UNIVERSITY OF TURKU

EFFECTS OF SHORT-TERM EXERCISE TRAINING ON LIVER, PANCREAS, AND INTESTINAL METABOLISM

Kumail Kumar Motiani

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA - SER. D OSA - TOM. 1432 | MEDICA - ODONTOLOGICA | TURKU 2019

UNIVERSITY OF TURKU

EFFECTS OF SHORT-TERM EXERCISE TRAINING ON LIVER, PANCREAS, AND INTESTINAL METABOLISM

Kumail Kumar Motiani

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA - SER. D OSA - TOM. 1432 | MEDICA - ODONTOLOGICA | TURKU 2019

University of Turku

Faculty of Medicine
Department of Clinical Physiology and Isotope Medicine
Doctoral Programme in Clinical Research (DPCR)
Turku PET Centre

Supervised by

Adjunct Professor Jarna Hannukainen, PhD
Turku PET Centre
University of Turku
Turku, Finland

Adjunct Professor Kari Kalliokoski, PhD
Turku PET Centre
University of Turku
Turku, Finland

Reviewed by

Adjunct Professor Satu Pekkala, PhD
Faculty of Sport and Health Sciences
University of Jyväskylä
Jyväskylä, Finland

Associate Professor Nils Wierup
Department of Clinical Sciences
University of Lund
Lund, Sweden

Opponent

Professor Anna Krook
Department of Physiology and
Pharmacology
Karolinska Institutet
Stockholm, Sweden

ISBN 978-951-29-7662-1 (PRINT)
ISBN 978-951-29-7663-8 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Grano Oy - Turku, Finland 2019

If we knew what we were doing,
it would not be called research,
would it?

Albert Einstein

To my parents, Kishore and Bina Motiani

ABSTRACT

Kumail Kumar Motiani

Effects of short-term exercise training on liver, pancreas and intestinal metabolism

University of Turku, Faculty of Medicine, Department of Clinical Physiology and Isotope Medicine, Doctoral Programme in Clinical Research (DPCR), Turku PET Centre
Annales Universitatis Turkuensis, Turku, Finland, 2019

The health benefits of exercise have been known as early as the 5th century. Since then many studies have corroborated these benefits involving both animal and human subjects. But still, the scientific community is uncertain on whether exercise intensity or volume is more important for health benefits. Some studies indicate that vigorous intensity with low volume is adequate to improve whole-body insulin sensitivity and organ metabolism, while others indicate that moderate intensity and high volume exercise is required to achieve these benefits.

In this thesis, I have investigated the effects of two weeks of low volume and high intensity sprint interval training (SIT) and high volume and low intensity moderate intensity continuous training (MICT) on the liver, pancreas, and intestine in untrained healthy subjects and subjects with type 2 diabetes or prediabetes using a wide range of imaging modalities. Hepatic and pancreatic fat was measured using magnetic resonance spectroscopy (MRS). The hepatic, pancreas, and intestinal insulin-stimulated glucose and fasting free fatty acid uptake was measured using positron emission tomography (PET). Lipoprotein subfraction composition was analyzed with nuclear magnetic resonance (NMR) and gut microbiome was analyzed with 16S rRNA gene sequencing.

Both SIT and MICT reduced the liver and pancreatic fat content in subjects with high hepatic and pancreatic fat content. They also improved the lipoprotein subfraction profile with increasing the lipoproteins associated with protecting against diabetes and reducing the ones associated with acquiring diabetes. Moreover, both training modes also changed the gut microbiota profile with increasing microbes associated with protection against obesity. However, only MICT improved the hepatic and intestinal insulin-stimulated glucose and reduced fasting free fatty acid uptake.

In conclusion, two weeks of exercise improves fat content, lipoprotein, and the gut microbiota profile regardless of training intensity or volume; however, the changes in the liver and intestinal substrate uptake depend on exercise volume rather than intensity. This suggests that, in the short-term, MICT might be more beneficial in improving internal organ metabolism than SIT.

Keywords: Liver metabolism, hepatic fat content, lipoprotein subclasses, pancreatic metabolism, pancreatic fat content, intestinal metabolism, gut microbiome, exercise, sprint interval training, moderate intensity continuous training.

TIIVISTELMÄ

Kumail Kumar Motiani

Lyhyen aikavälin kuntoilun vaikutukset maksan, haiman ja suoliston aineenvaihduntaan

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

Turun Yliopiston julkaisuja, Turku, Suomi, 2019

Liikunnan terveyshyödyt on tunnettu jo pitkään. Lukuisat eläin- ja ihmistutkimukset ovat osoittaneet, että liikunta hyödyttää koko kehoa. Uusimmat tutkimukset puolestaan osoittavat, että suurin piirtein samat liikunnan hyödyt voi saada sekä kovatehoisella lyhytkestoisella että kohtuukuormitteisilla pidempikestoisella liikunnalla. Kumpi näistä on sitten hyödyllisempi terveyden kannalta?

Tässä väitöskirjatyössä vertasin kovatehoisen intervalliharjoittelun (SIT) ja kohtuukuormitteisen harjoittelun (MICT) vaikutuksia maksaan, haimaan ja suolistoon aiemmin vain vähän liikuntaa harrastaneilla terveillä ja tyypin 2 diabetesta tai sen esiastetta sairastavilla henkilöillä käyttäen erilaisia kuvantamismenetelmiä. Maksan ja haiman rasvan määrää mitattiin käyttäen magneettispektroskopiaa. Maksan, haiman ja suoliston insuliinistimuloitua glukoosin soluun ottoa ja vapaiden rasvahappojen soluun ottoa paastotilanteessa mitattiin käyttämällä positroniemissiotomografiaa (PET). Lisäksi lipoproteiinien alayksikkökoostumus analysoitiin ydinmagneettiresonanssispektroskopialla ja suoliston mikrobistoa analysoitiin 16S rRNA-geenisekvensoinnilla.

Sekä SIT että MICT vähensivät maksan ja haiman rasvapitoisuutta henkilöillä, joilla ne olivat ennen harjoittelua koholla. Molemmat harjoittelumuodot paransivat myös lipoproteiinien alayksikköprofiilia lisäämällä alentuneeseen diabetesriskiin ja vähentämällä lisääntyneeseen diabetesriskiin liittyviä lipoproteiineja sekä paransivat suoliston mikrobiprofiilia lisäämällä pienempään lihavuusriskiin liitettyjä mikrobeja. Ainoastaan MICT paransi maksan ja suolen insuliinistimuloitua glukoosinottoa ja vähensi vapaiden rasvahappojen ottoa paastotilanteessa.

Maksan ja haiman rasvapitoisuus, veren lipoproteiiniprofiili ja suoliston mikrobiprofiili parantuivat kahden viikon harjoittelun jälkeen harjoittelun intensiteetistä tai määrästä riippumatta. Vain MICT paransi maksan ja suolen glukoosin ja rasvahappojen soluunottoa. Näiden tulosten perusteella kohtuukuormitteinen harjoittelu näyttää olevan kovatehoista harjoittelua hyödyllisempää sisäelinten aineenvaihdunnalle.

Avainsanat: Maksan aineenvaihdunta, rasvapitoisuus, lipoproteiinialaluokat, haiman aineenvaihdunta, suoliston aineenvaihdunta, suoliston mikrobisto, liikunta, kovatehoinen intervalliharjoittelu, kohtuukuormitteinen harjoittelu.

TABLE OF CONTENTS

ABBREVIATIONS

[^{18}F]FDG	2-[^{18}F]fluoro-2-deoxy-D-glucose
[^{18}F]FTHA	14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid
ALAT	Alanine transaminase
ASAT	Aspartate transaminase
CD36	Cluster of differentiation 36
CI	Confidence intervals
CON	Control
CT	Computed tomography
EGP	Endogenous glucose production
FAU	Fatty acid uptake
FFA	Free fatty acids
GLUT	Glucose transporter
GU	Glucose uptake
HbA_1c	Glycosylated hemoglobin
HDL	High density lipoprotein
HFC	High hepatic fat content
HIIT	High intensity interval training
IDL	Intermediate density lipoprotein
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
LBP	Lipopolysaccharide binding protein
LDL	Low density lipoprotein
LFC	Low hepatic fat content
LPS	Lipopolysaccharide
MICT	Moderate intensity continuous training

MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
M-value	Whole-body insulin sensitivity
NAFLD	Non-alcoholic fatty liver disease
NMR	Nuclear magnetic resonance
OGTT	Oral glucose tolerance test
PET	Positron emission tomography
PFC	Pancreatic fat content
ROI	Region of interest
SGLT	Sodium-glucose linked transporter
SIT	Sprint interval training
T2D	Type 2 diabetes
VLDL	Very low density lipoprotein
VO_{2peak}	Aerobic capacity

LIST OF ORIGINAL PUBLICATIONS

I. Motiani KK, Savolainen AM, Eskelinen JJ, Toivanen J, Ishizu T, Yli-Karjanmaa M, Virtanen KA, Parkkola R, Kapanen J, Gronroos TJ, Haaparanta-Solin M, Solin O, Savisto N, Ahotupa M, Löyttyniemi E, Knuuti J, Nuutila P, Kalliokoski KK, Hannukainen JC. Two weeks of moderate intensity continuous training, but not high intensity interval training increases insulin-stimulated intestinal glucose uptake. Journal of Applied Physiology (2017), 122(5):1188-1197.

II. Heiskanen MA*, Motiani KK*, Mari A, Saunavaara V, Eskelinen JJ, Virtanen KA, Koivumäki M, Löyttyniemi E, Nuutila P, Kalliokoski KK, Hannukainen JC (2018). Exercise training decreases pancreatic fat content and improves beta cell function regardless of baseline glucose tolerance: a randomised controlled trial. Diabetologia (2018) 61: 1817.

III. Motiani KK, Savolainen AM, Toivanen J, Toivanen J, Eskelinen JJ, Yli-Karjanmaa M, Virtanen KA, Saunavaara V, Heiskanen MA, Parkkola R, Haaparanta-Solin M, Solin O, Savisto N, Löyttyniemi E, Knuuti J, Nuutila P, Kalliokoski KK, Hannukainen JC. Effects of short-term sprint interval and moderate-intensity continuous training on liver fat content, lipoprotein profile and substrate uptake: a randomized trial. Journal of Applied Physiology (2019) April 18.

IV. Motiani KK, Collado MC, Eskelinen JJ, Virtanen KA, Löyttyniemi E, Salminen S, Nuutila P, Kalliokoski KK, Hannukainen JC (2019). Exercise training modulates gut microbiota profile and improves endotoxaemia. Manuscript.

*Equal author contribution.

1 INTRODUCTION

Hippocrates has been attributed for stating that "…eating alone will not keep a man well; he must also take exercise. For food and exercise, while possessing opposite qualities, yet work together to produce health" (Jones WHS, 1931). These words of wisdom highlight the importance of physical activity in maintaining good health. Since then many studies have shown the benefits of exercise training, in fact, exercise has been shown to be a cornerstone in the prevention of at least 35 chronic diseases, such as obesity and insulin resistance type 2 diabetes(T2D) (Booth et al., 2012). Even though the benefits of exercise are well-known, for the majority of the population the amount of exercise performed has decreased and there has been an increase in sedentary lifestyle. This increase in sedentary behavior has led to a surge in obesity and lifestyle-induced diseases, which could have easily been avoided with exercise (Blair and Morris, 2009). Therefore, new exercise regimens (with lower duration and higher benefits) are needed to motivate the general population in order to avoid the increase in the prevalence of these lifestyle diseases associated with physical inactivity.

Traditional moderate intensity continuous exercise training (MICT) such as brisk walking, cycling, and jogging has long been known to improve insulin sensitivity and fitness (Colberg et al., 2010; Penedo and Dahn, 2005). Many studies have shown that there is a dose-response relationship between the length of physical activity and health benefits (increasing physical volume achieves the most benefits) (Garber et al., 2011a). However, MICT is a time consuming exercise regimen and therefore adherence to MICT among the general population remains low, with "lack of time" being cited as one of the main reasons (Trost et al., 2002). Subsequently, a lot of interest and research is being given to high intensity interval training (HIIT), particularly because HIIT with its substantially lower exercise volume has been shown to achieve the same health benefits as MICT (Gibala and McGee, 2008; Jelleyman et al., 2015; Milanovic et al., 2015). Normally, HIIT consists of short bursts of extremely intensive exercise bouts (be it cycling, rowing, skiing, walking) interposed with either light-intensity activity or complete rest recovery periods (Kemi and Wisloff, 2010). HIIT protocols include 1-4 min of exercise bouts performed at 80-100% of HR_{max}, while protocols consisting of ≤ 30 seconds of exercise bouts at maximal intensity (all-out) effort are referred to as sprint interval training (SIT) (Weston et al., 2014). Recently, both HIIT/SIT have been shown to induce superior cardiovascular benefits compared to MICT in healthy, sedentary, obese subjects and in subjects with lifestyle-induced diseases (Milanovic et al., 2015; Gillen et al., 2016a; Ramos et al., 2015a; Weston et al., 2014; Turk et al., 2017). Moreover, some

studies have shown HIIT/SIT have been perceived as more enjoyable compared to MICT even in obese individuals (Bartlett et al., 2011; Kong et al., 2016). Hence, HIIT/SIT with their short duration and higher health benefits might be the exercise regimens which can be acceptable to the general population.

Benefits of exercise training on insulin sensitivity have been known since the 5th century (Jones WHS, 1931). Most of the benefits of exercise on whole-body glucose metabolism are attributed to the exercise-induced adaptations on the muscle glucose uptake (GU) (Stanford and Goodyear, 2014). Acute exercise increases skeletal muscle uptake via translocating glucose transporter 4 (Glut4) via multiple signaling pathways and chronic exercise increases muscle GU by increasing mitochondria and Glut4 protein expression (Stanford and Goodyear, 2014). These exercise induced improvements in the skeletal muscle play a key role in the prevention and treatment of T2D because insulin resistance in the skeletal muscle plays a key role in the pathogenesis of T2D (DeFronzo and Tripathy, 2009; Kahn et al., 2014). In addition, to skeletal muscle the splanchnic region comprising of intestine, liver and pancreas, also play a key role in the regulation of the whole-body glucose homeostasis and perform a plethora of essential homeostatic functions.

Intestine in addition to absorbing nutrients, encompasses gut microbiome. The human gut microbiome consists of almost 10^{14} micro-organisms (bacteria, viruses, fungi and archaea). The gut microbiome performs a number of essential functions; secrete hormones, utilize glucose and free fatty acids, regulate insulin actions, whole-body metabolism, and adiposity (Clarke et al., 2012; Clarke et al., 2014a). It has been shown that impairments in the gut microbiome, i.e. dysbiosis can lead to systemic-low-grade inflammation and insulin resistance through the release of endotoxins, particularly lipopolysaccharides (LPS) (Cani et al., 2008a; Manco et al., 2010a; Cani et al., 2007a). The liver consumes almost one-third (Cherrington, 1999) of the ingested glucose, equaling the consumption by the skeletal muscle, and also functions as a glucose reservoir in the form of glycogen to be used during fasting (Human Anatomy & Physiology, 2012). The liver also has a key role in the lipid metabolism, through the disposal of plasma free fatty acids (FFA) and the release of lipoproteins (Nguyen et al., 2008). Impairments in the hepatic metabolism increase the risk of acquiring chronic diseases such as obesity and T2D (Basu et al., 2000a; Ferre et al., 2003a; Iozzo et al., 2010). Moreover, accumulation of excess hepatic fat has been shown to contribute in the pathogenesis of metabolic syndrome and T2D (Angulo and Lindor, 2002; Yki–Jarvinen, 2005). The pancreas maintains whole-body glucose homeostasis by secreting insulin and glucagon which maintain whole-body glucose homeostasis from being a net uptake (positive) to a net release (negative) after meal ingestion and fasting respectively. Similar to the liver, pancreatic fat accumulation has

been shown to impair the ability of pancreas to release insulin (Yaney and Corkey, 2003; Poitout and Robertson, 2008). All these essential physiological functions highlight the importance of splanchnic region on whole-body metabolism and health. Although the effects of exercise on muscle are well-known (Hamilton and Booth, 2000), the effects of exercise training on the splanchnic organs remains unclear. Furthermore, studies investigating the significance of exercise intensity on the splanchnic organs are needed, because it has been shown that HIIT/SIT might induce greater benefits in improving cardiovascular metabolism and hence HIIT/SIT might also have a greater impact on splanchnic metabolism.

Thus, the aim of this thesis was to examine the early adaptations of SIT and MICT on intestinal, liver, and pancreatic metabolism in both healthy subjects and subjects with prediabetes/T2D, and to establish whether there is a dose-response relationship (i.e. exercise intensity vs. exercise volume) in inducing greater benefits in improving internal organ metabolism.

2 REVIEW OF LITERATURE

2.1 Insulin sensitivity and insulin resistance

Insulin is one of the hormones released by the pancreas which has an important role in the regulation of nutrients' (carbohydrates, fats, proteins) metabolism in the body. Of these regulated nutrients', maintaining glucose homeostasis is the most important function (Accili, 2004). Insulin maintains whole-body glucose homeostasis by promoting glucose utilization in the peripheral organs (such as: skeletal muscle, adipose tissue) (Handbook of Diabetes, 2014), by suppressing the endogenous hepatic glucose production and by inhibiting adipose tissue lipolysis (Dimitriadis et al., 2011). The ability of insulin to increase whole-body glucose disposal is concentration dependent and saturable. The maximal response to insulin is defined as "insulin responsiveness" whereas the concentration of insulin required to achieve half of the maximal response is defined as "insulin sensitivity". Even though insulin also has a concentration dependent effects on the metabolism of nutrients', the term "insulin sensitivity" is typically reserved for referring to the action of insulin in stimulating whole-body glucose disposal.

The term insulin resistance (IR) is defined as a reduced responsiveness to the actions of insulin in stimulating whole-body glucose disposal and/or a reduced suppression of endogenous hepatic glucose production and adipose tissue lipolysis. This concept was first proposed by Himsworth HP in 1936 to describe diabetic subjects who required increased doses of insulin (Himsworth, 1936). IR is associated with many major health problems including hypertension, dyslipidemias, obesity, coronary artery disease, metabolic syndrome and has a major role in the pathogenesis of T2D (Reaven, 2005; Petersen et al., 2007; DeFronzo and Ferrannini, 1991). Insulin resistance is commonly associated with a variety of clinical manifestations: glucose intolerance, visceral adiposity, endothelial dysfunction, dyslipidemia, hypercoagulable state, and elevated markers of inflammation (Muniyappa and Madan, 2000). Subsequently, the presence of these clinical manifestations is frequently a characteristic of the "insulin resistant state". Therefore, insulin resistance as a whole poses a major challenge and it is of great importance to investigate methods to improve insulin sensitivity.

Even though the exact mechanisms for insulin resistance are not yet known there are many potential contributors and causes of insulin resistance. Numerous studies both preclinical and clinical have been conducted to identify the exact cellular mechanisms responsible for the impaired effects of insulin. The ones

responsible for the impaired insulin-stimulated GU are: 1) a reduction in the insulin receptor tyrosine kinase activity (Freidenberg et al., 1988), 2) a decrease in the Glut4 and mRNA levels (Zierath et al., 1996), 3) a delayed and reduced glycogen storage capacity in muscle (Shulman et al., 1990) and 4) a reduced phosphorylation of AS160 resulting in an inhibition of Glut4 translocation in the muscle cells (Kramer et al., 2006). All of these impairments result in the inefficiency of insulin to stimulate the peripheral GU and a reduced ability to inhibit endogenous glucose production in the liver (Kolterman et al., 1981; Ferrannini et al., 1988; Golay et al., 1988; DeFronzo et al., 1992). Moreover, the mechanisms responsible for impairing the insulin-mediated hepatic glucose production involve increased levels of gluconeogenetic precursors, higher activity of enzymes involved in the gluconeogenetic pathway and hepatic insulin resistance (DeFronzo et al., 1992).

Another contributor to the pathogenesis of insulin resistance are plasma FFA. In obesity, the inability of insulin to inhibit lipolysis leads to increased lipolytic activity of the enzyme lipoprotein lipase and reduced capacity of subcutaneous adipose tissue to store excess FFA in the form of triacylglycerol, results in the spillover of FFA in the blood (Sattar and Gill, 2014; Almandoz et al., 2013). Due to the increased availability of plasma FFA and the body's inability to utilize these fatty acids, they began to accumulate into metabolically active visceral adipose tissue (Virtanen et al., 2002) and other organs (the liver, the muscles, the pancreas). Impaired fatty acid utilization and their accumulation in the form of ectopic fat in the liver and muscles activates Randle cycle (increased FFA oxidation lowers gluco/hexokinase activity leading to a reduced glycolytic rate (RANDLE et al., 1963), impairing both insulin-dependent and independent glucose disposal (Shulman, 2014). This has been shown in the study done by Bajaj et al. (2002) where in 10 patients with type 2 diabetes who underwent an euglycemic hyperinsulinemic clamp, a reduction of almost 10% was found in the splanchnic GU by elevating the plasma FFA levels to 2.5 mM with an intralipid-infusion (Bajaj et al., 2002). Moreover, plasma FFA have also been suggested to play an important role in the regulation of endogenous glucose production (Boden, 1997). It has been shown that in vivo elevation of FFA in hyperinsulinemic clamp studies (by lipid/heparin infusions) impairs insulin's ability to suppress glucose production (Boden, 1997; Saloranta et al., 1991). This creates a viscous cycle wherein the increase in plasma FFA due to insulin resistance further contributes to hyperglycemia worsening the pathogenesis of diabetes. In addition to these known actors, new players such as the gut microbiome and intestinal insulin resistance have also been proposed to have a significant role in the pathogenesis of diabetes. Their potential roles are discussed later in the section 2.3.1 and 2.3.2.

Despite all these contributing factors (impaired peripheral glucose uptake and hepatic glucose production) the pancreatic β-cells maintain glucose homeostasis by augmenting their insulin secretion capacity. However, after a few years of continuous hyperglycaemia this compensatory augmented insulin secretion mechanism cannot be maintained due to impairments in β-cell function (Rorsman and Ashcroft, 2018). For further information regarding the β-cell function please see, 2.5.1.

2.2 Exercise and insulin sensitivity

One of the first pieces of research work linking the improvement in insulin sensitivity with exercise was presented in the study by Chauveau & Kaufman's (Chauveau A , 1887). Their observation in 1887 was that "When a horse chews on hay the concentration of glucose in the blood draining its masseter muscle substantially decreases", this led to many studies corroborating the role of exercise training in enhancing insulin sensitivity and its beneficial role in insulin-resistant states (Wilcox, 2005). In research context, the term exercise encompasses many variables such as modality (e.g. aerobic versus resistance), frequency, duration and intensity. Each of these variables are mitigating factors for exercise induced improvements in the metabolic and molecular processes (Egan and Zierath, 2013).

Recent joint guidelines published by the American College of Sports Medicine and American Heart association recommend at least 150 minutes of moderate intensity exercise (30 min, 5 days/week) or 60 minutes of vigorous exercise (20 min, 3 days/week) (Haskell et al., 2007; Nelson et al., 2007) in order for all adults to maintain and promote health. For subjects with T2D the recommendations are 150 minutes/week of moderate intensity or vigorous exercise as given by the American Diabetes Association and American College of Sports Medicine (Nelson et al., 2007). The benefits of these exercise guidelines have been illustrated in large scale randomized clinical trials, where it has been demonstrated that a 58% reduction in the progression of impaired glucose tolerance to T2D is possible by intensive lifestyle modifications; these modifications included a minimum of 20-30 min of exercise per day (Haskell et al., 2007). Even though the benefits of exercise training are well known, the "lack of time" is the most common reason given for not meeting the minimum recommendations (Trost et al., 2002). Moreover, meta-analysis suggests that exercise intensity has a greater role in improving blood glucose levels than exercise volume (Boule et al., 2003). This indicates that subjects with impaired glucose tolerance should shift from moderate intensity to vigorous high intensity

training in order to obtain additional benefits for improving blood glucose levels (Boule et al., 2003). Thus, many studies are now focusing on designing an exercise regimen with an intensity which would have the most optimal health benefits and would require the minimum amount of exercise; this would, in addition, also encourage a permanent lifestyle change.

2.2.1 Exercise intensity

Exercise intensity is an important determinant of the physiological training adaptations (Garber et al., 2011a). Exercise intensity is defined as energy needed to perform certain exercise. Exercise intensity can be measured in both absolute and relative terms. Absolute intensity is a measure that refers to the absolute energy required to perform a certain activity and is expressed as the metabolic equivalent (MET) or heart rate (HR). Relative intensity is proportional to an individual's maximal capacity and is expressed as a percentage of maximal HR (% HR_{max}) or percentage of maximal oxygen uptake (% VO_{2max}) (Garber et al., 2011b; Powell et al., 2011). As mentioned earlier, exercise intensity has a greater role in improving blood glucose levels than volume (Boule et al., 2003). One of the possible explanations for these higher benefits is that exercise intensity is applicable to the overload principal of training i.e. no improvement in any of the physiological parameters (e.g. VO_{2max}, HbA_{1c}) can be achieved if the training intensity does not exceed the individual's specific threshold intensity (Weston et al., 2014; Wisloff et al., 2009). This was also illustrated in the meta-analysis where exercise intensity and not exercise volume predicted the difference in HbA_{1c} (Boule et al., 2003). Moreover, exercise intensity has also been shown to be superior in improving the aerobic capacity (VO_{2max}) compared to endurance training (Milanovic et al., 2015). As VO_{2max} is an important prognostic determinant of cardiovascular disease and morbidity this will have additional cardiovascular benefits for individuals with T2D. However, there are safety concerns over the implementation of higher intensity exercise regimens clinically as it has been associated with cardiovascular events (Weston et al., 2014; Wisloff et al., 2009). Given the higher benefits of exercise intensity in improving insulin sensitivity, it is clinically important to compare different exercise intensities to determine which intensity would have the most optimal benefits for insulin sensitivity and can be implemented safely in any clinical population.

American College of Sports Medicine has classified exercise intensities according to an individual's HR_{max} (Table 1) and exercise protocols using various exercise intensities have been shown in (Figure 1)

Table 1: Classification of exercise intensities based on the HR_{max}

Intensity	HR_{max}
Low (e.g. Walking)	57-63%
Moderate (e.g. Endurance Training)	64-76%
Vigorous (e.g. High Intensity interval Training)	77-95%
All-out (Sprint interval training)	>95%

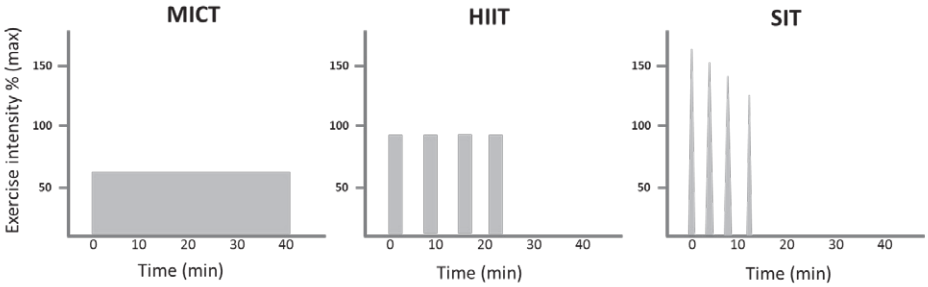

Figure 1: Different exercise intensities used in MICT, HIIT and SIT protocols. MICT consist of continuous exercise with constant work effort. HIIT consists of exercise bouts of a few minutes with short resting periods between. SIT consists of second exercise bouts with supra-maximal work effort. Edited with permission from (Gibala et al., 2014).

In this thesis, we have compared the effects of moderate intensity exercise (MICT) and sprint interval training (SIT) on liver, pancreas, and intestinal metabolism and the resulting effect on whole-body insulin sensitivity.

2.2.2 Sprint Interval training (SIT)

Interval training refers to series of short-burst of high intensity vigorous activity interspersed with periods of low activity or rest. Interval training is not a new idea because it has been around since the early 20th century. Its origins can be traced back to as early as 1912, when Hannes Kolehmainen a Finnish Olympic long-distance runner used interval training in his workouts (Billat, 2001). However, it was the German coach Woldemar Gerschler who is considered to be the pioneer of the modern based interval training. Gerschler training was essentially physiologically oriented (being based on heart rates). This was significant because his training method had a scientific approach which lead to a better cardiovascular conditioning resulting in maximizing the heart's

fitness and efficient. Since then interval training has been the preferred form of training among athletes because it has been shown to be effectively challenging and leads to higher improvements in the aerobic energy metabolism even in previously highly trained individuals (Koral et al., 2018). In addition, over the last decade interval training has been gaining interest among the general population due to that it is being expected to effectively counter the "lack of time" argument given by many individuals for not meeting the minimum recommendations for exercise training. Today, many scientific studies incorporating various interval training protocols have been conducted to elucidate the effects of interval training on both healthy and individuals with various diseases.

Because interval training can be performed using various exercise modes (cycling, rowing, skiing, walking) and with several different protocols (Table 2), there are many studies investigating the effects of interval training on the whole-body metabolism. When studying interval training there are nine variables which can be manipulated: number of repetitions (frequency), exercise intensity and duration, exercise modality, recovery period duration and intensity, number of repetitions and finally number of series, which also includes between series duration and intensity (Buchheit and Laursen, 2013). Generally, it is recommended to use the term high intensity interval training (HIIT) for interval training protocol which consists of 1-4 min of exercise bouts performed at 80-100% of HR_{max}. While the term sprint interval training (SIT) is used when the interval training protocol consists of ≤ 30 seconds of exercise bouts at maximal intensity (all-out) effort (Weston et al., 2014). As mentioned earlier "lack of time" is the most common argument given by most individuals, and therefore there has been considerable interest in the scientific community as regards SIT. One of most common interval training protocol used by many studies is the Wingate test protocol (SIT), which consists of 4-6 x 30 seconds of exercise bouts at supra maximal effort with a 4-minute rest after each bout (Burgomaster et al., 2005; Gibala et al., 2006). This SIT protocol has been shown to improve skeletal muscle oxidative capacity (Burgomaster et al., 2008), exercise performance (Gibala et al., 2006), aerobic and anaerobic performance (Hazell et al., 2010), and circulatory function (lower heart rate and higher stroke volume) (Trilk et al., 2011). Moreover, recently a similar SIT protocol with a lower exercise duration (20 seconds) and frequency (3 times) introduced by Gibala et al. has shown to improve both cardiometabolic health (Gillen et al., 2016b) and skeletal muscle oxidative capacity (Gillen et al., 2014). All these results suggest SIT with its short duration and numerous metabolic benefits can be a potential exercise intervention which could be recommended to the general population.

Table 2: Some examples of different interval training protocols

Protocol	Tabata (Tabata et al., 1996)	Wingate Test (Burgomaster et al., 2008)	Little (Little et al., 2011)	One-minute (Gillen et al., 2016b)
Frequency	8	4-6	10	3
Exercise duration	20	30 seconds	60 seconds	20 seconds
Total duration*	~ 4 minutes	~ 20-30 min	~ 10 minute	~ 1 minute
Intensity	All-out	All-out	~90% HR_{max}	All-out
Recovery (rest/exercise)	10 seconds	~ 4 minutes	60 seconds	2 minutes of low- intensity cycling
Modality	Cycle ergometer	Cycle ergometer	Cycle ergometer	Cycle ergometer
Main Outcomes	HIIT improved both aerobic and anaerobic capacity	HIIT improved skeletal muscle oxidative capacity	HIIT improved glucose control and muscle mitochondrial capacity	HIIT improved cardio metabolic health

% HR_{max}: percentage of maximal heart rate. *Total duration of exercise includes the total duration of exercise and the recovery period (either rest or low intensity exercise) and not the warm-up and cool-down period.

2.2.3 Moderate intensity continuous training (MICT)

Moderate intensity continuous training (MICT) is any form of endurance training performed at a constant intensity without any rest intervals. Typically MICT is performed at an exercise intensity which induces a heart rate response of 55-69% of HR_{max} or increases the oxygen consumption to 40-59% of VO_{2max} (Norton et al., 2010). MICT can be performed by both indoor and outdoor exercise modes such as jogging, cycling, running, rowing, skating, swimming etc. It is the preferred mode of exercise training recommended by professional organizations around the world for health promotion among the general population. The American Heart Association, American College of Sports Medicine, American Diabetes Associations all have recommended a minimum of

30 minutes of MICT per day to improve physical fitness and health-related parameters (Haskell et al., 2007; Nelson et al., 2007). One of reasons why MICT is being promoted by so many organizations is because it can easily be started and is very safe to perform.

Many studies have supported a dose-response relationship between health outcomes and chronic physical activity levels, meaning that an increase in the volume of exercise will lead to greater benefits to health (Garber et al., 2011a). However, because MICT requires more time and given that a "lack of time" is the most common reason given by the general population for not engaging in regular exercise (Trost et al., 2002), studies are now being conducted using different exercise protocols to find a suitable alternative for MICT that will still achieve the health benefits.

2.2.4 Studies comparing HIIT/SIT and MICT protocols

Recently, a lot of studies have compared the effects of different low-volume and high-intensity SIT/HIIT protocols to high-volume and moderate intensity MICT. They have shown that both HIIT/SIT induce superior cardiovascular benefits compared to MICT in sedentary, healthy and subjects with lifestyle-induced diseases (Milanovic et al., 2015; Ramos et al., 2015b; Weston et al., 2014) (Table 3). However, results comparing the effects SIT/HIIT and MICT on insulin sensitivity are less conclusive. Even though a recent meta-analysis showed that various SIT/HIIT protocols had significantly lowered the insulin resistance (defined as Homeostasis Model Assessment (HOMA), QUICKI, Matsuda index, Cederholm index) compared to MICT, no difference was observed between the protocols for the HbA_{1C}, fasting glucose or fasting insulin levels (Jelleyman et al., 2015) (Table 3). Moreover, most of the studies in the meta-analysis measured insulin resistance either from the HOMA or from models (QUICKI, Matsuda index, Cederholm index) which are not designed to address exercise intervention-based changes in glucose metabolism (Keshel and Coker, 2015). Thus, to fully understand the effects of SIT and MICT on insulin sensitivity, studies should use hyperinsulinemic-euglycemic clamp (as clamp is considered to be the gold standard in assessing insulin responsiveness). In this thesis, the effects on SIT and MICT on insulin sensitivity were assessed through the use of a clamp, measuring the β-cell function and liver, pancreas and intestinal metabolism in healthy subjects and those with insulin-resistance.

Table 3: Results of various parameters between HIIT/SIT and MICT protocols

Study	Number of studies	Number of participants	Exercise interventions	Main outcomes	Main results
Milanovic et al., 2015	28	723	HIIT/SIT and MICT	VO_{2max} ↑	HIIT/SIT > MICT
Ramos et al., 2015	7	182	HIIT and MICT	Brachial artery vascular function ↑	HIIT > MICT
Weston et al., 2014	10	273	HIIT and MICT	VO_{2peak} ↑	HIIT > MICT.
Jelleyman et al., 2015	50	2033	HIIT/SIT, MICT and CONTROL	Insulin resistance ↓ (HOMA-IR, QUICKI, Matsuda and Cederholm index)	HIIT/SIT > MICT and control

2.3 Intestine

The intestine is a long continuous tube which extends from the stomach to the anus. It is divided into two main parts, the small intestine and the large intestine (Grolier, 1999) (Figure 2). The small intestine is usually between 6 to 7 meters long and weighs around 600 grams. The small intestine is functionally and macroscopically divided into the duodenum, jejunum and ileum (Gray, 1918). While the duodenum is a retroperitoneal organ, both the jejunum and ileum are intraperitoneal. The main function of the small intestine is to aid in the enzymatic degradation and absorption of nutrients (such as carbohydrates, proteins, fats) (Collins and Badireddy, 2018). In addition to this, the small intestine also plays key role in regulating metabolism through releasing hormones which affect the glucose uptake as well as insulin sensitivity (Drucker, 2007). The large intestine is the most distal part of the digestive tract and on average it is about 166 cm in males and 155 cm in females. It is subdivided into cecum, ascending colon, transverse colon and descending colon (Gray H, 2000) (Figure 2). Its main function is to extract water and electrolytes, as well as absorb and produce vitamins (Azzouz and Sharma, 2018). In addition, to the absorption of water the large intestine also hosts a large amount of microbiome (gut microbiome), which aid in the fermentation of unabsorbed materials (Ganong W.F., 2005). Recently, there has been considerable interest in examining the potential role of the gut microbiota in the pathogenesis of impaired glucose homeostasis and diabetes (Vrieze et al., 2010). In this thesis, the effects of exercise on the intestinal substrate uptake (glucose and fatty acid uptake) and gut microbiome have been investigated.

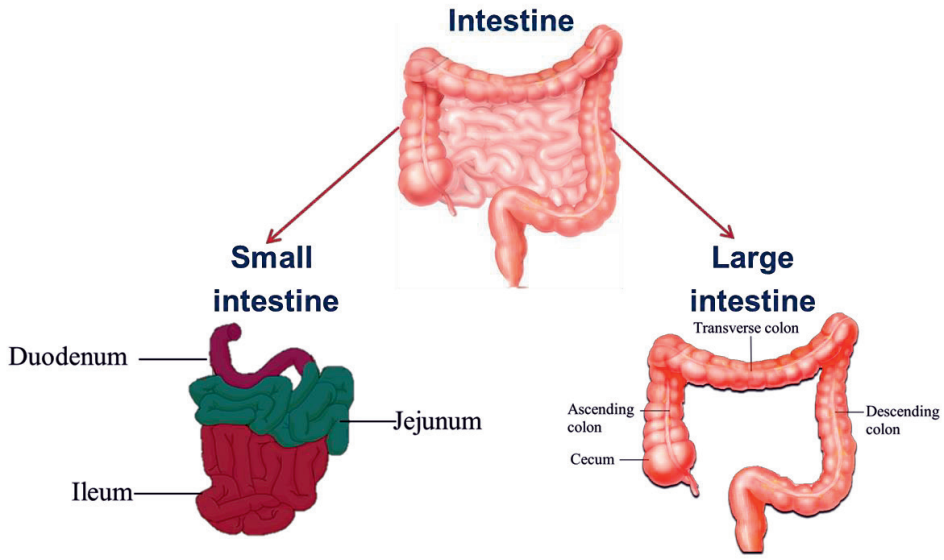

Figure 2: The intestine is divided into the small and large intestine. The small intestine has three regions the duodenum, jejunum and ileum. The large intestine includes the cecum, ascending, transverse and descending colon.

2.3.1 Intestinal substrate uptake

Intestinal enterocytes play a crucial role in the absorption of nutrients (carbohydrates, lipids and proteins), vitamins, bile acids and water from the gut lumen. Moreover, enterocytes also control trafficking of glucose and fatty acids between the gut lumen and circulation (Ross, 2003). The regulation of glucose fluxes inside the enterocytes is a highly complex process. During the post-absorptive stage the enterocytes absorb glucose from the luminal side and transport it towards the circulation via two glucose transporters; on the luminal side (brush border membrane) the sodium-dependent glucose transporter (SGLT1) absorbs glucose and the glucose transporter 2 (Glut2) on the basolateral membrane ensures glucose exits into the blood (Figure 3) (Wright et al., 2003). Additionally, Glut2 is also transiently translocated towards the brush border membrane, mainly to potentiate the glucose absorption from the luminal side (Figure 3) (Tobin et al., 2008). This transient translocation of Glut2 is regulated by insulin. Normally insulin removes Glut2 from the brush-border membrane and thus limits the absorption of glucose from the luminal side (Tobin et al., 2008). This effect of insulin was shown by Tobin et al. where they incubated human intestinal colonic carcinoma cells (Caco-2/TC7) in a glucose-containing medium and after insulin administration this lead to the internalization of Glut2 from the

brush-border membrane resulting in a reduction in the glucose absorption and an increase in the intestinal GU (Tobin et al., 2008). Recently, it has been shown that insulin also stimulates intestinal GU from the blood in humans (Honka et al., 2013) and that this effect is blunted in obese human subjects (Makinen et al., 2015). This blunted response in obesity is due to the reduced effect of insulin which results in the permanent incorporation of Glut2 in the brush border membrane and a constant blood-to-lumen efflux of glucose causing a pathological increase in blood sugar levels and lower accumulation of glucose in the intestine (Ait-Omar et al., 2011). In the same study, intestinal GU improved after weight loss induced by bariatric surgery (Makinen et al., 2015). Moreover, the use of insulin sensitizer, metformin (the most common drug prescribed for T2D) has been shown to increase the intestinal basolateral GU (Gontier et al., 2008) suggesting that insulin indeed plays a key role in the intestinal absorption of glucose from the blood. Exercise is known to improve the whole-body insulin sensitivity. However, it is not known whether the exercise induced improvement in the whole-body insulin sensitivity would also lead to the improvement in the intestinal GU.

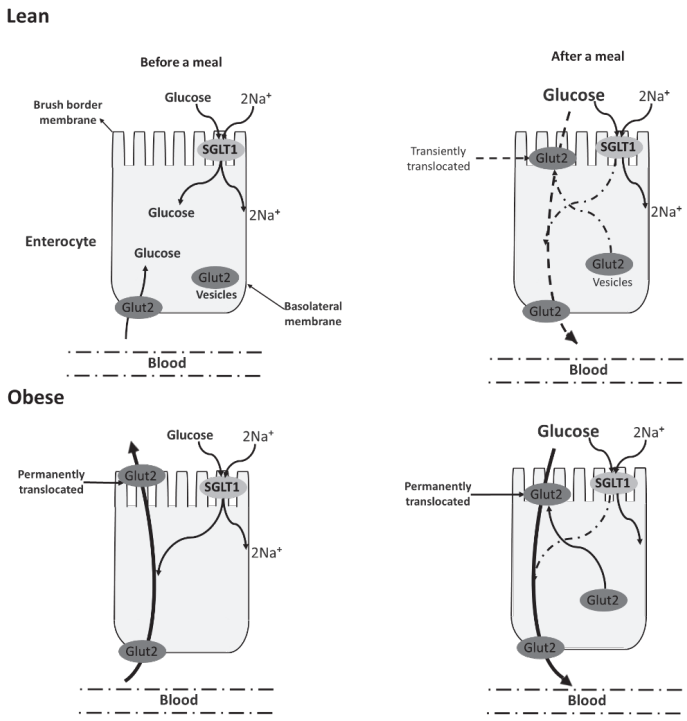

Figure 3: Intestinal Glut2 position in healthy (lean) and in obesity. The position of Glut2 depends on the glucose concentration difference between the intestinal lumen and blood. In the top panel, before a meal under (lean) conditions, sodium-dependent glucose transporter (SGLT1) absorb glucose from the lumen and Glut2 absorbs glucose from the blood inside the cells. While immediately after a meal, Glut2 is transiently translocated on the brush border membrane to complement the glucose uptake with SGLT1. In the bottom panel, under obese conditions the Glut2 is permanently translocated in the brush border membrane resulting in a pathological increase in the plasma glucose levels both before and after a meal. Modified from (Kellett and Brot-Laroche, 2005)

The regulation of FFA inside the enterocytes is also highly complex and compartmentalized. This compartmentalization ensures that the FFA absorbed from the luminal side and the basolateral side (blood) have different metabolic fates. FFA are absorbed from the luminal side by diffusion or by facilitated transport by cluster differentiation 36 (CD36) or fatty acid transporter protein 4 (FATP4) (Niot et al., 2009). These FFA are mostly esterified, combined with lipoproteins and are transported to other parts of the body through the lymphatic system, while the FFA absorbed from the plasma are mostly oxidized and are used to serve the structural and caloric requirements of the intestinal cells (Gangl and Ockner, 1975; Gangl and Renner, 1978). This duality in handling FFA can

be explained by the organelle compartmentalization inside the enterocytes (Trier J.S, 1968). FFA absorbed from the luminal side are predominantly esterified because of the preponderance of endoplasmic reticulum on the brush border membrane, while the FFA absorbed from the basolateral membrane are primarily oxidized because of the dominance of mitochondria on that side (Gangl and Ockner, 1975).

Although much is known about the mechanisms of FFA absorption from the luminal side (Abumrad and Davidson, 2012; Labbe et al., 2011), the mechanisms involved in the absorption of FFA from the basolateral membrane still remain to be elucidated. In a recent study by Koffert J et al. it was shown that the intestinal free fatty acid uptake (FAU) was higher in obese subjects with insulin resistance compared to healthy lean subjects (Koffert et al., 2018). This higher intestinal FAU after insulin resistance was attributed to the changes in the glucose-free fatty acid energy ratio and an increase in the plasma FFA due to an increase in the release of FFA from the adipocytes. Nevertheless, this increased intestinal FAU did not reduce after bariatric surgery (Koffert et al., 2018). Exercise training has been known to improve whole-body FFA metabolism by inducing lipolysis in adipocytes and facilitating the oxidation of FFA in muscles (Hashimoto, 2013). However, as with the intestinal GU it is not known whether exercise-induced improvements in the whole-body FFA metabolism would have an impact on the intestinal FAU.

2.3.2 Gut microbiome

The human gut microbiome consists of almost 10^{14} micro-organisms (bacteria, viruses, fungi and archaea). All these microorganisms colonize the human gut soon after birth through maternal and environmental bacteria (Guarner and Malagelada, 2003). The human gut microbiome has a biomass of more than 1.5 kg and exceed the human genome by more than 100-fold (Lepage et al., 2013; Flint et al., 2012). Microbiota composition varies across the digestive tract (from stomach to colon). There are relatively few species of gut microbiome present in the stomach and small intestine (Guarner and Malagelada, 2003). While in colon they are densely-populated and make up to 10^{12} cells per gram of intestinal content (Guarner and Malagelada, 2003). Between 300-1000 species of bacteria have been identified (Sears, 2005). Out of which 30 to 40 bacterial species make up 99% of these bacteria (Stephen and Cummings, 1980). Therefore, most of the gut microbiome is made up from bacteria (Beaugerie and Petit, 2004). While viruses, fungi and archaea also make up the rest of the gut microbiome, but less is known about their function (Lozupone et al., 2012). The

four most dominant bacterial phyla in the human gut are Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria (Khanna and Tosh, 2014). The most common bacterial genera are *Bacteroides, Clostridium, Feacalibacterium, Eubacterium, Ruminococcus, Peptococcus, Peptostreptococcus*, and *Bifidobacterium* (Guarner and Malagelada, 2003; Beaugerie and Petit, 2004). There are also other bacterial genera, such as *Escheridhia* and *Lactobacillus* but they are present to a lesser extent (Guarner and Malagelada, 2003). Out of these bacterial genera *Bacteroidetes* is the most important one as it makes up almost 30% of all the bacterial genera (Sears, 2005). Research in the field of human microbiome is relatively new and continuously growing with several preliminary and but promising studies showing a modulatory role of human microbiome in both wellness and disease.

Earlier studies have shown that the gut microbiome have multiple physiological functions which include: aiding in the digestion of complex carbohydrates, production of short-chain fatty acids, synthesis of vitamin B and K, development of innate and adaptive immunity, metabolism of sterols, xenobiotics and bile acids (Ganong W.F., 2005; 2012). However, recent studies have also suggested that the gut microbiome have a key role in regulating the metabolic pathways in different diseases (Tilg and Moschen, 2014). This is supported by animal studies where germ free mice lacking the gut microbiome are significantly leaner compared to mice with intact gut microbiome despite consuming more energy (Backhed et al., 2004). In addition, germ free mice are resistant to high-fat diet induced obesity (Rabot et al., 2010). Moreover, in the metagenome-wide association studies conducted in Europe it was suggested that a "gut signature" exist in T2D subjects. In the study, it was found that the butyrate-producing bacteria (*Roseburia* and *Feacalibacterium prausnitzi*) were lower in T2D patients (Karlsson et al., 2013). Butyrate has a trophic (growth) effect on colonocytes and has also been shown to have anti-inflammatory and anti-carcinogenic effects (Hamer et al., 2008). Moreover, butyrate producing bacterias' have been linked to improved insulin sensitivity and amelioration of diabetes in human feacal microbiome studies (Vrieze et al., 2012; Furet et al., 2010). Overall, these results suggest that in both obesity and diabetes an altered gut microbiome (gut dysbiosis) can have an important role in their pathogenesis.

In addition to increased energy harvest, one possible mechanism through which the gut microbiome contributes to the pathogenesis of T2D is through the release of a gut-derived chronic inflammatory marker lipopolysaccharide (LPS). LPS is a heat-stable endotoxin derived from the glycolipid component of the outer membrane of gram-negative bacteria (Hurley, 1995). Elevation of plasma LPS is associated with amplified proinflammatory and oxidant environment and is known as "metabolic endotoxaemia". Many recent studies have suggested that

LPS has an important role in the chronic low-grade inflammation observed in T2D diabetic subjects. This was first observed by Cani et al. who showed that mice fed with a high fat diet showed an increase in serum LPS, and in the same study a subcutaneous injection of LPS in the mice resulted in insulin resistance and obesity (Cani et al., 2007b). These findings have been corroborated by human studies where subjects with T2D and metabolic syndrome exhibit endotoxaemia (Pussinen et al., 2011; Lassenius et al., 2011). Furthermore, high levels of LPS have been negatively linked to high-density lipoprotein and positively with triglyceride and insulin levels, implying that high plasma LPS level induce both poor lipid and glucose homeostasis (Delzenne et al., 2011). Therefore, LPS might indeed prove to be one of the important players in gut-microbiome induced T2D pathogenesis.

2.3.3 Exercise, intestine and the gut microbiota

Most of the studies regarding exercise and the intestines have shown that exercise has both a protective and therapeutic role in gastrointestinal conditions such as inflammatory bowel syndrome and colonic cancer (Wolin et al., 2009; Cronin et al., 2016). To date, no prior studies have investigated the effects of exercise on the intestinal GU and FAU from the blood circulation. Moreover, the effects of exercise on the gut microbiome has been gaining attention. Many interventional studies done with animals have shown that exercise indeed has a positive effect on the gut microbiome composition (increased phylum Bacteriodetes, reduced phylum Firmicutes, increased Bifidobacterium spp.) (Lambert et al., 2015; Evans et al., 2014). Cross-sectional studies in humans have shown that physically active subjects had higher abundance of (Akkermansia, Feacalibacterium prausnitzi, Roseburia) and higher diversity (Bressa et al., 2017; Clarke et al., 2014b) compared to sedentary subjects. Moreover, interventional studies done with humans comparing the effects of exercise intervention on gut microbiome have shown an increase in abundance of 6 of the major bacterial phyla/genera (Shukla et al., 2015), an increase in (*Akkermansia*, Coriobacteriaceae, Succinivibrionaceae), a decrease in (*Protoeobacteria*) (Munukka et al., 2018; Zhao et al., 2018) and an increase in the butyrate producing bacteria (Allen et al., 2018). Additionally, interventional studies in animals have shown that exercise attenuates the systematic inflammatory response induced by LPS (Bagby et al., 1994; Kasawara et al., 2016; Littlefield et al., 2015; Yamada et al., 2018)

In this thesis the effects of SIT and MICT on the intestinal GU and FAU, gut microbiome and intestinal inflammatory marker (LPS) were examined.

2.4 Liver

The liver is both the heaviest organ (on average 1.6 kg) and largest gland inside the human body (Cotran et al., 2015). The liver is divided into four lobes (right, left, caudate and quadrate) of unequal shape and size. It plays a central role in regulating all the metabolic processes in the body. The basic functional unit of the liver is known as the lobule, which is made of up of millions of hepatocytes (Human Anatomy & Physiology, 2012). Hepatocytes are highly specialized cells which perform a wide array of biochemical reactions. They convert nutrients absorbed from intestine into substances which other organs in the body can use, they also store these substances and release them when the body requires it (IQWiG 2016). Moreover, hepatocytes take up harmful products, such as alcohol, medications and either convert them into harmless substances or ensure their excretion from the body (IQWiG 2016). The liver also plays a key role in regulating the whole-body glucose metabolism (Iozzo et al., 2003a). It ensures that blood glucose levels remain steady during fasting by releasing glucose in the circulation and after a meal removes excess glucose ingestion and stores it in the form of glycogen (Human Anatomy & Physiology, 2012). Furthermore, the liver also plays several roles in lipid metabolism: performs lipogenesis, synthesis of cholesterol, lipoproteins and triglycerides (Nguyen et al., 2008). Maladaptations in the liver's ability to perform all these functions have a profound effect on the pathogenesis of many diseases (Mannucci et al., 2012; Bjorntorp, 1992; Iozzo et al., 2003b; Basu et al., 2000b; Ferre et al., 2003b; Angulo and Lindor, 2002; Tilg and Moschen, 2014). In this thesis, alterations in hepatic fat content and hepatic GU and FAU were addressed.

2.4.1 Hepatic substrate uptake

The liver has a central role in the regulation of systematic glucose and lipid fluxes both during the post absorptive and postprandial states. It also relies on the oxidation of these substrates for meeting its own energy needs. Under normal conditions the liver has a fine tuned and tight regulatory mechanism through which it regulates glucose and fatty acid metabolism. In the progression of T2D and non-alcoholic fatty liver disease these tight regulatory mechanisms are loosened contributing to the systematic increase in glucose (hyperglycemia) and lipid (hyperlipidemia) (Rui, 2014). Both hyperglycemia and hyperlipidemia are associated with retinopathy, insulin resistance, hepatic steatosis (increased accumulation of fat inside the liver), cardiovascular disease and increased risk of

death (Mannucci et al., 2012; Bjorntorp, 1992). Thus, it is of great significance to understand the mechanisms behind the regulation of hepatic GU and FAU.

The liver is a major regulator of glucose metabolism, receiving glucose from the intestine via the portal vein and from the systematic circulation via the hepatic artery. Glucose enters the hepatocytes from the blood via facilitative diffusion powered by glucose transporter (Glut2) (Shepherd and Kahn, 1999; Thorens, 1996). Depending on the nutritional state of the body the liver is both a net glucose uptake and a net glucose output organ. During the postprandial stage, in response to the oral glucose load and the resulting increase in the plasma insulin and glucose levels, the liver becomes a net glucose uptake organ. Previous studies have estimated that the liver consumes almost one-third (~25 to 35%) of the oral glucose load (Ferrannini et al., 1985), while skeletal muscles together with adipose tissue consume another one-third and the remaining are taken up by the noninsulin-sensitive glucose using tissues (brain, red blood cells) (Ferrannini et al., 1988). This one-third of the glucose is mainly used to replenish the hepatic glycogen stores by converting the excess glucose to glycogen. It is estimated that almost 100g of glycogen is synthesized and stored by the liver through the process known as glycogenesis, i.e. formation of glycogen from glucose (Marieb and Hoehn, 2012). During the post absorptive stage the liver shifts to being a net glucose output organ by maintaining euglycemia through cleaving glycogen (glycolysis) and gluconeogenesis. Gluconeogenesis is the synthesis of glucose via precursors such as lactate, glycerol and certain amino acids (Marieb and Hoehn, 2012). It is estimated that the liver produces around 30-65 % of the total endogenous glucose after an overnight fast (Rothman et al., 1991). Evidence suggests that in patients with T2D there is a reduction in the hepatic GU (Iozzo et al., 2003b) and that impairments in hepatic glucose metabolism have been implicated in the pathogenesis of hypertriglyceridemia, hepatic steatosis and diabetes (Iozzo et al., 2003b; Basu et al., 2000b; Ferre et al., 2003b). Previously, a single bout of exercise in non-obese diabetic subjects has been shown to increase the hepatic GU (Tobin et al., 2008). However, it is not known which mode or intensity of exercise would have the most optimal effect on hepatic GU.

The liver also has a key role in regulating the whole-body lipid metabolism; it receives lipids from the portal vein and from the general circulation via the hepatic artery and the lymphatic system. Normally, hepatocytes have a high capacity for the removal of lipids from the circulation and releasing them at a moderate rate. Lipids arrive to the hepatocytes in various forms of lipoproteins and FFA (Figure 4). FFA enter the hepatocytes via a combination of facilitated transport and diffusion (Berk and Stump, 1999a). The lipoproteins are either taken up by receptor-mediated endocytosis (Lambert et al.,

2001) or broken down by hepatic lipase (HL) into FFA and transported inside the hepatocytes by facilitated transport and diffusion (Berk and Stump, 1999b) (Figure 4). Once inside the cells, FFA are either converted into triglycerides (TG) or are oxidized as fuels. Most FFA are converted into TG, TG are used for the production of VLDL (lipoproteins) and exported into the circulation or stored in the form of lipid droplets inside the cells depending on the availability of protein components (Bradbury, 2006a). While the rest of FFA are used as fuels to produce energy by undergoing β-oxidation inside the mitochondria, some of the FFA are used in the production of phospholipids, glucose, cholesterol and other compounds (Bradbury, 2006a). Under normal physiological conditions most of the FFA are metabolized as above and excess FFA are converted into TGs and stored. In obesity, an increased flux of FFA because of the inability of the adipose tissue to store FFA and increased lipolysis leads to an increased availability of FFA for the liver (Sattar and Gill, 2014; Almandoz et al., 2013). This increased availability of FFA results in a higher hepatic FAU and results in an accumulation of fat inside the liver cells (Figure 4) (Bradbury and Berk, 2004). Moreover, the increased FFA initiates a maladaptive response leading to an overproduction of reactive oxygen species that has been associated obesity-related metabolic syndrome (Iozzo et al., 2010). This accumulation of FFA inside the liver can lead to increased endogenous glucose production (Seppala-Lindroos et al., 2002) and an increased secretion of TG in VLDL (Westerbacka et al., 2004). All these aberrations further contribute to dyslipidemia and eventually lead to the development of type 2 diabetes (Petersen et al., 2005). Previous studies have shown that the reduction in hepatic FAU uptake leads to: an improvement in the hepatic insulin sensitivity, reduction in the accumulation of fat inside the liver (Viljanen et al., 2009), reduction in oxidative stress and liver injury (Liu et al., 2016). Moreover, studies by Iozzo et al. and Hannukainen et al. have shown that in subjects with a higher physical activity status had a reduced hepatic FAU uptake compared to subjects with lower physical activity status (Hannukainen et al., 2007; Iozzo et al., 2004b). However, as mentioned earlier for hepatic GU it is not known which mode of exercise or intensity would have the most optimal effects on the hepatic FAU uptake.

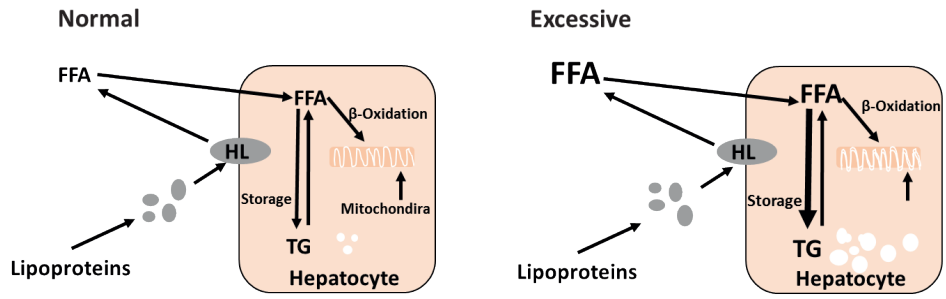

Figure 4: Lipid transportation inside the hepatocyte under normal and excessive conditions. In the left hand panel, lipids arrive as plasma free fatty acids (FFA) or in the form of lipoproteins which are converted into FFA by hepatic lipase (HL) enzyme, these FFA are then transported inside the hepatocyte with the plasma FFA. Inside the hepatocytes, the FFA undergo either oxidation inside the mitochondria or are esterified into triglycerides (TG), some of which is stored as lipid droplets (white circles). On the right panel, when there is excessive FFA most of it is esterified into TG and stored as large lipid droplets resulting in steatosis. Modified from (Bradbury, 2006b)

2.4.2 Hepatic fat content

Accumulation of fat in the liver is known as fatty liver or hepatic steatosis. The diagnosis criteria for fatty liver or hepatic steatosis is fat content inside the liver greater than 5.6 % of its weight (Szczepaniak et al., 2005). Fatty liver disease can be clinically classified into two broad entities 1) alcoholic liver disease (ALD) and 2) non-alcoholic fatty liver disease (NAFLD). ALD is caused by excessive consumption of alcohol whereas NAFLD is due to any other cause except excessive alcohol consumption (Table 4). Fatty liver has been associated with dyslipidemia, hypertension, obesity, metabolic syndrome and diabetes (Angulo and Lindor, 2002; Tilg and Moschen, 2014). Accumulation of fat inside the liver cells is a two-stage process. In the first-stage (microvesicular fatty change) the fats accumulate around the nucleus in the form of small vacuoles (liposomes), at this stage multiple liposomes accumulate inside the liver cells but do not displace the centrally placed nucleus (Figure 5). In the next stage (macrovesicular), the liposomes size increases drastically and the centrally placed nucleus is pushed to the periphery giving it a signet ring appearance (Figure 5). Regardless of the cause of fatty liver (either ALD or NAFLD), it has propensity to progress from fatty liver → steatohepatitis → cirrhosis → hepatocellular carcinoma (Figure 6). Moreover, because the progression of the disease is similar regardless of the aetiology (ALD or NAFLD), it suggests that

there is a possible convergence of the pathological mechanisms responsible at a critical juncture enabling them to progress from simple steatosis to liver inflammation and finally leading towards liver cancer (Angulo and Lindor, 2002). Fatty liver is considered innocuous and reversible at the steatohepatitis stage and therefore many interventions are being conducted to halt its progression.

Weight reduction of a minimum of 10% or more through diet and lifestyle modifications is considered to be an effective strategy to resolve steatohepatitis in over 90% of subjects (Vilar-Gomez et al., 2015). However, this weight loss threshold is very difficult to meet and clinical trials have shown that more than 50% of subjects are not able to achieve this reduction (Musso et al., 2012). Moreover, medicinal interventions with vitamin E, pioglitazone, and obeticholic acid have only been effective in 45% of patients (Sanyal et al., 2010; Neuschwander-Tetri et al., 2015). Physical activity has been shown to be an alternative intervention to reduce steatosis in fatty liver (van der Windt et al., 2018a). It has both preventive and a therapeutic value in improving NAFLD prognosis. Exercise has been recommended by The American college of Gastroenterology, The American Association for the Study of Liver Diseases and The American Gastroenterological Association as a treatment intervention for NAFLD (Chalasani et al., 2012). However, the current recommendations do not specify which exercise mode or intensity would have the most beneficial effects on hepatic fat.

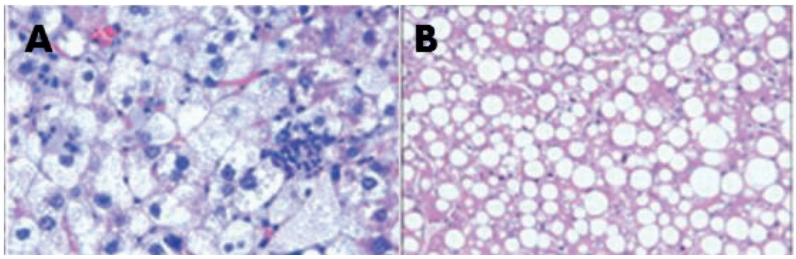

Figure 5: Histological appearances of microvesicular and macrovesicular changes inside the liver cells as a result of accumulation of fat. A) Microvesicular changes, the small fat vacuole (liposomes) fill the liver cell cytoplasm but do not displace the centrally placed nucleus. B) Macrovesicular changes, large fat vacuole displace the centrally placed nucleus to the periphery giving it a signet ring like appearance. Modified from (Reddy and Rao, 2006).

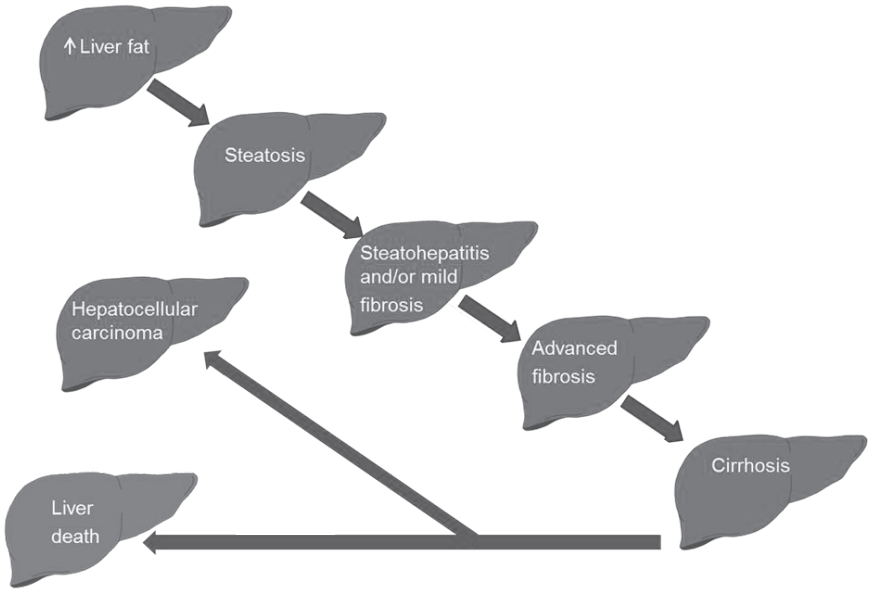

Figure 6: Different stages of progression of accumulation of fat inside the liver. Accumulation of fat regardless of the aetiology (ALD or NAFLD) leads to steatosis, inflammation (steatohepatitis) fibrosis, cirrhosis and finally results in either hepatocellular carcinoma or death.

Table 4: List of the various causes of non-alcoholic fatty liver disease

NUTRITIONAL	DRUGS*	METABOLIC OR GENETIC	OTHER
Protein-calorie malnutrition	Glucocorticoids	Lipodystrophy	Inflammatory bowel disease
Starvation	Synthetic estrogens	Dysbetalipoproteinemia	Small-bowel diverticulosis with bacterial overgrowth
Total parenteral nutrition	Aspirin	Weber-Christian disease	Human immunodeficiency virus infection
Rapid weight Loss	Calcium-channel blockers	Wolman's disease	Environmental hepatotoxins
Gastrointestinal surgery for obesity	Amiodarone	Cholesterol ester storage	Phosphorus
	Tamoxifen	Acute fatty liver of pregnancy	Petrochemicals
	Tetracycline		Toxic mushrooms
	Methotrexate		Organic solvents
	Perhexiline maleate		***Bacillus Cereus*** toxins
	Valproic acid		
	Cocaine		
	Antiviral agents		
	Zidovudine		
	Didanosine		
	Fialuridine		

* The list of drugs are a partial list of agents that cause fatty liver. Some of them also cause liver inflammation. Table modified from (Angulo and Lindor, 2002).

2.4.3 Lipoprotein subclasses

Lipoproteins are a complex biochemical array encompassing a central hydrophobic core containing non-polar lipids (mainly cholesterol esters and triglycerides) and surrounded by a hydrophilic core consisting of phospholipids, free cholesterol and apolipoproteins. This complex structure helps to emulsify fats. This emulsification is important as fats cannot be transported in the blood stream on their own as they are insoluble in water. Lipoproteins transport fats (triglycerides) and cholesterol from where they are synthesized (the small intestine and the liver) and transport them to all the other tissues in the body. With the advent in techonology it is now possible to analyze changes in the liporotein profile (dyslipoproteinemia) in a more efficient manner and on a larger scale using nuclear magnetic resonace (NMR) (Soininen et al., 2015). Until now, lipoproteins were only analyzed using tradional blood lipid tests (lipid profile, clolesterol tests), with no information on the lipoprotein particle size or its composition (Figure 7). NMR lipoproteins are classified according to their fat to protein ratio into extra-large, large, small and extra-small with larger lipoporteins being less dense while small lipoproteins being more dense. They are classified as follows (from large to small) chylomicrons [carry triglycerides from the intestine to the liver, muscle and adipose tissue], very low density lipoproteins (VLDL) (six subclasses) [carry newly synthesized triglycerides from the liver to the adipose tissue], intermediate density lipoprotein (IDL) [results from the hydrolysizes of VLDL], low density lipoprotein (LDL) (three subclasses) [carries lipid molecules and transports them to the peripheral tissues and is commonly referred as bad lipoprotein as higher concentration is commonly associated with atherosclerosis progression] and high density lipoprotein (HDL) (four subclasses) [transports lipids from peripheral tissues to the liver and is referred to as good lipoprotein as its higher concentration is associated with a lower progression of atheroscelrosis]. With NMR we also measured the lipid composition of each subclass; exterfied cholesterol, free cholesterol, triglyceridesm phopholipids, total cholesterol and total lipids (Figure 7).

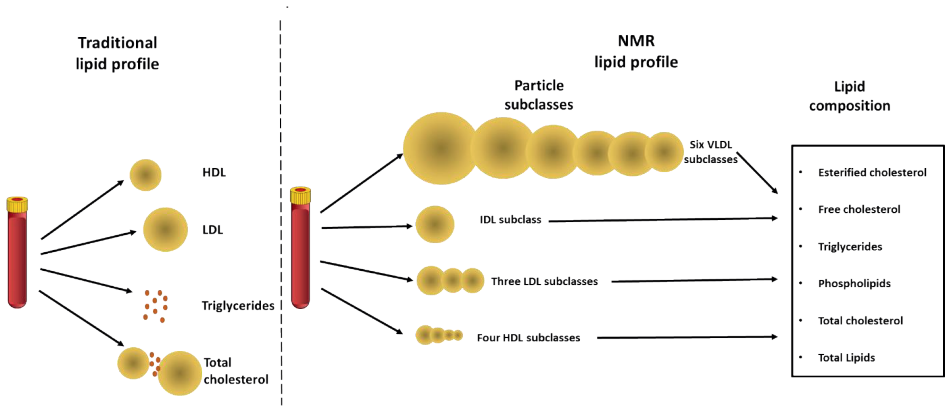

Figure 7: Lipid profile analysis using traditional blood lipid test and NMR. In the left-hand panel (traditional lipid profile), only measures the total high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides and total cholesterol. In the right-hand panel, nuclear magnetic resonance (NMR) analyses 14 different subclasses; six very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), three LDL and four HDL subclasses. From each subclass six different lipid composition parameters are also measured.

Recently, many studies have shown that analyzing the lipoproteins by particle size and compisition levels results in a better understanding of the pathogenesis of various diseases (Cromwell and Otvos, 2004; Carmena et al., 2004; Freedman et al., 1998; Magkos et al., 2008; Rizzo and Berneis, 2007). Accumation of fat inside in the liver in normoglycemic (Kim et al., 2004) and T2D subjects (Kelley et al., 2003) has been associated with dyslipidemia. This dyslipidemia is marked by alterations in both lipoprotein profile and composition. In fact, recent studies have shown that the risk of acquiring T2D depends on the distribution of HDL subclasses, with small HDL having a higher risk while large HDL reduce the risk of acquiring T2D (Mora et al., 2010). Exercise has been shown to improve the lipoprotien profile (Halverstadt et al., 2007; Brown et al., 2009; Sabaka et al., 2015; Munukka et al., 2018) however, as with liver fat it is currently there are no reccomendations on which exercise mode has the most optimum effect on lipoprotein subclasses.

2.4.4 Exercise and liver

Over the past decade, several exercise trials have been conducted to assess the effects of exercise training on the liver. Several randomized clinical trials using different exercise intensities and frequencies have provided evidence that both aerobic and resistance exercise training independent of weight loss do

indeed reduce hepatic fat content (Johnson et al., 2009; Bacchi et al., 2013; Lee et al., 2012; Monteiro et al., 2015; Sullivan et al., 2012; Finucane et al., 2010; van der Heijden et al., 2010) (Table 5). Additionally, a recent review by Golabi et al. focusing on studies published between 2011 and 2016 showed that exercise intervention lasting for at least 8 weeks resulted in a reduction of almost 30.2 % in hepatic fat content in 433 subjects (Golabi et al., 2016). Exercise reduces the hepatic fat content through various pathways: by improving peripheral insulin sensitivity it reduces the availability of both FFA and glucose to the liver and thereby reduces the fatty acid synthesis in the liver, and it increases fatty acid oxidation and prevents hepatocellular and mitochondrial damage (van der Windt et al., 2018b). Moreover, recent studies have shown that HIIT is also an effective intervention in reducing the hepatic fat content (Winn et al., 2018; Hallsworth et al., 2015; Oh et al., 2017). Furthermore, the results regarding the effects of exercise on lipoproteins profile and liver enzymes (alanine transaminase (ALAT), aspartate transaminase (ASAT) and gamma-glutamyl transpeptidase (GT)) also suggest that exercise is effective in improving both lipoprotein profile (Halverstadt et al., 2007; Brown et al., 2009; Sabaka et al., 2015) and hepatic damage (Pugh et al., 2013; Sreenivasa et al., 2006; Abd El-Kader et al., 2014; Sullivan et al., 2012; de et al., 2012) (Table 5). However, studies regarding the effects of exercise on hepatic GU and FAU are scarce. There are only two previous cross-sectional studies showing that more physically active subjects had lower hepatic FAU compared to less active subjects (Iozzo et al., 2004b; Hannukainen et al., 2007). However, for hepatic GU it has been shown that just a single bout of exercise increases GU both in humans (Kawamori et al., 1991) and in animals (Galassetti et al., 1999).

Although the effects of exercise on hepatic fat content, enzymes and lipoproteins have been well established, the effects of exercise on hepatic GU and FAU are less understood. Even though, some studies do indicate that exercise does have a positive effect on hepatic GU and FAU, there are no studies comparing the effects SIT and MICT on liver metabolism.

Table 5: Exercise induced changes in the hepatic fat content, lipoprotein profile and liver enzymes

Reference	N	Duration of exercise	Exercise intervention	Main results
			Effect of exercise on liver fat content	
Finucane et al (Finucane et al., 2010)	100	12 weeks	Aerobic exercise (3/week) vs. control	Aerobic exercise reduced hepatic fat content and improved cardiac function without any weight loss
Sullivan et al (Sullivan et al., 2012)	18	16 weeks	Aerobic exercise (5/week) vs. control	Aerobic exercise reduced the hepatic fat content without any weight loss
Lee et al (Lee et al., 2012)	45	3 months	Aerobic (3/week) vs. resistance exercise (3/week) vs. control	Both aerobic and resistance training reduced the hepatic fat content without any weight loss
Bacchi et al (Bacchi et al., 2013)	40	4 months	Aerobic (3/week) vs. resistance (3/week)	Both aerobic and resistance training reduced the hepatic fat content similarly without any weight loss
Johnson et al (Johnson et al., 2009)	19	4 weeks	Aerobic exercise (4/week) vs. control (home-based stretching exercise)	Aerobic exercise significantly reduced the hepatic fat content without any weight loss
Van der Heijden (van der Heijden et al., 2010)	29	12 weeks	Aerobic exercise (4/week)	Aerobic exercise reduced the hepatic fat content in obese but not in lean subjects without any weight loss

Effect of exercise on lipoproteins				
Halverstadt et al (Halverstadt et al., 2007)	100	24 weeks	Aerobic exercise (3/week)	Most lipoprotein particle concentrations improved (large and small VLDL, medium and small LDL particles reduced and HDL particle increased significantly)
Brown et al (Brown et al., 2009)	37	16-24 weeks	Aerobic (variable/week as exercise duration was individualized) vs. control	Significant improvements in lipoprotein profile (large VLDL, medium and small HDL reduced while the large HDL concentration improved significantly)
Sabaka et al (Sabaka et al., 2015)	10	1 week	Aerobic exercise (4/week)	VLDL, medium LDL concentrations and small HDL reduced Both in fasting and post prandial stage
Effects of exercise on liver enzymes				
Pugh et al (Pugh et al., 2013)	20	16 weeks	Aerobic exercise (3/week) vs. controls	Aerobic exercise improved the ALAT/ASAT levels
Sreenivasa et al (Sreenivasa et al., 2006)	16	12 weeks	Aerobic exercise	Aerobic exercise lead to an improvement in both ALAT and ASAT levels.
El-kader et al (Abd El-Kader et al., 2014)	50	3 months	Aerobic (3/week) vs. resistance (3/week)	Both aerobic and resistance training improved the ALAT and ASAT levels with aerobic exercise improving it more compared to resistance.
Sullivan et al (Sullivan et al., 2012)	18	16 weeks	Aerobic exercise (5/week) vs. control	Aerobic exercise improved the ALAT levels
De Piano A et al (de et al., 2012)	58	1 year	Aerobic (3/week) vs. aerobic + resistance (3/week)	Both aerobic and aerobic + resistance groups improved the ALAT and ASAT levels

2.5 Pancreas

The pancreas is an elongated organ (about 15 cm long) located behind the stomach at the level of the first and second lumber vertebrae. Anatomically, the pancreas is divided into four parts; head, neck, body and tail. Functionally, the pancreas has both digestive and endocrine functions. These functions are performed by two distinct types of cells in the pancreatic parenchyma exocrine acinar cells and the islets of the Langerhans respectively. The exocrine acinar cells constitute the majority of the pancreas parenchyma, they synthesize bicarbonate (produced by cells in the ductal epithelium) and enzymes (produced by acinar cells) which aid in the digestion of carbohydrates, proteins and lipids (Kasper et al., 2015). The islets of Langerhans are clustered together within the exocrine cells and constitute only 1-2 % of the entire pancreas. They release hormones (including insulin, somatostatin, glucagon and pancreatic polypeptide) all of which play a crucial role in the regulation of glucose metabolism in both the post prandial and fasting states (Barbara Young et al., 2006). It is estimated that a healthy human pancreas consists of ~ 3.2 million islets of Langerhans (Ionescu-Tirgoviste et al., 2015). The islets are composed of five different cells types; insulin, amylin, C-peptide secreting β-cells (65-80 %), glucagon secreting α-cells (15-20 %), somatostatin secreting δ-cells (3-10 %), pancreatic polypeptide secreting γ-cells (3-5 %) and ghrelin secreting ε-cells (< 1 %) (Roder et al., 2016). In this thesis, the effects of exercise on the β-cell function have been analyzed only and therefore only β-cell function will be addressed in the forthcoming chapters.

2.5.1 β-cell function

β-cells are the most common type of cells in the islets of Langerhans. Their main function is to store insulin and control the blood glucose levels through the release of insulin in the blood. Insulin is formed in the endoplasmic reticulum of the β-cells and is then transported in the form off membrane-bound granules in the cytoplasm. Inside the granules two polypeptides of active human insulin are bound to a connecting peptide (C-peptide). The release of insulin into the blood depends on the blood glucose levels outside the cells. When the blood glucose levels are high, glucose enters inside the β-cells by facilitated diffusion through the Glut2 transporter (De et al., 1995a) and is metabolized by aerobic glycolysis. This system for the release of insulin is commonly known as glucose-stimulated insulin secretion (GSIS) (Komatsu et al., 2013). There are four key

components of the "Consensus Model" of GSIS: i) Glut2 dependent glucose uptake, ii) glucose metabolism, iii) K_{ATP} channel closure and iv) opening of the voltage gated Ca^{2+} channels. Both the K_{ATP} and Ca^{2+} channels are embedded in the plasma membrane of β-cells (Ramadan et al., 2011; Ashcroft and Rorsman, 1990). Normally, when the blood sugar levels are low the K_{ATP} are open while the Ca^{2+} channels are closed (Boland et al., 2017). This keeps the β-cell membrane potential at a hyperpolarized state which prevents the Ca^{2+} influx and insulin release (Martin et al., 2013). When the glucose levels rise outside the cells, it enters inside the cells by facilitated diffusion (down its concentration gradient) via the Glut 2 transporter (De et al., 1995b). The metabolism of glucose leads to an increase in the ATP levels which increases the ATP/ADP ratio and closing of K_{ATP} channels (Santulli et al., 2015). This closer of K_{ATP} channels leads to depolarization of the β-cell membrane which opens the Ca2+-channels in the β-cell membrane causing further depolarization and exocytosis of granules to the outside of the cell and into the blood circulation (Ganong W.F., 2005). These secretory granules belong to at least two functionally distinct pools: the readily releasable pool (RRP) and the reserve pool (RP). The RRP are granules which are already "primed" to be released while the RP granules are not ready to be released and need to undergo a series of ATP-Ca^{2+} and temperature-dependent reactions (mobilization or priming) in order to be secreted out from the cells (Rorsman and Ashcroft, 2018). During the process of exocytosis, insulin and C-peptide are enzymatically separated and thus leads to an equimolar secretion of both insulin and C-peptide to the blood. In normal glucose tolerant subjects, the release of insulin is pulsatile in both the fasting and postprandial stage. In the fasting stage the release of insulin is stable and in the postprandial stage the release is biphasic in pattern. The first phase is transient but rapid and is followed by a slow and continuously increasing and modest peak. The first peak reflects the exocytosis of the RRP insulin granules while the second peak reflects the release of RP granules (Rorsman and Ashcroft, 2018). Impairments in the first phase of secretion is an early sign of β-cell dysfunction (Cerasi and Luft, 1967). It has been shown that the first phase is blunted in subjects with impaired glucose tolerance and completely absent in T2D subjects (Ward et al., 1984; Byrne et al., 1996). Moreover, severe impairments in the second phase have also been shown. This shift in the curve is due to the β-cell defect (β-cell function). Therefore, many studies concentrate on how to preserve or prevent the reduction in β-cell functions.

One of the most well-known insults which impair β-cell function is hyperglycemia, termed glucose toxicity. It has been established that chronic hyperglycemia impairs the previously reduced β-cell function. This is because chronic hyperglycemia can lead to oxidative stress and inflammation, causing a

change in the gene regulation which lead to impaired insulin secretion and amplified apoptosis (Gilbert and Liu, 2012). This phenomena can be observed in the study done by Toschi et al. (Toschi et al., 2002), where in normally glucose tolerant subjects they observed a decrease in the insulin secretion after step-wise increasing the glucose levels (2.8, 2.8 and 5.6 mM) by giving glucose boluses and following it with by glucose-insulin clamp. Another important insult for the β-cell dysfunction is plasma FFA levels. In obesity and insulin resistance, where there is an impairment in the ability of adipose tissue to store excess FFA, increased lipolysis leads to an increase in the plasma FFA levels (Sattar and Gill, 2014; Almandoz et al., 2013). The increased plasma FFA then start accumulating in non-adipose tissues (such as the liver, heart, pancreas). These FFA induce organ dysfunction, programmed cell-death and apoptosis due to their non-oxidative metabolism (Kusminski et al., 2009a). Long-term exposure to FFA levels suppresses the β-cell glucose-stimulated insulin secretion, reduces insulin biosynthesis and causes β-cell loss (Yaney and Corkey, 2003; Poitout and Robertson, 2008). Moreover, persistent hyperglycemia and increased FFA level induces another detrimental state for the β-cells known as glucolipotoxic, which leads to a severe reduction in β-cell function (reduced insulin synthesis and secretion) as they lead to increased oxidative stress (Cerf, 2013).

Since most of the insulin is extracted by the liver before being streamed into the peripheral circulation, quantification of β-cell function is not that straightforward as it is difficult to cannulate portal vein in humans. As a result, many mathematical models have been introduced to estimate the β-cell function during oral and intravenous glucose loads. The two most commonly used models are 1) C-peptide deconvolution (Eaton et al., 1980; Van et al., 1992) and 2) "Pisa-Padova" β-cell function model (Figure 8) (Mari and Ferrannini, 2008a). The C-peptide deconvolution method estimates the insulin secretions rate (ISR) based on the different pharmacokinetic profiles of C-peptide and insulin. As insulin and C-peptide are secreted in equimolar amounts and hepatic extraction of C-peptide is negligible, it is feasible to estimate ISR from the peripheral C-peptide levels using a two-compartment model. The "Pisa-Padova" model estimates β-cell function by taking into account the body surface area, age, sex, and plasma levels of glucose, insulin and C-peptide levels during fasting and glucose loading. The model estimates the insulin secretion rate in three distinct components 1) "glucose sensitivity", defined as the β-cell ability to sense the increase in glucose levels (a linear dose-response curve between plasma insulin secretion and glucose). 2) "potentiation ratio", defined as the insulin secretion potentiation which depends on several factors (neural modulation, non-glucose insulin secretagogues, prolonged hyperglycemia). 3) "rate sensitivity", defined as

components which affect the insulin secretion as a change in glucose levels typically at the early phases after glucose loading.

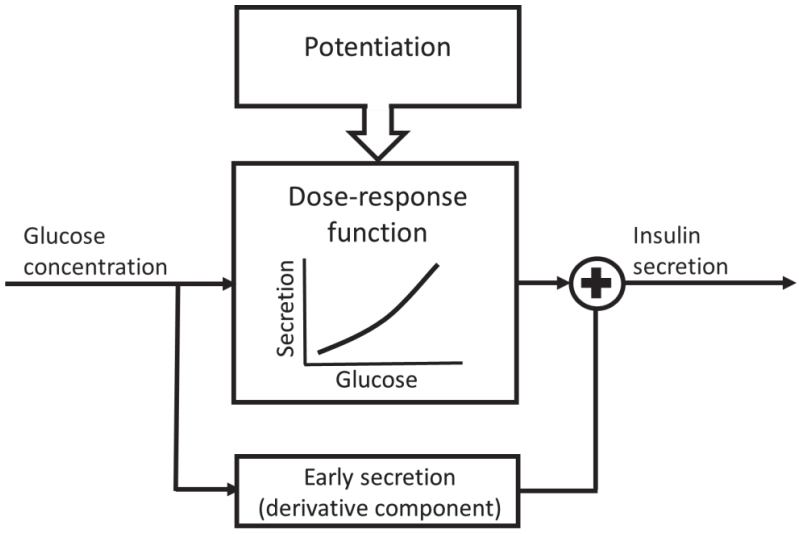

Figure 8: "Pisa-Padova" model of β-cell function. Modified from (Mari and Ferrannini, 2008a).

2.5.2 Pancreatic substrate uptake

Both islets and exocrine pancreas use glucose as their primary fuel for oxidative metabolism. In β-cells, however the GU is crucial as it adjusts for insulin secretion. Under normal conditions β-cells sense the glucose levels in the blood and release insulin accordingly. Glucose is first internalized by the insulin-independent glucose transporter (Glut2) inside the β-cells, after which it is phosphorylated by glucokinase (Matschinsky et al., 1993). Studies have shown that β-cells glucose sensing mechanism is mainly dependent on glucokinase (Matschinsky, 1996) and that impairments in the glucokinase enzyme could result in a diabetic phenotype (Matschinsky, 2009).

On the other hand, data from in vitro studies suggest an interrelation between pancreatic glucose and FFA metabolism. Pancreatic FAU depends on either passive diffusion or facilitated transport, which also depends on membrane bound fatty acid transporter (CD36), fatty acid binding protein (FABP) and fatty acid transporter proteins (FATPs). The study by Wallin et al. showed that by inducing an increase in the CD36 transporter in rat INS-1 cells, increased the oleate (fatty acid) uptake, inhibited the normal glucose mediated inhibition of

oleate oxidation and significantly impaired the glucose-mediated insulin secretion (Wallin et al., 2010). Moreover, in another study by Kim et al. it was shown that by incubating the rat INS-1 cells for 12 hours in a high glucose medium (30 mM) induced an increase in the CD36 and palmitate uptake and in parallel reduced the glucose mediated insulin secretion (Kim et al., 2012). Additionally, in the same study glucose mediated insulin secretion normalized in rat INS-1 cells through the siRNA inhibition of CD36 despite of the high glucose medium (Kim et al., 2012). These results suggest that the increased pancreatic FAU during hyperglycemia inhibits the pancreatic glucose sensing mechanism and metabolism. Furthermore, interventions leading to a reduced pancreatic FAU uptake might lead to an improved glucose sensing mechanism and improved glucose mediated insulin secretion. To verify this hypothesis, interventional studies are needed which measure both pancreatic substrate uptake and β-cell function.

2.5.3 Pancreatic fat content

Accumulation of ectopic fat in the pancreas also known as fatty pancreas (Lesmana et al., 2015) is a progressively important clinical entity owing to its association with several metabolic co-morbidities. Fatty pancreas has been shown to be associated with hypertension, metabolic syndrome and increasing the risk of acquiring T2D by two-fold (Singh et al., 2017b). Additionally, it has also been observed that there is an accumulation of fat in the pancreas with increasing age and weight as observed in human autopsies (Olsen, 1978). Moreover, further research in rodents has revealed that the accumulation of fat in the pancreas has different outcomes depending on whether the accumulation is in the exocrine part or within the islets. In the acinar cells, the accumulation of fat leads to the formation of large lipid droplets and a low rate of apoptosis (Higa et al., 1999). While in the islets the number of lipid droplets is lower compared to the acinar cells but the rate of apoptosis is higher (Kusminski et al., 2009b). This higher rate of apoptosis is due to the formation of reactive long-chain fatty acyl-CoA and toxic metabolites like ceramides, increased oxidative stress and by activating protein kinase C-δ (Robertson et al., 2004; Shimabukuro et al., 1998; Prentki and Nolan, 2006; Newsholme et al., 2007; Kharroubi et al., 2004). All these factors contribute to the loss of β-cells and further exacerbate the pathophysiology of T2D. Loss of pancreatic fat due to weight loss has been shown to reverse T2D and normalization of glucose metabolism (Steven et al., 2016). Even though it can be presumed that the accumulation of fat in the pancreas is associated with the pancreatic fatty acid uptake, not many studies have investigated this hypothesis. Exercise has been shown to improve whole-

body FFA metabolism by inducing active lipolysis in adipocytes and oxidation of fatty acids in muscles (Hashimoto, 2013). However, it is not known whether exercise-induced reduction in whole-body FFA metabolism would reduce pancreatic FFA uptake and lead to a reduction in pancreatic fat content.

2.5.4 Exercise and pancreas

The effects of exercise on pancreatic fat content have not been extensively studied, with only one study comparing three different training loads on pancreatic fat (Jennifer L.Kuk, 2007) (Table 6). In that study, pancreatic fat content tended to decrease after three different exercise interventions for six months (3-4 days/week expanding 4,8,12 Kcal/kg) (Jennifer L.Kuk, 2007). On the other hand, the effects of exercise on β-cell function have been studied comprehensively, with many studies actually concluding that exercise improves the β-cell function independent of weight loss (Bloem and Chang, 2008; Malin et al., 2013; Lee et al., 2015; Krotkiewski et al., 1985) (Table 6). Exercise-induced improvement in β-cell function is due to exercise-induced improvements in the whole-body lipid metabolism, which in turn reduces the lipotoxicity and improves the β-cell function (Malin et al., 2013; Bloem and Chang, 2008). Moreover, recent studies have also compared the effect of exercise intensity on β-cell function but with contrasting results; one study indicating that HIIT is more effective in improving it (Madsen et al., 2015) while another study suggesting that MICT is more effective (Slentz et al., 2009). However, no prior studies have investigated the effects of exercise on pancreatic GU and FAU, while many studies have shown that exercise training improves β-cell function.

Table 6: Exercise induced changes in pancreatic fat content and β-cell function

Reference	n	Duration of exercise	Exercise intervention	Main results
Effect of exercise on pancreatic fat content				
Kuk (Jennifer L.Kuk, 2007)	87	6 months	Three different Aerobic exercise loads (3-4/week) vs. controls	Tendency to reduce the pancreatic fat content in all three different exercise loads versus the controls
Effect of exercise on β-cell function				
Krotkiewski et al (Krotkiewski et al., 1985)	101	3 months	Aerobic exercise (3/week)	Improved insulin secretion attributed to exercise induced improvements in β-cell mass
Bloem and Chang (Bloem and Chang, 2008)	12	1 week	Aerobic exercise (7/week)	Short-term aerobic exercise improved the β-cell function.
Malin et al (Malin et al., 2013)	35	12 weeks	Aerobic exercise (5/week)	Aerobic exercise training improved the β-cell function with a linear dose-response (i.e. higher exercise doses will result in more improvement in β-cell function)
Lee et al (Lee et al., 2015)	120	1 year	Aerobic exercise (5/week) vs Accumulated 1 million-steps (10000 steps, 5/week) vs. control	Both the aerobic and 1 million-steps improved the β-cell function.

2.6 Summary of the literature

Exercise has long been known to improve insulin sensitivity but adherence to exercise training among the general population remains low. Recently, HIIT/SIT have gained considerable attention in both the general population and the scientific community particularly because of their short duration and greater health benefits. Moreover, exercise intensity has been shown to be an important determinant of the exercise induced physiological adaptations. Many studies have shown that HIIT/SIT lead to comparable or even sometimes superior cardiovascular benefits compared to MICT. However, the results regarding the effects of HIIT/SIT on insulin sensitivity are less conclusive. Furthermore, the role of the splanchnic organs (the liver, pancreas, and intestine) in the regulation of whole-body glucose metabolism cannot be ignored. Even though traditional exercise (MICT) has been shown to improve hepatic fat content, lipoprotein subclasses and pancreatic β-cell function, the effects of exercise intensity remain unclear on liver, pancreas, and intestinal metabolism, lipoprotein subclasses, and gut microbiome.

3 AIMS OF THE STUDY

Impairments in the metabolism and functions of muscle, liver and pancreas have a key role in the pathogenesis of diabetes (DeFronzo, 2004). Recent studies have suggested that impairments in intestinal metabolism, function and gut microbiome also play a key role in the pathogenesis of diabetes (Musso et al., 2010; Holst et al., 2009). While the benefits of different modes of exercise training and volume have been extensively studied concerning muscles, the benefits of different modes of training on the liver, pancreas, and intestines remains more poorly characterized. Although SIT with its short duration has been shown to be as effective in improving muscle metabolism as MICT (Gibala and McGee, 2008), it remains unclear which of these two training modes is optimal for inducing improvements in liver, pancreas, and intestinal metabolism and function.

Therefore, the main aims of this current thesis were to compare the adaptations of SIT and MICT on:

- Intestinal glucose and free fatty acid uptake, gut microbiome and LPS (I and IV)
- Pancreatic fat content, pancreatic glucose and free fatty acid uptake and beta cell function (II)
- Hepatic fat content, hepatic glucose, free fatty acid uptake and the lipoprotein profile (III)

4 MATERIALS AND METHODS

4.1 Subjects

The total number of study subjects for all four studies was composed of 56 subjects, of which 28 were healthy (n = 28) and 26 were prediabetic/T2D (n = 26). The baseline characteristics of the study subjects in different studies are given in (Table 7).

Table 7: Characteristics of subjects in different study groups

Parameter	Study I	Study II		Study III		Study IV
	Healthy	Normoglycemic	Prediabetic/T2D	Normoglycemic	Prediabetic/T2D	Prediabetic/T2D
N	28	28	16	28	26	18
Male/Female	28/0	28/0	16/0	28/0	16/10	13/5
SIT/MICT	14/14	14/14	9/7	14/14	13/13	10/8
Medication						
Metformin			9		11	8
DPP-4 inhibitors (sitagliptin)			4		5	4
Sulfonylurea (glimepiride)			1		1	1
Statins			5		7	6
Anthropometrics						
Age (y)	48 [46, 49]	48 [46, 49]	49 [48, 51]	48 [46, 49]	49[48, 51]	49 [47, 51]
Weight (kg)	83.6 [80.2, 87.1]	83.6 [80.2, 87.1]	96.3 [91.2, 101.3]	83.6 [80.2, 87.1]	91.8 [86.4, 97.2]	89.8 [84.4, 95.1]
BMI (kg/m^2)	26.1 [25.2, 57.1]	26.1 [25.2, 57.1]	30.4 [29.1, 31.8]	26.1 [25.2, 57.1]	30.5 [29.3, 31.6]	30.0 [28.7, 31.3]
Whole body fat (%)	22.6 [20.9, 24.3]	22.6 [20.9, 24.3]	28.8 [26.5, 31.2]	22.6 [20.9, 24.3]	31.6 [28.1, 35.5]	31.6 [28.1, 35.5]
VO_{2peak} (ml/kg/min)	34.2 [32.6, 35.9]	34.2 [32.6, 35.9]	29.3 [27.2, 31.4]	34.2 [32.6, 35.9]	28.2 [25.3, 29.1]	27.9 [25.4, 30.4]

All values are model based means [95% CI]. T2D, type 2 diabetes; SIT, sprint interval training; MICT, moderate intensity continuous training; DDP-4, dipeptidyl peptidase 4; BMI, body mass index; VO_{2peak}, aerobic capacity.

In the first phase, healthy normoglycemic but not previously physically active subjects (VO_{2peak} < 40 mL·kg^{-1}·min^{-1}) were recruited (through newspapers, personal contacts, electronic and traditional bulletin boards). The inclusion criteria was as follows: sex: male; age: 40-55 years; body mass index: 18.5-30 kg·m^{-2} and subjects had normal glycemic status. The exclusion criteria was: blood pressure > 140/90 mm Hg; history of rest or exercised induced asthma; history of anorexia or bulimia; any chronic medical defect or injury which would either hinder/interfere everyday life; previous use of anabolic steroids; additives or any other substrates; use of tobacco products; use of

narcotics; significant use of alcohol; presence of any ferromagnetic objects which could make MR imaging contraindicated; or any other condition which in the opinion of the investigator would hinder the interpretation of study results or would create a hazard to the subjects safety or endanger the study procedures. During the intervention one subject withdrew from the SIT group due to hip pain induced by the training and one from the MICT group due to personal reasons.

In the second phase, prediabetic/T2D (n = 26) subjects were recruited (through newspapers, personal contacts, electronic and traditional bulletin boards). For the prediabetic/T2D subjects the inclusion was slightly modified: sex: male/female; body mass index: 18.5-35 $kg \cdot m^{-2}$; all subjects had to meet the criteria of defective glucose tolerance set by the American Diabetes Association (ADA 2015) and have an HbA1c < 7.5 mmol·l-1. In the exclusion criteria two parameters were altered; blood pressure value was raised to ≤ 160/100 and history of chronic disease other than diabetes. Of the 26 subjects, 17 met the criteria for the diagnosis of T2D and nine met the criteria for prediabetes; having either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) (ADA 2015). Thirteen out 17 T2DM subjects were treated with oral hypoglycemic medication (11 metformin; 5 DPP-IV and 1 sulfonylurea) while 4 did not take any medication for T2DM. None of the subjects were on insulin. All diabetes medications were discontinued 48 hours before any studies were performed. Moreover, of the total 26, 7 were also taking statins (Table 7). In addition to the two subjects who withdrew from the healthy subjects there were also five who did not finish the studies in the prediabetic/T2D group. One withdrew due to claustrophobic feelings in the MRI, one due to migraine during the training session and three subjects could not continue the study due to personal reasons. This brought the total number of subjects to 7 who did not complete the training intervention in this study.

All studies were conducted according to the Declaration of Helsinki, and the study protocol was approved by the ethical committee of the Hospital district of Southwest Finland, Turku (decision 95/180/2010 §228).

Study I

Study I included the healthy normoglycemic subjects (n = 28). In this study, the effects of SIT and MICT on intestinal GU and FAU were investigated.

Study II

Study II included both the healthy normoglycemic subjects (n = 28) and prediabetic/T2D subjects (n = 26). In this study, the effects of SIT and MICT on pancreatic fat content (PFC), pancreatic GU and FAU were examined.

Study III

Study III also included all the subjects both the healthy normoglycemic (n = 28) and prediabetic/T2D (n = 26). The effects of SIT and MICT were investigated on the hepatic fat content, hepatic GU and FAU and lipoprotein subclasses.

Study IV

Study IV included only the prediabetic/T2D (n=26) subjects. The effects of SIT and MICT were examined on the gut microbiome and intestinal GU and FAU. Unfortunately, of the 26 subjects we could only acquire 18 feacal samples due to technical issues and therefore the results in this study are from 18 subjects. The gender, training groups, diabetic and medication status of the groups is explained in Table 7.

4.2 Study designs

This thesis is 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, date: April 28, 2011). All subjects were screened to determine their physical, aerobic (VO_{2peak}) and glycemic (β cell function) status (oral glucose tolerance test, OGTT), after which the subjects underwent magnetic resonance spectroscopy (MRS) to measure their pancreatic and hepatic fat content, and nuclear magnetic resonance (NMR) to measure their lipoprotein subclasses, a nucleic acid analysis of their feacal samples to determine their gut microbiome composition and finally two PET scans on two occasions after an overnight fast on separate days. This was to measure the GU and FAU in the intestines, pancreas, and liver. The subjects were than randomized into groups performing either SIT or MICT training for two weeks and six sessions (i.e. 3 sessions/week). Healthy subjects were randomized in two phases. Firstly, 24 participants were permuted randomly with a 1:1 allocation ratio. However, due to technical problems with the PET studies a second phase with a block of 4 participants was randomly permuted. Hence, the final group size for the healthy subjects was (n = 28) for SIT (n = 14) and MICT (n = 14). Randomization for the prediabetic/T2D group was done in a

single phase with blocks of 4 participants with a 1:1 ratio. The total number of subjects in the prediabetic/T2D group was (n = 26) for SIT (n = 13) and MICT (n = 13), males (n = 16) and females (n = 10). Finally, 48 hours after the last exercise session all the studies were repeated. This time duration was chosen to study the long-term effects of the exercise training and not the acute effects of exercise training (Figure 9).

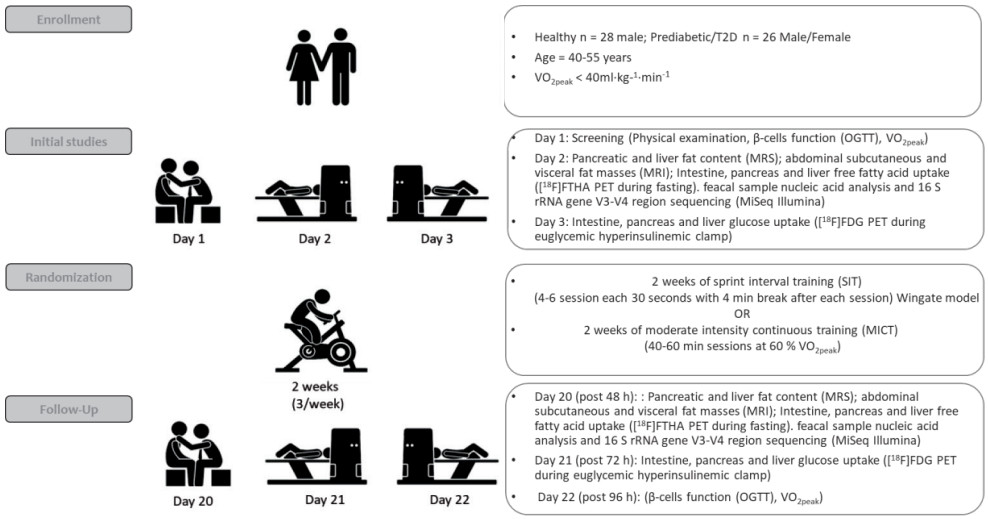

Figure 9: Study design for all the studies (I-IV).

4.3 Training interventions

All the participants (n= 54, i.e. healthy normoglycemic + prediabetic/T2D) performed six-sessions of either SIT or MICT over a period of two weeks (i.e. 3/week) under laboratory conditions. The SIT training protocol was similar to the one described by Burgomaster et al. (Burgomaster et al., 2005). SIT training was progressive and included 4-6 x 30 sec bouts of all-out cycling efforts with a 4 min recovery period after each bout in which the participant either remained still or performed unloaded cycling (Monark Ergomedic 828E; Monark, Vansbro, Sweden). All participants were familiarized with SIT training ~ one week before the start of the training intervention (2 x 30 sec bout). The initial number of bouts was 4 and increased to 5 and finally to 6 after every other exercise session. Each bout of exercise started with 5 sec of acceleration to maximal cadence without any resistance followed by a sudden increase of load (7.5% of whole body weight in kg for healthy subjects and 10% of fat free mass in kg for the prediabetic/T2D participants) and maximal cycling

for 30 sec (Wingate protocol). The MICT training protocol consisted of 40-60 min cycling bouts with electrically braked ergometer (Tunturi E85, Tunturi Fitness, Almere, the Netherlands) at moderate intensity (60% of peak workload VO_{2peak}). The duration of exercise was 40 min at the first exercise session which was then increased by 10 min after every other exercise session, thus finally reaching a 60 min duration in the second week.

4.4 Maximal exercise test

A maximal exercise test to measure aerobic capacity was performed at the Paavo Nurmi Center (Turku, Finland) approximately one week before the first training session and 96 hours after the last exercise session. The participants were asked to refrain from eating and drinking for two hours before the test. The test was performed on a bicycle ergometer (Ergoline 800s; VIASYS Healthcare, Germany) starting at 50 W followed by an increase of 30 W every 2 min until volatile exhaustion. Ventilation and gas exchange was measured with (Jaeger Oxycon Pro, VIASYS Healthcare, Germany) and reported as the mean value per minute. For all tests the peak respiratory exchange ratio was ≥1.15 and peak blood lactate concentration was ≥8.0 $mmol \cdot L^{-1}$, measured from capillary samples immediately and 1 min after exhaustion (YSI 2300 Stat Plus; YSI Incorporated Life Sciences, Yellow Springs, OH). In all except one participant in the MICT group and one in the SIT group the peak heart rate (HR) (RS800CX, Polar Electro Ltd., Kempele, Finland) was within 10 beats of the age-appropriate reference value (220-age) for both pre and post training tests. Therefore, the aerobic capacity (VO_{2peak}) was defined as the highest 1 min mean value for oxygen consumption. Maximal performance was measured by calculating the Peak workload ($Load_{peak}$) from the average workload during the last 2 min of the test.

4.5 Hyperinsulinemic euglycemic clamp

Subjects had to fast for at least 10 h before the euglycemic hyperinsulinemic clamp was performed. The technique used for the clamp has been used as previously and is described by Defronzo et al. (DeFronzo et al., 1979). The clamp started with cannulating both arms antecubital veins. One of the arms was heated with an electronic cushion to “arterialize” the venous blood samples. A primed-constant insulin infusion (Actrapid, 100 U/ml, Novo Nordisk, Bagsvaerd, Denmark) was then started at a rate of 120 $mU/min/m^2$ for the first 4 min. After 4 min, the infusion was reduced to 80 $mU/min/m^2$ until 7 min and

after 7 min until the end of the clamp the insulin infusion was further reduced to 40 mU/min/m^2. The exogenous glucose infusion started after 4 min of the insulin infusion at a rate depending on the subjects weight (in kg)·0.1^{-1}·g^{-1}·h^{-1}. Glucose infusion was then doubled at 10 min and after which it was adjusted to maintain plasma glucose concentration at a level of 5 mmol/L. To adjust the glucose infusion rate, plasma glucose levels were measured from blood samples taken every 5-10 min. Subjects were then moved to the PET scanner to perform the [^{18}F]FDG study. The scan started when the subject attained a steady state. Whole-body insulin-stimulated glucose uptake (M-value) was calculated from the steady-state plasma glucose values during the [^{18}F]FDG scan.

4.6 Positron emission tomography (PET)

PET studies were performed on two separate occasions one before and two after (48 and 72 hours) the last exercise session. The liver, pancreas, and intestinal (duodenum, jejunum and colon) FAU and GU was measured using radiotracer 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid (FTHA) and 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) respectively. [^{18}F]FTHA PET was done under fasting conditions and [^{18}F]FDG under a euglycemic hyperinsulinemic clamp. Before the PET scans started the antecubital veins of both arms were cannulated, one was used for radiotracer injection ([^{18}F]FTHA and [^{18}F]FDG) and infusions (insulin and glucose during [^{18}F]FDG PET scan for clamp), and the other arm for blood sampling. An electric cushion was used throughout the study to heat the arm used for blood sampling to "arterialize" the venous blood. A computed tomography (CT) scan was performed before the PET scan to obtain an anatomical reference. Both PET/CT scans were done using a GE Advance PET/CT scanner (General Electric Medical System, Milwaukee, WI, USA).

FAU was measured by injecting [^{18}F]FTHA radiotracer under a fasting state. Dynamic images of the abdominal region (3x300 sec frames) were acquired ~ 46 min after the tracer injection. During the scan, blood samples were collected at regular intervals to determine plasma radioactivity levels (Wizard 1480, Wallac, Turku, Finland) and were used to calculate the input function. As FTHA metabolizes (Takala et al., 2002), additional blood samples were also taken at ~ 5, 10, 20, 30, 40 and 50 min to measure the [^{18}F]FTHA metabolites. These metabolites were then corrected from the input function to obtain a pure plasma [^{18}F]FTHA input function (Maki et al., 1998).

GU was measured by using [^{18}F]FDG radiotracer under a euglycemic hyperinsulinemic clamp. The method used to perform the euglycemic hyperinsulinemic clamp was similar to the one explained previously by Defronzo

et al. (DeFronzo et al., 1979). After ~ 91 min [SEM 2] from the start of the clamp radiotracer [^{18}F]FDG was injected. Dynamic abdominal images were acquired similar to the one explained earlier for the [^{18}F]FTHA PET scan. Blood samples were also taken during the scan to measure the plasma radioactivity levels but no additional samples were required as [^{18}F]FDG is not metabolized.

4.7 Image analysis

Before the PET imaging data was analyzed it was corrected for decay, dead time and photo attenuation. All the images were then reconstructed using 3D-OSEM (ordered subset expectation maximization) method and analyzed with a Carimas version 2.7 (http://turkupetcentre.fi/carimas). Three dimensional regions of interests (ROIs) were drawn on the liver (carefully avoiding great vessels and ducts), intestinal sections duodenum, jejunum and transverse colon (carefully avoiding external metabolically active tissues and intestinal contents) as previously described (Honka et al., 2013), and in head, body and tail of the pancreas. An example of how these ROIs were drawn is shown in (Figure 10). Time activity curves (TAC) were extracted from these ROIs.

From the TAC and input function the rate constant (Ki) was calculated for the uptake of [^{18}F]FTHA and [^{18}F]FDG radiotracers into the cell. Graphical analysis was then used to calculate the fractional uptake rate (FUR) (Patlak and Blasberg, 1985). The liver, intestinal, and pancreas FAU and GU was calculated by multiplying their specific FUR by the corresponding plasma free fatty acid and glucose concentrations respectively. For GU, the values were further divided by a lumped constant (LC) (Liver = 1, intestine = 1.15 and pancreas = 1). Furthermore, for colonic GU, the value was divided with a recovery coefficient (2.5) (Honka et al., 2013) to take into account the partial volume effect (Aston et al., 2002). This was not required for duodenal and jejunal GU.

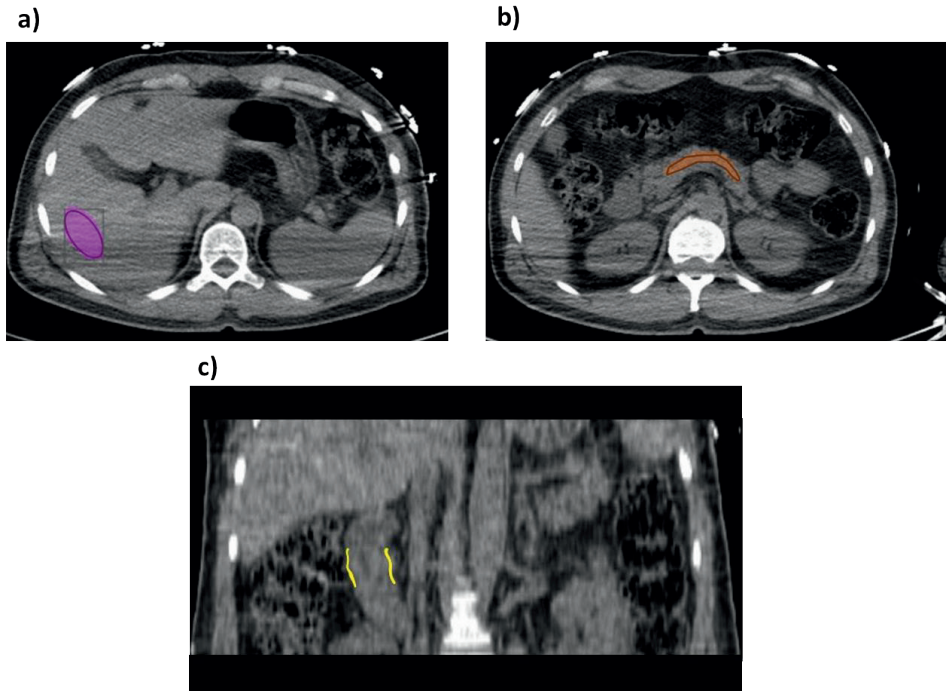

Figure 10: ROIs drawn in (a) the liver, (b), pancreas and (c) intestine. ROI, Region of interest.

4.8 Magnetic resonance imaging (MRI)

Abdominal adipose tissue depots masses (subcutaneous and visceral) and the liver volume were measured using Philips Gyroscan Intera 1.5 T CV Nova Dual MRI scanner (Philips Medical Systems, the Netherlands). Whole-body (head to knee) axial MRI images were obtained using the following parameters (TE 2.3 and 4.7 ms, TR 120 ms, slice thickness 10 mm without gap). Images were analyzed using SliceOmatic software version. 4.3 (http://www.tomovision.com/products/sliceomatic.html). Abdominal fat masses were measured from the xiphoid process till where the both heads of femur were visible. The abdominal depots were divided into subcutaneous and visceral fat depots according to the classification by Abate et al. (Abate et al., 1994). An example of how the abdominal depots were divided is shown in (Figure 11). From the images pixel surface area of each depot was obtained and multiplied with slice thickness and then with the density of the adipose tissue (0.9196 kg/L) (Abate et al., 1994) to obtain the subcutaneous and visceral depot mass.

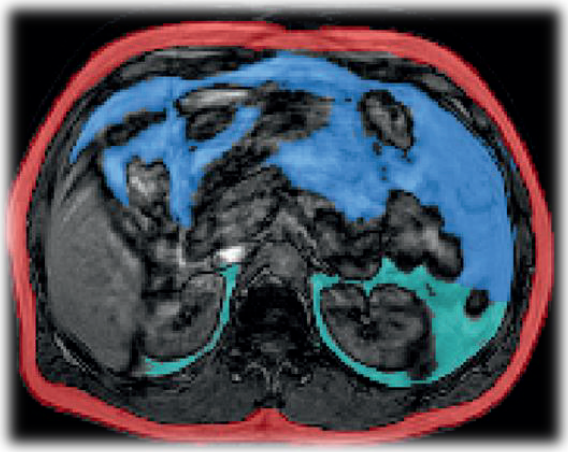

Figure 11: Division of different abdominal fat masses. The red colour is for subcutaneous tissue mass, the blue for intraperitoneal fat mass and the green for extraperitoneal fat mass. Visceral fat mass was (intraperitoneal +extraperitoneal fat mass).

4.9 Magnetic resonance spectroscopy (MRS)

The hepatic and pancreatic fat content was measured by proton magnetic resonance spectroscopy (^{1}H-MRS) using Philips Gyroscan Intera 1.5T CV Nova Dual scanner (Philips Medical Systems, the Netherlands). For the hepatic fat content a single voxel of 27 cm^3 was positioned in the liver parenchyma carefully avoiding the great vessels. Using point resolved spectroscopy (PRESS) ^{1}H-MRS sequence with the following parameters: echo time (TE) = 25 ms (milliseconds), repetition time (TR) = 3000 ms and lipid ratio (peaks at 0.9 ppm and 1.3 ppm) the lipid and water content was obtained from each voxel. From each breath-hold interval four data frames were obtained and the process was repeated for four consecutive breath-holds. Each frame consisted of the complete triglyceride and water content. To increase the reliability of the data frames, they were averaged to reduce the signal-to-noise (SNR) ratio. Linear combinations of the model spectra software package (LCModel version 3.3-0C) (Provencher, 1993) was used to analyze the data. The signal amplitudes were corrected because of different molar concentration of ^{1}H nuclei of fat and water and T2 decay times (Szczepaniak et al., 1999). Fat in relation to the total weight of the liver was defined as the hepatic fat content (Thomsen et al., 1994).

For pancreas fat content a volume of interest (12mm x 10mm x 15mm) was placed inside the body of the pancreas using sagittal, coronal and axial plane images. Similar to the liver, the PRESS sequence with the following parameters: TE = 27ms TR = 3000 ms and lipid ratio (peaks at 0.9 ppm, 1.3 ppm and 1.6

ppm) was used to measure the lipid and water content. Images were obtained in breath holds and a total of 10 spectra were collected as a time series, each with 4 measurements. For each subject the measurements were performed twice, one with water suppression and the other without it. Data was analyzed using LCModel software (Provencher, 1993). Similar to the liver the signal amplitudes were also corrected (Szczepaniak et al., 1999). The locations of both the voxel and the volume of interest were carefully documented to ensure that they were positioned in the same location before and after the exercise intervention.

4.10 Lipoprotein subclasses

High-throughput proton NMR proton metabolomics (Nightingale Health Ltd, Helsinki, Finland) was used to quantify lipid and lipoprotein metabolic biomarkers from fasting serum samples. Fourteen lipoprotein subclasses were quantified as follows: extremely-large very low density lipoprotein (XL-VLDL) with particle diameter 75nm upwards and a possible contribution of chylomicrons; five VLDL subclasses: extra-large, large, medium, small and extra-small; intermediate density lipoprotein (IDL); three low density lipoprotein (LDL) subclasses: large, medium and small; four high density lipoprotein (HDL) subclasses: extra-large, large, medium and small. Within each subclass the following components were quantified: total lipids, phospholipids, triglycerides, cholesterol, free cholesterol and cholesterol esters. The methods used for the quantification have been described previously (Soininen et al., 2015).

4.11 Feacal DNA extraction, 16S rRNA gene sequencing analysis

DNA extraction was done from stool samples which were collected before and after exercise intervention and kept at – 80 °C. MasterPure Complete DNA & RNA Purification Kit (Epicentre, Madison, WI, United States) was used according to the manufacturer's instructions with some modifications (including a bead-beater step and enzyme incubation with lysozyme and mutanolysis to increase DNA extraction) to isolate the total DNA from the feacal samples as described previously (Rodriguez-Diaz et al., 2017). Qubit® 2.0 Fluorometer (Life Technology, Carlsbad, CA, USA) was used to measure the DNA concentration and was diluted to 5 ng/μL. PCR was used to amplify 16S rRNA gene (V3-V4 region) by using Illumina adapter overhand nucleotide sequences and following Illumina protocols. After the gene amplification of 16S rRNA gene amplicon, a Nextera XT Index Kit (Illumina, San Diego, CA, USA) was used to perform the multiplexing step. Bioanalyzer DNA 100 chip (Agilent

Technologies, Santa Clara, CA, USA) was used to check 1µl of the PCR product and using 2x300 pb paired-end run (MiSeq Reagent Kit v3) according to the manufacturer's instruction the libraries were sequenced on a MiSeq-Illumina platform (FISABIO sequencing service, Valencia, Spain).

QIIME pipeline version 1.9.0 was used to process the data (Caporaso et al., 2010). From the data set chimeric sequences and sequences which could not be aligned were removed. In addition, OTUs with a relative frequency below 0.01 were removed. Furthermore, sequences which could be classified as Cyanobacteria and chloroplasts and sequences which could not be classified as domain levels were removed as they most likely represented ingested plant material.

4.12 LBP, CRP and TNF α measurements

Multiplex bead assay analysis (Luminex® Performance Assay Multiplex Kit; Procarta® Immunoassay Affymetrix, Santa Clara USA) was used to measure the tumor necrosis factor (TNF α), C-reactive protein (CRP) according to manufacturer's instructions. Luminex xPonent software (Luminex, USA) was used with LUMINEX® 200™ to analyze the samples. An ELISA Kit (FineTest® Ref- EH1560, Wuhan Fine Biotech Co., Ltd,) was used to determine the human lipopolysaccharide-binding protein (LBP).

4.13 Oral glucose tolerance test (OGTT)

A 2-h OGTT was performed on two separate occasions, one on the screening day before the exercise intervention and one 96 hours after the last exercise session. All subjects were required to fast for at least 10 h before the 2-h OGTT test was performed. The test started with an ingestion of a 330 ml solution containing glucose (Nutrical, Nutricia Medical, Turku, Finland). Glucose, insulin and C-peptide concentrations were measured from blood samples collected at baseline and at 15,30,60, and 120 min after the drinking the solution.

4.14 Beta cell function (β cell)

In the current study, various parameters were calculated from the 2-h OGTT to describe the β cell function using the "Pisa-Podova" model and C-peptide deconvolution method. C-peptide and insulin levels for every 5 min for

the whole 2-h OGTT was used to calculate the insulin secretion rate (ISR) (Van et al., 1992). ISR_{early} and ISR_{late} was calculated from an area under the curve (AUC) of ISR from 0 to 30 min and from 30 to 120 min respectively. AUC from 0-120 min denotes the total ISR (ISR_{total}). Another index was calculated as (ISR30-ISR0)/ (glucose30-glucose0) from the ISR_{early} normalized to glucose concentration (ΔISR0-30/ΔG0-30). Modeling as described by Mari et al. (Mari et al., 2002 and Mari and Ferrannini, 2008a) was used to determine other parameters. The mean slope of glucose-insulin secretion dose-response curve was defined as glucose sensitivity and it describes the degree of responsiveness of pancreatic β cells to blood glucose levels. A potentiation factor was calculated every 5 min from the 2-h OGTT and included various potentiating signals (secretory influence of gastrointestinal hormones, glucose-induced potentiation and neural factors). The ratio mean (potentiation 110-120)/mean (potentiation 0-10) was defined as the potentiation factor ratio. Finally, rate sensitivity defined as the dependence of insulin secretion on the rate of change in the plasma glucose levels (Mari and Ferrannini, 2008b).

4.15 Body composition and measurement of clinical variables

A Bioimpedence monitor (InBody 720, Mega Electronics, Kuopio, Finland) was used to measure the whole-body fat percentage. An automatic enzymatic method (Cobus 8000, Roche diagnostics GmbH Mannheim, Germany) was used to measure the liver enzymes (Alanine transaminase (ALAT), aspartate aminotransferase (ASAT) and gamma-glutamyl transpeptidase (GT)), total cholesterol, triglycerides and HDL. The Friedewald equation was used to calculate the LDL concentration (Friedewald et al., 1972).

4.16 Animal studies

In addition to the human studies, we also performed animal studies to investigate the changes at the cellular uptake level in the intestine (study I). Animal care complied with the guidelines set by International Council of Laboratory Animal Science (ICLAS). All procedures were approved by National Animal Experimental Board (ESAVI/5053/04.10.03/2011) and were performed in accordance with the guidelines of the European Community Council Directives 86/609/EEC. Wistar male rats (n = 24; aged between 8 to 12 weeks) were acquired from the Charles River German facility. The animals arrived at least 2 weeks before any exercise intervention to acclimatize them to the new environment and in order to minimize the stress of handling; they were handled

one week before any intervention was performed. They were housed in groups of 3 at the Central Animal Laboratory of the University of Turku under standard conditions (lights on from 6:00 am to 6:00 pm, temperature 21°C, humidity 55±5%). The animals had free access to tap water and a standard diet (from where?). They were randomized into three groups with n=8 in each group, (control (CON), SIT and MICT). Interventions were performed between 8 AM - 18 PM. Intestinal (duodenum) and liver samples were collected 5 days after the last exercise session for protein expression and mRNA expression analyses respectively.

4.16.1 Study design

The animals were also randomized into SIT, MICT and a control group. The SIT and MICT group performed 10 exercise sessions over the course of two weeks. While the CON group did not perform any exercise session. One day after the last training session, an OGTT was performed, which was followed by $\dot{V}O_{2max}$ tests on the second and third day after the last exercise session. Thereafter, the animals were kept in the metabolic cages for 2 days and sacrificed on the 5th day for protein and mRNA expression analyses (Figure 12).

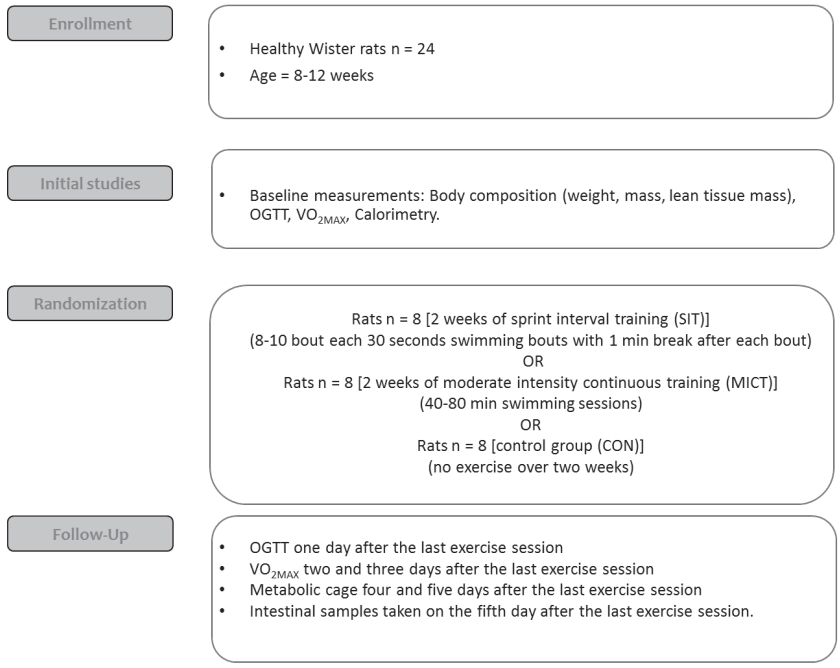

Figure 12: Animal study design.

4.16.2 Exercise interventions

In two weeks, animals in the SIT and MICT group performed 10 exercise sessions. Each SIT session consisted of 8-10 x about 30 sec swimming bouts with 1 min resting period after each exercise bout. To force the animals into performing all-out efforts extra weights of 30-50 grams were attached to their waists. In the MICT group the animal swam for 40-80 min. They started swimming for 40 min and then the duration was increased by 10 min after every other exercise session to finally reach 80 min in the last two exercise sessions. Rats in the MICT group did not bear any additional weights.

4.16.3 Western blot analysis

The intestinal (duodenal) samples were frozen and homogenized in ice with a lysis buffer (150 mM NaCl, 1% Np-40, 0.5% sodium-deoxycholate, 0.1% SDS, 50 mM Tris·HCL, pH 8.0) and supplemented with Ultra-Turrax T25 (Ika-Werke) a protease inhibitor cocktail. Samples were first denatured with SDS loading buffer containing β-mercaptoethanol (Sigma-Aldrich, St Louis, MO, USA) in +95°C for 5 min before the protein concentration was quantified with a Thermo Scientific Pierce BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). The samples were first run on a 10% SDS-polyacrylamide gel and after electrophoresis were transferred to a nitrocellulose membrane (Santa Cruz Biotechnology, Dallas, TX, USA). To block the unspecific binding sites the samples were incubated with 5% (wt/vol) milk diluted in Tris-buffered saline-Tween 20 (0.02 M Tris-buffered saline, 0.1% Tween 20). After this the samples were incubated overnight at +4°C with the following primary antibodies: vascular endothelial growth factor 2 (VEGFR2) (no. NB-100-530, Novus Biologicals, Centennial, CO, USA), β-actin (no. sc-8432, Santa Cruz Biotechnology), CD36 (no. sc-9154, Santa Cruz Biotechnology), and GLUT2 (no. 07–1402, MilliporeSigma, Burlington, MA, USA). LI-COR Odyssey CLx Imager (LI-COR, Lincoln, NE, USA) was used to detect the near-infrared fluorescent signals (bands) from the secondary antibodies IRDye 800CW Donkey anti-Mouse lgG (H+L) (LI-COR Biosciences) and IRDye 800CW Donkey anti-Rabbit lgG (H+L) that were incubated with the membranes for 1 h at RT???. CLx Imager software was used to quantify the resulting bands. Signal intensities were than normalized to a reference band (beta-actin) in each membrane and the relative values were used to calculate the fold-change.

4.16.4 Body composition, OGTT, VO_2max and whole body energy expenditure

Each animal's body fat mass and lean tissue mass was measured with EchoMRI-700 (Echo Medical Systems LLC, Houston, TX, USA) before the first and after the last exercise intervention. Animals fasted for at least 6 hours before OGTT was performed one day after the last exercise session. During the OGTT test glucose (20%, wt/vol, 1 ml /100g) was given orally and measured at time points 0, 30, 60, 90 and 120 min from the tail vein using a Precision Xceed Glucose Monitoring Device (Abbott Diabetes Care Ltd, Abbot Park, IL, USA). Aerobic capacity (VO_{2max}) was measured using a single lane treadmill (Panlab-Harvard Apparatus, Spain) before and then two and three days after the last exercise session. Animals were familiarized with the single lane treadmill three days before the VO_{2max} test. The test started by keeping the treadmill at an angle of 25° and increasing the speed by 3cm/s until volatile exhaustion. On the fourth and fifth day after the last exercise session the metabolic cage (Oxylet system, Panlab, Harvard Apparatus, Spain) was used to measure the whole-body energy expenditure over 48 hours. Carbon dioxide (CO_2) production and oxygen (O_2) consumption was measured and the energy expenditure was calculated from their averaged 24 h values.

4.17 Statistical analyses

Skeletal muscle GU was the primary outcome used for determining the sample size for the entire study (NCT01344928). A total of 24 subjects (SIT = 12 and MICT = 12) were needed to achieve > 90% power of detecting a 20 % unit change in insulin-stimulated GU in quardriceps femoris for healthy normoglycemic subjects. The estimated increase in GU for the SIT group was 40% vs. 20% for MICT group, SD 15 with a level of significance at 5%. While for prediabetic/T2D subjects the total of 20 subjects (SIT = 10 and MICT = 10) were required to achieve a similar statistical power. The estimated increase in GU for SIT group was 60% vs. 30% for MICT, SD 20. To accommodate for drop-outs and technical problems during the study, an extra four subjects and six subjects were recruited in the healthy (normoglycemic) and prediabetic/T2D groups respectively. This brought the total number to 28 (SIT = 14 and MICT = 14) healthy (normoglycemic) subjects and 26 subjects (SIT = 13 and MICT = 13) in prediabetic/T2D group. For the outcomes of studies (I-IV) no specific sample size calculations were performed.

Variable normal distribution was evaluated visually and tested with the Shapiro-Wilk test (SAS). When variables were not normally distributed appropriate transformations either logarithmic or square root were done. Statistical analysis for all studies (I-IV) were performed using a hierarchical mixed linear model. Compound symmetry covariance for repeated measurements were used, including one within-factor term (Time, indicating the mean change between baseline and post intervention values) (Study I-IV). Three between-factors (diabetic status (Dia); normoglycemic and prediabetic/T2D; Training: SIT and MICT) (study II) and (Liverfat: high fat and low fat group) (study III). Three interaction terms (Dia*time; indicating the mean change in the parameter was different between normoglycemic and prediabetic/T2D groups) (Study II-IV); (training*time; indicating the mean change in the parameter was different between SIT and MICT groups) (study I-IV) and (Liverfat*time; indicating the mean change in the parameter was different between high fat and low fat group) (study III). Women were excluded in the comparison between normoglycemic and prediabetic/T2D groups to avoid mixing the effects of gender and glucose tolerance (study II-III). For the comparison between high hepatic fat content (HFC) and low hepatic fat content (LFC) groups, all participants (men + women) were included as some women had both LFC and HFC, effectively removing the mixing effect of gender in the comparison (Study III). In addition, medication status (on oral glycemic medication (metformin) or on statins) were also used as factors in the statistical analyses (Study II-IV). Within the linear mixed models, the missing values for participants (due to drop-outs, technical issues) were accounted for by a restricted maximum likelihood estimation and Turkey's correction method was used for multiple comparisons. All parameters values reported in the tables and figures in this thesis are model-based means (SAS least square means) and with 95% confidence intervals (CI). Pearson's correlation coefficients were used to calculate correlations between parameters.

Statistics for the animal studies (Study I), two-way analysis of variance was used to evaluate (time) effect and (group*time) interaction. As measurements were taken from different rats, all observations from pre and post values are independent.

All statistical tests were performed as two-sided and the statistical significance level was set at 0.05. Statistical analyses were performed using the SAS system version 9.3 for Windows (SAS Institute Inc., Cary, NC, USA).

5 RESULTS

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

5.1 Effects of SIT and MICT on intestinal substrate uptake in healthy normoglycemic subjects (study I)

Basic characteristics: There were significant improvements in the anthropometrics, glucose and lipid profiles after two weeks of intervention with similar results in various parameters for both SIT and MICT except for LDL, which was reduced more significantly in the SIT group compared to the MICT group (Table 8).

Table 8: Characteristics of healthy normoglycemic subjects and their adaptations to two weeks of SIT and MICT. Table modified from Study I.

Parameter	SIT		MICT		p value	
	Pre	Post	Pre	Post	Time	Time*training
n	14	13	14	13		
Anthropometrics						
BMI (kg/m^2)	25.9 [24.5, 27.3]	25.7 [24.3, 27]	26.4 [25.0, 27,7]	26.4 [25.0, 27.7]	0.14	0.19
Whole body fat (%)	22.2 [19.8, 24.6]	21.2 [18.8, 23.6]	22.9 [20.5; 25.3]	22.1 [19.7, 24.5]	**<0.0001**	0.56
Subcutaneous fat mass (kg)	4.03 [3.3, 4.8]	3.93 [3.2, 4.7]	4.44 [3.7, 5.2]	4.38 [3.6, 5.1]	**0.04**	0.54
Visceral fat mass (kg)	2.91 [2.1, 3.8]	2.80 [1.9, 3.7]	2.66 [1.7, 3.5]	2.59 [1.8, 3.4]	**0.046**	0.73
VO_{2peak} (ml/kg/min)	34.7 [32.4, 37.1]	36.7 [34.3, 39.1]	33.7 [31.3, 36]	34.7 [32.4, 37.1]	**0.001**	0.27
Glucose Profile						
$Glucose_{fasting}$ (mmol/L)	5.5 [5.3, 5.7]	5.4 [5.2, 5.6]	5.7 [5.5, 5.9]	5.6 [5.4, 5,8]	0.43	0.77
$Glucose_{clamp}$ (mmol/L)	5.0 [4.7, 5.3]	4.9 [4.6, 5.2]	4.9 [4.5, 5.2]	5.0 [4.7, 5.3]	0.96	0.20
$Insulin_{fasting}$ (mU/L)§	5.2 [3.8, 7.2]	4.8 [3.4, 6.6]	5.8 [4.1, 8.1]	6.0 [4.3, 8.5]	0.80	0.46
$Insulin_{clamp}$ (mU/L)	75.3 [66.8, 83.9]	73.8 [65.1, 82.6]	75.4 [66.5, 84.3]	79.4 [70.3, 88.6]	0.64	0.31
HbA1c (mmol/mol)	36.5 [34.3, 38.6]	35.2 [33.0, 37.4]	37.4 [35.3, 39.5]	34.3 [32.1, 36.5]	**<0.001**	0.11
Whole-body insulin sensitivity (M-value) (µmol/kg/min)	38.2 [30.1, 46.4]	42.8 [34.5, 51.0]	31.9 [23.1, 40.7]	34.2 [25.4, 43.1]	**0.03**	0.45
Lipid Profile						
$FFA_{fasting}$ (mmol/L)	0.61 [0.50, 0.71]	0.59 [0.48, 0.70]	0.78 [0.67, 0.89]	0.67 [0.54, 0.79]	0.052	0.14
FFA_{clamp} (mmol/L)	0.06 [0.05, 0.08]	0.06 [0.05, 0.08]	0.08 [0.06, 0.10]	0.07 [0.05, 0.09]	0.41	0.43
Cholesterol (mmol/L)	5.3 [4.8, 5.7]	4.6[4.1, 5.0]	4.7 [4.3, 5.2]	4.4 [3.9, 4.9]	**<0.001**	0.06
HDL (mmol/L) §	1.4 [1.2, 1.6]	1.2 [1.1, 1.4]	1.4 [1.2, 1.5]	1.3 [1.1, 1.5]	**<0.001**	0.28
LDL (mmol/L)	3.4 [3.0, 3.8]	2.8 [2.4, 3.3]	2.9 [2.5, 2.3]	2.7 [2.3, 3.1]	**<0.001**	**0.03**
Triglycerides (mmol/L)	1.02 [0.85, 1.19]	0.97 [0.79, 1.15]	0.96 [0.78, 1.13]	0.80 [0.62, 0.98]	0.07	0.37

All values are model-based means [95% CI]. SIT, sprint interval training; MICT, moderate intensity continuous training; BMI, body mass index; VO_{2peak}, aerobic capacity; HbA1c, glycosylated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein. (§) Log transformation was performed to achieve normal distribution. Time p value Indicates the change between pre- and post-measurements in the whole study group. Time*training p value Indicates whether the change in the parameter was different between the SIT and MICT training modes.

Intestinal substrate uptake: Insulin-stimulated intestinal GU improved only in the colon after MICT (p = 0.02, Time*training) and there was a tendency to improve jejunal GU after MICT (p = 0.08, Time*training). Fasting intestinal FAU was reduced in the duodenum after both SIT and MICT (p = 0.001, Time) and there was a tendency to reduce the FAU uptake in the colon after MICT training (p = 0.08, Time*training). However, there was no significant change in duodenal GU and jejunal FAU after both SIT and MICT. (Figure 13)

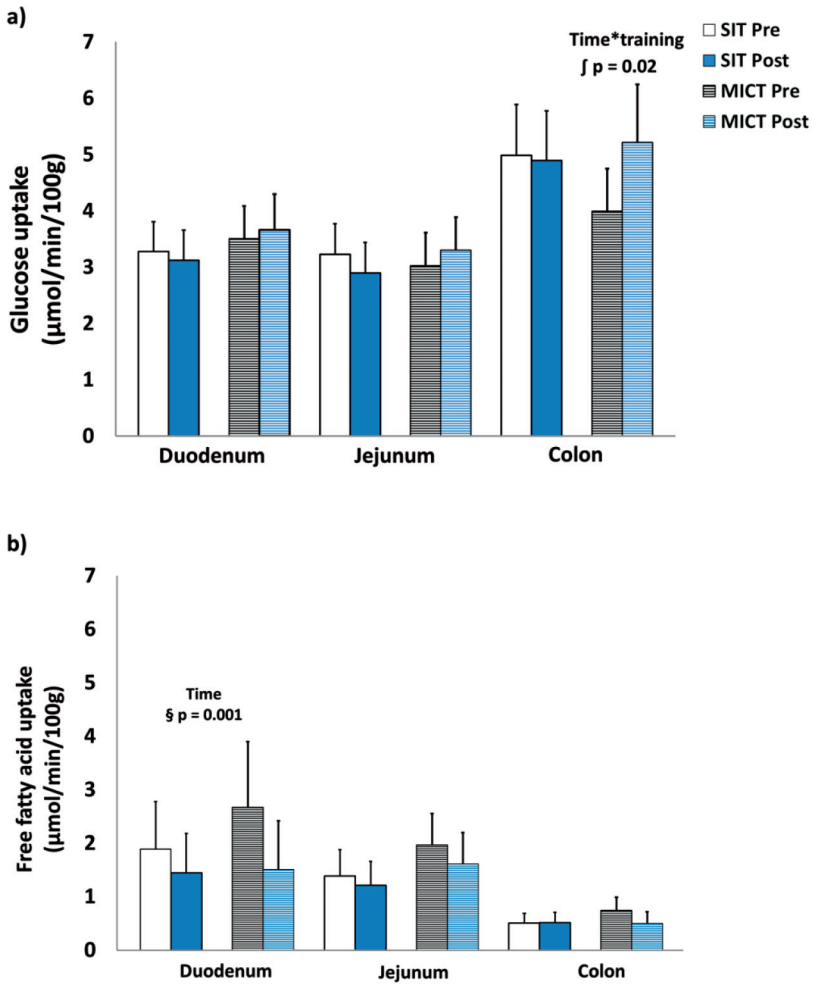

Figure 13: Effects of SIT and MICT on intestinal glucose and free fatty acid uptake. White bars (before the exercise intervention), filled bars (after the exercise intervention), no pattern filled bars are for sprint interval training (SIT) and pattern filled bars are for moderate intensity continuous training (MICT). a) Intestinal glucose uptake in the duodenum, jejunum and colon. All values are model based means with error bars representing [95 % confidence intervals]. ∫ p value (time*training) indicating that the change in the colonic glucose uptake was different between SIT and MICT, with MICT significantly increasing the colonic glucose uptake. b) Intestinal free fatty acid uptake in duodenum, jejunum and colon. § p value (time) indicates the change in the duodenal free fatty acid uptake is similar between SIT and MICT, both exercise modes significantly reduced the duodenal free fatty acid uptake.

Correlations: Jejunal GU correlated positively with aerobic capacity (VO_{2peak}) [before training (pre): r = 0.46, p = 0.03; after training (post): r = 0.45, p = 0.03] and negatively with the visceral fat mass [pre: r = -0.42, p = 0.05; post: r = -0.45, p = 0.03]. Finally, in the MICT group the whole-body insulin sensitivity (M-value) correlated positively with colonic GU [pre: r = 0.17, p = 0.63; post: r = 0.68, p = 0.03] (Figure 14).

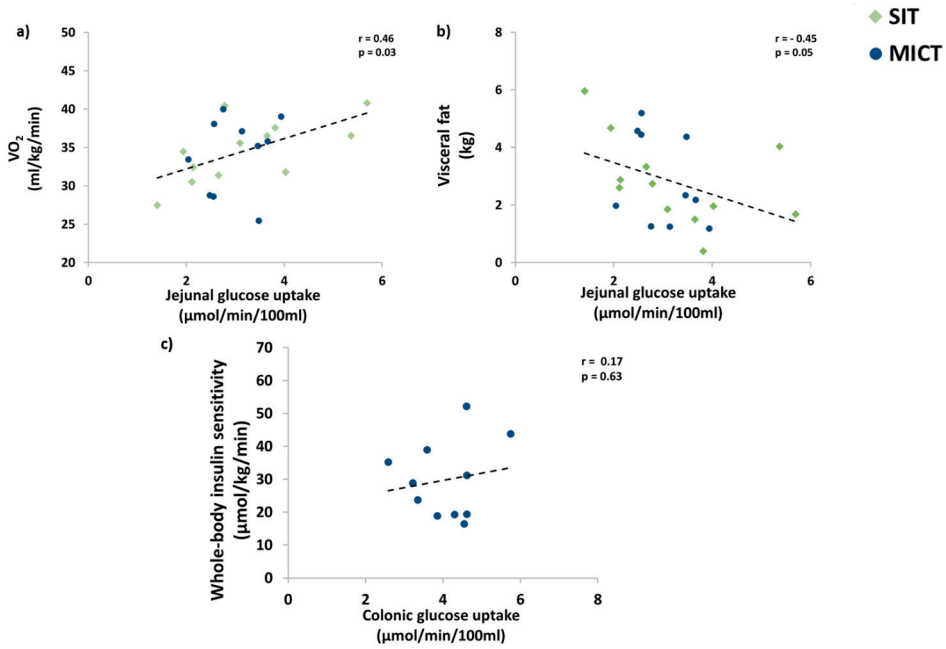

Figure 14: Intestinal associations with various parameters. Insulin-stimulated jejunal glucose uptake correlated with a) aerobic capacity (VO_{2peak}) and b) visceral fat. c) Insulin-stimulated colonic glucose uptake associates with whole-body glucose uptake. (○) sprint interval training (SIT) and (●) moderate intensity continuous training (MICT).

Animal results: In rats, due to age-induced growth there was a significant increase in the body weight and fat free mass in all groups (SIT, MICT and CON). Fat percentage was reduced in both the SIT and MICT group animals, while there was an increase in the CON group animals. Aerobic capacity (VO_{2max}) significantly improved in the MICT and SIT groups compared to the CON group (Table 9). There was also a significant increase in the rats intestinal Glut2 and CD36 protein expression in both SIT and MICT groups compared to the CON group. Between the SIT and MICT groups there was no significant difference in Glut2 and CD36 expression (Figure 15).

Table 9: Animal characteristics at baseline and the changes induced after the exercise intervention. Table modified from Study I.

Parameter	CON n = 8		SIT n = 8		MICT n = 8		P value	
	Pre	Post	Pre	Post	Pre	Post	Time	Time x group interaction
Anthropometrics								
Weight (g)	282 [269, 294]	351 [338, 364]*	297 [285, 309]	346 [331, 360]*	281 [269, 293]	350 [337, 364]*	**<.0001***	**0.002**
Fat free mass (%)	239 [229, 248]	282 [271, 294]	253 [244, 263]	296 [285, 307]	248 [238, 257]	291 [279, 302]	**<.0001***	0.99
Fat mass (g)$^{\tau}$	36.8 [33.6, 40.4]	47.2 [42.2, 52.7]*	38.4 [35.0, 42.1]	40.5 [36.3, 45.2]	35.9 [32.7, 39.4]	40.4 [36.2, 45.1]*	**<.0001***	**<0.001**
Fat (%)	11.9 [11.0, 12.9]	12.7 [11.6;13.8]*	11.7 [10.8, 12.7]	10.7 [9.7, 11.8]*	11.4 [10.4, 12.3]	10.8 [9.7, 11.9]*	0.09	**<.001**
VO_{2max} (ml/min/kg)	69.0 [65.1, 72.9]	68.9 [65.0, 72.8]	70.1 [66.2, 74.0]	72.9 [69.0, 76.8]*	71.2 [67.3, 75.1]	72.8 [68.9, 76.7]	**0.01***	**0.05**

All values are mean [95 % confidence intervals]. CON, control group no exercise; MICT, moderate intensity continuous training; SIT, sprint interval training. (§) Log transformation was done to achieve normal distribution. The p-value for time indicates the change in the whole study group. The p-value for time x group interaction indicates if the change in the parameter was different between the CON, SIT and MICT training modes and * pre vs. post p value < 0.05.

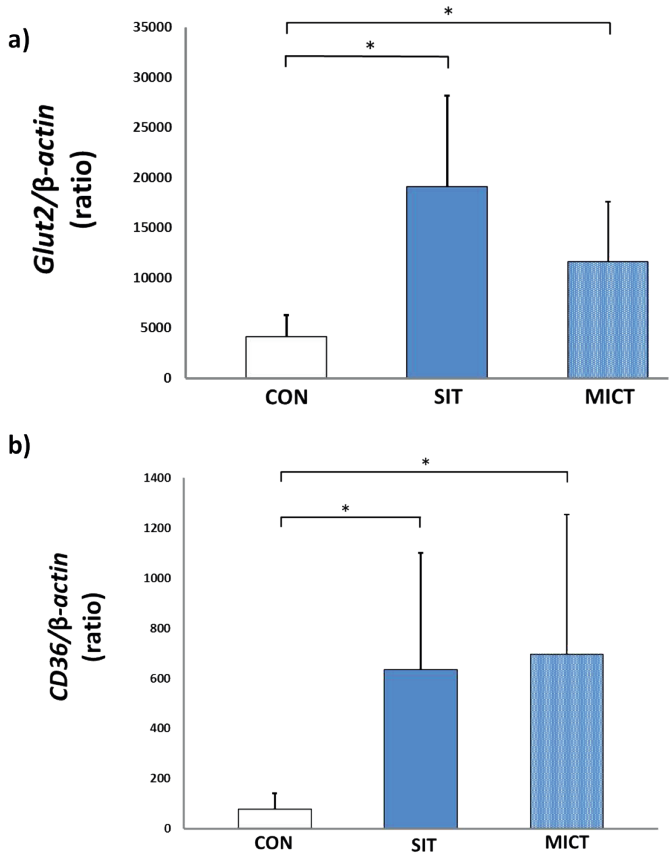

Figure 15: Relative expression of Glut2 and CD36 in rat intestinal cells. a) The CON animal group had significantly lower Glut2 expression compared to the SIT and MICT animal groups. b) CD36 expression was also significantly lower in the CON animal group compared to both SIT and MICT animal groups. White bars control group (CON), filled bars sprint interval training (SIT) and pattern filled bars moderate intensity continuous training (MICT). All values are model based means with error bars representing [95 % confidence intervals]. *p value < 0.05.

5.2 Effects of SIT and MICT on gut microbiome and intestinal substrate uptake in prediabetic/T2D subjects (study IV)

Basic characteristics: There was a significant reduction in the whole-body fat percentage, visceral fat mass and HbA1c after both SIT and MICT (all $p < 0.05$, Time). The aerobic capacity (VO_{2peak}) improved only after SIT training and not after MICT ($p = 0.03$, Time*training) (Table 10).

Table 10: Characteristics of SIT and MICT subjects at baseline and after the intervention. Table modified from Study IV.

Parameter	SIT		MICT		p value	
	Pre	Post	Pre	Post	Time	Time*training
n	10		8			
Anthropometrics						
BMI (kg/m^2)	29.3 [27.5, 31.0]	29.1 [27.3; 30.8]	30.7 [28.9,32.6]	30.7 [28.8, 32.6]	0.25	0.50
Whole body fat§ (%)	32.6 [27.8, 38.3]	31.6 [26.9, 37.1]	30.5 [25.8, 36.2]	29.7 [25.1, 35.2]	**0.04**	0.91
Subcutaneous fat mass§ (kg)	6.6 [5.2, 8.5]	6.5 [5.1, 8.4]	5.9 [4.5, 7.6]	5.8 [4.5, 7.6]	0.26	0.58
Visceral fat mass§ (kg)	2.81 [1.95;4.05]	2.76 [1.92, 3.98]	3.99 [2.71, 5.88]	3.70 [2.51, 5.46]	**0.04**	0.18
VO_{2peak} (ml/kg/min)	27.1 [23.7, 30.5]	28.7 [25.2, 32.1]	28.6 [25.0, 32.3]	28.2 [24.6, 31.9]	0.17	**0.03**
Glucose Profile						
$Glucose_{fasting}^{\S}$ (mmol/L)	7.0 [6.5 7.6]	7.1 [6.5, 7.7]	6.2 [5.7, 6.7]	6.2 [5.7, 6.7]	0.87	0.76
$Glucose_{clamp}$ (mmol/L)	4.7 [4.6, 4.9]	4.9 [4.7, 5.1]	4.9 [4.7, 5.1]	5.0 [4.8, 5.2]	0.19	0.59
$Insulin_{fasting}^{\S}$ (mU/L)	11.9 [7.2, 19.6]	10.7 [6.5, 17.8]	8.9 [5.4, 14.8]	9.7 [5.8, 16.1]	0.91	0.28
$Insulin_{clamp}$ (mU/L)	85.4 [76.1, 94.7]	87.0 [77.1, 97.0]	85.8 [76.5, 95.1]	85.0 [75.0, 94.9]	0.92	0.76
Whole body insulin sensitivity (M-value)(µmol/min/kg)	22.4 [14.1, 30.8]	26.8 [18.2, 35.4]	21.9 [13.5, 30.3]	21.9 [13.3, 30.4]	0.29	0.28
HbA1c (mmol/mol)	39.8 [36.2, 43.3]	37.5 [33.9, 41.1]	38.6 [34.6, 42.6]	37.0 [33.0, 41.1]	**0.003**	0.56
Lipid Profile						
$FFA_{fasting}$ (mmol/L)	0.73 [0.60, 0.80]	0.74 [0.60, 0.87]	0.81 [0.67, 0.95]	0.72 [0.57, 0.87]	0.22	0.16
$FFA_{clamp}^{\S}$ (mmol/L)	0.06 [0.04, 0.10]	0.06 [0.04, 0.08]	0.08 [0.05, 0.11]	0.07 [0.04, 0.10]	0.17	0.95

All values are model-based means [95% CI]. SIT, sprint interval training; MICT, moderate intensity continuous training; BMI, body mass index; VO_{2peak}, aerobic capacity; HbA1c, glycosylated hemoglobin. (§) Log transformation was performed to achieve normal distribution. Time p value indicates the change between pre- and post-measurements in the whole study group. Time*training p value Indicates if the change in the parameter was different between the SIT and MICT training modes.

Intestinal substrate uptake: MICT significantly reduced the jejunal FAU while there was no change in the duodenal and colonic FAU. Additionally, there was no change in the insulin-stimulated GU after SIT or MICT (Figure 16).

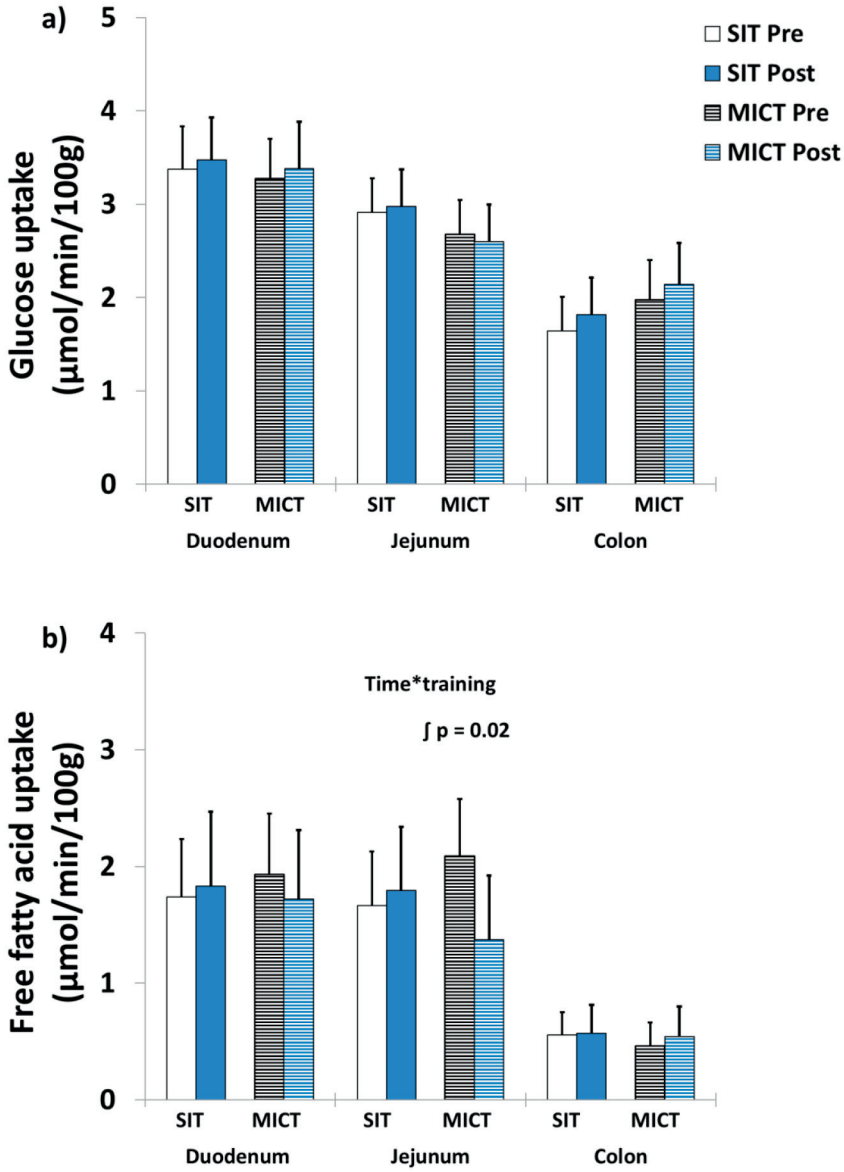

Figure 16: Effects of exercise on intestinal glucose and free fatty acid uptake in prediabetic/T2D subjects. White bars (before the exercise intervention), filled bars (after the exercise intervention), no pattern filled bars are for sprint interval training (SIT) and pattern filled bars are for moderate intensity continuous training (MICT). All values are model based means with error bars representing [95 % confidence intervals]. a) Intestinal glucose uptake in duodenum, jejunum and colon. b) Intestinal free fatty acid uptake in duodenum, jejunum and colon. ∫ p value (time*training) indicating that the change in the jejunal free fatty acid uptake was different between SIT and MICT, with MICT significantly decreasing the jejunal free fatty acid uptake.

Inflammatory markers: Both SIT and MICT significantly reduced tumor necrosis factor alpha (TNF α) and endotoxemic load as shown by reduced lipopolysaccharide binding protein (LBP) (Figure 17). Both training modes also tended to reduce the C-reactive protein (p = 0.08).

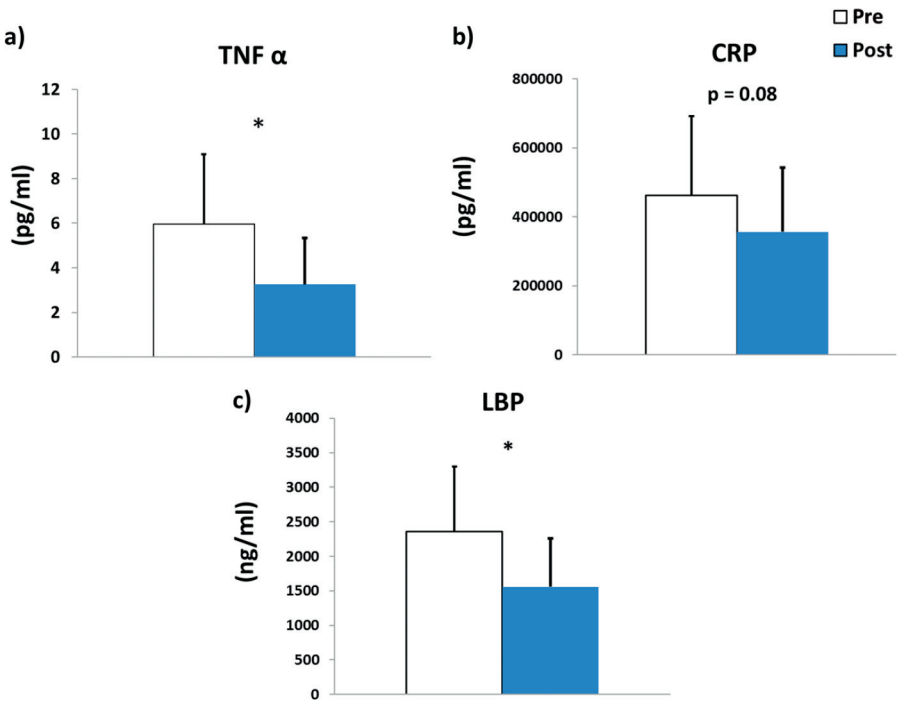

Figure 17: Effects of exercise on systematic (TNF α and CRP) and intestinal inflammatory markers (LBP). White bars (before the exercise intervention), filled bars (after the exercise intervention). All values are expressed as model-based means and the bars are confidence intervals [95% CI]. a) TNF α, b) CRP, c). LBP. * $p < 0.05$ value for time interaction (i.e. the change in the parameter was similar between the SIT and MICT groups. TNF α, tumor necorsis factor alpha; CRP, C-reactive protein; LBP, lipopolysaccharide binding protein; SIT, sprint interval training; MICT, moderate intensity continuous training.

Gut microbiota: Both SIT and MICT modified the gut microbiota composition similarly without any difference between them. There was a significant reduction in the ratio (Firmicutes/Bacteroidetes), an increase at the phylum level for Bacteroidetes, reduction in *Clostridium* at the genus level and a tendency to reduce *Blautia* at the genus level (Figure 18)

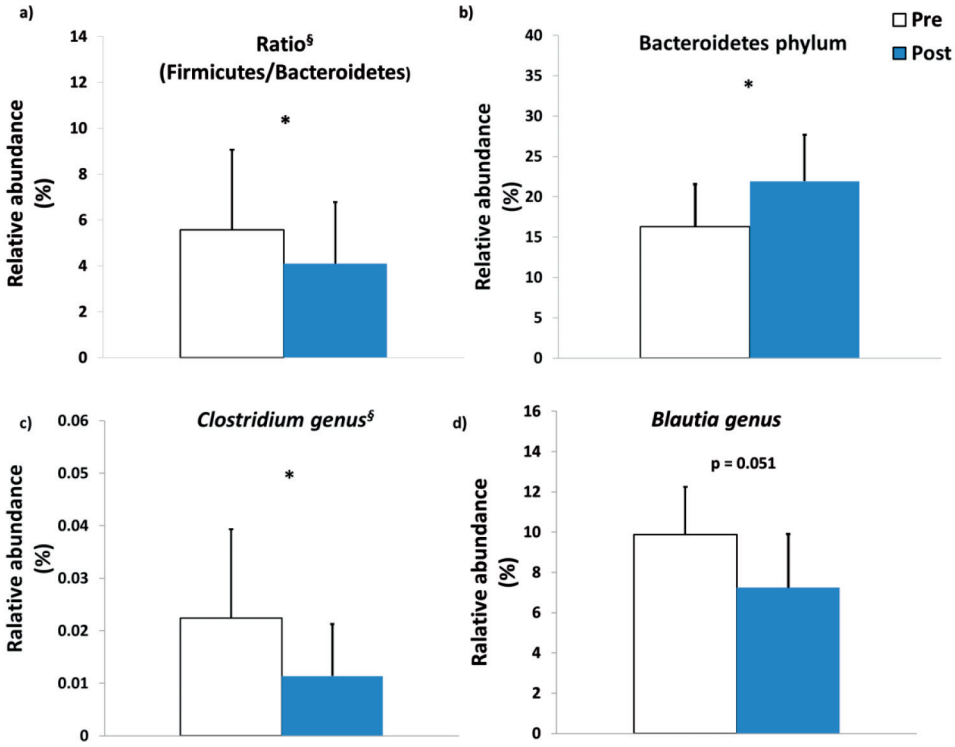

Figure 18: Effects of exercise on gut microbiota composition. All values are expressed as model-based means and bars are confidence intervals [95 % CI]. (§) Log transformation was performed to achieve normal distribution. * p value for time interaction (i.e. the groups (SIT+ MICT) behaved similarly for the change in parameter. SIT, sprint interval training; MICT, moderate intensity continuous training.

Correlations: Jejunal FAU correlated negatively with the whole-body insulin sensitivity (M-value) [(Pre) r = - 0.81, p = 0.049). LBP correlated positively with HbA_1c [(Pre) r = 0.51, p = 0.02]. With gut microbiome, there were significant associations between colonic GU with the ratio (Firmicutes/Bacteroidetes) [(pre) r = -0.62, p = 0.03], with Bacteroidetes [(Pre) r = 0.71, p = 0.007), with Firmicutes [(Pre) r = -0.59, p = 0.03] and Blautia [(Pre) r = -0.57, p = 0.049] (Figure 19). Moreover, *Blautia* associates negatively with whole-body insulin sensitivity (M-value) [(Pre) r = -0.53, p = 0.04].

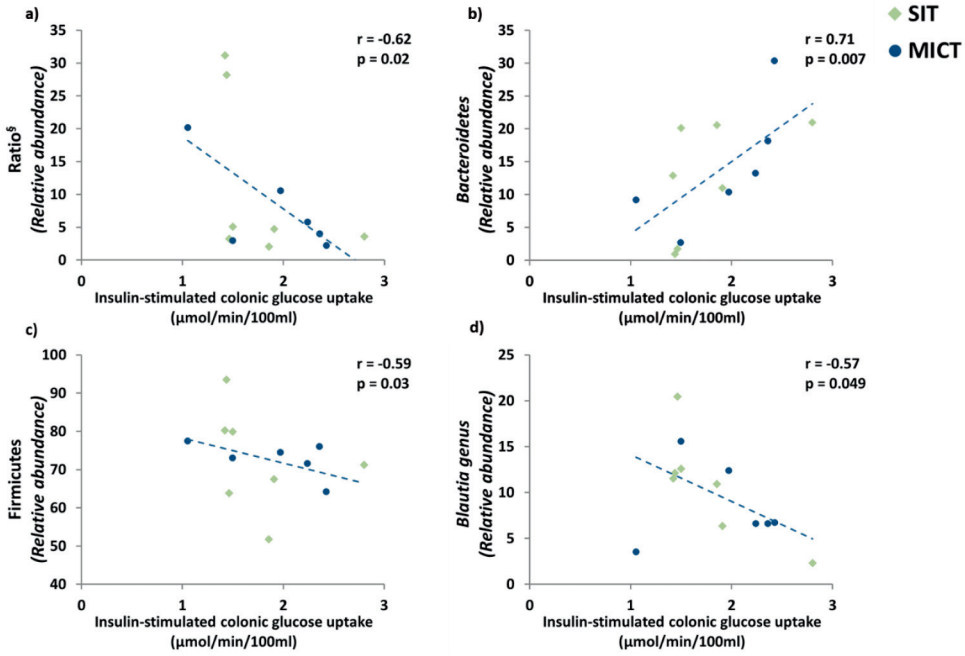

Figure 19: Associations between insulin-stimulated colonic glucose uptake and gut microbiome. a) Ratio (Firmicutes/Bacteroidetes), b) Bacteroidetes, c) Firmicutes and d) Blautia. (§) Log transformation was performed to achieve normal distribution. (◇) sprint interval training (SIT) and (●) moderate intensity continuous training (MICT).

5.3 Effects of SIT and MICT on pancreatic fat content, pancreatic substrate uptake and beta cell function (Study II)

Basic characteristics: The prediabetic/T2D group had significantly different anthropometrics, glucose and lipid profiles compared to the normoglycemic groups (all $p < 0.05$ baseline) (Table 11). After the training intervention, both SIT and MICT had reduced similarly whole-body adiposity, HbA1c and lipid profile (all time p value < 0.05) except for plasma $FFA_{fasting}$, which reduced more significantly in the MICT group ($p = 0.01$, Time*training). Additionally, both SIT and MICT groups increased whole-body insulin sensitivity (M-value) and aerobic capacity (VO_{2peak}), with SIT improving aerobic capacity more significantly than MICT ($p = 0.005$, Time*training) (Table 11).

Table 11: Characteristics of normoglycemic and prediabetic/T2D subjects and their adaptations to two weeks of SIT and MICT. Table modified from Study II.

Parameter	Normoglycemic		Prediabetic/T2D		p value		
	Pre	Post	Pre	Post	Baseline	Time	Training*time
n	28	26	16	13			
Anthropometrics							
BMI (kg/m^2)	26.1[25.1, 27.1]	26.0 [25.0, 27.0]	30.4 [29.1, 31.8]	30.4 [29.0, 31.7]	**<0.001**	**0.17**	0.30
Whole body fat§ (%)	22.6 [20.9, 24.3]	21.7 [20.0, 23.3]	28.8 [26.5, 31.2]	28.1 [25.7, 30.4]	**<0.001**	**<0.001**	0.62
Subcutaneous fat mass§ (kg)	4.1 [3.7, 4.5]	4.0 [3.6, 4.4]	5.6 [4.9, 6.4]	5.5 [4.9, 6.4]	**<0.001**	**0.03**	0.65
Visceral fat mass$^{\&}$ (kg)	3.1 [2.7, 3.4]	3.0 [2.6, 3.4]	4.2 [5.0, 3.6]	4.1 [4.8, 3.5]	**<0.001**	**0.002**	0.60
VO_{2peak} (mL/kg/min)	34.2 [32.7, 35.7]	35.7 [34.2, 37.2]	29.3 [27.2, 31.4]	30.0 [27.9, 32.1]	**<0.001**	**0.003**	**0.005**
Glucose profile							
$Glucose_{fasting}$ § (mmol/L)	5.6 [5.4, 5.8]	5.5 [5.3, 5.7]	6.6 [6.3, 7.0]	6.6 [6.3, 7.0]	**<0.001**	0.86	0.83
$Glucose_{clamp}$ (mmol/L)	4.9 [4.8, 5.1]	4.9 [4.8, 5.1]	4.8 [4.6, 5.1]	5.0 [4.7, 5.2]	0.40	0.35	0.86
$Insulin_{fasting}$ § (mU/L)	5.5 [4.3, 7.0]	5.4 [4.2, 6.9]	13.1 [9.3, 18.3]	12.0 [8.5, 17.0]	**<0.001**	0.46	0.14
$Insulin_{clamp}$ (mU/L)	75.4 [69.6, 81.2]	76.5 [70.5, 82.5]	87.6 [79.9, 95.4]	86.0 [77.8, 94.2]	**0.02**	0.92	0.46
Whole-body insulin sensitivity (M-value)§ (µmol/min/kg)	35.3 [30.0, 40.6]	38.7 [33.3, 44.1]	17.5 [10.3, 24.8]	21.6 [14.2, 29.0]	**<0.001**	**<0.001**	0.06
HBA_1c (mmol/mol)	36.9 [35.2, 38.6]	34.8 [33.0, 36.5]	39.6 [37.3, 41.8]	37.5 [35.2, 39.9]	0.08	**<0.001**	0.38
Lipid profile							
$FFA_{fasting}$ (mmol/L)	0.70 [0.62, 0.77]	0.62 [0.54, 0.70]	0.69 [0.60, 0.78]	0.68 [0.58, 0.78]	0.86	**0.04**	**0.01**
FFA_{clamp} $^{\&}$ (mmol/L)	0.065 [0.05, 0.08]	0.060 [0.05, 0.07]	0.093 [0.07, 0.12]	0.082 [0.06, 0.10]	**0.02**	0.15	0.76
Cholesterol (mmol/L)	5.0 [4.7, 5.3]	4.5 [4.1, 4.8]	4.8 [4.4, 5.3]	4.4 [3.9, 4.9]	0.51	**<0.001**	0.12
HDL§ (mmol/L)	1.4 [1.2, 1.5]	1.3 [1.2, 1.4]	1.2 [1.1, 1.4]	1.1 [1.0, 1.2]	0.10	**<0.001**	0.19
LDL (mmol/L)	3.1 [2.9, 3.4]	2.8 [2.5, 3.1]	2.7 [2.3, 3.1]	2.6 [2.2, 3.0]	0.09	**0.001**	0.12
Triglycerides§ (mmol/L)	0.9 [0.8, 1.1]	0.8 [0.7, 1.0]	1.7 [1.4, 2.1]	1.5 [1.2, 1.9]	**<0.001**	0.08	0.63

All values are model based means [95% confidence intervals]. T2D, type 2 diabetes; BMI, body mass index; VO_{2peak}, aerobic capacity; HbA1c, glycosylated hemoglobin; FFA, free fatty acids; HDL, high density lipoprotein; LDL, low density lipoprotein. (§) Log transformation and (&) square root transformation was performed to achieve normal distribution. p-value for baseline indicates the differences between the normoglycemic and prediabetic/T2D groups. The p-value for time indicates the change between pre- and post-measurements in the whole study group. The p-value for training* time interaction indicates if the change in the parameter was different between the SIT and MICT training modes.

Pancreatic fat content: At baseline, the pancreatic fat content was significantly higher in the prediabetic/T2D compared with the normoglycemic group. After two weeks of training both SIT and MICT significantly reduced the pancreatic fat content in both normoglycemic and prediabetic/T2D groups ($p = 0.036$, Time) (Figure 20). We observed that some males in both the normoglycemic and prediabetic/T2D groups had a pancreatic fat content higher than the normal pancreatic fat cut-off point 6.2% (Singh et al., 2017a). Thus, the participants were then divided into high (above 6.2%) and low (below 6.2%) groups according to their pancreatic fat content. In this comparison, both SIT and MICT reduced the fat content only in the high fat group ($p = 0.001$, PFC*time) (Figure 20).

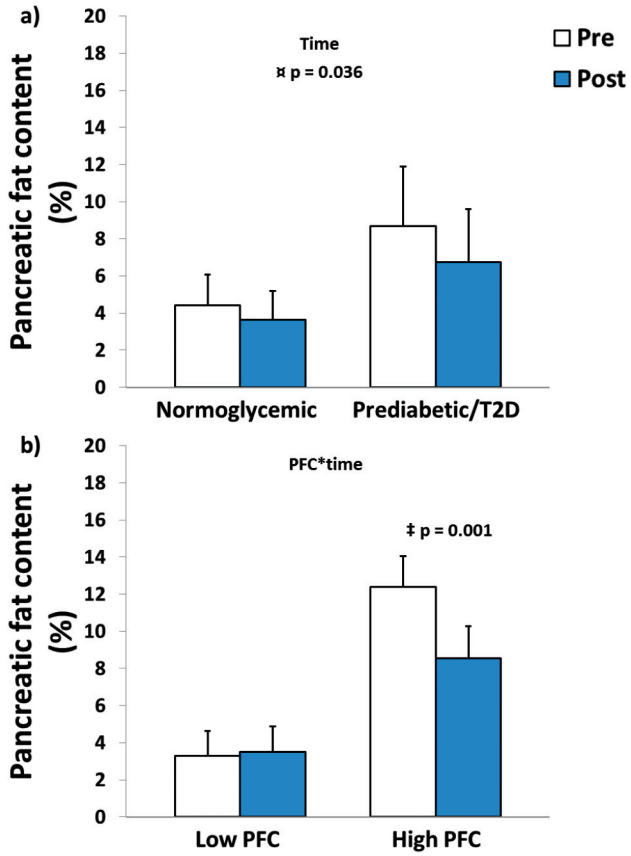

Figure 20: Effects of exercise on pancreatic fat content in normoglycemic prediabetic/T2D, low PFC (n = 20) and high PFC (n = 14) groups. White bars (before the exercise intervention), filled bars (after the exercise intervention). All values are model based means with error bars representing [95 % confidence intervals]. a) Pancreatic fat content in normoglycemic and prediabetic/T2D groups. ¤ p value (Time) indicating exercise reduced the pancreatic fat content in both the normoglycemic and prediabetic/T2D groups. b) Pancreatic fat content in low PFC and high PFC groups. ‡ p value (PFC*time) indicating that the change in the pancreatic fat content was significantly different between the high and low fat groups, with a significant reduction in the high PFC group. T2D, type 2 diabetes; PFC, pancreatic fat content.

Pancreatic substrate uptake: There was no significant difference between the pancreatic GU and FAU at baseline between normoglycemic and prediabetic/T2D groups and no change was observed after two weeks of training.

β-cell parameters: Almost all the β cell sensitivity markers were statistically different at baseline between the normoglycemic and prediabetic/T2D men

except for rate sensitivity (Table 12). After training: ISR_{basal} was significantly reduced in the prediabetic/T2D group, ISR_{early} improved significantly in the normoglycemic group. When the ISR_{early} was normalized to glucose concentrations (ΔISR0-30/ΔG0-30) it decreased similarly in both healthy normoglycemic and prediabetic/T2D groups (Table 12). The rate sensitivity decreased similarly in both normoglycemic and prediabetic/T2D men. Finally, there was a tendency for the potentiation factor to increase after training (Table 12).

Table 12: Effects of exercise on pancreatic β cell function. Table modified from study II,

Parameter	Normoglycemic		Prediabetic/T2D		p value		
	Pre	Post	Pre	Post	Baseline	Time	Dia*time
Beta cell function							
ISR_{basal} (pmol·min^{-1}·m^{-2})	81 [69, 94]	89 [76, 102]	152 [136, 169]	139 [122, 157]	**<0.001**	0.54	**0.006**
ISR_{early} (nmol·m^{-2})§	7.5 [6.4, 8.7]	9.1 [7.7, 10.7]	9.1 [7.4, 11.1]	8.5 [6.9, 10.6]	0.15	0.23	**0.028**
$\Delta ISR_{0-30}/\Delta G_{0-30}$ (nmol·m^{-2}/mmol·L^{-1})§	0.16 [0.13, 0.19]	0.12 [0.10, 0.15]	0.08 [0.06, 0.10]	0.07 [0.05, 0.09]	**<0.001**	**0.010**	0.71
ISR_{late} (nmol·m^{-2})	32 [28, 36]	32 [28, 36]	41 [36, 46]	41 [36, 47]	**0.005**	0.98	0.85
ISR_{total} (nmol·m^{-2})	40 [36, 45]	42 [37, 46]	50 [45, 56]	50 [44, 56]	**0.008**	0.75	0.65
Glucose sensitivity (pmol·min^{-1}·m^{-2}·[mmol/L]$^{-1}$)	114 [94, 133]	114 [94, 133]	61 [35, 86]	58 [31, 84]	**0.001**	0.81	0.81
Rate sensitivity (pmol·m^{-2}·[mmol/L]$^{-1}$)	1043 [836, 1250]	842 [620, 1065]	726 [453, 1000]	452 [156, 748]	0.12	**0.013**	0.69
Potentiation factor ratio&	2.0 [1.7, 2.4]	1.9 [1.6, 2.3]	1.3 [1.0, 1.7]	1.7 [1.3, 2.2]	**0.010**	0.29	0.086

All values are model based means [95% confidence intervals]. T2D, type 2 diabetes; ISR, Insulin secretion rate. (§) Log transformation and (&) square root transformation was performed to achieve normal distribution. P-value for baseline indicates the differences between the normoglycemic and prediabetic/T2D groups. The p-value for time indicates the change between pre- and post-measurements in the whole study group. The p-value for dia* time interaction indicates if the change in the parameter was different between the normoglycemic and prediabetic/T2D groups.

Correlations: Pancreatic fat content correlated positively with BMI [(pre) r = 0.42, p = 0.012], whole-body fat % [(pre) r = 0.45, p = 0.007], visceral fat mass [(pre) r = 0.59. p = <.001], ISR_{basal} [(pre) r = 0.41, p = 0.015] and ISR_{total} [(pre) r = 0.42, p = 0.014].

5.4 Effect of SIT and MICT on hepatic fat content, hepatic substrate uptake and lipoprotein subclasses (study III)

Basic characteristics: The basic characteristics of the subjects for this study are similar to those explained earlier in Study II.

Lipoprotein subclasses: Similar to the basic characteristics, there were significant differences in both lipoprotein subclass distribution (VLDL, IDL, LDL, and HDL) and their composition (total lipids, phospholipids, cholesterol, cholesterol esters, free cholesterol and triglycerides) at baseline between the normoglycemic and prediabetic/T2D groups (Appendix table 1). Both SIT and MICT improved all components of the extra-large HDL except triglycerides and reduced various sub components of IDL, LDL and small HDL subclasses (Figure 21). There was no significant change in the VLDL sub class or its composition.

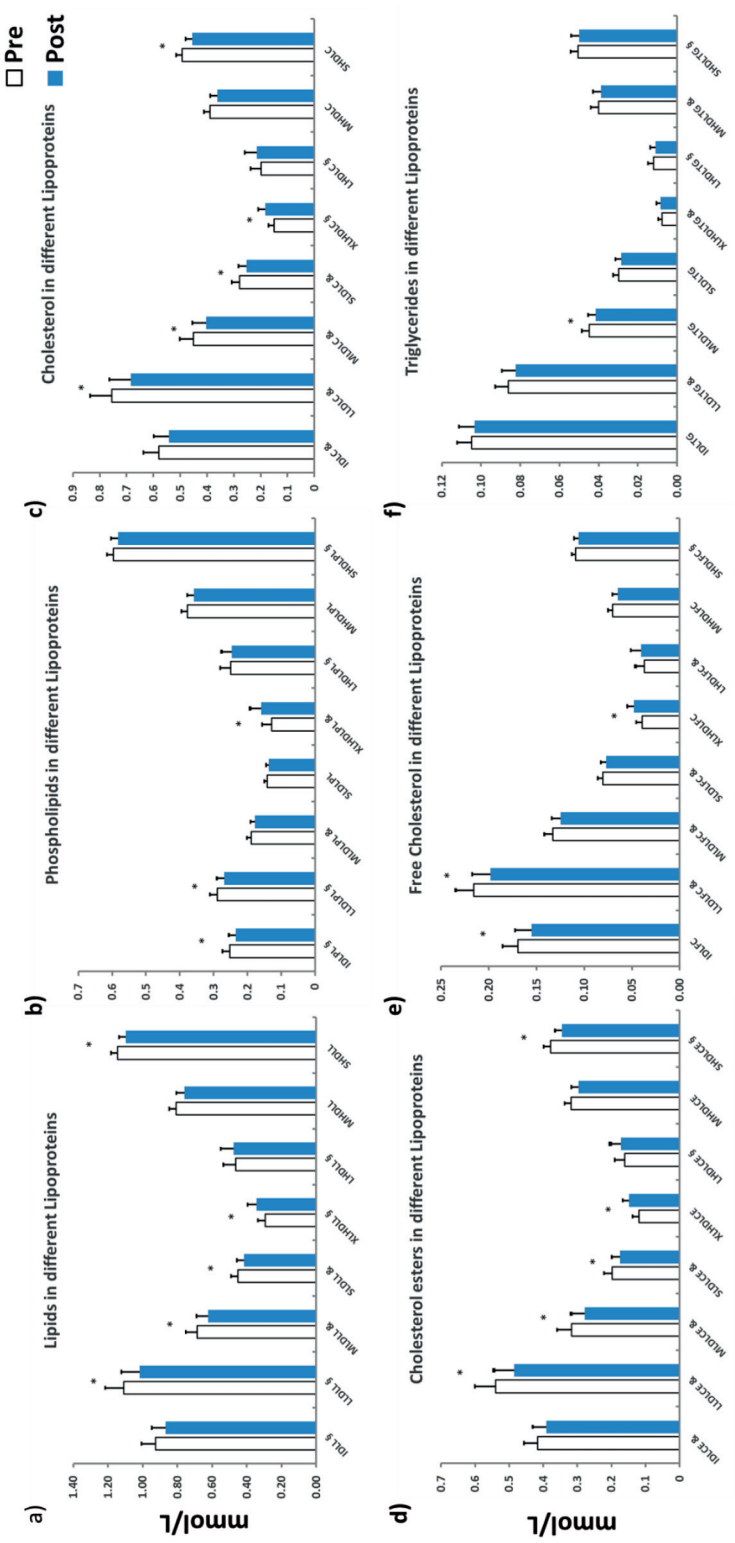

Figure 21: Effects of exercise on different lipoprotein subclasses and composition in the whole study group (normoglycemic + prediabetic/T2D).White bars (before the exercise intervention), filled bars (after the exercise intervention). All values are expressed as model-based means and bars are confidence intervals [95% CI]. a) Lipids, b) Phospholipids, c) Cholesterol, d) Cholesterol esters, e) Free cholesterol and f) Triglycerides. Intermediate density lipoprotein (IDL); LLDL (large low density lipoprotein); MLDL (medium low density lipoprotein); SLDL (small low density lipoprotein); XLHDL (extra-large high density lipoprotein); LHDL (large high density lipoprotein); MHDL (medium high density lipoprotein) and SHDL (small high density lipoprotein). (§) Log transformation and (&) square root transformation was performed to achieve normal distribution. *p < 0.05 value for time interaction (i.e. the change in the parameter was similar between the normoglycemic and prediabetic/T2D groups).

Hepatic fat content: Hepatic fat content was significantly higher in the prediabetic/T2D compared to the normoglycemic group at baseline ($p < .0001$). After the intervention, the reduction in hepatic fat content was significantly different between the normoglycemic and prediabetic/T2D groups ($p = 0.03$, Dia*time), with a tendency to reduce the hepatic fat content in the prediabetic/T2D ($p = 0.051$). Similar to the Study II, in Study III, during the analysis, we noticed that there were some normoglycemic subjects who had high hepatic fat content (HFC > 5.6%) and some prediabetic/T2D subjects who had low hepatic fat content (LFC < 5.6%) based on the cut-off point of 5.6% recommended for normal hepatic fat content (Szczepaniak et al., 2005). Consequently, all the subjects were pooled together (men + women) and were divided into HFC and LFC groups. At baseline the HFC group had 522 % more hepatic fat compared to the LFC group. After the intervention, the reduction in hepatic fat was different between the HFC and LFC groups ($p = 0.01$, Liverfat*time), with hepatic fat only being significantly reduced in the HFC group -13 % ($p = 0.009$) (Figure 22).

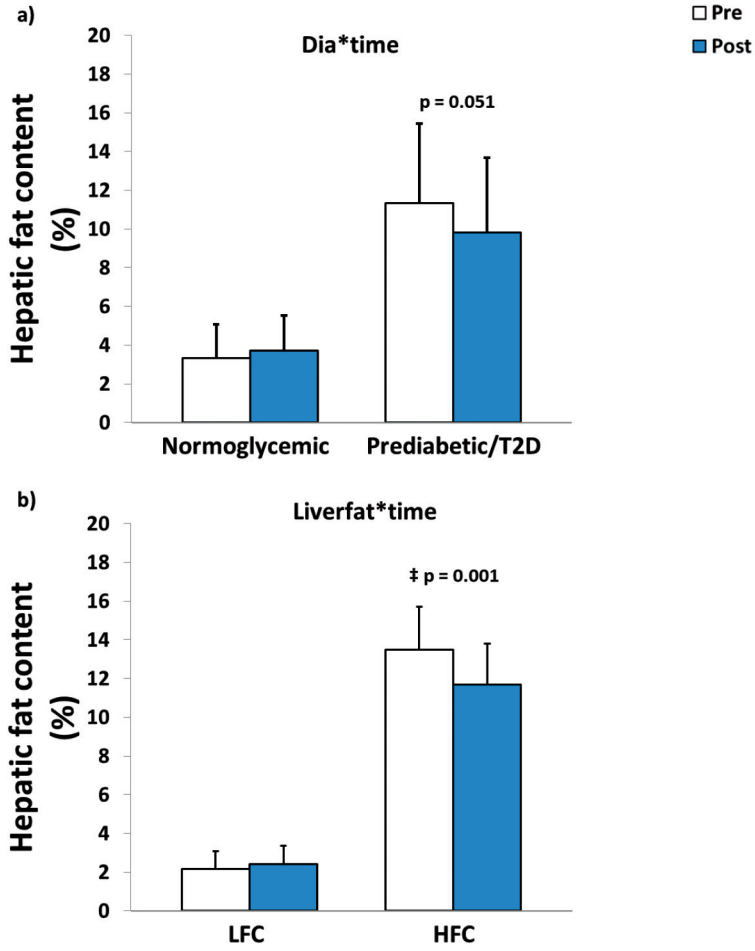

Figure 22: Effects of exercise on hepatic fat content in normoglycemic, prediabetic/T2D, LFC and HFC groups. White bars (before the exercise intervention), filled bars (after the exercise intervention). All values are model based means with error bars representing [95 % confidence intervals]. a) Hepatic fat content in normoglycemic and prediabetic/T2D groups. p value (Dia*time) indicating that the change in the hepatic fat content was different between normoglycemic and prediabetic/T2D, with a tendency to reduce the hepatic fat content in the prediabetic/T2D group. b) Hepatic fat content in LFC and HFC groups. ‡ p value (Liverfat*time) indicating that the change in the hepatic fat content was significantly different between the high and low fat groups, with a significant reduction in the HFC group. T2D, type 2 diabetes; HFC, high hepatic fat content and LFC, low hepatic fat content.

Hepatic substrate uptake: There was no difference in hepatic GU and FAU between the normoglycemic and prediabetic/T2D. Training induced no change in

either the hepatic GU or FAU. However, as mentioned earlier when we analyzed the subjects according to their hepatic fat content (HFC and LFC), we observed that MICT significantly improved the hepatic GU and tended to reduce the hepatic FAU in all subjects (HFC + LFC) (Figure 23). There was no difference in EGP in either the normoglycemic and prediabetic/T2D or HFC and LFC groups.

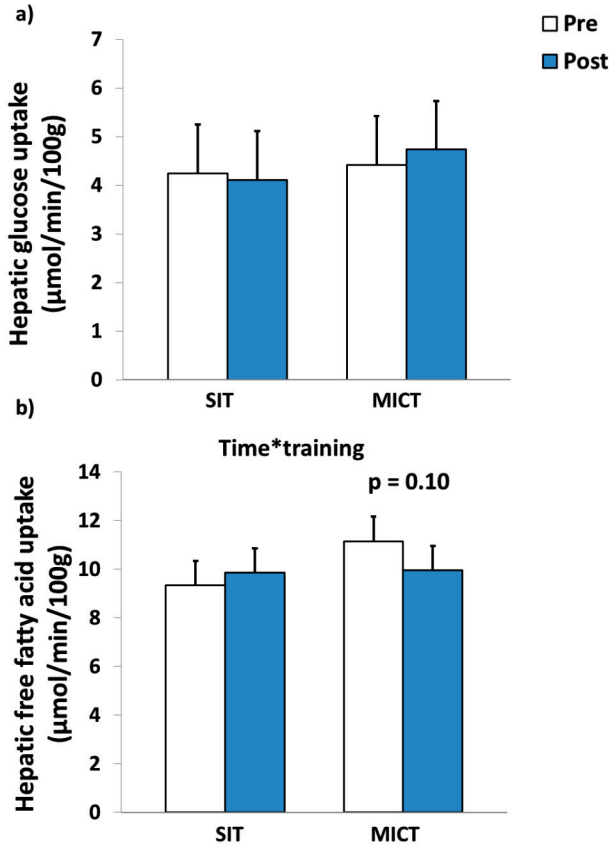

Figure 23: Effects of exercise on insulin-stimulated hepatic GU and FFA. White bars (before the exercise intervention), filled bars (after the exercise intervention). All values are model based means with error bars representing [95 % confidence intervals]. a) insulin-stimulated hepatic glucose uptake. ∫ p value (time*training) indicating that the change in the hepatic glucose uptake was significantly different between SIT and MICT groups, with a significant improvement in the MICT group. b) Fasting hepatic free fatty acid uptake. p value (time*training) indicating the change in hepatic free fatty acid uptake to be different between the SIT and MICT groups, with a tendency to reduce the free fatty acid uptake after MICT. SIT, sprint interval training: MICT, moderate intensity continuous training.

Liver enzymes: At baseline, ALAT, ASAT and GT were significantly higher in the prediabetic/T2D group compared to the healthy (normoglycemic) group. After training, there was a significant reduction in ALAT, ASAT and GT levels without any differences in the training modes (Table 13).

Table 13: Effects of exercise on the liver enzymatic profile (study III)**.**

Parameter	Normoglycemic		Prediabetic/T2D		p value		
	Pre	Post	Pre	Post	Baseline	Time	Training*time
Liver enzyme profile							
ALAT§ (U/L)	27.1 [23.1, 31.9]	23.3 [19.7, 27.6]	42.2 [34.0, 52.3]	34.5 [27.5, 43.2]	**<0.001**	**0.001**	0.27
ASAT§ (U/L)	26.0 [23.2, 29.1]	22.7 [20.1, 25.7]	31.8 [27.3, 37.0]	25.3 [21.4, 29.8]	**0.047**	**0.003**	0.23
GT§ (U/L)	24.0 [19.0, 30.3]	19.0 [15.0, 24.1]	47.7 [35.0, 65.0]	36.2 [26.3, 49.7]	**<0.001**	**<0.001**	0.59

All values are model based means [95% confidence intervals]. T2D, type 2 diabetes; ALAT, alanine transaminase; ASAT, aspartate transaminase; GT, gamma-glutamyltranspeptidase. (§) Log transformation was performed to achieve normal distribution. p-value for baseline indicates the differences between the normoglycemic and prediabetic/T2D groups. The p-value for time indicates the change between pre- and post-measurements in the whole study group. The p-value for training* time interaction indicates if the change in the parameter was different between SIT and MICT.

Correlations: At baseline, the hepatic fat content [(pre): $r = -0.67$, $p < 0.001$] and ALAT [(pre): $r = -0.33$, $p = 0.02$; (post): $r = -0.38$, $p = 0.02$] correlated negatively with the whole-body insulin sensitivity (M-value). Of the lipoproteins, extra-large HDL components correlated positively with whole-body insulin sensitivity (M-value) and negatively with the liver volume and ALAT, and small HDL components correlated negatively with LFC, aerobic capacity and whole-body insulin sensitivity (Appendix table 2).

6 DISCUSSION

The main findings for this thesis were:

- Improvements in the intestinal glucose and free fatty acid uptake in response to exercise depend on exercise volume rather than intensity. Both intestinal glucose and free fatty acid uptake improved after MICT training compared to SIT. These improvements in substrate uptake were similar in healthy normoglycemic as well as in subjects with prediabetic/T2D. Proposing that MICT might be more beneficial in improving intestinal substrate uptake.

- Exercise-induced reduction in hepatic and pancreatic fat content was independent of exercise volume or intensity. This reduction in fat content was also observed for whole-body fat percentage, subcutaneous and visceral fat masses. Therefore, these results suggest that both SIT and MICT are equally effective in reducing the ectopic and whole-body fat content.

- Exercise training leads to marked improvements in lipoprotein profile and composition. Moreover, improvements in the lipoprotein profile and composition were associated with protection against diabetes. This suggest that exercise volume and intensity seem to have a similar impact on the improvements in lipoprotein profile.

- Exercise-induced increase in hepatic glucose uptake was only seen after MICT and not SIT. Proposing again that MICT might be more beneficial for subjects with impaired hepatic metabolism.

- Exercise training altered the gut microbiome and reduced the endotoxaemia associated with the gut microbiome. In addition, the changes in the gut microbiome were associated with protection against obesity. The changes in the gut microbiome were also independent of exercise intensity and volume.

6.1 Exercise volume plays a key role in modulating substrate uptake in the intestines and the liver

One of the most important findings of this thesis was that exercise volume plays an important role in modulating the substrate uptake in both the intestine and the liver. Two weeks of MICT increased the insulin-stimulated intestinal and

hepatic GU and reduced the fasting intestinal and hepatic FAU. There was no change in the pancreatic substrate uptake in response to both SIT and MICT.

Previous studies have shown that insulin stimulates intestinal GU in both the duodenum and the jejunum of normal weight individuals (Honka et al., 2013; Makinen et al., 2015) and that this stimulation is blunted in obese subjects suggesting that the intestine is in fact an insulin sensitive organ. This intestinal insulin-resistance detected in obese individuals is ameliorated after bariatric surgery and rapid weight loss (Makinen et al., 2015). In the present study, GU tended to increase in the jejunum and increased significantly in colon of healthy normoglycemic subjects after two weeks of MICT. However, no significant change was found in any of the intestinal segments in the prediabetic/T2D groups. The tendency to improve the jejunal GU may be explained by the increased translocation of Glut2 to the cell membrane in the jejunal cells after exercise training. Exercise training increases the translocation of Glut4 to the cell membranes in muscles via increasing phosphorylation of AS160 (Castorena et al., 2014). Based on this, similar adaptations may have been induced in the jejunum after exercise training by increasing the phosphorylation of protein kinase C (responsible for translocating intestinal Glut2 from the brush border membrane) leading to the improved jejunal GU. Moreover, Glut2 levels were also measured in the intestines of healthy rats after SIT and MICT interventions. Both SIT and MICT increased the Glut2 levels in rats after the intervention. However, the improvement in the jejunal GU in humans was only seen in response to MICT and not in the SIT group, suggesting that even though two weeks of SIT was enough to improve the Glut2 expression in rats, SIT might need a longer time to induce similar improvements in intestinal GU in humans.

The colon has been suggested to play a role in glucose disposal (Bahler et al., 2016a). This hypothesis is supported by the fact that subjects with ileostomy display several characteristics of insulin resistance as compared to healthy subjects (Robertson et al., 2000). Additionally, metformin administration induces an increase in the colonic GU contributing to its glucose lowering effect (Bahler et al., 2017). Moreover, increased colonic GU has also been suggested to associate with weight loss or the weight maintaining effect induced by metformin in subjects with T2DM (Bahler et al., 2016a). Therefore, changes in the colonic GU also have an important role in glucose homeostasis. In our study, colonic GU improved only in the healthy normoglycemic subjects after MICT only and not in the prediabetic/T2D subjects. One of the possible explanations for this discrepancy can be the improvement in the whole-body insulin sensitivity in the healthy normoglycemic group (Table 8) and not in the prediabetic/T2D group (Table 10). Interestingly, in this thesis the insulin-stimulated colonic GU correlated positively with whole-body insulin sensitivity (Figure 14).

Both SIT and MICT reduced the duodenal FAU in healthy normoglycemic subjects while MICT also significantly reduced the jejunal FAU in prediabetic/T2D subjects. The reduction in the intestinal FAU can be due to the improvement in the fatty acid oxidation after exercise training. Recently, Hung and co-workers have shown that endurance training improves fatty acid oxidation and turnover in the intestinal cells of rodents (Hung et al., 2015). Our results regarding the reduction in duodenal FAU of the healthy normoglycemic subjects agrees with the study by Hung et al (Hung et al., 2015). However, the results do not explain the observed reduction in the jejunal FAU only after MICT in prediabetic/T2D subjects. One of the possible explanations for the observed difference in the prediabetic/T2D group may be the observed differences in the supply of plasma FFA to the intestine. In the prediabetic/T2D group there was a small but non-significant reduction in the plasma FFA levels only after MICT -11% (Table 10) and not after SIT.

Another important finding in the present study was the improvement in hepatic GU after MICT. The improvement in hepatic GU also agrees with findings in the intestine, where only MICT improved the colonic GU in healthy normoglycemic subjects but not SIT. The observed difference between the training modes may be explained by the difference in the plasma FFA levels. Previously, it has been shown that the plasma FFA levels correlate negatively with the hepatic GU (Iozzo et al., 2004a) and that an increase in plasma FFA levels impair the hepatic GU (Iozzo et al., 2004a). This is because plasma FFA has an allosteric inhibitory effect on the glucokinase enzyme (the enzyme responsible for phosphorylation of glucose once inside the cell), which results in less trapping of glucose inside the liver cells (because of less phosphorylation) leading to lower hepatic GU (Iozzo et al., 2004a). Interestingly, in the present study the plasma FFA levels were only reduced in the MICT group and the change in the plasma FFA levels correlated inversely with the hepatic GU again only in the MICT group [$r = -0.60$, $p = 0.01$].

In addition to regulating glucose metabolism, the liver also has a central role in regulating the whole-body FFA metabolism. It has been shown that each ml of the liver can utilize almost 50 times more FFA compared to 1 gram of muscle (Iozzo et al., 2003c). Consequently, even a small change in the hepatic FAU warrants attention. In the present study, there was a small but non-significant decrease in the hepatic FAU ($p = 0.10$) after MICT. The reduction in the hepatic FAU occurring only after MICT can be explained by the higher reduction in the fasting plasma FFA levels in the MICT group, which may be due to higher energy expenditure and training volume compared to SIT (Eskelinen et al., 2015). This finding also agrees with a previous study, where the reduction in the plasma FFA supply lead to a lower hepatic FAU uptake (Rigazio et al., 2008).

Thus, according to our results it seems that the short-term exercise volume has a role in improving both hepatic insulin-stimulated GU and fasting FAU.

6.2 Both SIT and MICT reduced the hepatic and pancreatic fat content

The reduction in both the hepatic and pancreatic fat content after just six training sessions is one of the key clinical findings of this study. The ectopic fat accumulation in the liver and pancreas is associated with an increased risk of acquiring (T2D, Cardiovascular disease, metabolic syndrome and hypertension) (Singh et al., 2017a; Angulo and Lindor, 2002).

Effects of exercise on hepatic fat content have been studied extensively compared to the effects of exercise on PFC. The results regarding the reduction in PFC are of particular interest as they demonstrate for the first time how two different modes of exercise can efficiently reduce the PFC in subjects with high PFC and prediabetic/T2D groups. This is important as previously it has been thought that weight loss induced decrease in PFC leads to normalization of glucose metabolism (Steven et al., 2016). In the current study, there were no differences at baseline or after the training intervention in either the insulin-stimulated pancreatic GU or FAU. Therefore, substrate uptake does not explain the observed reduction in the PFC. However, we did not measure the fatty acid postprandially (fat accumulation might occur during the postprandial period), possibly explaining why there was no association with substrate uptake and PFC in the current study. Another explanation can be that exercise improves the disposal and oxidation of FFA in the pancreatic cells thereby reducing the PFC. This hypothesis has not been tested and warrants further studies.

The pathogenesis of fatty liver is multifactorial and is mainly caused by an imbalance in the FFA influx to the liver. This increased influx in FFA is caused by alterations in the adipose tissue lipolysis, de novo lipogenesis, diet and disposal of FFA via VLDL secretion or beta oxidation (Nassir et al., 2015; Donnelly et al., 2005). All these imbalances are attributed to multi-organ insulin resistance (i.e. adipose tissue, muscle and the liver) (Petersen et al., 2007). Exercise training has been shown to ameliorate fatty liver by increasing the whole-body insulin sensitivity, reducing adipose tissue lipolysis as well as increasing fatty acid disposal and oxidation (Cuthbertson et al., 2016; Rabol et al., 2011). In our study, both SIT and MICT improved the whole-body insulin sensitivity and reduced plasma FFA levels (all time $p < 0.05$) (Table 11). Another explanation for the reduction in fatty liver could be the improvement in the fatty acid disposal and oxidation in the mitochondria of the liver cells. Accumulative

evidence suggests that exercise induced mitochondrial adaptations also play a key role in the amelioration of fatty liver (Sunny et al., 2017). However, this was not investigated in the current study and further studies are needed to determine whether there is a dose-response relationship (i.e. exercise intensity vs. exercise volume) for improving hepatic fat content via the changes in mitochondria.

Thus, in the short-term both the hepatic fat content and PFC were reduced irrespective of the exercise intensity or volume.

6.3 Both SIT and MICT had a similar effect on β cell function

In the current study, there were significant differences in the glucose sensitivity and potentiation factor between the healthy normoglycemic and prediabetic/T2D groups (Table 12). Both SIT and MICT decreased ISR_{basal} in the prediabetic/T2D groups and improved ISR_{early} in the healthy normoglycemic group (Table 12). The improvement in both ISR_{early} and ISR_{basal} may be attributed to the improvement in the whole-body insulin sensitivity after SIT and MICT. The increase in the ISR_{early} in the healthy normoglycemic subjects is likely explained by the increase in the muscle insulin sensitivity while the decrease in the ISR_{basal} in the prediabetic/T2D may be due to a compensatory response to maintaining or decreasing insulin secretion because of the improved insulin sensitivity. Interestingly, when the ISR_{early} was normalized to glucose concentrations ($\Delta ISR0\text{-}30/\Delta G0\text{-}30$) it decreased similarly in both healthy normoglycemic and prediabetic/T2D groups (Table 12). Moreover, there was a tendency to decrease ISR_{early} when it was normalized to the potentiation ratio in the prediabetic/T2D towards the healthy normoglycemic group (Table 12). This suggests that exercise training has the potential to improve the ability of β cells to detect potentiating signals, such as incretins and neural signals. Finally, rate sensitivity decreased similarly after SIT and MICT, probably because of the improvements in the whole-body insulin sensitivity.

To conclude, in the short-term both exercise intensity and volume seem to have a similar effect on β cell function.

6.4 Both SIT and MICT improve the liver enzyme profile and the lipoprotein profile

Regarding the liver enzymes, serum ALAT, ASAT and GT were higher in the prediabetic/T2D group compared to normoglycemic groups at baseline (Table

13). Training efficiently reduced the levels of all enzymes independently of the glycaemic status or training modes. Of the liver enzymes, an increase in serum ALAT has been shown to have the strongest association with hepatic fat accumulation and the development of non-alcoholic fatty liver disease (Kim et al., 2008). Moreover, ALAT has been suggested to be more closely associated to liver insulin resistance than either ASAT or GT (Chang et al., 2007). In the present study, serum ALAT correlated negatively with whole-body insulin sensitivity (Pre r= -0.33, p= 0.02; Post r= -0.38, p=0.02).

At baseline, the prediabetic/T2D group had significant impairments in the lipoprotein subclasses (VLDL, IDL, LDL and HDL) and the compositions of lipids, phospholipids, cholesterol, cholesterol esters, free cholesterol and triglycerides in them compared to healthy normoglycemic group (Appendix table 1). After training there was significant improvement in the IDL, LDL and HDL subclasses and compositions except for the VLDL subclasses. This is because VLDL has a faster turnover rate compared to IDL, LDL and HDL subclasses and therefore additional exercise sessions or longer intervention might be required to induce significant change in it (Magkos et al., 2006). Both SIT and MICT decreased all the IDL components (except triglyceride) and LDL components (Figure 21) without any differences between the healthy normoglycemic and prediabetic/T2D groups. The reduction in the LDL subclasses and components, particularly the small LDL is vital because it has been shown to be associated with the risk of acquiring diabetes (Mora et al., 2010). In HDL subclasses there was an increase in the large HDL and a decrease in the small HDL subclass components (Figure 21). This disparity among the HDL subclass is also worth mentioning as it has been shown that large HDL have a protective effect against acquiring diabetes while small HDL increases the risk of acquiring diabetes (Mora et al., 2010). Moreover, Garvey et al. have also shown an association with the progression of insulin resistance and VLDL, LDL and small HDL (Garvey et al., 2003).

In conclusion, regardless of the intensity and volume exercise has beneficial cardiovascular effects because both SIT and MICT improved the lipoprotein profile in subjects with normal and impaired lipoprotein profile. Moreover, both training modes also protected against diabetes by increasing the lipoprotein subclasses associated with prevention against diabetes and reducing the subclasses associated with the risk of acquiring diabetes.

6.5 Both SIT and MICT improve the gut microbiota profile and reduce endotoxaemia

The current study is one of the first studies to investigate the effects of two different exercise training modes on gut microbiome. Previous cross-sectional studies have suggested a link between physical activity and gut microbiome (Virtanen et al., 2002; Estaki et al., 2016; Clarke et al., 2014b). In the present study, both SIT and MICT reduced the Firmicutes/Bacteroidetes ratio (Figure 18). This reduction in the ratio is important as it has been shown that in obesity it is elevated (Murphy et al., 2010; Turnbaugh et al., 2006; Ley et al., 2005). Previous studies have also shown that the ratio improves after a dietary intervention and that it correlates with a loss of body weight (Ley et al., 2006). However, there are also studies showing that there were no difference in the Firmicutes/Bacteroidetes ratio between the lean and obese humans at baseline and after weight loss (Zhang et al., 2009; Duncan et al., 2008). The observed differences are most likely due to different environmental influences (diet, physical activity, socioeconomic status) (Dugas et al., 2016). In the current study, the reduction in the ratio was mainly due to the significant increase in the relative abundance of Bacteroidetes (Figure 18). Bacteroidetes has also been known to perform essential metabolic conversions (Rajilic-Stojanovic and de Vos, 2014), which may influence host metabolism. In addition to protecting against obesity, Bacteroidetes have also been suggested to have a role in inflammatory bowel diseases (IBD). It has been shown that in subjects with IBD the levels of Bacteroidetes are low (Rajilic-Stojanovic et al., 2011).

Both training modes also reduced the relative abundance of *Clostridium* (Figure 18) and tended to reduce the abundance of the *Blautia* (Figure 18) genus. *Clostridium* has been shown to play an essential role in the whole-body immune responses (Tuovinen et al., 2013) while *Blautia* has been shown to be one of the most abundant genus in subjects with prediabetes and T2D compared to healthy subjects (Egshatyan et al., 2016). Furthermore, *Blautia* has also been proposed to increase the release of proinflammatory cytokines (TNFα) (Tuovinen et al., 2013). Interestingly, in the current study even though there was a tendency to reduce the relative abundance of *Blautia* in response to exercise, there was a significant reduction in the plasma TNFα levels (Figure 17). Moreover, gut microbiota associated significantly with insulin-stimulated colonic GU (Figure 19), suggesting that changes in the intestinal substrate uptake might have an impact on the gut microbiota composition.

Both training modes also reduced the endotoxaemia as shown by the reduced serum LPS-binding protein LBP. Normally, gut microbiome releases a small amount of LPS which is essential for the host immune response

development. However, when LPS is released in substantial amounts it induces a pathophysiological reaction in various organs such as the adipose tissue (inflammation and insulin resistance), the endothelium (plaque formation and rupture), the liver (steatohepatitis) and in some cases causes irreversible shock (Manco et al., 2010a). Because measuring LPS has significant technical difficulties (Manco et al., 2010b), LBP (endogenous protein) is used as an alternative clinical marker for endotoxaemia (Gonzalez-Quintela et al., 2013; Albillos et al., 2003). LBP enhances the binding of LPS and the "Cluster of Differentiation 14" (CD14) (Tobias and Ulevitch, 1993). This LPS-CD14 translocates into the cell nucleus and initiates the inflammatory cascade response (Nassir et al., 2015). In the present study, both training modes significantly reduced the LBP levels (Figure 17). The reduction in the LBP levels can be due to the changes in the gut microbiota profile. Previously, Cani et al. showed that a high fat diet increases the LBP levels by changing the gram-negative to gram-positive ratio (Cani et al., 2008b). Compared to Cani et al. in the current study, exercise training increased the relative abundance of Bacteroidetes thus leading to an improvement in the gram-negative to gram-positive ratio that may have contributed to the reduction in the plasma LBP levels. In addition, the increase in the relative abundance of Bacteroidetes can also lead to a reduction in the intestinal inflammation as Bacteroidetes have been shown to release anti-inflammatory cytokine (IL-10) (Mazmanian et al., 2008).

In conclusion, the results of the present study suggest that the changes in the gut microbiota profile and endotoxaemia are independent of the exercise intensity or volume.

6.6 Which one is superior (SIT or MICT) and future direction

The increasing prevalence of lifestyle-induced diseases in the general population is alarming. Even though exercise training is a well established preventive measure against these lifestyle-induced diseases the prevalence of physical inactivity in the general population is worrying. The goal of exercise interventions should be to encourage healthier lifestyles and at the same time increase acceptability among the general population. The results of the current thesis highlight the importance of exercise intensity and volume in improving various parameters of the body. Just as medical prescriptions are individualized so as to get the most optimal benefits for each individual. Exercise prescriptions should also be individualized and based on the desired outcomes, this might also help in increasing the levels of physical activity in the general population.

Many studies have shown that SIT has superior benefits to MICT in improving cardiovascular health. The results of the current thesis indicate that both SIT and MICT bring about similar changes in lipoprotein profile, gut microbiome and whole-body health. Although it appears that at least in short-term MICT is superior in improving the intestinal and hepatic substrate uptake. Combination of both SIT and MICT might be more beneficial rather than having just one exercise mode. This could reduce the overall exercise duration and at the same time lessen the intensity of the exercise regimen. Moreover, as interval training can performed via different training protocols (Table 2). Future studies should concentrate on combining MICT with these different protocols in order to achieve the most optimal health benefits. This might lead to an exercise regimen which was more motivating and acceptable to the general population and thus encourage a healthier lifestyle.

All things considered, understanding the importance of exercise intensity and volume in achieving desired outcomes will provide new insights on how exercise can be used as an effective tool in health management.

6.7 Strength and limitations

The major strength of this thesis is the use of state-of-the art imaging facilities (positron emission tomography, magnetic resonance spectroscopy) and (nuclear magnetic resonance and 16S rRNA gene V3-V4 region sequencing (MiSeq Illumina) modalities to measure substrate uptake, fat content lipoproteins and gut microbiome in the study. The present thesis is novel in that it is one of the first studies where the effects of two different exercise regimens have been studied so extensively in various internal organs of the body in both healthy normoglycemic and prediabetic/T2D subjects. It is also among the first studies to investigate the effects of two different exercise training modes on pancreatic fat content, intestinal substrate uptake, gut microbiome and endotoxaemia. Moreover, by combining the results from whole-body parameters with organ specific parameters it provides novel data on how SIT and MICT differ in improving the whole-body metabolism.

One of the major limitations is the low number of subjects included in the study. While we had sufficient subjects to compare the effects of exercise on healthy normoglycemic and prediabetic/T2D subjects but we did not have significant number of subjects to compare the sex-related differences. Moreover, we had both males and females in the prediabetic/T2D group but no females in the healthy normoglycemic group. Ideally, both groups should have an age and sex-matched distribution. Initially, the purpose of the study was to compare the

effects of training only in healthy normoglycemic males and prediabetic/T2D men. However, due to recruitment problems females were included in the prediabetic/T2D group. Thus, there are slightly different comparisons between different study populations (Study II and III), which makes the interpretations of results a little challenging. Another limitation was the uneven distribution of prediabetic and T2D in the SIT and MICT groups. We addressed this issue by taking disease status (prediabetic or T2D) as a confounding factor in our statistical analyses to minimize the effect. Moreover, some of the subjects in the prediabetic/T2D group were on different oral hypoglycemic drugs which might have affected our results. For example, metformin has been shown to alter the gut microbiota profile (Forslund et al., 2015) and increase colonic glucose uptake (Bahler et al., 2016b), and therefore it is possible that metformin might have affected the training responses. We circumvented this issue by taking the medication status as a covariate in our analysis, again to minimize the effects of medication on the results.

Not all the PET studies could be completed in this study, especially the FTHA-PET, and this led to missing data for many of the subjects. This was mainly due to the technical difficulties in the production of the [^{18}F]FTHA radiotracer.

Another limitation is the length of the exercise intervention. Even though using a similar SIT protocol has been shown to be an affective regimen (Burgomaster et al., 2008; Gibala et al., 2006), additional time might be required to induce similar benefits in the organs studied here. However, it should be noted that SIT is an extremely intensive work-out and that increasing the duration of SIT might result in an increasing drop-out which would increase the overall length of the study.

To conclude, there is outstanding evidence that exercise training does have many health benefits on cardiovascular function, insulin resistance, weight loss, whole-body metabolism, aerobic capacity among others. However, exercise interventions should not be generalized and should be tailored individual according to goals and abilities of each person. They should include sufficient exercise intensity and volume to achieve the most optimal benefits for desired outcomes.

7 ACKNOWLEDGMENTS

I am extremely fortunate to be a part of HITPET project, it has changed my life in so many ways. I have found my passion in life as a researcher and have grown not only in professional but also my personal life. I am really thankful to all the people who have been a part of this family and have always supported and stood beside me throughout this years.

My warm thanks to all the participants without whom this study would have never been possible. Without your commitment to the study we would have not been able to get such interesting results. Director of Turku PET Centre Professor Juhani Knuuti MD, PhD for providing state of the art research facilities and relaxed working environment.

This study was financially supported by the Academy of Finland, European Foundation for the Study of Diabetes, Finnish Cultural Foundation, Varsinais-Suomi Regional Fund, Juho Vainio Foundation, Emil Aaltonen Foundation, Hospital District of Southwest Finland, Orion Research Foundation, Finnish Diabetes Foundation, the Ministry of Education of the State of Finland, Paavo Nurmi Foundation, Novo Nordisk Foundation, Paulo Foundation and Centre of Excellence funding. I would like to thank all the funding organizations and hope they will continue lending their support to research.

I would like to express my sincerest gratitude to my supervisors, Docent Jarna C. Hannukainen and Docent Kari K. Kalliokoski, for all their guidance, motivation and support during these last six years. Thank you, for giving me the opportunity to work with you it has been an immense pleasure. Especially, Jarna Hannukainen I am lucky to have the opportunity to have worked with such a wonderful and dedicated researcher (your limitless positive attitude towards science is truly inspiring). Your constant desire to think out of the box and be open to new ideas has inspired me to pursue my career in research. I have always appreciated our one on one meetings and your continuous feedback on how to improve my work. I have no words to describe of how much I respect you as my mentor. I hope I will have the opportunity to work with you in the future as well. Kari K. Kalliokoski, whom I always admired and look up to for guidance in difficult situations. I have always appreciated your approach and attitude towards research, it has helped me overcome the number of challenges I had to face during the years and motivated me to continue working even when it was truly hard to do so. I hope I can emulate your attitude towards research in my career.

I want to specially thank to all my work colleagues at the Turku PET Centre. I always felt blessed to be surrounded by such great minds. I had the privilege to work with such excellent co-aurthors and scientists: Pirjo Nuutila

(for always being the behind the scene motivator) Kirsi Virtanen MD, PHD (whose soft spoken words always motivated me), Tamiko Ishizu (for being the rock during the painstaking animal data analysis), Tove Grönross (for her valuable support in analyzing the animal data), Minna Yli-Karjanmaa (for completing the animal studies), Petri Elo (for sharing valuable insights during our little chit chats) Tiina Saanijoki (for your amazing baking skills which I will always remember and being a great friend over the years), Jussi Toivanen (for not losing hope with the animal data), Mika Teräs (for sharing his valuable experiences), Eliisa Löyttyniemi (for not losing hope in me learning statistics), Marja Heiskanen (for providing her excellent writing skills and support), Sanna Honkala (for always spreading the energetic skills she has to the group), Ronja Ojala (for sharing her positive energy), Joonas Eskelinen (for welcoming me in the group and sharing his friendship), Mikko Koivumäki (without you we would have been nowhere), Miikka Honka (for providing his valuable insights and being a great friend), Marco Bucci (for sharing his knowledge and rejuvenating my interest in board games), Mueez u Din (for all the interesting talks we had while working together), Harri Merisaari (for his sharing his interesting attitude towards research) Minna Lahesmaa (for those interesting lunches and positive attitude, Aida Kiviniemi, Heidi Immonen (both for having those small pep talks) and Henri Honka (for not only teaching me a lot but also for motivating me in pursuing my career as a researcher).

I am extremely thankful to Professor Anna Krook for accepting my invitation to be my opponent in the public defense. My reviewers Satu Pekkala, PhD and Nils Wierup, PhD for their constructive criticism in improving my book but also for meeting the close deadlines. I really appreciate all the work we did together and hope that I would have the pleasure of working with you in the future as well.

I also want to thank Sauli Pirola and Timo Laitinen for providing me constant support with handling Carimas and helping me resolve the issues with PET data. Rami and Marko for their IT support, especially Rami for resolving all the software issues I had over the years promptly. Kristiina Nuutila, Outi Irjala and Eeva Raino for guiding me through the graduate school and making the whole process smother.

My friends in Finland: Thomas Keller, Kamil, Suvi, Ania, Marjukka, Kalen Lin, Sina, Salla, Aleksi, Jenni, Eerik, Cheng-Bin Yim, Margad, Noemie Nowak-Foki and Jaakko. You guys are the reason why Finland felt home and I was able to survive the cold dark winters over the years. My old university friends back in Pakistan: Asjad, Hannan, Imran and Bushra without whom life would have not been that joyful during those hot summers.

Finally, I like to thank my parents Dr. Kishore Motiani and Dr. Bina Motiani for their continuous and invaluable support and guidance. It is because of their continuous guidance and reassurance that I have been able to achieve so much in my life. You have always been by my side and for that I have no words to express my gratitude. My uncle Karu Motiani and aunt Veena Motiani, you have always been the cornerstone to our family and without your blessing we would have not been able to achieve anything in life. My wives parents (Mohan Lal and Sushila Kumari) for their blessings and support. My siblings Priyanka and Sagar Motiani who have been an invaluable part of my life and making me so proud by finding their purpose in life. My son Pranay Motiani for bringing me overwhelming joy and gratitude of being a father. I hope I will be able to provide you the same guidance and support as my parents did for me so that you continue to pursue your life dreams and ambition. My wife Amrita Kumari for her constant support not only during the good times but also during the rough days when writing seemed impossible. It always gave me overwhelming comfort and joy knowing that you and Pranay are waiting at home for me at home. I am so grateful to be able to share this experience and life with you.

Turku, May 2019

Kumail K Motiani

8 REFERENCES

(2012). Structure, function and diversity of the healthy human microbiome. Nature *486*, 207-214.

(2015). (2) Classification and diagnosis of diabetes. Diabetes Care *38 Suppl*, S8-S16.

How does the liver work? InformedHealth.org [Internet].Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG); 2006- . 22-8-2016.

Ref Type: Online Source

Abate,N., Burns,D., Peshock,R.M., Garg,A., and Grundy,S.M. (1994). Estimation of Adipose-Tissue Mass by Magnetic-Resonance-Imaging - Validation Against Dissection in Human Cadavers. Journal of Lipid Research *35*, 1490-1496.

Abd El-Kader,S.M., Al-Jiffri,O.H., and Al-Shreef,F.M. (2014). Markers of liver function and inflammatory cytokines modulation by aerobic versus resisted exercise training for nonalcoholic steatohepatitis patients. Afr. Health Sci. *14*, 551-557.

Abumrad,N.A. and Davidson,N.O. (2012). Role of the gut in lipid homeostasis. Physiol Rev. *92*, 1061-1085.

Accili,D. (2004). Lilly lecture 2003: the struggle for mastery in insulin action: from triumvirate to republic. Diabetes *53*, 1633-1642.

Ait-Omar,A., Monteiro-Sepulveda,M., Poitou,C., Le,G.M., Cotillard,A., Gilet,J., Garbin,K., Houllier,A., Chateau,D., Lacombe,A., Veyrie,N., Hugol,D., Tordjman,J., Magnan,C., Serradas,P., Clement,K., Leturque,A., and Brot-Laroche,E. (2011). GLUT2 accumulation in enterocyte apical and intracellular membranes: a study in morbidly obese human subjects and ob/ob and high fat-fed mice. Diabetes *60*, 2598-2607.

Albillos,A., de la Hera,A., Gonzalez,M., Moya,J.L., Calleja,J.L., Monserrat,J., Ruiz-del-Arbol,L., and Alvarez-Mon,M. (2003). Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. Hepatology *37*, 208-217.

Allen,J.M., Mailing,L.J., Niemiro,G.M., Moore,R., Cook,M.D., White,B.A., Holscher,H.D., and Woods,J.A. (2018). Exercise Alters Gut Microbiota Composition and Function in Lean and Obese Humans. Med. Sci. Sports Exerc. *50*, 747-757.

Almandoz,J.P., Singh,E., Howell,L.A., Grothe,K., Vlazny,D.T., Smailovic,A., Irving,B.A., Nelson,R.H., and Miles,J.M. (2013). Spillover of Fatty acids during dietary fat storage in type 2 diabetes: relationship to body fat depots and effects of weight loss. Diabetes *62*, 1897-1903.

Angulo,P. and Lindor,K.D. (2002). Non-alcoholic fatty liver disease. J.

Gastroenterol. Hepatol. *17 Suppl*, S186-S190.

Ashcroft,F.M. and Rorsman,P. (1990). ATP-sensitive K+ channels: a link between B-cell metabolism and insulin secretion. Biochem. Soc. Trans. *18*, 109-111.

Aston,J.A., Cunningham,V.J., Asselin,M.C., Hammers,A., Evans,A.C., and Gunn,R.N. (2002). Positron emission tomography partial volume correction: estimation and algorithms. J. Cereb. Blood Flow Metab *22*, 1019-1034.

Azzouz,L.L. and Sharma,S. (2018). Physiology, Large Intestine.

Bacchi,E., Negri,C., Targher,G., Faccioli,N., Lanza,M., Zoppini,G., Zanolin,E., Schena,F., Bonora,E., and Moghetti,P. (2013). Both resistance training and aerobic training reduce hepatic fat content in type 2 diabetic subjects with nonalcoholic fatty liver disease (the RAED2 Randomized Trial). Hepatology *58*, 1287-1295.

Backhed,F., Ding,H., Wang,T., Hooper,L.V., Koh,G.Y., Nagy,A., Semenkovich,C.F., and Gordon,J.I. (2004). The gut microbiota as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. U. S. A *101*, 15718-15723.

Bagby,G.J., Sawaya,D.E., Crouch,L.D., and Shepherd,R.E. (1994). Prior exercise suppresses the plasma tumor necrosis factor response to bacterial lipopolysaccharide. J. Appl. Physiol (1985.) *77*, 1542-1547.

Bahler,L., Holleman,F., Chan,M.W., Booij,J., Hoekstra,J.B., and Verberne,H.J. (2017). 18F-FDG uptake in the colon is modulated by metformin but not associated with core body temperature and energy expenditure. PLoS. One. *12*, e0176242.

Bahler,L., Stroek,K., Hoekstra,J.B., Verberne,H.J., and Holleman,F. (2016a). Metformin-related colonic glucose uptake; potential role for increasing glucose disposal?--A retrospective analysis of (18)F-FDG uptake in the colon on PET-CT. Diabetes Res. Clin. Pract. *114*, 55-63.

Bahler,L., Stroek,K., Hoekstra,J.B., Verberne,H.J., and Holleman,F. (2016b). Metformin-related colonic glucose uptake; potential role for increasing glucose disposal?--A retrospective analysis of (18)F-FDG uptake in the colon on PET-CT. Diabetes Res. Clin. Pract. *114*, 55-63.

Bajaj,M., Pratipanawatr,T., Berria,R., Pratipanawatr,W., Kashyap,S., Cusi,K., Mandarino,L., and DeFronzo,R.A. (2002). Free fatty acids reduce splanchnic and peripheral glucose uptake in patients with type 2 diabetes. Diabetes *51*, 3043-3048.

Barbara Young, James S.Lowe, Alan Stevens, John W.Health, and Drawing by Philip J.Deakin (2006). Wheater's Functional Histology: A Text and Colour Atlas. Churchill Livingstone/Elsevier).

Bartlett,J.D., Close,G.L., Maclaren,D.P., Gregson,W., Drust,B., and Morton,J.P. (2011).

High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: implications for exercise adherence. J. Sports Sci. *29*, 547-553.

Basu,A., Basu,R., Shah,P., Vella,A., Johnson,C.M., Nair,K.S., Jensen,M.D., Schwenk,W.F., and Rizza,R.A. (2000a). Effects of type 2 diabetes on the ability of insulin and glucose to regulate splanchnic and muscle glucose metabolism: evidence for a defect in hepatic glucokinase activity. Diabetes *49*, 272-283.

Basu,A., Basu,R., Shah,P., Vella,A., Johnson,C.M., Nair,K.S., Jensen,M.D., Schwenk,W.F., and Rizza,R.A. (2000b). Effects of type 2 diabetes on the ability of insulin and glucose to regulate splanchnic and muscle glucose metabolism: evidence for a defect in hepatic glucokinase activity. Diabetes *49*, 272-283.

Beaugerie,L. and Petit,J.C. (2004). Microbial-gut interactions in health and disease. Antibiotic-associated diarrhoea. Best. Pract. Res. Clin. Gastroenterol. *18*, 337-352.

Berk,P.D. and Stump,D.D. (1999a). Mechanisms of cellular uptake of long chain free fatty acids. Mol. Cell Biochem. *192*, 17-31.

Berk,P.D. and Stump,D.D. (1999b). Mechanisms of cellular uptake of long chain free fatty acids. Mol. Cell Biochem. *192*, 17-31.

Billat,L.V. (2001). Interval training for performance: a scientific and empirical practice. Special recommendations for middle- and long-distance running. Part I: aerobic interval training. Sports Med. *31*, 13-31.

Bjorntorp,P. (1992). Abdominal fat distribution and the metabolic syndrome. J. Cardiovasc. Pharmacol. *20 Suppl 8*, S26-S28.

Blair,S.N. and Morris,J.N. (2009). Healthy hearts--and the universal benefits of being physically active: physical activity and health. Ann. Epidemiol. *19*, 253-256.

Bloem,C.J. and Chang,A.M. (2008). Short-term exercise improves beta-cell function and insulin resistance in older people with impaired glucose tolerance. J. Clin. Endocrinol. Metab *93*, 387-392.

Boden,G. (1997). Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes *46*, 3-10.

Boland,B.B., Rhodes,C.J., and Grimsby,J.S. (2017). The dynamic plasticity of insulin production in beta-cells. Mol. Metab *6*, 958-973.

Booth,F.W., Roberts,C.K., and Laye,M.J. (2012). Lack of exercise is a major cause of chronic diseases. Compr. Physiol *2*, 1143-1211.

Boule,N.G., Kenny,G.P., Haddad,E., Wells,G.A., and Sigal,R.J. (2003). Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in Type 2 diabetes mellitus. Diabetologia *46*, 1071-1081.

Bradbury,M.W. (2006a). Lipid metabolism and liver inflammation. I. Hepatic fatty acid uptake: possible

role in steatosis. Am. J. Physiol Gastrointest. Liver Physiol *290*, G194-G198.

Bradbury,M.W. (2006b). Lipid metabolism and liver inflammation. I. Hepatic fatty acid uptake: possible role in steatosis. Am. J. Physiol Gastrointest. Liver Physiol *290*, G194-G198.

Bradbury,M.W. and Berk,P.D. (2004). Lipid metabolism in hepatic steatosis. Clin. Liver Dis. *8*, 639-71, xi.

Bressa,C., Bailen-Andrino,M., Perez-Santiago,J., Gonzalez-Soltero,R., Perez,M., Montalvo-Lominchar,M.G., Mate-Munoz,J.L., Dominguez,R., Moreno,D., and Larrosa,M. (2017). Differences in gut microbiota profile between women with active lifestyle and sedentary women. PLoS. One. *12*, e0171352.

Brown,A.J., Setji,T.L., Sanders,L.L., Lowry,K.P., Otvos,J.D., Kraus,W.E., and Svetkey,P.L. (2009). Effects of exercise on lipoprotein particles in women with polycystic ovary syndrome. Med. Sci. Sports Exerc. *41*, 497-504.

Buchheit,M. and Laursen,P.B. (2013). High-intensity interval training, solutions to the programming puzzle: Part I: cardiopulmonary emphasis. Sports Med. *43*, 313-338.

Burgomaster,K.A., Howarth,K.R., Phillips,S.M., Rakobowchuk,M., Macdonald,M.J., McGee,S.L., and Gibala,M.J. (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. J. Physiol *586*, 151-160.

Burgomaster,K.A., Hughes,S.C., Heigenhauser,G.J., Bradwell,S.N., and Gibala,M.J. (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. J. Appl. Physiol (1985.) *98*, 1985-1990.

Byrne,M.M., Sturis,J., Sobel,R.J., and Polonsky,K.S. (1996). Elevated plasma glucose 2 h postchallenge predicts defects in beta-cell function. Am. J. Physiol *270*, E572-E579.

Cani,P.D., Amar,J., Iglesias,M.A., Poggi,M., Knauf,C., Bastelica,D., Neyrinck,A.M., Fava,F., Tuohy,K.M., Chabo,C., Waget,A., Delmee,E., Cousin,B., Sulpice,T., Chamontin,B., Ferrieres,J., Tanti,J.F., Gibson,G.R., Casteilla,L., Delzenne,N.M., Alessi,M.C., and Burcelin,R. (2007a). Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes *56*, 1761-1772.

Cani,P.D., Amar,J., Iglesias,M.A., Poggi,M., Knauf,C., Bastelica,D., Neyrinck,A.M., Fava,F., Tuohy,K.M., Chabo,C., Waget,A., Delmee,E., Cousin,B., Sulpice,T., Chamontin,B., Ferrieres,J., Tanti,J.F., Gibson,G.R., Casteilla,L., Delzenne,N.M., Alessi,M.C., and Burcelin,R. (2007b). Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes *56*, 1761-1772.

Cani,P.D., Bibiloni,R., Knauf,C., Waget,A., Neyrinck,A.M., Delzenne,N.M., and Burcelin,R.

(2008a). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes *57*, 1470-1481.

Cani,P.D., Bibiloni,R., Knauf,C., Waget,A., Neyrinck,A.M., Delzenne,N.M., and Burcelin,R. (2008b). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes *57*, 1470-1481.

Caporaso,J.G., Kuczynski,J., Stombaugh,J., Bittinger,K., Bushman,F.D., Costello,E.K., Fierer,N., Pena,A.G., Goodrich,J.K., Gordon,J.I., Huttley,G.A., Kelley,S.T., Knights,D., Koenig,J.E., Ley,R.E., Lozupone,C.A., McDonald,D., Muegge,B.D., Pirrung,M., Reeder,J., Sevinsky,J.R., Turnbaugh,P.J., Walters,W.A., Widmann,J., Yatsunenko,T., Zaneveld,J., and Knight,R. (2010). QIIME allows analysis of high-throughput community sequencing data. Nat. Methods *7*, 335-336.

Carmena,R., Duriez,P., and Fruchart,J.C. (2004). Atherogenic lipoprotein particles in atherosclerosis. Circulation *109*, III2-III7.

Castorena,C.M., Arias,E.B., Sharma,N., and Cartee,G.D. (2014). Postexercise improvement in insulin-stimulated glucose uptake occurs concomitant with greater AS160 phosphorylation in muscle from normal and insulin-resistant rats. Diabetes *63*, 2297-2308.

Cerasi,E. and Luft,R. (1967). The plasma insulin response to glucose infusion in healthy subjects and in diabetes mellitus. Acta Endocrinol. (Copenh) *55*, 278-304.

Cerf,M.E. (2013). Beta cell dysfunction and insulin resistance. Front Endocrinol. (Lausanne) *4*, 37.

Chalasani,N., Younossi,Z., Lavine,J.E., Diehl,A.M., Brunt,E.M., Cusi,K., Charlton,M., and Sanyal,A.J. (2012). The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology *55*, 2005-2023.

Chang,Y., Ryu,S., Sung,E., and Jang,Y. (2007). Higher concentrations of alanine aminotransferase within the reference interval predict nonalcoholic fatty liver disease. Clinical Chemistry *53*, 686-692.

Chauveau A ,K.M. (1887). Expériences pour la determination du coefficient de l'activité nutritive et respiratoire des muscles en respos et en travail. Compt Rend Ac Sci *104*, 1126-1132.

Cherrington,A.D. (1999). Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes *48*, 1198-1214.

Clarke,G., Stilling,R.M., Kennedy,P.J., Stanton,C., Cryan,J.F., and Dinan,T.G. (2014a). Minireview: Gut microbiota: the neglected endocrine organ. Mol. Endocrinol. *28*, 1221-1238.

Clarke,S.F., Murphy,E.F., Nilaweera,K., Ross,P.R., Shanahan,F., O'Toole,P.W., and Cotter,P.D. (2012). The gut microbiota and its relationship to diet and obesity: new insights. Gut Microbes. *3*, 186-202.

Clarke,S.F., Murphy,E.F., O'Sullivan,O., Lucey,A.J., Humphreys,M., Hogan,A., Hayes,P., O'Reilly,M., Jeffery,I.B., Wood-Martin,R., Kerins,D.M., Quigley,E., Ross,R.P., O'Toole,P.W., Molloy,M.G., Falvey,E., Shanahan,F., and Cotter,P.D. (2014b). Exercise and associated dietary extremes impact on gut microbial diversity. Gut *63*, 1913-1920.

Colberg,S.R., Albright,A.L., Blissmer,B.J., Braun,B., Chasan-Taber,L., Fernhall,B., Regensteiner,J.G., Rubin,R.R., and Sigal,R.J. (2010). Exercise and type 2 diabetes: American College of Sports Medicine and the American Diabetes Association: joint position statement. Exercise and type 2 diabetes. Med. Sci. Sports Exerc. *42*, 2282-2303.

Collins,J.T. and Badireddy,M. (2018). Anatomy, Abdomen and Pelvis, Small Intestine.

Cotran, Ramzi S., Kumar, Vinay, Fausto, Nelson, Nelso Fausto, Robbins, Stanley L., and Abbas, Abul K. Robbins and Cotran pathologic basis of disease (7th ed.). [(7th ed.)], 878. 2015. Robbins and Cotran pathologic basis of disease.

Ref Type: Online Source

Cromwell,W.C. and Otvos,J.D. (2004). Low-density lipoprotein particle number and risk for cardiovascular disease. Curr. Atheroscler. Rep. *6*, 381-387.

Cronin,O., Molloy,M.G., and Shanahan,F. (2016). Exercise, fitness, and the gut. Curr. Opin. Gastroenterol. *32*, 67-73.

Cuthbertson,D.J., Shojaee-Moradie,F., Sprung,V.S., Jones,H., Pugh,C.J., Richardson,P., Kemp,G.J., Barrett,M., Jackson,N.C., Thomas,E.L., Bell,J.D., and Umpleby,A.M. (2016). Dissociation between exercise-induced reduction in liver fat and changes in hepatic and peripheral glucose homoeostasis in obese patients with non-alcoholic fatty liver disease. Clin. Sci. (Lond) *130*, 93-104.

de,P.A., de Mello,M.T., Sanches,P.L., da Silva,P.L., Campos,R.M., Carnier,J., Corgosinho,F., Foschini,D., Masquio,D.L., Tock,L., Oyama,L.M., do Nascimento,C.M., Tufik,S., and Damaso,A.R. (2012). Long-term effects of aerobic plus resistance training on the adipokines and neuropeptides in nonalcoholic fatty liver disease obese adolescents. Eur. J. Gastroenterol. Hepatol. *24*, 1313-1324.

De,V.A., Heimberg,H., Quartier,E., Huypens,P., Bouwens,L., Pipeleers,D., and Schuit,F. (1995a). Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. J. Clin. Invest *96*, 2489-2495.

De,V.A., Heimberg,H., Quartier,E., Huypens,P., Bouwens,L., Pipeleers,D., and Schuit,F. (1995b). Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. J. Clin. Invest *96*, 2489-2495.

DeFronzo,R.A. (2004). Pathogenesis of type 2 diabetes mellitus. Med. Clin. North Am. *88*, 787-835, ix.

DeFronzo,R.A., Bonadonna,R.C., and Ferrannini,E. (1992). Pathogenesis of NIDDM. A balanced overview. Diabetes Care *15*, 318-368.

DeFronzo,R.A. and Ferrannini,E. (1991). Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care *14*, 173-194.

DeFronzo,R.A., Tobin,J.D., and Andres,R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am. J. Physiol *237*, E214-E223.

DeFronzo,R.A. and Tripathy,D. (2009). Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care *32 Suppl 2*, S157-S163.

Delzenne,N.M., Neyrinck,A.M., Backhed,F., and Cani,P.D. (2011). Targeting gut microbiota in obesity: effects of prebiotics and probiotics. Nat. Rev. Endocrinol. *7*, 639-646.

Dimitriadis,G., Mitrou,P., Lambadiari,V., Maratou,E., and Raptis,S.A. (2011). Insulin effects in muscle and adipose tissue. Diabetes Res. Clin. Pract. *93 Suppl 1*, S52-S59.

Donnelly,K.L., Smith,C.I., Schwarzenberg,S.J., Jessurun,J., Boldt,M.D., and Parks,E.J. (2005). Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J. Clin. Invest *115*, 1343-1351.

Drucker,D.J. (2007). The role of gut hormones in glucose homeostasis. J. Clin. Invest *117*, 24-32.

Dugas,L.R., Fuller,M., Gilbert,J., and Layden,B.T. (2016). The obese gut microbiome across the epidemiologic transition. Emerg. Themes. Epidemiol. *13*, 2.

Duncan,S.H., Lobley,G.E., Holtrop,G., Ince,J., Johnstone,A.M., Louis,P., and Flint,H.J. (2008). Human colonic microbiota associated with diet, obesity and weight loss. Int. J. Obes. (Lond) *32*, 1720-1724.

Eaton,R.P., Allen,R.C., Schade,D.S., Erickson,K.M., and Standefer,J. (1980). Prehepatic insulin production in man: kinetic analysis using peripheral connecting peptide behavior. J. Clin. Endocrinol. Metab *51*, 520-528.

Egan,B. and Zierath,J.R. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab *17*, 162-184.

Egshatyan,L., Kashtanova,D., Popenko,A., Tkacheva,O., Tyakht,A., Alexeev,D., Karamnova,N., Kostryukova,E.,

Babenko,V., Vakhitova,M., and Boytsov,S. (2016). Gut microbiota and diet in patients with different glucose tolerance. Endocr. Connect. *5*, 1-9.

Eskelinen,J.J., Heinonen,I., Loyttyniemi,E., Saunavaara,V., Kirjavainen,A., Virtanen,K.A., Hannukainen,J.C., and Kalliokoski,K.K. (2015). Muscle-specific glucose and free fatty acid uptake after sprint interval and moderate-intensity training in healthy middle-aged men. Journal of Applied Physiology *118*, 1172-1180.

Estaki,M., Pither,J., Baumeister,P., Little,J.P., Gill,S.K., Ghosh,S., Ahmadi-Vand,Z., Marsden,K.R., and Gibson,D.L. (2016). Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct metagenomic functions. Microbiome. *4*, 42.

Evans,C.C., LePard,K.J., Kwak,J.W., Stancukas,M.C., Laskowski,S., Dougherty,J., Moulton,L., Glawe,A., Wang,Y., Leone,V., Antonopoulos,D.A., Smith,D., Chang,E.B., and Ciancio,M.J. (2014). Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. PLoS. One. *9*, e92193.

Ferrannini,E., Bjorkman,O., Reichard,G.A., Jr., Pilo,A., Olsson,M., Wahren,J., and DeFronzo,R.A. (1985). The disposal of an oral glucose load in healthy subjects. A quantitative study. Diabetes *34*, 580-588.

Ferrannini,E., Simonson,D.C., Katz,L.D., Reichard,G., Jr., Bevilacqua,S., Barrett,E.J., Olsson,M., and DeFronzo,R.A. (1988). The disposal of an oral glucose load in patients with non-insulin-dependent diabetes. Metabolism *37*, 79-85.

Ferre,T., Riu,E., Franckhauser,S., Agudo,J., and Bosch,F. (2003a). Long-term overexpression of glucokinase in the liver of transgenic mice leads to insulin resistance. Diabetologia *46*, 1662-1668.

Ferre,T., Riu,E., Franckhauser,S., Agudo,J., and Bosch,F. (2003b). Long-term overexpression of glucokinase in the liver of transgenic mice leads to insulin resistance. Diabetologia *46*, 1662-1668.

Finucane,F.M., Sharp,S.J., Purslow,L.R., Horton,K., Horton,J., Savage,D.B., Brage,S., Besson,H., De Lucia,R.E., Sleigh,A., Martin,H.J., Aihie,S.A., Cooper,C., Ekelund,U., Griffin,S.J., and Wareham,N.J. (2010). The effects of aerobic exercise on metabolic risk, insulin sensitivity and intrahepatic lipid in healthy older people from the Hertfordshire Cohort Study: a randomised controlled trial. Diabetologia *53*, 624-631.

Flint,H.J., Scott,K.P., Louis,P., and Duncan,S.H. (2012). The role of the gut microbiota in nutrition and health. Nat. Rev. Gastroenterol. Hepatol. *9*, 577-589.

Forslund,K., Hildebrand,F., Nielsen,T., Falony,G., Le,C.E., Sunagawa,S., Prifti,E., Vieira-Silva,S., Gudmundsdottir,V., Pedersen,H.K., Arumugam,M., Kristiansen,K., Voigt,A.Y., Vestergaard,H., Hercog,R.,

Costea,P.I., Kultima,J.R., Li,J., Jorgensen,T., Levenez,F., Dore,J., Nielsen,H.B., Brunak,S., Raes,J., Hansen,T., Wang,J., Ehrlich,S.D., Bork,P., and Pedersen,O. (2015). Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature *528*, 262-266.

Freedman,D.S., Otvos,J.D., Jeyarajah,E.J., Barboriak,J.J., Anderson,A.J., and Walker,J.A. (1998). Relation of lipoprotein subclasses as measured by proton nuclear magnetic resonance spectroscopy to coronary artery disease. Arterioscler. Thromb. Vasc. Biol. *18*, 1046-1053.

Freidenberg,G.R., Reichart,D., Olefsky,J.M., and Henry,R.R. (1988). Reversibility of defective adipocyte insulin receptor kinase activity in non-insulin-dependent diabetes mellitus. Effect of weight loss. J. Clin. Invest *82*, 1398-1406.

Friedewald,W.T., Levy,R.I., and Fredrickson,D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. *18*, 499-502.

Furet,J.P., Kong,L.C., Tap,J., Poitou,C., Basdevant,A., Bouillot,J.L., Mariat,D., Corthier,G., Dore,J., Henegar,C., Rizkalla,S., and Clement,K. (2010). Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. Diabetes *59*, 3049-3057.

Galassetti,P., Coker,R.H., Lacy,D.B., Cherrington,A.D., and Wasserman,D.H. (1999). Prior exercise increases net hepatic glucose uptake during a glucose load. Am. J. Physiol *276*, E1022-E1029.

Gangl,A. and Ockner,R.K. (1975). Intestinal metabolism of plasma free fatty acids. Intracellular compartmentation and mechanisms of control. J. Clin. Invest *55*, 803-813.

Gangl,A. and Renner,F. (1978). In vivo metabolism of plasma free fatty acids by intestinal mucosa of man. Gastroenterology *74*, 847-850.

Ganong W.F. (2005). Review of Medical Physiology. The McGrawhill Companies).

Garber,C.E., Blissmer,B., Deschenes,M.R., Franklin,B.A., Lamonte,M.J., Lee,I.M., Nieman,D.C., and Swain,D.P. (2011a). American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med. Sci. Sports Exerc. *43*, 1334-1359.

Garber,C.E., Blissmer,B., Deschenes,M.R., Franklin,B.A., Lamonte,M.J., Lee,I.M., Nieman,D.C., and Swain,D.P. (2011b). American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently

healthy adults: guidance for prescribing exercise. Med. Sci. Sports Exerc. *43*, 1334-1359.

Garvey,W.T., Kwon,S., Zheng,D., Shaughnessy,S., Wallace,P., Hutto,A., Pugh,K., Jenkins,A.J., Klein,R.L., and Liao,Y. (2003). Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes *52*, 453-462.

Gibala,M.J., Gillen,J.B., and Percival,M.E. (2014). Physiological and health-related adaptations to low-volume interval training: influences of nutrition and sex. Sports Med. *44 Suppl 2*, S127-S137.

Gibala,M.J., Little,J.P., van,E.M., Wilkin,G.P., Burgomaster,K.A., Safdar,A., Raha,S., and Tarnopolsky,M.A. (2006). Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. J. Physiol *575*, 901-911.

Gibala,M.J. and McGee,S.L. (2008). Metabolic adaptations to short-term high-intensity interval training: a little pain for a lot of gain? Exerc. Sport Sci. Rev. *36*, 58-63.

Gilbert,E.R. and Liu,D. (2012). Epigenetics: the missing link to understanding beta-cell dysfunction in the pathogenesis of type 2 diabetes. Epigenetics. *7*, 841-852.

Gillen,J.B., Martin,B.J., MacInnis,M.J., Skelly,L.E., Tarnopolsky,M.A., and Gibala,M.J. (2016a). Twelve Weeks of Sprint Interval Training Improves Indices of Cardiometabolic Health Similar to Traditional Endurance Training despite a Five-Fold Lower Exercise Volume and Time Commitment. PLoS. One. *11*, e0154075.

Gillen,J.B., Martin,B.J., MacInnis,M.J., Skelly,L.E., Tarnopolsky,M.A., and Gibala,M.J. (2016b). Twelve Weeks of Sprint Interval Training Improves Indices of Cardiometabolic Health Similar to Traditional Endurance Training despite a Five-Fold Lower Exercise Volume and Time Commitment. PLoS. One. *11*, e0154075.

Gillen,J.B., Percival,M.E., Skelly,L.E., Martin,B.J., Tan,R.B., Tarnopolsky,M.A., and Gibala,M.J. (2014). Three minutes of all-out intermittent exercise per week increases skeletal muscle oxidative capacity and improves cardiometabolic health. PLoS. One. *9*, e111489.

Golabi,P., Locklear,C.T., Austin,P., Afdhal,S., Byrns,M., Gerber,L., and Younossi,Z.M. (2016). Effectiveness of exercise in hepatic fat mobilization in non-alcoholic fatty liver disease: Systematic review. World J. Gastroenterol. *22*, 6318-6327.

Golay,A., DeFronzo,R.A., Ferrannini,E., Simonson,D.C., Thorin,D., Acheson,K., Thiebaud,D., Curchod,B., Jequier,E., and Felber,J.P. (1988). Oxidative and non-oxidative glucose metabolism in non-obese type 2 (non-insulin-dependent) diabetic patients. Diabetologia *31*, 585-591.

Gontier,E., Fourme,E., Wartski,M., Blondet,C., Bonardel,G., Le,S.E.,

Mantzarides,M., Foehrenbach,H., Pecking,A.P., and Alberini,J.L. (2008). High and typical 18F-FDG bowel uptake in patients treated with metformin. Eur. J. Nucl. Med. Mol. Imaging *35*, 95-99.

Gonzalez-Quintela,A., Alonso,M., Campos,J., Vizcaino,L., Loidi,L., and Gude,F. (2013). Determinants of serum concentrations of lipopolysaccharide-binding protein (LBP) in the adult population: the role of obesity. PLoS. One. *8*, e54600.

Gray H,L.W. (2000). Gray's Anatomy of the Human Body. New York, NY:Bartleby).

Gray,H. (1918). Gray's Anatomy. Philadelphia: Lea & Febiger).

Grolier (1999). "Intestine.". In The Encyclopedia Americana., Danbury, CT), p. 323.

Guarner,F. and Malagelada,J.R. (2003). Gut flora in health and disease. Lancet *361*, 512-519.

Hallsworth,K., Thoma,C., Hollingsworth,K.G., Cassidy,S., Anstee,Q.M., Day,C.P., and Trenell,M.I. (2015). Modified high-intensity interval training reduces liver fat and improves cardiac function in non-alcoholic fatty liver disease: a randomized controlled trial. Clin. Sci. (Lond) *129*, 1097-1105.

Halverstadt,A., Phares,D.A., Wilund,K.R., Goldberg,A.P., and Hagberg,J.M. (2007). Endurance exercise training raises high-density lipoprotein cholesterol and lowers small low-density lipoprotein and very low-density lipoprotein independent of body fat phenotypes in older men and women. Metabolism *56*, 444-450.

Hamer,H.M., Jonkers,D., Venema,K., Vanhoutvin,S., Troost,F.J., and Brummer,R.J. (2008). Review article: the role of butyrate on colonic function. Aliment. Pharmacol. Ther. *27*, 104-119.

Hamilton,M.T. and Booth,F.W. (2000). Skeletal muscle adaptation to exercise: a century of progress. J. Appl. Physiol (1985.) *88*, 327-331.

Handbook of Diabetes. Handbook of Diabetes. [4th Edition, Excerpt #4:Normal Physiology of Insulin Secretion and Action". Diabetes In Control]. 28-7-2014.

Ref Type: Online Source

Hannukainen,J.C., Nuutila,P., Borra,R., Kaprio,J., Kujala,U.M., Janatuinen,T., Heinonen,O.J., Kapanen,J., Viljanen,T., Haaparanta,M., Ronnemaa,T., Parkkola,R., Knuuti,J., and Kalliokoski,K.K. (2007). Increased physical activity decreases hepatic free fatty acid uptake: a study in human monozygotic twins. J. Physiol *578*, 347-358.

Hashimoto,T. (2013). Lipid metabolism and exercise. The Journal of Physical Fitness and Sports Medicine *2*, 149-154.

Haskell,W.L., Lee,I.M., Pate,R.R., Powell,K.E., Blair,S.N., Franklin,B.A., Macera,C.A., Heath,G.W., Thompson,P.D., and Bauman,A. (2007). Physical activity

and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Circulation *116*, 1081-1093.

Hazell,T.J., Macpherson,R.E., Gravelle,B.M., and Lemon,P.W. (2010). 10 or 30-s sprint interval training bouts enhance both aerobic and anaerobic performance. Eur. J. Appl. Physiol *110*, 153-160.

Higa,M., Zhou,Y.T., Ravazzola,M., Baetens,D., Orci,L., and Unger,R.H. (1999). Troglitazone prevents mitochondrial alterations, beta cell destruction, and diabetes in obese prediabetic rats. Proc. Natl. Acad. Sci. U. S. A *96*, 11513-11518.

Himsworth,H.P. (1936). MANAGEMENT OF DIABETES MELLITUS. Br. Med. J. *2*, 137-141.

Holst,J.J., Vilsboll,T., and Deacon,C.F. (2009). The incretin system and its role in type 2 diabetes mellitus. Mol. Cell Endocrinol. *297*, 127-136.

Honka,H., Makinen,J., Hannukainen,J.C., Tarkia,M., Oikonen,V., Teras,M., Fagerholm,V., Ishizu,T., Saraste,A., Stark,C., Vahasilta,T., Salminen,P., Kirjavainen,A., Soinio,M., Gastaldelli,A., Knuuti,J., Iozzo,P., and Nuutila,P. (2013). Validation of [18F]fluorodeoxyglucose and positron emission tomography (PET) for the measurement of intestinal metabolism in pigs, and evidence of intestinal insulin resistance in patients with morbid obesity. Diabetologia *56*, 893-900.

Human Anatomy & Physiology. Human Anatomy & Physiology + New Masteringa&p With Pearson Etext. 2012. Benjamin-Cummings Pub Co.

Ref Type: Online Source

Hung,Y.H., Linden,M.A., Gordon,A., Rector,R.S., and Buhman,K.K. (2015). Endurance exercise training programs intestinal lipid metabolism in a rat model of obesity and type 2 diabetes. Physiol Rep. *3*.

Hurley,J.C. (1995). Endotoxemia: methods of detection and clinical correlates. Clin. Microbiol. Rev. *8*, 268-292.

Ionescu-Tirgoviste,C., Gagniuc,P.A., Gubceac,E., Mardare,L., Popescu,I., Dima,S., and Militaru,M. (2015). A 3D map of the islet routes throughout the healthy human pancreas. Sci. Rep. *5*, 14634.

Iozzo,P., Bucci,M., Roivainen,A., Nagren,K., Jarvisalo,M.J., Kiss,J., Guiducci,L., Fielding,B., Naum,A.G., Borra,R., Virtanen,K., Savunen,T., Salvadori,P.A., Ferrannini,E., Knuuti,J., and Nuutila,P. (2010). Fatty acid metabolism in the liver, measured by positron emission tomography, is increased in obese individuals. Gastroenterology *139*, 846-56, 856.

Iozzo,P., Geisler,F., Oikonen,V., Maki,M., Takala,T., Solin,O., Ferrannini,E., Knuuti,J., and Nuutila,P. (2003a). Insulin stimulates liver glucose uptake in humans: an 18F-FDG PET Study. J. Nucl. Med. *44*, 682-689.

Iozzo,P., Hallsten,K., Oikonen,V., Virtanen,K.A., Kemppainen,J., Solin,O., Ferrannini,E., Knuuti,J., and Nuutila,P. (2003b). Insulin-mediated hepatic glucose uptake is impaired in type 2 diabetes: evidence for a relationship with glycemic control. J. Clin. Endocrinol. Metab *88*, 2055-2060.

Iozzo,P., Lautamaki,R., Geisler,F., Virtanen,K.A., Oikonen,V., Haaparanta,M., Yki-Jarvinen,H., Ferrannini,E., Knuuti,J., and Nuutila,P. (2004a). Non-esterified fatty acids impair insulin-mediated glucose uptake and disposition in the liver. Diabetologia *47*, 1149-1156.

Iozzo,P., Takala,T., Oikonen,V., Bergman,J., Gronroos,T., Ferrannini,E., Nuutila,P., and Knuuti,J. (2004b). Effect of training status on regional disposal of circulating free fatty acids in the liver and skeletal muscle during physiological hyperinsulinemia. Diabetes Care *27*, 2172-2177.

Iozzo,P., Turpeinen,A.K., Takala,T., Oikonen,V., Solin,O., Ferrannini,E., Nuutila,P., and Knuuti,J. (2003c). Liver uptake of free fatty acids in vivo in humans as determined with 14(R, S)-[18F]fluoro-6-thia-heptadecanoic acid and PET. Eur. J. Nucl. Med. Mol. Imaging *30*, 1160-1164.

Jelleyman,C., Yates,T., O'Donovan,G., Gray,L.J., King,J.A., Khunti,K., and Davies,M.J. (2015). The effects of high-intensity interval training on glucose regulation and insulin resistance: a meta-analysis. Obes. Rev. *16*, 942-961.

Jennifer L.Kuk. ASSOCIATIONS BETWEEN ABDOMINAL ADIPOSITY, EXERCISE,

MORBIDITY AND MORTALITY. 2007. Queen's University

Kingston, Ontario, Canada.

Ref Type: Thesis/Dissertation

Johnson,N.A., Sachinwalla,T., Walton,D.W., Smith,K., Armstrong,A., Thompson,M.W., and George,J. (2009). Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. Hepatology *50*, 1105-1112.

Jones WHS,W.ET. (1931). Works of Hippocrates. Cambridge, MA, Harvard Univeristy Press), p. 229.

Kahn,S.E., Cooper,M.E., and Del,P.S. (2014). Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet *383*, 1068-1083.

Karlsson,F.H., Tremaroli,V., Nookaew,I., Bergstrom,G., Behre,C.J., Fagerberg,B., Nielsen,J., and Backhed,F. (2013). Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature *498*, 99-103.

Kasawara,K.T., Cotechini,T., Macdonald-Goodfellow,S.K., Surita,F.G., Pinto E Silva JL, Tayade,C., Othman,M., Ozolins,T.R., and Graham,C.H. (2016). Moderate Exercise Attenuates Lipopolysaccharide-Induced Inflammation and Associated Maternal and Fetal Morbidities in Pregnant Rats. PLoS. One. *11*, e0154405.

Kasper,D., Fauci,A., Hauser,S., Longo,D., Jameson,J., and Loscalzo,J. (2015). Harrison's Principles of Internal Medicine. McGraw-Hill Professional).

Kawamori,R., Kubota,M., Ikeda,M., Matsuhisa,M., Kubota,M., Morishima,T., and Kamada,T. (1991). Quantitative determination of hepatic glucose uptake using an innovative approach: effect of strict glycemic regulation and exercise in diabetic subjects. J. Nutr. Sci. Vitaminol. (Tokyo) *37 Suppl*, S35-S42.

Kellett,G.L. and Brot-Laroche,E. (2005). Apical GLUT2: a major pathway of intestinal sugar absorption. Diabetes *54*, 3056-3062.

Kelley,D.E., McKolanis,T.M., Hegazi,R.A., Kuller,L.H., and Kalhan,S.C. (2003). Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. Am. J. Physiol Endocrinol. Metab *285*, E906-E916.

Kemi,O.J. and Wisloff,U. (2010). High-intensity aerobic exercise training improves the heart in health and disease. J. Cardiopulm. Rehabil. Prev. *30*, 2-11.

Keshel,T.E. and Coker,R.H. (2015). Exercise Training and Insulin Resistance: A Current Review. J. Obes. Weight. Loss. Ther. *5*.

Khanna,S. and Tosh,P.K. (2014). A clinician's primer on the role of the microbiome in human health and disease. Mayo Clin. Proc. *89*, 107-114.

Kharroubi,I., Ladriere,L., Cardozo,A.K., Dogusan,Z., Cnop,M., and Eizirik,D.L. (2004). Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress. Endocrinology *145*, 5087-5096.

Kim,H.J., Kim,H.J., Lee,K.E., Kim,D.J., Kim,S.K., Ahn,C.W., Lim,S.K., Kim,K.R., Lee,H.C., Huh,K.B., and Cha,B.S. (2004). Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. Arch. Intern. Med. *164*, 2169-2175.

Kim,W.R., Flamm,S.L., Di Bisceglie,A.M., and Bodenheimer,H.C. (2008). Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology *47*, 1363-1370.

Kim,Y.W., Moon,J.S., Seo,Y.J., Park,S.Y., Kim,J.Y., Yoon,J.S., Lee,I.K., Lee,H.W., and Won,K.C. (2012). Inhibition of fatty acid translocase cluster determinant 36 (CD36), stimulated by hyperglycemia, prevents glucotoxicity in INS-1 cells. Biochem. Biophys. Res. Commun. *420*, 462-466.

Koffert,J., Stahle,M., Karlsson,H., Iozzo,P., Salminen,P., Roivainen,A., and Nuutila,P. (2018). Morbid obesity and type 2 diabetes alter intestinal fatty acid uptake and blood flow. Diabetes Obes. Metab *20*, 1384-1390.

Kolterman,O.G., Gray,R.S., Griffin,J., Burstein,P., Insel,J., Scarlett,J.A., and Olefsky,J.M.

(1981). Receptor and postreceptor defects contribute to the insulin resistance in noninsulin-dependent diabetes mellitus. J. Clin. Invest *68*, 957-969.

Komatsu,M., Takei,M., Ishii,H., and Sato,Y. (2013). Glucose-stimulated insulin secretion: A newer perspective. J. Diabetes Investig. *4*, 511-516.

Kong,Z., Fan,X., Sun,S., Song,L., Shi,Q., and Nie,J. (2016). Comparison of High-Intensity Interval Training and Moderate-to-Vigorous Continuous Training for Cardiometabolic Health and Exercise Enjoyment in Obese Young Women: A Randomized Controlled Trial. PLoS. One. *11*, e0158589.

Koral,J., Oranchuk,D.J., Herrera,R., and Millet,G.Y. (2018). Six Sessions of Sprint Interval Training Improves Running Performance in Trained Athletes. J. Strength. Cond. Res. *32*, 617-623.

Kramer,H.F., Witczak,C.A., Taylor,E.B., Fujii,N., Hirshman,M.F., and Goodyear,L.J. (2006). AS160 regulates insulin- and contraction-stimulated glucose uptake in mouse skeletal muscle. J. Biol. Chem. *281*, 31478-31485.

Krotkiewski,M., Lonnroth,P., Mandroukas,K., Wroblewski,Z., Rebuffe-Scrive,M., Holm,G., Smith,U., and Bjorntorp,P. (1985). The effects of physical training on insulin secretion and effectiveness and on glucose metabolism in obesity and type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia *28*, 881-890.

Kusminski,C.M., Shetty,S., Orci,L., Unger,R.H., and Scherer,P.E. (2009a). Diabetes and apoptosis: lipotoxicity. Apoptosis. *14*, 1484-1495.

Kusminski,C.M., Shetty,S., Orci,L., Unger,R.H., and Scherer,P.E. (2009b). Diabetes and apoptosis: lipotoxicity. Apoptosis. *14*, 1484-1495.

Labbe,S.M., Grenier-Larouche,T., Croteau,E., Normand-Lauziere,F., Frisch,F., Ouellet,R., Guerin,B., Turcotte,E.E., and Carpentier,A.C. (2011). Organ-specific dietary fatty acid uptake in humans using positron emission tomography coupled to computed tomography. Am. J. Physiol Endocrinol. Metab *300*, E445-E453.

Lambert,J.E., Myslicki,J.P., Bomhof,M.R., Belke,D.D., Shearer,J., and Reimer,R.A. (2015). Exercise training modifies gut microbiota in normal and diabetic mice. Appl. Physiol Nutr. Metab *40*, 749-752.

Lambert,M.S., Avella,M.A., Berhane,Y., Shervill,E., and Botham,K.M. (2001). The fatty acid composition of chylomicron remnants influences their binding and internalization by isolated hepatocytes. Eur. J. Biochem. *268*, 3983-3992.

Lassenius,M.I., Pietilainen,K.H., Kaartinen,K., Pussinen,P.J., Syrjanen,J., Forsblom,C., Porsti,I., Rissanen,A., Kaprio,J., Mustonen,J., Groop,P.H., and Lehto,M. (2011). Bacterial endotoxin activity in human serum is associated with dyslipidemia, insulin resistance,

obesity, and chronic inflammation. Diabetes Care *34*, 1809-1815.

Lee,S., Bacha,F., Hannon,T., Kuk,J.L., Boesch,C., and Arslanian,S. (2012). Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys: a randomized, controlled trial. Diabetes *61*, 2787-2795.

Lee,S.F., Pei,D., Chi,M.J., and Jeng,C. (2015). An investigation and comparison of the effectiveness of different exercise programmes in improving glucose metabolism and pancreatic beta cell function of type 2 diabetes patients. Int. J. Clin. Pract. *69*, 1159-1170.

Lepage,P., Leclerc,M.C., Joossens,M., Mondot,S., Blottiere,H.M., Raes,J., Ehrlich,D., and Dore,J. (2013). A metagenomic insight into our gut's microbiome. Gut *62*, 146-158.

Lesmana,C.R., Pakasi,L.S., Inggriani,S., Aidawati,M.L., and Lesmana,L.A. (2015). Prevalence of Non-Alcoholic Fatty Pancreas Disease (NAFPD) and its risk factors among adult medical check-up patients in a private hospital: a large cross sectional study. BMC. Gastroenterol. *15*, 174.

Ley,R.E., Backhed,F., Turnbaugh,P., Lozupone,C.A., Knight,R.D., and Gordon,J.I. (2005). Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. U. S. A *102*, 11070-11075.

Ley,R.E., Turnbaugh,P.J., Klein,S., and Gordon,J.I. (2006). Microbial ecology: human gut microbes associated with obesity. Nature *444*, 1022-1023.

Little,J.P., Gillen,J.B., Percival,M.E., Safdar,A., Tarnopolsky,M.A., Punthakee,Z., Jung,M.E., and Gibala,M.J. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. J. Appl. Physiol (1985.) *111*, 1554-1560.

Littlefield,A.M., Setti,S.E., Priester,C., and Kohman,R.A. (2015). Voluntary exercise attenuates LPS-induced reductions in neurogenesis and increases microglia expression of a proneurogenic phenotype in aged mice. J. Neuroinflammation. *12*, 138.

Liu,J., Han,L., Zhu,L., and Yu,Y. (2016). Free fatty acids, not triglycerides, are associated with non-alcoholic liver injury progression in high fat diet induced obese rats. Lipids Health Dis. *15*, 27.

Lozupone,C.A., Stombaugh,J.I., Gordon,J.I., Jansson,J.K., and Knight,R. (2012). Diversity, stability and resilience of the human gut microbiota. Nature *489*, 220-230.

Madsen,S.M., Thorup,A.C., Overgaard,K., and Jeppesen,P.B. (2015). High Intensity Interval Training Improves Glycaemic Control and Pancreatic beta Cell Function of Type 2 Diabetes Patients. PLoS. One. *10*, e0133286.

Magkos,F., Mohammed,B.S., and Mittendorfer,B. (2008). Effect of obesity on the plasma lipoprotein subclass profile in normoglycemic and normolipidemic men and women. Int. J. Obes. (Lond) *32*, 1655-1664.

Magkos,F., Wright,D.C., Patterson,B.W., Mohammed,B.S., and Mittendorfer,B. (2006). Lipid metabolism response to a single, prolonged bout of endurance exercise in healthy young men. Am. J. Physiol Endocrinol. Metab *290*, E355-E362.

Maki,M.T., Haaparanta,M., Nuutila,P., Oikonen,V., Luotolahti,M., Eskola,O., and Knuuti,J.M. (1998). Free fatty acid uptake in the myocardium and skeletal muscle using fluorine-18-fluoro-6-thia-heptadecanoic acid. J. Nucl. Med. *39*, 1320-1327.

Makinen,J., Hannukainen,J.C., Karmi,A., Immonen,H.M., Soinio,M., Nelimarkka,L., Savisto,N., Helmio,M., Ovaska,J., Salminen,P., Iozzo,P., and Nuutila,P. (2015). Obesity-associated intestinal insulin resistance is ameliorated after bariatric surgery. Diabetologia *58*, 1055-1062.

Malin,S.K., Solomon,T.P., Blaszczak,A., Finnegan,S., Filion,J., and Kirwan,J.P. (2013). Pancreatic beta-cell function increases in a linear dose-response manner following exercise training in adults with prediabetes. Am. J. Physiol Endocrinol. Metab *305*, E1248-E1254.

Manco,M., Putignani,L., and Bottazzo,G.F. (2010a). Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. Endocr. Rev. *31*, 817-844.

Manco,M., Putignani,L., and Bottazzo,G.F. (2010b). Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. Endocr. Rev. *31*, 817-844.

Mannucci,E., Monami,M., Lamanna,C., and Adalsteinsson,J.E. (2012). Post-prandial glucose and diabetic complications: systematic review of observational studies. Acta Diabetol. *49*, 307-314.

Mari,A. and Ferrannini,E. (2008a). Beta-cell function assessment from modelling of oral tests: an effective approach. Diabetes Obes. Metab *10 Suppl 4*, 77-87.

Mari,A. and Ferrannini,E. (2008b). Beta-cell function assessment from modelling of oral tests: an effective approach. Diabetes Obes. Metab *10 Suppl 4*, 77-87.

Mari,A., Schmitz,O., Gastaldelli,A., Oestergaard,T., Nyholm,B., and Ferrannini,E. (2002). Meal and oral glucose tests for assessment of beta - cell function: modeling analysis in normal subjects. Am. J. Physiol Endocrinol. Metab *283*, E1159-E1166.

Marieb,E.N. and Hoehn,K. (2012). Human Anatomy & Physiology + New MasteringA&P With Pearson Etext. Benjamin-Cummings Pub Co).

Martin,G.M., Chen,P.C., Devaraneni,P., and Shyng,S.L.

(2013). Pharmacological rescue of trafficking-impaired ATP-sensitive potassium channels. Front Physiol *4*, 386.

Matschinsky,F., Liang,Y., Kesavan,P., Wang,L., Froguel,P., Velho,G., Cohen,D., Permutt,M.A., Tanizawa,Y., Jetton,T.L., and . (1993). Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. J. Clin. Invest *92*, 2092-2098.

Matschinsky,F.M. (1996). Banting Lecture 1995. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. Diabetes *45*, 223-241.

Matschinsky,F.M. (2009). Assessing the potential of glucokinase activators in diabetes therapy. Nat. Rev. Drug Discov. *8*, 399-416.

Mazmanian,S.K., Round,J.L., and Kasper,D.L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. Nature *453*, 620-625.

Milanovic,Z., Sporis,G., and Weston,M. (2015). Effectiveness of High-Intensity Interval Training (HIT) and Continuous Endurance Training for VO2max Improvements: A Systematic Review and Meta-Analysis of Controlled Trials. Sports Med. *45*, 1469-1481.

Monteiro,P.A., Chen,K.Y., Lira,F.S., Saraiva,B.T., Antunes,B.M., Campos,E.Z., and Freitas,I.F., Jr. (2015). Concurrent and aerobic exercise training promote similar benefits in body composition and metabolic profiles in obese adolescents. Lipids Health Dis. *14*, 153.

Mora,S., Otvos,J.D., Rosenson,R.S., Pradhan,A., Buring,J.E., and Ridker,P.M. (2010). Lipoprotein particle size and concentration by nuclear magnetic resonance and incident type 2 diabetes in women. Diabetes *59*, 1153-1160.

Muniyappa,R. and Madan,R. (2000). Assessing Insulin Sensitivity and Resistance in Humans.

Munukka,E., Ahtiainen,J.P., Puigbo,P., Jalkanen,S., Pahkala,K., Keskitalo,A., Kujala,U.M., Pietila,S., Hollmen,M., Elo,L., Huovinen,P., D'Auria,G., and Pekkala,S. (2018). Six-Week Endurance Exercise Alters Gut Metagenome That Is not Reflected in Systemic Metabolism in Over-weight Women. Front Microbiol. *9*, 2323.

Murphy,E.F., Cotter,P.D., Healy,S., Marques,T.M., O'Sullivan,O., Fouhy,F., Clarke,S.F., O'Toole,P.W., Quigley,E.M., Stanton,C., Ross,P.R., O'Doherty,R.M., and Shanahan,F. (2010). Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. Gut *59*, 1635-1642.

Musso,G., Cassader,M., Rosina,F., and Gambino,R. (2012). Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. Diabetologia *55*, 885-904.

Musso,G., Gambino,R., and Cassader,M. (2010). Obesity, diabetes, and gut microbiota: the

hygiene hypothesis expanded? Diabetes Care *33*, 2277-2284.

Nassir,F., Rector,R.S., Hammoud,G.M., and Ibdah,J.A. (2015). Pathogenesis and Prevention of Hepatic Steatosis. Gastroenterol. Hepatol. (N. Y.) *11*, 167-175.

Nelson,M.E., Rejeski,W.J., Blair,S.N., Duncan,P.W., Judge,J.O., King,A.C., Macera,C.A., and Castaneda-Sceppa,C. (2007). Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. Circulation *116*, 1094-1105.

Neuschwander-Tetri,B.A., Loomba,R., Sanyal,A.J., Lavine,J.E., Van Natta,M.L., Abdelmalek,M.F., Chalasani,N., Dasarathy,S., Diehl,A.M., Hameed,B., Kowdley,K.V., McCullough,A., Terrault,N., Clark,J.M., Tonascia,J., Brunt,E.M., Kleiner,D.E., and Doo,E. (2015). Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet *385*, 956-965.

Newsholme,P., Keane,D., Welters,H.J., and Morgan,N.G. (2007). Life and death decisions of the pancreatic beta-cell: the role of fatty acids. Clin. Sci. (Lond) *112*, 27-42.

Nguyen,P., Leray,V., Diez,M., Serisier,S., Le,B.J., Siliart,B., and Dumon,H. (2008). Liver lipid metabolism. J. Anim Physiol Anim Nutr. (Berl) *92*, 272-283.

Niot,I., Poirier,H., Tran,T.T., and Besnard,P. (2009). Intestinal absorption of long-chain fatty acids: evidence and uncertainties. Prog. Lipid Res. *48*, 101-115.

Norton,K., Norton,L., and Sadgrove,D. (2010). Position statement on physical activity and exercise intensity terminology. J. Sci. Med. Sport *13*, 496-502.

Oh,S., So,R., Shida,T., Matsuo,T., Kim,B., Akiyama,K., Isobe,T., Okamoto,Y., Tanaka,K., and Shoda,J. (2017). High-Intensity Aerobic Exercise Improves Both Hepatic Fat Content and Stiffness in Sedentary Obese Men with Nonalcoholic Fatty Liver Disease. Sci. Rep. *7*, 43029.

Olsen,T.S. (1978). Lipomatosis of the pancreas in autopsy material and its relation to age and overweight. Acta Pathol. Microbiol. Scand. A *86A*, 367-373.

Patlak,C.S. and Blasberg,R.G. (1985). Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations. J. Cereb. Blood Flow Metab *5*, 584-590.

Penedo,F.J. and Dahn,J.R. (2005). Exercise and well-being: a review of mental and physical health benefits associated with physical activity. Curr. Opin. Psychiatry *18*, 189-193.

Petersen,K.F., Dufour,S., Befroy,D., Lehrke,M., Hendler,R.E., and Shulman,G.I. (2005). Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight

reduction in patients with type 2 diabetes. Diabetes *54*, 603-608.

Petersen,K.F., Dufour,S., Savage,D.B., Bilz,S., Solomon,G., Yonemitsu,S., Cline,G.W., Befroy,D., Zemany,L., Kahn,B.B., Papademetris,X., Rothman,D.L., and Shulman,G.I. (2007). The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. Proc. Natl. Acad. Sci. U. S. A *104*, 12587-12594.

Poitout,V. and Robertson,R.P. (2008). Glucolipotoxicity: fuel excess and beta-cell dysfunction. Endocr. Rev. *29*, 351-366.

Powell,K.E., Paluch,A.E., and Blair,S.N. (2011). Physical activity for health: What kind? How much? How intense? On top of what? Annu. Rev. Public Health *32*, 349-365.

Prentki,M. and Nolan,C.J. (2006). Islet beta cell failure in type 2 diabetes. J. Clin. Invest *116*, 1802-1812.

Provencher,S.W. (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson. Med. *30*, 672-679.

Pugh,C.J., Cuthbertson,D.J., Sprung,V.S., Kemp,G.J., Richardson,P., Umpleby,A.M., Green,D.J., Cable,N.T., and Jones,H. (2013). Exercise training improves cutaneous microvascular function in nonalcoholic fatty liver disease. Am. J. Physiol Endocrinol. Metab *305*, E50-E58.

Pussinen,P.J., Havulinna,A.S., Lehto,M., Sundvall,J., and Salomaa,V. (2011). Endotoxemia is associated with an increased risk of incident diabetes. Diabetes Care *34*, 392-397.

Rabol,R., Petersen,K.F., Dufour,S., Flannery,C., and Shulman,G.I. (2011). Reversal of muscle insulin resistance with exercise reduces postprandial hepatic de novo lipogenesis in insulin resistant individuals. Proc. Natl. Acad. Sci. U. S. A *108*, 13705-13709.

Rabot,S., Membrez,M., Bruneau,A., Gerard,P., Harach,T., Moser,M., Raymond,F., Mansourian,R., and Chou,C.J. (2010). Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. FASEB J. *24*, 4948-4959.

Rajilic-Stojanovic,M., Biagi,E., Heilig,H.G., Kajander,K., Kekkonen,R.A., Tims,S., and de Vos,W.M. (2011). Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. Gastroenterology *141*, 1792-1801.

Rajilic-Stojanovic,M. and de Vos,W.M. (2014). The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol. Rev. *38*, 996-1047.

Ramadan,J.W., Steiner,S.R., O'Neill,C.M., and Nunemaker,C.S. (2011). The central role of calcium in the effects of cytokines on beta-cell function: implications for type 1 and type 2 diabetes. Cell Calcium *50*, 481-490.

Ramos,J.S., Dalleck,L.C., Tjonna,A.E., Beetham,K.S., and Coombes,J.S. (2015a). The impact of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. Sports Med. *45*, 679-692.

Ramos,J.S., Dalleck,L.C., Tjonna,A.E., Beetham,K.S., and Coombes,J.S. (2015b). The impact of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. Sports Med. *45*, 679-692.

RANDLE,P.J., GARLAND,P.B., HALES,C.N., and NEWSHOLME,E.A. (1963). The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet *1*, 785-789.

Reaven,G.M. (2005). The insulin resistance syndrome: definition and dietary approaches to treatment. Annu. Rev. Nutr. *25*, 391-406.

Reddy,J.K. and Rao,M.S. (2006). Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. Am. J. Physiol Gastrointest. Liver Physiol *290*, G852-G858.

Rigazio,S., Lehto,H.R., Tuunanen,H., Nagren,K., Kankaanpaa,M., Simi,C., Borra,R., Naum,A.G., Parkkola,R., Knuuti,J., Nuutila,P., and Iozzo,P. (2008). The lowering of hepatic fatty acid uptake improves liver function and insulin sensitivity without affecting hepatic fat content in humans. American Journal of Physiology-Endocrinology and Metabolism *295*, E413-E419.

Rizzo,M. and Berneis,K. (2007). Small, dense low-density-lipoproteins and the metabolic syndrome. Diabetes Metab Res. Rev. *23*, 14-20.

Robertson,M.D., Livesey,G., Hampton,S.M., and Mathers,J.C. (2000). Evidence for altered control of glucose disposal after total colectomy. Br. J. Nutr. *84*, 813-819.

Robertson,R.P., Harmon,J., Tran,P.O., and Poitout,V. (2004). Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. Diabetes *53 Suppl 1*, S119-S124.

Roder,P.V., Wu,B., Liu,Y., and Han,W. (2016). Pancreatic regulation of glucose homeostasis. Exp. Mol. Med. *48*, e219.

Rodriguez-Diaz,J., Garcia-Mantrana,I., Vila-Vicent,S., Gozalbo-Rovira,R., Buesa,J., Monedero,V., and Collado,M.C. (2017). Relevance of secretor status genotype and microbiota composition in susceptibility to rotavirus and norovirus infections in humans. Sci. Rep. *7*, 45559.

Rorsman,P. and Ashcroft,F.M. (2018). Pancreatic beta-Cell Electrical Activity and Insulin Secretion: Of Mice and Men. Physiol Rev. *98*, 117-214.

Ross,M.H.&.P.W. (2003). Histology: A Text and Atlas. Lippincott Williams & Wilkins, Philadelphia.).

Rothman,D.L., Magnusson,I., Katz,L.D., Shulman,R.G., and

Shulman,G.I. (1991). Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with 13C NMR. Science *254*, 573-576.

Rui,L. (2014). Energy metabolism in the liver. Compr. Physiol *4*, 177-197.

Sabaka,P., Kruzliak,P., Balaz,D., Komornikova,A., Celovska,D., Cammarota,G., Kusendova,K., Bendzala,M., Rodrigo,L., Dukat,A., Kwon,T.K., Dvorakova,M.C., and Gaspar,L. (2015). Effect of short term aerobic exercise on fasting and postprandial lipoprotein subfractions in healthy sedentary men. Lipids Health Dis. *14*, 151.

Saloranta,C., Franssila-Kallunki,A., Ekstrand,A., Taskinen,M.R., and Groop,L. (1991). Modulation of hepatic glucose production by non-esterified fatty acids in type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia *34*, 409-415.

Santulli,G., Pagano,G., Sardu,C., Xie,W., Reiken,S., D'Ascia,S.L., Cannone,M., Marziliano,N., Trimarco,B., Guise,T.A., Lacampagne,A., and Marks,A.R. (2015). Calcium release channel RyR2 regulates insulin release and glucose homeostasis. J. Clin. Invest *125*, 4316.

Sanyal,A.J., Chalasani,N., Kowdley,K.V., McCullough,A., Diehl,A.M., Bass,N.M., Neuschwander-Tetri,B.A., Lavine,J.E., Tonascia,J., Unalp,A., Van,N.M., Clark,J., Brunt,E.M., Kleiner,D.E., Hoofnagle,J.H., and Robuck,P.R. (2010). Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N. Engl. J. Med. *362*, 1675-1685.

Sattar,N. and Gill,J.M. (2014). Type 2 diabetes as a disease of ectopic fat? BMC. Med. *12*, 123.

Sears,C.L. (2005). A dynamic partnership: celebrating our gut flora. Anaerobe. *11*, 247-251.

Seppala-Lindroos,A., Vehkavaara,S., Hakkinen,A.M., Goto,T., Westerbacka,J., Sovijarvi,A., Halavaara,J., and Yki-Jarvinen,H. (2002). Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J. Clin. Endocrinol. Metab *87*, 3023-3028.

Shepherd,P.R. and Kahn,B.B. (1999). Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. N. Engl. J. Med. *341*, 248-257.

Shimabukuro,M., Zhou,Y.T., Levi,M., and Unger,R.H. (1998). Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. Proc. Natl. Acad. Sci. U. S. A *95*, 2498-2502.

Shukla,S.K., Cook,D., Meyer,J., Vernon,S.D., Le,T., Clevidence,D., Robertson,C.E., Schrodi,S.J., Yale,S., and Frank,D.N. (2015). Changes in Gut and Plasma Microbiome following Exercise Challenge in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). PLoS. One. *10*, e0145453.

Shulman,G.I. (2014). Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. N. Engl. J. Med. *371*, 1131-1141.

Shulman,G.I., Rothman,D.L., Jue,T., Stein,P., DeFronzo,R.A., and Shulman,R.G. (1990). Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. N. Engl. J. Med. *322*, 223-228.

Singh,R.G., Yoon,H.D., Poppitt,S.D., Plank,L.D., and Petrov,M.S. (2017a). Ectopic fat accumulation in the pancreas and its biomarkers: A systematic review and meta-analysis. Diabetes Metab Res. Rev. *33*.

Singh,R.G., Yoon,H.D., Wu,L.M., Lu,J., Plank,L.D., and Petrov,M.S. (2017b). Ectopic fat accumulation in the pancreas and its clinical relevance: A systematic review, meta-analysis, and meta-regression. Metabolism *69*, 1-13.

Slentz,C.A., Tanner,C.J., Bateman,L.A., Durheim,M.T., Huffman,K.M., Houmard,J.A., and Kraus,W.E. (2009). Effects of exercise training intensity on pancreatic beta-cell function. Diabetes Care *32*, 1807-1811.

Soininen,P., Kangas,A.J., Wurtz,P., Suna,T., and Ala-Korpela,M. (2015). Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circ. Cardiovasc. Genet. *8*, 192-206.

Sreenivasa,B.C., Alexander,G., Kalyani,B., Pandey,R., Rastogi,S., Pandey,A., and Choudhuri,G. (2006). Effect of exercise and dietary modification on serum aminotransferase levels in patients with nonalcoholic steatohepatitis. J. Gastroenterol. Hepatol. *21*, 191-198.

Stanford,K.I. and Goodyear,L.J. (2014). Exercise and type 2 diabetes: molecular mechanisms regulating glucose uptake in skeletal muscle. Adv. Physiol Educ. *38*, 308-314.

Stephen,A.M. and Cummings,J.H. (1980). The microbial contribution to human faecal mass. J. Med. Microbiol. *13*, 45-56.

Steven,S., Hollingsworth,K.G., Small,P.K., Woodcock,S.A., Pucci,A., Aribisala,B., Al-Mrabeh,A., Daly,A.K., Batterham,R.L., and Taylor,R. (2016). Weight Loss Decreases Excess Pancreatic Triacylglycerol Specifically in Type 2 Diabetes. Diabetes Care *39*, 158-165.

Sullivan,S., Kirk,E.P., Mittendorfer,B., Patterson,B.W., and Klein,S. (2012). Randomized trial of exercise effect on intrahepatic triglyceride content and lipid kinetics in nonalcoholic fatty liver disease. Hepatology *55*, 1738-1745.

Sunny,N.E., Bril,F., and Cusi,K. (2017). Mitochondrial Adaptation in Nonalcoholic Fatty Liver Disease: Novel Mechanisms and Treatment Strategies. Trends Endocrinol. Metab *28*, 250-260.

Szczepaniak,L.S., Babcock,E.E., Schick,F., Dobbins,R.L., Garg,A., Burns,D.K., McGarry,J.D., and

Stein,D.T. (1999). Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. Am. J. Physiol *276*, E977-E989.

Szczepaniak,L.S., Nurenberg,P., Leonard,D., Browning,J.D., Reingold,J.S., Grundy,S., Hobbs,H.H., and Dobbins,R.L. (2005). Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am. J. Physiol Endocrinol. Metab *288*, E462-E468.

Tabata,I., Nishimura,K., Kouzaki,M., Hirai,Y., Ogita,F., Miyachi,M., and Yamamoto,K. (1996). Effects of moderate-intensity endurance and high-intensity intermittent training on anaerobic capacity and VO2max. Med. Sci. Sports Exerc. *28*, 1327-1330.

Takala,T.O., Nuutila,P., Pulkki,K., Oikonen,V., Gronroos,T., Savunen,T., Vahasilta,T., Luotolahti,M., Kallajoki,M., Bergman,J., Forsback,S., and Knuuti,J. (2002). 14(R,S)-[18F]Fluoro-6-thia-heptadecanoic acid as a tracer of free fatty acid uptake and oxidation in myocardium and skeletal muscle. Eur. J. Nucl. Med. Mol. Imaging *29*, 1617-1622.

Thomsen,C., Becker,U., Winkler,K., Christoffersen,P., Jensen,M., and Henriksen,O. (1994). Quantification of liver fat using magnetic resonance spectroscopy. Magn Reson. Imaging *12*, 487-495.

Thorens,B. (1996). Glucose transporters in the regulation of intestinal, renal, and liver glucose fluxes. Am. J. Physiol *270*, G541-G553.

Tilg,H. and Moschen,A.R. (2014). Microbiota and diabetes: an evolving relationship. Gut *63*, 1513-1521.

Tobias,P.S. and Ulevitch,R.J. (1993). Lipopolysaccharide binding protein and CD14 in LPS dependent macrophage activation. Immunobiology *187*, 227-232.

Tobin,V., Le,G.M., Fioramonti,X., Stolarczyk,E., Blazquez,A.G., Klein,C., Prigent,M., Serradas,P., Cuif,M.H., Magnan,C., Leturque,A., and Brot-Laroche,E. (2008). Insulin internalizes GLUT2 in the enterocytes of healthy but not insulin-resistant mice. Diabetes *57*, 555-562.

Toschi,E., Camastra,S., Sironi,A.M., Masoni,A., Gastaldelli,A., Mari,A., Ferrannini,E., and Natali,A. (2002). Effect of acute hyperglycemia on insulin secretion in humans. Diabetes *51 Suppl 1*, S130-S133.

Trier J.S (1968). Morphology of the epithelium of the small intestine. Handb. Physiol *3 (Sect 6)*, 1125-1175.

Trilk,J.L., Singhal,A., Bigelman,K.A., and Cureton,K.J. (2011). Effect of sprint interval training on circulatory function during exercise in sedentary, overweight/obese women. Eur. J. Appl. Physiol *111*, 1591-1597.

Trost,S.G., Owen,N., Bauman,A.E., Sallis,J.F., and Brown,W. (2002). Correlates of adults' participation in physical activity: review and update.

Med. Sci. Sports Exerc. *34*, 1996-2001.

Tuovinen,E., Keto,J., Nikkila,J., Matto,J., and Lahteenmaki,K. (2013). Cytokine response of human mononuclear cells induced by intestinal Clostridium species. Anaerobe. *19*, 70-76.

Turk,Y., Theel,W., Kasteleyn,M.J., Franssen,F.M.E., Hiemstra,P.S., Rudolphus,A., Taube,C., and Braunstahl,G.J. (2017). High intensity training in obesity: a Meta-analysis. Obes. Sci. Pract. *3*, 258-271.

Turnbaugh,P.J., Ley,R.E., Mahowald,M.A., Magrini,V., Mardis,E.R., and Gordon,J.I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. Nature *444*, 1027-1031.

van der Heijden,G.J., Wang,Z.J., Chu,Z.D., Sauer,P.J., Haymond,M.W., Rodriguez,L.M., and Sunehag,A.L. (2010). A 12-week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, Hispanic adolescents. Obesity. (Silver. Spring) *18*, 384-390.

van der Windt,D.J., Sud,V., Zhang,H., Tsung,A., and Huang,H. (2018a). The Effects of Physical Exercise on Fatty Liver Disease. Gene Expr. *18*, 89-101.

van der Windt,D.J., Sud,V., Zhang,H., Tsung,A., and Huang,H. (2018b). The Effects of Physical Exercise on Fatty Liver Disease. Gene Expr. *18*, 89-101.

Van,C.E., Mestrez,F., Sturis,J., and Polonsky,K.S. (1992). Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes *41*, 368-377.

Vilar-Gomez,E., Martinez-Perez,Y., Calzadilla-Bertot,L., Torres-Gonzalez,A., Gra-Oramas,B., Gonzalez-Fabian,L., Friedman,S.L., Diago,M., and Romero-Gomez,M. (2015). Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. Gastroenterology *149*, 367-378.

Viljanen,A.P., Iozzo,P., Borra,R., Kankaanpaa,M., Karmi,A., Lautamaki,R., Jarvisalo,M., Parkkola,R., Ronnemaa,T., Guiducci,L., Lehtimaki,T., Raitakari,O.T., Mari,A., and Nuutila,P. (2009). Effect of weight loss on liver free fatty acid uptake and hepatic insulin resistance. J. Clin. Endocrinol. Metab *94*, 50-55.

Virtanen,K.A., Lonnroth,P., Parkkola,R., Peltoniemi,P., Asola,M., Viljanen,T., Tolvanen,T., Knuuti,J., Ronnemaa,T., Huupponen,R., and Nuutila,P. (2002). Glucose uptake and perfusion in subcutaneous and visceral adipose tissue during insulin stimulation in nonobese and obese humans. J. Clin. Endocrinol. Metab *87*, 3902-3910.

Vrieze,A., Holleman,F., Zoetendal,E.G., de Vos,W.M., Hoekstra,J.B., and Nieuwdorp,M. (2010). The environment within: how gut microbiota may influence

metabolism and body composition. Diabetologia *53*, 606-613.

Vrieze,A., Van,N.E., Holleman,F., Salojarvi,J., Kootte,R.S., Bartelsman,J.F., Dallinga-Thie,G.M., Ackermans,M.T., Serlie,M.J., Oozeer,R., Derrien,M., Druesne,A., Van Hylckama Vlieg,J.E., Bloks,V.W., Groen,A.K., Heilig,H.G., Zoetendal,E.G., Stroes,E.S., de Vos,W.M., Hoekstra,J.B., and Nieuwdorp,M. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology *143*, 913-916.

Wallin,T., Ma,Z., Ogata,H., Jorgensen,I.H., Iezzi,M., Wang,H., Wollheim,C.B., and Bjorklund,A. (2010). Facilitation of fatty acid uptake by CD36 in insulin-producing cells reduces fatty-acid-induced insulin secretion and glucose regulation of fatty acid oxidation. Biochim. Biophys. Acta *1801*, 191-197.

Ward,W.K., Bolgiano,D.C., McKnight,B., Halter,J.B., and Porte,D., Jr. (1984). Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. J. Clin. Invest *74*, 1318-1328.

Westerbacka,J., Corner,A., Tiikkainen,M., Tamminen,M., Vehkavaara,S., Hakkinen,A.M., Fredriksson,J., and Yki-Jarvinen,H. (2004). Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. Diabetologia *47*, 1360-1369.

Weston,K.S., Wisloff,U., and Coombes,J.S. (2014). High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. Br. J. Sports Med. *48*, 1227-1234.

Wilcox,G. (2005). Insulin and insulin resistance. Clin. Biochem. Rev. *26*, 19-39.

Winn,N.C., Liu,Y., Rector,R.S., Parks,E.J., Ibdah,J.A., and Kanaley,J.A. (2018). Energy-matched moderate and high intensity exercise training improves nonalcoholic fatty liver disease risk independent of changes in body mass or abdominal adiposity - A randomized trial. Metabolism *78*, 128-140.

Wisloff,U., Ellingsen,O., and Kemi,O.J. (2009). High-intensity interval training to maximize cardiac benefits of exercise training? Exerc. Sport Sci. Rev. *37*, 139-146.

Wolin,K.Y., Yan,Y., Colditz,G.A., and Lee,I.M. (2009). Physical activity and colon cancer prevention: a meta-analysis. Br. J. Cancer *100*, 611-616.

Wright,E.M., Martin,M.G., and Turk,E. (2003). Intestinal absorption in health and disease--sugars. Best. Pract. Res. Clin. Gastroenterol. *17*, 943-956.

Yamada,M., Hokazono,C., and Okutsu,M. (2018). Maternal exercise training attenuates endotoxin-

induced sepsis in mice offspring. Biochem. Biophys. Rep. *15*, 19-24.

Yaney,G.C. and Corkey,B.E. (2003). Fatty acid metabolism and insulin secretion in pancreatic beta cells. Diabetologia *46*, 1297-1312.

Yki–Jarvinen,H. (2005). Fat in the liver and insulin resistance. Annals of Medicine *37*, 347-356.

Zhang,H., DiBaise,J.K., Zuccolo,A., Kudrna,D., Braidotti,M., Yu,Y., Parameswaran,P., Crowell,M.D., Wing,R., Rittmann,B.E., and Krajmalnik-Brown,R. (2009). Human gut microbiota in obesity and after gastric bypass. Proc. Natl. Acad. Sci. U. S. A *106*, 2365-2370.

Zhao,X., Zhang,Z., Hu,B., Huang,W., Yuan,C., and Zou,L. (2018). Response of Gut Microbiota to Metabolite Changes Induced by Endurance Exercise. Front Microbiol. *9*, 765.

Zierath,J.R., He,L., Guma,A., Odegoard,W.E., Klip,A., and Wallberg-Henriksson,H. (1996). Insulin action on glucose transport and plasma membrane GLUT4 content in skeletal muscle from patients with NIDDM. Diabetologia *39*, 1180-1189.

APPENDICES

Appendix table 1: The baseline differences between normoglycemic and prediabetic/T2D men in different lipoprotein subclasses and components.

Parameter	Normoglycemic	Prediabetic/T2D*	Baseline p value
Lipids (mmol/L)			
Extremely-Large VLDL	0.01 [0.01, 0.02]	0.04 [0.03, 0.05]	<.0001
Extra-large VLDL	0.03 [0.02, 0.05]	0.09 [0.06, 0.13]	<.001
Large VLDL	0.17 [0.13, 0.22]	0.35 [0.26, 0.48]	<.0001
Medium VLDL	0.41 [0.34, 0.50]	0.67 [0.52, 0.87]	<.01
Small VLDL	0.047 [0.42, 0.54]	0.61 [0.51, 0.72]	0.047
IDL	1.06 [0.95, 0.17]	0.81 [0.70, 0.93]	<.01
Large LDL	1.29 [1.15, 1.44]	0.95 [0.82, 1.11]	<.001
Medium LDL	0.79 [0.71, 0.87]	0.59 [0.50, 0.69]	<.01
Small LDL	0.51 [0.46, 0.56]	0.40 [0.34, 0.46]	<.01
Small HDL	1.10 [1.06, 1.14]	1.19 [1.14, 1.25]	<.01
Phospholipids (mmol/L)			
Extremely-Large VLDL	0.001 [0.001, 0.001]	0.004 [0.003, 0.007]	<.0001
Extra-large VLDL	0.005 [0.003, 0.009]	0.012 [0.007, 0.022]	<.0001
Large VLDL	0.03 [0.02, 0.04]	0.06 [0.04, 0.08]	<.0001
Medium VLDL	0.006 [0.004, 0.009]	0.015 [0.009, 0.025]	<.001
Small VLDL	0.11 [0.10, 0.13]	0.15 [0.13, 0.17]	0.01
Extra-small VLDL	0.14 [0.13, 0.15]	0.12 [0.10, 0.13]	0.02
IDL	0.29 [0.27, 0.33]	0.22 [0.19, 0.25]	<.0001
Large LDL	0.33 [0.30, 0.36]	0.25 [0.23, 0.29]	<.001
Medium LDL	0.20 [0.19, 0.22]	0.17 [0.15, 0.19]	0.02
Extra-large HDL	0.16 [0.13, 0.20]	0.10 [0.07, 0.14]	0.02
Small HDL	0.54 [0.52, 0.56]	0.65 [0.62, 0.69]	<.0001
Cholesterol (mmol/L)			
Extremely-Large VLDL	0.001 [0.001, 0.002]	0.004 [0.002, 0.007]	<.001
Extra-large VLDL	0.005 [0.003, 0.008]	0.011 [0.007, 0.018]	<.001
Large VLDL	0.03 [0.02, 0.04]	0.06 [0.04, 0.09]	<.001
Extra-small VLDL	0.21 [0.19, 0.23]	0.17 [0.15, 0.19]	<.001
IDL	0.68 [0.61, 0.75]	0.49 [0.41, 0.57]	<.01
Large LDL	0.90 [0.80, 1.01]	0.62 [0.51, 0.74]	<.0001
Medium LDL	0.54 [0.48, 0.61]	0.37 [0.30, 0.45]	<.01
Small LDL	0.33 [0.29, 0.37]	0.23 [0.19, 0.27]	<.01

Cholesterol esters (mmol/L)			
Extra-Large VLDL	0.003 [0.002, 0.004]	0.006 [0.004, 0.010]	<.001
Large VLDL	0.02 [0.01, 0.03]	0.03 [0.02, 0.04]	<.001
Extra-small VLDL	0.14 [0.13, 0.16]	0.11 [0.10, 0.13]	<.001
IDL	0.48 [0.43, 0.53]	0.36 [0.30, 0.42]	<.01
Large LDL	0.64 [0.57, 0.73]	0.44 [0.36, 0.53]	<.001
Medium LDL	0.39 [0.34, 0.45]	0.25 [0.20, 0.31]	<.01
Small LDL	0.24 [0.21, 0.27]	0.16 [0.13, 0.19]	<.001
Small HDL	0.40 [0.38, 0.43]	0.36 [0.33, 0.39]	0.02
Free Cholesterol (mmol/L)			
Extremely-Large VLDL	0.0004 [0.0003, 0.0007]	0.0024 [0.0014, 0.0043]	<.0001
Extra-large VLDL	0.002 [0.001, 0.004]	0.005 [0.003, 0.009]	<.001
Large VLDL	0.01 [0.01, 0.02]	0.04 [0.02, 0.05]	<.001
Medium VLDL	0.05 [0.03, 0.06}	0.08 [0.06, 0.10]	<.001
Small VLDL	0.07 [0.06, 0.07]	0.08 [0.07, 0.10]	0.04
Extra-small VLDL	0.07 [0.06, 0.07]	0.06 [0.05, 0.07]	0.03
IDL	0.20 [0.18, 0.22]	0.13 [0.11, 0.16]	<.0001
Large LDL	0.26 [0.23, 0.28]	0.18 [0.15, 0.21]	<.0001
Medium LDL	0.15 [0.14, 0.16]	0.12 [0.10, 0.13]	<.0001
Small LDL	0.09 [0.08, 0.10]	0.07 [0.06, 0.08]	<.01
Extra-large HDL	0.05 [0.04, 0.06]	0.03 [0.02, 0.04]	<.01
Large HDL	0.05 [0.04, 0.06]	0.03 [0.02, 0.04]	0.04
Small HDL	0.10 [0.10, 0.11]	0.12 [0.11, 0.12]	<.0001
Triglycerides (mmol/L)			
Extremely-Large VLDL	0.01 [0.008, 0.013]	0.03 [0.020, 0.039]	<.0001
Extra-large VLDL	0.02 [0.02, 0.03]	0.06 [0.04, 0.09]	<.0001
Large VLDL	0.10 [0.08, 0.13]	0.22 [0.17, 0.30]	<.0001
Medium VLDL	0.22 [0.18, 0.27]	0.39 [0.31, 0.51]	<.0001
Small VLDL	0.19 [0.17, 0.22]	0.28 [0.24, 0.34]	<.0001
Extra-small VLDL	0.09 [0.08, 0.10]	0.12 [0.10, 0.13]	0.02
Medium HDL	0.03 [0.03, 0.04]	0.05 [0.04, 0.05]	<.001
Small HDL	0.04 [0.04, 0.05]	0.06 [0.05, 0.07]	<.0001

T2D, type 2 diabetes; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein and HDL, high density lipoprotein. Baseline p value indicates the difference between the lipoprotein subclass and components at baseline between the normoglycemic and prediabetic/T2D men.

Appendix table 2: Correlations at baseline between the different sub-components of large and small HDL lipoprotein classes and general parameters.

Components	Lipoprotein components	General parameters	r	p
Lipids	Extra-large HDL§	M-value§	0.49	<.001
	Extra-large HDL§	ALAT§	-0.38	0.01
	Extra-large HDL§	Liver volume§	-0.50	<.001
	Small HDL	M-value§	-0.46	<.001
	Small HDL	VO_{2peak}	-0.37	0.01
	Small HDL	Hepatic fat content&	0.35	0.02
	Small HDL	Liver volume§	0.36	0.01
Phospholipids	Extra-large HDL&	M-value§	0.35	0.01
	Extra-large HDL&	Liver volume§	-0.39	<.001
	Small HDL§	M-value§	-0.59	<.001
	Small HDL§	VO_{2peak}	-0.54	<.001
	Small HDL§	Hepatic fat content&	0.50	<.001
	Small HDL§	Liver volume§	0.46	<.001
Cholesterol	Extra-large HDL§	M-value§	0.42	<.01
	Extra-large HDL§	ALAT§	-0.37	0.01
	Extra-large HDL§	Liver volume§	-0.43	<.001
Cholesterol esters	Extra-large HDL§	Liver volume§	-0.29	0.03
Free cholesterol	Extra-large HDL§	M-value§	0.44	<.01
	Extra-large HDL§	ALAT§	-0.29	0.03
	Extra-large HDL§	Liver volume§	-0.44	<.001
	Small HDL§	M-value§	-0.50	<.001
	Small HDL§	Hepatic fat content&	0.41	0.01
	Small HDL§	Liver volume§	0.40	<.01

Triglycerides	Small HDL§	M-value§	-0.66	<.001
	Small HDL§	VO_{2peak}	-0.42	<.001
	Small HDL§	ALAT§	0.41	<.001
	Small HDL§	Hepatic fat content&	0.54	<.001
	Small HDL§	Liver volume§	0.52	<.001

HDL, high density lipoprotein; M-value, whole-body insulin sensitivity; ALAT, alanine transaminase; VO_{2peak}, aerobic capacity. (§) Log transformation and (&) square root transformation was performed to achieve normal distribution.

Annales Universitatis Turkuensis

UNIVERSITY OF TURKU

Grano Oy - Turku, Finland 2019

ISBN 978-951-29-7662-1 (PRINT)
ISBN 978-951-29-7663-8 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)