performed. Thirdly, the CT image was aligned with a CT-template in MNI coordinates using rigid registration, and the mapping was subsequently written to PET data as well. CT-based normalization

(non-rigid registration) was conducted with a Clinical Toolbox (Rorden *et al.*, 2012) and the PET imaging data was subsequently warped into the MNI space using the result deformation. The quality of the non-rigid registration was visually inspected, and only a small number of failures were detected. Final normalization of the PET data was obtained using the ligand-specific template as the target and individual PET image as the source in SPM-Normalize. Both the target and source images were smoothed with a 3D Gaussian filter using an 8 mm kernel (FWHM).

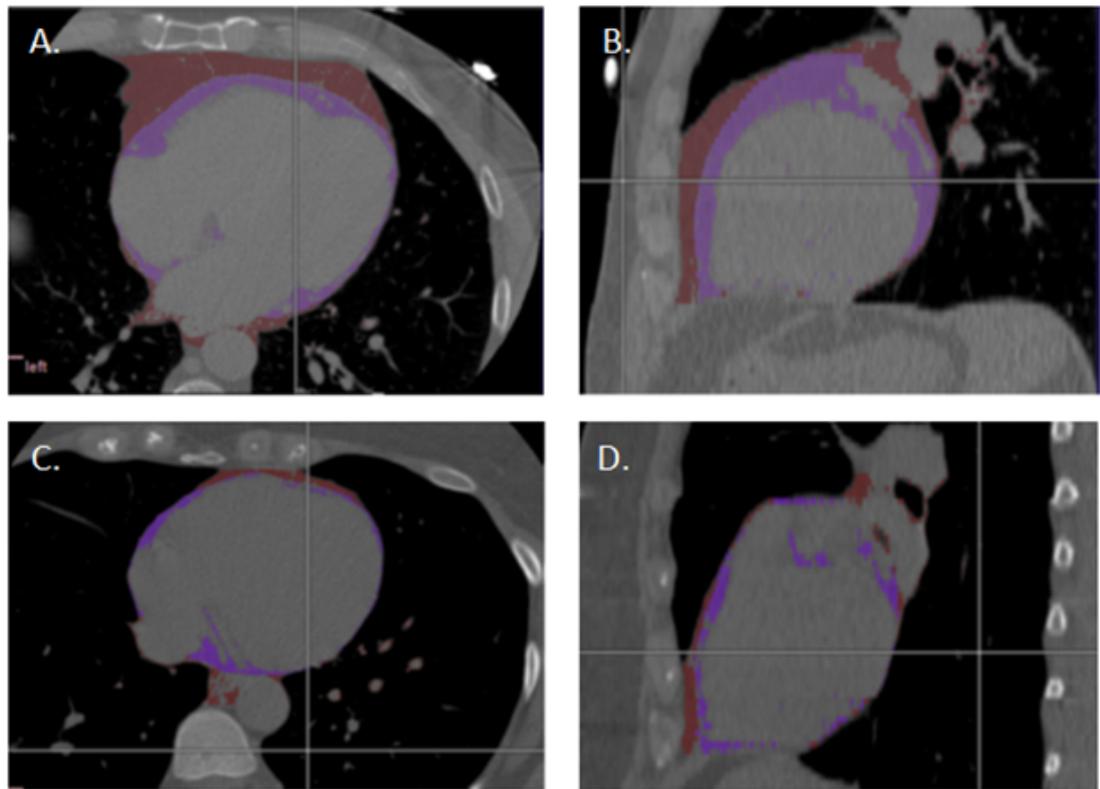
Fractional uptake rates (FUR) were calculated regionally relative to the concentration of the tracer in the plasma, and subsequently the metabolic rate of the glucose, or GU, was calculated relative to individual glucose concentration in the blood. For the calculation of FAU, an [<sup>18</sup>F]FTHA metabolite correction was performed for the radioactivity curves (Eskelinen *et al.*, 2015). Plasma and tissue time-radioactivity curves were analyzed graphically by linear graphical analysis of brain uptake relative to the metabolite-corrected arterial input function (Camici *et al.*, 1986). To obtain brain GU and FAU, the fractional uptake rate values were multiplied by the serum glucose and FFA concentration during the [<sup>18</sup>F]FDG and [<sup>18</sup>F]FTHA PET scanning and corrected for brain density (1.04 g·mL<sup>-1</sup>), respectively. A LC 0.65 was used for the brain GU (Wu *et al.*, 2003).

Region-of-interest (ROI) analysis was conducted using common manually delineated ROIs in MNI space. The ROIs were delineated on an MRI-template using a Carimas (version 2.9, Turku PET Centre, Finland). Similar to Kemppainen and colleagues, the ROIs were placed on the anterior cingulate cortex (ACC), the medial frontal cortex (MFC), the dorsal superior frontal gyrus (SFC), the temporal cortex (TC), the occipital cortex (OC), the thalamus (THA), the cerebellum (CER) and the pons (Kemppainen *et al.*, 2005). Whole brain uptake was measured using ROI covering the frontal, temporal, occipital, and parietal lobes. The brain networks were visualized with the BrainNet Viewer (<http://www.nitrc.org/projects/bnv/>) (Xia, Wang and He, 2013).

## 5.2 Computed tomography (CT)

The volumes of epi- and pericardial fat were determined using a CT with a 64-row GE Discovery VCT PET/CT scanner (General Electric Medical Systems, Milwaukee, WI, USA) using the fat volume quantification by Mahabadi and colleagues (Mahabadi *et al.*, 2009). The fat depots were measured from the calcium score CT images. A gated cine CT was done with a 512·512 matrix and a total of 64 slices were acquired with a rotation time of 0.4s, 120kV and 200mA. The axial thickness was 2.5mm. Images were reconstructed with an Std reconstruction algorithm using a DFOV of 25cm. The data analyst was blinded for the order of pre vs. post images

and analyzed images in a random order using a Carimas (version 2.9, Turku PET Centre, Finland). First, the pericardium was outlined at each cross-section. The fat inside the ROI was defined as the epicardial fat. Secondly, intrathoracic fat was determined, including both epi- and pericardial fat, by drawing regions of interests on the thoracic wall. The pericardial fat volume was determined as the difference between the intrathoracic fat and epicardial fat volume. Finally, the fat volume was defined as pixels within a window of -195 to -45 Hounsfield units (Yang *et al.*, 2013). Quantification of epi- and pericardial fat from the CT scans can be considered to be one of the most accurate methods in use due to its high spatial and temporal resolution and 3D-viewing (Davidovich, Gastaldelli and Sicari, 2013).



**Figure 9.** CT-analysis view of epi- and pericardial fat depots after drawing the ROIs and adjusting the intensity. Purple color stands for epicardial fat and red color for pericardial fat. Figures A and C are horizontal plane and B and D sagittal plane. Figures A and B are from a subject with high volume of epi- and pericardial fat and C and D from subject with low volume of fat.

### 5.3 Magnetic resonance spectroscopy (MRS)

The MRS was performed after an 8 hour fast during the first scanning day. The myocardial triglyceride content (MTC) was determined with a  $^1\text{H}$ -MRS (Philips Gyroscan Intera 1.5 T CV Nova Dual Scanner, Philips Medical Systems, Netherlands with a SENSE body coil). This method is based on different chemical shifts

of water and fat. The volumes of interests were placed on an interventricular septum using both 4ch and short-axis images. The Voxel size was 12mm·10mm·15mm. The molecular contents of lipids and water were determined using single-voxel proton spectroscopy with a PRESS sequence and using a TE of 30 msec and TR of 3000 msec. The measurement was triggered by the heartbeat, a typical triggering delay time was 350 msec. Spectras were collected as a time series with ten separate measurements done in breath holds. Four spectras were used to calculate the average MTC. The measurement was performed twice, once with water suppression and once without it. Thus, the total number of averages was 40 for the spectrum with water suppression and 40 for the spectrum without water suppression. The data was analyzed using a linear combination of the model spectra software package (LCModel) version 6.3-0C with the LCMgui (Provencher, 1993). The results were corrected as described earlier (Lehto *et al.*, 2012). The data quality was inspected both visually (fit quality and residue) and numerically (fit standard deviation of  $\leq 30\%$ ). <sup>1</sup>H-MRS evaluation of MTC has been previously validated by Felblinger and colleagues. (Felblinger *et al.*, 1999).

## 5.4 Magnetic resonance imaging (MRI)

The MRI studies were done to measure the masses of SAT and VAT depots. Scans were done using Philips Gyroscan Intera 1.5 T CV Nova Dual scanner (Philips Medical Systems, the Netherlands). Whole body (from head to knee) axial T1 weighted, dual fast field, echo images (TE 2.3 and 4.7 ms, TR 120 msec, slice thickness 10 mm without gap) were obtained. To measure different adipose tissue masses the images were analyzed using SliceOmatic software v. 4.3 (<http://www.tomovision.com/products/sliceomatic.htm>). To obtain the weight, the pixel surface area was multiplied by the slice thickness and the density of adipose tissue 0.9196 kg·l<sup>-1</sup> (Abate *et al.*, 1994).

## 5.5 Other measurements

### 5.5.1 Aerobic fitness test

Aerobic fitness was determined with a peak oxygen uptake ( $VO_{2\text{peak}}$ ) cycling ergometer test (ergoline 800 s; VIASYS Healthcare, Germany). The test was performed at the Paavo Nurmi Center (Turku, Finland) approximately one week before the first training session and 96 hours after the last training session. The subjects were asked to fast for two hours before testing. The initial workload was 50

W and was increased by 30 W every two minutes until volitional exhaustion. Ventilation and gas exchange (Jaeger Oxycon Pro, VIASYS Healthcare, Germany) were measured and reported as the mean value per minute. The peak respiratory exchange ratio was  $\geq 1.15$  and peak blood lactate (measured from the fingertip capillary samples; YSI2300 Stst Plus, YSI Incorporated Life Sciences, USA) was  $\geq 8.0 \text{ mmol}\cdot\text{l}^{-1}$  for all tests obtained immediately and 1 minute after exhaustion (YSI 2300 Stst Plus, YSI Incorporated Life Sciences, USA). A peak heartrate (RS800CX, Polar Electro Ltd., Kemple, Finland) was within 10 beats of an age-appropriate reference value ( $220 - \text{age}$ ) except for one subject. The highest 1-minute mean value of oxygen consumption ( $\text{VO}_{2\text{peak}}$ ) related to bodyweight ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) 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 measure of maximal performance.

### **5.5.2 Analysis of body composition**

Body composition was determined using the bioimpedance monitor (InBody 720; Mega Electronics Ltd., Kuopio, Finland) in a fasted state at the same time of the day before and 96 hours after the training intervention. The measurement was performed at the Paavo Nurmi Centre, University of Turku.

### **5.5.3 Oral glucose tolerance test (OGTT)**

The oral glucose tolerance test (OGTT) was performed after at least 10 hours of fasting. A 330 ml solution containing 75 grams of glucose (Nutrical®, Nutricia Medical, Turku, Finland) was given and blood samples were taken at the baseline and at 15, 30, 60, 90, 120 minutes during the test, in order to determine glucose and insulin concentrations for the glycemic status.

### **5.5.4 Framingham risk score (FRS)**

FRS, which is a 10-year cardiovascular disease risk score, was calculated based on age, systolic blood pressure, treatment of hypertension, diabetes, HDL, and total cholesterol (D'Agostino *et al.*, 2008).

### 5.5.5 Blood sampling

Plasma total and HDL cholesterol, TG, and fasting glucose were measured from the venous blood samples with an automatized enzymatic assay and insulin using automatized electrochemiluminescence immunoassay (Cobas 8000; Roche Diagnostics GmbH, Mannheim, Germany). LDL cholesterol concentration was calculated using the Friedewald formula. Blood samples were collected before and 96 h after the last training session.

The concentration of D- $\beta$ -hydroxybutyrate was quantified from serum using high-throughput proton NMR metabolomics (Brainshake Ltd, Helsinki, Finland). Details of the experimentation and applications of the NMR metabolomics platform have been described previously (Soininen *et al.*, 2015). Blood lactate concentration was measured from fingertip capillary samples before and within 1 minute after each training session with a handheld lactate analyzer (Lactate Pro, Arkray KDK, Kyoto, Japan) and in fasting conditions from blood samples during the [ $^{18}\text{F}$ ]FDG and [ $^{18}\text{F}$ ]FTHA PET measurements (Lactate Pro, Arkray KDK, Kyoto, Japan).

## 5.6 Statistical analysis

Sample size was calculated for the whole study (NCT01344928) based on its primary outcome (skeletal muscle GU). 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%. There are no previous studies regarding the effects of SIT and MICT in brain and adipose tissue metabolism and therefore the sample size calculated for the entire study was used. The final number of subjects recruited was 28 and 26 for the healthy and IR groups, respectively.

Together, there were seven drop-outs in the study, of which two were from the healthy group and five from the IR group. In the healthy group, both subjects dropped out during the training intervention, one from the MICT group because of hip pain and one from the SIT group because of personal reasons. In the IR group, there were two drop-outs in the SIT group; of which one discontinued before the intervention because of claustrophobic feelings in the PET scanner and one during the intervention, and three dropped-out of the MICT group; all for personal reasons.

Statistical analyses were performed using SAS (version 9.3 for Windows, SAS institute Inc., Cary, NC, USA). The normal distribution of the variables was tested with the Shapiro-Wilkin test and evaluated visually. Logarithmic or square root transformations were performed when appropriate, to achieve normal distribution in parameters. The baseline characteristics of the groups were compared by a two-way analysis of variance including the main effect of health status (healthy and IR, studies I-II), training mode (SIT and MICT, studies I-III) and their interaction (health status\*training mode, studies I-II). Pre- and post- measurements were analyzed using a hierarchical linear mixed model suitable for repeated measurements (PROX MIXED procedure). In the model, the IR (I-II), training mode (I-III) and time (I-III) effects were included as well as all interactions (most important results are seen in table 3). When a significant interaction was observed, pre-determined contrasts were calculated (the Fisher's LSD test) within the model in order to study the group-wise differences. Subjects with missing values (drop outs and those with technical problems) were all included in this model. Hence, the model-based mean (SAS least square means) values [95% CI] from all the parameters measured before and after the training were reported. Correlation analyses were carried out using Pearson's Correlation. A p-value of less than 0.05 (two-tailed) was considered statistically significant. All the data are presented as mean values [95% confidence interval, CI].

## 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 (I-III)

At the baseline, the IR men had higher body adiposity, impaired blood lipid profile and glucose homeostasis, higher blood pressure and an increased Framingham risk score (FRS) compared to the healthy controls (Table 3). The exercise groups were well matched at the baseline. Body mass, BMI, glucose homeostasis, NEFAs and FRS scores remained unchanged after the training intervention (Table 4). However, cholesterol values and whole-body fat percentage improved after both training modes, but not differently between healthy and IR subjects. Diastolic and systolic blood pressure were positively affected by exercise training. The glucose and plasma FFA concentrations during both PET scans were similar between healthy and IR groups and did not change after the intervention (data not shown).

**Table 3. Descriptive statistics and results of two-way analysis of variance for baseline characteristics for healthy subjects and subjects with insulin resistance (IR) in both SIT and MICT groups.** The p-value for 'health status' describes the statistical significance between healthy (n=28) and IR (n=16) men and 'training group' compares all SIT-trained (n=23) to all MICT-trained (n=21). 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  $\square$  to achieve the normal distribution. The values are LSmeans translated into the original unit. SIT (sprint interval training), MICT (moderate intensity continuous training), IR (insulin resistance), BMI (Body mass index), BP (blood pressure), SAT (subcutaneous adipose tissue), MTC (myocardial triglyceride content), HDL (high density lipoprotein), LDL (low density lipoprotein), FFA (free fatty acid), fP (fasting plasma), fS (fasting serum), HbA1c (glycated hemoglobin),  $VO_{2peak}$  (aerobic capacity), FRS (Framingham risk score).

	Healthy men		IR men		P-values	
	SIT	MICT	SIT	MICT	Health status	Training group
N	14	14	9	7	-	-
Age(y)	47 [45;50]	48 [45;51]	47 [44;50]	47 [44;51]	0.8	0.8
Body mass(kg)	83.1 [77.4;88.8]	84.1 [78.4;89.8]	96.2 [89.1;103.4]	96.5 [88.4;104.5]	<0.001	0.8
BMI(kg·m <sup>-2</sup> )	25.9 [24.4;27.3]	26.4 [25.0;27.8]	29.8 [27.9;31.7]	31.1 [29.1;33.2]	<0.001	0.3
BP systolic(mmHg)	124 [120;128]	128 [124;132]	133 [128;138]	146 [140;151]	<0.001	<0.001
BP diastolic(mmHg)	78 [75;81]	80 [77;83]	87 [82;91]	88 [83;93]	<0.001	0.3
Body fat(%)	22.2 [19.8;24.7]	22.9 [20.4;25.3]	29.3 [26.0;32.5]	28.4 [24.9;31.8]	<0.001	0.9
Abdominal SAT(kg) <sup>□</sup>	3.9 [3.1;4.8]	4.3 [3.5;5.2]	6.4 [5.1;7.9]	5.8 [4.5;7.4]	<0.001	0.96
Visceral fat(kg) <sup>□</sup>	2.7 [1.9;3.6]	2.5 [1.8;3.3]	4.2 [3.0;5.7]	4.5 [3.1;6.1]	0.003	0.99
Epicardial fat(ml)*	92 [77;110]	78 [64;94]	131 [107;161]	157 [124;199]	<0.001	0.98
Pericardial fat(ml)	157 [123;191]	139 [103;174]	228 [189;267]	211 [167;256]	<0.001	0.4
MTC(%)*	0.42 [0.26;0.68]	0.53 [0.33;0.86]	0.65 [0.35;1.22]	0.48 [0.25;0.89]	0.6	0.9
Cholesterol(mmol·l <sup>-1</sup> )*	5.2 [4.7;5.8]	4.6 [4.2;5.1]	4.5 [4.0;5.2]	4.9 [4.2;5.7]	0.5	0.7
HDL(mmol·l <sup>-1</sup> )*	1.4 [1.2;1.6]	1.4 [1.2;1.6]	1.2 [1.0;1.4]	1.2 [1.0;1.5]	0.1	0.97
LDL(mmol·l <sup>-1</sup> )*	3.4 [3.0;3.8]	2.9 [2.5;3.3]	2.5 [2.0;3.0]	3.0 [2.4;3.5]	0.1	0.9
FFAs (mmol·l <sup>-1</sup> )*	0.6 [0.5;0.8]	0.7 [0.6;0.9]	0.9 [0.7;1.1]	0.9 [0.6;1.2]	0.04	0.5
Triglycerides <sub>tr</sub> (mmol·l <sup>-1</sup> )*	1.0 [0.8;1.2]	0.9 [0.7;1.1]	1.6 [1.2;2.2]	1.8 [1.3;2.4]	<0.001	0.98
Glucose <sub>tr</sub> (mmol·l <sup>-1</sup> )*	5.3 [5.0;5.7]	5.6 [5.2;6.0]	7.5 [7.1;8.0]	7.0 [6.4;7.5]	<0.001	0.5
Insulin <sub>tr</sub> (mmol·l <sup>-1</sup> )*	4.7 [3.4;6.5]	4.7 [3.4;6.5]	14.6 [9.7;21.8]	14.4 [9.1;22.8]	<0.001	0.99
HbA1c(%)	5.5 [5.3;5.7]	5.6 [5.3;5.8]	5.7 [5.4;6.0]	5.8 [5.5;6.1]	0.07	0.5
HbA1c(mmol·l <sup>-1</sup> )*	36.6 [34.1;40.0]	37.5 [35.0;40.0]	39.2 [36.1;42.3]	40.2 [36.8;43.8]	0.07	0.5
M-value(μmol·kg <sup>-1</sup> ·min <sup>-1</sup> )*	35.0 [26.9;45.6]	29.5 [22.2;39.2]	14.1 [9.7;20.5]	15.6 [10.8;22.7]	<0.001	0.8
VO <sub>2peak</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	34.7 [32.6;36.9]	33.7 [31.5;35.8]	28.0 [25.1;30.8]	30.9 [27.8;33.9]	0.001	0.5
VO <sub>2peak</sub> (l·min <sup>-1</sup> )	2.9 [2.7;3.1]	2.8 [2.6;3.0]	2.5 [2.3;2.8]	3.0 [2.7;3.3]	0.04	0.1
FRS score(%)*	6.1 [5.0;7.5]	6.0 [4.9;7.3]	12.8 [9.8;16.7]	15.5 [11.7;20.6]	<0.001	0.5

**Table 4. Intervention induced within- group changes and different responses between the healthy subjects and subjects with insulin resistance (IR) and SIT (sprint interval training) and MICT (moderate intensity continuous training) groups.** The 'Change' describes the difference between pre-intervention (table 3) and post-intervention value in percentages. The p-value for 'time' describes the effect of the intervention on each variable in the whole group (n=44). The interactions of groups and intervention are represented in columns 'Time\*health status', 'Time\*training group' and 'Time\*health status\*training group'. 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 normal distribution. The values are L:means translated into original unit. BMI (Body mass index), BP (blood pressure), HDL (high density lipoprotein), LDL (low density lipoprotein), FFA (free fatty acid), RP (fasting plasma), fS (fasting serum HbA1c, (Glycated hemoglobin), FRS (Framingham risk score).

Variable	Healthy				IR				Interactions			
	SIT POST	Change (%)	MICT POST	Change (%)	SIT POST	Change (%)	MICT POST	Change (%)	Time	Time*health status	Time*training group	Time*health status*training group
Body mass (kg)	82.6 [76.9;88.3]	-1 %	84.1 [78.4;89.8]	±0 %	96.0 [88.8;103.1]	±0 %	96.4 [88.3;103.5]	±0 %	0.2	0.8	0.4	0.7
BMI (kg/m <sup>2</sup> )	25.7 [24.3;27.1]	-1 %	26.4 [24.9;27.8]	±0 %	29.7 [27.8;31.6]	±0 %	31.1 [29.1;33.1]	±0 %	0.2	0.7	0.3	0.6
BP systolic (mmHg)	125 [120;129]	1 %	131 [127;135]	2 %	131 [126;136]	-2 %	137 [131;143]	-6 %	0.2	<b>0.02</b>	0.5	0.2
BP diastolic (mmHg)*	78 [75;82]	±0 %	81 [77;83]	±0 %	81 [76;84]	-8 %	83 [78;87]	-7 %	<b>0.03</b>	<b>0.03</b>	0.9	0.9
Body fat (%)	21.2 [18.7;23.6]	-5 %	22.1 [19.7;24.6]	-4 %	28.2 [25.0;31.5]	-4 %	27.9 [24.4;31.4]	-2 %	< <b>0.001</b>	0.7	0.4	0.8
Subcutaneous fat (kg)□	3.8 [3.0;4.7]	-3 %	4.3 [3.4;5.2]	-1 %	6.3 [5.0;7.8]	-1 %	5.8 [4.4;7.3]	-1 %	<b>0.03</b>	0.9	0.7	0.8
Visceral fat (kg)□	2.6 [1.8;3.5]	-4 %	2.4 [1.7;3.2]	-5 %	4.1 [2.9;5.6]	-2 %	4.1 [2.8;5.7]	-8 %	<b>0.002</b>	0.5	0.2	0.3
Total cholesterol (mmol•l <sup>-1</sup> )*	4.5 [4.1;5.0]	-16 %	4.3 [3.9;4.8]	-8 %	4.0 [3.5;4.5]	-14 %	4.7 [4.0;5.5]	-4 %	< <b>0.001</b>	0.5	<b>0.04</b>	0.8
HDL (mmol•l <sup>-1</sup> )*	1.2 [1.1;1.4]	-11 %	1.3 [1.1;1.5]	-5 %	1.0 [0.9;1.2]	-14 %	1.1 [0.9;1.4]	-8 %	< <b>0.001</b>	0.6	0.2	0.99
LDL (mmol•l <sup>-1</sup> )	2.8 [2.4;3.3]	-19 %	2.7 [2.3;3.1]	-7 %	2.3 [1.8;2.9]	-9 %	2.8 [2.2;3.5]	-4 %	<b>0.001</b>	0.2	0.1	0.3
FFA <sub>fast</sub> (mmol•l <sup>-1</sup> )*	0.6 [0.5;0.8]	-1 %	0.6 [0.5;0.8]	-18 %	0.9 [0.7;1.3]	6 %	0.7 [0.5;1.0]	-19 %	0.3	0.8	0.2	0.8
Triglycerides <sub>fast</sub> (mmol•l <sup>-1</sup> )*	0.9 [0.7;1.2]	-6 %	0.7 [0.6;0.9]	-23 %	1.4 [1.0;1.9]	-19 %	1.6 [1.1;2.2]	-12 %	0.051	0.9	0.8	0.4
Glucose <sub>fast</sub> (mmol•l <sup>-1</sup> )	5.7 [5.3;6.1]	6 %	5.8 [5.4;6.2]	4 %	7.2 [6.7;7.7]	-4 %	7.2 [6.7;7.8]	4 %	0.2	0.1	0.2	0.07
Insulin <sub>fast</sub> (mmol•l <sup>-1</sup> )*	5.7 [4.0;5.1]	18 %	6.0 [4.3;8.4]	21 %	13.7 [8.9;21.2]	-6 %	13.4 [8.3;21.7]	-7 %	0.4	0.1	0.9	0.9
HbA1c (%)	5.4 [5.2;5.6]	-2 %	5.3 [5.1;5.5]	-5 %	5.5 [5.2;5.8]	-4 %	5.7 [5.3;6.0]	-3 %	< <b>0.001</b>	0.8	0.3	0.1
HbA1c (mmol•l <sup>-1</sup> )	35.3 [32.8;37.8]	-3 %	34.3 [31.8;36.9]	-8 %	37 [33.8;40.2]	-6 %	38.5 [34.9;42.1]	-4 %	< <b>0.001</b>	0.8	0.3	0.1
VO <sub>2peak</sub> (L•min <sup>-1</sup> )	3.0 [2.8;3.2]	4 %	2.9 [2.7;3.1]	3 %	3.0 [2.8;3.2]	6 %	2.9 [2.6;3.2]	-3 %	<b>0.007</b>	0.2	<b>0.006</b>	<b>0.04</b>
FRS Score %*	5.9 [4.8;7.3]	-3 %	6.2 [5.1;7.6]	4 %	12.0 [9.1;15.8]	-6 %	14.0 [10.4;18.5]	-13 %	0.2	0.2	0.9	0.4

## 6.2 Aerobic capacity and whole-body insulin sensitivity (I-III)

*Aerobic capacity.* At the baseline, IR subjects had a 16% lower aerobic capacity compared to healthy men ( $p < 0.001$ , Fig. 10A). Exercise training improved aerobic capacity in the whole study population ( $n = 54$ ) by 3% with no significant differences in the training response between the healthy and IR groups (4% and 2%, respectively, time  $p = 0.003$ , time\*health status  $p = 0.1$ , Fig. 10A). The improvement in  $VO_{2peak}$  was seen also when calculated per whole body both in IR and healthy subjects (from  $2.8 \text{ l}\cdot\text{min}^{-1}$  to  $2.9 \text{ l}\cdot\text{min}^{-1}$ , time  $p = 0.007$ , time\*health status  $p = 0.2$ ). After intervention, the aerobic capacity remained lower in IR subjects compared to the healthy subjects ( $p < 0.001$ ). However, when studied according to the exercise mode in the whole study population, only SIT increased the aerobic capacity significantly and no significant change was seen after MICT (SIT 6% vs. MICT 0.3%, time\*training group  $p = 0.003$ , Fig. 10B). The same finding was seen in the sub-study III, where only IR subjects were included ( $n = 26$ ) and only SIT led to improvement in aerobic capacity (SIT from  $26.6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  to  $27.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , 5%,  $p = 0.002$ ) while no change was seen after MICT.

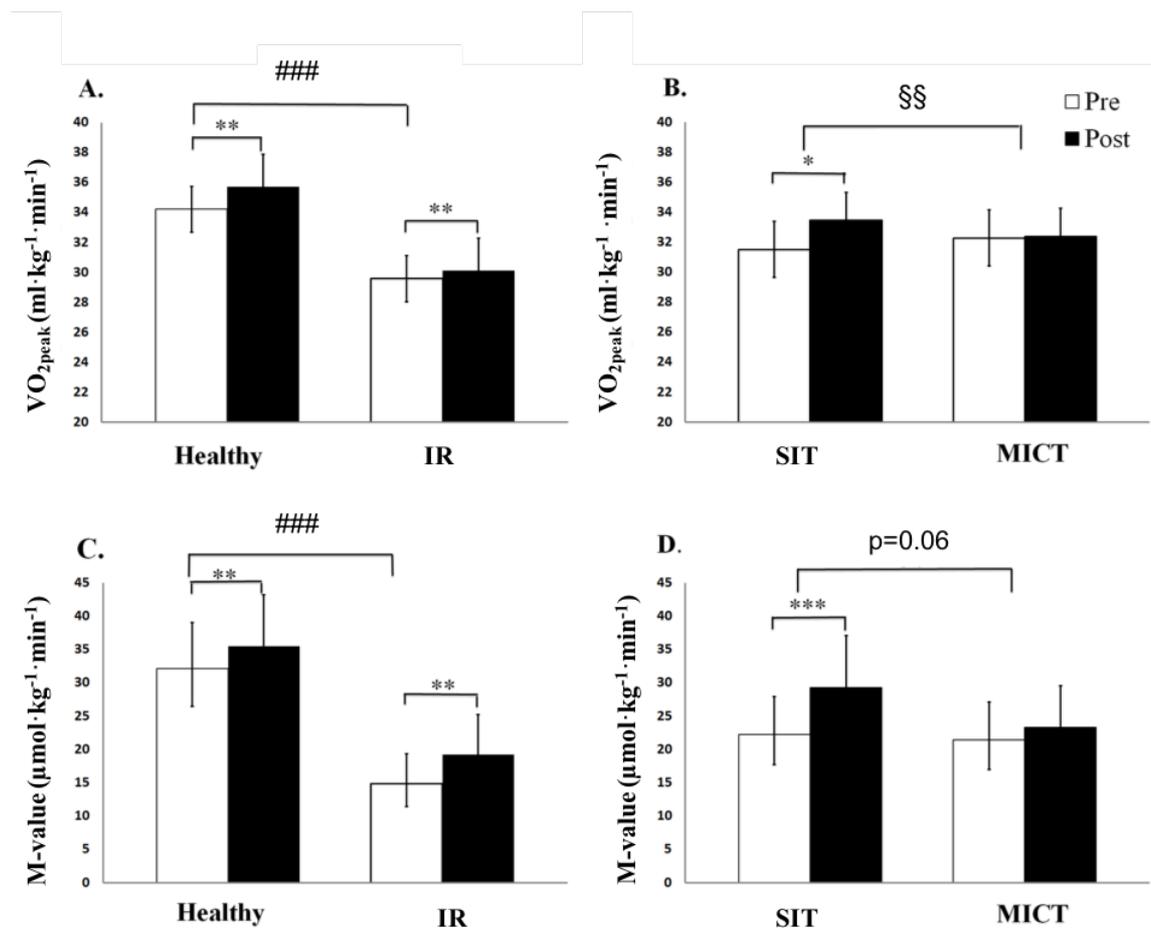


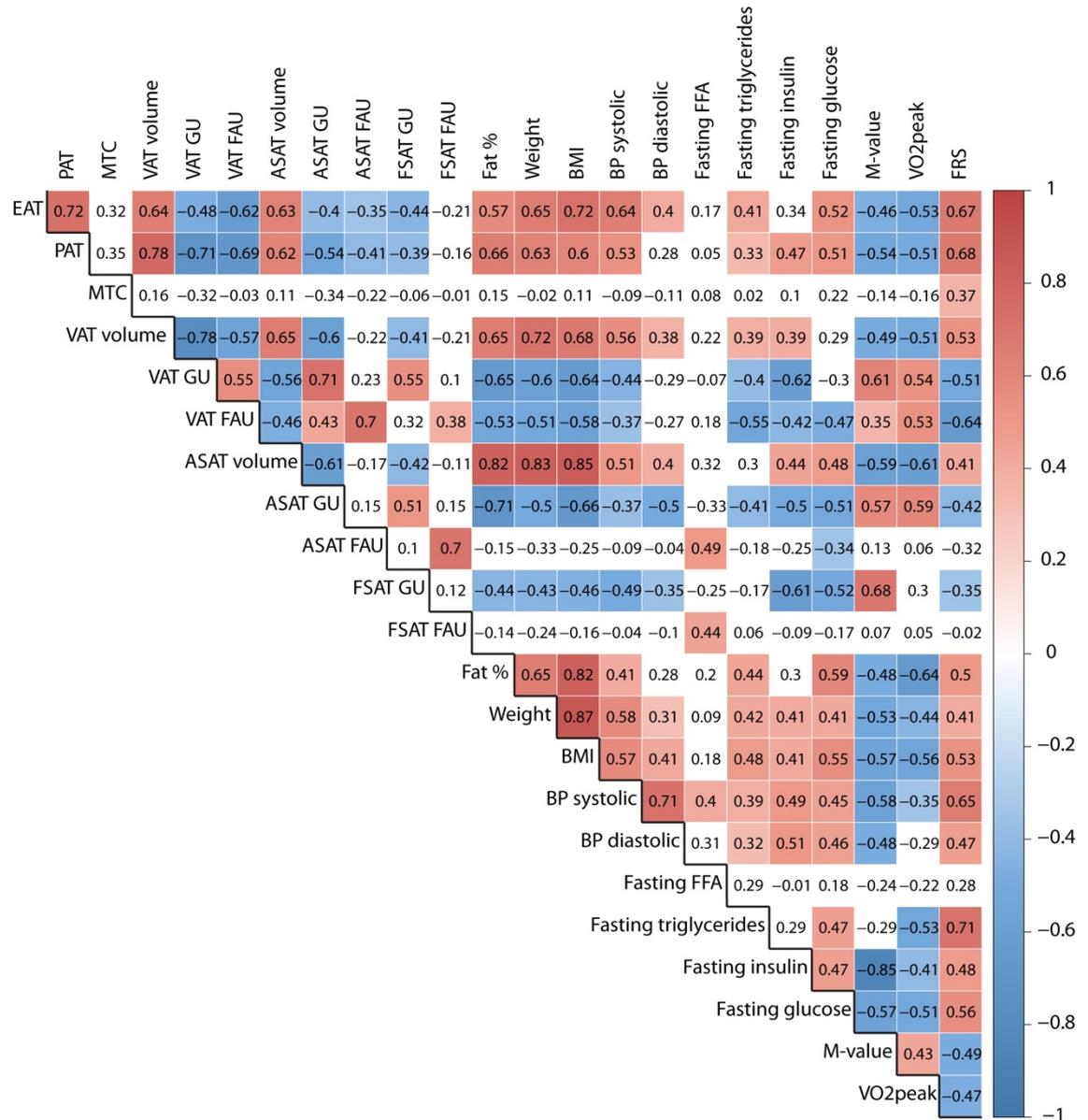
Figure 10. Aerobic capacity ( $VO_{2peak}$ ) and whole-body insulin sensitivity (M-value) at baseline (white bars) and after the training intervention (black bars) in healthy subjects and in subjects with

**insulin resistance (IR) (A and C) according to the training mode (B and D).** SIT, sprint interval training and MICT, moderate intensity continuous training. All the data are presented as mean values [95% confidence interval, CI]. ### $p < 0.001$ ; difference at baseline, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , the effect of exercise training over time (pre-post) within the group, §§ $p < 0.01$ , different response after SIT and MICT.

*Whole-body insulin sensitivity.* Before the intervention, the IR subjects had an impaired whole-body insulin sensitivity with a 54% lower M-value compared to the healthy men ( $p < 0.001$ , Fig. 10C). Insulin sensitivity improved by 17% after the training intervention, both SIT and MICT, in the whole study population with no significant differences in the training response between the healthy and IR groups (10% and 23%, respectively, time  $p = 0.001$ , time\*health status  $p = 0.1$ ). After the intervention, the insulin sensitivity remained lower in the IR subjects compared to the healthy subjects ( $p = 0.004$ ). When studied according to the exercise mode in the whole group ( $n = 54$ ), SIT tended to improve M-value more than MICT (SIT 23% vs. MICT 9%; time  $p = 0.001$ , time\*training group,  $p = 0.06$ , Fig. 10D). However, when comparing only IR subjects, both SIT and MICT led to improvements in M-value (SIT 17% and MICT 24%; time  $p = 0.003$ , time\*training group = 0.9).

### **6.3 Effects of SIT and MICT on adipose tissue glucose and FFA metabolism (I)**

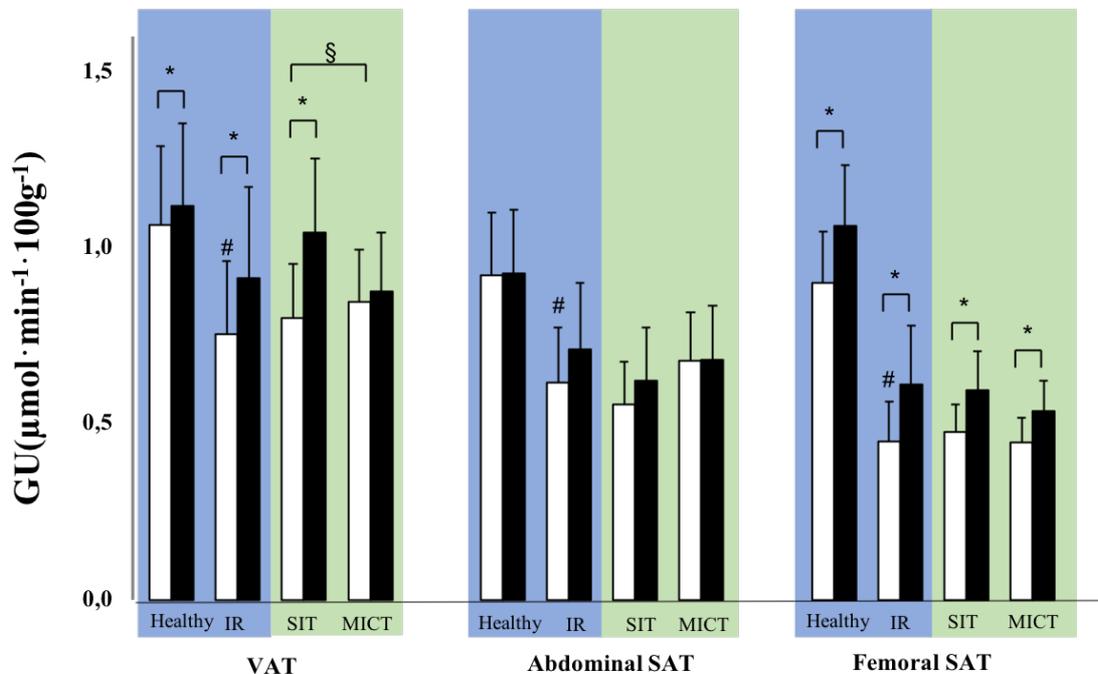
*Baseline data on GU.* At the baseline, when measured per unit, the IR subjects had significantly lower VAT GU (-41%), femoral SAT GU (-49%) and abdominal SAT GU (-101%) levels than the healthy subjects (all  $p < 0.001$ , Fig. 12A). These baseline differences were not seen when comparing the GU per whole tissue in VAT and abdominal SAT (Fig. 12B). Before the intervention, GU correlated positively with whole body insulin sensitivity and aerobic capacity, but negatively with parameters of body adiposity, VAT and abdominal SAT volume, fasting glucose, fasting insulin, and triglycerides in all measured adipose tissue (Fig. 11).



**Figure 11. Pairwise correlations for the measured variables at baseline in the male subjects of the study population (n=44).** The numbers indicate the correlation coefficient for a given pair of variables. Statistically significant correlations ( $p < 0.05$ ) are highlighted with red (positive correlations) or blue (negative correlations) according to the color key on the right. EAT (epicardial adipose tissue), PAT (pericardial adipose tissue), MTC (myocardial triglyceride content), VAT (visceral adipose tissue), ASAT (abdominal subcutaneous adipose tissue), FSAT (femoral subcutaneous adipose tissue), GU (glucose uptake), FAU (fatty acid uptake), fat% (whole body fat percentage), BMI (body mass index), BP (blood pressure), FFA (free fatty acid), M-value (whole body insulin sensitivity),  $VO_{2peak}$  (aerobic capacity).

*Exercise-induced responses in GU.* Exercise training increased VAT GU (time  $p < 0.001$ , time\*health status  $p = 0.10$ ) and femoral SAT GU (time  $p < 0.001$ , time\*health status  $p = 0.27$ ) similarly in the healthy and IR subjects (Fig. 12A). No

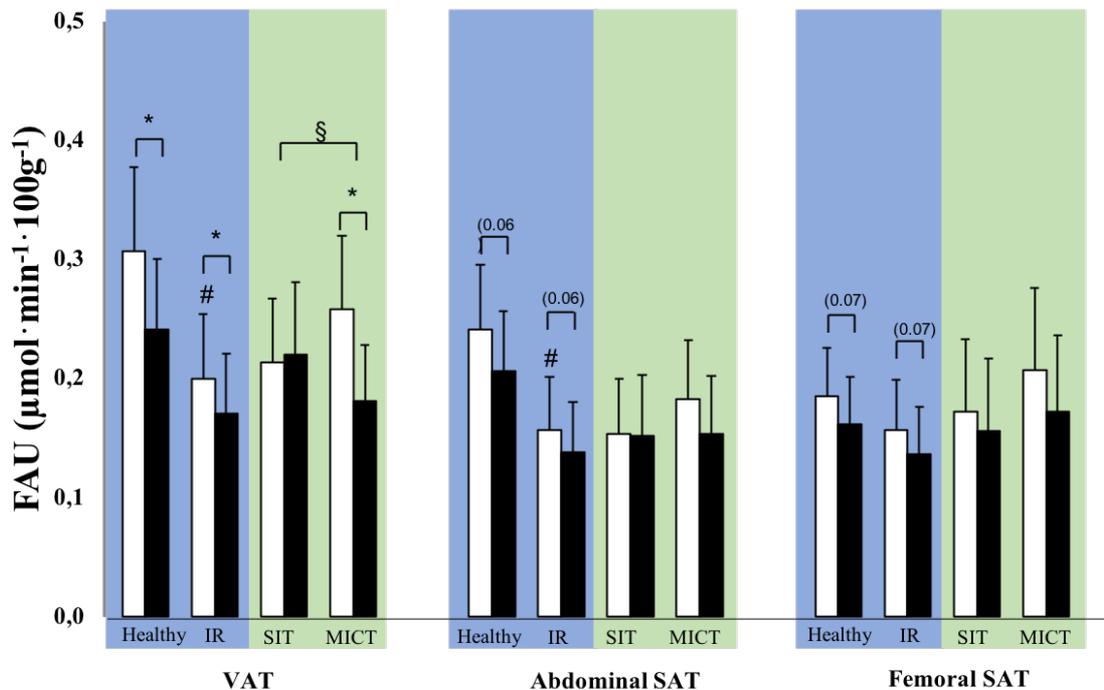
training response was seen in abdominal SAT GU in a whole group level nor when divided according to the disease status or the training mode. Only femoral SAT GU remained lower in the IR group compared to the healthy subjects after the two-week training (74%,  $p < 0.001$ ). The change in VAT GU and abdominal SAT GU was positively associated with the change in whole-body insulin sensitivity ( $r = 0.35$ ,  $p = 0.02$  and  $r = 0.37$ ,  $p = 0.03$ , respectively).



**Figure 12. Insulin stimulated glucose uptake (GU) before (white bars) and after (black bars) the training intervention in visceral adipose tissue, abdominal subcutaneous adipose tissue (SAT) and femoral SAT.** GU is compared in two different comparisons: healthy vs insulin resistant (IR) men (blue) and sprint interval training (SIT) vs moderate intensity continuous training (MICT) in IR subjects (green). All data is expressed as means and (95% CI). # $p < 0.001$ ; difference at baseline between the healthy and IR. \* $p < 0.05$ ; the effect of exercise training over time (pre-post) within the group, § $p < 0.05$ , different response after SIT and MICT.

*Baseline data on FAU.* Before the intervention, when measured per unit, the IR subjects had lower VAT (-209%) and abdominal SAT FAU (-54%) levels than the healthy subjects (both  $p < 0.001$ , Fig. 13A) but no difference in femoral SAT FAU ( $p = 0.3$ ). When FAU was studied per whole depot, the baseline differences were not seen (Fig 13B). In the whole group level, VAT FAU was positively associated with whole-body insulin sensitivity and aerobic capacity and negatively with parameters of body adiposity and fasting values of TGs, insulin, and glucose (Fig. 11). Similar correlations were not seen with abdominal and femoral SAT FAU.

*Exercise-induced responses in FAU.* Exercise training decreased FAU in VAT (time  $p=0.01$ , time\*health status  $p=0.58$ ) and tended to decrease FAU in abdominal and femoral SAT depots (time  $p=0.06$ , time\*health status  $p=0.84$  and time  $p=0.07$ , time\*health status  $p=0.97$ , respectively) similarly in healthy and IR subjects (Fig. 13). After the intervention, FAU remained lower only in abdominal SAT in the IR group compared to the healthy group (54%,  $p<0.001$ ).



**Figure 13.** Fasting free fatty acid uptake (FAU) per 100g before (white bars) and after (black bars) the training intervention in visceral adipose tissue (VAT), abdominal subcutaneous adipose tissue (SAT) and femoral SAT. GU is compared in two different comparisons: healthy vs insulin resistant (IR) men (blue) and sprint interval training (SIT) vs moderate intensity continuous training (MICT) in IR subjects (green). All data is expressed as means and (95% CI). # $p<0.001$ ; difference at baseline between the healthy and IR. \* $p<0.05$ ; the effect of exercise training over time (pre-post) within the group, § $p<0.01$ , different response after SIT and MICT.

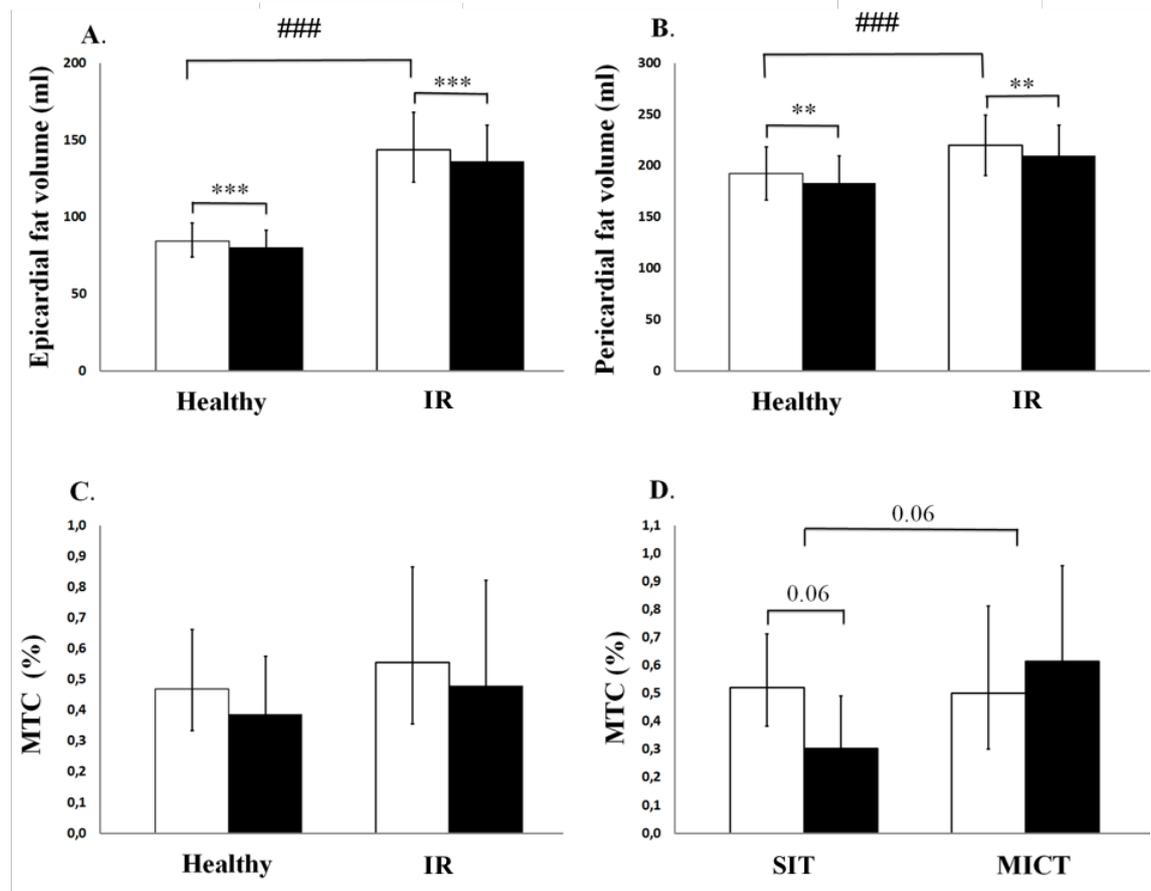
*SIT vs MICT.* When studied according to the training mode, both SIT and MICT improved femoral SAT GU (25% and 20%, respectively, time  $p=0.004$ , time\*training group  $p=0.8$ ). However, the training response was different between the two training modes with only SIT increasing VAT GU (30% vs. 4%, time\*training group,  $p=0.03$ , Fig. 12) and only MICT reducing VAT FAU (-30% vs. 3%,  $p=0.01$ , Fig 13). No response was seen after either training mode in abdominal SAT GU or FAU when only the IR subjects were included.

## 6.4 Effects of SIT and MICT on intrathoracic ectopic fat content (II)

*Baseline data.* At the baseline, the IR subjects had higher epicardial (41%) and pericardial (12%) adipose tissue volumes than the healthy subjects (both  $p < 0.001$ , Fig. 14A&B). MTC did not differ between the healthy and IR subjects (Fig. 14C). The epi- and pericardial fat volumes correlated positively with each other and with markers of adiposity (VAT and abdominal SAT volume, weight, BMI, and whole body fat content) and blood pressure, but negatively with aerobic capacity, whole-body insulin sensitivity and VAT and SAT FAU and GU (Fig. 11).

*Exercise-induced responses.* Training decreased both epi- and pericardial fat volumes by 5% both in the healthy and IR subjects (time  $p < 0.001$ ; time\*health status  $p = 0.8$ ; Fig. 14A&B). Both epi- and pericardial fat remained higher in the IR group compared to the healthy subjects after the two-week training period (39% and 13%,  $p < 0.001$  and  $p = 0.01$ , respectively) and no difference in MTC levels was observed after the intervention between IR and healthy subjects.

*SIT vs MICT.* No difference was observed in the training response between the training modes either in epi- or pericardial fat (time\*training group;  $p = 0.8$  and  $p = 0.9$ , respectively, Fig. 14C). However, the training response tended to be different between the two training modes (time\*training group;  $p = 0.06$ ) with SIT tending to decrease MTC ( $p = 0.06$ , Fig. 14D). Using MTC as the covariant, the different training response between SIT and MICT in  $VO_{2\text{peak}}$  (time\*training group  $p = 0.10$ ) and M-value (time\*training group  $p = 0.23$ ) was no longer significant.



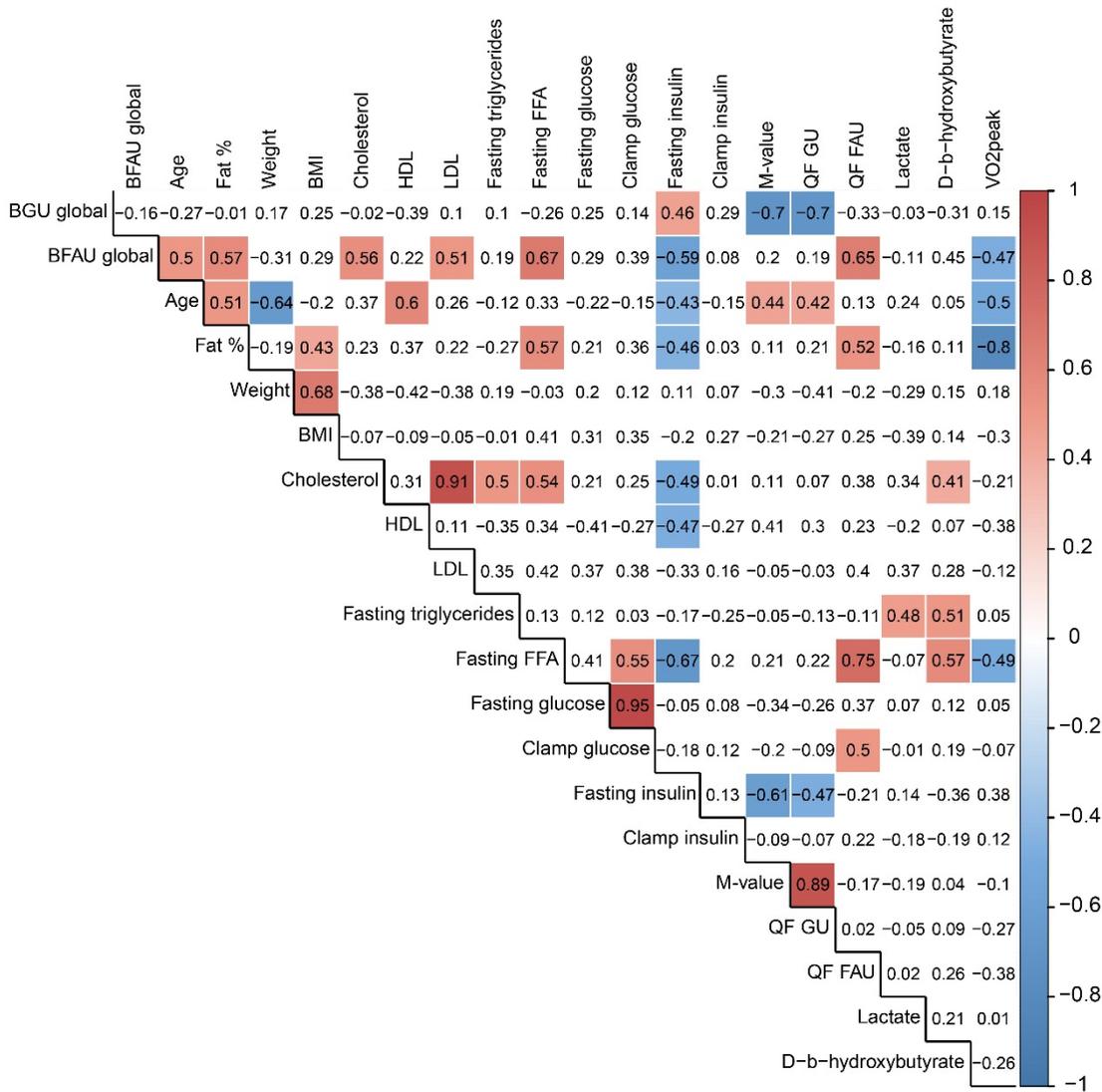
**Figure 14.** Epicardial fat volume, pericardial fat volume, and myocardial triglyceride content (MTC) at baseline (white bars) and after the training intervention (black bars) in healthy subjects and in subjects with insulin resistance (IR) (A, B and C) and MTC, according to the training mode (D). SIT, sprint interval training and MICT, moderate intensity continuous training. All the data are presented as mean values [95% confidence interval, CI]. ### $p < 0.001$ ; difference at baseline, \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , the effect of exercise training over time (pre-post) within the group,  $p = 0.06$ , tendency for different response after SIT and MICT.

## 6.5 Effects of SIT and MICT on brain metabolism in subjects with IR (III)

*Study subjects.* Brain metabolism was measured only in subjects with IR, because the head area was not imaged in healthy subjects. However, both IR women and IR men with a successful image of the head were included, leading to a final number of 21 subjects with pre- image and 15 subjects with pre- and post- image (Table 2).

*Baseline data.* In the whole study group ( $n = 21$ ), at baseline, the global insulin-stimulated brain GU correlated inversely with the whole-body insulin sensitivity (M-value, Fig. 15), positively with the serum insulin concentration in a fasting

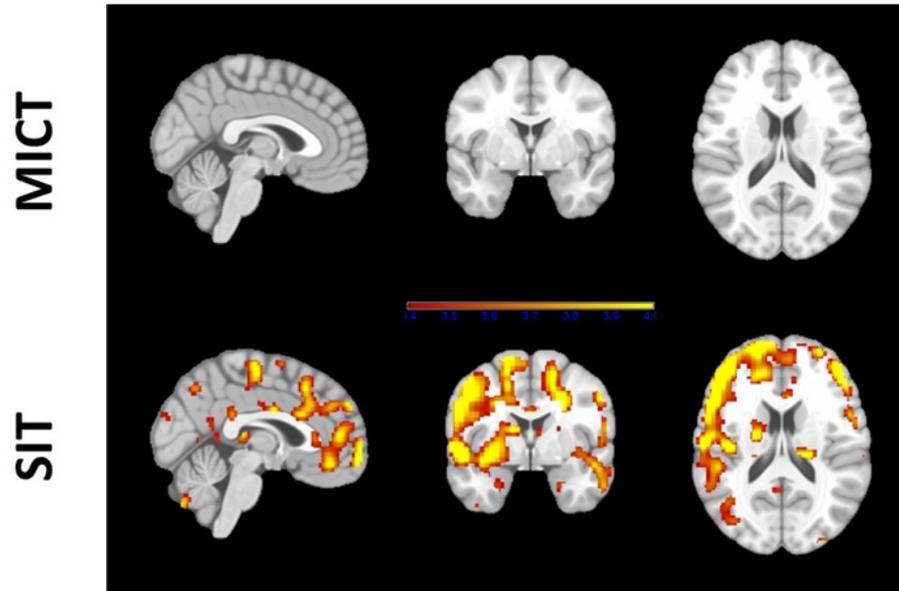
state and tended to correlate with the D- $\beta$ -hydroxybutyrate ( $r=0.4$ ,  $p=0.07$ ). Before the training, the brain FAU correlated positively with the whole-body fat percent, muscle FAU and age (Fig. 15) and inversely with aerobic capacity. In addition, brain FAU correlated with LDL total cholesterol and fasting glucose and insulin levels.



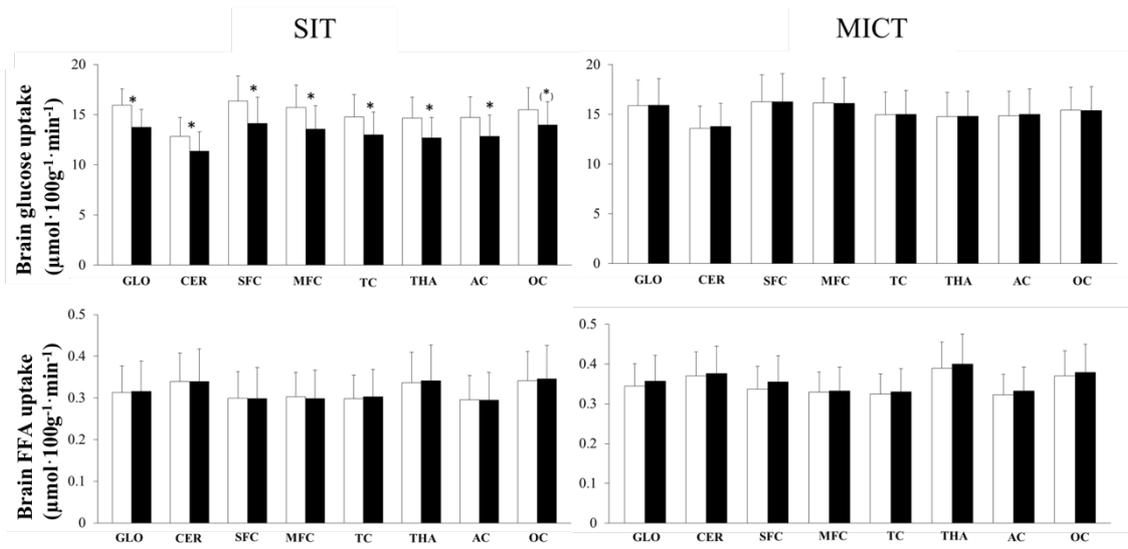
**Figure 15. Pairwise correlations for all measured variables at baseline in male subjects of the study population (n=44).** The numbers indicate the correlation coefficient for a given pair of variables. Statistically significant correlations ( $p<0.05$ ) are highlighted with red (positive correlations) or blue (negative correlations) according to the color key on the right. BGU (brain glucose uptake), BFAU (brain fatty acid uptake), fat% (whole body fat percentage), BMI (body mass index), HDL (high-density lipoprotein), LDL (low-density lipoprotein), FFA (free fatty acid), M-value (whole body insulin sensitivity), QF GU (quadriceps femoris glucose uptake), QF FAU (quadriceps femoris fatty acid uptake), VO<sub>2peak</sub> (aerobic capacity).

*Exercise-induced responses on brain GU and FAU.* Insulin-stimulated brain GU was measured both globally in the gray matter and when divided into discrete brain regions in IR subjects. SIT training induced a decrease in the insulin-stimulated

brain GU globally by 14% ( $p=0.03$ ) and in all regions of the cortex (except the occipital cortex). No change was observed in brain insulin-stimulated GU after MICT (Fig 16 and 17). We did not observe change in the brain FAU in global or regional lever after training (Fig 17).



**Figure 16. Pre vs. post change in brain glucose uptake in different areas of the brain in sprint-interval training (SIT) and moderate intensity continuous training (MICT) groups.**  $P<0.01$  voxel level uncorrected; which is equal to  $T=3.365$  in SIT and  $T=2.896$  in MICT. The bar represents T values.



**Figure 16.** Brain glucose uptake and brain FFA uptake globally and in different areas of the brain in the SIT and MICT training group before and after the training intervention. GLO, global; CER, cerebellum; SFC, superior frontal gyrus; MFC, medial frontal gyrus; TC, temporal cortex; THA, thalamus; AC, cingulate gyrus; OC, occipital cortex. Values are model-based means (95% confidence interval). \* $P < 0.05$  for the time effect within the training mode (pre vs. post comparison).

## 7 DISCUSSION

The present thesis investigated the effects of a two-week exercise training intervention, with either SIT or MICT, on adipose tissue volume and metabolism, and brain metabolism in healthy subjects and in subjects with IR.

The main findings of the thesis were:

1. At the baseline, IR subjects had impaired glucose uptake in their visceral adipose tissue, abdominal and femoral SAT, and increased fat volume in all measured adipose tissue depots (epicardial fat, pericardial fat, abdominal SAT, femoral SAT and visceral fat).
2. Two weeks of either SIT or MICT produced similar increases in GU in femoral SAT, but only after SIT was the VAT GU improved. In contrast, VAT FAU decreased only in response to MICT. Adipose tissue GU in all measured fat depots correlated with aerobic capacity suggesting that a poor aerobic capacity is a marker of defect in adipose tissue glucose metabolism.
3. Both training modes decreased epicardial and pericardial fat volume similarly both in healthy and IR middle-aged men. It appears that exercise training effectively decreases intrathoracic fat volume and thus reduces the risk for cardiovascular diseases. SIT tended to reduce MTC more than SIT.
4. At baseline, brain glucose uptake correlated negatively with whole body insulin sensitivity. Short-term exercise training decreased insulin-stimulated brain glucose metabolism in IR subjects. That result raises the question of whether exercise-induced reduction in brain glucose uptake is beneficial for the brain or not.

## 7.1 The effect of SIT and MICT on different adipose tissue depots

### 7.1.1 Both exercise modes have a beneficial effect on adipose tissue metabolism (I)

The baseline data on adipose tissue GU is in line with the previous studies. First, the thesis showed an inverse relationship between adipose tissue mass and adipose tissue glucose uptake in the measured fat depots, indicating a decreased insulin sensitivity in the expanding fat depots. Similar association has also been found in a previous study with obese and T2DM participants (Oliveira *et al.*, 2015). Secondly, the present results demonstrated a decreased GU in all measured adipose tissues in IR subjects compared to the healthy subjects. The GU was 41% lower in visceral, 49% lower in abdominal SAT and 101% lower in femoral SAT in IR men compared to healthy men. Another study conducted in our laboratory found 60% lower GU per mass in obese subjects compared to lean subjects both in VAT and abdominal SAT (Virtanen *et al.*, 2002). Interestingly, both in the present study and in the previous study by Virtanen *et al.*, when GU was calculated per tissue depot, it was found to be similar in the IR/obese and healthy group. This was observed both in VAT and abdominal SAT depot due to the significantly higher volume of the fat depots in the IR/obese group. Our baseline data of adipose tissue FAU showed partly controversial result compared to previous study conducted in our laboratory (Bucci *et al.*, 2015). The recent finding was that fasting FAU per gram was decreased in IR subjects compared to healthy subjects both in VAT and in abdominal SAT, while the study by Bucci and colleagues showed increased VAT FAU and no difference in abdominal SAT FAU in obese subjects with metabolic syndrome compared to non-obese subjects (Bucci *et al.*, 2015).

A two-week training intervention induced improvements in VAT GU, FAU, and femoral SAT GU both in healthy and IR subjects and tendency to decrease FAU in both SAT depots. Interestingly, the intervention did not have an effect on abdominal SAT GU. As discussed previously, exercise training has been shown to produce a greater reduction in VAT volume compared to SAT volume, which, based on the present results, seems also to be the same in the improvement in glucose metabolism of the adipose tissue. These are novel findings, as no previous study has been studying the exercise training induced responses on adipose tissue metabolism in healthy and IR subjects. Together with the decrease in VAT and abdominal SAT volume after the training intervention, abdominal SAT and VAT GU in the IR group did not differ from the healthy subjects after the intervention.

Most of the previous life-style related studies on adipose tissue metabolism have focused on diet-induced weight loss. Two separate studies measuring GU and FAU

using PET studied the effect of diet-induced weight-loss on adipose tissue metabolism. A study by Viljanen and colleagues did not find improvement in GU in VAT nor in abdominal SAT (both per kg and per tissue) despite significant weight-loss after a 6-week diet ( $p < 0.001$ ) (Viljanen *et al.*, 2009). Furthermore, a study by Bucci and colleagues did not observe significant change in FAU in VAT nor abdominal SAT after a 6-week diet intervention with significant weight loss ( $p < 0.001$ ) (Bucci *et al.*, 2015). Hence, present results could indicate that exercise training improves adipose tissue GU independent of weight loss. To support that suggestion, the present results also showed that both in healthy men and men with IR baseline GU in all measured adipose tissue depots was negatively correlated with  $VO_{2peak}$ .

In addition to exercise-induced adipose tissue responses in subjects with different baseline characteristics (healthy vs IR), one aim was to compare the effects of high-volume MICT and short-term SIT. Due to the fact, that the IR group included both males and females, the effects of MICT and SIT training were compared on the IR subjects only ( $n=26$ ). When comparing different training modes in this group, we observed that only SIT increased both VAT GU and aerobic capacity. As discussed in the literature review, previous studies have found that SIT/HIIT may decrease the volume of VAT and increase expression of GLUT4 more effectively compared to MICT. Thus, training with high intensities activates  $\beta$ -adrenergic receptors in larger extent and results in the higher secretion of lipolytic hormones, which are associated with fat oxidation and post-exercise energy expenditure (Horowitz, 2003). On the other hand, only MICT improved FAU in VAT. The different responses after SIT and MICT might also be explained by different pathways of ATP production during exercise. The ATP used during the MICT training is mainly produced via betaoxidation (aerobic pathway) while during SIT via fast glycolysis (anaerobic pathway). Hence, as SIT has higher effect on VAT GU, it may increase the GLUT4 receptor content more than MICT, and as MICT has a greater effect on VAT FAU, it may increase use of fats as energy source. However, both SIT and MICT led to similar reduction in weight; however, VAT and abdominal SAT, despite both the time spent during the training intervention (SIT 15 vs MICT 300 minutes) and the average calculated energy consumption during the training sessions (SIT 421 vs MICT 2907 kcal) were much less in SIT than MICT. This finding further supports the suggestion that SIT leads to higher delayed oxygen consumption (EPOC) and raises the post-training basal energy metabolism for several hours after the SIT when compared with MICT and thus, leads to similar reduction in body weight (Skelly *et al.*, 2014).

The improvement in SAT GU was only seen in the femoral area. Interestingly, when using femoral muscle GU as a covariant, the increase in femoral SAT GU was no longer significant ( $p=0.8$ ). This, therefore, suggests that improved muscle

GU after exercise training could play a role in the improvement in femoral SAT glucose metabolism. Eskelinen and colleagues has previously published a finding from this same dataset, which showed that the skeletal muscle GU improved only in the femoral muscle while no change was seen with the upper body muscles, which is a similar finding as in the previous study with adipose tissue glucose metabolism (Eskelinen *et al.*, 2015). Thus, differential response in lower and upper body might be explained by higher exercise volume on the lower body during cycling exercise. Probably longer intervention would have been needed to increase GU in abdominal SAT.

In conclusion (I), short-term training improves adipose tissue metabolism similarly in healthy and IR men. SIT is more effective in improving aerobic capacity and VAT insulin sensitivity in IR whereas MICT is preferable for VAT fat metabolism.

### ***7.1.2 Both exercise modes decrease epi- and pericardial fat volumes, but only SIT tends to decrease myocardial triglyceride content (II)***

The baseline data on epicardial and pericardial fat volumes showed increase in fat accumulation in IR men compared to healthy men. This finding is supported by several previous studies (Yang *et al.*, 2013; Vasques *et al.*, 2015; Guglielmi and Sbraccia, 2017). However, in contrast to previous studies, no difference in baseline MTC in healthy and IR groups was observed. Previous studies have shown positive correlation between epicardial fat and VAT volume, which was also seen in the present study (Kim *et al.*, 2009). In addition, other significant correlations between BMI, weight, SAT volume and epicardial fat volume were observed confirming the close relation between epi- and pericardial fat depots and body adiposity. Further, significant correlations between epi- and pericardial fat and markers of insulin resistance (M-value, SAT and VAT GU, fasting insulin, and glucose) were also observed, which supports the previous findings of untypical accumulation of epi- and pericardial fat as a risk factor for IR. Interestingly, we also found negative baseline correlations between aerobic capacity and volumes of epi- and pericardial fat, VAT and SAT and GU were found in all measured adipose tissues. This finding suggests that poor aerobic capacity is a strong predictor of adipose tissue dysfunction.

To further investigate the association between epi- and pericardial fat and coronary heart disease, the Framingham risk score (FRS) was calculated, which is a predictor of a 10-year coronary heart disease risk. FRS has previously shown to be positively correlated with pericardial fat (D'Agostino *et al.*, 2008). As expected, the FRS was higher in the IR compared to the healthy group both before and after the intervention. Furthermore, in the present study both epi- and pericardial fat volume

correlated with the FRS. It has been suggested that pericardial fat would be a better marker than epicardial fat for cardiometabolic diseases (Sicari *et al.*, 2011), but the present data indicates that both epi- and pericardial fat are relevant markers for coronary heart disease risk.

Similar decrease was seen in epi- and pericardial fat volume was seen in healthy and IR men after the training intervention. Epicardial fat decreased by 5.3% and pericardial fat by 5.5% in the whole group with no difference between the two training modes. The reduction in epi- and pericardial fat volumes were at the same level as in VAT (-4.9%) and higher as in abdominal SAT (-1.3%), which suggests that the exercise-induced response in epi- and pericardial fat is similar to VAT. The same finding was observed previously in a 12-week intervention study by Kim and colleagues (Kim *et al.*, 2009). As discussed earlier, the relatively higher reductions in epi- and pericardial fat and VAT are probably due to the higher  $\beta$ -adrenergic receptor density in these fat depots, which leads to higher exercise-induced lipolysis compared to SAT (Nielsen *et al.*, 1991; Goedecke and Micklesfield, 2014).

Previous studies on exercise-induced responses in epi- and pericardial fat in obese and T2DM subjects are scarce. However, as was shown in Table 3 in the literature review, few studies have studied the training responses after endurance and/or resistance training. Two of these studies included a 12-week intervention with moderate-intensity exercise training and were conducted with obese subjects and both found reduction in epicardial fat (Kim and colleagues 9% and Wu and colleagues 7%). Pericardial fat volume was measured only in a study by Wu and colleagues and pericardial fat decreased by 12% after the training. The third study on exercise training and epi- and pericardial fat showed a 20% decrease in pericardial fat, but no change in epicardial fat after a 6-month of MICT intervention in subjects with T2DM (Jonker *et al.*, 2013). In the present study both epi- and pericardial fat was reduced by 5% after only two-week intervention. Wu and colleagues also compared the exercise-induced reduction in epi- and pericardial fat to reduction seen after bariatric surgery. In the exercise group, the subjects performed a 12-week endurance training intervention with 40-minute sessions 3 to 5 times per week combined with a restricted diet. The post measurements were performed 3 months after the interventions. Both interventions led to weight loss (exercise 7.5% and surgery 16.0%). They also found that both exercise training and bariatric surgery lead to similar reduction in epicardial fat, but the reduction in pericardial fat, VAT and SAT was higher after bariatric surgery. Interestingly, they also found that reduction in BMI and reduction in different adipose tissues (epicardial, pericardial, visceral and subcutaneous fat) correlated with the exercise group but not in the bariatric surgery group, suggesting a different mechanism after weight loss. (Wu *et al.*, 2016). Bariatric surgery leads to rapid weight-loss and weight is also reduced

before the surgery by diet. Therefore, the epicardial fat may have reduced already before the bariatric surgery. The present study is the first short-term exercise training study showing that exercise-induced response is rapid as no more than six sessions of training was needed to see the reduction in epi- and pericardial fat. The present study also compared healthy and IR subjects and concludes that the training response is similar despite the higher level of these fat depots in IR subjects before the intervention.

Only one previous study has shown a decrease in MTC after 21-weeks of moderate-intensity exercise training in obese subjects (Schrauwen-Hinderling *et al.*, 2010) but the same research group did not find any decrease in MTC in T2DM subjects after a similar training intervention protocol (Schrauwen-Hinderling *et al.*, 2011). In addition, no change in MTC was observed after a 6-month moderate-intensity training with T2DM subjects (Jonker *et al.*, 2013). However, a single session of exercise has been shown to reduce MTC acutely, supporting the fat storage's role of MTC (Bucher *et al.*, 2014). In the present study, no difference was observed in MTC between healthy and IR subjects at baseline and no training response was seen after the intervention either in healthy or IR subjects. Interestingly, MTC tended to respond differently to the two different training modes (time\*training group  $p=0.06$ ), SIT reducing the MTC more efficiently compared to MICT (Fig.3D). The present study suggests, for the first time, that SIT may be more beneficial in reducing MTC, compared to MICT. It can be carefully speculated that one explanation for this finding is that extremely intense training is needed to strain the myocardium to the point of myocardial energy deficit and further, during a rapid and maximal energy need MTC are mobilized inside the myocyte. As a support for this theory, cross-sectional study by Sai and colleagues showed that endurance trained athletes, who usually use different HIIT protocols in training, had a lower MTC than healthy subjects matched for age, BMI, and body fat percentage (Sai *et al.*, 2013). In the present study, at baseline, MTC correlated with age and FRS but not with other variables, suggesting that MTC is not that strongly associated with body adiposity and metabolism as epi- and pericardial fat are.

In conclusion (II), both SIT and MICT reduce epicardial and pericardial fat volume effectively both in healthy and IR subjects. However, SIT tends to decrease MTC more than MICT.

## 7.2 Exercise training decreases glucose uptake in brain (III)

This study was the first one to investigate the effects of exercise training on brain metabolism in subjects with IR. Studies by Hirvonen and colleagues and Tuulari

and colleagues showed that basal brain GU during insulin stimulation is increased in subjects with IR and metabolic syndrome compared to healthy controls (Hirvonen *et al.*, 2011; Tuulari *et al.*, 2013). This finding suggests that IR subjects need a higher dose of insulin to increase GU in the brain, whereas the effect of insulin is already maximal in healthy subjects. In addition, Kami and colleagues showed that brain FAU is also increased in subjects with metabolic syndrome (Karmi *et al.*, 2010). Finally, Tuulari and colleagues showed that bariatric surgery induced weight-loss led to a decrease in insulin-stimulated GU along with improved whole-body insulin sensitivity (Tuulari *et al.*, 2013). These findings suggest that IR leads to metabolic changes in the brain and that the changes can be altered by weight-loss induced improvements in insulin sensitivity.

Another aim of the present study was to study if short-term exercise training, either SIT or MICT, could lead to a similar response in brain GU as seen after weight loss and that if the exercise training has an effect on brain FAU. At the baseline, brain GU was inversely associated with whole-body insulin sensitivity and muscle GU, meaning that better insulin sensitivity leads to lower brain GU during insulin stimulation. Brain GU was also positively associated with insulin levels during fasting and during hyperinsulinemia. Interestingly, the brain FAU correlated inversely with aerobic capacity and fasting plasma glucose but positively with whole body fat percent, FRS, and total and LDL cholesterol levels. These associations suggest that brain metabolism is linked to metabolic risk factors.

Two-weeks of SIT led to a decrease in insulin-stimulated GU, but interestingly no change was seen after MICT. The reduction in brain GU was seen globally (14%) and also when divided into smaller regions in all areas (12-14%) except in the occipital cortex (9%,  $p=0.08$ ). Similarly, in a study by Kemppainen and colleagues, the decrease in insulin-stimulated brain GU was seen both globally and in all measured regions acutely after exercise with high intensity (Kemppainen *et al.*, 2005). Yet, no change in brain FAU was observed in the present study. The mechanisms behind increased insulin sensitivity in the brain after exercise training and weight loss are not known.

In addition to glucose, the brain can utilize lactate and ketone bodies as a energy substrate. In fact, when lactate is available, the brain prefers lactate over glucose as an energy source and during intense exercise the brain uses lactate in proportion to the arterial concentration. During exercise, lactate and ketone bodies are produced and their level in the blood increases exponentially with an increasing intensity. Thus, the decreased brain GU during acute exercise with increasing intensity has been linked to an increased uptake of lactate in the brain (Ide *et al.*, 2000; Kemppainen *et al.*, 2005). In the present study, SIT led to markedly higher lactate concentrations after each training session compared to MICT (SIT  $14,4 \pm 0,9$  vs

MICT  $3,8 \pm 1,4$  mmol·l<sup>-1</sup>). However, FDG PET scanning was performed at least 72 hours after the last training session, and the acute increase in lactate levels had already disappeared. Thus, no correlation between brain GU and lactate levels measured acutely after the training sessions or on the PET study day 72-hours after the last training session was observed. In addition, the concentration of the ketone body D-  $\beta$  -hydroxybutyrate (DBHB) before and after the training intervention was measured in the present study. Interestingly, DBHB correlated negatively with the brain GU after the training in the SIT group, but not in the MICT group. Thus, it might be that the decrease in glucose uptake after SIT is partly explained by the increased utilization of other energy substrates, such as DBHB.

A very recent [<sup>18</sup>F]FDG-PET study by Robinson and colleagues (2017) showed that 12 weeks of combined HIIT (90% of VO<sub>2max</sub>) and vigorous-intensity exercise (70% of VO<sub>2max</sub>) increased brain GU in healthy participants in selected regions, but not globally, measured in the fasted state. The decrease was seen in the brain areas which are most linked to cognitive impairments and Alzheimer's disease and thus the authors speculate that the increase in brain metabolism may contribute to the previously observed benefits of aerobic exercise in the brain, such as increased brain volume and blood flow and a decreased risk for cognitive disorders (Robinson, Lowe and Nair, 2017). In the present study with the IR subjects, reduced insulin-stimulated brain GU was observed after SIT training. These two studies have been measured under two different physiological conditions and in different study populations and thus cannot be directly compared. In the present study [<sup>18</sup>F]FDG PET was performed during hyperinsulinemia and in the study by Robinson and colleagues during a fasting state and in the presence of low insulin and euglycemia.

In conclusion (III), brain GU is inversely associated with whole body insulin sensitivity at baseline and brain GU decreases in response to SIT training, whereas no changes are seen in brain FAU after short term training. The mechanism of regulating brain GU are not known and need to be studied in the near future.

### **7.3 Which one to choose, SIT or MICT? (I-III)**

SIT led a higher increase (6%) in aerobic capacity compared with MICT (0.3%) at the whole group level (n=54) and individually in the IR group (n=26). Previously, SIT has been shown to be an effective method for improving aerobic capacity in a various populations with a similar Wingate protocol than used in the present thesis (Burgomaster *et al.*, 2005; Sloth *et al.*, 2013; Weston, Wisløff and Coombes, 2014). Some of the earlier studies have also found greater improvement in aerobic capacity after SIT compared to MICT. Thus, the suggestion that MICT might not

be able to reach an intensity level that will lead to physiological processes leading to better cardio-respiratory fitness in the short-term might be accurate. However, not all exercise studies have found improved aerobic capacity after short-term SIT/HIIT. Yet, due to the different training protocols used in high-intensity interval training studies makes the comparison of different studies demanding. The optimal SIT/HIIT protocol is yet to be found and also the individual responses to exercise training should be taken into account.

In the present study, SIT tended to improve whole-body insulin sensitivity more than MICT. The literature is limited regarding the metabolic health benefits of SIT in overweight or T2DM subjects (Martin J. Gibala and McGee, 2008; Milanovic, Sporis and Weston, 2015). Recent meta-analysis, including various HIIT protocols, has indicated that HIIT may improve insulin sensitivity only in subjects with insulin resistance (Jelleyman *et al.*, 2015). However, the present results indicate that a 2-week training regime already enhances insulin sensitivity, and for up to 2 weeks at least, the enhancement is similar in both healthy and IR subjects.

In the adipose tissue depots, only SIT improved VAT GU and tended to decrease MTC. However, MICT was more effective in decreasing VAT FAU. In the brain, only SIT decreased insulin-stimulated brain GU (Table 4). However, in most of the parameters, SIT and MICT had similar effects on the outcomes. These results indicate that both SIT and MICT have beneficial effects on metabolic health. In the present study, the duration of intervention was only 2 weeks, and thus longer intervention studies are needed in the near future to clarify if SIT and MICT differs related to these metabolic responses.

Based on this study and the previous research in obese and T2DM subjects, both SIT and MICT can be effective methods to prevent and manage IR and T2DM. Thus, the combination of MICT, SIT and likely together with resistance training, could lead to optimal health outcomes. As the lack of time is the leading reason for physical inactivity, SIT could potentially be the most productive method of exercise training. SIT has also been suggested to increase the commitment to regular exercise training and therefore could offer a motivating training option.

**Table 4. Synopsis of the training-induced responses after sprint interval training (SIT) and moderate intensity continuous training (MICT) in the most important parameters of the studies I-III.** SAT (subcutaneous adipose tissue); VAT (visceral adipose tissue); GU (glucose uptake); FAU (fatty acid uptake); MTC (myocardial triglyceride content). ↑ increase after training, ↓ decrease after training.

Variable	SIT	MICT	p-value Time*training
M-value	↑	↑	<b>0.06</b> (all) 0.8 (IR)
Aerobic capacity	↑		<b>0.002</b>
Abdominal SAT GU			NS
Abdominal SAT FAU	↓	↓	0.8
Femoral SAT GU	↑	↑	0.7
Femoral SAT FAU	↓	↓	0.8
VAT GU	↑		<b>0.03</b>
VAT FAU		↓	<b>0.01</b>
Epicardial fat volume	↓	↓	0.8
Pericardial fat volume	↓	↓	0.9
MTC	↓		<b>0.06</b>
Brain GU	↓		0.1
Brain FAU			NS

## 7.4 Strengths and limitations

The strengths of this study are the modern imaging technologies, especially PET, which enables quantitative measurements of metabolism in different tissues. The use of [ $^{18}\text{F}$ ]FTHA and [ $^{18}\text{F}$ ]FDG PET tracers in determination of glucose and FFA uptake in different tissues has been validated in multiple studies. In addition, CT and MRI can be considered as most accurate methods to measure SAT, VAT and epi- and pericardial fat volumes (Kankaanpaa *et al.*, 2006) and MRS has been validated in measurement of MTC (Hocking *et al.*, 2013). The strength of the study is the dataset, with comparisons of training effects on healthy and IR subjects. This study provides novel data on adipose tissue volumes as well as adipose tissue and brain metabolism both in healthy subjects and in subjects with IR. Previous exercise training studies on myocardial adiposity have studied training effects on healthy individuals only with moderate intensity training. Adipose tissue and brain

GU and FAU have not been previously studied after exercise training with IR subjects.

The major limitation of our study is the relatively small number of subjects, although it is in line with previous exercise training studies with similar technically and financially demanding and detailed designs. Some technical difficulties during the PET measurements due to the problems in tracer production, which led to subjects with missing data, is also a limitation. To complete the whole study, subjects had to participate in four extensive scanning days, which led to a relatively high drop-out rate. The missing PET-scans and drop-outs mainly affected the sub-study III, in which 21 subjects performed pre-scans and only 15 subjects with both pre and post scans. Therefore, the number of subjects with pre-post comparison in the SIT group was 6 and in the MICT group 9.

In the sub-study III, the main limitation of the study was the lack of a control group. In the first phase of the study, the healthy subjects were not scanned from the head area. However, previous studies have shown that IR subjects have higher insulin-stimulated brain GU and fasting FAU than healthy controls, which most likely is also true in our study with IR subjects (Karmi *et al.*, 2010; Hirvonen *et al.*, 2011)

The study subjects in the IR group included both pre-diabetic and type 2 diabetic subjects. Omitting subjects with pre-diabetes (IFG, IGT) from the analysis or running the analysis using grouping according to diabetes status (pre-diabetes/T2DM) did not alter the results. Medication was used as a covariate in the SAS analysis, but however, it did not explain the difference between the pre-intervention and post-intervention. The IR group also included both men and women. As sex/gender has a large effect on body adiposity and may also have a large effect on epicardial fat, pericardial fat and MTC, and, as the healthy group consisted of only males, the females (n=10) were excluded from the IR group in the analyses comparing healthy and IR subjects (papers I and II). Thus, all three papers (I-III) had a slightly different study populations and comparisons, which makes the comparison of these papers challenging. Ideally, these groups should have been gender-matched. The original idea was to recruit only men in both groups, but the recruitment of the IR men turned out to be challenging, and therefore women were also included in order to complete the study.

In addition, the short duration of the intervention can be considered a limitation. The length of the intervention was decided based on the previous studies with similar SIT protocols (Burgomaster *et al.*, 2005). Moreover, considering the extremely intense nature of SIT, engaging study subjects in a longer intervention would have been challenging. The drop-out rate was relatively high already during the 2-week intervention and probably would have increased with an increasing length of in-

tervention. The subjects were instructed to maintain their normal eating habits during the study, but nutrition was not controlled. Thus, it might be that some of the study subjects increased their food intake as the energy expenditure increased and some ate healthier during the intervention.

## 8 CONCLUSIONS

The results of the current thesis showed that IR subjects have increased volume of epicardial and pericardial fat, and decreased adipose tissue GU and FAU in all measured adipose tissue depots. At baseline, whole-body insulin sensitivity was found to be linked to volumes and GU in all measured fat depots, and brain GU.

Two-week training, both MICT and SIT, can already within two weeks reduce epi- and pericardial fat and improve adipose tissue metabolism in previously inactive middle-aged healthy men and in men with IR. Furthermore, SIT increases aerobic capacity, decreases brain GU, and tends to improve insulin sensitivity and reduce MTC more effectively compared to MICT. Remarkably, these changes were seen without significant change in body weight and BMI. However, whole body fat content reduced after the training, supporting the previous findings of adipose tissue's important role in whole-body metabolism.

This study provides the first evidence that short-term exercise training alters brain glucose metabolism in subjects with IR. The finding of the decreased brain insulin-stimulated GU after weight-loss and exercise training raises the question of the importance of this finding. Thus, in the future it is important to study the mechanisms by which improved insulin sensitivity affects the brain metabolism and confirm the finding of reduced brain GU in IR subjects after exercise training; this can be done through longer intervention studies and with a higher number of subjects to increase the validity of the finding.

Based on this thesis, both in healthy and IR subjects, exercise training leads to similar responses in adipose tissue as well as in whole-body metabolic health. Although SIT appeared more effective in improving VAT and brain GU, aerobic capacity, and whole-body insulin sensitivity, both exercise modes similarly improved several parameters of whole-body health. The present thesis aimed to study metabolic responses to short-term exercise training and thus the responses into prolonged training remain unclear. Hence, longer training intervention studies are needed to discover whether the different responses after SIT and MICT are seen after prolonged exercise training.

Overall it can be stated that exercise training, both SIT and MICT, are effective strategies to prevent and manage IR and T2DM. In a modern world, where many people suffer from lack of time in everyday life, SIT offers an alternative time-saving exercise strategy. Thus, individual preferences should also be considered when planning an exercise prescription for each patient.

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A handwritten signature in blue ink that reads "Sana Hatab". The signature is written in a cursive style with a large initial 'S'.

Turku, June 2018

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# **ORIGINAL PUBLICATIONS**

Exercise training improves depot specific adipose tissue metabolism regardless of baseline glucose tolerance and sex

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## ABSTRACT

**Background.** Effects of exercise training on muscle metabolism are well characterized whereas the adipose tissue responses on exercise training are poorly understood. We studied the effects of sprint interval training (SIT) and moderate intensity continuous training (MICT) on adipose tissue substrate metabolism and tested whether the responses are similar regardless of baseline glucose tolerance and sex.

**Methods.** We randomized totally 54 sedentary subjects of whom 26 were insulin resistant (IR) men and women (BMI  $30.1 \pm 2.5$ ; age  $49 \pm 4$ ) and 28 were healthy men (BMI  $26.1 \pm 2.4$ ; age  $48 \pm 5$ ) into SIT and MICT for two weeks. AT masses were quantified by MRI, glucose uptake (GU) during euglycemic hyperinsulinemia and fasting free fatty acid uptake (FAU) in visceral (VAT), abdominal and femoral subcutaneous adipose tissue (SAT) with PET imaging technique.

**Results.** At baseline, compared to healthy men, IR men were insulin resistant, had higher body adiposity and lower insulin-stimulated GU in all adipose tissue depots and lower FAU in abdominal SAT. Considering the sex, women had 59% lower VAT mass and 50% higher abdominal SAT mass, higher VAT GU and FAU and femoral SAT GU compared to men. Training increased whole body insulin sensitivity and VAT and femoral SAT GU and decreased VAT and abdominal SAT FAU ( $p < 0.05$ , all) irrespective of baseline glucose tolerance and sex. SIT was superior in increasing aerobic capacity ( $VO_{2max}$ ) and VAT GU in IR group whereas MICT reduced VAT FAU more than SIT.

**Conclusion.** Although females have higher body adiposity, they have less VAT and higher adipose tissue substrate uptake rate compared to males. Short-term training improves adipose tissue metabolism similarly in healthy and IR men and independently of the sex. SIT is more effective in improving aerobic capacity and VAT insulin sensitivity in IR whereas MICT is preferable for VAT lipid metabolism.

## INTRODUCTION

The old perception of adipose tissue as a passive storage for FAs has been replaced by the notion that adipose tissue is an active endocrine organ and plays a major role in the regulation of glucose homeostasis and insulin sensitivity. Previous studies have provided consistent evidence that excess accumulation of white adipose tissue is associated with type 2 diabetes mellitus (T2DM) and other metabolic abnormalities. Although the total volume of adipose tissue is a risk factor for development of insulin resistance (IR) and T2DM as such, it is believed that the major driver of adipose tissue function is the quantity of visceral adipose tissue (VAT) and ectopic fat in and around internal organs.<sup>1-3</sup> Because its unique anatomical relation to the hepatic portal circulation, VAT releases FFAs directly into portal vein, making liver the first target of unsuppressed lipolysis and hepatic TG synthesis and therefore might have role in developing liver dysfunction and IR.<sup>4,5</sup> Previous studies using positron emission tomography (PET) have shown that VAT is metabolically more active than subcutaneous adipose tissue (SAT) with both higher glucose uptake (GU)<sup>6</sup> and fatty acid uptake (FAU)<sup>7</sup>. The metabolic activity of the cell is depended on its mitochondrial content and visceral adipocytes have higher number of mitochondria compared to subcutaneous adipocytes. VAT has also higher density of  $\beta$ -adrenergic receptors, which are stimulated by exercise training and once activated leading to higher lipolysis. Obesity is demonstrated to lead to several dysfunctions in adipose tissue. Studies with obese subjects have reported decreased adipose tissue insulin sensitivity and increased rate of fat oxidation, declined mitochondrial function, increased adipose tissue inflammation and adipocyte hypertrophy and hyperplasia compared to healthy lean subjects.<sup>8-11</sup>

Adipose tissue is highly insulin sensitive organ. Glucose uptake (GU) and fatty acid uptake (FAU) in adipose tissue might reflect metabolic activity of the adipose tissue and give pathophysical perception into relation between adipose tissue and insulin resistance. Prior studies on the effect of obesity on adipose tissue glucose and fatty acid metabolism have given controversial results. In a study done in our laboratory obese subjects had more than 50% lower visceral and abdominal SAT GU compared to lean subjects during insulin clamp.<sup>12</sup> However, in fasting conditions, Christen et al did not observe difference between lean and obese subjects in abdominal SAT or VAT GU.<sup>6</sup> Previous life-style intervention studies have mainly been focusing on diet-induced weight-loss. Surprisingly, despite reduction in VAT and abdominal SAT masses, weight reduction did not change VAT or SAT GU during insulin-stimulation or FAU during fasting in 6-week very-low calorie diet intervention. Yet, abdominal SAT and VAT masses correlated inversely with insulin-stimulated GU in the same tissue and positively with whole-body insulin sensitivity.<sup>13,14</sup>

While the main benefits of being physically active are clear, many unsolved issues surround the optimal prescription for these benefits. Sprint interval training (SIT) has been in focus of interest as time-saving and motivating training method for modern, busy people. Although different exercise methods have been under active research, there are no prior studies about exercise training and adipose tissue metabolism. However,

previous studies have shown that adipose tissue inflammation is decreased, and mitochondrial function, lipolysis and fatty acid oxidation increased after regular exercise training.

The present study was designed to address three major comparisons relating to exercise and adipose tissue metabolism in middle-aged, sedentary subjects. 1) Do healthy subjects and subjects with insulin resistance (IR) respond similarly to exercise training? 2) Is there different training response after two weeks of SIT and moderate-intensity continuous training (MICT)? 3) Do women and men response to the exercise training similarly? These findings should improve the ability to more accurately understand the benefits of different exercise modes and to target exercise recommendations to specific outcomes. This report summarizes the effects of SIT and MICT on VAT and SAT glucose and fatty acid metabolism in healthy subjects and subjects with IR.

## **METHODS AND PROCEDURES**

The study was a part of a larger study (NCT01344928) 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’. We have previously published several reports from this study.<sup>15-19</sup> The study was performed at the Turku PET Centre, the University of Turku and Turku University Hospital (Turku, Finland) and the Paavo Nurmi Centre (Turku, Finland) between March 2011 and September 2015. The study was approved by the Ethical Committee of the Hospital District of Southwest Finland (Turku, Finland, decision 95/180/2010 §228) and was carried out according to the Declaration of Helsinki. All participants gave written informed consent.

### **Subjects**

Middle-aged, physically inactive, healthy subjects and subjects with insulin resistance (IR) were recruited for the study via newspaper advertisements, personal contacts, and electronic and traditional bulletin boards. We recruited 28 healthy men and 26 men and women with IR. The inclusion criteria for the healthy subjects were male sex, age 40 – 55 years, BMI 18.5–30 kg·m<sup>2</sup>, VO<sub>2peak</sub> < 40 ml/kg/min and normal glycemic control.<sup>20</sup> The exclusion criteria were any chronic disease, a medical defect or injury that interfered with everyday life; a history of eating disorders; a history of asthma; use of tobacco products; use of anabolic steroids, additives or any other substrates; significant use of alcohol; current or a history on regular and systematic exercise training of any other condition that in the opinion of the investigator could create a hazard to the participant’s safety, endanger the study procedures or interfere with the interpretation of the study results. For the IR subjects, the inclusion criteria were the same as in healthy subjects except for a BMI of 18.5–35 kg/m<sup>2</sup> and an impaired glucose tolerance according to the criteria of the American Diabetes Association,<sup>20</sup> an HbA1c of less than 7.5 mmol/L and no insulin treatment in case of T2DM. Of the 26 IR subjects, 16 had T2DM and 10 impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). At the screening, five subjects were newly diagnosed

with T2DM and had no previous medication. In the other eleven T2DM subjects, the median diabetes duration was 4.2 years and they were treated by oral hypoglycaemic agents (11 with metformin; 5 with sitagliptin and 1 with glimepiride). Anti-diabetic treatment was with-held for 72 hours and the subjects were asked to avoid exhausting exercise 48 hours prior to the euglycemic hyperinsulinemic clamp.

### **Study design**

The physical examination, OGTT,  $VO_{2peak}$  test, magnetic resonance imaging (MRI), hyperinsulinemic clamp and positron-emission tomography (PET) with 14(R,S)-[ $^{18}F$ ]fluoro-6-thia-heptadecanoic acid ([ $^{18}F$ ]FTHA) and FDG, 2-[ $^{18}F$ ]fluoro-2-deoxy-D-glucose ([ $^{18}F$ ]FDG) tracers were performed as described in Figure 1. After all the pre-training measurements, the subjects were randomly divided into SIT and MICT training groups within both the healthy and IR groups. All the studies were repeated after the training intervention starting with PET-FTHA and MRI scans at 48 hours, FGD-PET and a euglycemic hyperinsulinemic clamp at 72 hours and an aerobic fitness at 96 hours after the last training session. (Fig.1).

Randomization of the healthy subjects was performed in two phases. First, a random permuted block of 24 subjects with a 1:1 allocation ratio was generated. Because of technical problems in the PET studies, another random permuted block of 4 subjects was also generated. Therefore, the final group sizes were  $n=14$  for the SIT and  $n=14$  for the MICT in the healthy subjects (in total  $n = 28$ ). Randomization of the IR subjects ( $n = 26$ ) was performed in blocks of four subjects with a 1:1 ratio. Power calculations were calculated based on the main outcomes and have been described previously.<sup>16,18</sup>

### **Exercise intervention**

Both training interventions took two weeks and included six supervised training sessions. The duration of the intervention was based on previous studies showing improvements in aerobic fitness and insulin sensitivity after only six training sessions. Each session was performed in laboratory conditions at the Turku PET Centre with the supervision of a member of the study group.

The MICT training protocol was planned to meet the current health-enhancing exercise recommendation of 150 minutes of moderate-intensity exercise per week. Each MICT session consisted of 40-60 minutes of cycling at a moderate intensity, which was determined as 60% of the measured  $VO_{2peak}$  workload (Tunturi E85, Tunturi Fitness, Almere, Netherlands) based on previous MICT protocols. The duration of the MICT increased progressively starting with 40 minutes and increasing by ten minutes every other session up to 60 minutes. The SIT protocol was based on the study originally described by Burgomaster and colleagues showing that only 15 minutes and two weeks of SIT improves exercise capacity and muscle insulin sensitivity. During the intervention, each SIT sessions consisted of 4-6  $\times$  30 seconds' all-out cycling bouts with a 4 minutes recovery

between each bout. The number of bouts increased progressively starting with four bouts and increasing by one every other session up to six bouts. The training load was individually determined (for healthy subjects 7.5% of whole body weight in kg, for IR subjects 10% of lean body mass in kg). Each bout started with prompt acceleration to maximum frequency without resistance. As the maximum frequency was accomplished, a sudden increase of load was applied with a special cycling ergometer (Monark Ergonomic 894E, MONARK, Vnasbro, Sweden), and cycling was carried out for 30 seconds at the maximum speed. Subjects cycled without resistance during a 4 minutes recovery period.

## **MRI**

VAT and SAT depot masses were measured with MRI (Philips Gyroscan Intera 1.5 T CV Nova Dual scanner, Philips Medical Systems). Abdominal area axial T1 weighted dual fast field echo images were obtained (echo time 2.3 and 4.7 ms, repetition time 120 ms, slice thickness 10 mm without gap). Images were analyzed using SliceOmatic software version 4.3 (<http://www.tomovision.com/products/sliceomatic.html>) to measure different adipose tissue masses. To obtain the mass, the pixel surface area was multiplied with the slice thickness and the density of adipose tissue, 0.9196 kg/l.<sup>21</sup>

## **PET**

A euglycemic-hyperinsulinemic clamp was performed after a 10-hour fast based on the original protocol by DeFronzo et al.<sup>22</sup> During the first 4 minutes, a primed-constant insulin (Actrapid, 100 U/ml, Novo Nordisk, Bagsvaerd, Denmark) infusion was started at a rate of 40 mU/m<sup>2</sup> of body surface area. After the first 4 minutes, the infusion rate was decreased to 20 mU/m<sup>2</sup> for 3 minutes, and then further decreased to 10 mU/m<sup>2</sup> for the rest of the clamp. An exogenous glucose infusion was started at 4 minutes after the beginning of the insulin infusion at a rate of the subject's 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 5 mmol/l as possible. Blood samples were collected before the clamp and every 5 minutes 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 the measured glucose values collected during the study.

### *Image acquisition and processing*

Participants underwent four PET sessions: one [<sup>18</sup>F]FTHA PET and one [<sup>18</sup>F]FDG PET before and after the training intervention. Antecubital veins of both arms were cannulated for the PET studies. One catheter was used to inject the radiotracers [<sup>18</sup>F]FTHA and [<sup>18</sup>F]FDG, whereas the second one was for blood sampling. To

arterialize the venous blood samples, the arm was heated using an electrically powered cushion. The subjects were positioned supine in a GE Discovery TM ST System, Milwaukee, WI, USA) scanner. On the first day, an [ $^{18}\text{F}$ ]FTHA-bolus (155 [SEM 0.4] 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). On the second PET study day, ~87 minutes after the start of the euglycemic-hyperinsulinemic clamp, [ $^{18}\text{F}$ ]FDG (156 [SEM 0.5] MBq) was injected, and dynamic scanning of the abdominal area started ~48 minutes after the injection. Thereafter, scanning of the femoral region followed as before the intervention. To measure the plasma radioactivity for tracer input function, arterialized venous blood samples were collected repeatedly during [ $^{18}\text{F}$ ]FTHA and [ $^{18}\text{F}$ ]FDG scanning. Plasma radioactivity was measured with an automatic gamma counter (Wizard 1480 3", Wallac, Turku, Finland)

### *Regions of interest*

All the imaging data was corrected for dead time, decay, and measured photon attenuation, and then reconstructed by scanner software using 3D-OSEM. Carimas 2.9 software (Turku PET Centre, Turku, Finland) was used to analyze all acquired PET-CT images. The regions of interest (ROIs) were drawn manually on abdominal subcutaneous white adipose tissue, on planes superior to the umbilicus, visceral adipose tissue at the level of the umbilicus and in femoral subcutaneous tissue at the mid-region of the thigh using CT as anatomical reference. The rate constant ( $K_i$ ) for the uptake of radiotracer ([ $^{18}\text{F}$ ]FTHA, [ $^{18}\text{F}$ ]FDG) into the cells was calculated using tissue time activity curves obtained from the abdominal subcutaneous, visceral and femoral subcutaneous adipose tissue using a fractional uptake method. Regional glucose and free fatty acid uptakes were calculated by multiplying regional specific  $K_i$  by corresponding plasma glucose or free fatty acid concentration, respectively. For GU the products were further divided by lumped constant (LC) value of 1.0 in adipose tissue.<sup>23</sup> LC is a correction factor, which accounts for the difference in transport and phosphorylation between [ $^{18}\text{F}$ ]FDG and glucose.

### **Other measurements**

Aerobic capacity was determined with a  $\text{VO}_{2\text{peak}}$  cycling ergometer test (ergoline 800s, VIASYS Healthcare, Germany) at the Paavo Nurmi Center (Turku, Finland) about one week before the first training session and 96 hours after the last training session. The  $\text{VO}_{2\text{peak}}$  test is previously described in detail by Kiviniemi et al.<sup>19</sup> Body composition was determined using the bioimpedance method (InBody 720, Mega Electronics Ltd., Kuopio, Finland).

## Statistical analysis

Statistical analyses were performed using SAS (version 9.3 for Windows, SAS institute Inc., Cary, NC, USA). The normal distribution of the variables was tested with the Shapiro-Wilkin test. Logarithmic transformations were performed for whole body fat percent, total cholesterol, HDL, triglycerides, fasting glucose and fasting insulin, VAT GU and femoral SAT FAU. Square root transformations were performed for VAT and SAT volume. 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) or training mode (SIT vs. MICT) or sex (women vs. men). Pre- and post- measurements were analyzed using a hierarchical linear mixed model suitable for repeated measurements (PROX MIXED procedure). Subjects with missing values (drop outs and those with technical problems) are all included in this model. Hence, we report the model-based mean (SAS least square means) values [95% CI] from all the parameters measured before and after the training. Correlation analyses were carried out using Pearson's Correlation. A p-value of less than 0.05 (two-tailed) was considered statistically significant.

## RESULTS

### *Healthy vs Insulin Resistant (IR)*

The IR group had significantly higher body mass, BMI, whole body fat, abdominal SC and visceral adipose tissue mass, plasma FFAs, plasma TGs, fasting plasma insulin values and tended to have higher HbA1c levels. They also differed from the healthy group by having lower fasting plasma insulin and insulin sensitivity. Both interventions reduced body adiposity and improved cholesterol values both in the healthy and IR subjects (Table 1). Aerobic capacity was 16% lower in IR subjects compared to healthy subjects at baseline ( $p < 0.001$ ). Training improved aerobic capacity in the whole study population by 3% with no significant differences in the training response between the healthy and IR groups (time  $p = 0.003$ , time\*IR  $p = 0.1$ ). After intervention, the aerobic capacity remained lower in IR subjects compared to healthy subjects ( $p < 0.01$ ).

At baseline, the IR subjects had significantly lower VAT, femoral and abdominal SAT GU levels than the healthy subjects (41%, 49% and 101%, respectively, all  $p < 0.001$ , Fig. 2). These baseline differences were not seen when comparing the GU per whole tissue in VAT and abdominal SAT (supplemental data 1). Training increased both VAT GU (time  $p < 0.001$ , time\*IR  $p = 0.10$ ) and femoral SAT GU (time  $p < 0.001$ , time\*IR  $p = 0.27$ ) similarly in the healthy and IR subjects. Only femoral SAT GU remained lower in the IR group compared to the healthy subjects after two-week training (74%,  $p < 0.001$ ). GU in all measured adipose tissue depots correlated positively with whole body insulin sensitivity and aerobic capacity, but negatively with parameters of body adiposity, fasting glucose, fasting insulin and triglycerides (Fig. 4). The change in VAT GU and abdominal SAT

GU was positively associated with the change whole body insulin sensitivity ( $p=0.02$ ,  $r=0.35$  and  $p=0.03$ ,  $r=0.37$ , respectively, data not shown).

Before the intervention, the IR subjects had lower visceral and abdominal SAT FAU levels than the healthy subjects (209% and 54%, respectively, both  $p<0.001$ , Fig. 3) but no difference in femoral SAT FAU ( $p=0.3$ ). Both in healthy and IR subjects, training further decreased FAU in VAT (time  $p=0.01$ , time\*IR  $p=0.58$ ) and tended to decrease FAU in abdominal and femoral SAT depots (time  $p=0.06$ , time\*IR  $p=0.84$  and time  $p=0.07$ , time\*IR  $p=0.97$ , respectively). FAU remained lower only in abdominal SAT in the IR group compared to the healthy (54%,  $p<0.001$ ). VAT and abdominal SAT FAU did not correlate with aerobic capacity, whole body insulin sensitivity or parameters of body adiposity. However, we found a negative correlation with femoral SAT FAU and aerobic capacity & whole-body insulin sensitivity (data not shown).

### *SIT vs MICT*

No differences were observed in basic characteristics between the SIT and MICT training group either at baseline or after the intervention (Supplemental data 3). As a whole group training significantly decreased visceral fat mass, HDL, LDL, total cholesterol, HbA1c and increased insulin sensitivity without any significant differences between the two training modes. There was a tendency towards reduction of whole body mass and abdominal SAT mass in the whole group no change was found in other parameters (Supplemental data 3). When studied according to the exercise mode, only SIT increased the aerobic capacity significantly (HIIT +5% vs. MICT 0.0%, time\*training  $p=0.047$ ).

Both SIT and MICT improved femoral SAT GU (25% and 20%, respectively, time  $p=0.004$ , time\*training  $p=0.8$ ). However, the training response was different between the two training modes with only SIT increasing VAT GU (30% vs. 4%, time\*training,  $p=0.03$ ) and only MICT reducing VAT FAU (-30% vs. 3%,  $p=0.01$ ) (Fig 2). No response was seen in abdominal SAT GU or FAU when only the IR subjects were included.

### *IR males and females*

IR females differed markedly from males, being older and shorter with significantly higher whole-body fat content, abdominal SAT mass and plasma FFAs with tendency of higher HDL. In addition, they also had significantly lower body mass, abdominal SAT and VAT mass, fasting plasma insulin and aerobic capacity when compared to males (Supplemental data 4).

At baseline, in contrast to IR males, VAT GU & FAU (26%,  $p=0.04$  and 56%,  $p=0.002$ , respectively) and femoral SAT FAU (64%,  $p=0.02$ ) were significantly higher in females with no significant differences found in abdominal SAT GU (Fig. 2). The training response in VAT was similar between men and women with both

increasing GU (21 % and 8 %, respectively, time  $p=0.04$ , time\*sex  $p=0.32$ ) and decreasing FAU (-15 % and -17%, respectively, time  $p=0.03$ , time\*sex  $p=0.84$ ) but differed in femoral SAT, with only men showing improved GU after exercise training (21% vs. 8%, time\*sex  $p=0.02$ ).

## DISCUSSION

The present data shows that exercise training increases insulin-stimulated GU in visceral and femoral SAT and reduces fasting FAU in VAT and tends to reduce femoral and abdominal SAT FAU both in healthy subjects and subjects with IR. We also show the different response after short-term SIT and MICT, while only SIT increasing GU and MICT reducing FAU in VAT in subjects with IR. To our knowledge, this is the first study to assess the effect of exercise training on depot-specific adipose tissue metabolism using positron emission tomography.

### *Healthy vs IR*

Our baseline data demonstrates an inverse relationship between adipose tissue mass and adipose tissue GU in the VAT, abdominal and femoral SAT depots indicating decreased insulin sensitivity in expanding fat depots. The present data is in line with the previous data in obese and T2DM participants.<sup>24</sup> Virtanen et al. showed 60 % lower GU per tissue mass in obese subjects compared to lean subjects both in VAT and abdominal SAT. In the present study, GU per 100g was 41 % lower in visceral, 49 % lower in abdominal SAT and 101 % lower in femoral SAT in IR men compared to healthy men. However, when calculated per tissue depot, GU in each scanned fat depot (VAT, abdominal and femoral SAT) was similar between healthy and IR subjects both in the present study and in the previous study by Virtanen et al despite significantly higher adipose tissue mass in IR/obese subjects. This was observed both in VAT and abdominal SAT depot due to the significantly higher volume of the fat depots in the IR/obese group. Interestingly, previous study done in our laboratory showed more than 100 % higher fasting FAU per 100 g in VAT and abdominal SAT in obese subjects with metabolic syndrome compared to lean subjects, while our study showed reduced fasting FAU in IR compared to healthy subjects (VAT 209 % and abdominal SAT 54 %).<sup>14</sup>

A two-week training intervention induced improvements in VAT GU, FAU, and femoral SAT GU both in healthy and IR subjects and tendency to decrease FAU in both SAT depots. Interestingly, the intervention did not have an effect on abdominal SAT GU. As discussed previously, exercise training has been shown to produce a greater reduction in VAT volume compared to SAT volume, which, based on the present results, seems also to be the same in the improvement in glucose metabolism of the adipose tissue. The finding may be explained by the higher adrenergic receptor density seen in VAT while exercise training is a stimulus for adrenergic receptors and the activation of the receptors lead to higher lipolysis. These are novel findings, as no previous study has been studying the exercise training induced responses on adipose tissue metabolism in healthy and IR subjects.

Together with the decrease in VAT and abdominal SAT volume after the training intervention, abdominal SAT and VAT GU in the IR group did not differ from the healthy subjects after the intervention.

The previous study by Viljanen et al. did not observe improvement in fasting GU in visceral or abdominal SAT after diet-induced weight loss.<sup>13</sup> This discrepancy indicates that exercise training improves adipose tissue GU independent of weight loss. Interestingly, we found that when using femoral muscle GU as a covariant, the increase in femoral SAT tissue was no longer significant ( $p=0.8$ ). This, therefore, suggests that the ability to rapidly improve muscle GU after exercise training could play a role in the improvement in femoral SAT glucose metabolism. In the same study data, similarly to the adipose tissue, the muscle GU improved only in the femoral muscle, while no change was seen with the upper body muscles.<sup>18</sup> Thus, differential response in the lower and upper body might be explained by the higher exercise volume on the lower body during cycling exercise.

### *SIT vs MICT*

Exercise has been shown to improve insulin sensitivity and decrease ectopic fat accumulation in several tissues, such as muscle, liver and heart.<sup>18,26-28</sup> Studies on adipose tissue and exercise have shown that exercise increases the blood flow and lipolysis in adipose tissue during exercise<sup>29-31</sup>; however, thus far no study has concentrated on exercise training and adipose tissue metabolism. Here, we show for the first time the different responses in adipose tissue metabolism both per 100g and per depot after SIT and MICT training.

When comparing different training modes in IR subjects, only SIT led to increase in aerobic capacity and visceral adipose tissue GU. Also previous studies have suggested that SIT lead to similar or higher improvements in  $VO_{2max}$  and insulin sensitivity.<sup>32,33</sup> Currently, the mechanisms of the superior effects of short-term SIT on aerobic capacity as well as on glucose homeostasis are unclear. It has been suggested that the repetition of the marked depletion of the working muscles glycogen stores during SIT could be one of the mechanisms behind the effectiveness of SIT in improving insulin sensitivity.<sup>34,35</sup> Study by Little et al (2011) first showed that already two weeks of HIIT (1 minute bouts of cycling at 90% maximal effort with 60 s recovery) increased skeletal muscle GLUT4 expression and markers of mitochondrial activity, and decreases blood glucose concentration in subjects with type 2 diabetes.<sup>36</sup> Thus, it might be that SIT has similar effect on adipose tissue as on skeletal muscle. On the other hand, we observed that only MICT improved FAU in VAT. The energy used during the MICT training is produced mainly via lipolysis while during SIT via fast glycogen stores, which may explain the different response in FAU after SIT and MICT. Similar finding has been also observed in skeletal muscle, further suggesting that adipose tissue might respond to exercise training similar to skeletal muscle.<sup>16</sup>

Whole body fat content, VAT and femoral & abdominal SAT volume decreased similarly after both training modes despite both the time spent during the training intervention (time SIT 15 min vs MICT 300 min) and the

average calculated energy consumption during the training sessions (421 vs 2907 kcal, respectively) were much less in SIT than MICT. However, it has been shown that SIT leads to higher delayed oxygen consumption for several hours after training and thus probably also raises the post-training basal energy metabolism more as compared with MICT.<sup>37,38</sup> Hence, this may explain the comparable or greater changes in body composition reported despite lower total training volume and time commitment after SIT.

#### *Women vs men*

It is well recognized that the fat accumulation in different depots is diverse with men and women. In humans, men accumulate more VAT, whereas women accumulate more SAT and have a higher percentage of body fat compared with men.<sup>39</sup> In the present study women had 42% higher body fat content, 51% higher abdominal SAT volume and 45% lower VAT volume (Supplemental data 4). However, our results as well as previous animal and human studies demonstrate that despite the higher level of total body fat, female are more insulin sensitive than males.<sup>40</sup> In the present study, women had significantly higher VAT GU and FAU and femoral SAT FAU (Fig 2). These findings may be related to sex steroids, which are known to play a role in the regulation of adipose tissue development and function as well as whole body insulin sensitivity as estrogen might have protective role against insulin resistance. This is the first study to compare the exercise training induced response in white adipose tissue in men and woman with insulin resistance. Despite the significant baseline differences, the only difference in the training response was observed in the femoral SAT GU, which improved only in men. This finding might be explained by higher muscle mass and pain tolerance in men, which allows men to push the limits further, especially during maximal effort in SIT training.

#### *Limitations of the study*

The study subjects included both pre-diabetic and type 2 diabetic subjects. Omitting subjects with pre-diabetes (IFG, IGT) from the analysis or running the analysis using grouping according to diabetes status (pre-diabetes/type 2 diabetes) did not alter the results. Hence, T2DM subjects were quite newly diagnosed with average duration of 4,2 years and therefore did not differentiate remarkably from the IR subjects. Medication was used as a covariate in the SAS analysis, however, it did not explain the difference between the pre-intervention and post-intervention decrease in glucose or fatty acid uptake. To complete the whole study, subjects had to participate in four extensive scanning days, which led to a relatively high drop-out rate.

The mechanisms by which GU and FAU are improved in adipose tissue were not studied. For the future studies, it would be interesting to study gene expression of markers of glucose and fatty acid transporters, mitochondrial function, vascularization and inflammation and further study the relationship between these parameters and metabolic changes after exercise training.

### *Conclusion*

In conclusion, already two weeks of exercise training improves glucose and fatty acid metabolism in visceral and subcutaneous adipose tissue compartments both in healthy subjects and subjects with IR and thus leads to similar improvements that are seen after surgery- or diet-induced weight loss. These improvements are seen despite the baseline glucose tolerance and sex. However, SIT seems to improve aerobic fitness and visceral adipose tissue GU more than MICT and in opposite, MICT seems to improve visceral adipose tissue FAU more than SIT. These findings are similar to skeletal muscle and suggest that VAT responds to exercise training similarly as skeletal muscle.

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### **DISCLOSURE**

The authors declare that there are no conflicts of interest associated with this manuscript.

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## Table legends

Table 1. Descriptive statistics and results of two-way analysis of variance for characteristics of healthy and insulin resistant (IR) men. The p-value for 'Baseline' describes the baseline differences between healthy (n=28) and IR (n=16) groups and 'Time' shows all healthy and IR males after training 'Time\*IR' demonstrates if 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 the normal distribution. The values are LSmeans translated into the original unit. SAT, subcutaneous adipose tissue; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free fatty acid; HbA1c, glycated hemoglobin; VO<sub>2</sub>peak, aerobic capacity.

	Healthy men		Insulin resistant (IR) men		Baseline	Time	Time* IR
	pre	post	pre	post			
N	28	26	16	13	-	-	-
Age (y)	48	-	49	-	-	-	-
Body mass (kg)	83.61 [79.60;87.63]	83.33 [79.31;87.35]	96.36 [91.01;101.71]	96.18 [90.82;101.53]	<0.001	0.22	0.78
BMI (kg/m <sup>2</sup> )	26.12 [25.11;27.12]	26.01 [25.01;27.02]	30.48 [29.10;31.86]	30.42 [29.04;31.80]	<0.001	0.18	0.69
Whole body fat (%) <sup>*</sup>	22.17[20.55;23.91]	21.12 [19.57;22.79]	28.48 [25.67;31.59]	27.68 [24.93;30.73]	<0.001	<0.001	0.30
Abdominal SAT (kg)	4.12 [3.53;4.75]	4.04 [3.46;4.67]	6.14 [5.18;7.18]	6.06 [5.11;7.10]	<0.001	0.04	0.84
Visceral fat (kg) <sup>□</sup>	2.59 [2.05;3.20]	2.48 [1.95;3.08]	4.33 [5.4;3.4]	4.11 [3.19;5.15]	0.002	0.002	0.48
<b>Cholesterol (mmol/L)<sup>*</sup></b>	4.92 [4.57;5.29]	4.40 [4.08;4.74]	4.71[4.27;5.19]	4.31 [3.90;4.77]	0.44	<0.001	0.52
<b>HDL cholesterol (mmol/L)<sup>*</sup></b>	1.37 [1.25;1.50]	1.27 [1.15;1.39]	1.20 [1.06;1.35]	1.09 [0.96;1.23]	0.08	<0.001	0.66
<b>LDL cholesterol (mmol/L)</b>	3.14 [2.85;3.43]	2.78 [2.48;3.08]	2.73 [2.34;3.12]	2.58 [2.18;2.98]	0.09	<0.001	0.16
<b>Triglycerides(mmol/L)<sup>*</sup></b>	0.94 [0.81;1.11]	0.83 [0.70;0.98]	1.70 [1.38;2.10]	1.50 [1.19;1.90]	<0.001	0.08	0.96
<b>Fasting FFA</b>	0.70 [0.62;0.77]	0.62 [0.54;0.69]	0.69 [0.60;0.78]	0.68 [0.59;0.78]	0.86	0.04	0.11
<b>HbA1c (mmol/L)</b>	36.93 [35.19;38.66]	34.72 [32.94;36.50]	39.64 [37.33;41.96]	37.65 [35.27;40.02]	0.07	<0.001	0.81
<b>Fasting glucose (mmol/L)<sup>*</sup></b>	5.44 [5.25;5.64]	5.74[5.52;5.96]	7.20 [6.86;7.56]	7.16 [6.80;7.55]	<0.001	0.15	0.80
<b>Fasting insulin (mmol/L)<sup>*</sup></b>	4.70 [3.75;5.90]	5.85 [4.61;7.43]	14.49 [10.72;19.57]	13.58 [9.87;18.68]	0.001	0.37	0.10
<b>M-value (μmol/kg/min)</b>	32.2 [26.6;39.0]	35.5 [29.2;43.2]	14.8 [11.4;19.2]	19.2 [14.7;25.19]	<0.001	0.001	0.1
<b>VO<sub>2</sub>peak (ml/kg/min)</b>	34.2 [32.7;35.7]	35.7 [34.2;35.7]	29.4 [27.3;31.5]	30.0 [27.9;32.1]	<0.001	0.003	0.14

## Figure legends

Screening DAY 1	Pre-scanning		Intervention 14 DAYS	Post-scanning			
	DAY 2	DAY 3		POST 48H	POST 72H	POST 96H	
<ul style="list-style-type: none"> <li>Physical examination</li> <li>OGTT</li> <li>VO<sub>2</sub>peak test</li> <li>Body composition</li> </ul>	<ul style="list-style-type: none"> <li>[<sup>18</sup>F] FTHA PET (FFA uptake)</li> <li>MRI</li> </ul>	<ul style="list-style-type: none"> <li>[<sup>18</sup>F] FDG PET-CT (glucose uptake) during hyperinsulinemic-euglycemic-clamp</li> </ul>	SIT 4-6 of 30 seconds all-out cycling bouts (Wingate's test)	MICT 40-60 minutes of cycling at an intensity of 60% of VO <sub>2</sub> peak	<ul style="list-style-type: none"> <li>[<sup>18</sup>F] FTHA-PET</li> <li>MRI</li> </ul>	<ul style="list-style-type: none"> <li>[<sup>18</sup>F] FDG-PET, hyperinsulinemic-euglycemic-clamp</li> </ul>	<ul style="list-style-type: none"> <li>VO<sub>2</sub>peak test</li> <li>OGTT</li> <li>Body composition</li> </ul>

Fig 1. The study design. VO<sub>2</sub>peak; aerobic capacity, OGTT, oral glucose tolerance test; [<sup>18</sup>F] FTHA, 14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid; PET, positron emission tomography; MRI, magnetic resonance imaging;

[<sup>18</sup>F] FDG, 2-[<sup>18</sup>F]fluoro-2-deoxy-D-glucose; SIT, sprint interval training, MICT, moderate-intensity continuous training.

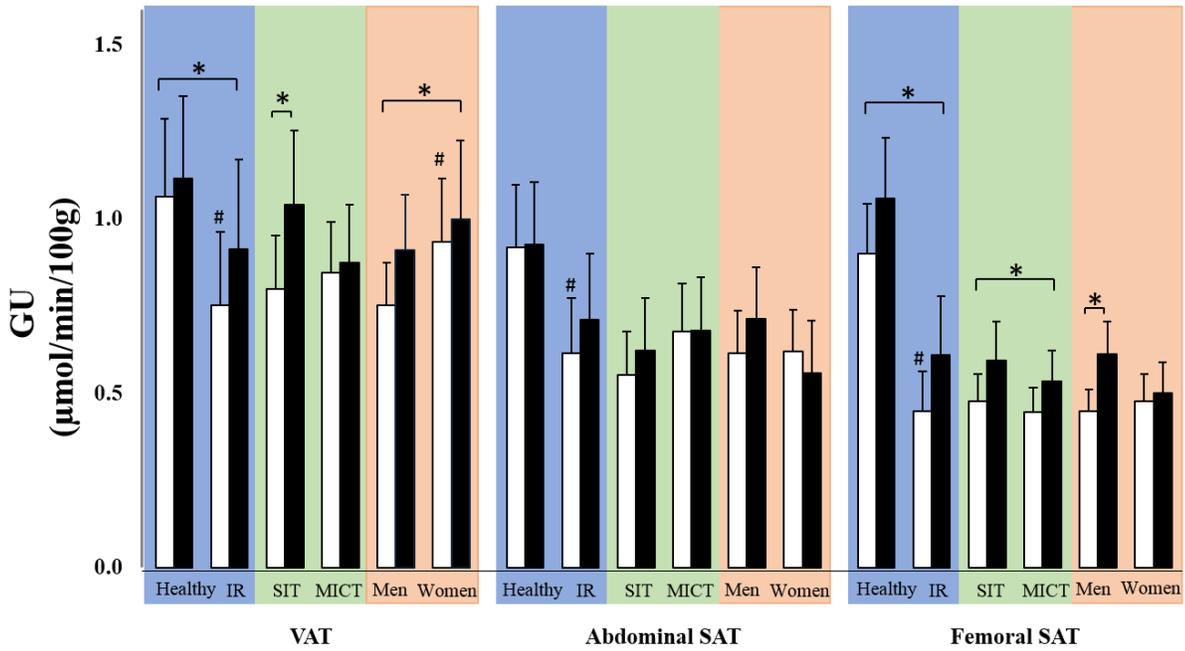


Fig 2. Insulin stimulated glucose uptake (GU) per 100g before (white bars) and after (black bars) the training intervention in visceral adipose tissue (VAT), abdominal subcutaneous adipose tissue (SAT) and femoral SAT. GU is compared in three different comparisons: healthy vs insulin resistant (IR) men (blue), sprint interval training (SIT) vs moderate intensity continuous training (MICT) in IR subjects (green) and men vs women in IR subjects (red). All data is expressed as means and (95% CI). #p<0.05; difference at baseline. \*p<0.05; the effect of exercise training over time in the whole group or a sub-group.

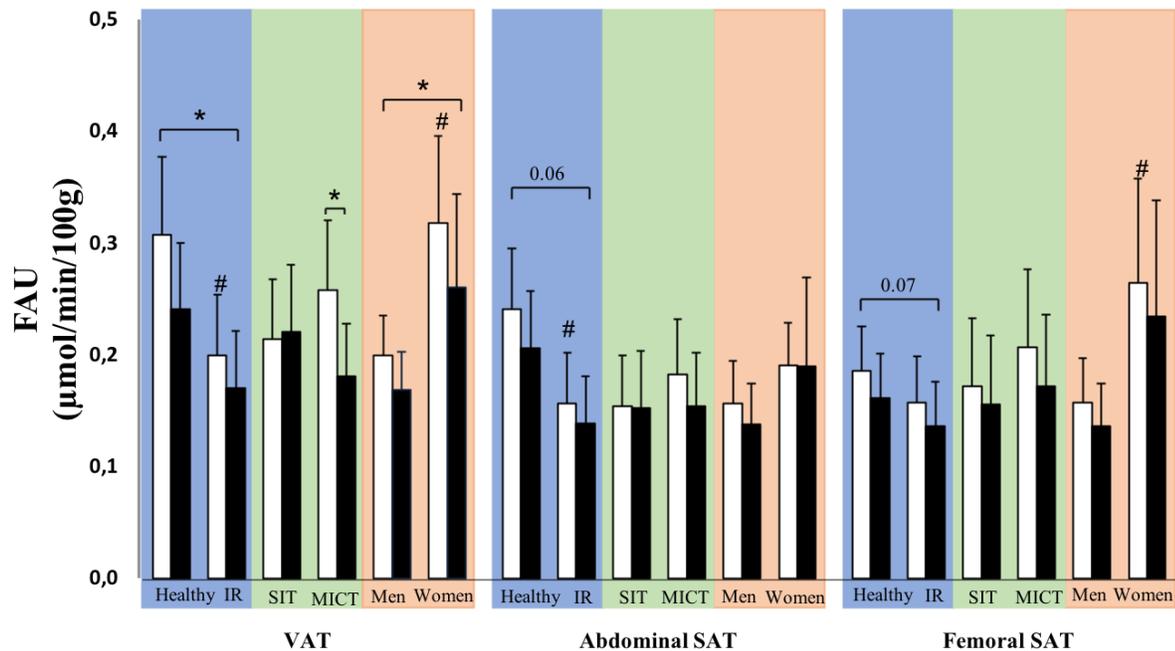


Fig 3. Fasting free fatty acid uptake (FAU) per 100g before (white bars) and after (black bars) the training intervention in visceral adipose tissue (VAT), abdominal subcutaneous adipose tissue (SAT) and femoral SAT. GU is compared in three different comparisons: healthy vs insulin resistant (IR) men (blue), sprint interval training (SIT) vs moderate intensity continuous training (MICT) in IR subjects (green) and men vs women in IR subjects (red). All data is expressed as means and (95% CI). #p<0.05; difference at baseline. \*p<0.05; the effect of exercise training over time in the whole group or a sub-group.

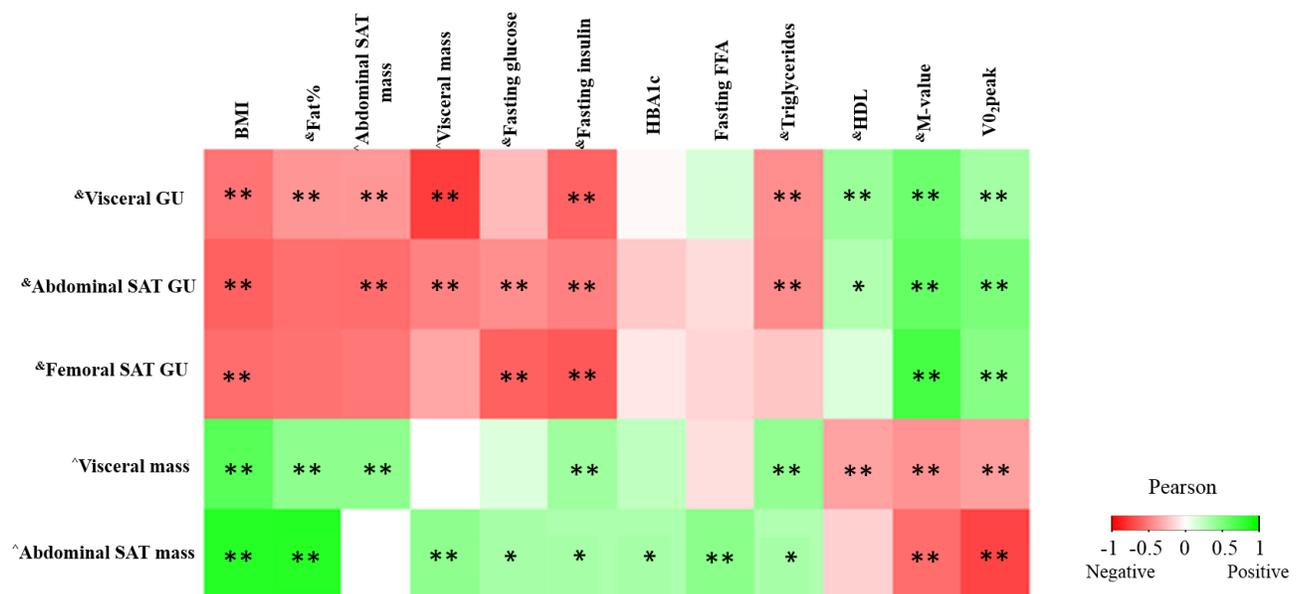
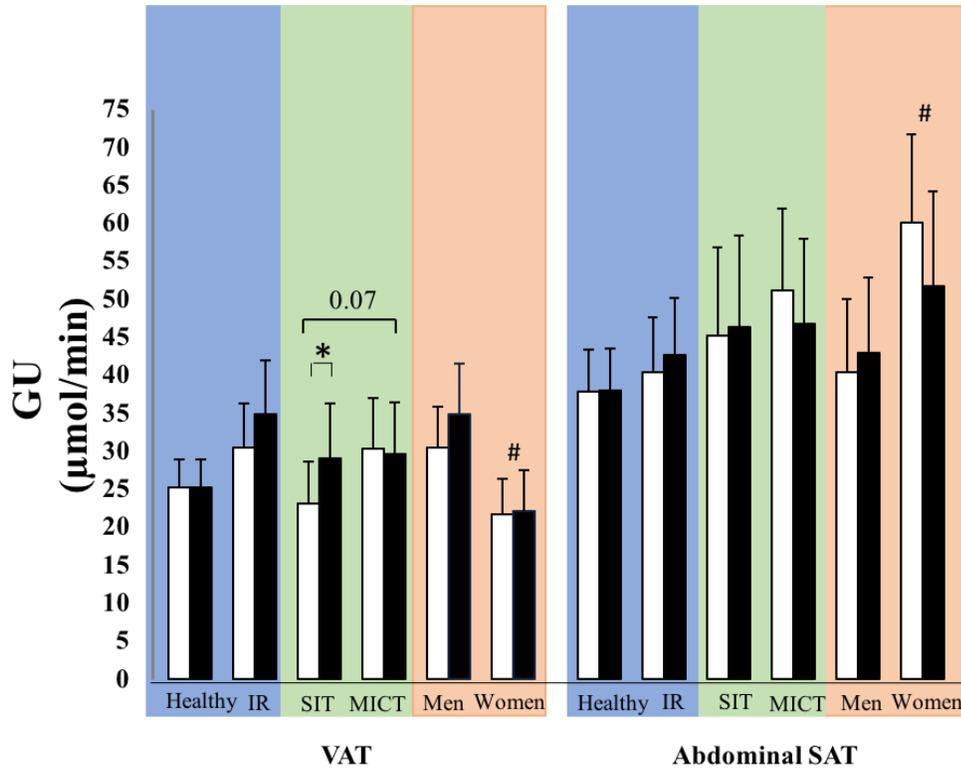
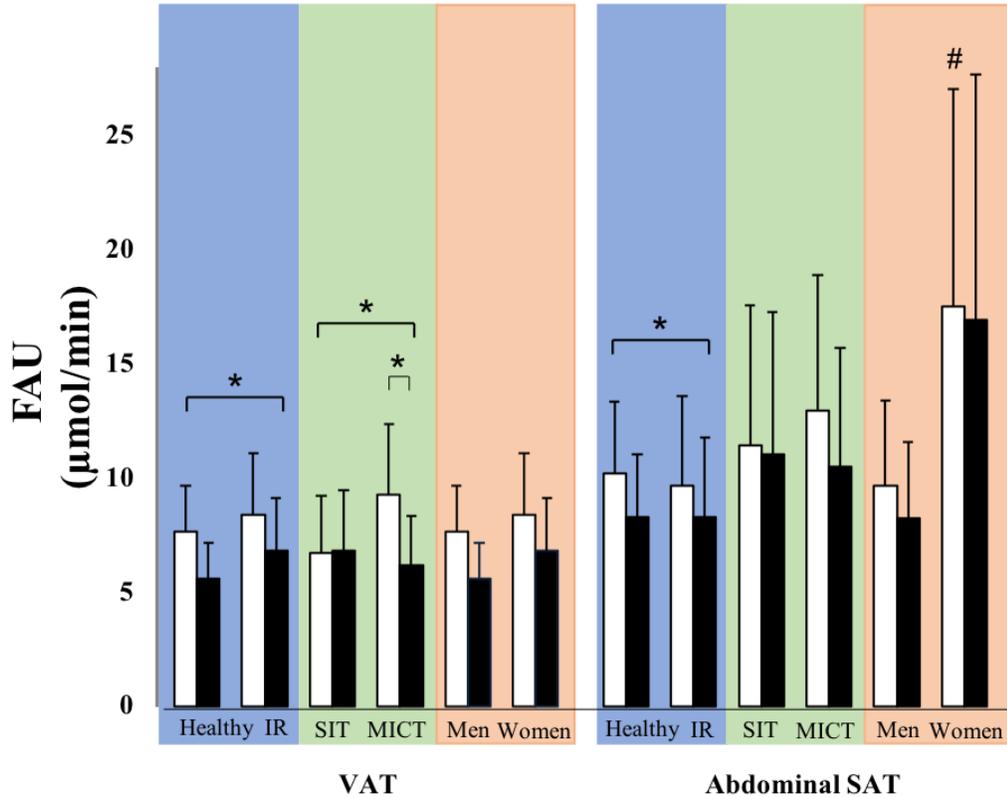


Fig 4. Pairwise correlations for variables at baseline. Statistically significant correlations ( $p < 0.05$ ) are highlighted with green (positive correlations) or red (negative correlations) according to the color key on the right. GU, glucose uptake; FAU, fatty acid uptake; SAT, subcutaneous adipose tissue; BMI, body mass index; HBA1c, glycated hemoglobin; FFA, free fatty acid; HDL, high-density lipoprotein;  $VO_{2peak}$ ; aerobic capacity. \* $p < 0.05$ , \*\* $p < 0.01$ .



Supplement 1. Insulin stimulated glucose uptake (GU) per tissue before (white bars) and after (black bars) the training intervention in visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (SAT). GU is compared in three different comparisons: healthy vs IR men, sprint interval training (SIT) vs moderate intensity continuous training (MICT) in IR subjects and men vs women in IR subjects. All data is expressed as means and (95% CI). # $p < 0.05$ ; difference at baseline. \* $p < 0.05$ ; the effect of exercise training over time in the whole group or a sub-group.



Supplement 2. Fasting fatty acid uptake (FAU) per tissue before (white bars) and after (black bars) the training intervention in visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (SAT). FAU is visualized in three different comparisons: healthy vs IR men, sprint interval training (SIT) vs moderate intensity continuous training (MICT) in IR subjects and men vs women in IR subjects. All data is expressed as means and (95% CI). # $p < 0.05$ ; difference at baseline. \* $p < 0.05$ ; the effect of exercise training over time in the whole group or a sub-group.

	IR SIT		IR MICT		Baseline	Time	Time* training
	Pre	Post	Pre	Post			
<b>N (M/F)</b>	13 (9/4)	11(7/4)	13 (7/6)	10 (6/4)	-	-	-
<b>Age (y)</b>	48 [46;49]	-	47 [44;49]	-	-	-	-
<b>Body mass (kg)</b>	91.6 [84.0;99.2]	91.2 [83.6;98.8]	92.0 [84.4;99.6]	91.5 [83.9;99.1]	0.9	0.09	0.96
<b>BMI (kg/m<sup>2</sup>)</b>	29.9 [28.3;31.5]	29.8 [28.2;31.4]	31.0 [29.4;32.6]	30.8 [29.2;32.4]	0.4	0.08	0.8
<b>Whole body fat (%)*</b>	32.2 [28.1;36.9]	31.2 [27.2;35.8]	33.0 [28.8;37.8]	32.0 [27.9;36.8]	0.8	<b>0.01</b>	0.9
<b>Abdominal SAT (kg)*</b>	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	<b>0.046</b>	0.9
<b>Visceral fat (kg)*</b>	3.1 [2.3;4.1]	3.0 [2.2;3.9]	3.6 [2.7;4.7]	3.4 [2.6;4.4]	0.4	<b>0.01</b>	0.4
<b>Cholesterol (mmol/L)*</b>	4.7 [4.2;5.2]	4.0 [3.6;4.5]	5.0 [4.4;5.6]	4.7 [4.2;5.3]	0.4	<b>0.006</b>	0.1
<b>HDL (mmol/L)</b>	1.3 [1.1;1.5]	1.2 [1.0;1.4]	1.3 [1.1;1.6]	1.3 [1.1;1.5]	0.8	<b>0.01</b>	0.8
<b>LDL (mmol/L)</b>	2.6 [2.2;3.0]	2.3 [1.8;2.7]	3.0 [2.5;3.5]	2.8 [2.2;3.2]	0.2	<b>0.02</b>	0.6
<b>Fasting Triglycerides (mmol/L)*</b>	1.5 [1.1;2.0]	1.3 [1.0;1.8]	1.5 [1.1;2.1]	1.5 [1.1;2.0]	0.9	0.4	0.7
<b>Fasting FFA (mmol/L)</b>	0.74 [0.61;0.85]	0.75 [0.62;0.88]	0.83 [0.72;0.95]	0.77 [0.65;0.89]	0.1	0.4	0.2
<b>HbA1c (mmol/L)</b>	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	<b>0.001</b>	0.8
<b>Fasting glucose (mmol/L) <sup>□</sup></b>	6.9 [6.4;7.4]	6.9 [6.4;7.4]	6.5 [6.0;6.9]	6.3 [5.8;6.8]	0.09	0.4	0.4
<b>Fasting insulin (mmol/L) <sup>□</sup></b>	12.6 [8.4;18.8]	11.0 [7.3;16.5]	9.5 [6.6;13.7]	9.8 [6.7;14.2]	<b>&lt;0.001</b>	0.4	0.2
<b>M-value (μmol/kg/min)*</b>	16.8 [11.9;23.9]	22.2 [15.4;32.0]	14.4 [10.4;19.8]	17.6 [12.5;24.8]	0.5	<b>0.02</b>	0.7
<b>VO<sub>2peak</sub> (ml/kg/min)</b>	27.0 [24.4;29.7]	28.4 [25.7;31.1]	27.4 [24.7;30.1]	27.2 [24.5;29.9]	0.9	0.1	<b>0.048</b>

Supplement 3. Descriptive statistics and results of two-way analysis of variance for characteristics of SIT and MICT training groups in insulin resistant (IR) subjects. 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 training 'Time\*training' demonstrates if there is an interaction between the change and the 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 <sup>□</sup> to achieve the normal distribution. The values are LSmeans translated into the original unit. SAT, subcutaneous adipose tissue; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free fatty acid; HbA1c, glycated hemoglobin; VO<sub>2peak</sub>, aerobic capacity.

	IR Men ♂		IR Women ♀		Baseline	Time	Time* sex
	Pre	Post	Pre	Post			
N	16	13	10	8	-	-	-
Age (y)	47 [45;49]	-	52 [20;55]	-	<b>0.002</b>	-	-
Height (m)	1.8 [1.7;1.8]	-	1.7 [1.6;1.7]	-	<b>&lt;0.001</b>	-	-
Body weight (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]	<b>0.02</b>	<b>0.04</b>	0.1
BMI (kg/m <sup>2</sup> )	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	<b>0.03</b>	0.09
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]	<b>&lt;0.001</b>	<b>0.02</b>	0.8
Abdominal SAT (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]	<b>0.003</b>	<b>0.03</b>	0.2
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]	<b>0.01</b>	0.8	0.8
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]	<b>0.002</b>	<b>0.01</b>	0.8
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]	<b>&lt;0.001</b>	0.4	0.9
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]	0.3	<b>0.01</b>	0.5
HDL (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]	<b>0.048</b>	<b>0.03</b>	0.2
LDL (mmol/L)	2.7 [2.3;3.1]	2.6 [2.1;3.0]	2.9 [2.4;3.5]	2.4 [1.9;3.0]	0.4	<b>0.01</b>	0.1
Fasting 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]	0.1	0.6	0.7
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]	<b>&lt;0.001</b>	0.2	0.4
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	<b>0.003</b>	0.9
Fasting glucose (mmol/L)	6.7 [6.2;7.2]	6.7 [6.2;7.2]	6.7 [6.1;7.2]	6.5 [5.9;7.1]	0.8	0.4	0.5
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]	0.1	0.6	0.6
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]	0.7	<b>0.04</b>	0.5
VO <sub>2peak</sub> (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]	<b>0.002</b>	0.1	0.2

Supplement 4. Descriptive statistics and results of two-way analysis of variance for characteristics of women and men in insulin resistant (IR) subjects. The p-value for 'Baseline' describes the baseline differences between sexes and 'Time' shows all IR subjects after training 'Time\*sex' demonstrates if there is an interaction between the change and the 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 the normal distribution. The values are LSmeans translated into the original unit. SAT, subcutaneous adipose tissue; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free fatty acid; HbA1c, glycated hemoglobin; VO<sub>2peak</sub>, aerobic capacity.

# Exercise Training Reduces Intrathoracic Fat Regardless of Defective Glucose Tolerance

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<sup>1</sup>Turku PET Centre, University of Turku, Turku, FINLAND; <sup>2</sup>Turku PET Centre, Turku University Hospital, Turku, FINLAND; <sup>3</sup>Department of Medical Physics, Turku University Hospital, Turku, FINLAND; <sup>4</sup>Department of Biostatistics, University of Turku, Turku, FINLAND; and <sup>5</sup>Paavo Nurmi Centre, University of Turku, Turku, FINLAND

## ABSTRACT

HONKALA, S. M., K. K. MOTIANI, J.-J. ESKELINEN, A. SAVOLAINEN, V. SAUNAVAARA, K. A. VIRTANEN, E. LÖYTTYNIEMI, J. KAPANEN, J. KNUUTI, K. K. KALLIOKOSKI, and J. C. HANNUKAINEN. Exercise Training Reduces Intrathoracic Fat Regardless of Defective Glucose Tolerance. *Med. Sci. Sports Exerc.*, Vol. 49, No. 7, pp. 1313–1322, 2017. **Purpose:** Epicardial (EAT) and pericardial (PAT) fat masses and myocardial triglyceride content (MTC) are enlarged in obesity and insulin resistance. We studied whether the high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) similarly decrease ectopic fat in and around the heart and whether the decrease is similar in healthy subjects and subjects with defective glucose tolerance (DGT). **Methods:** A total of 28 healthy men (body mass index = 20.7–30.0 kg·m<sup>-2</sup>, age = 40–55 yr) and 16 men with DGT (body mass index = 23.8–33.5 kg·m<sup>-2</sup>, age = 43–53 yr) were randomized into HIIT and MICT interventions for 2 wk. EAT and PAT were determined by computed tomography and MTC by <sup>1</sup>H-MRS. **Results:** At baseline, DGT subjects had impaired aerobic capacity and insulin sensitivity and higher levels of whole body fat, visceral fat, PAT, and EAT ( $P < 0.05$ , all) compared with healthy subjects. In the whole group, HIIT increased aerobic capacity (HIIT = 6%, MICT = 0.3%; time × training  $P = 0.007$ ) and tended to improve insulin sensitivity (HIIT = 24%, MICT = 8%) as well as reduce MTC (HIIT = -42%, MICT = +23%) (time × training  $P = 0.06$ , both) more efficiently compared with MICT, and without differences in the training response between the healthy and the DGT subjects. However, both training modes decreased EAT (-5%) and PAT (-6%) fat (time  $P < 0.05$ ) and not differently between the healthy and the DGT subjects. **Conclusion:** Whole body fat, visceral fat, PAT, and EAT masses are enlarged in DGT. Both HIIT and MICT effectively reduce EAT and PAT in healthy and DGT subjects, whereas HIIT seems to be superior as regards improving aerobic capacity, whole-body insulin sensitivity, and MTC. **Key Words:** TYPE 2 DIABETES MELLITUS, MYOCARDIAL FAT CONTENT, EPICARDIAL FAT, PERICARDIAL FAT, CT, H-MRS

**O**besity and physical inactivity are major risk factors for the development of insulin resistance and type 2 diabetes mellitus (T2DM), together with its

complications. In obesity, excess triglyceride (TG) accumulate not only into the peripheral fat depots but also in and around the internal organs such as liver, muscle, pancreas, and heart (22,42). It has been suggested that the regional fat distribution is independent and an important risk factor for metabolic and cardiovascular diseases as the whole body fat quantity as such (14,42).

The untypical accumulation of TG in and around the heart associates with body adiposity, circulation of nonesterified fatty acid and TG concentrations, insulin resistance, blood pressure, and an increased risk of cardiomyopathy and heart failure (18,22,27,35,45). In the heart, TG can accumulate either inside the myocardium (myocardial triglyceride content [MTC]) or outside the myocardium located between the myocardium and the pericardium (epicardial fat), or between the pericardium and the chest wall (pericardial fat). Both fat depots inside and outside the myocardium have been proposed to have a cardioprotective role by acting as a buffer to protect the myocardium from a high TG load and, in contrast, to act as a rapid energy source when needed (e.g., during intense exercise) (12,15,45). Epicardial and pericardial fat depots

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share some characteristics but also differ regarding anatomy, biochemistry, embryonic cell differentiation, and function and have thus been suggested to be two distinct fat depots (19). Epicardial fat, derived from the splanchnopleuric mesoderm, is not separated from the myocardium by any fascia and shares the microcirculation from the coronary arteries with the myocardium (18). Compared with other fat deposits, epicardial fat has a smaller cell size, a distinct fatty acid composition, and a higher fatty acid and lower glucose metabolism (15). Epicardial fat also secretes defensive cytokines and adipokines (e.g., adiponectin and interleukin-10) and abates vascular tension and thus is not considered harmful under normal physiological conditions (15,45). Pericardial fat is derived from the primitive thoracic mesenchyme, and its blood is supplied by branches of the internal mammary artery (18). The metabolic role of pericardial fat is still unclear, but increased pericardial fat may evolve into a higher risk for metabolic diseases compared with epicardial fat (43).

The mechanisms relating myocardial fats to increased cardiovascular and metabolic risks factors are complex and incompletely studied (8). Under pathological circumstances, such as obesity and T2DM, epi- and pericardial fat masses have been suggested to release adipokines and proinflammatory cytokines, which may lead to the development and progression of atherosclerotic lesions and coronary artery disease (3,15). Increased MTC has been shown to have a positive association with mitochondrial dysfunction and increased oxidative stress, which especially increases the risk of cardiac dysfunctions (27,29).

Exercise training has been shown to reduce MTC (38,39) and epicardial fat (23) accompanied by enhanced left ventricular function (39) in nondiabetic obese individuals. However, the few studies performed with T2DM patients have not revealed promising results. In the study by Schrauwen-Hinderling et al. (40), the 3-month combined endurance and strength training improved insulin sensitivity and the left ventricular ejection fraction but not MTC. Similarly, in the study by Jonker et al. (21), no response was observed in MTC or epicardial fat after 6 months of endurance training in T2DM subjects, although hepatic TG content, visceral fat, and pericardial fat decreased. Overall, the data on the effects of exercise training on MTC are limited. It is also unclear whether exercise training could increase MTC as it occurs in skeletal muscle, which is the phenomenon known as the “athlete's paradox.”

Recently, several studies have compared the benefits of high-intensity interval training (HIIT) over moderate-intensity continuous training (MICT). We and others have shown that short-term HIIT induces at least similar improvements in aerobic capacity and insulin sensitivity compared with MICT in healthy middle-age men (9,44,46). Furthermore, for patients with cardiovascular diseases and cardiac dysfunctions, high-intensity aerobic training seems to be the most efficient mode to maximize the cardiac benefits of exercise training (35,38). However, it is unclear whether high-intensity training is an effective method to reduce myocardial ectopic fat accumulation along with improvements in general

metabolic health. Thus, the aim of this study was to elucidate whether HIIT and MICT similarly decrease ectopic fat in and around the heart and whether the decrease is similar in healthy subjects and subjects with defective glucose tolerance (DGT). We hypothesized that exercise training reduces ectopic fats in both healthy subjects and subjects with DGT and that MICT reduces these fat depots more than HIIT.

## METHODS

The study was a part of a larger study titled “The Effects of Short-Term HIIT on Tissue Glucose and Fat Metabolism in Healthy Subjects and in Patients with Type 2 Diabetes” (NCT01344928). We have previously published several reports from this study (9,10,16,17,24,25,37). The main parameters of the present study, epi- and pericardial fat masses and MTC, have not been published before neither in healthy nor in DGT subjects. Also there are no prior published or accepted reports from the DGT group. The study was performed at the Turku PET Centre, the University of Turku and Turku University Hospital (Turku, Finland), and the Paavo Nurmi Centre (Turku, Finland) between March 2011 and September 2015. The study was approved by the ethical committee of the Hospital District of Southwest Finland (Turku, Finland, decision 95/180/2010 §228) and was conducted according to the Declaration of Helsinki. All participants gave written informed consent.

**Subjects and study design.** Middle-age physically inactive healthy subjects and subjects with DGT were recruited for the study via newspaper advertisements, personal contacts, and electronic and traditional bulletin boards. The inclusion criteria for healthy subjects ( $n = 28$ ) were male sex, 40–55 yr old, body mass index (BMI) of 18.5–30 kg·m<sup>-2</sup>,  $\dot{V}O_{2peak} < 40$  mL·kg<sup>-1</sup>·min<sup>-1</sup>, and normal glycemic control (1). The exclusion criteria were as follows: any chronic disease, a medical defect, or injury that interfered with everyday life; a history of eating disorders; a history of asthma; use of tobacco products; use of anabolic steroids, additives, or any other substrates; significant use of alcohol; and current or a history on regular and systematic exercise training of any other condition that in the opinion of the investigator could create a hazard to the participant's safety, endanger the study procedures, or interfere with the interpretation of the study results. For the DGT subjects, the inclusion criteria were the same as in healthy subjects except for a BMI of 18.5–35 kg·m<sup>-2</sup> and an impaired glucose tolerance according to the criteria of the American Diabetes Association (1), an HbA1c of less than 7.5 mmol·L<sup>-1</sup>, and no insulin treatment in case of T2DM. Of the 16 DGT subjects, 13 had T2DM and 3 had impaired fasting glucose and/or impaired glucose tolerance, and they were regarded as a DGT group. At the screening, three subjects were newly diagnosed with T2DM and had no previous medication. In the other 10 T2DM subjects, the median diabetes duration was 3.5 yr, and they were treated by oral hypoglycemic agents (9 with metformin, 4 with sitagliptin, and 1 with glimepiride). Antidiabetic

treatment was withheld for 72 h, and the subjects were asked to avoid exhausting exercise 48 h before the euglycemic hyperinsulinemic clamp.

The physical examination, oral glucose tolerance test (OGTT),  $\dot{V}O_{2peak}$  test, magnetic resonance imaging (MRI) and spectroscopy (MRS), hyperinsulinemic clamp, and computed tomography (CT) were performed as described in Figure 1. After all the pretraining measurements, the subjects were randomly divided into HIIT and MICT training groups within both the healthy and the DGT groups. All the studies were repeated after the training intervention, starting with MRS, MRI, and CT scans at 48 h, a euglycemic hyperinsulinemic clamp at 72 h, and an aerobic fitness and blood sampling at 96 h after the last training session (Fig. 1).

In previous long-term moderate-intensity training studies, epicardial fat has shown a decrease of 9% (23) and pericardial fat a decrease of 20% (21). As the present training intervention was shorter than used in the previous studies, we assumed that the training response would be at a level of 5% per unit

(assuming the SD to be 10% per unit). We calculated that a sample size of 34 subjects with decreases of 5% in epi- and pericardial fat masses would give an 80% power of detecting significant change after the training intervention with a level of significance at 5% (two-sided).

Randomization of the healthy subjects was performed in two phases. First, a random permuted block of 24 subjects with a 1:1 allocation ratio was generated. Because of technical problems in the PET studies, another random permuted block of four subjects was also generated. Therefore, the final group sizes were  $n = 14$  for the HIIT and  $n = 14$  for the MICT in the healthy subjects (totally  $n = 28$ ). Randomization of the DGT subjects ( $n = 26$ ) was performed in blocks of four subjects with a 1:1 ratio. Originally, the DGT group consisted of 26 subjects with T2DM or prediabetes of which 10 were females. As sex has large effect on body adiposity and may also have large effect on epicardial fat, pericardial fat, and MTC and as the healthy group consisted of only males, we decided to leave out the females from the DGT group in the analyses of this

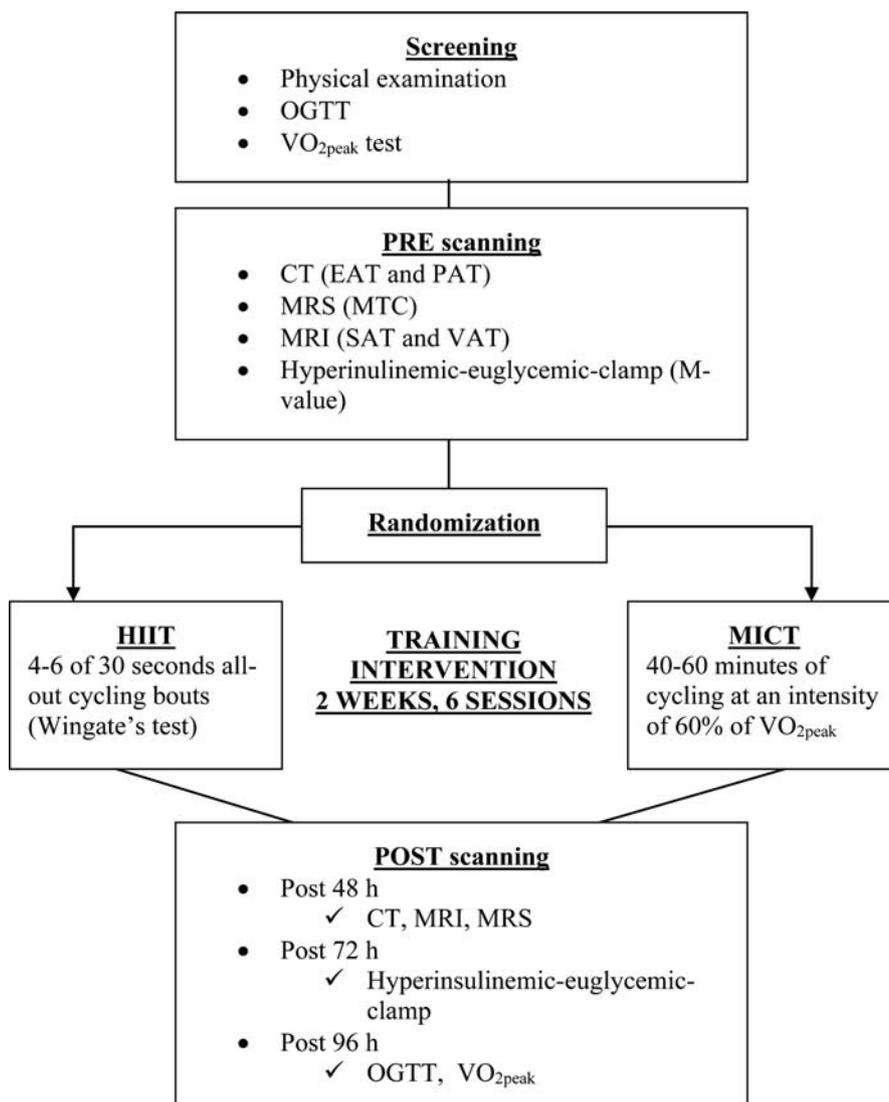


FIGURE 1—The study design. EAT, epicardial fat; PAT, pericardial fat.

report. Thus, the number of subjects in the DGT group was 16 and in the whole study 44; all of the subjects were males.

**Euglycemic–hyperinsulinemic clamp.** A euglycemic–hyperinsulinemic clamp was performed after a 10-h fast based on the original protocol by DeFronzo et al. (7). During the first 4 min, a primed-constant insulin (Actrapid, 100 U·mL<sup>-1</sup>; Novo Nordisk, Bagsvaerd, Denmark) infusion was started at a rate of 40 mU·m<sup>-2</sup> of body surface area. After the first 4 min, the infusion rate was decreased to 20 mU·m<sup>-2</sup> for 3 min and then further decreased to 10 mU·m<sup>-2</sup> for the rest of the clamp. An exogenous glucose infusion was started at 4 min after the beginning of the insulin infusion at a rate of the subject's weight (kg)/0.1 g·h<sup>-1</sup>. The glucose infusion rate was doubled after 10 min and then adjusted based on the blood glucose concentration to maintain it as close to 5 mmol·L<sup>-1</sup> as possible. Blood samples were collected before the clamp and every 5 min during the first 30 min to adjust the glucose infusion rate. After 30 min, the samples were collected every 5 to 10 min to check the glucose levels. The whole-body glucose uptake (M-value) was calculated from the glucose infusion rate, and the measured glucose values were collected during the study (7).

**OGTT.** The OGTT was performed after at least 10 h of fasting. Liquid containing 75 g of glucose (Nutrional®; Nutricia Medical, Turku, Finland) was given, and blood samples were taken at the baseline and at 15, 30, 60, 90, 120 min during the test to determine glucose and insulin concentrations for the glycemic status.

**VO<sub>2peak</sub> test and bioimpedance analysis.** Aerobic capacity was determined with a VO<sub>2peak</sub> cycling ergometer test (ergoline 800 s; VIASYS Healthcare, Germany). The test was performed at the Paavo Nurmi Center (Turku, Finland) approximately 1 wk before the first training session and 96 h after the last training session. The VO<sub>2peak</sub> test was performed as previously described by Kiviniemi et al. (25). Fat percentage was determined using the bioimpedance method (InBody 720; Mega Electronics Ltd., Kuopio, Finland).

**Other measurements.** Plasma total and HDL cholesterol, TG, and fasting glucose were measured from the venous blood samples with an automatized enzymatic assay and insulin using automatized electrochemiluminescence immunoassay (Cobas 8000; Roche Diagnostics GmbH, Mannheim, Germany). LDL cholesterol concentration was calculated using the Friedewald formula. Blood samples were collected before and 96 h after the last training session.

We also calculated the 10-yr cardiovascular disease risk score, based on age, systolic blood pressure, treatment of hypertension, diabetes, HDL, and total cholesterol using the Framingham risk score (FRS) (5).

**Training intervention.** Both training interventions took 2 wk and included six supervised training sessions. The duration of the intervention was based on previous studies showing improvements in aerobic fitness and insulin sensitivity after only six training sessions (2,4) and also considering the extremely intense nature of the HIIT intervention. The HIIT sessions consisted of 4–6 × 30 s maximal all-out cycling bouts

(Monark Ergonomic 894E; MONARK, Vnasbro, Sweden) with a 4-min recovery between each bout. The number of bouts increased progressively starting with four bouts and increasing by one every other session. The training load was individually determined (healthy 7.5% of whole body weight in kg, DGT 10% of lean body mass in kg). The MICT session consisted of 40–60 min of cycling at a moderate intensity, which was 60% of the VO<sub>2peak</sub> intensity (Tunturi E85; Tunturi Fitness, Almere, Netherlands). The duration of the MICT increased progressively starting with 40 min and increasing by 10 min every other session.

**CT.** Epi- and pericardial fat volumes were determined using a CT with a 64-row GE Discovery VCT PET/CT scanner (General Electric Medical Systems, Milwaukee, WI) using the fat volume quantification by Mahabadi et al. (28). The fat depots were measured from the calcium score CT images. A gated cine CT was done with a 512 × 512 matrix, and a total of 64 slices were acquired with a rotation time of 0.4 s, 120 kV, and 200 mA. The axial thickness was 2.5 mm. Images were reconstructed with an SD reconstruction algorithm using a display field of view of 25 cm. The data analyst (author SMH) was blinded for the order of before versus after images and analyzed images in random order using a Carimas 2.9 (Turku PET Centre, Finland). First, we outlined the pericardium at each cross section. The fat inside the region of interest was defined as the epicardial fat. Second, we defined the intrathoracic fat, including both epi- and pericardial fat masses, by drawing regions of interests on the thoracic wall. The pericardial fat volume was determined as the difference between the intrathoracic fat and the epicardial fat volume. Finally, the fat volume was defined as pixels within a window of -195 to -45 Hounsfield units (48). Quantification of epi- and pericardial fat masses from the CT scans can be considered to be one of the most accurate methods in use because of its high spatial and temporal resolution and 3-D viewing (6).

**MRS.** The MTC was determined with a <sup>1</sup>H-MRS (Philips Gyroscan Intera 1.5T CV Nova Dual Scanner, with a SENSE body coil; Philips Medical Systems, the Netherlands). This method is based on different chemical shifts of water and fat. The MRS was performed after an 8-h fast. The volume of interests were placed on an interventricular septum using both 4ch and short-axis images. The voxel size was 12 × 10 × 15 mm. The molecular contents of lipids and water were determined using single-voxel proton spectroscopy with a PRESS sequence and using an echo time of 30 ms and a repetition time of 3000 ms. The measurement was triggered by the heartbeat, and a typical triggering delay time was 350 ms. Spectra were collected as a time series with 10 separate measurements done in breath holds. Four spectra were used to calculate the average MTC. The measurement was performed twice, once with water suppression and once without it. Thus, the total number of averages was 40 for the spectrum with water suppression and 40 for the spectrum without water suppression. The data were analyzed using a linear combination of the model spectra software package (LCModel) version 6.3-0C with the LCMgui (34). The results

were corrected as described earlier (26). The data quality was inspected both visually (fit quality and residue) and numerically (fit SD of  $\leq 30\%$ ).  $^1\text{H-MRS}$  evaluation of MTC has been previously validated by Felbliner et al. (11).

**MRI.** The MRI studies were done to measure the masses of subcutaneous and visceral adipose tissue depots. Scans were done using Philips Gyroscan Intera 1.5T CV Nova Dual scanner (Philips Medical Systems). Whole-body (from head to knee) axial T1-weighted dual fast field echo images (echo time = 2.3 and 4.7 ms, repetition time = 120 ms, slice thickness = 10 mm without gap) were obtained. To measure different adipose tissue masses, the images were analyzed using SliceOmatic software version 4.3 (<http://www.tomovision.com/products/sliceomatic.htm>). To obtain the weight, the pixel surface area was multiplied by the slice thickness and the density of adipose tissue  $0.9196 \text{ kg}\cdot\text{L}^{-1}$ .

**Statistical analysis.** All the data are presented as mean values (95% confidence interval). Statistical analyses were performed using SAS for Windows (version 9.3; SAS institute Inc., Cary, NC). The normal distribution of the variables was tested using the Shapiro–Wilkin test. Logarithmic transformations were performed for MTC, epicardial fat, M-value, fasting plasma insulin, HDL, and fasting plasma TG, and a square root transformation was performed for visceral and subcutaneous fat to achieve normal distribution in these parameters. The baseline characteristics of the groups were compared by a two-way ANOVA, including the main effect of health status (healthy and DGT), training mode (HIIT and MICT), and their interaction (health status  $\times$  training mode). Before and after measurements were analyzed using a

hierarchical linear mixed model suitable for repeated measurements (PROX MIXED procedure). In the model, the DGT, training mode, and time effects were included as well as all interactions (most important results are seen in Table, Supplemental Digital Content 1, Intervention induced within-group changes and different responses between healthy subjects and subjects with DGT and HIIT and MICT groups, <http://links.lww.com/MSS/A863>). When a significant interaction was observed, we calculated predetermined contrasts (the Fisher's least significant difference test) within the model to study more about groupwise differences. Subjects with missing values (drop outs and those with technical problems) are all included in this model. Hence, we report the model-based mean (SAS least square means) values (95% confidence interval) from all the parameters measured before and after the training. We also tested the main results ( $\dot{V}\text{O}_{2\text{peak}}$ , M-value, epicardial fat, pericardial fat) using pre-diabetic/diabetic status, whole body fat content, and MTC added as a covariant otherwise using the same method as previously mentioned. Correlation analyses were conducted using Pearson's correlation.  $P$  value  $< 0.05$  (two-tailed) was considered statistically significant.

## RESULTS

At the baseline, the DGT subjects had increased body adiposity, impaired blood lipid values and glucose homeostasis, higher blood pressure, and increased FRS compared with the controls (Table 1). Both interventions improved cholesterol values in both healthy and DGT subjects but did not have any significant effect on glucose homeostasis, nonesterified fatty

TABLE 1. Descriptive statistics and results of two-way ANOVA for baseline characteristics for healthy subjects and subjects with DGT.

N	Healthy		DGT		P		
	HIIT	MICT	HIIT	MICT	DGT	Training	DGT $\times$ Training
Age (yr)	14 47 (45–50)	14 48 (45–51)	9 47 (44–50)	7 47 (44–51)	—	—	—
Body mass (kg)	83.1 (77.4–88.8)	84.1 (78.4–89.8)	96.2 (89.1–103.4)	96.5 (88.4–104.5)	<b>&lt;0.001</b>	0.8	0.9
BMI ( $\text{kg}\cdot\text{m}^{-2}$ )	25.9 (24.4–27.3)	26.4 (25.0–27.8)	29.8 (27.9–31.7)	31.1 (29.1–33.2)	<b>&lt;0.001</b>	0.3	0.6
BP systolic (mm Hg)	124 (120–128)	128 (124–132)	133 (128–138)	146 (140–151)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.09
BP diastolic (mm Hg)	78 (75–81)	80 (77–83)	87 (82–91)	88 (83–93)	<b>&lt;0.001</b>	0.3	0.9
Body fat (%)	22.2 (19.8–24.7)	22.9 (20.4–25.3)	29.3 (26.0–32.5)	28.4 (24.9–31.8)	<b>&lt;0.001</b>	0.9	0.6
Subcutaneous fat ( $\text{kg}$ )**	3.9 (3.1–4.8)	4.3 (3.5–5.2)	6.4 (5.1–7.9)	5.8 (4.5–7.4)	<b>&lt;0.001</b>	0.96	0.4
Visceral fat ( $\text{kg}$ )**	2.7 (1.9–3.6)	2.5 (1.8–3.3)	4.2 (3.0–5.7)	4.5 (3.1–6.1)	<b>0.003</b>	0.99	0.7
Epicardial fat (mL)*	92 (77–110)	78 (64–94)	131 (107–161)	157 (124–199)	<b>&lt;0.001</b>	0.98	0.09
Pericardial fat (mL)	157 (123–191)	139 (103–174)	228 (189–267)	211 (167–256)	<b>&lt;0.001</b>	0.4	0.9
MTC (%)*	0.42 (0.26–0.68)	0.53 (0.33–0.86)	0.65 (0.35–1.22)	0.48 (0.25–0.89)	0.6	0.9	0.3
Tot cholesterol ( $\text{mmol}\cdot\text{L}^{-1}$ )*	5.2 (4.7–5.8)	4.6 (4.2–5.1)	4.5 (4.0–5.2)	4.9 (4.2–5.7)	0.5	0.7	0.1
HDL ( $\text{mmol}\cdot\text{L}^{-1}$ )*	1.4 (1.2–1.6)	1.4 (1.2–1.6)	1.2 (1.0–1.4)	1.2 (1.0–1.5)	0.1	0.97	0.9
LDL ( $\text{mmol}\cdot\text{L}^{-1}$ )*	3.4 (3.0–3.8)	2.9 (2.5–3.3)	2.5 (2.0–3.0)	3.0 (2.4–3.5)	0.1	0.9	0.06
NEFA <sub>FS</sub> ( $\text{mmol}\cdot\text{L}^{-1}$ )*	0.6 (0.5–0.8)	0.7 (0.6–0.9)	0.9 (0.7–1.1)	0.9 (0.6–1.2)	<b>0.04</b>	0.5	0.6
TG <sub>FP</sub> ( $\text{mmol}\cdot\text{L}^{-1}$ )*	1.0 (0.8–1.2)	0.9 (0.7–1.1)	1.6 (1.2–2.2)	1.8 (1.3–2.4)	<b>&lt;0.001</b>	0.98	0.6
Glucose <sub>EP</sub> ( $\text{mmol}\cdot\text{L}^{-1}$ )*	5.3 (5.0–5.7)	5.6 (5.2–6.0)	7.5 (7.1–8.0)	7.0 (6.4–7.5)	<b>&lt;0.001</b>	0.5	0.08
Insulin <sub>FP</sub> ( $\text{mmol}\cdot\text{L}^{-1}$ )*	4.7 (3.4–6.5)	4.7 (3.4–6.5)	14.6 (9.7–21.8)	14.4 (9.1–22.8)	<b>&lt;0.001</b>	0.99	0.96
HbA1c (%)	5.5 (5.3–5.7)	5.6 (5.3–5.8)	5.7 (5.4–6.0)	5.8 (5.5–6.1)	0.07	0.5	0.95
HbA1c ( $\text{mmol}\cdot\text{L}^{-1}$ )	36.6 (34.1–40.0)	37.5 (35.0–40.0)	39.2 (36.1–42.3)	40.2 (36.8–43.8)	0.07	0.5	0.95
M-value ( $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )*	35.0 (26.9–45.6)	29.5 (22.2–39.2)	14.1 (9.7–20.5)	15.6 (10.8–22.7)	<b>&lt;0.001</b>	0.8	0.4
$\dot{V}\text{O}_{2\text{peak}}$ ( $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	34.7 (32.6–36.9)	33.7 (31.5–35.8)	28.0 (25.1–30.8)	30.9 (27.8–33.9)	<b>0.001</b>	0.5	0.1
FRS score (%)*	6.1 (5.0–7.5)	6.0 (4.9–7.3)	12.8 (9.8–16.7)	15.5 (11.7–20.6)	<b>&lt;0.001</b>	0.5	0.4

The  $P$  value for DGT describes the difference between healthy ( $n = 28$ ) and impaired glucose tolerance ( $n = 16$ ) groups, and "training" compares all HIIT trained ( $n = 23$ ) to all MICT trained ( $n = 21$ ). DGT  $\times$  training demonstrates if there is an interaction between the DGT and the training mode. All the data are presented as model based means (95 % confidence interval). The values are LSmeans translated into original unit. Boldfaced values are significantly different between the groups,  $P < 0.05$ . EXE, exercise mode; BP, blood pressure; NEFA, nonesterified fatty acid; FFA, free fatty acid; fP, fasting plasma; fS, fasting serum; HbA1c, glycated hemoglobin.

\*Variables with logarithmic transformation to achieve the normal distribution.

\*\*Variables with square transformation to achieve the normal distribution.

acid, or FRS score (see Table, Supplemental Digital Content 1, Intervention induced within-group changes and different responses between healthy subjects and subjects with DGT and HIIT and MICT groups, <http://links.lww.com/MSS/A863>). HIIT reduced total cholesterol more compared with MICT, but this difference was no longer significant when whole body fat percentage was used as the covariant (training  $\times$  pre-post  $P = 0.08$ ). Also, whole body fat percentage (healthy =  $-4\%$  and DGT =  $-3\%$ ), visceral (healthy =  $-4\%$  and DGT =  $-5\%$ ) and subcutaneous fat (healthy =  $-2\%$  and DGT =  $-1\%$ ) masses reduced, but not differently between the groups.

Aerobic capacity was 16% lower in DGT subjects compared with healthy subjects at baseline ( $P < 0.001$ , Fig. 2A). Training improved aerobic capacity in the whole study population from 31.8 (30.5–33.1) to 32.8 (31.5–34.1)  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  (+3%) with no significant differences in the training response between the healthy (+4%) and the DGT (+2%) groups (time  $P = 0.003$ , time  $\times$  DGT  $P = 0.1$ , Fig. 2A). After intervention, the aerobic capacity remained lower in DGT subjects compared with healthy subjects ( $P < 0.001$ ). When studied according to the exercise mode, only HIIT increased the aerobic capacity significantly (HIIT = +6% vs MICT = +0.3%, time  $\times$  training  $P = 0.003$ ; Fig. 2B). In the whole study group, the aerobic capacity correlated negatively with parameters of body adiposity and positively with insulin

sensitivity at the baseline, but not with MTC (see Supplemental Digital Content 2, Pairwise correlations for all measured variables at baseline, <http://links.lww.com/MSS/A864>).

Before the intervention, the DGT subjects had attenuated insulin sensitivity with 54% lower M-value compared with the controls ( $P < 0.001$ , Fig. 2C). Exercise training improved insulin sensitivity in the whole study population significantly from 21.9 (18.6–25.7) to 26.1 (22.1–30.9)  $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  (+17%) with no difference between the healthy (10%) and the DGT (23%) groups (time  $P = 0.001$ , time  $\times$  DGT  $P = 0.1$ ) in the training response. After the intervention, the insulin sensitivity stayed lower in DGT compared with the healthy subjects ( $P = 0.004$ ). HIIT tended to improve M-value more than MICT (23% vs 9%; time  $\times$  training,  $P = 0.06$ ; Fig. 2D). In the whole study group, the M-value correlated positively with aerobic capacity and negatively with the parameters of body adiposity, but not with MTC (see Supplemental Digital Content 2, Pairwise correlations for all measured variables at baseline, <http://links.lww.com/MSS/A864>). The change in insulin sensitivity correlated inversely with the changes in the whole body fat percentage ( $R = -0.38$ ,  $P = 0.02$ ) and subcutaneous fat ( $R = -0.34$ ,  $P = 0.047$ ).

At the baseline, the DGT subjects had higher epi- and pericardial fat volumes than the healthy subjects (41% and 12%, respectively, both  $P < 0.001$ ; Fig. 3A and B). Training

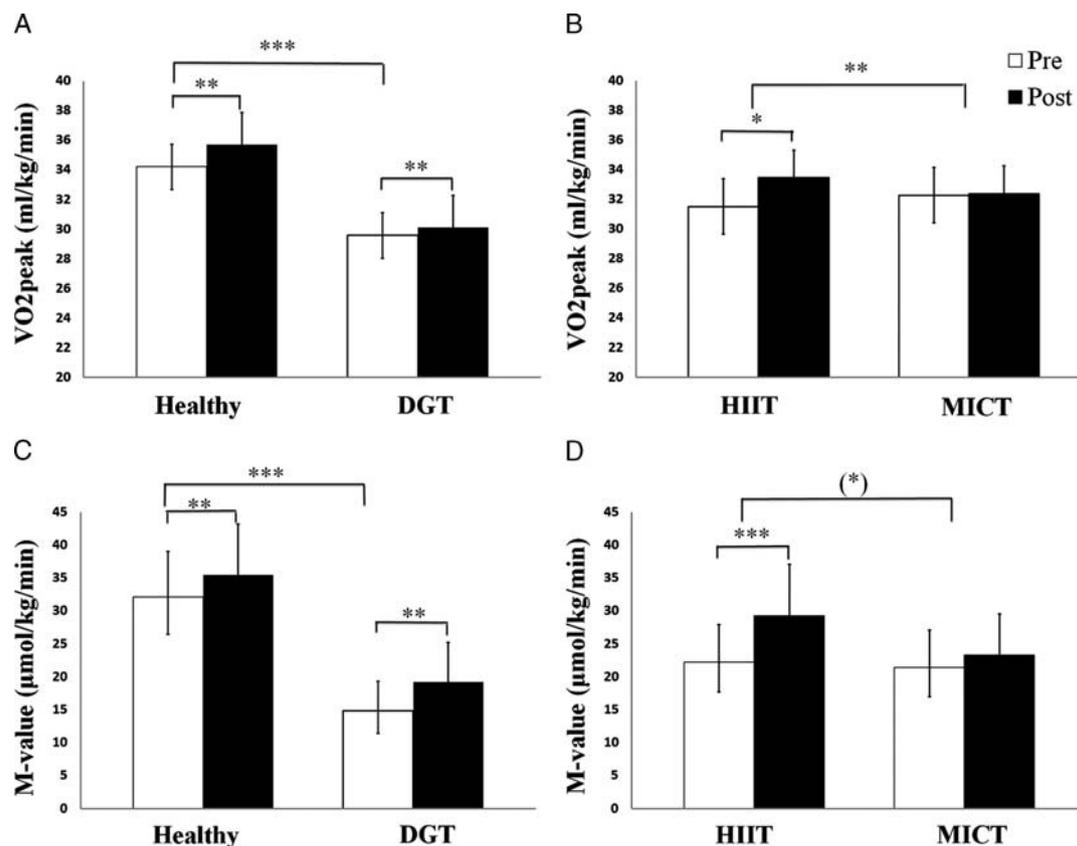
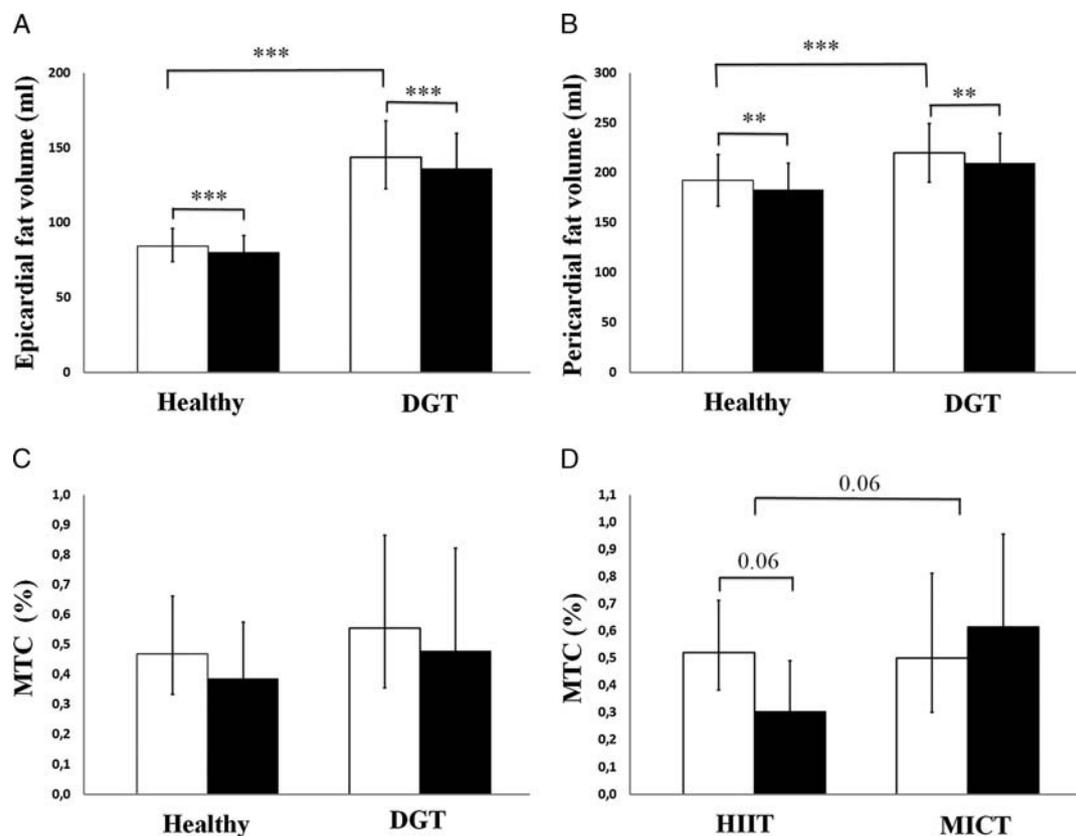


FIGURE 2—Aerobic capacity ( $\dot{V}O_{2\text{peak}}$ ) and whole-body insulin sensitivity (M-value) at baseline (white bars) and after the training intervention (black bars) in healthy subjects and in subjects with DGT (A and C) according to the training mode (B and D). All the data are presented as mean values (95% confidence interval). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



**FIGURE 3**—Epicardial fat (EAT), pericardial fat (PAT), and MTC at baseline (white bars) and after the training intervention (black bars) in healthy subjects and in subjects with DGT (A, B, and C) and MTC, according to the training mode (D). All the data are presented as mean values (95% confidence interval). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

decreased both epi- and pericardial fat volumes by 5% in both healthy and DGT subjects (time  $P < 0.001$ , time  $\times$  DGT  $P = 0.8$ ; Fig. 3A and B). No difference was observed in the training response between the training modes in either epi- or pericardial fat (time  $\times$  training;  $P = 0.8$  and  $P = 0.9$ , respectively). Both epi- and pericardial fat volumes remained higher in the DGT group compared with the healthy subjects after the 2-wk training (39% and 13%,  $P < 0.001$  and  $P = 0.01$ , respectively). Epi- and pericardial fat volumes correlated positively with each other and with body adiposity but negatively with aerobic capacity and insulin sensitivity (see Supplemental Digital Content 2, Pairwise correlations for all measured variables at baseline, <http://links.lww.com/MSS/A864>).

No differences were observed in MTC between the healthy and the DGT subjects either at the baseline or after exercise (Fig. 3C). However, the training response tended to be different between the two training modes (time  $\times$  training;  $P = 0.06$ ) with HIIT tending to decrease MTC ( $P = 0.06$ , Fig. 3D). Using MTC as the covariant, the different training response between HIIT and MICT in  $\dot{V}O_{2\text{peak}}$  (time  $\times$  training  $P = 0.10$ ) and M-value (time  $\times$  training  $P = 0.23$ ) was no longer significant.

## DISCUSSION

The aim of this study was to evaluate whether HIIT and MICT have a similar effect on decreasing the ectopic fat in

and around the heart and whether the decrease is similar in healthy subjects and subjects with DGT. We found that both HIIT and MICT effectively reduce epicardial (5%) and pericardial (6%) fat in both healthy and DGT subjects, whereas HIIT seems to be superior in improving aerobic capacity, whole-body insulin sensitivity, and MTC.

Before the intervention, the DGT subjects had higher epi- and pericardial fat volumes than those of the healthy subjects (both  $P < 0.001$ ), but no differences were observed in MTC between these groups. Pericardial fat has been shown to correlate with different metabolic and cardiovascular risk factors, and it has been suggested to be a better marker for cardiometabolic diseases than epicardial fat within a wide range of body adiposity (BMI = 21–40  $\text{kg}\cdot\text{m}^{-2}$ ) (43). In the present study, before the intervention, both fat deposits correlated with metabolic risk markers such as visceral adiposity, systolic blood pressure, insulin sensitivity, and aerobic capacity, but only epicardial fat correlated with plasma TG (Figure 1, Supplemental Digital Content 2, Pairwise correlations for all measured variables at baseline, <http://links.lww.com/MSS/A864>). We also calculated the 10-yr coronary heart disease risk score using the FRS, which has previously shown to be positively correlated with pericardial fat (5). The DGT subjects had a higher FRS at baseline than the healthy subjects ( $P < 0.001$ ), and this remained unchanged by the 2-wk exercise training. In the present study, both epi- and pericardial

fat masses correlated with FRS. Thus, our data suggest that both epi- and pericardial fat masses are relevant correlates for coronary heart disease risk.

In previous exercise training studies, the exercise response on epi- and pericardial fat masses has been studied in obese and T2DM subjects (21,23,47). In obese men, a 4-month intervention decreased epicardial fat on average by 9% (no data regarding pericardial fat or MTC), and this change correlated positively with the reduction in visceral fat (23). In a recent study with morbidly obese subjects, a 12-wk training intervention decreased epi- and pericardial fat masses 7% and 12%, respectively (no data regarding MTC) (47). Jonker et al. (21) showed a 20% decrease in pericardial fat along with a reduction in visceral fat mass, but no change in epicardial fat in T2DM subjects after a 6-month exercise intervention. In the present study, training decreased epi- and pericardial fat volumes in both healthy and DGT subjects (both  $-5\%$ ), thus showing no difference in the training response between the healthy and the DGT groups. Our results also agree with the findings that training induces relatively higher reductions in epicardial fat ( $-5.3\%$ ), pericardial fat ( $-5.5\%$ ), and visceral fat ( $-4.9\%$ ) compared with subcutaneous fat ( $-1.3\%$ ) as previously shown by Kim et al. (23). In addition to these previous results, we now show that this response is rapid as no more than 2 wk of training was needed for these changes. The relatively higher reductions in epi- and pericardial fat masses and visceral fat are probably due to the higher  $\beta$ -adrenergic receptor density in these fat depots, and thus a higher exercise induced lipolysis compared with subcutaneous fat (14,33). It is also interesting that although epi- and pericardial fat masses have been shown to differ as regards embryologic origin, local circulation, function, and metabolic activity, and that epicardial fat is more similar to visceral fat, our data suggest that exercise training induces a similar mass reduction in each of these deposits (19).

Data on the effect of regular training on MTC are limited to one study showing decreased MTC in overweight men (39), whereas no training responses have been observed in T2DM (21,40). In the present study, we did not observe differences in MTC between healthy and DGT subjects at baseline or in any training responses in either healthy or DGT subjects. However, MTC tended to respond differently to the two different training modes (time  $\times$  training  $P = 0.06$ ), HIIT reducing the MTC more efficiently compared with MICT (Fig. 3D). Thus, our data suggest that high-intensity training may be more beneficial in reducing MTC than low- to moderate-intensity training. One explanation for this finding could be that extremely high-intensity training is needed to strain the myocardium to the point of myocardial energy deficit and during the rapid and maximal energy need MTC are mobilized inside the myocyte. In previous exercise training studies showing no change in MTC, moderate-intensity training protocols have been used (21,40). In a recent cross-sectional study, endurance-trained athletes, who usually use different HIIT protocols in training, had a lower MTC than those of healthy male subjects matched for

age, BMI, and body fat percentage (38). In the present study, at baseline, MTC correlated with age and FRS but not with other variables.

Subjects with T2DM have higher levels of skeletal muscle intramyocellular lipids (IMCL) compared with healthy subjects in relation to insulin resistance. On the other hand, regular exercise training increases IMCL along with improving insulin sensitivity and aerobic capacity, which is a phenomenon known as the “athlete's paradox.” An increase in athlete's IMCL content provides an energy reservoir for rapid energy release during exercise (32). However, whether a similar “athlete's paradox” also occurs in the myocardium is unclear. In a cross-sectional study by Sai et al. (38), MTC was lower in endurance athletes compared with healthy controls. In addition, our present data together with previous exercise training studies (38,39) suggest more of a decrease than an increase in MTC posttraining. The possible differences in the exercise response between MTC and IMCL might be the fact that the myocardium relies on free fatty acid uptake and beta-oxidation at rest and during exercise as well as on the differences in mitochondrial function (41).

Despite the differences at baseline, the 2-wk training demonstrated a similar improvement in both the aerobic capacity (3%) and the insulin sensitivity (16%) of healthy and DGT subjects; both aerobic capacity and insulin sensitivity also remained lower in the DGT subjects after the 2-wk training intervention. Interestingly, HIIT induced a higher increase (6%) in aerobic capacity compared with MICT (0.3%) and also tended to induce a higher response in insulin sensitivity (Fig. 2). HIIT using the Wingate protocol (4) has been shown to be an effective method of improving aerobic capacity in various populations (44,46), but the literature is limited regarding the metabolic health benefits of HIIT in overweight or T2DM subjects (13,31). Recent meta-analysis, including various high-intensity interval training protocols, has indicated that high-intensity exercise may improve insulin sensitivity only in subjects with insulin resistance (20). However, our results indicate that 2 wk training already enhances insulin sensitivity, and for up to 2 wk at least, the enhancement is similar in both healthy and DGT subjects. Currently, the mechanisms of the superior effects of HIIT on aerobic capacity as well as on glucose homeostasis are unclear. It is suggested that the repetition of the marked depletion of the working muscles' glycogen stores during HIIT could be one of the mechanisms behind the effectiveness of HIIT in improving insulin sensitivity (30,36). We studied whether the greater improvements in  $\dot{V}O_{2\text{peak}}$  and whole-body insulin sensitivity after HIIT could be due to the changes in total body adiposity or observed trend toward the greater decrease in MTC after HIIT than MICT. Using myocardial MTC as a covariant, we indeed found that the difference in training response between HIIT and MICT in  $\dot{V}O_{2\text{peak}}$  (training  $\times$  time  $P = 0.10$ ) and insulin sensitivity (training  $\times$  time  $P = 0.23$ ) was no longer significant. This, therefore, suggests that the ability to rapidly reduce MTC could play a role in the superior effects of HIIT to

improve aerobic capacity and glucose metabolism. In the present study, we studied HIIT and MICT responses, and the aim was not to standardize the total amount of work done or energy consumed between the training interventions but rather to compare the two totally different training methods. Both the time spent during the training intervention (time HIIT 15 min vs MICT 300 min) and the average calculated energy consumption during the training sessions (421 vs 2907 kcal, respectively) were much less in HIIT than MICT. However, HIIT leads to higher delayed oxygen consumption for several hours after the training and thus probably also raises the posttraining basal energy metabolism more as compared with MICT. Unfortunately, we did not give any specific diet instructions but only instructed the subjects to maintain their typical diet. Moreover, we were not able to measure 24 h energy consumption and thus evaluate the effect of the possible difference in training-induced energy consumption or intake on the studied parameters.

The major limitation of our study is the relatively small number of subjects, although it is in line with previous exercise training studies with similar technically and financially demanding and detailed designs. Also the fact that DGT group included both subjects with pre-T2DM ( $n = 5$ ) and T2DM ( $n = 11$ ) can be considered as a limitation. However, the statistical analyses were performed also as diabetes status (T2DM or prediabetes) as a covariant, with no influence on the outcomes. In the present study, the whole body fat was measured with bioelectrical impedance analysis, which is commonly used in research although it is not as reliably as dual-energy X-ray absorptiometry or MRI. However, the subjects were instructed to prepare for the studies similarly before and after the training intervention and the same BIA

machine was used before and after measurement. In addition, all the participants were males, which exclude the possible variation in the results due to menstrual cycles.

## CONCLUSION

In conclusion, we have demonstrated that both MICT and HIIT can already, within 2 wk, reduce epi- and pericardial fat masses in middle-age healthy men and in men with DGT. Furthermore, HIIT increases aerobic capacity and tends to improve insulin sensitivity and reduce MTC more effectively compared with MICT. This study suggests that time-saving HIIT training is tolerable and can be recommended as an exercise mode as well as MICT to reduce enlarged myocardial adiposity.

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The authors declare no conflict of interest. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The authors have nothing to disclose. The results of the present study do not constitute an endorsement by the American College of Sports Medicine.

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# Short-term interval training alters brain glucose metabolism in subjects with insulin resistance

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## Abstract

Brain insulin-stimulated glucose uptake (GU) is increased in obese and insulin resistant subjects but normalizes after weight loss along with improved whole-body insulin sensitivity. Our aim was to study whether short-term exercise training (moderate intensity continuous training (MICT) or sprint interval training (SIT)) alters substrates for brain energy metabolism in insulin resistance. Sedentary subjects ( $n=21$ , BMI 23.7–34.3 kg/m<sup>2</sup>, age 43–55 y) with insulin resistance were randomized into MICT ( $n=11$ , intensity  $\geq 60\%$  of  $VO_{2peak}$ ) or SIT ( $n=10$ , all-out) groups for a two-week training intervention. Brain GU during insulin stimulation and fasting brain free fatty acid uptake (FAU) was measured using PET. At baseline, brain GU was positively associated with the fasting insulin level and negatively with the whole-body insulin sensitivity. The whole-body insulin sensitivity improved with both training modes (20%,  $p=0.007$ ), while only SIT led to an increase in aerobic capacity (5%,  $p=0.03$ ). SIT also reduced insulin-stimulated brain GU both in global cortical grey matter uptake (12%,  $p=0.03$ ) and in specific regions ( $p < 0.05$ , all areas except the occipital cortex), whereas no changes were observed after MICT. Brain FAU remained unchanged after the training in both groups. These findings show that short-term SIT effectively decreases insulin-stimulated brain GU in sedentary subjects with insulin resistance.

## Keywords

Insulin resistance, exercise training, brain glucose metabolism, brain lipid metabolism, positron emission tomography

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## Introduction

Although the brain represents only a small proportion of the entire body mass, the brain's energy consumption is much higher than the energy consumption of other organs at rest.<sup>1</sup> The brain has an extremely limited capacity to store energy in the form of ATP and glycogen and therefore maintaining an uninterrupted supply of ATP is pivotal for the survival of brain tissue and thus the entire organism.<sup>1</sup> Glucose has long been considered to be the sole substrate for energy production in the brain, and ATP arising from aerobic metabolism of glucose is essential for maintaining ion gradients across the plasma membrane of neurons, as well as subserving general metabolism. During strenuous exercise or prolonged starvation, glucose can be

supplemented, for example with lactate or ketone bodies,<sup>2,3</sup> and it is now appreciated that fatty acids (FAs) can contribute significantly to brain energy metabolism in the developing brain.<sup>4,5</sup>

Disturbed glucose sensing in the central nervous system (CNS), insulin signaling, and cerebral hypoperfusion has been linked to the pathophysiology of

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obesity and type 2 diabetes.<sup>6–9</sup> Compared to healthy subjects, subjects with insulin resistance have increased insulin-stimulated brain glucose uptake (GU), while insulin-stimulated GU is decreased in peripheral tissues (e.g. skeletal muscle),<sup>10–13</sup> thus brain GU in response to insulin challenge behaves in a contrary manner to GU uptake in peripheral tissues such as skeletal muscle of individuals with insulin resistance or type 2 diabetes. The molecular mechanisms of the increased brain GU are currently unclear and need to be studied in detail. However, increased metabolic sensitivity of the brain to insulin could be a sign of brain insulin resistance, which could be caused by a disturbance in the insulin transported through the blood–brain barrier (BBB) or a weakened neuronal responses to insulin.<sup>14,15</sup> It is well known that weight loss with or without concomitant exercise training improves skeletal muscle and whole-body insulin sensitivity.<sup>16,17</sup> In our recent study with morbidly obese subjects, we further showed that bariatric surgery, which caused marked weight loss and improved whole-body insulin sensitivity, led to decreased insulin-stimulated brain GU.<sup>13</sup> Furthermore, free fatty acid uptake (FAU) in the brain of subjects with metabolic syndrome is similarly increased as in peripheral tissues and decreases substantially after rapid weight loss.<sup>18</sup>

Exercise training lessens insulin resistance and especially increases skeletal muscle insulin sensitivity; however, there have only been a few studies on the effects of exercise on brain glucose metabolism in humans. The possible positive effects of exercise training on the brain may be of particular interest given the increasing awareness of the increased risk for neurodegenerative disease among patients with diabetes. It is known that increasing acute exercise intensity decreases brain GU, most probably because of the increased use of lactate as an energy source.<sup>19</sup> We and others have shown that two weeks of extremely demanding sprint interval training (SIT), consisting only of 15 min of total working time, can already improve aerobic capacity<sup>21,21</sup> and peripheral insulin sensitivity;<sup>21–23</sup> this improvement is similar or superior to moderate intensity continuous training (MICT) in healthy men and in subjects with insulin resistance. However, the effects of exercise training and different training intensities on brain metabolism are unclear.

This paper presents a study of the effects of two weeks SIT and MICT on brain GU and FAU using positron emission tomography (PET) in sedentary middle-aged men and women with insulin resistance. Based on the result from the previous studies,<sup>13,18,21</sup> our hypothesis was that SIT would decrease brain GU and FAU more than MICT due to its superior effects on whole-body insulin sensitivity in the short-term.

## Materials and methods

### Study subjects

The study was a part of a larger randomized controlled clinical HITPET trial comparing the effects of short-term SIT on tissue specific glucose and fat metabolism (<https://clinicaltrials.gov/ct2/show/NCT01344928>). The study was approved by the ethical committee of the Hospital District of Southwest Finland (Turku, Finland, decision 95/180/2010 §228) and was carried out according to the Declaration of Helsinki. All the participants gave their written informed consent. The study was performed at Turku PET Centre, University of Turku and Turku University Hospital (Turku, Finland) and the Paavo Nurmi Centre (Turku, Finland) between February 2013 and October 2015 and all the subjects were from the Southwest region of Finland.

In the current study, 21 of the 26 sedentary, non-smoking, middle-aged subjects were included. These subjects had been brain scanned and had pre-diabetes (impaired glucose tolerance/impaired fasting glucose,  $n = 8$ , females  $n = 5$ ) or type 2 diabetes ( $n = 13$ , average duration 4.4 years, females  $n = 4$ ). The inclusion criteria were as follows: an age of 40–55 years, a BMI of 18.5–35 kg/m<sup>2</sup>, a  $VO_{2peak} < 40$  ml/kg/min and no insulin treatment. The exclusion criteria were as follows: the use of insulin treatment in the case of T2DM, other chronic diseases or defects which might hinder daily life, smoking or the use of narcotics, a history of anorexia nervosa or bulimia, a history of asthma, current, regular, and systematic exercise training or a history of such training, any other condition that in the opinion of the investigator could create a hazard to the participant's safety, endanger the study procedures, or interfere with the interpretation of the study results. The screening and physical examination of the study subjects were performed by the physicians JJE and KAV.

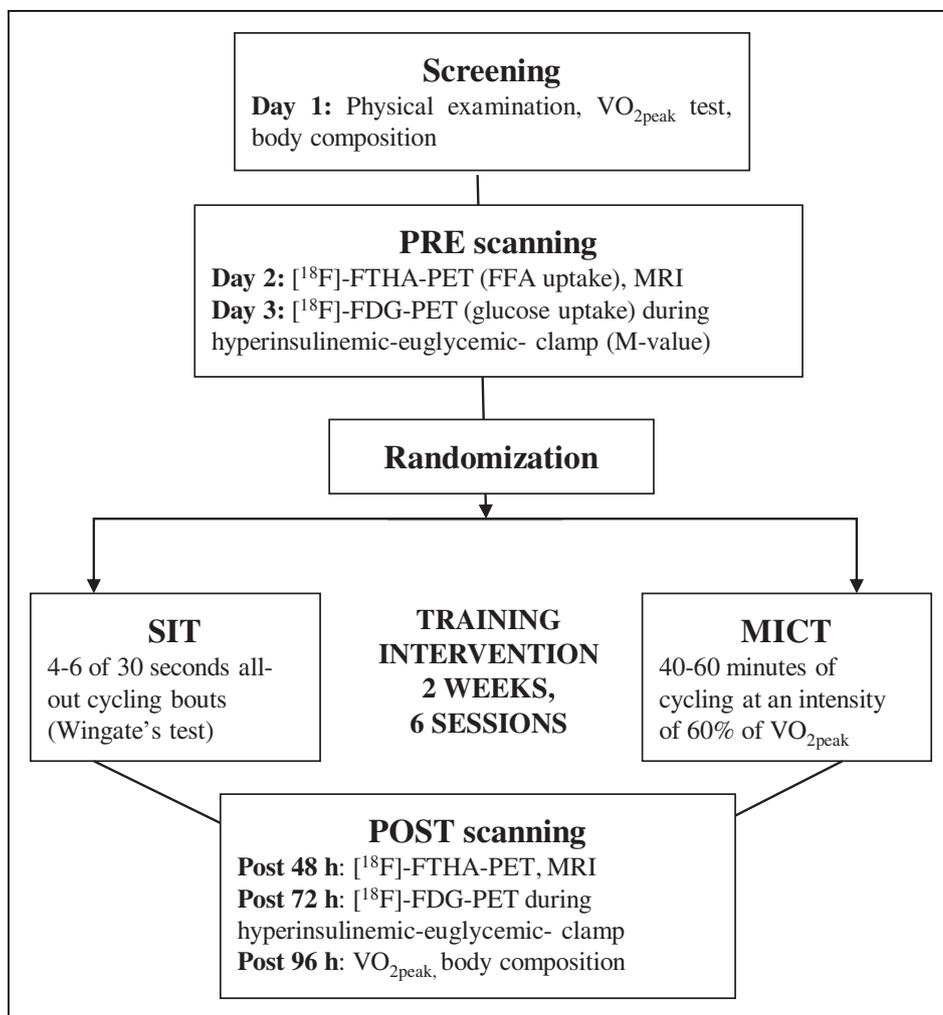
Subjects were randomized into two different training modes, either SIT or MICT, with a ratio of 1:1 in blocks of four subjects. Given the nature of the interventions, no blinding was used. Nine of the subjects in the SIT group and four in the MICT group were treated by oral hypoglycemic agents (metformin/sitagliptin/glimepiride) (Table 1). The subjects were instructed not to alter their eating habits or daily activities during the intervention. During the intervention, one participant left the study due to migraine and four for personal reasons. Due to technical problems with the PET-scanner, the radiotracer production, and the drop-outs, the brain scans were performed before the intervention for 21 subjects and after the intervention for 15 subjects. (Figure 1)

**Table 1.** Basic characteristics of the subjects at before and after training intervention and comparison of training response between SIT and MICT groups (time × training).

	SIT			MICT			Results p-values	
	Pre	Post	Post	Pre	Post	Post	Baseline	Time
	n = 10	n = 6	n = 9	n = 11	n = 9	n = 9	Time	Time × Training
Age (y)	50 (46–54)			48 (44–52)			–	–
Gender (M/F)	6/4	3/3		5/6	4/5		–	–
Diabetes (pre-T2D/T2D)	2/8	1/5		6/5	5/4		–	–
Diabetes medication (yes/no)	7/3	5/1		3/11	2/7		–	–
VO <sub>2peak</sub> (ml/kg/min)	26.6 (24.2–29.0)	27.8 (25.4–30.3)		26.7 (24.4–28.9)	26.7 (24.3–29.0)		0.97	0.13
Body mass (kg)	88.3 (80.3–97.1)	87.6 (79.6–96.3)		89.9 (82.2–98.3)	89.0 (81.4–97.3)		0.81	<b>0.032</b>
BMI (kg/m <sup>2</sup> )	29.9 (27.9–32.0)	29.8 (27.7–31.8)		30.9 (29.0–32.8)	30.6 (28.7–32.5)		0.46	<b>0.025</b>
Whole body fat (%)	33.9 (30.8–37.2)	33.1 (30.0–36.3)		34.2 (31.3–37.3)	33.1 (30.2–36.2)		0.87	<b>0.007</b>
M-value (mmol/mL/kg)	21.6 (14.5–28.8)	25.2 (17.9–32.4)		15.8 (9.1–22.4)	19.6 (12.4–26.4)		0.23	<b>0.007</b>
HbA1c (%)	5.7 (5.5–6.0)	5.6 (5.4–5.9)		5.7 (5.5–6.0)	5.5 (5.3–5.8)		0.99	<b>0.010</b>
HbA1c (mmol/mol)	39 (37–42)	38 (35–41)		39 (37–42)	37 (34–40)		0.99	<b>0.010</b>
fP Glucose (mmol/L)	7.0 (6.3–7.6)	6.9 (6.3–7.6)		6.6 (6.0–7.1)	6.3 (5.7–6.9)		0.36	0.26
P-Glucose (mmol/L)(clamp)	4.8 (4.6–5.0)	4.9 (4.7–5.0)		5.0 (4.8–5.1)	5.0 (4.8–5.2)		0.18	0.63
fS Insulin (mmol/L) <sup>a</sup>	10.6 (7.2–15.6)	9.4 (6.4–14.0)		10.7 (7.5–15.4)	10.4 (7.2–15.1)		0.96	0.34
S-Insulin (mmol/L)(clamp)	88.1 (80.0–96.3)	89.5 (80.6–98.5)		85.3 (77.7–92.9)	83.6 (75.4–91.9)		0.62	0.62
fS FFA (mmol/L)	0.82 (0.69–0.95)	0.79 (0.66–0.93)		0.84 (0.73–0.95)	0.80 (0.68–0.92)		0.77	0.31
Lactate <sub>48h</sub> (mmol/l) <sup>a</sup>	1.6 (1.3–2.0)	1.6 (1.3–2.0)		1.4 (1.1–1.7)	1.2 (0.9–1.5)		0.41	0.32
Lactate <sub>72h</sub> (mmol/L) <sup>a</sup>	1.1 (0.9–1.2)	1.1 (1.0–1.3)		1.1 (0.9–1.2)	1.0 (0.9–1.2)		0.98	0.48
fS Acetone <sub>48h</sub> (mmol/L) <sup>a</sup>	0.043 (0.038–0.048)	0.039 (0.034–0.044)		0.038 (0.034–0.043)	0.039 (0.034–0.044)		0.95	0.18
fS Acetoacetate <sub>48h</sub> (mmol/L) <sup>a</sup>	0.038 (0.027–0.054)	0.040 (0.029–0.057)		0.036 (0.026–0.049)	0.036 (0.025–0.051)		0.85	0.79
fS D-β-Hydroxybutyrate <sub>48h</sub> (mmol/L) <sup>a</sup>	0.24 (0.15–0.35)	0.26 (0.17–0.40)		0.23 (0.15–0.48)	0.23 (0.15–0.34)		0.90	0.50

Note: The P value for baseline describes the difference between SIT and MICT groups before training, and 'time' describes training effect in the whole group (n = 21). 'Training × time' compares all SIT trained (n = 10) to all MICT trained (n = 11). All the data are presented as model based means (95% confidence interval, CI). The values are LSmeans transformed into original unit. SIT: sprint interval training; MICT: moderate intensity continuous training; T2D: type 2 diabetes; fS: fasting serum value; S: serum value; fP: fasting plasma value; P: plasma value; FFA: free fatty acid; HbA1c: glycated hemoglobin.

<sup>a</sup>Variables with logarithmic transformation to achieve the normal distribution. Boldfaced values are significantly different after intervention, P < 0.05.



**Figure 1.** Consort flow of the study subjects.

### Study design

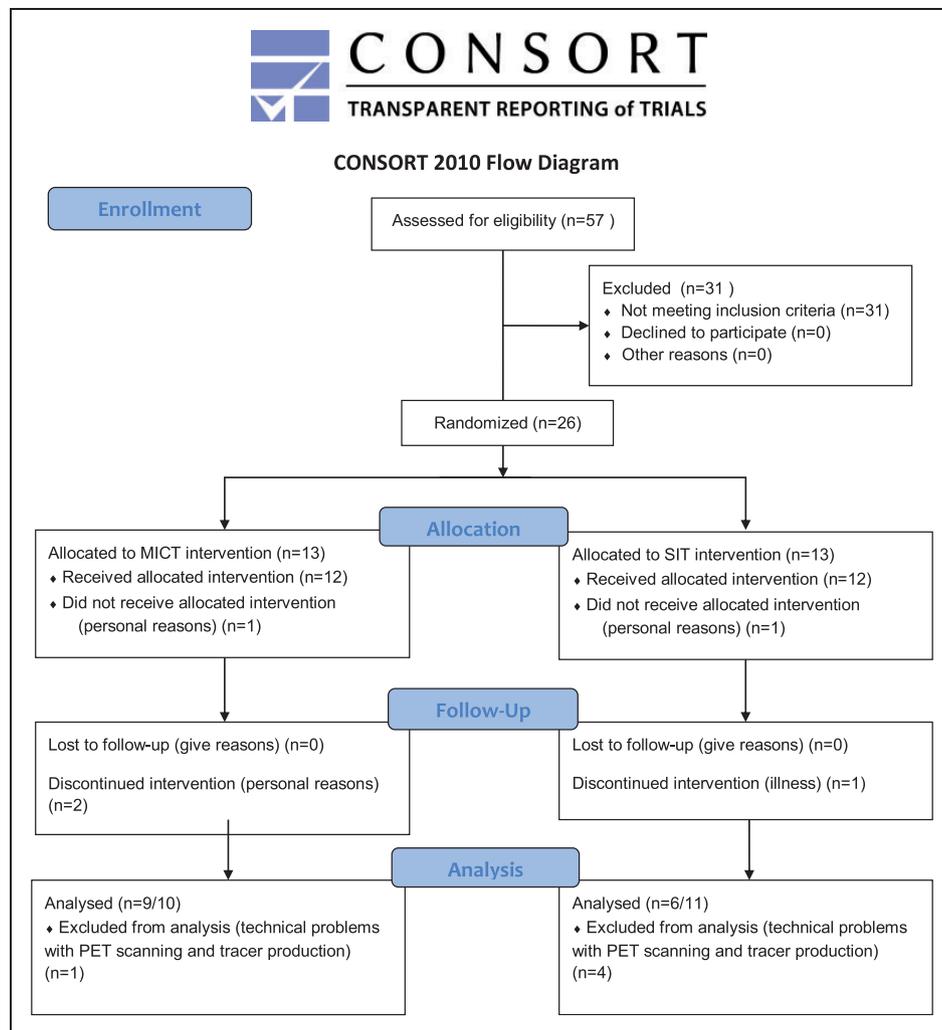
Initial screening included a physical examination, a bioimpedance measurement, an oral glucose tolerance test (OGTT), and a cycling  $VO_{2peak}$  test to assess the participant's health, glycemic status, body composition and aerobic capacity (day 1, Figure 2). The participants then underwent two PET imaging sessions on two different days. On the first study day, the fasting brain FAU was studied and on the second day the brain GU was studied during an euglycemic hyperinsulinemic clamp using PET and radiotracers 14(R,S)-[ $^{18}F$ ]fluoro-6-thia-heptadecanoic acid ([ $^{18}F$ ]FTHA) and FDG, 2-[ $^{18}F$ ]fluoro-2-deoxy-D-glucose ([ $^{18}F$ ]FDG), respectively, at resting conditions (Figure 2). All PET studies were conducted after an overnight fast. Subjects were instructed to refrain from caffeine-containing nutrients and strenuous physical activity for 12 and 24 h prior to the studies and anti-diabetic medication for 48 h.

In both training modes, the training intervention took two weeks and included six supervised training

sessions. After the two-week training intervention, either SIT or MICT, all the studies were repeated after the last exercise session starting with an [ $^{18}F$ ]FTHA PET after 48 h, an [ $^{18}F$ ]FDG PET during an euglycemic hyperinsulinemic clamp after 72 h, and finally after 96 h OGTT and  $VO_{2peak}$  tests were conducted.

### Exercise intervention

The SIT sessions consisted of 4–6 × 30 s supra-maximal all-out cycling bouts (Wingate protocol) (Monark Ergonomic 828E, MONARK, Vansbro, Sweden) with 4 min recovery time between each. The number of bouts increased progressively starting with four bouts and increasing by one every other session up to six bouts. The total duration of training was only 15 min including all six training sessions. The training load was individually determined (10 % of lean body mass in kg). The SIT protocol was based on a previous study by



**Figure 2.** The study design.

VO<sub>2peak</sub>: aerobic capacity; PET: positron emission tomography; FDG: 2-[<sup>18</sup>F]fluoro-2-deoxy-D-glucose; FTHA: 14(R,S)-[<sup>18</sup>F]fluoro-6-thia-heptadecanoic acid; FFA: free fatty acid; SIT: high-intensity interval training; MICT: moderate-intensity continuous training.

Burgomaster et al.<sup>20</sup> All the participants were familiarized with the SIT training protocol (2 × 30 s bouts) before they were randomized into the training groups. The MICT sessions consisted of 40–60 min of cycling at moderate intensity, which was ≥60 % of the maximal output calculated from the individual VO<sub>2peak</sub> (Tunturi E85, Tunturi Fitness, Almere, Netherlands). The duration of MICT-training increased progressively starting with 40 min and increasing by 10 min every other session up to 60 min. The total duration of training was 300 min including all six training sessions.

### PET imaging and analysis

PET imaging was conducted using a Ge Discovery VCT PET/CT scanner (General Electric Medical Systems, Milwaukee, WI, USA) as previously described.<sup>23</sup> PET scanning of the brain was started ~80 min after the

[<sup>18</sup>F]FDG / [<sup>18</sup>F]FTHA injection and during the [<sup>18</sup>F]FDG PET study ~170 min after the start of the euglycemic clamp. Eskelinen et al.<sup>21</sup> have previously described in detail the technical aspects of the PET imaging procedures. The euglycemic hyperinsulinemic clamp was performed based on the original protocol by DeFronzo et al.<sup>24</sup> The insulin-stimulated whole-body GU (M-value) was calculated from the glucose infusion rate and the measured glucose values collected during the PET scan. Arterialized venous plasma glucose was determined in duplicate by the glucose oxidase method and the mean of these values was taken to represent the plasma glucose. (Analox GM9 Analyzer; Analox Instruments LTD, London, United Kingdom).

The production of radiotracers [<sup>18</sup>F]FDG and [<sup>18</sup>F]FTHA has been previously described.<sup>21,25</sup> [<sup>18</sup>F]FTHA is a palmitate analogue for fatty acid metabolism. [<sup>18</sup>F]FTHA is injected into the circulation

from the point where it crosses the BBB and enters the brain.<sup>26</sup> In the brain, it either subsequently infiltrates the mitochondria or is incorporated into complex lipids,<sup>25</sup> mostly triglycerides. In mitochondria, [<sup>18</sup>F]FTHA undergoes the initial steps of  $\beta$ -oxidation and is thereafter trapped as further  $\beta$ -oxidation is blocked by its sulfur heteroatom.<sup>25</sup> The half-life of [<sup>18</sup>F]FTHA is 109 min.

All PET imaging data were preprocessed using SPM8 (Wellcome institute, London, UK) and manual volumes-of-interest were delineated using Carimas (version 2.9, Turku PET Centre). Fractional uptake rates (FUR) were calculated regionally relative to the concentration of the tracer in the plasma, and subsequently the metabolic rate of the glucose, or GU, was calculated relative to individual glucose concentration in the blood.

For the calculation of FAU, an [<sup>18</sup>F]FTHA metabolite correction was performed for the radioactivity curves.<sup>21</sup> Plasma and tissue time-radioactivity curves were analyzed graphically by linear graphical analysis of brain uptake relative to the metabolite-corrected arterial input function.<sup>27</sup> To obtain brain GU/FAU, the fractional uptake rate values were multiplied by the serum glucose/FFA concentration during the [<sup>18</sup>F]FDG/[<sup>18</sup>F]FTHA PET scanning and corrected for brain density (1.04 g/mL). A Lumped constant 0.65 was used for the brain GU.<sup>28</sup>

All imaging data were preprocessed using SPM8 (Wellcome institute, London, UK). Firstly, all DICOM data were converted into Nifti-format using SPM-DICOMimport. Secondly, within each PET session, the frame-to-frame misalignments were compensated for by using a mutual information-(MI) based rigid registration. A visual inspection was deemed adequate for the alignment of the PET and CT data, and thus no re-alignment was performed. Thirdly, the CT image was aligned with a CT-template in MNI coordinates using rigid registration, and the mapping was subsequently written to PET data as well. CT-based normalization (non-rigid registration) was conducted with a Clinical Toolbox<sup>29</sup> and the PET imaging data were subsequently warped into the MNI space using the result deformation. The quality of the non-rigid registration was visually inspected and a small number of failures were detected. Final normalization of the PET data was obtained using the ligand-specific template as the target and individual PET image as the source in SPM-Normalize. Both the target and source images were smoothed with a 3D Gaussian filter using an 8 mm kernel (FWHM).

Volume-of-interest (VOI) analysis was conducted using common manually delineated VOIs in MNI space. The VOIs were delineated on an MRI-template using Carimas (version 2.9, Turku PET Centre). Similar to Kemppainen et al.,<sup>19</sup> the VOIs were placed

on the anterior cingulate cortex (ACC), the medial frontal cortex (MFC), the dorsal superior frontal gyrus (SFC), the temporal cortex (TC), the occipital cortex (OC), the thalamus (THA), the cerebellum (CER), and pons. Whole brain uptake was measured using VOI covering the frontal, temporal, occipital, and parietal lobes. The brain networks were visualized with the BrainNet Viewer (<http://www.nitrc.org/projects/bnv/>).<sup>30</sup>

### Other measurements

The  $VO_{2peak}$  was determined via a maximal bicycle ergometer test.<sup>21</sup> The test was performed at the Paavo Nurmi Center (Turku, Finland) about one week before the first training session and 96 h after the last training session. D- $\beta$ -hydroxybutyrate was quantified from serum using high-throughput proton NMR metabolomics (Brainshake Ltd, Helsinki, Finland). Details of the experimentation and applications of the NMR metabolomics platform have been described previously.<sup>31</sup> Blood lactate concentration was measured from capillary samples before each training session and within 1 min after each session using a handheld lactate analyzer (Lactate Pro, Arkray KDK, Kyoto, Japan); it was also measured in fasting conditions from blood samples during the [<sup>18</sup>F]FDG and [<sup>18</sup>F]FTHA days. Fat percentage was determined using the bioimpedance method (InBody 720, Mega Electronics Ltd, Kuopio, Finland).

### Statistical analysis

Statistical analyses were performed using SAS (version 9.3 for Windows, SAS institute Inc., Cary, NC, USA). The normal distribution of the variables was tested with the Shapiro–Wilk test. Logarithmic transformations were performed for the variables fasting insulin, lactate, acetone, acetoacetate and D- $\beta$ -hydroxybutyrate in order to achieve normal distribution. The baseline characteristics of the training groups were compared by a one-way ANOVA, which included the main effect of the training mode (SIT and MICT). The mean value changes between pre and post measurements were analyzed using a hierarchical linear mixed model. In the model, the training mode and time effects were included as well as all interactions. In addition, linear contrasts were programmed within the model to estimate the overall mean value change within group (Table 1). Missing data points were accounted for by restricted maximum likelihood estimation within the linear mixed models. Hence, we report model-based mean (SAS least square means) values (95% CI) from all the parameters measured before and after the training. Correlation analyses were carried out using Pearson's Correlation. A *p*-value of less than 0.05 was considered

statistically significant. The sample size was calculated for the whole study (NCT01344928) based on its primary outcome and has been described previously.<sup>23</sup>

## Results

Aerobic capacity improved 5% with SIT (time  $p=0.03$ ) but stayed unchanged after MICT (Table 1). Overall, in the whole study population, training increased the whole-body insulin sensitivity (M-value) by 20 % (time  $p=0.007$ ) and decreased slightly, but significantly, the whole-body adiposity and the HbA1c; however, no differences were observed between the training modes (Table 1).

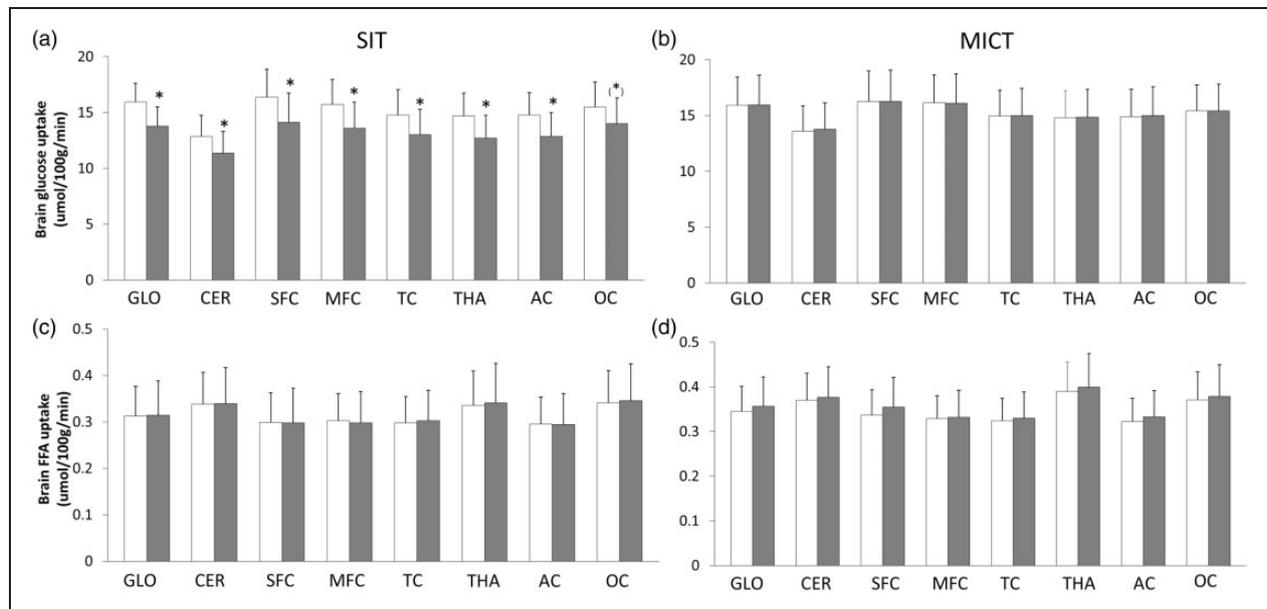
We measured insulin-stimulated brain GU both globally in the gray matter and when divided into discrete brain regions. SIT decreased the insulin-stimulated brain GU globally by 14% ( $p=0.03$ ) and in all regions of the cortex except the occipital cortex, whereas no changes were observed after MICT (Figures 3(a) and 4). In the whole study group, at baseline, the insulin-stimulated brain GU correlated inversely with the whole-body insulin sensitivity (M-value;  $r=-0.68$ ,  $p=0.001$ , Figure 5(a)), and positively with the serum insulin concentration in a fasted state ( $r=0.46$ ,  $p=0.04$ ) and during the clamp procedure ( $r=0.47$ ,  $p=0.04$ ), but did not reach a significant level for D- $\beta$ -hydroxybutyrate ( $r=-0.40$ ,  $p=0.07$ , Figure 5(b)).

We found no change in the brain FAU in either group after training (Figure 3(b)). Before the training, the brain FAU correlated positively with the whole-body fat percent ( $r=0.59$ ,  $p=0.04$ , Figure 5(d)) and inversely with aerobic capacity ( $r=-0.47$ ,  $p=0.04$ , Figure 5(c)). In addition, brain FAU correlated with LDL ( $r=0.51$ ,  $p=0.03$ ), total cholesterol ( $r=0.56$ ,  $p=0.02$ ), and plasma glucose during the FTHA scan ( $r=-0.57$ ,  $p=0.01$ ) and tended to correlate with the serum insulin during the FTHA scan ( $r=-0.44$ ,  $p=0.06$ ) (data not shown).

## Discussion

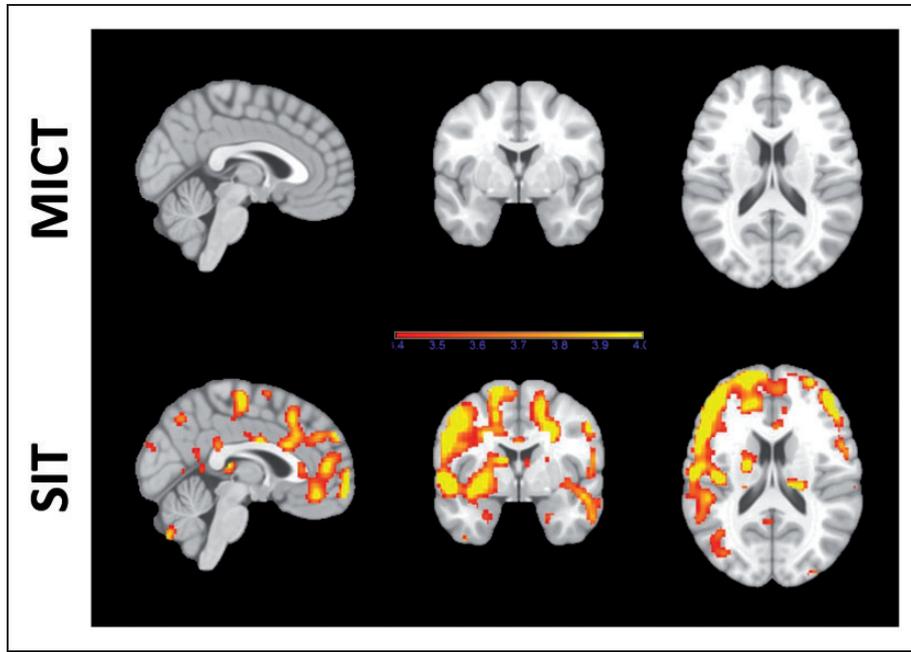
These results show that insulin-stimulated brain GU correlates negatively with whole-body insulin sensitivity. However, although both SIT and MICT enhanced whole-body insulin sensitivity, only SIT decreased the insulin-stimulated brain GU in the sedentary, middle-aged subjects with impaired glucose tolerance.

The human brain is an insulin-sensitive organ, as has been shown using the [<sup>18</sup>F]FDG-PET method with and without insulin-stimulation.<sup>3,32</sup> In the study by Hirvonen et al.,<sup>33</sup> conducted in our laboratory, it was shown that brain GU is increased in insulin resistant subjects but not in healthy controls during hyperinsulinemia. This suggests that whereas the effect of insulin on brain GU in healthy subjects is

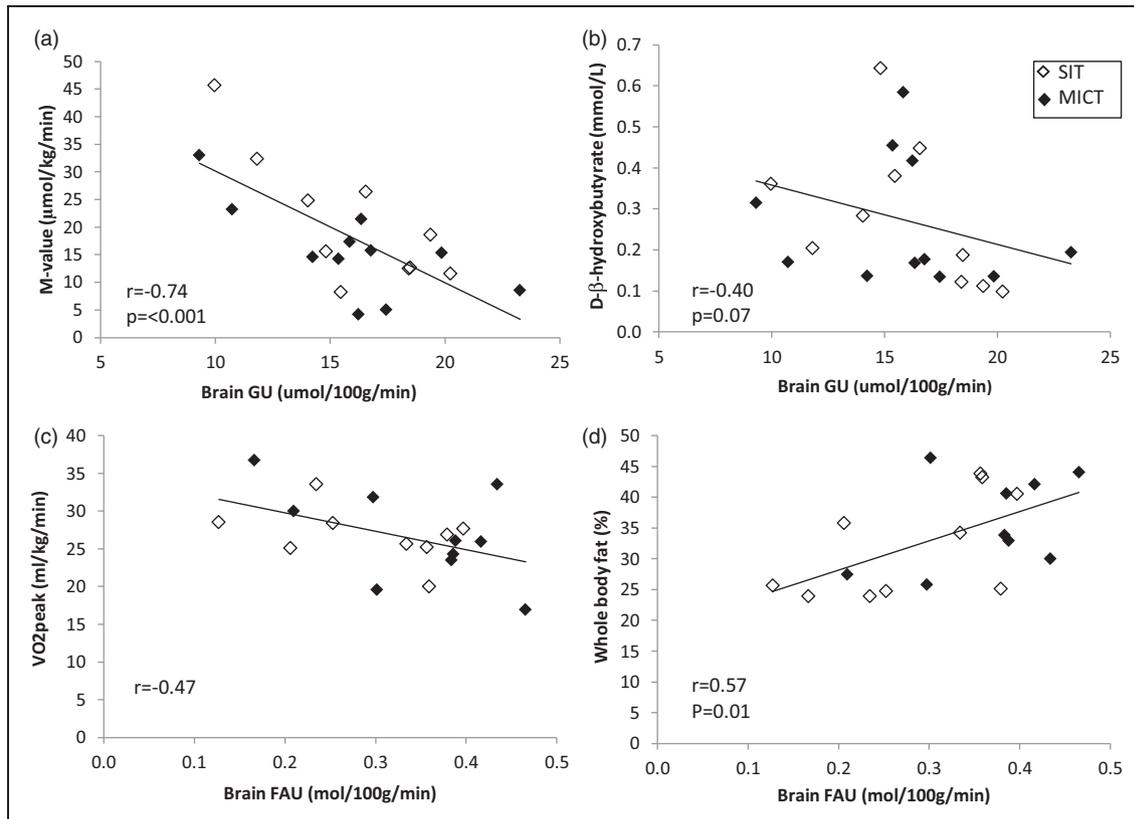


**Figure 3.** Brain glucose uptake (a and b) and brain FFA uptake (c and d) globally and in different areas of the brain in SIT (a and c) and MICT (b and d) training group before and after the training intervention.

GLO: global; CER: cerebellum; SFC: superior frontal gyrus; MFC: medial frontal gyrus; TC: temporal cortex; THA: thalamus; AC: cingulate gyrus; OC: occipital cortex. Values are model-based means (95% confidence interval). \* $P < 0.05$  for the time effect within the training mode (pre vs. post comparison).



**Figure 4.** Pre vs. post change in brain glucose uptake in different areas of the brain in sprint-interval training (SIT) and moderate intensity continuous training (MICT) groups.  $P < 0.01$  voxel level uncorrected, which is equal to  $T = 3.365$  in SIT and  $T = 2.896$  in MICT. The bar represents T values.



**Figure 5.** Correlations between insulin-stimulated brain glucose uptake (GU) and whole-body insulin sensitivity (M-value) (a), brain GU and 3-hydroxybutyrate (b), brain free fatty acid uptake (FAU) and  $VO_{2peak}$  (c) and brain FAU and whole-body fat content (d) before the intervention in the whole study group ( $n = 21$ ).

already maximal in a fasting state, a higher dose of insulin is needed to increase brain GU in insulin resistant subjects.<sup>33</sup> Insulin-stimulated brain GU is also increased in morbidly obese compared to healthy subjects, and it decreases after bariatric surgery induced-weight loss and improvement in whole-body insulin sensitivity.<sup>13</sup> The exact mechanism explaining the increased brain insulin sensitivity after the weight loss is unclear, but may be related to the decreased ratio of insulin between cerebrospinal fluid and plasma.<sup>34</sup> This decreased ratio may be caused by (1) impaired insulin transport through the BBB, (2) declined neuronal response to insulin, or (3) increased insulin clearance due to an adaptation to chronic hyperinsulinemia in insulin resistance.<sup>13,14</sup>

In the present study, we show that brain GU correlates positively with serum insulin in a fasting state and during hyperinsulinemia, but strongly negatively with whole-body insulin sensitivity (Figures 5(a) and 6(a)). Furthermore, we show that there is a reduction in insulin-stimulated brain GU in insulin resistant subjects after SIT but not after MICT. Training improved whole-body insulin sensitivity in both groups, but aerobic capacity improved only after SIT. We found no correlation between the improvement in brain GU and the improvement in whole-body insulin sensitivity or aerobic capacity.

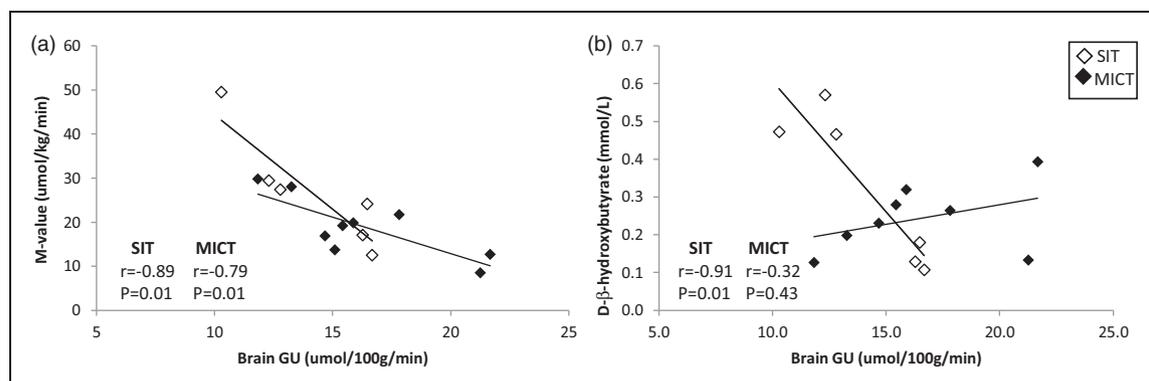
When available, the brain prefers lactate over glucose as an energy source and during exercise, the brain takes up lactate in proportion to the arterial concentration. Thus, the decreased brain GU during acute exercise with increasing intensity has been linked to an increased uptake of lactate in the brain.<sup>19,35–37</sup> In the present study, the SIT consisted of extremely high intensity interval cycling, which led to markedly higher lactate concentrations after the training sessions compared to MICT sessions (SIT  $14.4 \pm 0.9$  vs. MICT  $3.8 \pm 1.4$  mmol/l). However, we did not find

correlations between blood lactate levels measured after the training sessions or at rest on PET study days and the decrease in brain GU. On the other hand, the concentration of the ketone body D- $\beta$ -hydroxybutyrate (DBHB) correlated negatively with the brain GU after the training in the SIT group (Figure 6(b)). DBHB is an energy metabolite of which level increases in the liver when glucose levels are reduced, for example, after caloric restriction, fasting, or prolonged exercise.<sup>38</sup> DBHB is believed to serve as a signaling molecule in response to metabolic changes and as an energy source. In the brain, the levels of ketone bodies can reach levels as high as 1–5 mmol/L.<sup>39</sup> Thus, it might be that the decrease in GU after SIT is partly explained by the increased utilization of other energy substrates, such as DBHB.

Interestingly, at baseline, the brain FAU correlated inversely with aerobic capacity and fasting plasma glucose but positively with whole body fat percent and total and LDL cholesterol; thus, linking the high brain FAU to known metabolic risk factors. However, we did not find any changes in brain FAU that would suggest that training induces alterations more rapidly in brain glucose than lipid metabolism.

### Limitations of the study

The study subjects included both pre-diabetic and type 2 diabetic subjects. Omitting subjects with pre-diabetes (IFG, IGT) from the analysis or running the analysis using grouping according to diabetes status (pre-diabetes/type 2 diabetes) did not alter the results. Medication was used as a covariate in the SAS analysis; however, it did not explain the difference between the pre-intervention and post-intervention decrease in GU. To complete the whole study, subjects had to participate in four extensive scanning days, which led to a relatively high drop-out rate. In addition, healthy controls were not studied for a comparison.



**Figure 6.** Correlations between insulin-stimulated brain glucose uptake (GU) and whole-body insulin sensitivity (M-value) (a) and brain GU with 3-hydroxybutyrate (b) after the intervention.

## Conclusions

This study provides the first evidence that short-term exercise training alters brain glucose metabolism in subjects with impaired glucose tolerance. Our results suggest that in addition to the well-known beneficial effects of exercise training on whole-body insulin sensitivity, SIT decreases insulin-stimulated brain GU in subjects with insulin resistance.

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## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Authors' contributions

SMH analyzed and interpreted the data and wrote the manuscript. JJ and KKM analyzed the data and edited the manuscript. JE and KV collected the data and edited the manuscript. EL contributed to statistical analysis and edited the manuscript. PN and JK interpreted and edited the manuscript. KK planned the experiments and edited the manuscript. JCH planned the experiments, interpreted the data and wrote the manuscript. All authors approved the version to be published. JCH is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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