UNIVERSITY OF TURKU

TISSUE-SPECIFIC INSULIN SENSITIVITY IN HUMANS – WITH SPECIAL REFERENCE TO THE LIVER

Miikka-Juhani Honka

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
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ABSTRACT

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Tissue-specific insulin sensitivity in humans - with special reference to the liver

University of Turku, Faculty of Medicine, Internal Medicine, Doctoral Programme in Clinical Research, Turku PET Center, Turku, Finland

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The number of individuals affected by Type 2 diabetes mellitus (T2DM) is increasing rapidly nearly everywhere in the world. Insulin resistance, which means reduced impact of insulin in its target tissues, especially in the liver, skeletal muscle and adipose tissue, is the defining hallmark of T2DM. Insulin resistance in these tissues manifests as impaired ability to take up glucose and fatty acids from blood after a meal, increased production of glucose and triglycerides into the blood circulation by the liver and increased release of free fatty acids into the blood by adipose tissue.

This thesis work focuses on studying insulin resistance using a cross-sectional cohort of a wide range of individuals of different ages and body mass index (BMI). P.Pro12Ala polymorphism of the PPARG gene reduces diabetes risk, and the aim of this study was to determine whether this variant affects liver insulin sensitivity. Further goals were to study associations between insulin sensitivity in different tissues and to develop inexpensive and fast models to identify muscle and whole-body insulin resistance. Moreover, possible benefits of resistance training to liver and adipose tissue insulin sensitivity among elderly women were evaluated.

It was found that overweight and obese carriers of the p.Pro12Ala polymorphism of the PPARG gene display higher insulin-stimulated liver glucose uptake when compared to carriers of the common p.Pro12Pro genotype. It was discovered that insulin resistance is more likely to be present simultaneously in skeletal muscle and adipose tissue than in the liver, and that insulin sensitivity is affected by obesity, sex and age. The developed regression models based on serum metabolomics for identifying whole-body and skeletal muscle insulin resistance correlated with insulin sensitivity better than the currently used fasting surrogate markers for insulin resistance. Moreover, it was revealed resistance training does not affect adipose tissue glucose uptake but instead improves insulin suppression of endogenous glucose production in elderly women and may thus prevent glucose levels rising too high after a meal.

In conclusion, this thesis work shows that genetic mutations can alter tissue insulin sensitivity, and insulin resistance tends to be simultaneously present in several tissues. The newly developed models for identifying insulin resistance may improve the possibility of finding persons at risk of diabetes and ultimately cardiovascular disease. In addition, resistance training is an effective tool for improving diabetes and cardiovascular disease risk factors.

Keywords: PET, insulin sensitivity, liver, muscle, adipose tissue, obesity

TIIVISTELMÄ

Miikka-Juhani Honka
Insuliinin vaikutus ihmisen kudoksissa – erityisesti maksassa

Turun yliopisto, Lääketieteellinen tiedekunta, Sisätautioppi, Turun kliininen tohtoriohjelma, PET-keskus, Turku, Suomi

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Tyypin 2 diabetes lisääntyy nopeasti lähes kaikkialla maailmassa. Tyypin 2 diabetekselle on tunnusomaista erityisesti lihaksessa, maksassa sekä rasvakudoksessa esiintyvä insuliiniresistenssi. Insuliiniresistenssi esiintyy näissä kudoksissa heikentyneenä kykynä ottaa sokeria ja rasvahappoja verenkierrosta aterian jälkeen. Lisäksi maksassa se esiintyy lisääntyneenä glukoosin ja triglyseridien tuottamisena ja rasvakudoksessa lisääntyneenä rasvahappojen erityksenä verenkiertoon.

Tässä väitöskirjatyössä tutkittiin insuliiniresistenssin esiintymistä eri kudoksissa positroniemissiotomografiaa hyödyntäen kohortissa, jonka ikä- ja painoindeksijakauma on laaja. Työssä selvitettiin vaikuttaako diabetekselta suojaava PPARG-geenin p.Pro12Ala-alleeli maksan insuliiniherkkyyteen sekä kehitettiin yksinkertaisia malleja, joita voidaan hyödyntää koko kehon ja luurankolihaksen insuliiniresistenssin tunnistamisessa. Lisäksi tutkittiin voidaanko lihasvoimaharjoittelun avulla parantaa maksan ja rasvakudoksen insuliiniherkkyyttä iäkkäillä naisilla.

Insuliiniherkkyyden geneettistä taustaa selvitettäessä havaittiin maksan glukoosinkäyttökyvyn olevan suurempi ylipainoisilla ja lihavilla PPARG-geenin p.Pro12Ala-alleelin kantajilla verrattuna p.Pro12Pro-genotyyppiä kantaviin. Työssä havaittiin lisäksi, että insuliiniresistenssi esiintyy useammin yhtäaikaisesti luurankolihaksessa ja rasvakudoksessa kuin maksassa sekä todettiin lihavuuden, sukupuolen ja iän olevan insuliiniherkkyyteen vaikuttavia tekijöitä. Työssä koko kehon ja luurankolihaksen insuliiniherkkyyden mittaamiseksi luodut metabolomiikkaan perustuvat mallit ovat tarkempia, kuin tällä hetkellä suurissa kliinisissä tutkimuksissa käytössä olevat insuliiniherkkyyttä mittaavat epäsuorat indeksit. Lihasvoimaharjoittelua käsittelevässä työssä havaittiin, että harjoittelu ei vaikuta rasvakudoksen insuliiniherkkyyteen, mutta parantaa insuliinin kykyä vähentää maksan glukoosintuotantoa iäkkäillä naisilla, mikä puolestaan voi ehkäistä verensokerin kohoamista.

Tässä työssä osoitettiin, että p.Pro12Ala polymorfia parantaa maksan insuliiniherkkyyttä ja insuliiniresistenssi esiintyy usein useissa kudoksissa yhtäaikaisesti. Työssä kehitettiin lupaavia malleja insuliiniresistenssin tunnistamiseen, jotka saattavat auttaa löytämään henkilöitä, joilla on kohonnut riski tyypin 2 diabeteksen ja lopulta sydän- ja verenkiertosairauksien kehittymiseen. Lisäksi työssä näytettiin, kuinka tyypin 2 diabeteksen ja sydän- ja verisuonitautien riskitekijöitä voidaan vähentää voimaharjoittelun avulla.

Avainsanat: PET, insuliiniherkkyys, maksa, rasvakudos, luurankolihas, lihavuus

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ABBREVIATIONS

18:3n−6	γ-linolenic acid
20:3n−6	Dihomo-γ-linolenic acid
^{18}F-FDG	2-deoxy-2-(^{18}F)fluoro-D-glucose
ADA	American Diabetes Association
ALT	Alanine aminotransferase
ANKRD55	Ankyrin Repeat Domain 55
ANOVA	Analysis of variance
ApoC-III	Apolipoprotein C-III
ARL15	ADP-ribosylation factor-like 15
AST	Aspartate amino transferase
ATP	Adenosine triphosphate
AUC	Area under the curve
BCAA	Branched-chained amino acid
BCL2	B-cell lymphoma 2
BMI	Body mass index
CoA	Coenzyme A
COBLL1	Cordon-Bleu WH2 Repeat Protein Like 1
CPT1	Carnitine palmitoyltransferase
CT	Computed tomography
CTR	Control group
DNA	Deoxyribonucleic acid
DNL	*De novo* lipogenesis
EGP	Endogenous glucose production
eNOS	Endothelial nitric oxide synthase
FA	Fatty acid
FAM13A	Family with sequence similarity 13, member A
FAM19A2	Family with sequence similarity 19, member A2
FFA	Free fatty acid
FFA2, -3	Free fatty acid receptor 2 and 3
FI1	Fat index 1
FoxO	Forkhead box-containing protein O
FSIVGTT	Frequently sampled intravenous glucose tolerance test
G1P	Glucose-1-phosphate
G6P	Glucose-6-phosphate
GCKR	Glucokinase regulatory protein

GIP	Gastric inhibitory peptide
GKRP	Glucokinase regulatory protein
GLP-1	Glucagon-like peptide 1
GLUT1, -2, -4	Glucose transporters 1, 2, and 4
GRB14	Growth factor receptor-bound protein 14
GSK3	Glycogen synthase kinase 3
GU	Glucose uptake
HDL	High-density lipoprotein
HL	Hepatic lipase
HMG-CoA	3-hydroxy-3-methyl glutaryl coenzyme A
HOMA-IR	Homeostatic model assessment of insulin resistance
HSL	Hormone-sensitive lipase
IDF	International Diabetes Federation
IDL	Intermediate-density lipoprotein
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
ILT	Inside lean tissue method
INTR	Intervention group
IQR	Interquartile range
IR	Insulin resistant group
IRS	Insulin receptor substrate
IST	Insulin suppression test
JNK	cJun-N-terminal-kinase
KLF14	Krüppel-like factor 14
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
LYPLAL1	Lysophospholipase-like 1
MAP3K1	Mitogen-Activated Protein Kinase Kinase Kinase 1
MAPK	Mitogen-activated protein kinase
MEK	Mitogen-activated protein kinase kinase
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
mTOR	Mammalian target of rapamycin
MUFA	Monounsaturate fatty acid
Muscle-ISI	Muscle insulin sensitivity index
NAFLD	Non-alcoholic fatty liver disease
ND	Non-diabetic group
NMR	Nucleic magnetic resonance

OGIS	Oral glucose insulin sensitivity
OGTT	Oral glucose tolerance test
OLM	Offspring of lean/normal-weight mothers
OOM	Offspring of overweight/obese mothers
PCA	Principal component analysis
PDGFC	Platelet-derived growth factor C
PDK	Phosphoinositide-dependent protein kinase
PEPCK	Phophoenolpyruvate carboxykinase
PEPD	Peptidase D
PET	Positron emission tomography
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PIP	Phophatidylinositol-3,4,5-trisphosphate
PKC	Protein kinase C
PNPLA3	Patatin-like phospholipase domain-containing protein 3
PPARG	Peroxisome proliferator receptor gamma (gene)
PPARγ	Peroxisome proliferator receptor gamma
PPP1R3B	Protein phosphatase 1 regulatory subunit 3B
PREDIM	PREDIcted M
PTB	Phosphotyrosine binding
PUFA	Polyunsaturated fatty acid
QUICKI	Quantitative insulin sensitivity check index
ROC	Receiver-operating characteristics
ROI	Region of interest
SCFA	Short-chain fatty acid
SFA	Saturated fatty acid
SD	Standard deviation
SH2	Src-homology 2
T2DM	Type 2 diabetes mellitus
TCA	Tricarboxylic acid
TET2	Tet methylcytosine dioxygenase 2
TM6SF2	Transmembrane 6 superfamily 2
TG	Triglycerides
UDP	Uridine diphosphate
UHRF1BP1	UHRF1 binding protein 1
USD	Unites States dollar
VLDL	Very low-density lipoprotein
WB-ISI	Whole-body insulin sensitivity index
WHO	World Health Organisation

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are later referred by using Roman numerals I-IV.

I. Honka MJ*, Vänttinen M*, Iozzo P, Virtanen KA, Lautamäki R, Hällsten K, Borra RJ, Takala T, Viljanen AP, Kemppainen J, Pihlajamäki J, Knuuti J, Nuutila P, Laakso M. The Pro12Ala polymorphism of the PPARgamma2 gene is associated with hepatic glucose uptake during hyperinsulinemia in subjects with type 2 diabetes mellitus. Metabolism. 2009 Apr;58(4):541-6.

II. Honka MJ, Latva-Rasku A, Bucci M, Virtanen KA, Hannukainen JC, Kalliokoski KK, Nuutila P. Insulin stimulated glucose uptake in skeletal muscle, adipose tissue and liver: a positron emission tomography study. Eur J Endocrinol. 2018 May;178(5):523-531.

III. Klén R*, Honka MJ*, Hannukainen JC, Huovinen V, Bucci M, Latva-Rasku A, Venäläinen MS, Kalliokoski KK, Virtanen KA, Lautamäki R, Iozzo P, Elo LL, Nuutila P. Predicting skeletal muscle insulin sensitivity - a study combining [^{18}F]FDG-PET and NMR-based metabolomic profiling. Manuscript.

IV. Honka MJ, Bucci M, Andersson J, Huovinen V, Guzzardi MA, Sandboge S, Savisto N, Salonen MK, Badeau RM, Parkkola R, Kullberg J, Iozzo P, Eriksson JG, Nuutila P. Resistance training enhances insulin suppression of endogenous glucose production in elderly women. J Appl Physiol. 2016 Mar 15;120(6):633-9.

*equal contribution

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1 INTRODUCTION

Type 2 diabetes is currently one of the largest global health concerns. Over 425 million adults between the ages of 20 and 79 have been estimated to have diabetes, 90% of whom suffer from type 2 diabetes mellitus (T2DM) (International Diabetes Federation 2017). Diabetes is associated with major morbidity and high mortality. Its complications include, for example, cardiovascular diseases, nephropathy, neuropathy and retinopathy. Diabetes and its complications are estimated to cause 4 million annual deaths among adults (10.7% of all deaths in this age group) and require 727 billion USD in health care costs (International Diabetes Federation 2017). Cardiovascular diseases are the leading cause of death worldwide, with 17.7 million deaths annually (31% of all deaths) (World Health Organization 2018a).

Insulin resistance is a major pathophysiological feature behind the development of T2DM and cardiovascular diseases. It is characterised as reduced insulin stimulated glucose uptake (GU) into insulin sensitive organs, such as skeletal muscle, the liver and adipose tissue and deteriorated suppression of glucose production from the liver and kidney as well as lower suppression of lipolytic release of free fatty acids (FFAs) from triglycerides by insulin. Reduced skeletal muscle GU and increased endogenous glucose production after a meal together with impaired insulin secretion from the pancreas are the central factors leading to hyperglycemia and type 2 diabetes (DeFronzo 2009). In addition to disrupting glucose homeostasis, insulin resistance causes atherogenic dyslipidemia by overproduction of triglyceride-rich VLDL particles and reduction of lipoprotein lipase (LPL)-mediated removal of triglycerides from these particles (Taskinen and Borén 2015).

Western-style diet, physical inactivity, genetic risk variants and epigenetic DNA modifications are factors affecting insulin sensitivity. Central obesity with ectopic fat accumulation is a significant risk factor for insulin resistance. In 2016 more than 650 million people in the world were obese (World Health Organization 2018b). Thus, the widespread obesity pandemic has predisposed hundreds of millions of people to insulin resistance and its consequent comorbidities.

Lack of exercise is a considerable risk factor for obesity and insulin resistance. More than one quarter of the world's population is insufficiently physically active (less than 150 min of moderate-intensity or 75 min vigorous-intensity physical activity per week) (Guthold et al. 2018). Insufficient physical activity is especially common among older adults (Berkemeyer et al. 2016; NatCen Social Research and UCL 2017). This is exemplified in the frailty syndrome where aged individuals suffer from reduced muscle strength, endurance and physiologic function (Morley et al. 2013), which is often preceded by insulin resistance (Barzilay et al. 2007; Perez-Tasigchana et al. 2017).

Genetic association studies have shown that most of the mutations that increase T2DM risk affect insulin secretion and only a small fraction of the known common variants affect insulin sensitivity (Stancakova and Laakso 2016). In addition to genetic inheritance, epigenetic programming which does not affect the DNA sequence, but alters gene expression, is getting recognition as a factor affecting risk of cardiovascular and metabolic diseases. An example of epigenetic programming is maternal obesity before or during gestation, which predisposes offspring to obesity, insulin resistance, type 2 diabetes and cardiovascular disease (Eriksson et al. 2014; Godfrey et al. 2017; Hochner et al. 2012; Z. Yu et al. 2013). There is currently very limited knowledge about the effects that the genetic risk variants or maternal obesity may have on insulin sensitivity in different tissues of the predisposed persons.

Lifestyle interventions, such as diet and physical exercise, have shown to be effective for alleviating insulin resistance and preventing T2DM. However, the best prognosis is achieved when intervening early and identifying persons with high disease risk (Tuomilehto and Schwarz 2016). Despite huge prevalence of insulin resistance, there is no simple test used to detect it in a clinical setting at the moment. Instead, the treatment decisions are based on the patient's level of obesity, plasma glucose level and serum lipid profiles. Although these measures are associated with insulin resistance (Alberti et al. 2005), more specific measures may provide additional benefit.

This work was focused on studying tissue-specific insulin sensitivity by using state-of-the-art metabolic imaging, evaluating factors affecting insulin sensitivity and investigating methods to identify or treat insulin resistance in at-risk individuals.

2 REVIEW OF LITERATURE

2.1 Insulin action and energy metabolism

2.1.1 Glucose as an energy source

Glucose is one of the main substrates providing energy for body functions. It is especially important for the brain function because neurons cannot use fatty acids efficiently as an energy source (Schönfeld and Reiser 2013). In addition, glucose is needed as a rapid energy source for intensive skeletal muscle work. In the brain, muscles and white adipose tissue, adenosine triphosphate (ATP), a carrier of cellular energy, is generated from glycolysis and oxidation of glucose. Glycolysis is a fast process occurring in the cytosol, whereby glucose is metabolised to pyruvate or lactate, yielding two molecules of ATP for immediate energy needs. The resulting pyruvate or lactate can be further processed in a citric acid cycle (tricarboxylic acid or Krebs cycle) in mitochondria to gain additional 32-34 ATPs or released to circulation (Brooks 2018). Lactate released from skeletal muscle is an important substrate for the formation of glucose in gluconeogenesis in the liver. The cycling of carbons as lactate from skeletal muscle to the liver and back as glucose is called the Cori cycle. In addition, lactate produced from glucose by glycolysis and released to blood is an important systemic energy carrier to feed the citric acid cycle in various tissues (Brooks 2018; Hui et al. 2017; van Hall 2010).

2.1.2 Glucose homeostasis in fasting

Plasma glucose is under tight hormonal control to meet the constant need of glucose by the brain, on one hand, and to avoid harmful effects of hyperglycaemia, on the other hand. At a postabsorptive state glucose needed for body functions is provided at an approximate rate of 10-12 µmol/kg body weight/min by gluconeogenesis and glycogenolysis in the liver and gluconeogenesis in the kidneys. Both gluconeogenesis and glycogenolysis yield around 50% of the total production (Nuttall, Ngo, Gannon 2008; Shrayyef and Gerich 2010). Contributions of different tissues to glucose appearance and disappearance at a postabsorptive state are shown in Figure 2.1.

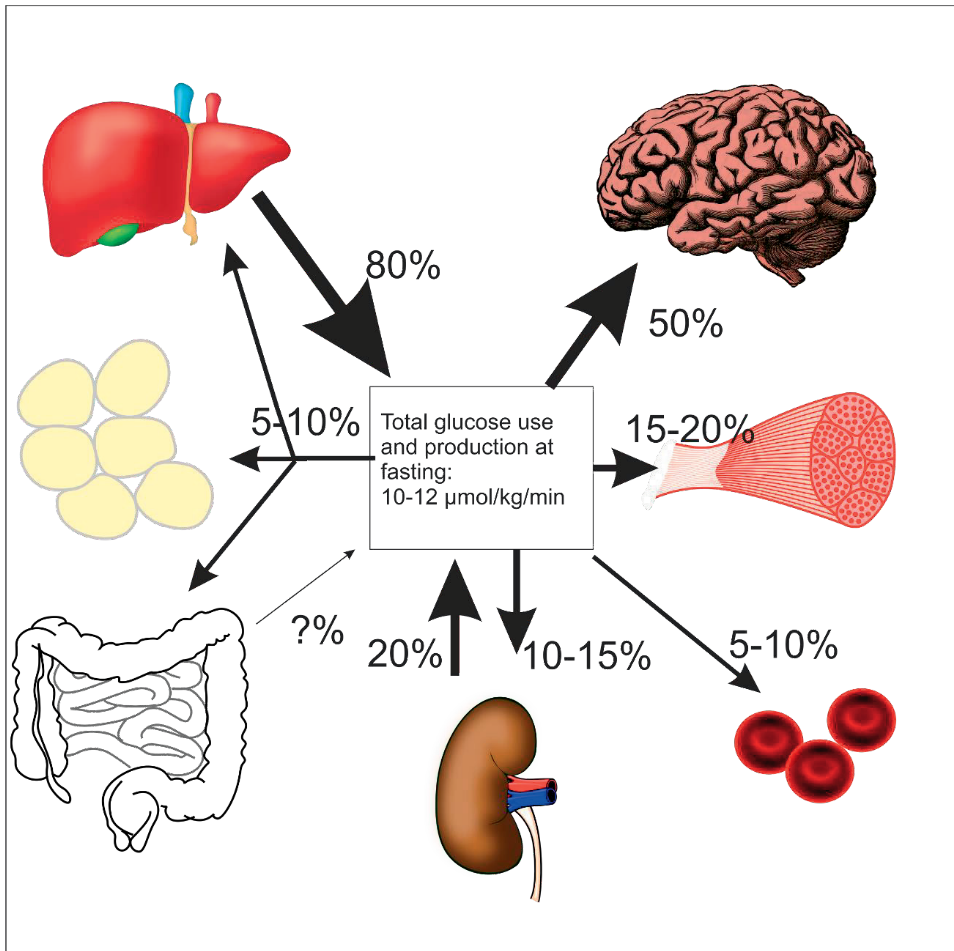


Figure 2.1. Fasting glucose production and disposal. Approximately half of the glucose at a postabsorptive state is used by the brain, 15-20% by skeletal muscle, 10-15% by the kidneys, 5-10% by blood cells and 5-10% by splanchnic organs and adipose tissue. The liver is responsible for approximately 80% and the kidneys for 20% of the glucose produced at a postabsorptive state, although the existence of possible intestinal glucose production remains unknown. Based on (Shrayyef and Gerich 2010).

In humans with normal glucose homeostasis, daily plasma glucose averages around 5.0-5.5 mmol/l (Freckmann et al. 2007). Lowering the plasma glucose level to below 4 mmol/l activates the release of glucagon, catecholamines, cortisol and growth hormone to increase glucose release into plasma and reduce GU into tissues. Simultaneously, the insulin level is decreased when the glucose level is lowered to below 4.5 mmol/l in order to prevent further reduction in plasma glucose. By contrast, an increase of plasma glucose level to over 5.6 mmol/l activates the release of insulin and suppresses glucagon release to lower the glucose level. In a

healthy person, an insulin level around 10 mU/l (60 pmol/l) is usually sufficient to maintain blood glucose around 5 mmol/l (Shrayyef and Gerich 2010; Sprague and Arbeláez 2011). Glycogenolysis releases glucose from the branched chain of glycogen molecules that contain reserve for glucose in the body. Glycogen molecules have a branched structure to enable rapid release of glucose from terminal ends of glycogen chains by glycogen phosphorylase. The first step in glycogen breakdown is separating glucose residues into glucose-1-phosphate from the terminal ends of glycogen chains by glycogen phosphorylase. Glucose-1-phosphate is then converted to glucose-6-phosphate (G6P) by phosphoglucomutase. The liver, the kidneys and possibly the intestine, but not skeletal muscles, have the glucose-6-phosphatase enzyme that converts G6P to glucose, which can then be released into blood circulation by diffusion. Gluconeogenesis is a process whereby new glucose is synthesized from lactate, glycerol, amino acids such as alanine and glutamine, pyruvate and metabolites of the citric acid cycle (Nuttall, Ngo, Gannon 2008).

Liver glycogen reserves are at approximately 100g after an overnight fast (Wasserman 2009). After prolonged fasting the glycogen deposits in the liver become smaller until completely depleted. After 24h, the contribution of gluconeogenesis to total EGP is approximately 70%, after 42h 90% and after 60h the contribution of gluconeogenesis is close to 100% (J. Katz and Tayek 1998; Landau et al. 1996; Magnusson et al. 1992; Rothman et al. 1991). Initially when fasting, gluconeogenesis is slightly elevated to compensate decreased glycogenolysis, even though total EGP is still reduced. The lower EGP results in lower glucose availability and thus decreased glycolysis in glucose target tissues which means that less lactate is available for gluconeogenesis. Muscle glycogen remains relatively stable after a few days' fast (Nieman et al. 1987; Vendelbo et al. 2012), but declines after several days of starvation (Greenhaff, Hultman, Harris 2004). In addition, muscle proteolysis is initially increased in the fasting state providing substrates for gluconeogenesis, but over a longer period of starvation, metabolism is shifted towards the use of fatty acids and ketone bodies as fuel to preserve muscle functionality, and muscle proteolysis is diminished to a less than third of the level at early starvation. Although, fatty acid oxidation is increased in the fasting state to compensate for the lack of glucose as a fuel source providing more glycerol, the lower lactate and amino acid availability causes a decrease in gluconeogenesis. This in combination with depletion of glycogen causes the plasma glucose level to decrease over a prolonged period of fasting, although generally remaining above 2.8 mmol/l. A reduced requirement for insulin is reflected by an insulin level of 3.5 mU/l after prolonged fasting. (Shrayyef and Gerich 2010)

2.1.3 Glucose homeostasis during exercise

The purpose of the skeletal muscle glycogen storage is to be an energy source for intense skeletal muscle work, which can increase muscle glucose oxidation tenfold. Muscle lacks the glucose-6 phosphatase, so it cannot release glucose into circulation. However, when exercising liver glucose production can be increased several-fold in comparison to a resting state by increased glucagon secretion and inhibition of insulin secretion to the portal vein. Even though muscle cannot release glucose directly, it is an important source of lactate, alanine, glutamine and other amino acids for the gluconeogenesis in the liver and the kidneys. Lactate is produced from glucose by glycolysis, alanine is built from pyruvate and glutamate is made by transamination catalysed by alanine aminotransferase, whereas glutamine is formed from muscle proteolysis or synthesis from glutamate and ammonia. Glycogen storages and enhanced gluconeogenesis allow blood glucose to remain close to 5 mmol/l for up to 2h of exercising, ensuring sufficient glucose supply to the brain (Goodwin 2010; Wasserman 2009). In addition, the brain uses lactate released to circulation during intense exercise as an energy source (Quistorff, Secher, Van Lieshout 2008). The relative contribution of gluconeogenesis to the liver glucose output increases over the course of prolonged exercise when body glycogen storages become depleted.

2.1.4 Postprandial glucose metabolism

Complete absorption of carbohydrates, fats and protein from a mixed meal takes at least 6 h, while meal containing only carbohydrates is fully absorbed faster, in approximately 4.5 h. Blood glucose appearance peaks at about 10 min after meal ingestion, reaching a rate of 70-90 μmol/kg body weight/min (Bock et al. 2006). Increased glucose concentration in the portal vein and secretion of incretins, such as glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP), from the intestine stimulate insulin secretion from the pancreas, so that the plasma insulin level increases several-fold compared to the fasting state as well as glucagon secretion is inhibited. The rate of insulin secretion is proportional to the ingested glucose load (Bagger et al. 2011; Kowalski et al. 2017; Tillil et al. 1988). Insulin suppresses EGP and increases glucose disposal to insulin sensitive tissues. Glucose disposal reaches its maximum rate at around 80-120 min after a meal and remains elevated until the glucose received from the meal has been used (Bock et al. 2006). Contributions of different tissues to glucose appearance and disappearance after a meal are described in the Figure 2.2. The purpose of increased uptake in the liver and skeletal muscle compared to a fasting state is to lower the glucose level in the blood, fill the glycogen storages which have been depleted during a fasting and/or

exercise and channel energy for long-term storage by lipogenesis in the liver, coupled to hepatic VLDL-triglyceride production. Approximately 45% of the glucose taken up after a meal ends up oxidised and 45% goes through glycogen synthesis. The rest of the glucose ends up back to circulation through gluconeogenesis, while lipogenesis has a negligible role (Shrayyef and Gerich 2010).

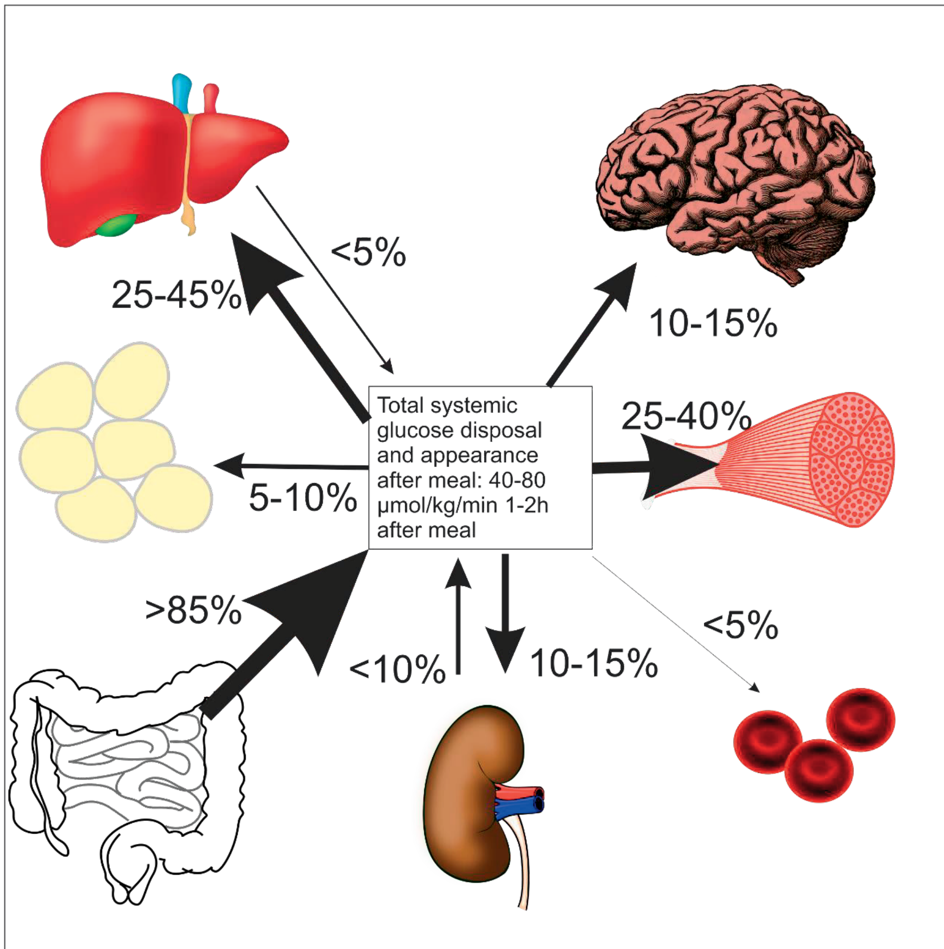


Figure 2.2. Postprandial systemic glucose disposal and appearance. Most of the glucose appearance derives from the intestine, while gluconeogenesis in the liver and the kidneys contribute less than 15%. The liver takes up to 25-45% of the glucose load after a meal whereas skeletal muscle takes anyhere from 25 to 40%. The rest of the glucose load is divided amongst the brain (10-15%), the kidneys (10-15%), adipose tissue (5-10%) and other organs, such as red blood cells, which take less than 5% of the glucose load. Based on (Bock et al. 2006; Gerich 2010; Moore et al. 2012; Shrayyef and Gerich 2010).

Liver glucose uptake

GU from the small intestine causes a rapid rise in the glucose level in the portal vein, which routes nutrients to the sinusoids of the liver where a high glucose concentration gradient drives glucose through GLUT2 into hepatocytes. GLUT2 transporters on the hepatocyte cell membranes have low affinity but have a high capacity for transporting glucose from a high concentration to a lower one. In the hepatocytes, glucose is rapidly phosphorylated to G6P by glucokinase (hexokinase IV liver specific isoforms 2 and 3 (O'Leary et al. 2016)) when the glucose level is high. This enzyme is very effective in promoting liver GU after eating since the enzyme has an s-shaped activity pattern with a half-maximal activity at 6-8 mmol/l and absence of allosteric inhibition by G6P (Burgess 2015; Massa, Gagliardino, Francini 2011). Increased cytosolic glucose concentration effectively releases glucokinase from the cell nucleus where it is bound to the glucokinase regulatory protein (GKRP) in the fasting state. In addition, the elevated insulin level increases the glucokinase gene expression twenty to thirty- fold, which translates to increases enzyme activity 30-60 min after carbohydrate ingestion. In fact, portal glucose concentration is responsible for most of the hepatic GU right after glucose delivery, but insulin is required to reach the maximal uptake (Pagliassotti et al. 1996).

Liver glycogen synthesis

The hepatocytes' rise in glucose level deactivates GKRP and glycogen synthase regulatory protein leading to activation of glucokinase and glycogen synthase, which in turn increases the incorporation of glucose into glycogen. Glycogen synthesis starts from the conversion of G6P to G1P by a phosphoglucomutase. This is followed by the conversion of G1P to UDP-glucose by UDP-glucose pyrophosphorylase and binding UDP-glucose molecules to the C-4 position of glucose residues at the ends of glycogen chains by glycogen synthase (α-1,4-glycosidic linkages). Glycogen molecules have a branched structure, in which the branching of the chains is performed by the glycogen branching enzyme. The enzyme removes chains that are approximately seven glucose residues in length from the outer ends of the building glycogen molecules. It then attaches them to the existing glycogen cores by α-1,6-glycosidic linkage, which allows further lengthening of the glycogen core with 1,4-glycosidic bonds (Figure 2.3) (Burgess 2015; Froese et al. 2015). Glycogen synthesis rates are increased when glycogen stores are low to ensure replenishment of glycogen reserves (Gonzalez et al. 2016). Although, a glucose signal can promote hepatic glycogen synthesis, insulin provides a stronger and additive activation of glycogen build-up (Pagliassotti et al. 1996).

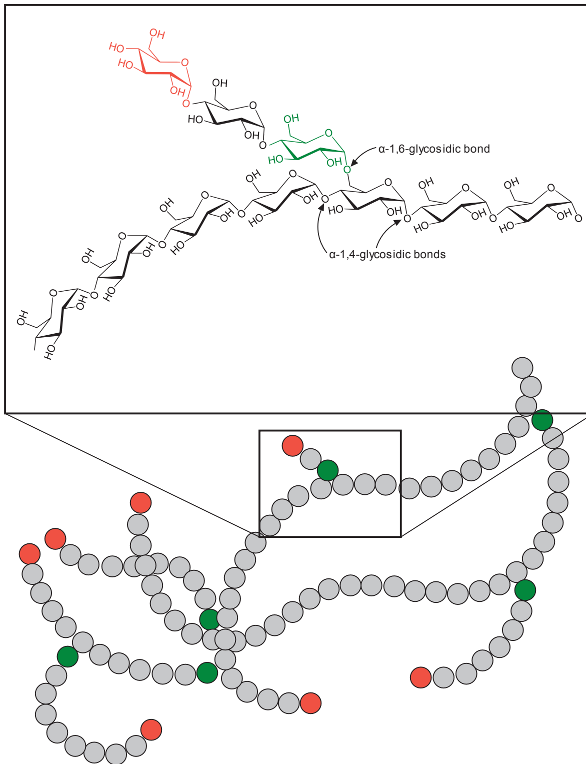


Figure 2.3 Structure of a glycogen molecule. Public domain image from Wikipedia. No copyright, CC0 1.0 (Creative Commons 2018a).

Endogenous glucose production

Increased levels of glucose and insulin effectively inhibit EGP. A lower insulin level is sufficient for EGP suppression than is required to increase GU and maximal suppression is reached at concentrations around 100 mU/l (Bonadonna et al. 1990; DeFronzo et al. 1983). After an oral glucose load EGP is gradually suppressed following increases in glucose appearance and insulin level during the first 30 min. EGP reaches maximal suppression at around 60 min (Abdul-Ghani et al. 2007; Bock et al. 2006; Kowalski et al. 2017). The suppression of glucose production is caused mostly by the inhibition of glycogenolysis or alternatively the channelling of glucose into glycogen synthesis instead of releasing it to circulation, whereas hyperinsulinemia has little impact on gluconeogenesis (Adkins et al. 2003; Gastaldelli et al. 2001).

Skeletal muscle glucose uptake and glycogen synthesis

Hyperinsulinemia in the fed state can increase skeletal muscle GU several times compared to the fasting state. This is due to the recruitment of GLUT4, hexokinase

II and muscle perfusion by insulin. The effect of insulin on muscle GU has an S-shaped dose-response curve where the response starts to raise after 10 mU/l and reaches near maximum around 400 mU/l. After 400 mU/l the incremental stimulatory effect of higher insulin doses is small (Bonadonna et al. 1990; DeFronzo et al. 1983; Natali et al. 2000; Rizza, Mandarino, Gerich 1981). Insulin levels for the maximal stimulation of muscle GU are much higher than typical insulin concentrations after a mixed meal (40-50 mU/l) in healthy nonobese persons (Tchobroutsky et al. 1973). After an overnight fast approximately 10-20% of an oral glucose load is stored as skeletal muscle glycogen 4-5 hours after glucose ingestion (D. Kelley et al. 1988; Mårin et al. 1992). Skeletal muscle can store approximately 500g of glucose as glycogen after which excess glucose is channelled into de novo lipogenesis (DNL) (J. Jensen et al. 2011). Insulin is an anabolic hormone that promotes muscle protein synthesis and suppresses proteolysis (Fujita et al. 2006; Greenhaff et al. 2008). This limits the flux of glucogenic amino acids from skeletal muscle into the liver and thus also the gluconeogenesis.

Adipose tissue glucose uptake and suppression of lipolysis

Visceral adipose tissue has been shown to be more metabolically active when compared to subcutaneous adipose tissue showing a higher rate of glucose uptake in both fasting and insulin-stimulated state (Ferrannini et al. 2018; Stolic et al. 2002; Virtanen et al. 2002). Even though the contribution of adipose tissue GU to the whole-body GU is relatively small in lean humans, it is higher in very obese persons due to an expanded fat mass (Dadson et al. 2016; Mitrou et al. 2009). In healthy humans, insulin suppresses most lipolytic activity already at 20 mU/l (150 pmol/l) (Conte et al. 2012; Kowalski et al. 2017). The antilipolytic effect of insulin has been reported to be lower in abdominal visceral and subcutaneous adipose tissue than in femoral adipose tissue in some studies (Meek, Nair, Jensen 1999), while others have not found such differences between abdominal and femoral subcutaneous depots (Hickner et al. 1999). The contribution of brown fat GU to the whole-body GU is small but there is evidence that brown fat thermogenesis is activated after a meal (U Din et al. 2018).

Intestinal glucose uptake and glucose release

The gut is responsible for absorption of nutrients and their release into circulation. The gut has the capacity to regulate glucose release so that the resulting blood glucose remains relatively similar despite the amount of glucose ingested. These control mechanisms include the regulation of gastric emptying, the secretion of incretins, which stimulate insulin secretion and regulate food intake and the other hormones affecting appetite (Holst et al. 2016; Kooijman et al. 2015; Seino, Fukushima, Yabe 2010). Sodium/glucose cotransporter 1 (SGLT1) mediates GU into

enterocytes from intestinal lumen, while GLUT2 transports glucose from enterocytes into circulation. Moreover, GLUT2 has been suggested to travel into the apical membrane to enhance GU from lumen in response to meal containing simple sugars (Gorboulev et al. 2012; Gouyon et al. 2003; Kellett and Helliwell 2000; Schmitt et al. 2017), although other studies found no evidence for this suggestion (Röder et al. 2014; Sala-Rabanal Monica et al. 2018). In humans apical GLUT2 has been found to be present in morbidly obese subjects but not their lean counterparts (Ait-Omar et al. 2011). In addition, a mouse study has provided evidence suggesting that insulin stimulates the removal of GLUT2 from the apical surface of the intestine in order to reduce the GU rate from the lumen to prevent an excessively high glucose flux into circulation (Tobin et al. 2008).

2.1.5 Fatty acid metabolism

FAs are an important energy source for almost all tissues in the human body, except for the brain, and the body fat reserves can supply the body functions for extensive periods of time. In general, FAs are the major energy source for the liver and heart, and for skeletal muscle at fasting or during low-intensity or long-lasting exercise (Birkenfeld and Shulman 2014; Frayn, Arner, Yki-Järvinen 2006).

After a meal FAs are released from the small intestine into circulation mainly as part of chylomicron triglycerides. FAs are hydrolysed rapidly from chylomicron triglycerides by LPL located on the endothelial cells lining capillaries and transported actively or by diffusion to underlying tissue. In addition, large portion of the FAs are released into blood circulation as FFAs (Teusink et al. 2003). Fatty acids taken up into the tissues are esterified into acyl-CoA which can be channelled to mitochondria for oxidation or used for glycerolipid synthesis. Most FFAs in the liver and white and brown adipose tissue are converted into triglycerides for (temporary) storage. Insulin promotes the uptake and storage of FAs into adipose tissue after a meal by stimulating adipose tissue LPL and suppressing lipolysis of intracellular triglyceride stores, whereas skeletal muscle LPL is slightly downregulated. In the liver insulin promotes synthesis of FAs by DNL and incorporation of FAs into triglyceride stores but suppresses their release into circulation as VLDL-triglycerides. Moreover, insulin inhibits FA oxidation in the liver indirectly by stimulation DNL which produces malonyl-CoA, an inhibitor of carnitine palmioyltransferase (CPT1). CPT1 is a key step in the oxidation of long-chain FAs (Frayn, Arner, Yki-Järvinen 2006; Taskinen and Borén 2015).

Hormone sensitive lipase (HSL), adipose triglyceride lipase (ATGL) and monoacylglycerol lipase cooperate to release FAs into the blood circulation from adi-

pose tissue triglyceride stores to provide energy to body tissues at fasting and during physical exercise. In addition, the liver releases FAs as part of VLDL-triglycerides at fasting state. LPL hydrolyses FAs from VLDL-triglycerides for the use by the underlying tissues, although this process is slower than with chylomicrons. Within the liver, hepatic lipase (HL) is responsible for hydrolysis of triglycerides from small VLDL, LDL and HDL particles. In addition, remnants of chylomicrons and VLDL, which are generated via the concerted action of LPL and HL and have therefore released most of their triglycerides, are taken up by the liver (Frayn, Arner, Yki-Järvinen 2006).

2.1.6 Insulin signal transduction

Insulin signal transduction occurs in cells via a network of multiple pathways (Figure 2.4). A common starting point for these pathways is the insulin binding to its receptor on the cell surface. An insulin receptor is a transmembrane glycoprotein consisting of two extracellular α subunits and two transmembrane/intracellular β subunits which are connected by disulfide bonds. The extracellular 135kDa α subunits contain a cysteine-rich insulin binding domain, whereas the 95 kDa β subunits contain domains for ATP binding and a catalytic loop for tyrosine kinase activity. The binding of insulin moves α subunits closer together and enables ATP binding. In addition, insulin binding activates insulin receptor tyrosine kinase by triphosphorylation of its activation loop. The tyrosine kinase phosphorylates tyrosine residues outside the kinase domain, which creates binding sites for either molecules containing src-homology 2- (SH2) or phosphotyrosine-binding (PTB) domains. These molecules include among others insulin receptor substrates (IRS) 1-6, adapter Shc, CBL and APS (Langlais, Mandarino, Garvey 2015). Of these molecules IRS1 and -2 and Shc are considered to be the most important ones for insulin signaling. Bound IRS is activated by the insulin receptor by means of phosphorylation.

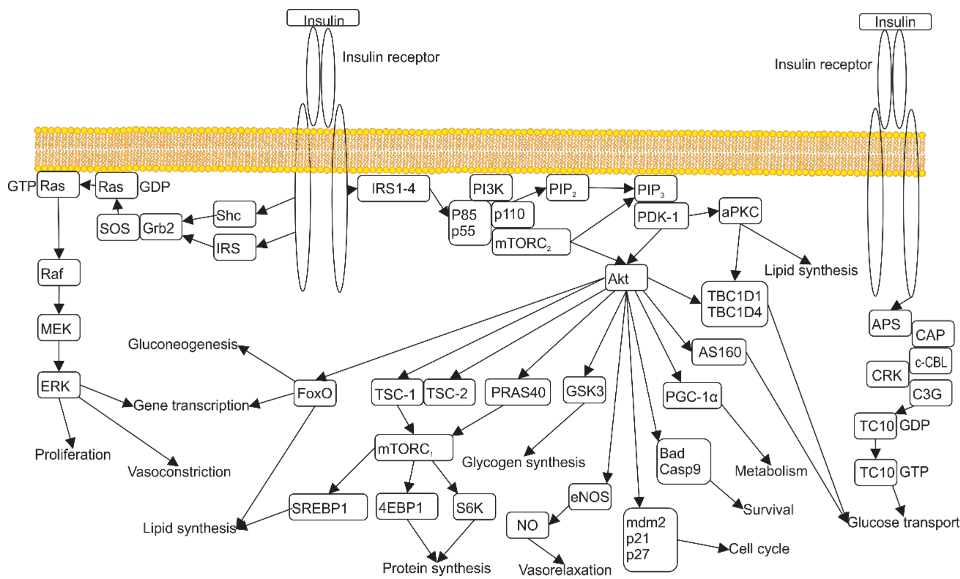


Figure 2.4. Insulin signalling pathways. Based on (Boucher, Kleinridders, Kahn 2014; Leto and Saltiel 2012; Muniyappa, Iantorno, Quon 2008)

The phophatidylinositol 3-kinase (PI3K)/Akt pathway is the main route for insulin signalling to affect metabolism, while the Raf/ras/MEK/MAPK pathway is mediating signal for cell growth and differentiation. PI3K is bound to IRS1 and IRS2 by regulatory subunits p85 and p55, causing activation of the catalytic subunit p110, which can generate phophatidylinositol-3,4,5-trisphosphate (PIP) from phophatidylinositol-4,5-bisphosphate. PIP is then bound to phosphoinositide-dependent protein kinase (PDK) 1 and -2, which can activate Akt1. Akt, also known as protein kinase B, is a serine/threonine protein kinase that has three structurally homologous isoforms, Akt1-3, which arise from different genes. Akt2 activates GLUT4 translocation to the cell surface in muscle and adipose tissue to increase glucose uptake. In the liver Akt2 mediates enhanced glycogen synthesis and reduces glycogen breakdown (Wan et al. 2013). The important role of Akt2 in insulin sensitivity in different tissues is highlighted by the low-frequency partial loss-of-function p.P50T/AKT2 variant which causes reduction of insulin-stimulated GU in multiple organs as well as impaired suppression of EGP (Latva-Rasku et al. 2018). The insulin signal is further transducted into at least four different pathways: 1) the mammalian target of rapamycin (mTOR), 2) the glycogen synthase kinase 3 (GSK3), 3) the forkhead box-containing protein O subfamily (FoxO) and 4) the AKT substrate of 160kDa (AS160) pathway. The mTOR-pathway mediates regulation of protein synthesis and stimulates hepatic DNL (S. Li, Brown, Goldstein 2010; Porstmann et al. 2008; Titchenell et al. 2016; Wan et al. 2011; Yecies et al. 2011). The GSK3 is an inhibitor of glycogen synthase which is inhibited by

Akt, whereas the FoxO1 is a transcription factor which stimulates gluconeogenesis by increasing the expression of phophoenolpyruvate carboxykinase (PEPCK) and inhibits cell cycle by stimulating cyclin G2. The Akt-mediated insulin signal deactivates FoxO1 and thus inhibiting gluconeogenesis (Nakae et al. 2001) and stimulating cell growth (Pauta et al. 2016). In addition, Akt signalling is required for the insulin-mediated vasodilatation caused by endothelial nitric oxide synthase (eNOS) in endothelium (Dimmeler et al. 1999; Luo et al. 2000).

CAP/Cbl/TC10 pathway has been shown to affect insulin-stimulated glucose transport in studies using adipose tissue cells. This pathway does not involve IRSs but is instead activated by the insulin receptor kinase -mediated phosphorylation of APS. TC10 has been shown to affect GLUT4 translocation via the cell cytoskeleton. However, the precise underlying mechanisms remain unclear as does the role of CAP/Cbl/TC10 pathway in insulin-stimulated GU in muscle cells (Langlais, Mandarino, Garvey 2015).

2.2 Insulin resistance

Insulin resistance means reduced insulin action on its targets on whole-body level, tissue level or cell level when compared to a normal physiological response (Petersen and Shulman 2018). More precisely, decreased insulin sensitivity may be described as a shift of insulin dose-response curve to the right, while a decreased insulin responsiveness means a lower maximal response compared to a normal state (Kahn 1978). Insulin resistance shows as lowered stimulation of GU in skeletal muscle, the liver, white and brown adipose tissue, the gut and the cardiac muscle as well as decreased suppression of lipolysis in adipose tissue and impaired suppression of EGP from the liver and the kidneys (Iozzo 2010; Mäkinen et al. 2015; Orava et al. 2013; Petersen and Shulman 2018). In the liver insulin resistance causes increased production triglyceride-rich VLDL particles and apolipoprotein C-III (ApoC-III) which inhibits catabolism of these particles by LPL (Taskinen and Borén 2015).

2.2.1 Skeletal muscle insulin resistance

Skeletal muscle insulin resistance is a key contributing factor to hyperglycaemia after a meal. This is due to the fact that in humans with normal glucose metabolism, postprandial hyperinsulinemia increases muscle GU several fold and muscle takes up 25-40% of an oral glucose load. Even though the liver can take up even more glucose after a meal, the contribution of insulin to the liver GU is lower since most

of the uptake is driven by high glucose gradient between the portal vein and liver cells. Isolated impaired glucose tolerance has been shown to be mostly dependent on insulin stimulated glucose disposal and not EGP (Varghese et al. 2016). Furthermore, there is some evidence in support of decreased GU, rather than impaired suppression of EGP, explaining increased glucose levels in obese individuals even in the fasting state (Jani et al. 2008).

Muscle glucose transport and phosphorylation are lowered in the insulin resistant states of obesity and type 2 diabetes (Sesti 2006). An elegant study by Cline et al. has shown that defective glucose transport is the rate-limiting step in the lowered ability of skeletal muscle to store glucose (Cline et al. 1999). Studies using limb arteriovenous catheterisation have suggested that an impaired effect of insulin on blood flow contributes to a reduced skeletal muscle GU in insulin resistance (Baron et al. 1991; Laakso et al. 1990; K. Mather et al. 2000). Although, this interpretation is disputed by studies using PET (Ferrannini et al. 2018). There is also evidence for defective transport of insulin to muscle in obesity (Broussard et al. 2016; Sandqvist et al. 2011).

A high glucose level that is present in diabetes decreases the whole-body insulin sensitivity by itself, which phenomenon is known as glucose toxicity. Studies using hyperglycaemic clamp have shown that this effect is present only when glucose level is above ~9 mmol/l (Boden et al. 1996; Leung et al. 2004; Solomon et al. 2012). Possible mechanisms for the glucotoxic effects on skeletal muscle insulin sensitivity include reduced glycogen synthesis, diacylglycerol accumulation (Hoy et al. 2007), decreased GLUT4 translocation (Huang et al. 2002) and oxidative stress (Meugnier et al. 2007). While hyperglycaemia interferes with insulin signalling, high glucose in itself promotes muscle GU, which may compensate for the defects in glycogen synthesis (D. E. Kelley and Mandarino 1990; Mandarino et al. 1990; Vaag et al. 1992). However, evidence on this matter is conflicting (Beck-Nielsen Henning 2012; Vind et al. 2012). In addition to hyperglycaemia, hyperinsulinemia may cause insulin resistance as shown in studies on chronic hyperinsulinemia (Poy et al. 2002; Rizza et al. 1985; Shanik et al. 2008).

Slow-twitch type I skeletal muscle fibres have a higher protein content of insulin receptor, GLUT4, hexokinase II, glycogen synthase and pyruvate dehydrogenase-E1α than type II fibres (Albers et al. 2015). The fraction of slow-twitch muscle fibres and type I fibre GLUT4 content is lower in obesity, metabolic syndrome and T2DM patients. This is accompanied by reduced muscle oxidative capacity and insulin-stimulated whole body GU (Gaster et al. 2001; Oberbach et al. 2006; Stuart et al. 2013). In addition, men born with a low birth weight, who are predisposed to T2DM, have proportionally less oxidative type IIa fast-twitch fibres and increased glycolytic IIx fibres than men with a normal birth weight (C. B. Jensen et al. 2007).

There is some evidence of insulin resistance with respect to protein synthesis in both males with T2DM (S. Pereira et al. 2008) and elderly persons (Fujita et al. 2009; Rasmussen et al. 2006). Insulin resistant protein synthesis could, therefore, contribute to the lower muscle mass observed in T2DM patients and aged individuals. Increased triglyceride content in muscle is associated with insulin resistance among sedentary lean and obese humans and in T2DM sufferers, but on the other hand, muscle triglyceride content is also increased in endurance athletes (Amati et al. 2011).

An artificial increase of plasma FFAs decreases the insulin-stimulated whole-body glucose disposal and insulin signalling in a dose dependent manner (Belfort et al. 2005). Studies employing ^{18}F-FDG and PET have shown that a high level of circulating FFAs reduce insulin-stimulated GU in skeletal muscle and the heart (Nuutila et al. 1992). The elevated FFA level reduces GU within hours suggesting that metabolic intermediates from FA metabolism are responsible for the decreased GU (Bonadonna et al. 1989). Fatty acids can induce insulin resistance by interfering with the IRS1 tyrosine phosphorylation and the subsequent PI3K activity. In addition, diacylglycerols deteriorate the insulin-stimulated GU by way of increased IRS1 serine phosphorylation and insulin receptor phosphorylation at Thr-1336, Thr-1348, and Ser-1305/1306 (Boucher, Kleinridders, Kahn 2014).

2.2.2 Liver insulin resistance

Insulin resistance of EGP is the key factor contributing to fasting hyperglycemia (DeFronzo, Ferrannini, Simonson 1989). Moreover, impaired suppression of EGP by insulin is a major determinant of glucose tolerance (Båvenholm et al. 2001). Insulin resistance affects hepatic GU and glycogen synthesis, which are impaired in type 2 diabetes (Basu et al. 2000; Basu, Basu, Shah, Vella, Johnson et al. 2001; Hwang et al. 1995; Iozzo, Hällsten et al. 2003a; Krssak et al. 2004). An important contributing factor to hepatic insulin resistance is the accumulation of lipid to the liver, known as nonalcoholic fatty liver disease (NAFLD), which is associated with both impaired stimulation of hepatic glucose uptake and glycogen synthesis and lower suppression of glucose production. A possible mechanism for increased fat content in the liver causing insulin resistance is accumulation of diacylglycerol, which can activate novel protein kinase C (PKC) isoforms that decrease phosphorylation of IRS1 and IRS2 by means of insulin receptor kinase. Hyperinsulinemia promotes hepatic accumulation of diacylglycerol by stimulating DNL (Taskinen and Borén 2015). In addition, ceramides have been associated with impaired Akt2 activation. Accumulation of diacylglycerol could be a result from increased diacylglycerol synthesis and too slow incorporation of FAs to diacylglycerol to form

triglycerides or increased hydrolysis of triglycerides (Samuel and Shulman 2012). It is unclear whether FAs interfere with insulin signalling directly or only by other, lipotoxic, mediators such as diacyglycerols and ceramides. Nevertheless, saturated FAs (SFAs) have been associated with hepatic insulin resistance (Petersen and Shulman 2018). Proinflammatory cytokines can also interfere with insulin signalling through cJun-N-terminal-kinase (JNK) (Solinas and Becattini 2017) or inhibition of IκB kinase-β.

Lipolysis and gluconeogenesis from glycerol are increased in obesity and T2DM (Bortz et al. 1972; Puhakainen, Koivisto, Yki-Järvinen 1992). Studies in canines and rodents have shown that insulin resistance in adipose tissue can indirectly deteriorate the suppression of glucose production in the liver as a result of failure to inhibit lipolysis (Perry et al. 2015; Rebrin et al. 1996; Titchenell et al. 2016). Further, an elegant study in dogs showed that this defect is caused by failure to channel the extra glucose formed by FFA-driven gluconeogenesis to glycogen synthesis during insulin-stimulation (Edgerton et al. 2017). In fact, in healthy humans, FFA infusion has only a minor effect on insulin suppression of EGP (Ferrannini et al. 1983; Lewis et al. 1997). The key role of impaired suppression of lipolysis in increased glucose production is further supported by studies using the lipolysis suppressing drug, acipimox. As part of these studies, EGP was suppressed in obese individuals and T2DM sufferers after a 12 day treatment (Daniele et al. 2014) and fasting glucose and insulin levels were decreased after 6 months treatment in obese subjects without changes in muscle insulin sensitivity (Makimura et al. 2016). Although FFA infusion seems to have little impact on EGP suppression in healthy humans, it does lower liver GU likely by impairing glucokinase activity (Iozzo et al. 2004).

Unlike the effects of insulin on liver glucose metabolism, insulin's ability to promote DNL is not impaired in an insulin resistant state. This means that the hyperinsulinemia caused as a response to disturbed glucose homeostasis may promote production of VLDL triglycerides by stimulating DNL, thus contributing to hypertriglyceridemia (Choi and Ginsberg 2011; Sanders and Griffin 2016). Moreover, the FAs generated by DNL contribute also to the hepatic lipid accumulation (Donnelly et al. 2005) where bioactive lipids interfere with insulin signalling controlling glucose metabolism thus creating a vicious cycle (Sanders and Griffin 2016). Another contributing factor to increased VLDL triglycerides in obesity and insulin resistance is increased ApoC-III production in the liver (Cohn et al. 2004). ApoC-III promotes hypertriglyceridemia by inhibiting hydrolysis of VLDL triglycerides by LPL (Larsson et al. 2013).

2.2.3 Adipose tissue insulin resistance

Insulin-stimulated GU into visceral adipose tissue and subcutaneous adipose tissue depots is decreased in obesity and T2DM when measuring uptake per litre of tissue indicating insulin resistance. However, the large fat mass compensates for this defect, so that the GU, when expressed per whole depots is actually similar to or higher than that of a lean or normal-weight person (Dadson et al. 2016; Mitrou et al. 2009; Virtanen et al. 2002). In addition to white adipose tissue GU, also brown adipose tissue GU has been shown to be insulin resistant in obesity (Orava et al. 2013).

Several studies have shown that the antilipolytic effect of insulin is impaired in obesity, insulin resistance and type 2 diabetes (Basu, Basu, Shah, Vella, Rizza et al. 2001; Hickner et al. 1999; Jansson, Smith, Lönnroth 1990; Nellemann et al. 2012; Robinson et al. 1998). In addition, there is evidence for higher postprandial FFA levels in obesity and persons on a high fat diet. Some studies attribute increased lipolysis in obese subjects to a higher fat mass and not to lower antilipolytic effect of insulin (Bolinder et al. 2000; Robinson et al. 1998). Adipose tissue expansion by adipocyte hypertrophy is characterised by insulin resistance and metabolic disturbances while expansion by hyperplasia that produces new small adipocytes is connected to a more benign phenotype (Lafontan 2014). A study of lipolytic activity in large and small adipocytes has shown that lipolytic activity is higher in large adipocytes, but not in proportion to lipid weight (Laurencikiene et al. 2011). In fact, circulating FFA levels are only meagrely related to fat mass and are actually lower per kg of adipose tissue with increasing fat mass (Karpe, Dickmann, Frayn 2011). One likely contributing factor to higher FFA levels is inflammation, which has been shown to increase adipose tissue lipolysis (Perry et al. 2015).

Lower adipose tissue blood flow has been shown to be a major factor in decreased insulin-stimulated adipose tissue GU in case of insulin resistance (Ferrannini et al. 2018; Raitakari et al. 1996). In addition, there is evidence for impaired transport of insulin through vascular endothelium in obesity (Sandqvist et al. 2011).

Mendelian randomisation studies have identified a possible causal pathway whereby insulin resistance increases branched-chained amino acid (BCAA) concentrations (Mahendran et al. 2017; Q. Wang et al. 2017). Studies in humans and rodents suggest that the defect behind high circulating BCAA is the decreased catabolism of branched-chain amino acids in adipose tissue (Mardinoglu et al. 2014; She et al. 2007). This is especially important considering that BCAAs are likely to contribute to T2DM pathogenesis (Lotta et al. 2016).

2.2.4 Insulin resistance in other tissues

PET studies have shown that GU from blood into intestinal mucosa is insulin resistant in morbid obesity and T2DM (Koffert et al. 2017; Mäkinen et al. 2015). Insulin-stimulated intestinal GU may be of importance in controlling blood glucose both in the fasting state and after a meal. However, more studies are needed to clarify the effects of insulin on the intestine and the role of the intestine in regulating glucose homeostasis. On the other hand, patients with T2DM and cardiovascular disease have impaired insulin-stimulated myocardial GU. Even though the heart has only a small impact on the whole-body GU after a meal due to its small size, lower myocardial GU is associated with lower left ventricular ejection fraction suggesting increased risk of heart failure (Iozzo 2010). Unlike in other tissues, in the brain whole-body insulin resistant state is reflected by a paradoxical increase in the brain's GU during insulin stimulation (Hirvonen et al. 2011; Tuulari et al. 2013). Mechanisms behind increased brain GU are currently unknown.

2.2.5 Associations between different tissues

Insulin sensitivity in skeletal muscle, the liver and adipose tissue has been studied simultaneously in the same subjects in regard to skeletal muscle GU as well as suppression of EGP and lipolysis (Conte et al. 2012; Korenblat et al. 2008). The study by Korenblat et al. (Korenblat et al. 2008) showed that insulin sensitivity in these tissues is closely linked to the liver's fat content. In addition, hyperinsulinemia in the physiological range is sufficient to compensate for insulin resistance in the EGP and lipolysis suppression but not for stimulation of skeletal muscle GU. However, it is unclear if insulin resistance of skeletal muscle, adipose tissue and liver GU is present simultaneously and to what extent. Mechanisms and mediators of interorgan crosstalk are discussed in detail elsewhere (Gancheva et al. 2018; Oh et al. 2016; Petersen and Shulman 2018).

2.3 Insulin resistance risk factors

2.3.1 Obesity and lifestyle-related risk factors

Obesity and ectopic fat accumulation

Obesity and low physical activity are the key environmental risk factors for insulin resistance (Reaven 2011). Together these factors explain a major share of the variation in the whole-body insulin-stimulated GU (Bogardus et al. 1985; Huth et al. 2016; Jukarainen et al. 2017; Yki-Järvinen and Koivisto 1983). Visceral fat mass has the strongest association with insulin resistance among different fat depots while leg fat shows no association (M. Zhang et al. 2015). Visceral fat is thought to confer liver insulin resistance by providing high amounts of FFAs directly to the liver through the portal vein. In fact, the contribution of visceral depot to hepatic FFA delivery increases with a larger depot size (Nielsen et al. 2004). In addition to vast epidemiological evidence and weight loss interventions, Mendelian randomisation analyses give support for a causal role of obesity in insulin resistance (Dale et al. 2017; Emdin et al. 2017; Holmes et al. 2014; Tao et al. 2018; T. Wang et al. 2016).

The liver's fat content predicts type 2 diabetes irrespective of BMI and age (Lallukka and Yki-Järvinen 2016) and is an independent predictor of hepatic insulin resistance (Kotronen et al. 2008). However, it is important to note that liver fat accumulation does not always cause insulin resistance. The p.Ile148Met polymorphism of the Patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene and p.Glu167Lys of the transmembrane 6 superfamily 2 (TM6SF2) gene predispose the liver to increased fat accumulation when a person is subject to nutrient excess (Anstee and Day 2015). A fatty liver caused by such mutations does not directly cause insulin resistance (Kantartzis et al. 2009; Kotronen et al. 2009; Romeo et al. 2010; Zhou et al. 2015) but may do so indirectly through liver damage (Dongiovanni et al. 2018). A possible explanation to the lack of association between fatty liver and insulin resistance in carriers of these mutations is accelerated incorporation of FAs into triglycerides and decreased hydrolysis of triglycerides which may limit the amounts of cytosolic bioactive lipid species (J. Z. Li et al. 2012).

Diet and microbiota

A diet leading to an intake of more calories than the person's energy expenditure, a so-called 'positive energy balance' causes obesity and thus indirectly insulin resistance. Apart from overfeeding, diet nutrients have also independent effects on insulin sensitivity. A diet rich in carbohydrates and saturated FAs, instead of unsaturated FAs, predispose to insulin resistance (Imamura et al. 2016). On the other hand, reduced polyunsaturated FAs and increased glycerol, monounsaturated FAs and saturated FAs in blood have been associated with current and future hyperglycemia and T2DM (Djoussé et al. 2011; Laaksonen et al. 2002; Mahendran et al. 2013). Some studies investigating plasma cholesteryl esters have come to similar conclusions, but in these studies dihomo-γ-linolenic (20:3n−6) and γ-linolenic

(18:3n−6) acids were associated with higher T2DM risk and worse glycemic control (Vessby et al. 1994; L. Wang et al. 2003). While many metabolites are produced endogenously, lipids reflect diet quality relatively well (Akbaraly et al. 2018). Moreover, replacing carbohydrates with a higher fructose consumption has been associated with hepatic insulin resistance in nondiabetic subjects (ter Horst et al. 2016). However, in T2DM fructose when replacing other carbohydrates seems actually beneficial for glycaemic control (Cozma et al. 2012). More studies are needed to clarify this seeming paradox.

Gut microbiota is emerging as a major element affecting insulin sensitivity. Microbial genera Dorea, Oscillospira and Ruminococcus that ferment acetate, propionate and butyrate from complex carbohydrates are enriched in high fat diet-induced obese rodents and may thus promote obesity by providing extra energy (Jiao et al. 2018). However, apart from energy from complex carbohydrates, these FAs have a more complex role in regulating insulin sensitivity and body weight. For example, SCFAs have been shown to reduce food intake and increase FA oxidation (Chambers et al. 2018; Z. Li et al. 2018). Short-chain FAs (SCFAs) are substrates for the G-protein coupled receptor 41 (free fatty acid receptor 3) and -43 (FFA2) and butyrate for GPR109a (hydroxycarboxylic acid receptor 2) which are present in various tissues in the body and can thus mediate direct tissue-specific effects on these FAs (Canfora, Jocken, Blaak 2015; Chambers et al. 2018). Furthermore, gut microbiota can synthesize BCAAs (Deguchi et al. 1978; Metges et al. 1999; Torrallardona, Harris, Fuller 2003). Increased BCAA synthesis and lowered breakdown by gut microbes may contribute to the higher BCAA levels observed in obesity and insulin resistance, which in turn, is likely to contribute to T2DM pathophysiology (Lotta et al. 2016).

Physical inactivity

Amount of work-related physical activity has decreased considerably over the past decades in high-income countries such as Finland and Unites States (Church et al. 2011; Husu et al. 2011). In concordance, the amount of time spent sitting down in European countries is generally the highest in high-income North-Western European countries and lower in lower-income Southern Europe (Bennie et al. 2013). Being sedentary increases the risk of T2DM and cardiovascular diseases (Biswas et al. 2015; van der Berg et al. 2016), whereas a low amount of physical activity is associated with insulin resistance (Balkau et al. 2008).

Smoking

Smoking is an independent predictor of insulin resistance (Eliasson et al. 1994; Facchini et al. 1992; Rönnemaa et al. 1996). Smoking increases central fat depots, sympathetic activity, cortisol levels, inflammation and oxidative stress, all of

which may contribute to higher insulin resistance (Grøndahl et al. 2018). In addition, there is evidence that nicotine itself induces insulin resistance (Eliasson, Taskinen, Smith 1996). However, nicotine use increases energy expenditure and leads to reduction in bodyweight which oppose the harmful effects (Rupprecht et al. 2018).

Sleep

Sleep duration has a U-shaped association with T2DM risk. The lowest risk of T2DM is observed in persons of 7-8 h sleep per day (Shan et al. 2015). Sleep deprivation causes insulin resistance, impairs glucose homeostasis and increases appetite and caloric intake (Mesarwi et al. 2013). In fact, reduced insulin sensitivity can be observed already after one night of short sleep (Donga et al. 2010). Moreover, having a preserved circadian rhythm is important because circadian misalignment is associated with obesity and T2DM as well as reduces skeletal muscle insulin sensitivity (Wefers et al. 2018). Mice exposed to prolonged day length by increased light exposure are prone to increased adiposity (Kooijman et al. 2015). In addition, short sleep duration or poor sleep quality have been associated with hepatic lipid accumulation, albeit these association may be explained by higher BMI and sleep apnea in persons with these conditions (Bos et al. 2018).

2.3.2 Genetic and epigenetic risk factors

T2DM is markedly heritable. With one affected parent, the lifetime risk of being afflicted with T2DM is estimated to be two-fold to four-fold compared to persons whose parents do not have T2DM (Pierce M., Keen H., Bradley C. 1995). An estimated heritability of T2DM by the age 80 has been calculated to be 40% with one affected parent and 70% when both parents have T2DM (Köbberling and Tillil 1982). Similarly, studies on twin pairs have shown a heritability of 70% (Willemsen et al. 2015). Studies assessing heritability of insulin sensitivity applying the frequently sampled intravenous glucose tolerance test (FSIVGTT), the euglycemic-hyperinsulinemic clamp or the hyperglycaemic clamp have yielded estimates of 20-60% (Goodarzi et al. 2014). In addition, persons with African or Hispanic Mexican genetic background tend to be more insulin resistant than Caucasians and East Asians, and East Asians are generally more insulin sensitive than Caucasians (Golden et al. 2012; Kodama et al. 2013).

The identified common gene mutations (minor allele frequency >5%) predisposing to T2DM explain only a minor portion of T2DM heritability. Most of the found mutations affect insulin secretion and not insulin sensitivity (Stancakova and

Laakso 2016). The found common variants affecting insulin sensitivity are listed in Table 2.1.

Table 2.1. Loci associated with fasting insulin/insulin sensitivity when accounting for BMI (A. K. Manning et al. 2012; Scott et al. 2012; Walford et al. 2016)

Single nucleotide polymorphisms (SNPs) with a plausible mechanistic link to insulin sensitivity/metabolism		
Locus	SNP	Protein function(s)
ARL15	rs4865796	Promotes phosphorylation of members of the IR/IRS1/PDPK1/AKT insulin signalling pathway (J. Zhao et al. 2017); important for adipocyte differentiation (Rocha et al. 2017)
BCL2	rs12454712	Prevents adipocyte apoptosis (Nagel et al. 2014; Tinahones et al. 2012)
GCKR	rs780094	Inhibits glucokinase (Massa, Gagliardino, Francini 2011)
GRB14	rs10195252	Inhibition of insulin receptor catalytic activity (Popineau et al. 2016)
FAM13A	rs3822072	Improves insulin signalling by protecting IRS1 from proteasomal degradation (Wardhana et al. 2018)
IRS1	rs7578326, rs2943634	Insulin signal mediator (Langlais, Mandarino, Garvey 2015) (Chapter 2.1.6)
KLF14	rs972283	Female-specific effect on adipocyte proliferation and lipogenesis (Small et al. 2018)
PDGFC	rs6822892, rs4691380	Activation of platelet-derived growth factor receptor α. Cell proliferation, migration, differentiation and survival through members of insulin signalling pathway. Promotes angiogenesis and adipose tissue beiging (C. Lee and Li 2018; Seki et al. 2016).

PPARG	rs1801282, rs17036328	Regulation of lipid and glucose metabolism, FA transport, adipogenesis and inflammation (Ahmadian et al. 2013).
PPP1R3B	rs2126259, rs4841132	Protein phosphatase-1 catalytic subunit. Activates and promotes glycogen synthase (Mehta et al. 2017; Ragolia and Begum 1998; Stender et al. 2018)
SNPs without any known mechanistic link to insulin sensitivity/metabolism		
Locus	SNP	Protein function(s)
ANKRD55-MAP3K1	rs459193	Functions of ANKRD55 are unknown. MAP3K1 regulates cell death, survival, migration and differentiation (Suddason and Gallagher 2015)
COBLL1-GRB14	rs7607980	COBLL1 modulates cytoskeleton structure by actin assembly and remodelling (Takayama et al. 2018)
FAM19A2	rs10506418	Unknown
LYPLAL1	rs4846565, rs2785980	Unknown (Watson et al. 2017)
PEPD	rs731839	Hydrolysis of di- and tripeptides with carboxy-terminal proline (Tanoue, Endo, Matsuda 1990)
TET2	rs974801	Oxidation of 5-methylcytosine in DNA to 5-hydroxymethylcytosine. Hematopoietic stem and progenitor cell self-renewal (Fuster et al. 2017)
UHRF1BP1	rs6912327, rs4646949	Regulation of inflammation and cytosine methylation (Payne et al. 2018)

In addition to the common variants there are many rare variants of which a small number have a high effect size. Identified rare variants affecting insulin sensitivity include mutations affecting the peroxisome proliferator-activated receptor γ (PPARγ) and Akt2.

PPARγ is a nuclear transcription factor that regulates lipid and glucose metabolism, FA transport, adipogenesis and inflammation (Ahmadian et al. 2013). The

PPARG gene produces two protein isoforms by alternative mRNA splicing: PPARγ1 and PPARγ2. PPARγ1 is present ubiquitously while PPARγ2 is concentrated in adipose tissue and to a lesser amount in the liver (Fajas et al. 1997). PPARγ2 has a key role in facilitating adipose tissue expansion, preventing lipotoxicity and moderating the effect of nutrition on insulin sensitivity (Medina-Gomez et al. 2005; Medina-Gomez et al. 2007). Rare loss-of-function mutations of PPARγ have been found to cause defective adipocyte differentiation, partial lipodystrophy, especially in the gluteofemoral region, adipose tissue dysfunction and severe insulin resistance. Such agency highlights the important role of this gene in adipose tissue and energy metabolism (Majithia et al. 2014; Semple, Chatterjee, O'Rahilly 2006). The rs1801282 variant of the PPARG gene causes substitution of alanine for proline at position 12 (p.Pro12Ala) in the NH_2-terminus of PPARγ2 and results in reduced receptor activity and improved insulin sensitivity (Deeb et al. 1998). The p.Pro12Ala-variant is relatively common in Caucasians (minor allele frequency approximately 13%) and less frequent in East Asians (4.5%). This polymorphism reduces the risk of T2DM (Gouda et al. 2010) but increases the risk of cardiovascular disease (Z. Wu et al. 2012). The diabetes lowering effect of the Ala12 allele is mediated through improved insulin sensitivity, although this effect is modified by a person’s BMI and ethnicity (Dimas et al. 2014; Tönjes et al. 2006; Vänttinen et al. 2005). Higher skeletal muscle insulin sensitivity has been observed among non-diabetic lean/normal-weight carriers of the Ala12 allele but not in obese subjects (Vänttinen et al. 2005). However, it is unclear whether the Ala12 allele affects tissue-specific insulin sensitivity in T2DM.

The serine/threonine kinase Akt2 is a key mediator in the insulin signalling pathway (Chapter 2.1; Figure 2.3). A partial loss-of-function mutation p.Pro50Thr of the AKT2 gene is distinctive of the Finnish ancestry. This mutation causes impaired insulin-stimulated GU in several insulin sensitive organs, skeletal muscle, the liver, brown adipose tissue and bone marrow (Latva-Rasku et al. 2018) and increases the risk of diabetes (A. Manning et al. 2017). Similarly, as observed between obese/insulin resistant and insulin sensitive persons (Tuulari et al. 2013), brain GU was higher during insulin stimulation in p.Pro50Thr-induced insulin resistance. Also, EGP is less suppressed in carriers of this mutation (Latva-Rasku et al. 2018). Rare mutations of AKT2 have been found to affect insulin sensitivity, including the loss-of-function p.Arg274His mutation causing severe insulin resistance (George et al. 2004) and the p.Glu17Lys mutation causing constitutive insulin-independent Akt2 activation and severe fasting hypoinsulinemic hypoglycaemia (Hussain et al. 2011).

Epigenetic and structural modifications to DNA occurring during pregnancy are getting recognition as important factors affecting the health of the offspring (Iozzo

et al. 2014). Large cohort studies have shown that maternal obesity before or during gestation, predisposes offspring to obesity, insulin resistance and type 2 diabetes (Eriksson et al. 2014; Hochner et al. 2012; Z. Yu et al. 2013). Low birthweight and low muscle mass predict low muscle mass and strength also in adulthood (Du et al. 2010; Sayer et al. 2008). Low muscle mass in relation to body weight is associated with lower insulin sensitivity over the whole range of muscle mass and additionally correlates with worse glycemia (Srikanthan and Karlamangla 2011).

2.3.3 Other risk factors

Sex

Women are generally considered more insulin sensitive than men. This is supported by evidence pointing to women having higher insulin-stimulated skeletal muscle and whole-body glucose disposal (Lundsgaard and Kiens 2014; Nuutila et al. 1995) as well as better insulin suppression of EGP than men (Ter Horst et al. 2015). In addition, there is evidence of lower effect of insulin on adipocyte insulin signalling in male rodents (Macotela et al. 2009), but studies about sex differences in human adipose tissue GU are scarce. The female body type favours gluteofemoral fat deposition instead of androgenic abdominal fat deposition, which is likely a major contributor to the found differences in insulin sensitivity between sexes. Insulin sensitivity in women decreases when visceral fat deposition increases after menopause, suggesting that oestrogen is the reason behind the favourable deposition and protection from insulin resistance in women (Hocking et al. 2013).

Aging

Insufficient physical activity, weight-gain (Husu et al. 2011; NatCen Social Research and UCL 2017) and loss of muscle mass are typical characteristics of aging (Morley et al. 2001). Aged individuals tend to be more insulin resistant compared to younger persons (DeFronzo 1979; Rowe et al. 1983). In addition, loss of oxidative capacity and mitochondrial mass per volume of muscle occurs with aging (Conley, Jubrias, Esselman 2000). A hallmark of the problems associated with muscle loss is the frailty syndrome whereby aged individuals suffer from reduced muscle strength, endurance and physiologic function (Morley et al. 2013). The frailty syndrome is often preceded by whole-body insulin resistance (Barzilay et al. 2007; Perez-Tasigchana et al. 2017). Elderly patients with T2DM have lower muscle mass and strength when compared to nondiabetic individuals of the same age (Leenders et al. 2013; Park et al. 2009). Based on this information and other research (Amati et al. 2009; Ferrannini et al. 1996; Karakelides et al. 2010), it

seems that low physical activity and weight gain explain the most, if not all, of the effects of aging on loss of insulin sensitivity.

Cellular aging, measured by telomere length, is an aspect of aging which is not necessarily tied to chronological aging (K. A. Mather et al. 2011). Telomeres are repetitive DNA sequences at the ends of chromosomes which protect the coding and regulatory elements of the genome from degradation. Cellular aging is marked by telomeres becoming shorter due to oxidative stress and copying errors during cell division (K. A. Mather et al. 2011). Shorter leukocyte telomere length is associated with obesity (S. Chen et al. 2014; M. Lee et al. 2011) and predicts development of insulin resistance (Verhulst et al. 2016) as well as T2DM (J. Zhao et al. 2014). Additionally, low telomere length is associated with whole-body insulin resistance in young adults (Gardner et al. 2005) and with skeletal muscle insulin resistance in elderly women (Bucci et al. 2016). It is unclear whether shorter telomeres are associated with insulin resistance in other organs.

Statins

Statin treatment increases the risk of T2DM by decreasing insulin sensitivity and insulin secretion (Cederberg et al. 2015). 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase activity, which is the target of statins, is at least partly the reason behind the increased diabetes risk (Swerdlow et al. 2015). Moreover, cholesterol overload caused by statin-induced increase in LDL receptor on pancreatic β cells may be the mechanistic link behind impaired insulin secretion (Q. Yu, Chen, Xu 2017).

2.4 Methods of assessing insulin sensitivity

2.4.1 Measurement of whole-body insulin sensitivity

Euglycemic-hyperinsulinemic clamp

The euglycemic-hyperinsulinemic clamp technique (Defronzo, Tobin, Andres 1979) is widely considered the gold standard method for measuring insulin sensitivity and has been established as the standard method. The euglycemic-hyperinsulinemic clamp is carried out after an overnight fast in order to ascertain that no glucose is emerging from the gastro-intestinal tract. In the clamp, insulin is infused at a constant rate, which is above the fasting insulin levels. Plasma glucose is monitored using 5-10 min intervals and glucose is given intravenously at a rate which is sufficient to keep plasma glucose at a steady level. The usual target level in

euglycemic clamp is 5 mmol/l, which is around the normal fasting plasma glucose level and, therefore, does not cause an increase in glucose uptake by glucose mass action. Keeping the glucose level steady allows measuring insulin sensitivity without confounding by other homeostatic control mechanisms affecting blood glucose. In the euglycemic-hyperinsulinemic clamp, insulin infusion rate of 40-120 mU/m^2/min is usually used to stimulate GU into insulin sensitive tissues and to suppress EGP. Obese and insulin resistant subjects require a rate at the higher end of the range to be used in order to completely suppress EGP (Prager, Wallace, Olefsky 1986; Rizza, Mandarino, Gerich 1981). Only if EGP is completely suppressed, the amount of infused glucose reflects glucose uptake of the whole body.

There are several ways to express the whole-body glucose disposal rate (M-value) by the euglycemic-hyperinsulinemic clamp, which can make comparisons between studies difficult. The glucose infusion rate is usually reported per kg body weight or per kg fat-free mass to describe whole-body insulin sensitivity. Rationale for using fat-free mass is that the majority (~75-80%) of infused glucose during the clamp is taken up by skeletal muscle, and thus a large fat mass would exaggerate peripheral insulin resistance. A counterargument to this is that the contribution of adipose tissue GU to the whole-body glucose disposal is increased by the existence of a large fat mass (Dadson et al. 2016; Mitrou et al. 2009). M-value may be further divided by the clamp insulin level to describe insulin's effectiveness. However, the effect of insulin on M-value is not linear (Prager, Wallace, Olefsky 1986; Rizza, Mandarino, Gerich 1981), which should be taken into account when using the described approach.

There are a couple of limitations to using the clamp. First, frequent blood glucose measurements and adjustments to the glucose infusion rate that are crucial to a successful study require a highly trained staff or sophisticated equipment. Secondly, the clamp does not create the high insulin and glucose concentrations occurring physiologically in the portal vein at the postprandial state, thus limiting its use in measuring liver metabolism.

Insulin suppression test

The insulin suppression test (IST) (Shen, Reaven, Farquhar 1970) is a method of directly assessing insulin sensitivity of whole-body glucose disposal. IST is based on suppressing endogenous secretion of insulin and infusing insulin and glucose at a constant rate. Typical rates of insulin infusion are 25 or 30 mU/body surface m^2/min and of glucose infusion they are 240 or 260 mg/body surface m^2/min. Somatostatin, its analogue octreotide or alternatively epinephrine in combination with propranolol are used to block pancreatic insulin release (Harano et al. 1978; Pei et al. 1994; Shen, Reaven, Farquhar 1970). IST is conducted after an overnight

fast and insulin sensitivity is determined by the glucose level at steady state. Usually steady state is achieved between 150 to180 minutes after the commencement of the study. Insulin sensitivity assessed by IST is highly correlated ($r > 0.9$) with insulin sensitivity measured using euglycemic-hyperinsulinemic clamp (Greenfield et al. 1981; Mimura et al. 1994).

The required amount of labour and time as well as demand for equipment set limits to using the insulin suppression test and prohibit its large-scale use. In addition, EGP should be completely blocked to avoid overestimating insulin resistance. Moreover, the glucose level reached during the test may be relatively low in highly insulin sensitive subjects or high in subjects with insulin resistance. The former can cause confounding to the estimated insulin sensitivity due to insulin-independent glucose disposal and the latter due to glucose released to urine. These limitations may be addressed by adjusting the steady-state glucose level by fasting plasma glucose and collecting urine samples during the study (Pacini and Mari 2007). In general, IST is much less popular than the euglycemic-hyperinsulinemic clamp.

Intravenous glucose tolerance test

There are various types of intravenous glucose tolerance tests. The most commonly used is the frequently sampled intravenous glucose tolerance test (FSIVGTT) developed by Bergman and others (Bergman et al. 1979; Y. J. Yang, Youn, Bergman 1987). The test is performed after an overnight fast. The procedure starts with giving the subject an intravenous bolus of glucose over a period of 2 min. In a typical procedure, plasma glucose and insulin samples are collected every 1-2 min until 30 min from the start of the study and continued every 10 min until 180 min have passed. Plasma glucose and insulin concentrations are entered into a computer program called MINMOD, which calculates an index for insulin sensitivity (Pacini and Bergman 1986).

Dependence on the endogenous insulin secretion and difficulties in differentiating the effects of insulin-stimulation from the effect of high glucose concentration on glucose disposal are limitations to applying the original FSIVGTT (Y. J. Yang, Youn, Bergman 1987). To overcome this issue, a common modification to the protocol is to include a 5 min insulin infusion, which is started 20 min after the commencement of the glucose infusion (Finegood, Hramiak, Dupre 1990). Another solution is to use tolbutamide in order to increase insulin secretion (Beard et al. 1986). With these modifications, the insulin sensitivity assessment from FSIVGTT has a reasonably good correlation with the insulin sensitivity measured using the euglycemic-hyperinsulinemic clamp (Saad et al. 1997).

Fasting surrogate measures of insulin sensitivity

The simplest way to assess insulin sensitivity is measuring fasting plasma glucose and insulin and using surrogate insulin sensitivity indices derived from these measurements, such as the Homeostatic model assessment for insulin resistance (HOMA-IR) (D. R. Matthews et al. 1985) and the Quantitative insulin sensitivity check index (QUICKI) (A. Katz et al. 2000). Fasting FFAs provide information about insulin sensitivity of lipolysis. A modified version of the QUICKI, the revised QUICKI (Perseghin et al. 2001), combines information from fasting glucose, insulin and FFAs and provides the highest correlation with the insulin sensitivity measured using the hyperinsulinemic euglycemic clamp among common fasting surrogate indices of insulin sensitivity (Otten, Ahren, Olsson 2014). Even though, these measures provide a reasonably good estimate of whole-body insulin sensitivity, they only explain approximately 35-50% of the variation in directly measured whole body insulin sensitivity (Otten, Ahren, Olsson 2014). Thus, it is possible that the newly recognised metabolite markers for insulin resistance may provide additional predictive power for estimating insulin sensitivity using fasting blood samples. A natural limitation to making use of these measurements is that the fasting measurements can provide only a limited estimate of insulin sensitivity at postprandial state.

Oral glucose tolerance test

The oral glucose tolerance test is the standard test used in the diagnosis of impaired glucose tolerance and T2DM (American Diabetes Association 2015; WHO and IDF Technical Advisory Group 2006). The OGTT is performed after an overnight fast. In OGTT, an oral glucose load is given to the subject, after which plasma glucose is measured at predetermined points in time. The usual glucose load is 75 g and time point 120 min is used to diagnose impaired glucose tolerance T2DM. When conducting the OGTT to measure insulin resistance, insulin levels during the test need to be measured since pancreatic insulin secretion is a major factor affecting the glucose levels. High glucose levels during the test can reflect impaired insulin secretion without marked insulin resistance. Additional limitations to the accuracy of OGTT measurements to identify insulin resistance are differences in gastric emptying, intestinal absorption and motility as well as splanchnic blood flow (Hücking et al. 2008). Several surrogate insulin sensitivity indices are based on the measurements received from the OGTT, such as Matsuda (Matsuda and DeFronzo 1999), Stumvoll (Stumvoll et al. 2000), Gutt (Gutt et al. 2000) and OGIS indices (Mari et al. 2001). These indices are generally better correlated with the insulin sensitivity measurements from the gold standard method of euglycemic-hyperinsulinemic clamp than measurements from fasting state, although the

revised QUICKI (Perseghin et al. 2001) performs equally well (Otten, Ahren, Olsson 2014). A new promising insulin sensitivity index is PREDIM, which is based on OGIS, BMI and 2 h glucose and fasting insulin. PREDIM has a high association with clamp-derived whole-body GU (Tura et al. 2018).

2.4.2 Measurement of tissue specific insulin sensitivity

Measurement of tissue glucose uptake and lipolysis

Arteriovenous catheterisation in glucose uptake and lipolysis measurement

The arteriovenous difference technique is the oldest method of assessing insulin sensitivity at tissue level. This method is based on the concentration difference of a metabolite between an artery delivering the tissue of interest and a vena leaving from the tissue (Zierler 1961). GU and lipolysis during insulin stimulation can be determined based on the difference in the blood level of glucose and glycerol or the FFAs between artery and venous blood by using the Fick's principle when blood flow is steady. A common limitation to the arteriovenous catheterisation is the difficulty of catheterisation of the right vein(s) since a tissue can be drained with multiple veins or alternatively a single vein may drain from multiple tissues. In addition, internal organs or the brain are usually not available for measurement of metabolism by using arteriovenous difference method in humans due to the invasiveness of the procedure.

Microdialysis in measurement of tissue glucose uptake and lipolysis

Microdialysis is a method for assessing biochemical processes inside various tissues. In this method, a catheter with an inner and an outer tube is inserted into the tissue of interest. The tissue is then slowly perfused with dialysis fluid through the inner tube. The outer tube has a semipermeable membrane, which allows the analyte of interest, for example glucose or glycerol, to pass through according to a concentration gradient with interstitial space (Jansson, Smith, Lönnroth 1990; Lönnroth, Jansson, Smith 1987; Rooyackers et al. 2004). Subsequently, dialysate from the outer tube is collected and the analyte of interest is measured. GU and lipolysis can be measured using the Fick's equation when the arterial glucose and glycerol level, the regional blood flow and the permeability surface area product are known (Jansson et al. 1992; Virtanen et al. 2001). Thus, this method can be used to assess insulin sensitivity of GU and lipolysis. Limitations to the microdialysis technique include the inability to measure large molecules or hydrophobic molecules such as FFAs, the relatively high cost of the procedure, the limited access to organs deeper inside the body and the need for a recovery period to prevent

inflammation caused by insertion of the catheter, which may influence findings. In addition, one catheter only gives information about a tissue in its close vicinity, thus any heterogeneous organs would require multiple catheters to provide a more balanced view of tissue metabolism (Rooyackers et al. 2004).

Positron emission tomography used in measuring tissue specific glucose upake and blood flow

The usability of the arteriovenous difference technique and microdialysis in measuring insulin sensitivity in tissues deep inside the body is limited because of restricted access to these tissues. This limitation can be overcome by imaging techniques such as the positron emission tomography (PET). PET is a highly sensitive medical imaging technique that is based on measuring radioactive decay of labelled positron emitting compounds injected into humans or animals. A portion of gamma rays resulting from annihilation of the emitted positrons go through body tissues and are measured with the detector ring of a tomograph and computationally constructed to form three-dimensional images of tracer activity. Labelling glucose with a positron emitting isotope ^{18}F allows the measurement of the localisation of positron decay and thus GU. Furthermore, combining a positron-emitting glucose analogue, the ^{18}F-labelled fluorodeoxyglucose (^{18}F-FDG), with PET and the euglycemic-hyperinsulinemic clamp allows the measurement of tissue specific insulin-stimulated GU (Nuutila et al. 1992).

The structure of ^{18}F-FDG is favourable to measuring tissue GU due to its limited metabolism. ^{18}F-FDG transported into a cell is either phosphorylated to ^{18}F-fluorodeoxyglucose-6-phosphate (^{18}F-FDG6P) or transported back out of the cell. ^{18}F-FDG6P is not metabolized further to glycogen synthesis (Iozzo et al. 2007a) or glycolysis, and its metabolism into the pentose phosphate pathway is slow. Metabolites 2-^{18}F-fluoro-2-deoxy-6-phospho-d-gluconolactone (^{18}F-FD6PGL) and 2-^{18}F-fluoro-2-deoxy-6-phosphogluconate (^{18}F-FD6PG1) may become important when study times are longer than 60-90 min. However, like ^{18}F-FDG6P, these metabolites are not transported out of the cell (Bender et al. 2001). The low expression glucose-6-phosphatase in tissues other than the liver, the kidneys and the intestine (The Human Protein Atlas project 2018; Uhlén et al. 2015) cause the ^{18}F label to be effectively trapped into tissues until the positron decay of ^{18}F, which allows recording GU over time.

In addition, liver GU can be reliably measured during the hyperinsulinemic clamp. Such reliability is due to two different factors: First, the activation of glucokinase and inhibition of glucose-6-phosphatase by insulin cause GU to be much higher than glucose release. Second, phosphorylation of glucose just entering the cells and the dephosphorylation of G6P derived from gluconeogenesis, glycogen breakdown or cycling back from glycolysis are compartmentalised (Iozzo, Geisler et al.

2003; Iozzo et al. 2007a). Similarly, GU in the intestines can be measured with PET (H. Honka et al. 2013), although the possible role of intestinal glucose production, which could affect GU measurements, is unknown.

Since ^{18}F-FDG is trapped after phosphorylation, a three-compartment model consisting of blood, intracellular space and phosphorylated state can be used to model GU (Phelps et al. 1979) using the following equation:

$$MR_{glucose} = \frac{C_{glucose}}{LC} \times \frac{K_1^* \times k_3^*}{k_2^* + k_3^*} \tag{2.1}$$

Where $MR_{glucose}$ is the metabolic rate of glucose (GU rate), K_1^* is the rate of tracer forward membrane transport, k_2^* the rate of backward membrane transport and k_3^* the rate constant of ^{18}F-FDG's phosphorylation. Dephosphorylation of ^{18}F-FDG6P (k_4^*) is assumed to be 0. $C_{glucose}$ is the plasma glucose average from the ^{18}F-FDG injection until end of the PET scan. LC is a lumped constant adjusting for differences between transfer rates of ^{18}F-FDG and glucose of the tissue which is studied. Importantly, measuring $MR_{glucose}$ does not require blood flow measurement, thus providing an advantage over the arteriovenous difference and microdialysis techniques, which require information about blood flow.

Another way to calculate the GU rate is to use a graphical analysis method, the Patlak plot (Figure 2.5) (Patlak and Blasberg 1985a) whereby a net transfer rate is described using term K_i^* which combines K_1^*, k_2^* and k_3^*:

$$K_i^* = \frac{K_1^* \times k_3^*}{k_2^* + k_3^*} \tag{2.2}$$

$$MR_{glucose} = \frac{C_{glucose}}{LC} \times K_i^* \tag{2.3}$$

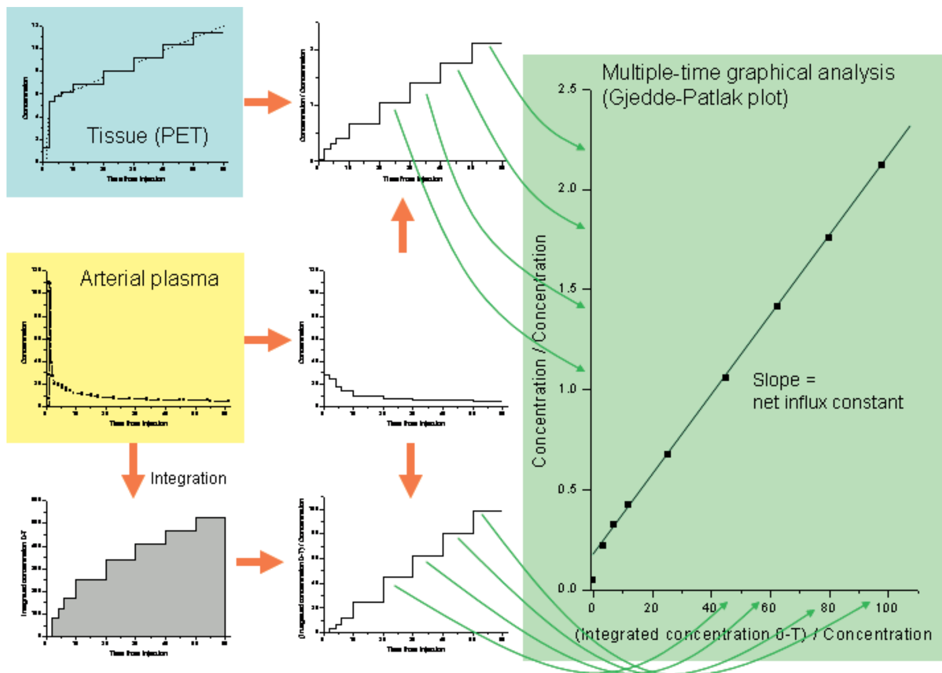


Figure 2.5. Patlak plot becomes linear after the tracer concentrations in reversible compartments and in plasma are in equilibrium. The slope of plot's linear phase is the net uptake (influx) rate constant K_i. K_i is presented in units of min^{-1}, or (ml tissue)/((ml plasma)*min). Note that although the x axis consists of time units (min or sec), the values do not represent the PET sample times. © 2013-2018 Turku PET Centre, University of Turku. Reproduced with permission under CC BY 4.0 (Creative Commons 2018b) from (Oikonen 2018a).

More simple models for measuring GU rates include fractional uptake rate (FUR) and standardised uptake value (SUV). FUR is a simple case of the Patlak plot, where blood activity measurement is required as well, but only one timepoint (static image) is used to measure tissue activity and the effective distribution volume is assumed to be 0 (Thie 1995). FUR yields an estimate of the tracer net transfer rate, which can be translated to a GU rate by multiplying FUR with the plasma glucose average and dividing by a lumped constant. Using FUR leads to some overestimation of the true net transfer rate. The bias is over than 20% within the first 20 min from the ^{18}F-FDG injection and become less than 5% after 60 min of scan time (Oikonen 2018b). Therefore, FUR is an accurate alternative for the Patlak plot when only static images are available, and the scanning time is long.

SUV is a semiquantitative method for assessing tissue GU, which is calculated by dividing tissue activity by injected dose and body weight. SUV values are affected by several factors which can lead to large errors and hamper the use of SUV for

measurement of glucose metabolism: 1) body composition and habitus, 2) length of uptake period, 3) plasma glucose, 4) recovery coefficient and partial volume effects (Keyes 1995). Although these matters can be often addressed by standardising scanning times, correcting for anthropometric features and plasma glucose, accuracy of SUV is inferior to the quantitative three-compartment modelling, Patlak plot and FUR (Bertoldo, Rizzo, Veronese 2014). Adjusting tissue SUV by blood or liver activity as improves accuracy of SUV measurement (Keramida et al. 2015; van den Hoff et al. 2013).

The effects of insulin on blood flow can be measured using positron emitting oxygen-15 labelled water (^{15}O-H_2O), which technique provides reliable information about tissue perfusion. This is due to the tracer being diffusible and not becoming metabolised or trapped. ^{15}O has a short half-life of only 122 seconds, which allows multiple measurements in a short period of time. This is a major improvement over Xenon-133 (half-life 5.3 days) often used in blood flow measurements (Ruotsalainen et al. 1997).

Limitations to the use of PET for measuring tissue specific GU and blood flow include the high cost of tomographs and tracer production as well as the need for highly trained personnel. The radiation dose emitted by the tracers limits the repeated use of PET especially with ^{18}F-FDG.

Magnetic resonance spectroscopy (MRS) in measuring tissue metabolism

MRS can be used to measure tissue glycogen content at baseline and after insulin stimulation to assess the effect of insulin on glycogen synthesis. This is done by measuring the naturally occurring carbon-13 glycogen spectra. A significant benefit of the described method is that it is completely non-invasive. Limitations include the use of ferromagnetic metal implants, which prohibits studies on subjects with metallic implants because such implants may distort imaging. MRS usually has a relatively low sensitivity, which requires long acquisition times. The signal-to-noise ratio may be improved by using an MR tomograph with higher field strength or an improved coil design. In a repeated measurement, similar ROI positioning is critical (Buehler et al. 2016). Hyperpolarised MRS is an interesting emerging improvement over the conventional MRS measurement, and it has great potential for the measurement of energy metabolism. In hyperpolarised MRS, the compounds labelled with NMR-active nuclei such as (1-^{13}C)lactate or (1-^{13}C)pyruvate are hyperpolarised and injected into a human or animal. Hyperpolarisation can increase the MR signal-to-noise ratio for detecting these compounds anywhere from 10,000 to 100,000-fold, thus allowing a fast measurement of the injected compounds' metabolism (Zaccagna et al. 2018).

Tracer methods of EGP measurement

One commonly used tracer technique to measure EGP is tracer dilution, whereby stable isotope-labelled glucose molecules are infused at a constant speed. At fasting state, glucose level, appearance and disappearance are steady, due to which EGP is easy to calculate by dividing the tracer infusion rate by the plasma tracer's and glucose concentration's ratio and subtracting the tracer infusion rate from the result after achieving a steady state of tracer in the blood. The equation can also be used during the euglycemic-hyperinsulinemic clamp after reaching a steady state. However, more complex models are needed for measuring glucose production at a non-steady state such as the postprandial state (Radziuk and Pye 2002; Vella and Rizza 2009). Stable isotopes ^{2}H and ^{13}C have the benefit of yielding no radioactivity, which means they can be used in larger study populations and in pregnant or lactating women as well as children who need special protection from radioactivity. They are also safe for the environment. However, these stable isotopes are more expensive than their radioactive alternatives, ^{3}H and ^{14}C. The measurement of stable isotopes from blood is based on using mass-spectrometry, while radioactivity is measured using a scintillation counter (Choukem and Gautier 2008). EGP during the euglycemic-hyperinsulinemic clamp and the ^{18}F-FDG study can be measured by subtracting the glucose infusion rate from the glucose disappearance rate measured from ^{18}F-FDG clearance which is corrected for activity lost to urine (Iozzo et al. 2006).

2.5 Type 2 diabetes

Diabetes is diagnosed by measuring either glycated haemoglobin (HbA1C) or fasting plasma glucose, using an oral glucose tolerance test or observing clear hyperglycemia. Diagnostic criteria are further described in Table 2.2.

Table 2.2. Diagnostic criteria for diabetes. Modified from (American Diabetes Association 2018a)

HbA1C ≥ 6.5% (48 mmol/mol)*
Plasma glucose ≥ 7.0 mmol (126 mg/dl) after at least 8 hours fast*
Plasma glucose ≥ 11.1 mmol/l (200 mg/dl) two hours after taking an oral bolus of 75g glucose dissolved in water*
Plasma glucose ≥ 11.1 mmol (200 mg/dl) anytime with combination of classic symptoms of hyperglycemia or hyperglycemic crisis

A diagnosis is based on fulfilling at least one of these criteria. *Unless the hyperglycemia is clear, the test should be repeated for confirmation.

The condition where fasting glucose is over 5.6 mmol/l or over 6.1 mmol/l but under 7.0 mmol/l is called impaired fasting glucose (IFG) and having 2h OGTT glucose concentration higher than 7.8 mmol/l but lower than 11.1 mmol/l is called impaired glucose tolerance (IGT)(American Diabetes Association 2018a; WHO and IDF Technical Advisory Group 2006). Together these conditions are called prediabetes, since the persons in these categories have high risk to develop T2DM. In addition to the previously mentioned criteria, persons having HbA1C above 5.7%, but below 6.5% may be diagnosed to have prediabetes (American Diabetes Association 2018a).

Persons eventually developing T2DM typically have insulin resistance with only minor changes in blood glucose levels for several years or more than a decade before T2DM onset because insulin resistance is compensated by increased insulin secretion (Færch et al. 2013; Tabák et al. 2009). Persons in the upper normal range of fasting glucose and 2h OGTT glucose show considerable insulin resistance (Stančáková et al. 2009). If insulin resistance is given the chance to progress further, eventually the insulin secretion capacity of the pancreas cannot fully compensate for the weaker insulin action or will start to decline allowing glycaemic control to deteriorate. Compensatory insulin secretion is still demonstrated in persons with IGT, while individuals with T2DM have impaired insulin release (Stančáková et al. 2009).

Although an insufficient inhibition of EGP by insulin is the main cause for increased fasting glucose in T2DM (DeFronzo, Ferrannini, Simonson 1989), glucose clearance has also been shown to be lower in obesity and T2DM. This is possibly due to a lower expression of the constitutive GLUT1-transporter in skeletal muscle (Ciaraldi et al. 2005; Henry et al. 1995; Jani et al. 2008).

2.6 Cardiovascular disease

IGT, hypertriglyceridemia and low HDL-cholesterol are characteristics of syndrome X, a cluster of coronary artery disease risk factors, which is likely caused by insulin resistance (Reaven et al. 1988). Insulin resistance increases the risk of incident cardiovascular events in persons without diabetes (Gast et al. 2012). Insulin resistance also predicted coronary heart disease, stroke and transient ischemic attack in a population-based follow-up study in elderly men (Wiberg et al. 2009; Zethelius et al. 2005). Similarly, a Mendelian randomisation study including 11 loci associated with insulin resistance found evidence of an increased cardiovascular disease risk in subjects with a higher insulin resistance (Ross et al. 2015). It is unclear whether insulin resistance is an independent risk factor for cardiovascu-

lar disease in type 2 diabetes (Laakso 2015). However, a cluster of hyperinsulinemia with other risk factors predicts coronary heart disease in men with T2DM (Kuusisto et al. 2001).

Triglyceride-rich lipoprotein remnants are a likely causal link between insulin resistance and cardiovascular disease. These particles contain large amounts of cholesteryl esters and smaller remnant particles can cross the endothelium to advance atherosclerosis. In addition to higher production of triglyceride-rich VLDL particles, slower LPL-dependent removal of remnant particles is typical for the insulin resistant state (Taskinen and Borén 2015). Moreover, lipolysis of the large triglycerides-rich VLDL particles results in formation of small dense LDL particles, which are associated with increased cardiovascular disease risk (Taskinen and Borén 2015).

2.7 Improving insulin sensitivity

2.7.1 Lifestyle changes

Regular exercise is known to improve insulin sensitivity and glycaemic control and to reduce the risk of T2DM (Colberg et al. 2010; Way et al. 2016). In fact, exercise may be as effective in preventing T2DM as diet or combined exercise and diet as shown in the Chinese Da Qing Diabetes Prevention Study (G. Li et al. 2008). Increased sedentary time is shown to heighten the risk of T2DM regardless of physical activity (Biswas et al. 2015; van der Berg et al. 2016). Studies in sedentary individuals and patients with T2DM have shown that frequent light activities, which reduce the amount of sedentary time are superior in improving insulin sensitivity compared to less frequently performed energy-matched but more intense structured exercise (Duvivier et al. 2013; Duvivier et al. 2017). Regular exercise can mitigate the risks conveyed from foetal environment to adulthood (Bucci et al. 2016; Eriksson et al. 2004). Moreover, endurance exercise improves insulin-stimulated GU and EGP suppression regardless of aging (Lanza et al. 2008) and may reverse age-related resistance to anabolic action of insulin by improving endothelial function and Akt/mTOR signalling (Fujita et al. 2007). Findings from a 12-week combined endurance and resistance training program suggest that mitochondrial function and increased intracellular lipid contribute to enhanced insulin sensitivity (Meex et al. 2010). Furthermore, proteomics analyses have identified that exercise increases the number of enzymes of the malate-aspartate shuttle, which moves NADH to the mitochondria, the amount of mitochondrial electron transport and respiratory chain complex 1 assembly as well as the tricarboxylic

acid (TCA) cycle. Moreover, exercise increases the number of NADH ubiquinone oxidoreductace subunits of complex 1, glutamic-oxaloacetic transaminase 2 and ATP synthase β, which are low in obesity and T2DM. Exercise also increases the amount of proteins related to oxidative phosphorylation. Interestingly, some proteins related to fatty acid and amino acid metabolism and the TCA cycle are increased in number in both obesity and T2DM as well as after exercise training (Srisawat et al. 2017). In addition to an improved mitochondrial function and biogenesis, exercise improves muscle GU capacity by an increased GLUT4 expression (Stanford and Goodyear 2014). Even though the benefits of aerobic exercise to insulin sensitivity are clear, there is limited knowledge about any possible benefits of resistance training to insulin sensitivity (Heinonen et al. 2014).

Diet is an effective tool for improving insulin sensitivity. Different diets targeting weight loss are generally equally effective (B. C. Johnston et al. 2014). ADA recommends an individualised diet with a 500-750 kcal daily energy deficit, exercise and behavioural strategies for 6 months to achieve a higher than 5% weight loss in overweight and obese patients with T2DM. A short-term very-low-calorie diet may be used in selected patients with close medical monitoring. These types of interventions should be followed by a comprehensive weight maintenance program with a reduced-calorie diet, 200-300 min of weekly physical activity and regular counselling and monitoring of body weight (American Diabetes Association 2018b). At least 10% weight loss improves whole-body insulin-stimulated glucose disposal and suppression of EGP (Viljanen, Lautamäki et al. 2009; Viljanen, Iozzo et al. 2009; Vitola et al. 2009).

A meta-analysis of randomised controlled isocaloric feeding studies has shown that replacing carbohydrates or SFAs with polyunsaturated FAs (PUFAs) in diet increases insulin sensitivity and insulin secretion capacity. Moreover, substituting carbohydrates with monounsaturated FAs (MUFAs) has a positive impact on insulin sensitivity and replacing MUFA with PUFA improves the acute insulin response (Imamura et al. 2016). There are several intervention studies showing the benefit of increased fibre consumption to insulin sensitivity in healthy volunteers (Robertson et al. 2005), overweight or obese subjects (Marinangeli and Jones 2011; M. A. Pereira et al. 2002; Weickert et al. 2006), subjects with insulin resistance (K. L. Johnston et al. 2010; Robertson et al. 2012) and type 2 diabetes (Dainty et al. 2016). Not all interventions have shown a benefit, however (Andersson et al. 2007; Bodinham et al. 2014). A meta-analysis of randomised controlled trials evaluating the effects of whole grain intake versus the intake of refined products did not yield evidence for any benefits of whole grain intake to insulin resistance in medium/long-term trials, although postprandial glycaemic and insulin response are better controlled by consuming whole grain products in acute studies (Marventano et al. 2017). Similarly, a meta-analysis evaluating total fibre intake

and higher vs. lower whole grain intake did not confirm the benefit of higher fibre or whole-grain intake, although both showed a tendency for reduction in insulin resistance (Reynolds et al. 2019). This may be due to low number of participants with measurements of insulin sensitivity, because higher fibre and whole grain intakes showed robust reductions in bodyweight, T2DM indidence as well as cardiovascular disease incidence and mortality in larger numbers of participants. Resistant starch is fermented into SCFAs by microbes in the colon, which possibly contributes to the benefits of fibre intake (Canfora, Jocken, Blaak 2015). An example of the benefits of fibre intake and SCFAs is a recent study where enrichment of SCFA producing bacteria by dietary fiber caused a reduction HbA1c levels among persons with T2DM (L. Zhao et al. 2018).

Quitting smoking improves insulin sensitivity (Eliasson et al. 1997). However, measures to prevent weight-gain should be applied when quitting smoking, since the commonly occurring weight-gain after smoking cessation increases the risk of T2DM (Hu et al. 2018).

2.7.2 Medications

Metformin is the first-line drug therapy for T2DM. Its main function is to suppress EGP. Metformin has been used as a therapy for T2DM for several decades, even though its mechanisms of affecting EGP are still uncovering. While there are probably several mechanisms whereby metformin affects EGP, a likely mechanism improving hepatic insulin sensitivity is the inhibition of acetyl-CoA carboxylase 1 by AMPK-mediated phosphorylation. Acetyl-CoA carboxylase 1 catalyses conversion of acetyl-CoA to malonyl-CoA, which is a substrate for FA synthesis and inhibits CPT1-mediated FA transport to mitochondria for oxidation. Blocking this phosphorylation of acetyl-CoA carboxylase 1 leads to hepatic fat accumulation and insulin resistance (Fullerton et al. 2013; Rena, Hardie, Pearson 2017). In addition to the liver, the intestine is an important target for the insulin sensitizing effects of metformin. Studies in humans and rodents have shown that metformin treatment increases insulin-stimulated GU from the blood circulation into the small intestine and the colon, an effect which is localised to the enterocytes of the mucosal layer (Koffert et al. 2017). This effect is likely mediated via AMPK (Massollo et al. 2013). Moreover, metformin has been shown to improve glucose homeostasis by causing alterations in gut microbiota, decreasing amounts of Bacteroides fragilis, increasing concentration of the bile acid glycourodeoxycholic acid and inhibition of intestinal farnesoid receptor X signalling (Sun et al. 2018; H. Wu et al. 2017).

Thiazolidinediones are a class of drugs that improve insulin sensitivity by activating the nuclear receptor PPARγ. PPARγ is a key regulator for expression of genes controlling adipogenesis as well as glucose and lipid metabolism. Alternative splicing of the PPARG mRNA results in two proteins PPARγ1 and -2, of which PPARγ1 is ubiquitously expressed while the latter is normally mostly expressed in adipose tissue (Fajas et al. 1997). PPARγ agonism has an insulin-sensitizing effect on multiple tissues. PPARγ-agonist, rosiglitazone, improves insulin-stimulated skeletal muscle GU both at rest and during exercise in T2DM patients (Hällsten et al. 2002; Karlsson et al. 2005). In addition, rosiglitazone enhances the insulin-stimulated uptake into visceral and subcutaneous adipose tissue, the liver and the myocardium (Hällsten et al. 2004; Iozzo, Hällsten et al. 2003b; Lautamäki et al. 2005; Viljanen et al. 2005; Virtanen et al. 2003). Altogether, three different thiazolidinediones have been on the market: troglitazone, rosiglitazone and pioglitazone. Troglitazone was withdrawn due to toxic effects on the liver (Gale 2001), whereas the use of rosiglitazone is limited due to cardiovascular risks, especially heart failure (Graham et al. 2010; Loke, Kwok, Singh 2011; Mannucci et al. 2010; Nissen and Wolski 2007). Pioglitazone is still on the market, as it does not increase all-cause mortality (Mannucci E. et al. 2008) and is protective against major cardiovascular events (Liao et al. 2017), atherosclerosis (Nissen et al. 2008) and stroke (M. Lee et al. 2017). Both rosiglitazone and pioglitazone are associated with an increased fracture risk, heart failure, oedema and weight gain (Aubert et al. 2010; Liao et al. 2017; Mannucci et al. 2010; T. Yang and Soodvilai 2008). In addition, a novel PPARγ agonist, lobeglitazone, has been recently released on the market in South Korea. Lobeglitazone has shown a similar performance in improving glycemia as pioglitazone with a much lower treatment dose in phase III trials (Jin et al. 2015). The lower required dose is due to favourable structural properties, which allow better interaction with PPARγ (Jang et al. 2018). Lobeglitazone has resulted in reduced insulin resistance and liver fat along with improved lipid profiles in T2DM patients in early trials (Kim et al. 2014; Y. H. Lee et al. 2017).

GLP-1 analogues likely do not directly improve insulin sensitivity (Vella et al. 2000), although they have a direct anti-inflammatory effect which may lead to improved insulin sensitivity (Rakipovski et al. 2018; Y. Wang et al. 2014). In addition, GLP-1 analogues may improve insulin sensitivity indirectly by weight loss and visceral fat reduction (F. Zhang et al. 2015) as well as resolved hyperglycaemia or reduced adipose tissue lipolysis (Zander et al. 2002). Sodium/glucose co-transporter 2 (SGLT2) inhibitors have shown improvement in whole-body insulin sensitivity in some studies (Merovci et al. 2014; Mudaliar et al. 2014). Since SGLT2 is not expressed in skeletal muscle (J. Chen et al. 2010), it is likely that the observed insulin sensitising effects are due to resolved hyperglycaemia and hyperinsulinemia or weight loss.

2.7.3 Bariatric surgery

Bariatric surgery (also called metabolic surgery) is the most effective way to treat morbid obesity and diabetes (Ribaric, Buchwald, McGlennon 2014) and improves insulin sensitivity in the short term as well as the long term (Rao, Yanagisawa, Kini 2012). Bariatric surgery enhances skeletal muscle, adipose tissue and liver insulin sensitivity to GU (Dadson et al. 2016; Immonen et al. 2014; Mäkinen et al. 2015) and normalises the increased insulin-stimulated brain GU (Tuulari et al. 2013). According to a meta-analysis the decrease in BMI five years or more after a bariatric surgery is 10-15 kg/m^2 (Golzarand, Toolabi, Farid 2017). Studies with an average follow-up time of 17 months after surgery have shown a T2DM remission rate of approximately 60% (Ribaric, Buchwald, McGlennon 2014). The effect of bariatric surgery diminishes over time (Peterli et al. 2018; Salminen et al. 2018; Sjöström et al. 2014), but surgery-treated patients have still much higher diabetes remission rates and less cardiovascular complications at least 5 years after surgery when compared to those who received treatment without surgery (Sheng et al. 2017). Bariatric surgery is the recommended treatment option for obesity and T2DM for persons with BMI $\geq$ 40 kg/m^2 and is recommended also for individuals with BMI in the range of 35.0-39.9 kg/m^2 when a lifestyle change and optimal medical therapy are ineffective to reduce hyperglycemia. In addition, bariatric surgery should be considered for persons with BMI 30.0-34.9 kg/m^2 when hyperglycemia cannot be controlled with a lifestyle change and optimal medical therapy (American Diabetes Association 2018b).

3 AIMS OF THE STUDY

I. To study effects of the p.Pro12Ala polymorphism of the PPARG gene on liver and skeletal muscle insulin sensitivity (Study I)

II. To measure how insulin sensitivity in the liver, skeletal muscle and adipose tissue associate with each other (Study II)

III. To study the associations between serum metabolites and skeletal muscle insulin sensitivity and to use metabolomic measures to develop inexpensive surrogate models for skeletal muscle and whole-body metabolism (Study III)

IV. To evaluate the effects of resistance training on liver and adipose tissue insulin sensitivity among elderly women (Study IV)

4 MATERIALS AND METHODS

4.1 Study subjects (I-IV)

The study subjects in Studies I, II, III and IV comprised participants of the CMgene study (NCT03310502; 499 individual participants), in which associations were investigated between tissue metabolism, genetic background, adipose tissue deposition and circulating metabolites in a cross-sectional cohort of individuals with a wide range of body fat mass, age and insulin sensitivity. The subjects were participants of an independent PET metabolic study project and were subsequently recruited to the CMgene study, at which point they gave a separate signed informed consent. The overview of the study population is presented in Table 4.1. Figure 4.1 shows the number of cases overlapping between the different studies.

Table 4.1. Baseline characteristics of the subjects

Study	**Group**	**N**	**M/F**	**Age (years)**	**BMI (kg/m^2)**	**Fasting glucose (mmol/l)**	**Fasting insulin (mU/l)**
I	ND	68	45/23	35 [28-47]	26 [23-30]	5.3 [5.1-5.6]	5 [4-8]
I	T2DM	105	73/32	61 [55-67]	30 [27-32]	7.5 [6.4-8.9]	7 [5-9]
II	ND	326	217/111	42 [29-59]	26 [23-30]	5.5 [5.2-5.9]	7 [5-11]
III	NFG	122	88/34	45 [38-54]	26 [23-32]	5.2 [5.0-5.4]	6 [4-8]
III	IR	182	94/88	61 [52-69]	29 [26-32]	6.2 [5.8-7.1]	8 [6-13]
IV	CTR	9	0/9	71 [69-75]	27 [25-31]	6.0 [5.9-6.6]	8 [7-14]
IV	Frail	35	0/35	72 [69-74]	27 [24-30]	5.8 [5.4-6.4]	8 [6-12]

CTR: control, IR: insulin resistant, ND: no diabetes, NFG: normal fasting glucose, T2DM: type 2 diabetes; M: men, F: women. Data represented as median [interquartile range].

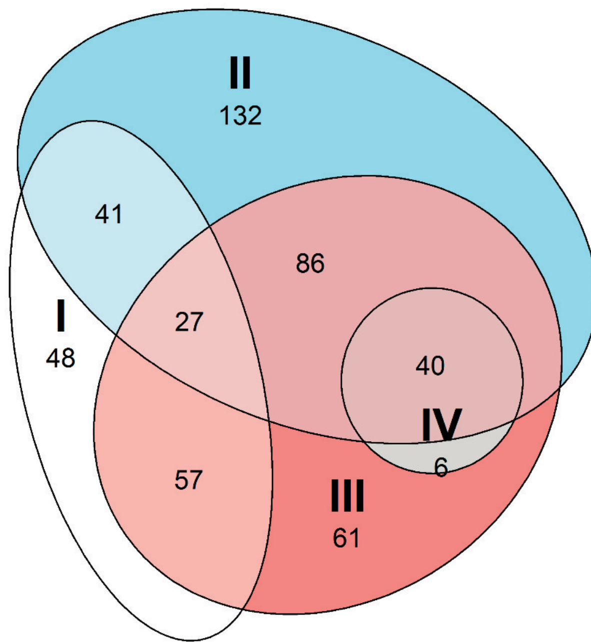


Figure 4.1. Number of subjects overlapping between each study. Total number of subjects in study I: 173, II: 326, III: 277, IV: 46.

The subjects from the CMgene study cohort consisted of individuals who had participated in PET metabolic studies at Turku PET Centre (Turku, Finland) between 1990 and 2017. The study protocol was approved by the Ethics Committee of the Hospital District of South-West Finland. The CMgene study has been registered on ClinicalTrials.gov (NCT03310502).

Study I included 173 volunteers (68 nondiabetic subjects and 105 subjects with type 2 diabetes) studied between 1990 and 2007. The subjects' T2DM diagnosis was confirmed according to the World Health Organization criteria (Alberti and Zimmet 1998). Out of the 105 subjects with T2DM, 46 were receiving metformin, sulfonylurea, or both at the time of study. None of the subjects with T2DM were having insulin or thiazolidinedione treatment. The subjects were divided into two groups, nonobese and obese, using a BMI cutoff point of 27 kg/m^2 as in our previous study (Vänttinen et al. 2005). This cutoff point was used because there was a statistically significant interaction with the effect the p.Pro12Ala polymorphism of the PPARG gene and this BMI threshold on skeletal muscle (Vänttinen et al. 2005) and liver GU.

Study II included 326 nondiabetic volunteers who had their insulin-stimulated skeletal muscle, adipose tissue or liver GU measured between 1990 and 2017.

Study III included 277 volunteers, who had participated in PET research studies between 2002 and 2017. The population was divided into subjects with 1) normal fasting glucose (NFG) and 2) insulin resistance (IR; persons with IFG or T2DM). IFG and T2DM diagnoses were confirmed by using the American Diabetes Association criteria (American Diabetes Association 2015). The oral glucose tolerance test (OGTT) was not available for all participants, hence it was not used to diagnose prediabetes in this study.

The subjects in Study IV (Developmental ORIgins of Healthy and Unhealthy AgeiNg, DORIAN; NCT01931540) were recruited from the Helsinki Birth Cohort Study (HBCS), which is a longitudinal cohort study of subjects born in Helsinki (Finland) between 1934 and 1944 (Eriksson et al. 2014). Forty-six elderly women were recruited. Thirty-five subjects, who were characterised as frail using a hand grip strength test, participated in the resistance exercise intervention, and nine women with normal strength served as controls and did not participate in the intervention. Two additional frail participants, of whom no postintervention data existed, were used in baseline comparisons. The inclusion criteria for the whole study population were having an age between 68 and 78 years and for participants with low muscle strength: 1) having grip strength that is in the lower half of HBCS, 2) having either a lean/normal-weight (BMI ≤ 26.3 kg/m^2, lower half of BMI) or an overweight/obese (BMI ≥ 28.1 kg/m^2, highest quartile of BMI) mother at the time of birth. Inclusion criteria for the control group were having grip strength in the upper half of HBCS and having a lean/normal-weight mother. Six of the participants had oral diabetes medication and 16 had statin medication. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland. The study was conducted according the principles of Declaration of Helsinki and all participants gave their signed informed consent for the study.

4.2 Study principles and designs

CMgene cohort

Studies I-III used subjects from the CMgene cohort. Subjects in this cohort were recruited from participants of independent PET metabolic study projects which evaluated insulin-stimulated skeletal muscle, adipose tissue and liver GU, insulin-mediated suppression of EGP, body adiposity and ectopic fat accumulation. Measurements were performed similarly in all the studies as described later in the Methods section, thus allowing combining data from different studies. Although many of the included studies had an interventional design, only measurements from the baseline were included, resulting in a cross-sectional design. In addition, a whole blood sample was collected from the participants.

Study I

In Study I, whole blood samples were used to determine carriers of the p.Pro12Ala polymorphism of the PPARG gene. This study was aimed to investigate the hypothesis that the p.Pro12Ala polymorphism of the PPARG gene improves liver insulin sensitivity in both healthy subjects and T2DM patients and skeletal muscle insulin sensitivity among T2DM patients. This hypothesis was based on earlier findings that had shown the p.Pro12Ala polymorphism to be protective against T2DM and to improve peripheral insulin sensitivity in nondiabetic subjects (Tönjes et al. 2006; Vänttinen et al. 2005).

There were only 9 persons homozygous for the Ala12 allele, therefore persons carrying one or two Ala2 alleles were combined into one group and compared to the carriers of the p.Pro12Pro genotype. Liver, skeletal muscle and adipose tissue GUs were measured for each participant when possible.

Study II

In Study II, data of liver, skeletal muscle and adipose tissue GUs and EGP among nondiabetic individuals in the CMgene database were used to determine how insulin sensitivity associates between the different tissues simultaneously in single individuals. The hypothesis in this study was that the insulin sensitivity of GU in these tissues are correlated as insulin sensitivity in skeletal muscle (stimulation of whole-body GU), adipose tissue (suppression of lipolysis) and the liver (suppression of glucose production) had shown consistent patterns when comparing lean and obese individuals or studying associations with liver fat content (Conte et al. 2012; Korenblat et al. 2008). In addition, reference values for tissue-specific insulin resistance were determined.

Study III

Study III aimed to develop prediction models for whole-body and skeletal muscle GU by using metabolomics and common clinical measurements. This was based on the hypothesis that lipoprotein subclass lipids, amino acids and other metabolomic measures would improve prediction of insulin sensitivity when added to the traditional fasting insulin, glucose and FA measurements as many of these metabolomic measurements had been associated with measures of insulin sensitivity and T2DM risk in other studies (Festa et al. 2005; Fizelova et al. 2015; Guasch-Ferre et al. 2016; Hodge et al. 2009; Mackey et al. 2015; Mora et al. 2010).

Study III included subjects whose insulin-stimulated skeletal muscle or whole-body GU had been measured and who had given a serum sample for metabolomic

profiling. NFG participants were used to create training prediction models for skeletal muscle and whole-body GU using metabolomic and common clinical measurements and persons with insulin resistance were used to test the prediction models.

Study IV

Study IV focused on investigating the hypothesis that resistance training improves liver and adipose tissue insulin sensitivity among elderly women and reverses the possible harmful effects of maternal obesity on tissue-specific insulin sensitivity. These hypotheses were based on findings from earlier studies that had shown improvement in insulin-stimulated GU and EGP suppression regardless of aging with endurance exercise (Lanza et al. 2008) and mitigation of risks conveyed from foetal environment to adulthood with regular exercise (Bucci et al. 2016; Eriksson et al. 2004).

PET-CT (PET with computed tomography), magnetic resonance imaging (MRI) and MRS studies were conducted twice in the frail group; before and after the four-months exercise intervention in the frail group, and once in the non-frail control group (Figure 4.2). The second PET and magnetic resonance measurements were performed approximately 2-3 days after the last exercise training session.

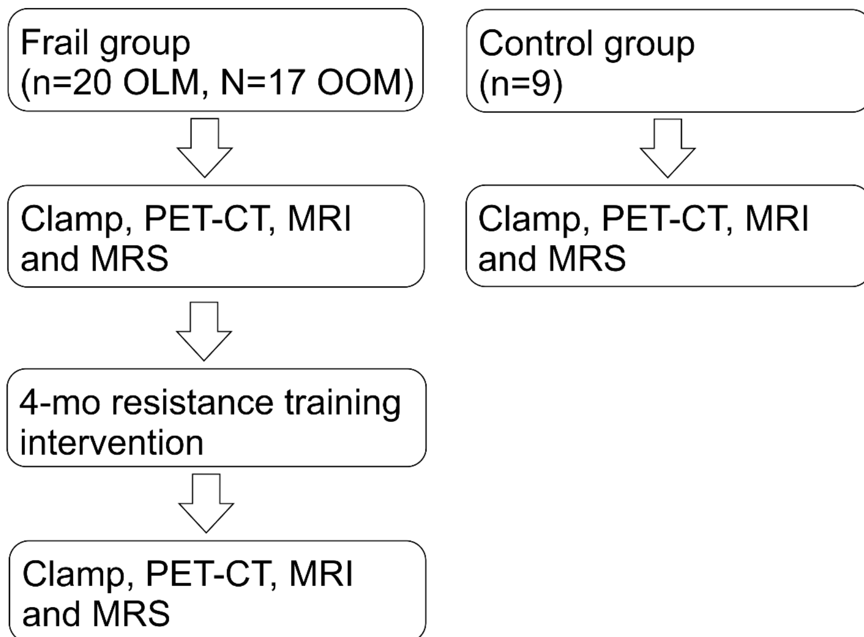


Figure 4.2. Design of the Dorian study (IV). OLM: offspring of lean/normal-weight mothers, OOM: offspring of overweight/obese mothers.

4.3 Methods

4.3.1 PET-studies (I-IV)

The PET studies were conducted following a 10-hour fast. The clamp protocol started at approx. 11:10 (range 8:40-15:45). The use of alcohol and caffeine were prohibited for a period of 12 hours before the study and subjects were instructed to avoid heavy physical exercise in the preceding 24 hours. The PET studies were conducted during an euglycemic-hyperinsulinemic clamp with subjects resting in a supine position. Infusions of insulin, glucose and ^{18}F-FDG were given using a cannula inserted into an antecubital vein. Blood samples were collected from the opposite side radial artery or the antecubital vein warmed with a heating pillow. In the clamp study, plasma glucose was maintained at 5 mmol/l by infusing insulin at the rate of 40 mU/body surface m^2/min (after a 7 min priming) and 20% glucose when needed. Whole-body GU rates were measured after achieving a steady plasma glucose level (in average from 60 min onwards). The M-value was calculated by using three 20 min time intervals.

4.3.2 Exercise intervention in elderly women (IV)

The subjects participated in a four-months individualised circuit-type training program three times a week under the supervision of a physiotherapist (Wasenius et al. 2018). The exercise program consisted of eight different exercises aimed training different large muscle groups in the upper and lower body (leg press, chest press, seated row, abdominal crunch, back extension, seated leg extension, seated leg curl and hip abduction). Three sets of 8 to 15 repetitions were executed at medium intensity. Medium intensity was defined as being 50-80% of estimated one-repetition maximum (Epley 1985). Progress in muscle strength was measured once per month, and training loads were updated accordingly.

4.3.3 Skeletal muscle, liver and adipose tissue glucose uptake measurement (I-IV)

A computer-controlled device was used to synthesise ^{18}F-FDG using a modified method of Hamacher et al. (Hamacher, Coenen, Stocklin 1986). The studies were done by using several PET-scanners: ECAT 931/08 (Siemens Molecular Imaging, Inc., Knoxville, TN), GE Advance, PET/CT Discovery VCT and PET/CT Discov-

ery 690 (General Electric Medical Systems, Milwaukee, WI). In order to get consistent results, the PET-scanners were cross calibrated against the same VDC-404 Dose calibrator (COMECER Netherlands, Joure, the Netherlands). Obtained data were all corrected for decay, photon attenuation and dead time. The images were reconstructed by using median root prior with iterations and a Bayesian coefficient 0.3 whenever possible (Alenius and Ruotsalainen 1997). Tissue ^{18}F-FDG activity was measured by drawing ROIs to the quadriceps femoris muscle, the right lobe of the liver and abdominal subcutaneous adipose tissue. MRI and CT images were used as references whenever available. Tissue fractional phosphorylation rates (Ki) of the tracer were calculated by using the Patlak-plot of plasma and tissue time-activity curves (Gjedde 1982; Patlak and Blasberg 1985b). The tissue GUs were then calculated by multiplying Ki by the average plasma glucose concentration during the period between the point of injection of ^{18}F-FDG and the end of the PET scan of each tissue and dividing the result by tissue density and a lumped constant. A lumped constant value of 1.0 for the liver, 1.2 for skeletal muscle, and 1.14 for adipose tissue was used, as previously described (Iozzo et al. 2007b; D. E. Kelley et al. 1999; Peltoniemi et al. 2000; Virtanen et al. 2001). Fifty-seven subjects in Study I performed intermittent isometric exercise with one leg during the scan; but only the measurements of the noncontracting, that is, control, leg were used to determine skeletal muscle GU.

EGP measurement (II-III)

EGP was calculated by subtracting the exogenous glucose infusion rate from the rate of disappearance of glucose during the euglycemic hyperinsulinemic clamp:

$$\mathrm{EGP} = \mathrm{R}_d + \mathrm{V}_{glucose} \times \frac{\Delta_{\mathrm{glucose}}}{\Delta_{\mathrm{T}}} - \mathrm{GIR} \qquad 4.1$$

Where R_d is the rate of disappearance and GIR is the glucose infusion rate. GIR is corrected by a space correction (Defronzo, Tobin, Andres 1979) where $V_{glucose}$ is the estimated glucose distribution volume (0.19 l/kg), $\Delta_{glucose}$ is the change in glucose from ^{18}F-FDG injection to the end of sampling (mmol/l), and Δ_T is the time of ^{18}F-FDG injection to the end of sampling (min).

Glucose disappearance rate (R_d) was calculated using ^{18}F-FDG clearance corrected by tracer lost to urine (Iozzo et al. 2006):

$$\mathrm{R}_d = \frac{\mathrm{dose}_{\mathrm{FDG}} - \mathrm{urine}_{\mathrm{FDG}}}{\mathrm{AUC}_{\mathrm{FDG}}} * \mathrm{avg}_{\mathrm{glucose}} \qquad 4.2$$

Where $dose_{FDG}$ is the activity of the injected ^{18}F-FDG, $urine_{FDG}$ is ^{18}F-FDG secreted to urine from the tracer injection until voiding bladder at the end of the study, AUC_{FDG} is the area under the curve representing ^{18}F-FDG from the tracer injection

to infinity and $avg_{glucose}$ is the average glycemia during the interval between the time of ^{18}F-FDG injection and the end of sampling.

4.3.4 Liver fat measurement (IV)

Liver fat was measured by means of MRS using a 1.5T MR imager (Gyroscan Intera CV Nova Dual, Philips Medical Systems, Best, the Netherlands) with a flexible surface and body coils. The LCModel software package (http://s-provencher.com/lcmodel.shtml) was used to measure proton MR spectra (28). A previously described fat index (FI1) (Borra et al. 2009) based on liver or whole-body MR images was used to determine liver fat content in cases where both before- and after-intervention liver MRS measurements were not available. In each liver image, the median FI1 of the pixels was used. A linear, least-squares fit between FI1 and liver fat content measured using MRS was performed. This was done in all cases where both MR images and spectroscopic measurements were available for the same subject. The fitting was done separately for the data from the whole-body scans and the data from dedicated liver scans. The coefficient of determination was $r^2 = 0.884$ for the whole-body scans and $r^2 = 0.929$ for the liver scans. Liver fat content values were used in the following order of preference if both baseline and after-intervention values were available: MRS (n = 35) > liver MRI (n = 4) > whole-body MRI (n = 9).

4.3.5 Measurement of visceral adipose tissue volume (IV)

In Study IV, a visceral adipose tissue volume was computed semiautomatically. For the purposes of the study, the abdominal region was defined to extend from the top of the diaphragm to the waist. The automated segmentations were performed in four steps: 1) whole-body in-phase T1-weighted images were corrected for intensity inhomogeneity using the simultaneous correction method (Vovk, Pernus, Likar 2006); 2) Otsu's method (Otsu 1979) with two thresholds was used to classify the pixels in the area as representing either adipose tissue, nonadipose tissue or background; 3) arms were removed using simple morphological operations and the removal of small objects in axial 2D slices; and 4) the inside lean tissue method was used to separate subcutaneous tissue from other adipose tissue as described below. Pixels that had been incorrectly classified as representing adipose tissue were manually removed. Finally, adipose tissue volumes were converted to masses using a density of 0.916 kg/l.

The inside lean tissue (ILT) method

The ILT method has been developed and evaluated using CT data (Kullberg et al. 2017). However, in Study IV, the ILT method was adapted for analysing MRI data. For validation, manual reference segmentations of volumes containing VAT and SAT without other adipose tissue were performed in three slices near the umbilicus in five subjects. The volume of fat in these volumes was then calculated using thresholding. Linear regression between these volumes and the volumes determined using the ILT method was performed, giving r the values 0.9993 for VAT and 0.9995 for SAT. The volumes calculated using the ILT method were on average 2.40% greater for VAT and 0.32% greater for SAT when compared to the results from the manual segmentations.

4.3.6 Biochemical measurements (I-IV)

Fasting plasma glucose were measured with an automatised enzymatic assay (Roche Diagnostics GmbH, Mannheim, Germany). Insulin was measured using a double antibody radioimmunoassay (Phadeseph Insulin RIA kit, Pharmacia & Upjohn, Uppsala, Sweden), fluoroimmunometric assay (AutoDELFIA, PerkinElmer Inc., Turku, Finland) or automatised electro-chemiluminescence immunoassay (Cobas 8000, Roche Diagnostics GmbH, Mannheim, Germany). Plasma glucose measurements for the clamp were done in duplicate using the glucose oxidase method (Analox GM7 or GM9, Analox Instruments, London, UK). FFAs were measured with an automatised enzymatic assay (NEFA-HR2, ACS-ACOD, Wako Chemicals GmbH, Neuss, Germany; Cobas 8000, Roche Diagnostics GmbH, Mannheim, Germany).

4.3.7 Metabolomic measurements (III)

Metabolic biomarkers were quantified from the serum of 277 individuals at a fasting state using high-throughput proton NMR metabolomics (Nightingale Health Ltd, Helsinki, Finland; University of Eastern Finland, Kuopio, Finland). This method provides simultaneous quantification of routine lipids, fatty acid composition and various low-molecular metabolites including amino acids, ketone bodies and gluconeogenesis-related metabolites in molar concentration units as well as a lipoprotein subclass profiling with lipid concentrations within 14 subclasses. The 14 lipoprotein subclass sizes were defined as follows: extremely large VLDL with particle diameters from 75 nm upwards and a possible contribution of chylomicrons, five VLDL subclasses (average particle diameters of 64.0 nm, 53.6 nm, 44.5

nm, 36.8 nm, and 31.3 nm), IDL (28.6 nm), three LDL subclasses (25.5 nm, 23.0 nm, and 18.7 nm) and four HDL subclasses (14.3 nm, 12.1 nm, 10.9 nm, and 8.7 nm). The following components of the lipoprotein subclasses were quantified: phospholipids, triglycerides, cholesterol, free cholesterol and cholesteryl esters. The mean sizes of VLDL, LDL and HDL particles were calculated by weighting the corresponding subclass diameters with their particle concentrations. Details of the experimentation and applications of the NMR metabolomics platform have been discussed previously (Inouye et al. 2010; Soininen et al. 2009; Soininen et al. 2015).

4.3.8 Statistical methods (I-IV)

The effects of the exercise intervention were tested using a paired t-test. Differences between independent groups were tested using either the t-test or ANOVA. ANCOVA was used to adjust for any possible effects of covariates (e.g. diabetes medication or statin use). Logarithmic or square root transformation was used to convert right-tailed distributions to normal. In cases where normal distribution was not attainable, the Mann-Whitney U-test was applied to compare independent groups and the Wilcoxon Signed rank test was used for paired comparisons. Associations between study variables were tested using the either the Pearson or the Spearman correlation tests. P-values ≤ 0.05 were considered statistically significant. Results are expressed as a mean [standard deviation (SD)] value for normally distributed variables or a median [interquartile range (IQR)] value for non-normally distributed variables.

Statistical methods in the study II

In Study II, the Principal component analysis (PCA) was used to assess the associations between tissue glucose uptake rates. A variable was included in a PCA only if it had significant bivariate correlations with all the other variables used in the analysis. Eigenvalue 1.0 was used as a threshold value in PCA (Kaiser 1960). Receiver-operating characteristics (ROC) curve analysis was used to find cutoffs to identify insulin sensitive and insulin resistant individuals based on tissue-specific insulin-stimulated GUs and EGP. An earlier study has identified 28 µmol/kg fat-free mass/min (Stern et al. 2005) as the optimal cutoff point for determining whole-body insulin resistance. A body fat measurement was not available for all the subjects taking part in Study II and therefore ROC analysis was used to recognise a corresponding value expressed per kg body weight. 21 µmol/kg body weight/min produced 92% sensitivity and 99% specificity for determining whole-body insulin resistance and was used as a reference for the insulin resistant state in

the above analyses. The area under the ROC curve (AUC) of over 0.7 was considered acceptable for discrimination (Hosmer and Lemeshow 2000) and the Youden index which gives equal weight to sensitivity and specificity was applied to determine the optimal cutoff values (Greiner, Pfeiffer, Smith 2000).

Statistical methods in the Study III

Three regression models were constructed in order to predict GU of the skeletal muscle, M-value and liver fat percentage. These models are called Muscle-ISI, WB-ISI and Liver-FI, respectively. The regression models were built using a linear model with lasso regularisation (Tibshirani 1996). All the models were trained using subjects with normal fasting glucose (NFG). Independent insulin resistant subjects (IR) were used for testing.

Features of the final models were selected using the following nested cross-validation. For each of the three models, the feature ranking was determined by creating 100 models using a ten-fold cross-validation and defining the frequency of each feature of the models. Features with a frequency higher than 75% and ranking higher than 11 were selected to be included in the final model.

The models were tested with the independent testing data which were not used for model building. The models Muscle-ISI, WB-ISI, HOMA-IR and revised QUICKI were evaluated with the Spearman correlations by comparing the estimated values with the measured values. HOMA-IR was calculated as being equal to fasting plasma glucose (mmol/l) * fasting serum insulin (mU/l) / 22.5 (D. R. Matthews et al. 1985) and revised QUICKI as equal to 1/(log plasma glucose (mmol/l) + log serum insulin (mU/l) + log serum free fatty acids (mmol/l)) (Perseghin et al. 2001).

Receiver-operating characteristic (ROC) analysis was used to determine the optimal cutoffs for Muscle-ISI, WB-ISI, HOMA-IR and revised QUICKI for diagnosing skeletal muscle or whole-body insulin resistance as well as comparing the discrimination performance of these indices. Cutoff points 33 µmol/kg tissue/min for skeletal muscle GU and 21 µmol/kg body weight/min for M-value in Study II were used to determine insulin resistance. The Youden index, which gives equal weight to sensitivity and specificity, was used to determine the cutoff points in NFG subjects (Greiner, Pfeiffer, Smith 2000). The performance of the different cutoff points was subsequently tested in the IR group using McNemar's test.

Associations between circulating metabolites and whole-body and skeletal muscle insulin-stimulated GU were tested using the Spearman correlations. The mathematical modelling and the statistical analysis were done using an R statistical computing environment (version 3.4) (R Core Team 2017). The R package glmnet

(Friedman, Hastie, Tibshirani 2010) was applied for the linear and logistic regression models with lasso regularisation. Heatmaps were created with the gplots package (Warnes et al. 2016), correlation coefficients were compared using the WRS package (Wilcox 2011) and AUCs in the ROC analysis were compared using the pROC package (Robin et al. 2011).

5 RESULTS

5.1 Effects of the p.Pro12Ala polymorphism of the PPARG gene on liver and skeletal muscle insulin sensitivity (I)

There was a significant interaction between BMI and the effects of the p.Pro12Ala polymorphism on liver GU. Therefore, the populations were divided into nonobese and obese according to the determined cutoff point (BMI < 27 kg/m^2 and BMI ≥27.0 kg/m^2, respectively; P = 0.01 for interaction with this cutoff point).

Carriers of the p.Pro12Pro genotype had 28% lower liver GU compared to the Ala12 allele carriers in obese subjects with T2DM (Figure 5.1A). A similar, although non-significant, trend of 22% difference was observed in obese nondiabetic subjects (Figure 5.1B). There were no differences in liver GU among nonobese subjects irrespective of having T2DM.

Skeletal muscle, subcutaneous adipose tissue or whole-body GU did not differ between carriers of the Ala12 allele and p.Pro12Pro genotype among subjects with T2DM (Figure 5.2).

Glucose and insulin levels during the euglycemic hyperinsulinemic clamp were 5.1 ± 0.4 mmol/l and 63 ± 14 mU/l, respectively. There were no differences in FFA levels between the genotype groups in obese subjects with or without T2DM after correcting for confounding factors.

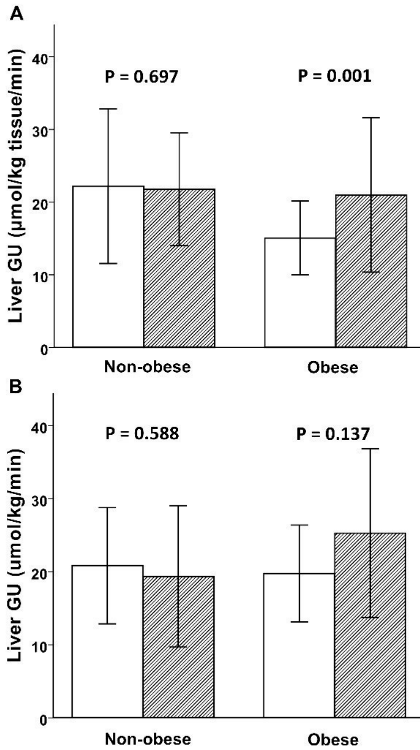


Figure 5.1. The effect of the p.Pro12Ala polymorphism of PPARG2 on the rates of hepatic GU in nonobese (n=23) and obese (n=72) diabetic (A) and nonobese (n=38) and obese (n=30) nondiabetic (B) subjects (analysis of covariance; adjusted for sex, age, the use of oral antidiabetic drugs, and one leg isometric exercise (yes/no) during the clamp in diabetic subjects, and adjusted for sex, age, and one leg isometric exercise (yes/no) during the clamp in nondiabetic subjects). White bars indicate the p.Pro12Pro genotype carriers; striped bars indicate carriers of the Ala12 allele. Values are means ± SD.

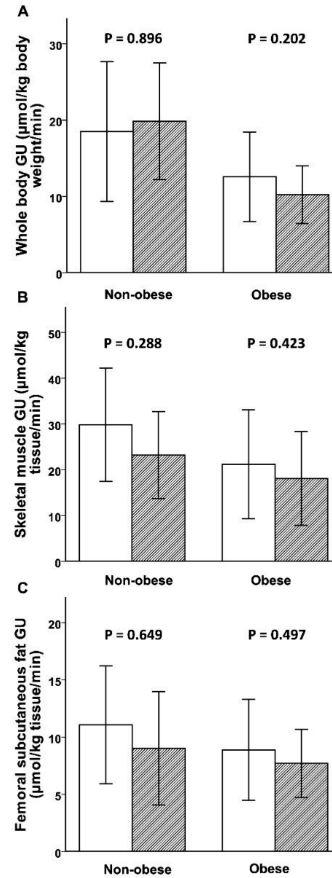


Figure 5.2. The effect of the p.Pro12Ala polymorphism of PPARG2 on the rates of whole-body (n=27 and n=78 for the nonobese and obese subjects, respectively) (A), skeletal muscle (n=25 and n= 74) (B), and subcutaneous adipose tissue (n=26 and n=74) (C) GU in nonobese and obese diabetic subjects (analysis of covariance; adjusted for sex, age, the use of oral antidiabetic drug treatment, and one leg isometric exercise (yes/no) during the clamp). White bars indicate the p.Pro12Pro genotype carriers; striped bars indicate carriers of the Ala12 allele. Values are means ± SD.

5.2 Association of insulin sensitivity across skeletal muscle, the liver and adipose tissue among nondiabetic subjects (II)

Insulin-stimulated GU by skeletal muscle, liver and abdominal subcutaneous fat, as well as the M-value, correlated significantly with each other in subjects with no diabetes (Figure 5.3). Furthermore, EGP correlated with the M-value as well as skeletal muscle and adipose tissue GU (Figure 5.4).

In addition, BMI correlated significantly with all the examined tissue GUs (skeletal muscle: $r = -0.583$, $P < 0.001$; liver: $r = -0.173$, $P = 0.009$: abdominal subcutanous fat: $r = -0.364$, $P < 0.001$). Correlation testing showed that there are differences between genders in associations of tissue GUs.

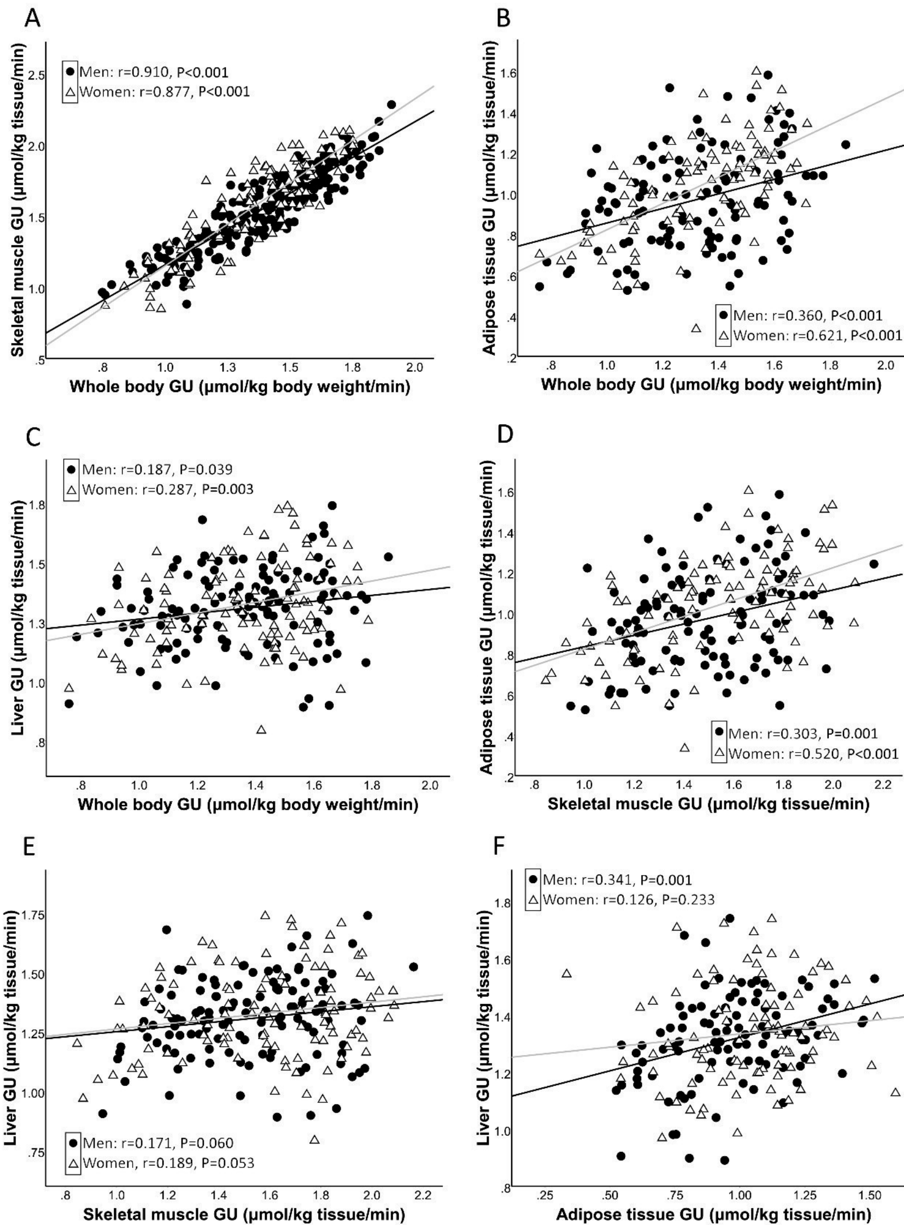


Figure 5.3. Correlation between whole-body glucose uptake (GU) and skeletal muscle GU (A), whole-body GU and subcutaneous adipose tissue GU (B) and whole-body GU and liver GU (C); correlation between skeletal muscle GU and subcutaneous adipose tissue GU (D) and skeletal muscle and liver GU (E); and correlation between subcutaneous adipose tissue GU and liver GU (F). GU values are derived from log10 transformed variables. Black regression line: men; grey regression line: women. Reproduced with permission from (M. J. Honka et al. 2018).

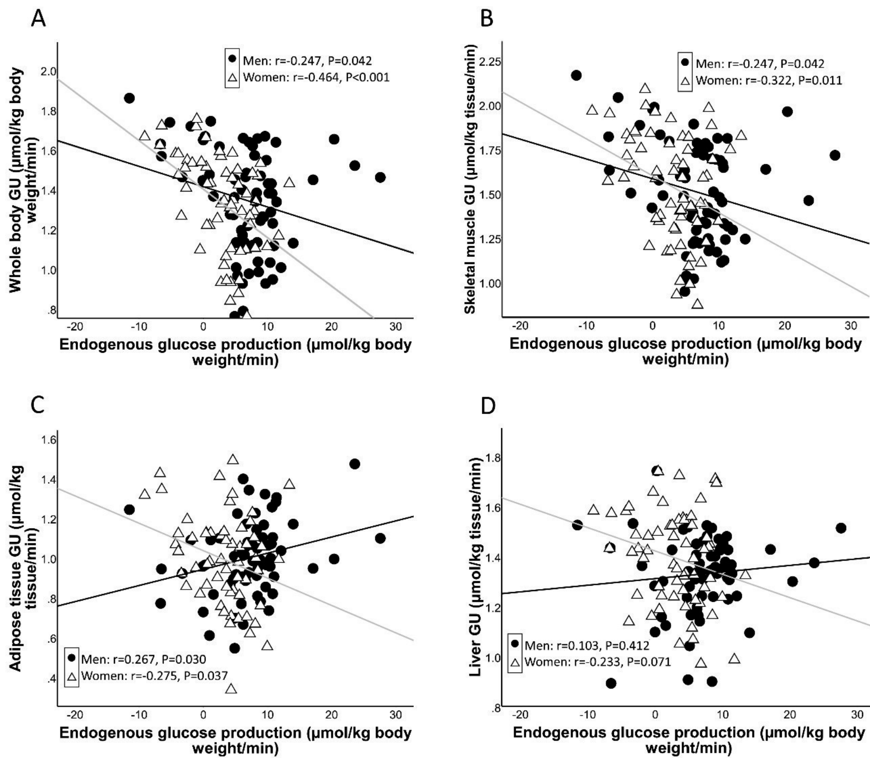


Figure 5.4. Correlation between EGP and whole-body glucose uptake (GU) (A), endogenous glucose production and skeletal muscle GU (B), EGP and subcutaneous adipose tissue GU (C) and EGP and liver GU (D). GU values are derived from log10 transformed variables. Black regression line: men; grey regression line: women. Reproduced with permission from (M. J. Honka et al. 2018).

Table 5.1. Principal component analysis. Loadings in men and women indicate the contribution of each variable to the principal components (PC1, PC2, PC3). Modified with permission from (M. J. Honka et al. 2018).

Model 1	**Men**		**Women**		**All**		
GU(µmol/kg tissue/min)	PC1	PC2	PC1	PC2	PC1	PC2	PC3
Age (years)	-0.205	0.895	-0.006	0.952	0.095	0.693	-
BMI (kg/m2)	-0.857	0.023	-0.724	-0.489	-0.829	0.108	-
Gender	-	-	-	-	-0.033	0.822	-

Skeletal muscle GU	0.833	-0.091	0.850	0.126	0.830	0.015	-
Subcutaneous adipose tissue GU	0.585	0.617	0.841	-0.206	0.703	0.240	-
Variance explained	45%	30%	49%	30%	38%	25%	-
Model 2							
Age (years)	-0.206	0.864	-0.039	0.881	-0.047	0.887	0.070
BMI (kg/m2)	-0.828	-0.057	-0.700	-0.442	-0.744	-0.278	0.292
Gender	-	-	-	-	0.061	0.076	0.959
Skeletal muscle GU	0.837	-0.051	0.844	0.162	0.848	0.039	0.047
Subcutanoeus adipose tissue GU	0.458	0.662	0.846	-0.130	0.724	0.065	0.233
Liver GU	0.513	0.423	0.193	0.768	0.322	0.684	0.011
Variance explained	38%	27%	39%	32%	32%	22%	18%

A separate PCA was performed in order to study the association of EGP with skeletal muscle GU and adipose tissue GU (Table 5.2). Again, muscle GU and adipose tissue GU had high loadings on PC1 in both sexes, whereas EGP had a high negative loading in women and no association in men. PC2 showed a positive effect of age on EGP.

Table 5.2. Principal component analysis. Loadings in men and women indicate the contribution of each variable to the principal components (PC1, PC2, PC3). Liver GU was replaced by endogenous glucose production (EGP). Modified with permission from (M. J. Honka et al. 2018).

Model 1	**Men**		**Women**		**All**		
GU/EGP (µmol/kg tissue/min)	PC1	PC2	PC1	PC2	PC1	PC2	PC3
Age (years)	-0.206	0.741	0.079	0.909	0.241	-0.131	0.884
Body mass index (kg/m2)	-0.901	-0.117	-0.541	-0.774	-0.855	0.157	-0.232
Gender	-	-	-	-	-0.156	0.667	0.602
Skeletal muscle GU	0.816	-0.314	0.783	0.294	0.804	0.341	-0.127
Subcutaneous adipose tissue GU	0.562	0.644	0.765	0.147	0.703	-0.079	0.119
EGP	0.024	0.719	-0.684	0.547	-0.056	-0.886	0.159
Variance explained	37%	32%	39%	37%	33%	23%	21%

ROC analysis yielded optimal cutoff points for identifying the insulin resistant state for muscle, abdominal subcutaneous adipose tissue and EGP in both sexes and visceral adipose tissue GU among women (Figure 5.5), whereas in the case of liver GU in both sexes and visceral adipose tissue GU in men, discrimination between insulin resistant and insulin sensitive individuals was poor.

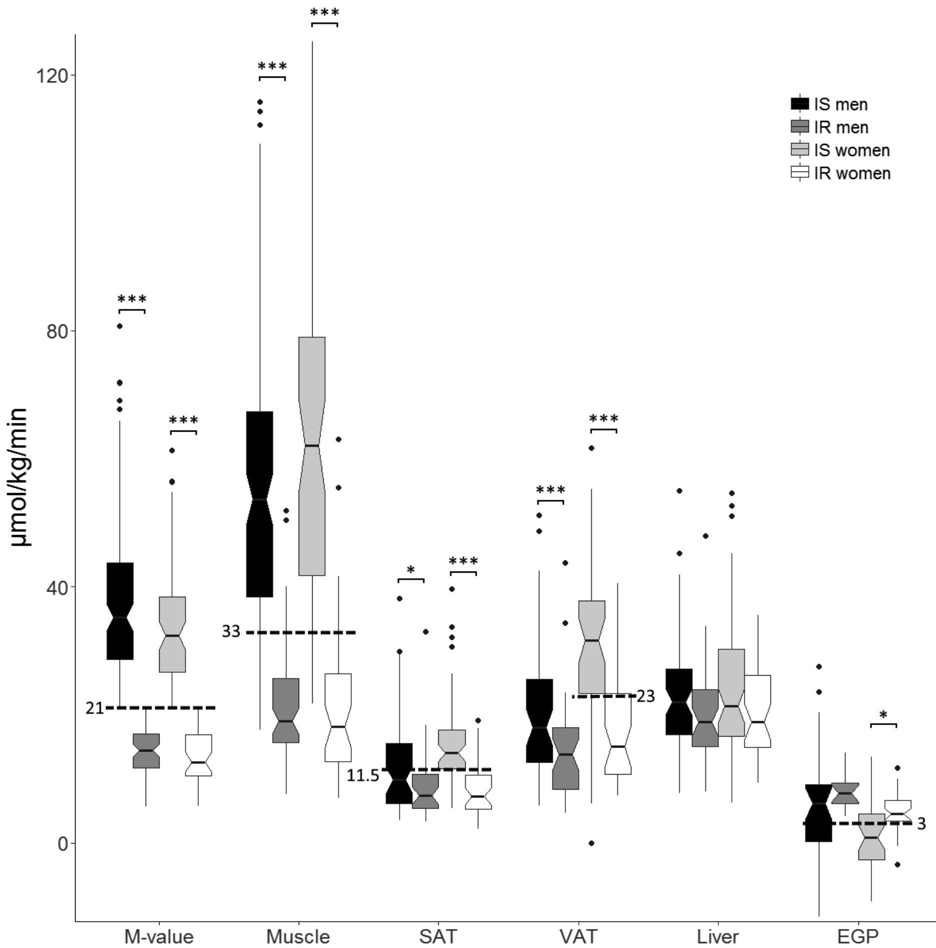


Figure 5.5. Whole-body (M value), muscle, subcutaneous adipose tissue (SAT), intraperitoneal adipose tissue (VAT) and liver glucose uptake and endogenous glucose production (EGP) between insulin sensitive (IS) and insulin resistant (IR) men and women. Dashed lines represent the optimal cutoff values between insulin resistant and insulin sensitive individuals. ***$P < 0.001$, *$P < 0.05$. Reproduced with permission from (M. J. Honka et al. 2018).

5.3 Predicting insulin sensitivity using serum NMR metabolomic measures (III)

The regression model of skeletal muscle GU (Muscle-ISI, model described in Table 5.4) correlated better to the actual GU measurement than HOMA-IR or revised QUICKI when studied using the whole dataset (Figure 5.6). However, the difference between Muscle-ISI and revised QUICKI was non-significant in the NFG group when the population was divided into NFG and IR groups (Table 5.3). The regression model whole-body GU (WB-ISI, Table 5.6) correlated better to the actual GU measurement than HOMA-IR or revised QUICKI when studied using the whole dataset (Figure 5.6), and a similar trend was observed when the study population was again divided into NFG and IR groups (Table 5.5).

Table 5.3. Spearman correlation between measured and predicted skeletal muscle GU values and P-values for comparisons of correlation coefficients between different indices. ***P < 0.001; **P < 0.01; *P < 0.05.

	Spearman correlation			**P-value**	**P-value**
	Muscle-ISI	HOMA-IR	revQUICKI	Muscle-ISI vs. HOMA-IR	Muscle-ISI vs. revQUICKI
NFG	0.58 ***	-0.3 **	0.39 ***	0.040	0.140
IR	0.68 ***	-0.47 ***	0.49 ***	0.017	0.010

Table 5.4. Coefficients in the final (Muscle-ISI) linear regression model of Skeletal muscle GU prediction. An estimate of Skeletal muscle GU is calculated as the sum of each row where the row values are obtained by multiplying the value of the variable by the coefficient. All metabolite and biochemical measurements were taken from fasting samples. LG10, logarithm to base 10.

Feature	Coefficient
	Muscle-ISI
Intercept	-211
Insulin (mU/l)	-1.45
LG10 phospholipids in medium-sized VLDL (% of total lipids)	254
Free fatty acids (mmol/l)	-19.5
Triglycerides in small HDL (mmol/l)	-287
LG10 Glycine (mmol/l)	38.5

BMI (kg/m^2)	-0.59
LG10 Serum triglycerides (mmol/l)	-4.72
Triglycerides in large VLDL (mmol/l)	-6.46
Triglycerides in medium-sized HDL (% of total lipids)	-0.0831

Table 5.5. Spearman correlation between measured and predicted M-value values and P-values for comparisons of correlation coefficients between different indices. ***P < 0.001; **P < 0.01; *P < 0.05.

	Spearman correlation			**P-value**	**P-value**
	WB-ISI	HOMA-IR	revQUICKI	WB-ISI vs. HOMA-IR	WB-ISI vs. revQUICKI
NFG	0.75 ***	-0.49 ***	0.60 ***	<0.001	0.080
IR	0.70 ***	-0.58 ***	0.59 ***	0.073	0.087

Table 5.6. Coefficients in the final (WB-ISI) linear regression model of M-value prediction. An estimate of M-value is calculated as the sum of each row where the row values are obtained by multiplying the value of the variable by the coefficient. All metabolite and biochemical measurements were taken from fasting samples.

Feature	
	WB-ISI
Intercept	73
LG10 HOMA-IR	-17.1
Free fatty acids (mmol/l)	-13.1
LG10 acetoacetate (mmol/l)	-10.8
LG10 acetate (mmol/l)	25.7
BMI (kg/m^2)	-0.305
Triglycerides in medium-sized HDL (% of total lipids)	-1.01
LG10 Omega-3 fatty acids (mmol/l)	-12.2
Triglycerides in large VLDL (mmol/l)	-11
Triglycerides in medium HDL (mmol/l)	-79.8

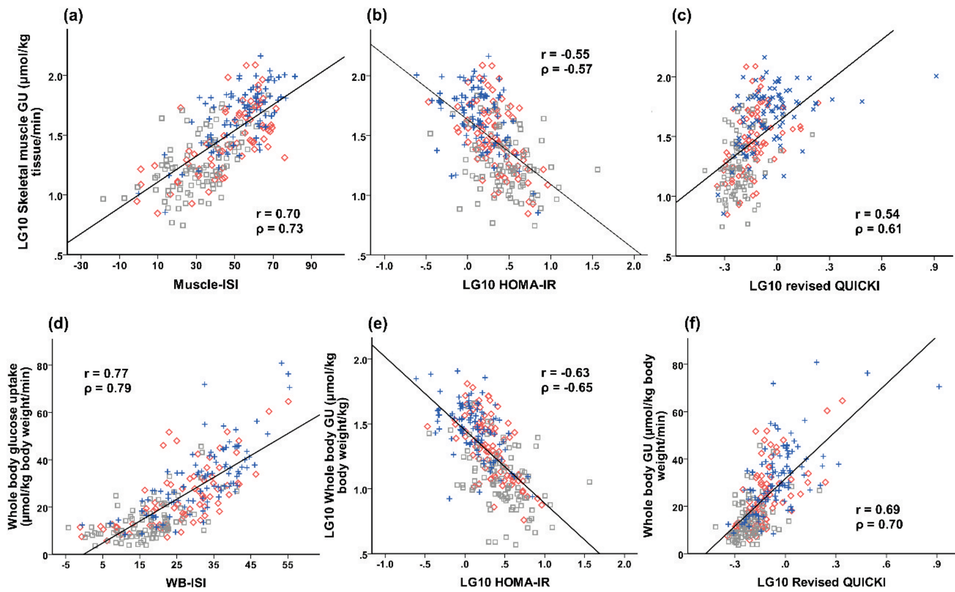


Figure 5.6. Correlations between skeletal muscle GU and Muscle-ISI (a), skeletal muscle GU and HOMA-IR (b) and skeletal muscle GU and revised QUICKI (c) and correlations between whole-body GU and WB-ISI (d), whole-body GU and HOMA-IR (e) and whole-body GU and revised QUICKI (f). Blue crosses represent NFG, red diamonds represent IR groups shown as IFG and grey squares represent T2DM. r: Pearson correlation coefficient, ρ: Spearman correlation coefficient. $P < 0.0001$ for all correlations. Spearman ρ was higher for Muscle-ISI and WB-ISI when compared to HOMA-IR or revised QUICKI ($P \leq 0.01$ for all comparisons).

NFG subjects were used to find optimal cutoffs for detecting skeletal muscle and whole-body insulin resistance. For Muscle-ISI, 50 was the cutoff that yielded 80% sensitivity and 84% specificity in detecting muscle insulin resistance. 1.46 for HOMA-IR gave 72% sensitivity and 76% specificity and 0.74 for revised QUICKI produced 85% sensitivity and 68% specificity. Using these cutoffs, there were no differences in sensitivity or specificity between the tests among NFG subjects. Next, the performance of these cutoffs was tested in IR subjects. For muscle-ISI, the cutoff of 50 yielded 88% sensitivity and 69% specificity in detection of skeletal muscle insulin resistance, 1.46 for HOMA-IR gave 87% sensitivity and 35% specificity and 0.74 for revised QUICKI produced 85% sensitivity and 49% specificity. There were no differences between sensitivities of the tests, but Muscle-ISI had a better specificity than HOMA-IR ($P < 0.001$) or revised QUICKI ($P = 0.013$).

Based on ROC analysis using the NFG group, the optimal cutoffs for detecting whole-body insulin resistance were 29 for WB-ISI, 1.68 for HOMA-IR and 0.74 for revised QUICKI. Using these cutoffs, WB-ISI had 84% sensitivity and 77% specificity, HOMA-IR had 74% sensitivity and 81% specificity and revised QUICKI had 74% sensitivity and 84% specificity in detecting whole-body insulin resistance. There appeared no differences between the tests in sensitivity or specificity. When testing among IR subjects, the cutoff of 29 for WB-ISI yielded 93% sensitivity and 62% specificity in detecting whole-body insulin resistance, 1.68 for HOMA-IR gave 84% sensitivity and 53% specificity and 0.74 for revised QUICKI produced 87% sensitivity and 56% specificity. WB-ISI had a higher sensitivity than HOMA-IR (P = 0.013) and tended to have a higher sensitivity than revised QUICKI (P = 0.065).

When comparing AUCs from ROC analysis using IR subjects, Muscle-ISI and WB-ISI were superior to HOMA-IR (P = 0.005 and P = 0.001, respectively) and revised QUICKI (P = 0.026 and 0.004) for discriminating between insulin resistant subjects and insulin sensitive subjects (5.7).

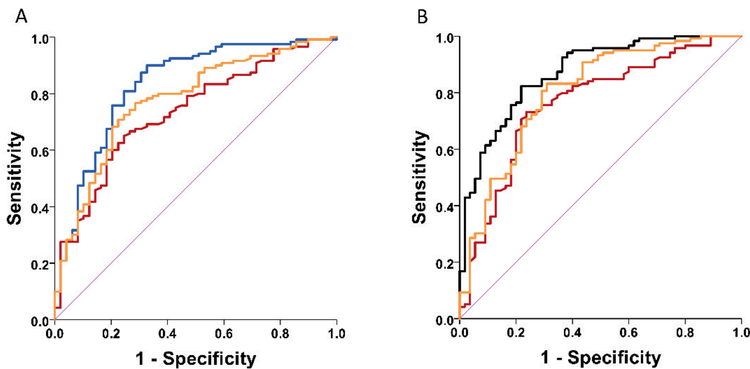
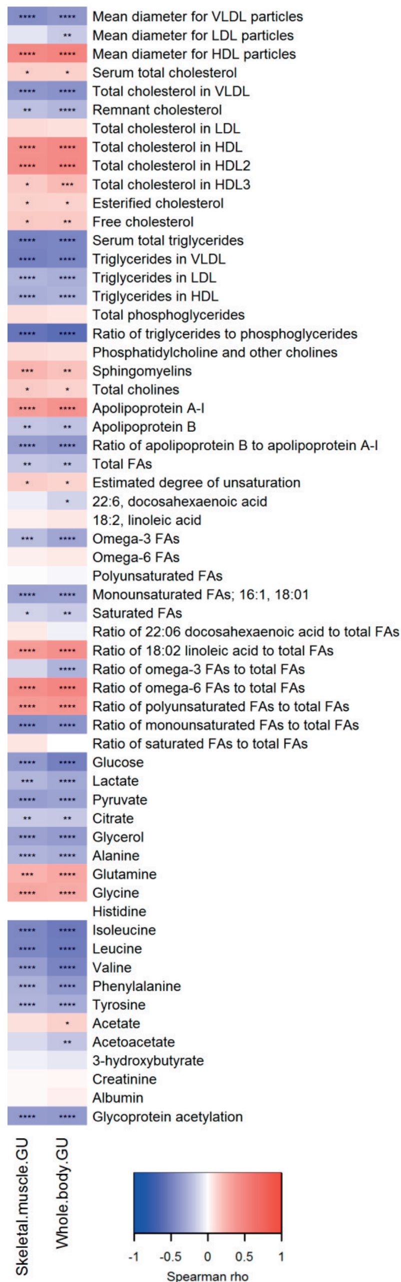


Figure 5.7. (A) ROC curve comparing sensitivity and specificity of Muscle-ISI (blue line), HOMA-IR (brown line) and revised QUICKI (yellow line) in determining skeletal muscle insulin resistance. AUC for Muscle-ISI: 0.83, HOMA-IR: 0.73 and revised QUICKI: 0.78 and (B) ROC curve comparing sensitivity and specificity of WB-ISI (black line), HOMA-IR (brown line) and revised QUICKI (yellow line) in determining whole-body insulin resistance. AUC for WB-ISI: 0.87, HOMA-IR: 0.76 and revised QUICKI: 0.80.



Correlations between insulin sensitivity and metabolites

Correlation testing using the whole population showed positive correlations between insulin sensitivity and glycine and glutamine, whereas alanine, isoleucine, leucine, valine, phenylalanine and tyrosine had a negative association with insulin sensitivity (Figure 5.9). Metabolites related to glycolysis were negatively associated with insulin sensitivity. Mono- and omega-3 fatty acid concentration was associated with insulin resistance, whereas linoleic acid, omega-6 fatty acids and total polyunsaturated fatty acids were positively associated with insulin sensitivity.

Figure 5.8. Heatmap showing how skeletal muscle GU and M-value correlate with metabolites. FAs = Fatty acids. ****P < 0.0001; ***P < 0.001; **P < 0.01; *P < 0.05.

Correlations between insulin sensitivity with lipoprotein subclass measures

Triglycerides were negatively associated with insulin sensitivity across all the studied lipoprotein subclasses, with the exception of triglycerides within large and very large HDL (Figures 5.9 and 5.10). The opposite finding regarding HDL can be explained by an increased concentration of large and very large HDL with increased insulin sensitivity, because fraction of triglycerides to total lipid content in these particles had negative association with insulin sensitivity (Figure 5.10C). In contrast to large HDL particle concentration, concentration of small HDL particles was negatively associated with insulin sensitivity. The evaluation of small HDL lipids showed that phospholipid fractions, free cholesterol fractions and triglyceride fractions of the total lipid contents were inversely associated with insulin sensitivity, whereas total cholesterol fractions and cholesteryl ester fractions had an opposite pattern.

All lipids in VLDL subclasses were negatively associated with insulin sensitivity. The associations were the strongest among the larger subclasses (Figure 5.9A). Further examination of the VLDL subclass lipid fractions revealed that the free cholesterol fractions and phospholipid fractions of the total lipid contents were associated with insulin resistance in the larger subclasses, but had an opposite pattern in the smaller VLDL (5.10A).

LDL and IDL subclass lipids did not show any associations with insulin sensitivity, apart from the triglycerides (Figure 5.9B), although phospholipid fractions of total lipid contents in medium and small LDL were negatively associated with insulin sensitivity similarly to the triglyceride fraction (Figure 5.10B).

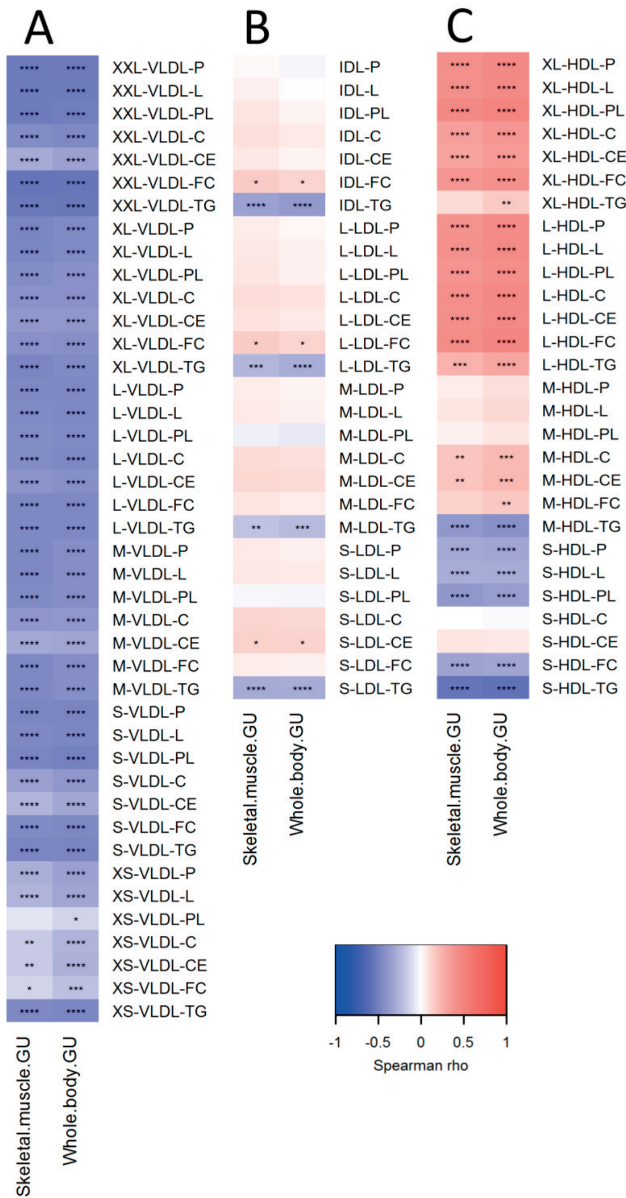


Figure 5.9. Heatmaps showing how skeletal muscle GU and M-value correlate with VLDL (A), IDL and LDL (B) and HDL (C) subclass lipids (i.e. absolute concentrations). P = particles; L = lipids; PL = phospholipids; C = total cholesterol, CE = cholesteryl esters; FC = free cholesterol; TG = triglycerides. ****P < 0.0001; ***P < 0.001; **P < 0.01; *P < 0.05.

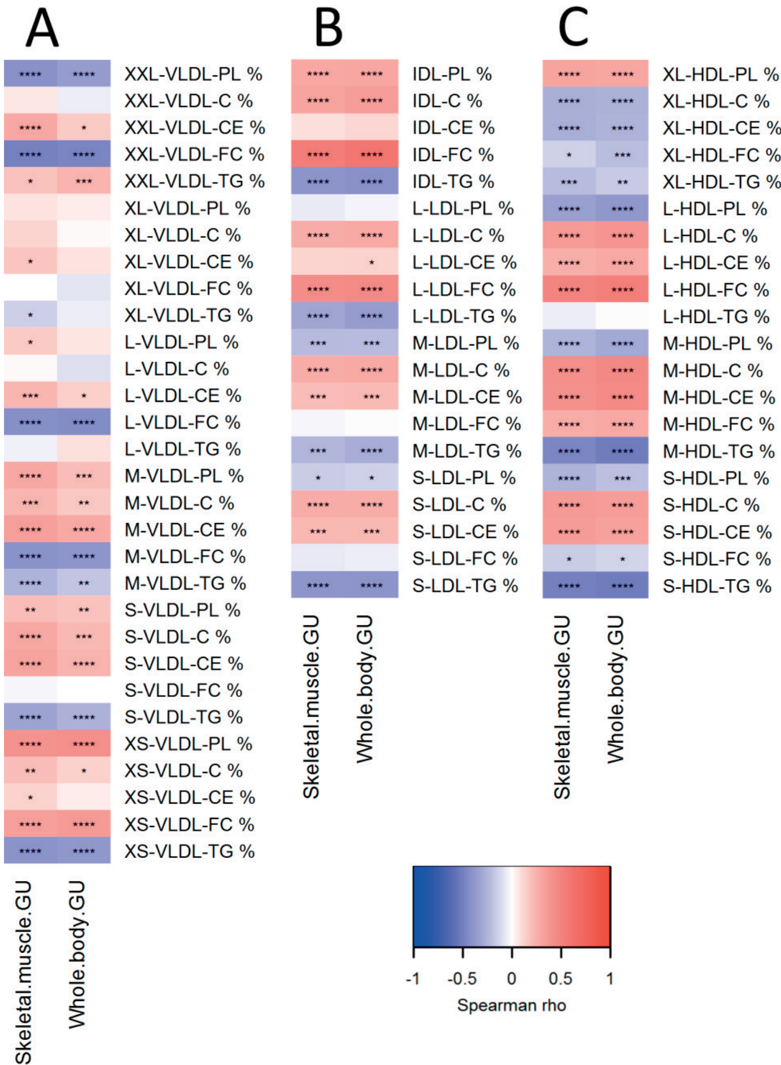


Figure 5.10. Heatmaps showing how skeletal muscle GU and M-value correlate with VLDL (A), IDL and LDL (B) and HDL (C) lipid subclass fractions (i.e. relative concetrations) . PL = phospholipids; C = total cholesterol, CE = cholesteryl esters; FC = free cholesterol; TG = triglycerides. ****P < 0.0001; ***P < 0.001; **P < 0.01; *P < 0.05.

5.4 Effects of resistance training on insulin sensitivity and ectopic fat deposition in elderly women (IV)

Previous data from this study showed that the resistance training intervention yielded considerable improvements on muscle strength and resulted in increased muscle mass (Bucci et al. 2016; Wasenius et al. 2018). Muscle strength based on different resistance exercises increased 35-75% (Wasenius et al. 2018). There were no differences between the offspring of lean/normal-weight mothers (OLM) and the offspring of obese mothers (OOM) in the improvement of resistance exercise results or increase in muscle mass (Bucci et al. 2016; Wasenius et al. 2018). OOM had worse insulin-stimulated skeletal muscle GU compared to OLM in the beginning of the study, which associated with shorter telomeres. However, the exercise intervention reversed these disadvantages so that, in the end, there were no differences in skeletal muscle insulin sensitivity. The improvement in muscle insulin sensitivity was higher among those with shorter telomeres at baseline (Bucci et al. 2016).

This study showed that insulin suppression of EGP was more effective after the resistance training intervention in the whole group of elderly women. When OLM and OOM groups were studied separately, there was a trend towards decreased EGP after the intervention among OOM, whereas the decrease was non-significant among OLM (Figure 5.11).

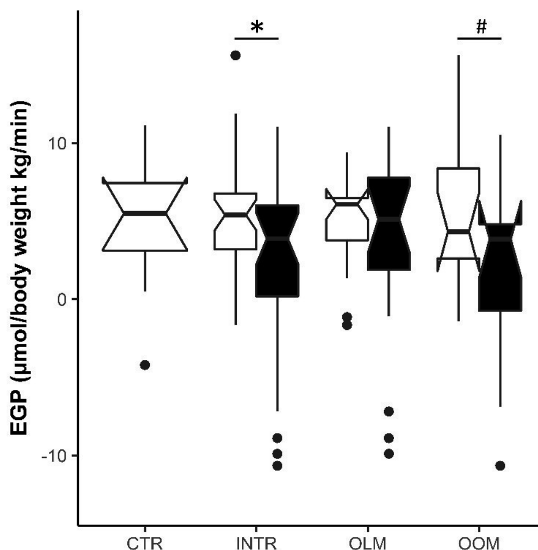


Figure 5.11. Endogenous glucose production (EGP) during clamp among controls (CTR) and frail participants before and after the resistance training intervention (INTR). Frail subjects are further divided into the offspring of lean/normal-weight mothers (OLM) and the offspring of obese mothers (OOM). White bars represent preintervention and black bars represent postintervention. The middle, bottom and top edges of the boxes represent medians with the first and third quartiles. Notches are calculated as 1.58 × interquartile range/square root (n) (95% confidence interval for the median). If notches do not

overlap, there is evidence of a difference between medians. The error bars extend to the furthest case from the box inside the 1.5 interquartile range. Values outside the error bars are presented as dots. *P = 0.042 and #P = 0.060 for change after the intervention. Reproduced with permission from (M. J. Honka et al. 2016).

There was a significant correlation between EGP and the previously reported skeletal muscle and whole-body insulin sensitivity (Bucci et al. 2016) (r = -0.457, P = 0.001 and r = -0.578, P = 2.76E-5, respectively). An improvement in EGP after the intervention was observed among those participants who had lower suppression of EGP at baseline (higher EGP than median) but not in subjects with higher suppression of EGP at baseline (-5.6 [7.1] vs. 0.1 [5.4] µmol/body kg/min, P = 0.015; Figure 5.12).

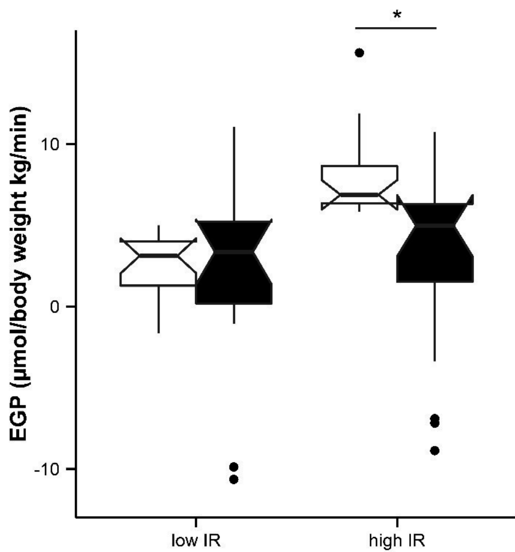


Figure 5.12. Endogenous glucose production (EGP) during clamp in the frail participants with low (low IR) and high (high IR) hepatic insulin resistance before the resistance training intervention. White bars represent preintervention and black bars represent postintervention. *P = 0.015 for change after the intervention. Reproduced with permission from (M. J. Honka et al. 2016).

Before the intervention, mesenteric fat GU, the antilipolytic effect of insulin and visceral adipose tissue mass did not differ between non-frail and frail offspring of lean/normal-weight or overweight/obese mothers. Neither mesenteric fat GU, antilipolytic effect of insulin nor visceral adipose tissue mass was changed during the resistance training intervention. Change in EGP was negatively correlated with change in telomere length after the resistance training intervention (r = -0.620, P =

0.001), meaning that improvement in EGP was associated with telomere elongation (Figure 5.14). Participants with telomere elongation improved their EGP suppression, whereas subjects with telomere shortening did not (-5.2 (5.8) vs 1.6 (4.3) μmol/kg body weight/min, P = 0.002). In addition, the subjects with telomere elongation tended to show improvement in visceral fat mass (-0.21 (0.36) vs. 0.02 (0.30) kg, P = 0.079).

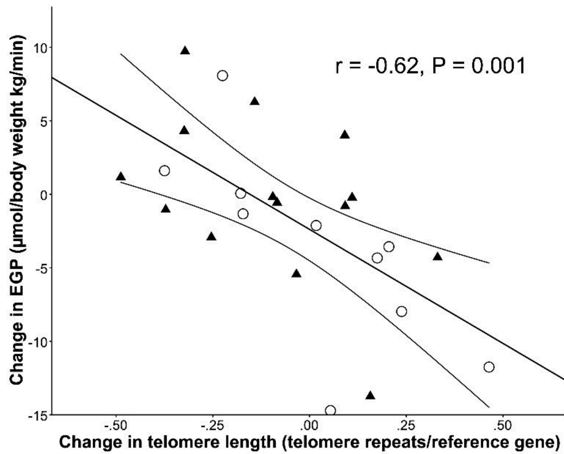


Figure 5.13. Association between the change in EGP and the change in telomere length after the resistance training intervention. Black triangles represent frail offspring of lean/normal-weight mothers and white circles represent frail offspring of overweight/obese mothers. The curves indicate a 95% confidence interval for the mean. Subjects with a full suppression of EGP before the intervention (EGP ≤ 0 μmol·kg body weight^{-1}·min^{-1}; n = 3) are not included in the graph (outliers). Spearman $\rho = -0.596$, P = 0.001 without the removal of outliers. Reproduced with permission from (M. J. Honka et al. 2016).

6 DISCUSSION

This thesis work focused on several key areas in insulin resistance research mentioned in the Review of Literature. Effects of various risk factors such as obesity, sex, aging and genetic and epigenetic predisposition on whole-body and tissue-specific insulin sensitivity were evaluated, and a resistance exercise intervention was performed to study its possible benefits on some of these risk factors. In addition, insulin sensitivity was measured directly in this work and new indirect insulin sensitivity indices were developed based on the direct measurements. Thus, this thesis work gave the author a well-rounded introduction to different aspects of the study of insulin resistance.

Before beginning of this thesis work, only a low number of variants affecting T2DM risk had been identified and understanding of their potential effects tissue-specific metabolism remained scarce. This thesis work shed light to this area by studying effects of p.Pro12Ala polymorphism of the PPARγ2 gene on tissue-specific insulin sensitivity (I). This variant is important since it is now known to be one of the relatively few common variants affecting insulin sensitivity (Table 2.1).

The second work was focused on studying insulin sensitivity simultaneously across multiple tissues (II). While this kind of research had been previously done focusing on skeletal muscle GU and suppression of EGP and lipolysis, associations between skeletal muscle, adipose tissue and liver GU and possible effects of age, sex and BMI had not been comprehensively evaluated in a single session in a large, heterogeneous cohort of people (Chapter 2.2.5). In addition, threshold values for determining tissue-specific insulin resistance were provided, which may be useful when trying to identify insulin resistant persons in future research.

At the moment fasting surrogate insulin sensitivity measures which are based on insulin, glucose and FFAs are widely used in large studies but provide only limited accuracy (Chapter 2.4.1). However, at same time, serum metabolomics is breaking through as a cost-effective tool to screen blood samples with variety of lipoprotein subclasses, lipids, amino acids and other small metabolites many of which has been associated with insulin resistance. The third study showed that serum metabolomic measurements can be used to improve identification of insulin resistance from fasting blood samples (III). This result is important since the best results for reversing insulin resistance and preventing T2DM are achieved when intervening early and that persons with high T2DM risk are correctly identified for intervention (Tuomilehto and Schwarz 2016).

The fourth study focused on studying the effects of resistance training intervention in a high-risk cohort of frail, elderly women half of whom had been born to overweight or obese mothers (IV). Resistance training has been often overlooked as a way of exercise to improve insulin sensitivity. However, this study provided evidence that in addition to improving muscle strength, resistance training improves insulin sensitivity and may even help to reverse epigenetic predisposition carried for decades after gestation.

Overall, the separate substudies succesfully answered the aims determined beforehand albeit some caveats on the findings still exist.

6.1 p.Pro12Ala polymorphism of the PPARγ2 gene affects insulin-stimulated liver glucose uptake in obesity (I)

The study showed that insulin-stimulated liver GU was higher among obese carriers of the Ala12 allele than the p.Pro12Pro genotype in subjects with T2DM. A similar trend was observed among obese nondiabetic subjects. However, these differences were not present among nonobese subjects, and thus the effect is modified by BMI. In addition, there were no differences in skeletal muscle, subcutaneous adipose tissue and whole-body GU or FFA levels during insulin stimulation between the genotype groups.

Using the CMgene cohort, it had been previously shown that insulin-stimulated skeletal muscle GU was higher among lean/normal-weight nondiabetic subjects carrying the p.Pro12Ala polymorphism of the PPARG gene than the common p.Pro12Pro genotype, but this difference was not present in obese subjects (Vänttinen et al. 2005). The p.Pro12Ala polymorphism has already been known to be protective against T2DM and to improve peripheral insulin sensitivity in nondiabetic subjects (Tönjes et al. 2006; Vänttinen et al. 2005), but its effect on insulin sensitivity in liver GU as well as its effects on T2DM patients was still unknown. The present work was done to extend the above-mentioned previous results by studying the possible effects of p.Pro12Ala polymorphism of the PPARG gene on liver, skeletal muscle, subcutaneous adipose tissue and whole-body GU in subjects with T2DM and liver GU in nondiabetic subjects.

Similarly to the previous study (Vänttinen et al. 2005), obese and nonobese subjects were studied separately using 27 kg/m^2 as a cutoff BMI value since there was a statistical interaction between this BMI threshold and the effect of p.Pro12Ala polymorphism on insulin sensitivity. This finding suggests that the effect of p.Pro12Ala is modified according to level of obesity determined by this threshold. Because there were no differences in skeletal muscle GU between the genotype

groups among subjects with T2DM, the previously found insulin-sensitizing effect of the Ala12 allele on skeletal muscle GU is either lost or masked in T2DM.

The finding of higher hepatic GU among the obese Ala12 allele carriers suggests that these subjects would benefit from this variant, which would lead to better glycemic control after a meal due to the liver's ability to take up 45% of an oral glucose load (Shrayyef and Gerich 2010). The hypothesis for better glycemic control after a meal among Ala12 allele carriers is supported by the finding that Ala12 carriers have better insulin suppression of EGP than individuals with the p.Pro12Pro genotype (Muller et al. 2003). Higher hepatic insulin sensitivity, along with the previously reported higher skeletal muscle GU (Vänttinen et al. 2005), may contribute to a reduced risk of T2DM in carriers of the Ala12 allele (Gouda et al. 2010).

Elevation of circulating FFAs impairs liver GU during hyperinsulinemia (Iozzo et al. 2004). In contrast with a previous study (Tschritter et al. 2003), which showed lower FFA levels during hyperinsulinemia among Ala12 carriers, no differences between the genotype groups in FFA levels were observed. Thus, it seems unlikely that the found difference between the Ala12 carriers and the p.Pro12Pro genetype in liver GU would be due to a difference adipose tissue lipolysis. However, it should be noted that circulating FFAs do not necessarily reflect hepatic FFA flux (Viljanen, Iozzo et al. 2009). Therefore, differences in hepatic FFA delivery and subsequent lipid-mediated interference of insulin signalling cannot be ruled out as a possible explanation behind the difference in liver GU between the genetype groups.

In conclusion, this study was able to show, in a relatively small number of subjects, that obese carriers of the p.Pro12Ala polymorphism of the PPARG gene have improved liver insulin sensitivity with respect to GU, which may be beneficial to glycemic control after a meal.

6.2 Insulin sensitivity in skeletal muscle, liver and adipose tissue are partly related (II)

Close to a half of the variance in the insulin-stimulated GU in skeletal muscle and adipose tissue was explained by a single principal component. Adding liver GU or EGP to the model decreased the amount of variance explained to below 40%, suggesting that liver insulin resistance occurs differently from skeletal muscle and adipose tissue insulin resistance. Not surprisingly, obesity had a negative effect on all examined tissue GUs. Interestingly, age was positively associated with liver

GU and EGP in both men and women and positively associated with adipose tissue GU in men only.

When determining cutoffs for insulin resistance, the study population was divided into insulin sensitive and insulin resistant subjects using a previously published threshold (Stern et al. 2005) and ROC curve analysis. Based on that threshold, the optimal cutoff values were 33 µmol/kg tissue/min for skeletal muscle GU, 11.5 µmol/kg tissue/min for abdominal subcutaneous adipose tissue GU and 3 µmol/kg body weight/min for EGP. These cutoffs may be useful when identifying insulin resistance in different tissues in future studies.

The main strength of this study is the direct measurement of insulin-stimulated GU in the liver, skeletal muscle and adipose tissue during a single session that allowed the assessment of insulin sensitivity in all these tissues. Such information regarding a large number of subjects has not been available earlier. Furthermore, the study dataset included persons of both sexes across a wide range of age and BMI, which enabled the investigation of any possible effects of these variables.

In summary, this study showed that approximately one third of the variation of insulin sensitivity in skeletal muscle, adipose tissue and the liver is similar. As expected, obesity had a strong negative impact on tissue insulin sensitivity, whereas age and gender associated differently between skeletal muscle, adipose tissue and the liver. The effect of obesity on insulin sensitivity is at least partly due to increased lipolysis, which creates FFA flux into other insulin sensitive organs that interferes with insulin signalling (Gancheva et al. 2018). Skeletal muscle and adipose tissue insulin sensitivity correlated better with each other than with liver insulin sensitivity, which suggests that they are partly regulated differently. One possible mechanism, which could dissociate liver insulin sensitivity from that of skeletal muscle and adipose tissue, is the release of FFAs from visceral adipose tissue to the portal vein, that causes a locally increased FFA flux to the liver (Nielsen et al. 2004). These FFAs may cause insulin resistance immediately by creation of bioactive lipid species that interfere insulin signalling or can contribute to hepatic insulin resistance in long term by promoting liver fat accumulation (Birkenfeld and Shulman 2014).

6.3 New models for predicting insulin sensitivity by using serum NMR-metabolomics (III)

In this study, models were created to predict insulin-stimulated skeletal muscle GU and whole-body GU using serum NMR-measurements as well as common clinical measurements. An important finding was that the Muscle-ISI model and the WB-

ISI model had higher associations with the skeletal muscle and whole-body glucose disposal measurements received from PET and euglycemic-hyperinsulinemic clamp studies than the HOMA-IR model and the revised QUICKI model. In addition, the new models provided improvements over HOMA-IR and revised QUICKI when determining if a person has either muscle or whole-body insulin resistance.

The models were created using NFG subjects and then tested using a separate group of persons with IR to confirm that the models work outside the sample group whereby they were created. The determined cutoff for Muscle-ISI had higher specificity than that of HOMA-IR or revised QUICKI when determining if a person has skeletal muscle insulin resistance. On the other hand, the cutoff for WB-ISI had higher sensitivity than that of HOMA-IR or revised QUICKI for detecting whole-body insulin resistance in the IR group. Moreover, AUCs for detecting skeletal muscle and whole-body insulin resistance were higher for Muscle-ISI and WB-ISI than HOMA-IR and revised QUICKI in the IR group. Muscle-ISI correlated better to the actual skeletal muscle GU than HOMA-IR or revised QUICKI. Furthermore, WB-ISI tended to have higher correlation than HOMA-IR or revised QUICKI when compared to measured whole-body glucose disposal in the IR group. Muscle-ISI and WB-ISI correlated better also to skeletal muscle GU and whole-body GU than HOMA-IR and tended to have higher correlation than revised QUICKI among NFG subjects.

An increase in VLDL particles and lipids, especially in large, very large and extremely large VLDL and chylomicrons, was associated with a decrease in skeletal muscle and whole-body insulin sensitivity. This is likely due to overproduction of VLDL particles and triglycerides from the liver as well as reduced hydrolysis of FAs from VLDL and chylomicron triglycerides by LPL in other tissues driven by the insulin resistant state (Larsson et al. 2013; Taskinen and Borén 2015). These findings agree with earlier reports connecting larger VLDL particles with lower insulin sensitivity, worse glycemic control and higher T2DM risk (Festa et al. 2005; Fizelova et al. 2015; Garvey et al. 2003; Goff et al. 2005; Hodge et al. 2009; Mackey et al. 2015; MacLean et al. 2000; Mora et al. 2010; J. Wang et al. 2012).

HDL triglyceride content was negatively associated with whole-body and skeletal muscle insulin sensitivity in this study. HDL triglyceride content increases by exchange of cholesteryl esters from HDL to triglycerides from chylomicrons and VLDL, which is facilitated by the cholesteryl ester transfer protein (CETP) (Oliveira and de Faria 2011; H. Wang and Peng 2011). Thus, high HDL triglyceride content is another manifestation of increased amounts of triglyceride-rich lipoprotein particles which are characteristics of hepatic and extrahepatic insulin re-

sistance. In addition, most of the plasma CETP is derived from the hepatic macrophages and is positively associated to the liver fat content which is another link to hepatic insulin resistance (Y. Wang et al. 2015). In the liver CETP provides protection from NAFLD by promotion of FA oxidation and inhibition of triglyceride synthesis, both of which are altered in an insulin resistant state (Palmisano et al. 2016; Zhu et al. 2018). In contrast, the contribution of adipose tissue to plasma CETP pool is low (Blauw et al. 2016).

Increased MUFAs were negatively associated with insulin sensitivity, whereas the omega 6-FAs to total FAs ratio was positively associated with insulin sensitivity. The finding agrees with a larger study where MUFAs were predictors of worse present and future glycemia and omega-6 FAs to total FAs was associated with better glycemic profiles (Mahendran et al. 2013). Surprisingly, omega-3 FAs were found to be predictors of insulin resistance. However, while observational and animal studies have revealed positive associations between omega-3 FAs and insulin sensitivity, trials using omega-3 FA supplementation in humans have shown no benefit in the form of a reduction of insulin resistance, which agrees with our finding (Lalia and Lanza 2016).

Sex did not appear as a predictor in the models of insulin sensitivity. This suggest that the effects of sex on skeletal muscle and whole-body insulin sensitivity can be explained by other variables in the models. It is likely that the effects of sex on insulin sensitivity are driven mainly by the differences in body fat deposition (Hocking et al. 2013) which affects most, if not all, variables in these models.

The strengths of this study are the use of a direct measurement of skeletal muscle insulin sensitivity and having a population which has persons of both sexes with a wide range of glycemic control, insulin sensitivity, BMI and age. Direct measuring of skeletal muscle GU provides additional precision compared to M-value, which may be confounded by non-suppressed EGP. In addition, studying a heterogeneous population shows that the created indices work well among different types of people.

In summary, the predictive models created in this study perform well when compared to the commonly used surrogate markers for insulin sensitivity and may thus be useful for determining insulin sensitivity in large clinical studies or in a clinical setting where direct measuring of insulin sensitivity is too laborious and costly. In addition, this study has provided cutoffs for HOMA-IR and revised QUICKI for determining skeletal muscle insulin resistance.

6.4 Resistance training improves liver insulin sensitivity in elderly women (IV)

This study showed that a resistance training according to the general exercise guidelines is sufficient to enhance insulin suppression of EGP in elderly women. Elderly women are at risk for developing T2DM due to muscle weakness and post-menopausal shift of adipose tissue deposition towards central obesity (Hocking et al. 2013; Morley et al. 2001). Although resistance training did not affect adipose tissue masses, it improved muscle strength (Wasenius et al. 2018), enhanced insulin suppression of EGP as well as improved insulin-stimulated skeletal muscle GU and mass (Bucci et al. 2016). These findings should result better glycemic control and thus protect from T2DM and cardiovascular disease.

Not surprisingly, the improvement in EGP after the intervention was higher among those who were more insulin resistant at baseline. Levels of FFAs during the euglycemic-hyperinsulinemic clamp did not differ before and after exercise intervention, suggesting that the enhanced suppression by insulin in EGP was not an indirect effect resulting from systemic adipose tissue lipolysis. However, the circulating FFA level does not necessarily reflect liver FFA uptake (Viljanen, Iozzo et al. 2009), especially because the visceral fat depot releases FFAs directly to the portal vein. Thus, it is possible that lower FFA flux to the liver may be behind the improved suppression of EGP. In addition, the exercise intervention may have improved lipid handling within hepatocytes as demonstrated earlier in mice (Jordy et al. 2015).

Shorter telomeres have been associated with cardiometabolic risk factors such as glucose intolerance, insulin resistance, high BMI, high waist-to-hip ratio, high levels of C-reactive protein and glycosylated haemoglobin and carotid intima-media thickness (Adaikalakoteswari et al. 2007; Olivieri et al. 2009; J. Zhao et al. 2014). Reactive oxygen species, oxidative stress and inflammation are likely the reason behind these associations (C. Matthews et al. 2006; Salpea and Humphries 2010; Serra et al. 2000). In the present study, it was discovered that telomere elongation was associated with enhanced insulin suppression of EGP. A trend of telomere elongation's association with reduction in visceral fat mass was also observed. Our findings are consistent with the study of Uziel et al., where improved glycemic control in type 2 diabetes patients and subjects with coronary artery disease was harmonious with lower telomere shortening (Uziel et al. 2007).

In summary, this study showed resistance training to be an effective way to improve insulin sensitivity among elderly women. In addition, the results suggest that improvement in insulin sensitivity is associated with slower cellular ageing based on telomere length.

6.5 Limitations and future directions

The PET studies using the CMgene cohort were performed during steady glycemia created using peripheral insulin and glucose infusions. This allowed measuring the independent effect of insulin on liver GU and glucose production. This said, the results do not reflect the postprandial state, where glucose and insulin concentrations are much higher in the portal vein than in periphery. At the moment there is no reliable model available to assess non-steady state kinetics of ^{18}F-FDG and glucose during oral ^{18}F-FDG administration.

Fasting GU and EGP measurements were not included in the current studies due to time constraints and radiation dose limitations related to the original PET study projects of the CMgene cohort. Nevertheless, insulin sensitivity is the major determinant of skeletal muscle and adipose tissue GU and suppression of EGP. It is also an important factor in liver GU since insulin stimulation can increase skeletal muscle GU up to ten-fold, adipose tissue GU several-fold (Dadson et al. 2016) and liver GU two-fold (Immonen et al. 2014) compared to the fasting state, and EGP can be completely suppressed.

Even though hepatic FFA flux could affect liver insulin sensitivity (Iozzo et al. 2004), it was not measured in the studies I, II or IV in part due to limitations on radiation exposure to the study subjects. Thus, alterations in FFA flux to the liver should be investigated in future studies as a potential mechanistic factor behind the higher liver insulin sensitivity among Ala12 carriers (I) and after resistance exercise intervention (IV) and as a possible contributing factor to differences in liver, skeletal muscle and adipose tissue insulin sensitivity along with other mediators of intertissue communication (II). Moreover, evaluation of FFA fluxes could give further insight on the observed differences in tissue insulin sensitivities between sexes.

Duration of insulin infusion could potentially change tissue insulin-stimulated GUs over time due to delay to achieve full insulin effect (Koopmans, Mandarino, DeFronzo 1998) or skeletal muscle activation aimed to retain same body position over time. Since this study was performed using pre-existing data from multiple PET studies, timing of the PET scans when compared to start of insulin infusion varied according to the used protocol. However, scan time relative to start of insulin infusion did not appear as significant predictor for skeletal muscle GU when building the Muscle-ISI prediction model and scan times relative to start of insulin infusion did not correlate with skeletal muscle, adipose tissue or liver GU. Thus, scan time relative to clamp protocol does not seem to be a major concern in the context of this study. Moreover, the studies in the CMgene cohort were conducted after at least a 10 hour fast but the actual study start time was dependent on design

of the original PET study projects as well as possible logistical restrictions. There were no associations between scanning time and tissue specific GUs or EGP. However, given the accumulating evidence that both glucose and fatty acid metabolism are subject to circadian rhythmicity (van den Berg et al. 2018), future studies should probably take into account time of scanning in relation to the research question asked.

Some of the participants to Study III used lipid or diabetes medication, but it is unlikely that the models for M-value and skeletal muscle GU were confounded by medication use because the models were created using nondiabetic participants of whom only 6% used lipid medication and none used diabetes medication. Similarly, nondiabetic subjects were used in studying associations between skeletal muscle, adipose tissue and liver GU (Study II). Medication use served as a covariate when analysing the effect of the p.Pro12Ala polymorphism on tissue GU in Study I. Some of the participants in the Dorian study (Study IV) took diabetes or lipid medication but it is unlikely that medication had a meaningful impact on the outcome of the study as the effects of exercise were compared within subjects. In addition, some of the participants of Study I were performing one-legged exercise. Only results from the noncontracting leg were used in comparisons between the genotype groups and one-legged exercise status (yes/no) was used as a covariate to ensure that doing the one-legged exercise does not affect the study results.

Additionally, insulin measurement is known to vary according to the used assay. Nevertheless, the associations found between M-value and HOMA-IR or revised QUICKI in Study III correspond well with prior literature (Otten, Ahren, Olsson 2014). Due to a limited sample size, there was no possibility to have a separate validation group of NFG subjects. However, the models were built using nested cross-validation to avoid selecting overfitted models. The models also performed better among IR subjects than the other indices, which shows that the models work well outside the sample whereby they were created.

Interesting associations between telomere length and skeletal muscle and liver insulin sensitivity were observed by Bucci et al. (Bucci et al. 2016) and in the study IV. Reactive oxygen species, oxidative stress and inflammation are potential mechanisms behind these associations (C. Matthews et al. 2006; Salpea and Humphries 2010; Serra et al. 2000). However, these factors were not measured in the study IV and, therefore, future mechanistic studies should evaluate the possible role of reactive oxygen species, oxidative stress and inflammation in the observed relationship between telomere length, exercise and insulin sensitivity.

In the future, it would be of interest to study whether the improved liver insulin sensitivity by the resistance exercise intervention in study IV or by the Ala12 allele found in study I actually translates to better glycemic control after a meal. Also,

the effects the Ala12 allele on insulin sensitivity in other tissues apart from the ones studied here are much unexplored area. Evaluating the benefits of using the models of insulin sensitivity created here for identifying persons with insulin resistance and high risk for T2DM and cardiovascular diseases is an important task for the future.

7 CONCLUSIONS

I. This thesis has provided evidence in support of the existence of a higher insulin-stimulated GU in the liver among the obese carriers of the minor Ala12 allele of the PPARG2 gene compared to carriers of the p.Pro12Pro genotype. The Ala12 allele is known to be protective against T2DM (Gouda et al. 2010). The liver takes up 25-45% of the glucose from a meal's carbohydrates. Therefore, it is possible that higher liver GU contributes to better glycemic control after a meal and thus protection from T2DM among Ala12 allele carriers.

II. Studying skeletal muscle, adipose tissue and liver insulin-stimulated GU and insulin-suppressed EGP in a single session among a large number of subjects revealed that the insulin sensitivity of GU in skeletal muscle and adipose tissue are tightly linked, while their association with liver insulin sensitivity is lower. Furthermore, the current study allowed us to provide cutoff points for tissue specific GUs and EGP between normal and insulin resistant states when using the euglycemic hyperinsulinemic clamp with the most commonly used insulin infusion rate. These cutoffs may be useful in future studies when evaluating how high of a GU or glucose production rate can be considered normal during hyperinsulinemia.

III. The models for predicting insulin sensitivity using fasting metabolite measures perform better than the commonly used fasting insulin sensitivity indices. Therefore, the models may be useful in large studies or in cases of clinical use that lack the possibility of using more precise but laborious and costly direct methods of insulin sensitivity assessment.

IV. This study showed that regular resistance exercise among elderly women with low muscle strength improves insulin suppression of EGP. The finding is important because low muscle strength and insulin resistance are characteristics of the frailty syndrome, which impairs daily functioning and increases the risks of attendant comorbidities such as T2DM (Veronese et al. 2016). Importantly, improved suppression of EGP was found among subjects who had poor suppression of EGP before the exercise intervention. In addition, the improvement in EGP suppression was associated with leukocyte telomere elongation. Shorter telomeres, a marker of cellular aging, are associated with an increased risk of T2DM as well as various cardiometabolic disease risk markers (J. Zhao et al. 2014). While resistance training is incorporated into common exercise guidelines, usually only aerobic exercise receives credit for improving insulin sensitivity. However, the above

findings show that the benefits of resistance training are not limited to increased muscle strength, but also include broader health improvements.

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REFERENCES

Abdul-Ghani MA, Matsuda M, Balas B, DeFronzo RA. 2007. Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. Diabetes Care 30(1):89-94.

Adaikalakoteswari A, Balasubramanyam M, Ravikumar R, Deepa R, Mohan V. 2007. Association of telomere shortening with impaired glucose tolerance and diabetic macroangiopathy. Atherosclerosis 195(1):83-9.

Adkins A, Basu R, Persson M, Dicke B, Shah P, Vella A, Schwenk WF, Rizza R. 2003. Higher insulin concentrations are required to suppress gluconeogenesis than glycogenolysis in nondiabetic humans. Diabetes 52(9):2213-20.

Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, Evans RM. 2013. PPAR-gamma signaling and metabolism: The good, the bad and the future. Nat Med 19(5):557-66.

Ait-Omar A, Monteiro-Sepulveda M, Poitou C, Le Gall M, Cotillard A, Gilet J, Garbin K, Houllier A, Chateau D, Lacombe A, et al. 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(10):2598-607.

Akbaraly T, Wurtz P, Singh-Manoux A, Shipley MJ, Haapakoski R, Lehto M, Desrumaux C, Kähönen M, Lehtimäki T, Mikkilä V, et al. 2018. Association of circulating metabolites with healthy diet and risk of cardiovascular disease: Analysis of two cohort studies. Sci Rep 8(1):8620,018-26441-1.

Albers PH, Pedersen AJT, Birk JB, Kristensen DE, Vind BF, Baba O, Nøhr J, Højlund K, Wojtaszewski J. 2015. Human muscle fiber Type–Specific insulin signaling: Impact of obesity and type 2 diabetes. Diabetes 64(2):485.

Alberti KG and Zimmet PZ. 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15(0742-3071; 7):539-53.

Alberti KG, Zimmet P, Shaw J, IDF Epidemiology Task Force Consensus Group. 2005. The metabolic syndrome--a new world-wide definition. Lancet 366(9491):1059-62.

Alenius S and Ruotsalainen U. 1997. Bayesian image reconstruction for emission tomography based on median root prior. Eur J Nucl Med 24(0340-6997; 3):258-65.

Amati F, Dube JJ, Alvarez-Carnero E, Edreira MM, Chomentowski P, Coen PM, Switzer GE, Bickel PE, Stefanovic-Racic M, Toledo FG, et al. 2011. Skeletal muscle triglycerides, diacylglycerols, and ceramides in insulin resistance: Another paradox in endurance-trained athletes? Diabetes 60(10):2588-97.

Amati F, Dubé JJ, Coen PM, Stefanovic-Racic M, Toledo FGS, Goodpaster BH. 2009. Physical inactivity and obesity underlie the insulin resistance of aging. Diabetes Care 32(8):1547.

American Diabetes Association. 2018a. 2. classification and diagnosis of diabetes: Standards of medical care in diabetes – *2018*. Diabetes Care 41:S13.

American Diabetes Association. 2018b. 7. obesity management for the treatment of type 2 diabetes: Standards of medical care in diabetes – *2018*. Diabetes Care 41:S65.

American Diabetes Association. 2015. (2) classification and diagnosis of diabetes. Diabetes Care 38 Suppl:S8-S16.

Andersson A, Tengblad S, Karlstrom B, Kamal-Eldin A, Landberg R, Basu S, Aman P, Vessby B. 2007. Whole-grain foods do not affect insulin sensitivity or markers of lipid peroxidation and inflammation in healthy, moderately overweight subjects. J Nutr 137(6):1401-7.

Anstee QM and Day CP. 2015. The genetics of nonalcoholic fatty liver disease: Spotlight on PNPLA3 and TM6SF2. Semin Liver Dis 35(3):270-90.

Aubert RE, Herrera V, Chen W, Haffner SM, Pendergrass M. 2010. Rosiglitazone and pioglitazone increase fracture risk in women and men with type 2 diabetes. Diabetes Obes Metab 12(8):716-21.

Bagger JI, Knop FK, Lund A, Vestergaard H, Holst JJ, Vilsbøll T. 2011. Impaired regulation of the incretin effect in patients with type 2 diabetes. The Journal of Clinical Endocrinology & Metabolism 96(3):737-45.

Balkau B, Mhamdi L, Oppert J, Nolan J, Golay A, Porcellati F, Laakso M, Ferrannini E. 2008. Physical activity and insulin sensitivity: The RISC study. Diabetes 57(10):2613-8.

Baron AD, Laakso M, Brechtel G, Edelman SV. 1991. Mechanism of insulin resistance in insulin-dependent diabetes mellitus: A major role for reduced skeletal muscle blood flow. J Clin Endocrinol Metab 73(3):637-43.

Barzilay JI, Blaum C, Moore T, Xue QL, Hirsch CH, Walston JD, Fried LP. 2007. Insulin resistance and inflammation as precursors of frailty: The cardiovascular health study. Arch Intern Med 167(7):635-41.

Basu A, Basu R, Shah P, Vella A, Rizza RA, Jensen MD. 2001. Systemic and regional free fatty acid metabolism in type 2 diabetes. Am J Physiol Endocrinol Metab 280(6):E1000-6.

Basu A, Basu R, Shah P, Vella A, Johnson CM, Jensen M, Nair KS, Schwenk WF, Rizza RA. 2001. Type 2 diabetes impairs splanchnic uptake of glucose but does not alter intestinal glucose absorption during enteral glucose feeding: Additional evidence for a defect in hepatic glucokinase activity. Diabetes 50(6):1351-62.

Basu A, Basu R, Shah P, Vella A, Johnson CM, Nair KS, Jensen MD, Schwenk WF, Rizza RA. 2000. 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(2):272-83.

Båvenholm PN, Pigon J, Östenson CG, Efendic S. 2001. Insulin sensitivity of suppression of endogenous glucose production is the single most important determinant of glucose tolerance. Diabetes 50(0012-1797; 6):1449-54.

Beard JC, Bergman RN, Ward WK, Porte D,Jr. 1986. The insulin sensitivity index in nondiabetic man. correlation between clamp-derived and IVGTT-derived values. Diabetes 35(3):362-9.

Beck-Nielsen Henning. 2012. The role of glycogen synthase in the development of hyperglycemia in type 2 diabetes – 'To store or not to store glucose, that's the question'. Diabetes Metab Res Rev 28(8):635-44.

Belfort R, Mandarino L, Kashyap S, Wirfel K, Pratipanawatr T, Berria R, DeFronzo RA, Cusi K. 2005. Dose-response effect of elevated plasma free fatty acid on insulin signaling. Diabetes 54(6):1640.

Bender D, Munk OL, Feng HQ, Keiding S. 2001. Metabolites of (18)F-FDG and 3-O-(11)C-methylglucose in pig liver. J Nucl Med 42(11):1673-8.

Bennie JA, Chau JY, van der Ploeg HP, Stamatakis E, Do A, Bauman A. 2013. The prevalence and correlates of sitting in european adults - a comparison of 32 eurobarometer-participating countries. Int J Behav Nutr Phys Act 10:107,5868-10-107.

Bergman RN, Ider YZ, Bowden CR, Cobelli C. 1979. Quantitative estimation of insulin sensitivity. Am J Physiol 236(6):E667-77.

Berkemeyer K, Wijndaele K, White T, Cooper AJM, Luben R, Westgate K, Griffin SJ, Khaw KT, Wareham NJ, Brage S. 2016. The descriptive epidemiology of accelerometer-measured physical activity in older adults. International Journal of Behavioral Nutrition and Physical Activity 13(1):2.

Bertoldo A, Rizzo G, Veronese M. 2014. Deriving physiological information from PET images: From SUV to compartmental modelling. Clinical and Translational Imaging 2(3):239-51.

Birkenfeld AL and Shulman GI. 2014. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology 59(2):713-23.

Biswas A, Oh PI, Faulkner GE, Bajaj RR, Silver MA, Mitchell MS, Alter DA. 2015. Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: A systematic review and meta-analysis. Ann Intern Med 162(2):123-32.

Blauw LL, de Mutsert R, Lamb HJ, de Roos A, Rosendaal FR, Jukema JW, Wang Y, van Dijk KW, Rensen PCN. 2016. Serum CETP concentration is not associated with measures of body fat: The NEO study. Atherosclerosis 246:267-73.

Bock G, Dalla Man C, Campioni M, Chittilapilly E, Basu R, Toffolo G, Cobelli C, Rizza R. 2006. Pathogenesis of pre-diabetes: Mechanisms of fasting and postprandial hyperglycemia in people with impaired fasting glucose and/or impaired glucose tolerance. Diabetes 55(12):3536-49.

Boden G, Ruiz J, Kim CJ, Chen X. 1996. Effects of prolonged glucose infusion on insulin secretion, clearance, and action in normal subjects. Am J Physiol 270(2 Pt 1):E251-8.

Bodinham CL, Smith L, Thomas EL, Bell JD, Swann JR, Costabile A, Russell-Jones D, Umpleby AM, Robertson MD. 2014. Efficacy of increased resistant starch consumption in human type 2 diabetes. Endocr Connect 3(2):75-84.

Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G. 1985. Relationship between degree of obesity and in vivo insulin action in man. Am J Physiol 248(3 Pt 1):E286-91.

Bolinder J, Kerckhoffs DA, Moberg E, Hagstrom-Toft E, Arner P. 2000. Rates of skeletal muscle and adipose tissue glycerol release in nonobese and obese subjects. Diabetes 49(5):797-802.

Bonadonna RC, Zych K, Boni C, Ferrannini E, DeFronzo RA. 1989. Time dependence of the interaction between lipid and glucose

in humans. Am J Physiol 257(1 Pt 1):E49-56.

Bonadonna RC, Groop L, Kraemer N, Ferrannini E, Del Prato S, DeFronzo RA. 1990. Obesity and insulin resistance in humans: A dose-response study. Metabolism 39(5):452-9.

Borra RJ, Salo S, Dean K, Lautamäki R, Nuutila P, Komu M, Parkkola R. 2009. Nonalcoholic fatty liver disease: Rapid evaluation of liver fat content with in-phase and out-of-phase MR imaging. Radiology 250(1):130-6.

Bortz WM, Paul P, Haff AC, Holmes WL. 1972. Glycerol turnover and oxidation in man. J Clin Invest 51(6):1537-46.

Bos MM, Noordam R, van dB, de Mutsert R, Rosendaal FR, Blauw GJ, Rensen PCN, Biermasz NR, van Heemst D. 2018. Associations of sleep duration and quality with serum and hepatic lipids: The netherlands epidemiology of obesity study. J Sleep Res 0(0):e12776.

Boucher J, Kleinridders A, Kahn CR. 2014. Insulin receptor signaling in normal and insulin-resistant states. Cold Spring Harb Perspect Biol 6(1):a009191. doi:10.1101/cshperspect.a009191.

Brooks GA. 2018. The science and translation of lactate shuttle theory. Cell Metabolism 27(4):757-85.

Broussard JL, Castro AV, Malini I, Paszkiewicz RL, Bediako IA, Szczepaniak LS, Szczepaniak EW, Bergman RN, Kolka CM. 2016. Insulin access to skeletal muscle is impaired during the early stages of diet-induced obesity. Obesity 24(9):1922-8.

Bucci M, Huovinen V, Guzzardi MA, Koskinen S, Raiko JR, Lipponen H, Ahsan S, Badeau RM, Honka MJ, Koffert J, et al. 2016. Resistance training improves skeletal muscle insulin sensitivity in elderly offspring of overweight and obese mothers. Diabetologia 59(1):77-86.

Buehler T, Bally L, Dokumaci AS, Stettler C, Boesch C. 2016. Methodological and physiological test-retest reliability of (13) C-MRS glycogen measurements in liver and in skeletal muscle of patients with type 1 diabetes and matched healthy controls. NMR Biomed 29(6):796-805.

Burgess SC. 2015. Regulation of glucose metabolism in liver. In: International textbook of diabetes mellitus. Defronzo RA, Ferrannini E, Zimmet P, et al, editors. 4th ed. Chichester, West Sussex, UK: John Wiley & Sons, Ltd. p. 193-210.

Canfora EE, Jocken JW, Blaak EE. 2015. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol 11(10):577-91.

Cederberg H, Stancakova A, Yaluri N, Modi S, Kuusisto J, Laakso M. 2015. Increased risk of diabetes with statin treatment is associated with impaired insulin sensitivity and insulin secretion: A 6 year follow-up study of the METSIM cohort. Diabetologia 58(5):1109-17.

Chambers ES, Preston T, Frost G, Morrison DJ. 2018. Role of gut microbiota-generated short-chain fatty acids in metabolic and cardiovascular health. Current Nutrition Reports 7(4):198-206.

Chen J, Williams S, Ho S, Loraine H, Hagan D, Whaley JM, Feder JN. 2010. Quantitative PCR tissue expression profiling of the human SGLT2 gene and related family members. Diabetes Therapy 1(2):57-92.

Chen S, Yeh F, Lin J, Matsuguchi T, Blackburn E, Lee ET, Howard BV, Zhao J. 2014. Short leukocyte telomere length is associated with obesity in american indians: The strong heart family study. Aging (Albany NY) 6(5):380-9.

Choi SH and Ginsberg HN. 2011. Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. Trends in Endocrinology and Metabolism: TEM 22(9):353-63.

Choukem SP and Gautier JF. 2008. How to measure hepatic insulin resistance? Diabetes Metab 34(6 Pt 2):664-73.

Church TS, Thomas DM, Tudor-Locke C, Katzmarzyk PT, Earnest CP, Rodarte RQ, Martin CK, Blair SN, Bouchard C. 2011. Trends over 5 decades in U.S. occupation-related physical activity and their associations with obesity. Plos One 6(5):e19657.

Ciaraldi TP, Mudaliar S, Barzin A, Macievic JA, Edelman SV, Park KS, Henry RR. 2005. Skeletal muscle GLUT1 transporter protein expression and basal leg glucose uptake are reduced in type 2 diabetes. J Clin Endocrinol Metab 90(1):352-8.

Cline GW, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z, Inzucchi S, Dresner A, Rothman DL, Shulman GI. 1999. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. N Engl J Med 341(4):240-6.

Cohn JS, Patterson BW, Uffelman KD, Davignon J, Steiner G. 2004. Rate of production of plasma and very-low-density lipoprotein (VLDL) apolipoprotein C-III is strongly related to the concentration and level of production of VLDL triglyceride in male sub-

jects with different body weights and levels of insulin sensitivity. J Clin Endocrinol Metab 89(8):3949-55.

Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, Chasan-Taber L, Albright AL, Braun B, American College of Sports Medicine, et al. 2010. Exercise and type 2 diabetes: The american college of sports medicine and the american diabetes association: Joint position statement. Diabetes Care 33(12):e147-67.

Conley KE, Jubrias SA, Esselman PC. 2000. Oxidative capacity and ageing in human muscle. J Physiol 526 Pt 1:203-10.

Conte C, Fabbrini E, Kars M, Mittendorfer B, Patterson BW, Klein S. 2012. Multiorgan insulin sensitivity in lean and obese subjects. Diabetes Care 35(6):1316-21.

Cozma AI, Sievenpiper JL, de Souza RJ, Chiavaroli L, Ha V, Wang DD, Mirrahimi A, Yu ME, Carleton AJ, Di Buono M, et al. 2012. Effect of fructose on glycemic control in diabetes: A systematic review and meta-analysis of controlled feeding trials. Diabetes Care 35(7):1611-20.

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Dadson P, Landini L, Helmio M, Hannukainen JC, Immonen H, Honka MJ, Bucci M, Savisto N, Soinio M, Salminen P, et al. 2016. Effect of bariatric surgery on adipose tissue glucose metabolism in different depots in patients with or without type 2 diabetes. Diabetes Care 39(2):292-9.

Dainty SA, Klingel SL, Pilkey SE, McDonald E, McKeown B, Emes MJ, Duncan AM. 2016. Resistant starch bagels reduce fasting and postprandial insulin in adults at risk of type 2 diabetes. J Nutr 146(11):2252-9.

Dale CE, Fatemifar G, Palmer TM, White J, Prieto-Merino D, Zabaneh D, Engmann JEL, Shah T, Wong A, Warren HR, et al. 2017. Causal associations of adiposity and body fat distribution with coronary heart disease, stroke subtypes, and type 2 diabetes mellitus: A mendelian randomization analysis. Circulation 135(24):2373-88.

Daniele G, Eldor R, Merovci A, Clarke GD, Xiong J, Tripathy D, Taranova A, Abdul-Ghani M, DeFronzo RA. 2014. Chronic reduction of plasma free fatty acid improves mitochondrial function and whole-body insulin sensitivity in obese and type 2 diabetic individuals. Diabetes 63(8):2812-20.

Deeb SS, Fajas L, Nemoto M, Pihlajamäki J, Mykkänen L, Kuusisto J, Laakso M, Fujimoto W, Auwerx J. 1998. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 20(1061-4036; 3):284-7.

DeFronzo RA, Ferrannini E, Simonson DC. 1989. Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: Contributions of excessive hepatic glucose production and impaired tissue glucose uptake. Metabolism 38(4):387-95.

Defronzo RA, Tobin JD, Andres R. 1979. Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237(0002-9513; 3):E214-23.

DeFronzo RA. 2009. From the triumvirate to the ominous octet: A new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 58(4):773-95.

DeFronzo RA. 1979. Glucose intolerance and aging: Evidence for tissue insensitivity to insulin. Diabetes 28(12):1095.

DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J. 1983. Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. Diabetes 32(1):35.

Deguchi E, Niiyama M, Kagota K, Namioka S. 1978. Incorporation of 15n administered to germfree and spf piglets as 15n-urea into amino acids of hydrolyzed liver and muscle proteins. Jap J Vet Res 26:68-73.

Dimas AS, Lagou V, Barker A, Knowles JW, Magi R, Hivert MF, Benazzo A, Rybin D, Jackson AU, Stringham HM, et al. 2014. Impact of type 2 diabetes susceptibility variants on quantitative glycemic traits reveals mechanistic heterogeneity. Diabetes 63(6):2158-71.

Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. 1999. Activation of nitric oxide synthase in endothelial cells by akt-dependent phosphorylation. Nature 399(6736):601-5.

Djoussé L, Biggs ML, Lemaitre RN, King IB, Song X, Ix JH, Mukamal KJ, Siscovick DS, Mozaffarian D. 2011. Plasma omega-3 fatty acids and incident diabetes in older adults. The American Journal of Clinical Nutrition 94(2):527-33.

Donga E, van Dijk M, van Dijk JG, Biermasz NR, Lammers GJ, van Kralingen KW, Corssmit EP, Romijn JA. 2010. A single night of par-

tial sleep deprivation induces insulin resistance in multiple metabolic pathways in healthy subjects. J Clin Endocrinol Metab 95(6):2963-8.

Dongiovanni P, Stender S, Pietrelli A, Mancina RM, Cespiati A, Petta S, Pelusi S, Pingitore P, Badiali S, Maggioni M, et al. 2018. Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver. J Intern Med 283(4):356-70.

Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. 2005. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest 115(5):1343-51.

Du M, Yan X, Tong JF, Zhao J, Zhu MJ. 2010. Maternal obesity, inflammation, and fetal skeletal muscle development. Biol Reprod 82(1):4-12.

Duvivier BM, Schaper NC, Bremers MA, van Crombrugge G, Menheere PP, Kars M, Savelberg HH. 2013. Minimal intensity physical activity (standing and walking) of longer duration improves insulin action and plasma lipids more than shorter periods of moderate to vigorous exercise (cycling) in sedentary subjects when energy expenditure is comparable. PLoS One 8(2):e55542.

Duvivier BM, Schaper NC, Hesselink MK, van Kan L, Stienen N, Winkens B, Koster A, Savelberg HH. 2017. Breaking sitting with light activities vs structured exercise: A randomised crossover study demonstrating benefits for glycaemic control and insulin sensitivity in type 2 diabetes. Diabetologia 60(3):490-8.

Edgerton DS, Kraft G, Smith M, Farmer B, Williams PE, Coate KC, Printz RL, O'Brien RM, Cherrington AD. 2017. Insulin's direct hepatic effect explains the inhibition of glucose production caused by insulin secretion. JCI Insight 2(6):e91863.

Eliasson B, Taskinen MR, Smith U. 1996. Long-term use of nicotine gum is associated with hyperinsulinemia and insulin resistance. Circulation 94(5):878-81.

Eliasson B, Attvall S, Taskinen MR, Smith U. 1997. Smoking cessation improves insulin sensitivity in healthy middle-aged men. Eur J Clin Invest 27(5):450-6.

Eliasson B, Attvall S, Taskinen MR, Smith U. 1994. The insulin resistance syndrome in smokers is related to smoking habits. Arterioscler Thromb 14(12):1946-50.

Emdin CA, Khera AV, Natarajan P, Klarin D, Zekavat SM, Hsiao AJ, Kathiresan S. 2017. Genetic association of waist-to-hip ratio with cardiometabolic traits, type 2 diabetes, and coronary heart disease. Jama 317(6):626-34.

Epley B. 1985. Poundage chart. In: Boyd epley workout. Lincoln, NE: Body Enterprises. p. 86.

Eriksson JG, Sandboge S, Salonen MK, Kajantie E, Osmond C. 2014. Long-term consequences of maternal overweight in pregnancy on offspring later health: Findings from the helsinki birth cohort study. Ann Med 46(6):434-8.

Eriksson JG, Ylihärsilä H, Forsen T, Osmond C, Barker DJ. 2004. Exercise protects against glucose intolerance in individuals with a small body size at birth. Prev Med 39(1):164-7.

Facchini FS, Hollenbeck CB, Jeppesen J, Chen YD, Reaven GM. 1992. Insulin resistance and cigarette smoking. Lancet 339(8802):1128-30.

Færch K, Witte DR, Tabák AG, Perreault L, Herder C, Brunner EJ, Kivimäki M, Vistisen D. 2013. Trajectories of cardiometabolic risk factors before diagnosis of three subtypes of type 2 diabetes: A post-hoc analysis of the longitudinal whitehall II cohort study. The Lancet Diabetes & Endocrinology 1(1):43-51.

Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, et al. 1997. The organization, promoter analysis, and expression of the human PPARgamma gene. J Biol Chem 272(30):18779-89.

Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA. 1983. Effect of fatty acids on glucose production and utilization in man. J Clin Invest 72(5):1737-47.

Ferrannini E, Iozzo P, Virtanen KA, Honka MJ, Bucci M, Nuutila P. 2018. Adipose tissue and skeletal muscle insulin-mediated glucose uptake in insulin resistance: Role of blood flow and diabetes. Am J Clin Nutr 108(4):749-58.

Ferrannini E, Vichi S, Beck-Nielsen H, Laakso M, Paolisso G, Smith U. 1996. Insulin action and age. european group for the study of insulin resistance (EGIR). Diabetes 45(7):947-53.

Festa A, Williams K, Hanley AJ, Otvos JD, Goff DC, Wagenknecht LE, Haffner SM. 2005. Nuclear magnetic resonance lipoprotein abnormalities in prediabetic subjects in the insulin resistance atherosclerosis study. Circulation 111(25):3465-72.

Finegood DT, Hramiak IM, Dupre J. 1990. A modified protocol for estimation of insulin

sensitivity with the minimal model of glucose kinetics in patients with insulin-dependent diabetes. J Clin Endocrinol Metab 70(6):1538-49.

Fizelova M, Miilunpohja M, Kangas AJ, Soininen P, Kuusisto J, Ala-Korpela M, Laakso M, Stancakova A. 2015. Associations of multiple lipoprotein and apolipoprotein measures with worsening of glycemia and incident type 2 diabetes in 6607 non-diabetic finnish men. Atherosclerosis 240(1):272-7.

Frayn KN, Arner P, Yki-Järvinen H. 2006. Fatty acid metabolism in adipose tissue, muscle and liver in health and disease. Essays Biochem 42:89-103.

Freckmann G, Hagenlocher S, Baumstark A, Jendrike N, Gillen RC, Rössner K, Haug C. 2007. Continuous glucose profiles in healthy subjects under everyday life conditions and after different meals. Journal of Diabetes Science and Technology (Online) 1(5):695-703.

Friedman J, Hastie T, Tibshirani R. 2010. Regularization paths for generalized linear models via coordinate descent. J Stat Softw 33(1):1-22.

Froese DS, Michaeli A, McCorvie TJ, Krojer T, Sasi M, Melaev E, Goldblum A, Zatsepin M, Lossos A, Álvarez R, et al. 2015. Structural basis of glycogen branching enzyme deficiency and pharmacologic rescue by rational peptide design. Hum Mol Genet 24(20):5667-76.

Fujita S, Glynn EL, Timmerman KL, Rasmussen BB, Volpi E. 2009. Supraphysiological hyperinsulinaemia is necessary to stimulate skeletal muscle protein anabolism in older adults: Evidence of a true age-related insulin resistance of muscle protein metabolism. Diabetologia 52(9):1889-98.

Fujita S, Rasmussen BB, Cadenas JG, Grady JJ, Volpi E. 2006. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. Am J Physiol Endocrinol Metab 291(4):E745-54.

Fujita S, Rasmussen BB, Cadenas JG, Drummond MJ, Glynn EL, Sattler FR, Volpi E. 2007. Aerobic exercise overcomes the age-related insulin resistance of muscle protein metabolism by improving endothelial function and akt/mammalian target of rapamycin signaling. Diabetes 56(6):1615.

Fullerton MD, Galic S, Marcinko K, Sikkema S, Pulinilkunnil T, Chen ZP, O'Neill HM, Ford RJ, Palanivel R, O'Brien M, et al. 2013. Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. Nat Med 19(12):1649-54.

Gale EA. 2001. Lessons from the glitazones: A story of drug development. Lancet 357(9271):1870-5.

Gancheva S, Jelenik T, Alvarez-Hernandez E, Roden M. 2018. Interorgan metabolic crosstalk in human insulin resistance. Physiol Rev 98(3):1371-415.

Gardner JP, Li S, Srinivasan SR, Chen W, Kimura M, Lu X, Berenson GS, Aviv A. 2005. Rise in insulin resistance is associated with escalated telomere attrition. Circulation 111(17):2171-7.

Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, Pugh K, Jenkins AJ, Klein RL, 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(2):453-62.

Gast KB, Tjeerdema N, Stijnen T, Smit JW, Dekkers OM. 2012. Insulin resistance and risk of incident cardiovascular events in adults without diabetes: Meta-analysis. PLoS One 7(12):e52036.

Gastaldelli A, Toschi E, Pettiti M, Frascerra S, Quinones-Galvan A, Sironi AM, Natali A, Ferrannini E. 2001. Effect of physiological hyperinsulinemia on gluconeogenesis in nondiabetic subjects and in type 2 diabetic patients. Diabetes 50(8):1807-12.

Gaster M, Staehr P, Beck-Nielsen H, Schroder HD, Handberg A. 2001. GLUT4 is reduced in slow muscle fibers of type 2 diabetic patients: Is insulin resistance in type 2 diabetes a slow, type 1 fiber disease? Diabetes 50(6):1324-9.

George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, Soos MA, Murgatroyd PR, Williams RM, Acerini CL, et al. 2004. A family with severe insulin resistance and diabetes due to a mutation in *AKT2*. Science 304(5675):1325.

Gerich JE. 2010. Role of the kidney in normal glucose homeostasis and in the hyperglycaemia of diabetes mellitus: Therapeutic implications. Diabet Med 27(2):136-42.

Gjedde A. 1982. Calculation of cerebral glucose phosphorylation from brain uptake of glucose analogs in vivo: A re-examination. Brain Res 257(2):237-74.

Godfrey KM, Reynolds RM, Prescott SL, Nyirenda M, Jaddoe VWV, Eriksson JG, Broekman BFP. 2017. Influence of maternal

obesity on the long-term health of offspring. The Lancet.Diabetes & Endocrinology 5(1):53-64.

Goff DC,Jr, D'Agostino RB,Jr, Haffner SM, Otvos JD. 2005. Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. results from the insulin resistance atherosclerosis study. Metabolism 54(2):264-70.

Golden SH, Brown A, Cauley JA, Chin MH, Gary-Webb T, Kim C, Sosa JA, Sumner AE, Anton B. 2012. Health disparities in endocrine disorders: Biological, clinical, and nonclinical factors--an endocrine society scientific statement. J Clin Endocrinol Metab 97(9):E1579-639.

Golzarand M, Toolabi K, Farid R. 2017. The bariatric surgery and weight losing: A meta-analysis in the long- and very long-term effects of laparoscopic adjustable gastric banding, laparoscopic roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy on weight loss in adults. Surg Endosc 31(11):4331-45.

Gonzalez JT, Fuchs CJ, Betts JA, Luc J C van L. 2016. Liver glycogen metabolism during and after prolonged endurance-type exercise. American Journal of Physiology-Endocrinology and Metabolism 311(3):E543-53.

Goodarzi MO, Langefeld CD, Xiang AH, Chen YD, Guo X, Hanley AJ, Raffel LJ, Kandeel F, Nadler JL, Buchanan TA, et al. 2014. Insulin sensitivity and insulin clearance are heritable and have strong genetic correlation in mexican americans. Obesity (Silver Spring) 22(4):1157-64.

Goodwin ML. 2010. Blood glucose regulation during prolonged, submaximal, continuous exercise: A guide for clinicians. J Diabetes Sci Technol 4(3):694-705.

Gorboulev V, Schurmann A, Vallon V, Kipp H, Jaschke A, Klessen D, Friedrich A, Scherneck S, Rieg T, Cunard R, et al. 2012. Na(+)-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. Diabetes 61(1):187-96.

Gouda HN, Sagoo GS, Harding AH, Yates J, Sandhu MS, Higgins JPT. 2010. The association between the peroxisome proliferator-activated receptor-γ2 (PPARG2) Pro12Ala gene variant and type 2 diabetes mellitus: A HuGE review and meta-analysis. Am J Epidemiol 171(6):645-55.

Gouyon F, Caillaud L, Carriere V, Klein C, Dalet V, Citadelle D, Kellett GL, Thorens B, Leturque A, Brot-Laroche E. 2003. Simple-sugar meals target GLUT2 at enterocyte apical membranes to improve sugar absorption: A study in GLUT2-null mice. J Physiol 552(Pt 3):823-32.

Graham DJ, Ouellet-Hellstrom R, MaCurdy TE, Ali F, Sholley C, Worrall C, Kelman JA. 2010. Risk of acute myocardial infarction, stroke, heart failure, and death in elderly medicare patients treated with rosiglitazone or pioglitazone. Jama 304(4):411-8.

Greenfield MS, Doberne L, Kraemer F, Tobey T, Reaven G. 1981. Assessment of insulin resistance with the insulin suppression test and the euglycemic clamp. Diabetes 30(5):387-92.

Greenhaff PL, Hultman E, Harris RC. 2004. Carbohydrate metabolism. In: Principles of exercise biochemistry. Portmans JR, editor. 3rd, revised ed. Basel: Karger AG. p. 108-51.

Greenhaff PL, Karagounis LG, Peirce N, Simpson EJ, Hazell M, Layfield R, Wackerhage H, Smith K, Atherton P, Selby A, et al. 2008. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. American Journal of Physiology-Endocrinology and Metabolism 295(3):E595-604.

Greiner M, Pfeiffer D, Smith RD. 2000. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev Vet Med 45(1-2):23-41.

Grøndahl MF, Bagger JI, Lund A, Faurschou A, Rehfeld JF, Holst JJ, Vilsbøll T, Knop FK. 2018. Effects of smoking versus nonsmoking on postprandial glucose metabolism in heavy smokers compared with nonsmokers. Diabetes Care 41(6):1260-7.

Guasch-Ferre M, Hruby A, Toledo E, Clish CB, Martinez-Gonzalez MA, Salas-Salvado J, Hu FB. 2016. Metabolomics in prediabetes and diabetes: A systematic review and meta-analysis. Diabetes Care 39(5):833-46.

Guthold R, Stevens GA, Riley LM, Bull FC. 2018. Worldwide trends in insufficient physical activity from 2001 to 2016: A pooled analysis of 358 population-based surveys with 1.9 million participants. Lancet Glob Health 6(10):e1077-86.

Gutt M, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, Schneiderman N, Skyler JS, Marks JB. 2000. Validation of the insulin sensitivity index (ISI(0,120)): Comparison with other measures. Diabetes Res Clin Pract 47(3):177-84.

Hällsten K, Virtanen KA, Lönnqvist F, Janatuinen T, Turiceanu M, Rönnemaa T, Viikari J, Lehtimäki T, Knuuti J, Nuutila P. 2004. Enhancement of insulin-stimulated

myocardial glucose uptake in patients with type 2 diabetes treated with rosiglitazone. Diabet Med 21(12):1280-7.

Hällsten K, Virtanen KA, Lönnqvist F, Sipilä H, Oksanen A, Viljanen T, Rönnemaa T, Viikari J, Knuuti J, Nuutila P. 2002. Rosiglitazone but not metformin enhances insulin- and exercise-stimulated skeletal muscle glucose uptake in patients with newly diagnosed type 2 diabetes. Diabetes 51(0012-1797; 12):3479-85.

Hamacher K, Coenen HH, Stocklin G. 1986. Efficient stereospecific synthesis of no-carrier-added 2-[18F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. J Nucl Med 27(0161-5505; 2):235-8.

Harano Y, Hidaka H, Takatsuki K, Ohgaku S, Haneda M, Motoi S, Kawagoe K, Shigeta Y, Abe H. 1978. Glucose, insulin, and somatostatin infusion for the determination of insulin sensitivity in vivo. Metabolism 27(9 Suppl 1):1449-52.

Heinonen I, Kalliokoski KK, Hannukainen JC, Duncker DJ, Nuutila P, Knuuti J. 2014. Organ-specific physiological responses to acute physical exercise and long-term training in humans. Physiology (Bethesda) 29(6):421-36.

Henry RR, Abrams L, Nikoulina S, Ciaraldi TP. 1995. Insulin action and glucose metabolism in nondiabetic control and NIDDM subjects. comparison using human skeletal muscle cell cultures. Diabetes 44(8):936-46.

Hickner RC, Racette SB, Binder EF, Fisher JS, Kohrt WM. 1999. Suppression of whole body and regional lipolysis by insulin: Effects of obesity and exercise. J Clin Endocrinol Metab 84(11):3886-95.

Hirvonen J, Virtanen KA, Nummenmaa L, Hannukainen JC, Honka MJ, Bucci M, Nesterov SV, Parkkola R, Rinne J, Iozzo P, et al. 2011. Effects of insulin on brain glucose metabolism in impaired glucose tolerance. Diabetes 60(2):443-7.

Hochner H, Friedlander Y, Calderon-Margalit R, Meiner V, Sagy Y, Avgil-Tsadok M, Burger A, Savitsky B, Siscovick DS, Manor O. 2012. Associations of maternal prepregnancy body mass index and gestational weight gain with adult offspring cardiometabolic risk FactorsClinical perspective. Circulation 125(11):1381-9.

Hocking S, Samocha-Bonet D, Milner K, Greenfield JR, Chisholm DJ. 2013. Adiposity and insulin resistance in humans: The role of the different tissue and cellular lipid depots. Endocr Rev 34(4):463-500.

Hodge AM, Jenkins AJ, English DR, O'Dea K, Giles GG. 2009. NMR-determined lipoprotein subclass profile predicts type 2 diabetes. Diabetes Res Clin Pract 83(1):132-9.

Holmes MV, Lange LA, Palmer T, Lanktree MB, North KE, Almoguera B, Buxbaum S, Chandrupatla HR, Elbers CC, Guo Y, et al. 2014. Causal effects of body mass index on cardiometabolic traits and events: A mendelian randomization analysis. Am J Hum Genet 94(2):198-208.

Holst JJ, Gribble F, Horowitz M, Rayner CK. 2016. Roles of the gut in glucose homeostasis. Diabetes Care 39(6):884.

Honka H, Mäkinen J, Hannukainen JC, Tarkia M, Oikonen V, Teräs M, Fagerholm V, Ishizu T, Saraste A, Stark C, et al. 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(4):893-900.

Honka MJ, Latva-Rasku A, Bucci M, Virtanen KA, Hannukainen JC, Kalliokoski KK, Nuutila P. 2018. Insulin-stimulated glucose uptake in skeletal muscle, adipose tissue and liver: A positron emission tomography study. Eur J Endocrinol 178(5):523-31.

Honka MJ, Bucci M, Andersson J, Huovinen V, Guzzardi MA, Sandboge S, Savisto N, Salonen MK, Badeau RM, Parkkola R, et al. 2016. Resistance training enhances insulin suppression of endogenous glucose production in elderly women. J Appl Physiol (1985) 120(6):633-9.

Hosmer DW and Lemeshow S. 2000. Assessing the fit of the model. In: Applied logistic regression. New York, Unites States: John Wiley & Sons, Inc. p. 143-202.

Hoy AJ, Bruce CR, Cederberg A, Turner N, James DE, Cooney GJ, Kraegen EW. 2007. Glucose infusion causes insulin resistance in skeletal muscle of rats without changes in akt and AS160 phosphorylation. American Journal of Physiology-Endocrinology and Metabolism 293(5):E1358-64.

Hu Y, Zong G, Liu G, Wang M, Rosner B, Pan A, Willett WC, Manson JE, Hu FB, Sun Q. 2018. Smoking cessation, weight change, type 2 diabetes, and mortality. N Engl J Med 379(7):623-32.

Huang C, Somwar R, Patel N, Niu W, Török D, Klip A. 2002. Sustained exposure of L6 myotubes to high glucose and insulin decreases insulin-stimulated GLUT4 translocation but upregulates GLUT4 activity. Diabetes 51(7):2090-8.

Hücking K, Watanabe RM, Stefanovski D, Bergman RN. 2008. OGTT-derived measures of insulin sensitivity are confounded by factors other than insulin sensitivity itself. Obesity (Silver Spring) 16(8):1938-45.

Hui S, Ghergurovich JM, Morscher RJ, Jang C, Teng X, Lu W, Esparza LA, Reya T, Le Z, Yanxiang Guo J, et al. 2017. Glucose feeds the TCA cycle via circulating lactate. Nature 551(7678):115-8.

Hussain K, Challis B, Rocha N, Payne F, Minic M, Thompson A, Daly A, Scott C, Harris J, Smillie BJL, et al. 2011. An activating mutation of *AKT2* and human hypoglycemia. Science 334(6055):474.

Husu P, Paronen O, Suni J, Vasankari T. 2011. Physical activity and fitness of Finns in 2010 - Current status and changes in health-enhancing physical activity. Finland: Ministry of Education and culture. Report nr 2011:15. p. 1-85. Available from: http://www.ukkinstituutti.fi/filebank/588-Suomalaisten_fyysinen_aktiivisuus_netti.pdf.

Huth C, Pigeon E, Riou ME, St-Onge J, Arguin H, Couillard E, Dubois MJ, Marette A, Tremblay A, Weisnagel SJ, et al. 2016. Fitness, adiposopathy, and adiposity are independent predictors of insulin sensitivity in middle-aged men without diabetes. J Physiol Biochem 72(3):435-44.

Hwang JH, Perseghin G, Rothman DL, Cline GW, Magnusson I, Petersen KF, Shulman GI. 1995. Impaired net hepatic glycogen synthesis in insulin-dependent diabetic subjects during mixed meal ingestion. A 13C nuclear magnetic resonance spectroscopy study. J Clin Invest 95(2):783-7.

Imamura F, Micha R, Wu JHY, de Oliveira Otto MC, Otite FO, Abioye AI, Mozaffarian D. 2016. Effects of saturated fat, polyunsaturated fat, monounsaturated fat, and carbohydrate on glucose-insulin homeostasis: A systematic review and meta-analysis of randomised controlled feeding trials. PLoS Med 13(7):e1002087. doi:10.1371/journal.pmed.1002087.

Immonen H, Hannukainen JC, Iozzo P, Soinio M, Salminen P, Saunavaara V, Borra R, Parkkola R, Mari A, Lehtimäki T, et al. 2014. Effect of bariatric surgery on liver glucose metabolism in morbidly obese diabetic and non-diabetic patients. J Hepatol 60(2):377-83.

Inouye M, Kettunen J, Soininen P, Silander K, Ripatti S, Kumpula LS, Hamalainen E, Jousilahti P, Kangas AJ, Mannisto S, et al. 2010. Metabonomic, transcriptomic, and genomic variation of a population cohort. Mol Syst Biol 6:441.

International Diabetes Federation. 2017. IDF Diabetes Atlas - 8th Edition. Brussels, Belgium: International Diabetes Federation Available from: http://diabetesatlas.org/resources/2017-atlas.html.

Iozzo P. 2010. Metabolic toxicity of the heart: Insights from molecular imaging. Nutrition, Metabolism and Cardiovascular Diseases 20(3):147-56.

Iozzo P, Hällsten K, Oikonen V, Virtanen KA, Kemppainen J, Solin O, Ferrannini E, Knuuti J, Nuutila P. 2003a. Insulin-mediated hepatic glucose uptake is impaired in type 2 diabetes: Evidence for a relationship with glycemic control. J Clin Endocrinol Metab 88(5):2055-60.

Iozzo P, Geisler F, Oikonen V, Mäki M, Takala T, Solin O, Ferrannini E, Knuuti J, Nuutila P. 2003. Insulin stimulates liver glucose uptake in humans: An 18F-FDG PET study. J Nucl Med 44(0161-5505; 5):682-9.

Iozzo P, Lautamäki R, Geisler F, Virtanen KA, Oikonen V, Haaparanta M, Yki-Järvinen H, Ferrannini E, Knuuti J, Nuutila P. 2004. Non-esterified fatty acids impair insulin-mediated glucose uptake and disposition in the liver. Diabetologia 47(7):1149-56.

Iozzo P, Hällsten K, Oikonen V, Virtanen KA, Parkkola R, Kemppainen J, Solin O, Lönnqvist F, Ferrannini E, Knuuti J, et al. 2003b. Effects of metformin and rosiglitazone monotherapy on insulin-mediated hepatic glucose uptake and their relation to visceral fat in type 2 diabetes. Diabetes Care 26(0149-5992; 7):2069-74.

Iozzo P, Holmes M, Schmidt MV, Cirulli F, Guzzardi MA, Berry A, Balsevich G, Andreassi MG, Wesselink JJ, Liistro T, et al. 2014. Developmental ORIgins of healthy and unhealthy AgeiNg: The role of maternal obesity--introduction to DORIAN. Obes Facts 7(2):130-51.

Iozzo P, Gastaldelli A, Järvisalo MJ, Kiss J, Borra R, Buzzigoli E, Viljanen A, Naum G, Viljanen T, Oikonen V, et al. 2006. 18F-FDG assessment of glucose disposal and production rates during fasting and insulin stimulation: A validation study. J Nucl Med 47(0161-5505; 6):1016-22.

Iozzo P, Järvisalo MJ, Kiss J, Borra R, Naum GA, Viljanen A, Viljanen T, Gastaldelli A, Buzzigoli E, Guiducci L, et al. 2007a. Quantification of liver glucose metabolism by positron emission tomography: Validation study in pigs. Gastroenterology 132(2):531-42.

Iozzo P, Järvisalo MJ, Kiss J, Borra R, Naum GA, Viljanen A, Viljanen T, Gastaldelli A, Buzzigoli E, Guiducci L, et al. 2007b.

Quantification of liver glucose metabolism by positron emission tomography: Validation study in pigs. Gastroenterology 132(0016-5085; 2):531-42.

Jang JY, Bae H, Lee YJ, Choi YI, Kim HJ, Park SB, Suh SW, Kim SW, Han BW. 2018. Structural basis for the enhanced anti-diabetic efficacy of lobeglitazone on PPARgamma. Sci Rep 8(1):31,017-18274-1.

Jani R, Molina M, Matsuda M, Balas B, Chavez A, DeFronzo RA, Abdul-Ghani M. 2008. Decreased non-insulin-dependent glucose clearance contributes to the rise in fasting plasma glucose in the nondiabetic range. Diabetes Care 31(2):311-5.

Jansson PA, Smith U, Lönnroth P. 1990. Interstitial glycerol concentration measured by microdialysis in two subcutaneous regions in humans. Am J Physiol 258(6 Pt 1):E918-22.

Jansson PA, Larsson A, Smith U, Lönnroth P. 1992. Glycerol production in subcutaneous adipose tissue in lean and obese humans. J Clin Invest 89(5):1610-7.

Jensen CB, Storgaard H, Madsbad S, Richter EA, Vaag AA. 2007. Altered skeletal muscle fiber composition and size precede whole-body insulin resistance in young men with low birth weight. The Journal of Clinical Endocrinology & Metabolism 92(4):1530-4.

Jensen J, Rustad PI, Kolnes AJ, Lai Y. 2011. The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise. Frontiers in Physiology 2:112.

Jiao N, Baker SS, Nugent CA, Tsompana M, Cai L, Wang Y, Buck MJ, Genco RJ, Baker RD, Zhu R, et al. 2018. Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: A meta-analysis. Physiol Genomics 50(4):244-54.

Jin SM, Park CY, Cho YM, Ku BJ, Ahn CW, Cha BS, Min KW, Sung YA, Baik SH, Lee KW, et al. 2015. Lobeglitazone and pioglitazone as add-ons to metformin for patients with type 2 diabetes: A 24-week, multicentre, randomized, double-blind, parallel-group, active-controlled, phase III clinical trial with a 28-week extension. Diabetes Obes Metab 17(6):599-602.

Johnston BC, Kanters S, Bandayrel K, al e. 2014. Comparison of weight loss among named diet programs in overweight and obese adults: A meta-analysis. Jama 312(9):923-33.

Johnston KL, Thomas EL, Bell JD, Frost GS, Robertson MD. 2010. Resistant starch improves insulin sensitivity in metabolic syndrome. Diabetic Med 27(4):391-7.

Jordy AB, Kraakman MJ, Gardner T, Estevez E, Kammoun HL, Weir JM, Kiens B, Meikle PJ, Febbraio MA, Henstridge DC. 2015. Analysis of the liver lipidome reveals insights into the protective effect of exercise on high-fat diet-induced hepatosteatosis in mice. Am J Physiol Endocrinol Metab 308(9):E778-91.

Jukarainen S, Holst R, Dalgård C, Piirilä P, Lundbom J, Hakkarainen A, Lundbom N, Rissanen A, Kaprio J, Kyvik KO, et al. 2017. Cardiorespiratory fitness and adiposity as determinants of metabolic Health—Pooled analysis of two twin cohorts. The Journal of Clinical Endocrinology & Metabolism 102(5):1520-8.

Kahn CR. 1978. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: A necessary distinction. Metabolism 27(12 Suppl 2):1893-902.

Kaiser HF. 1960. The application of electronic computers to factor analysis. Educ Psychol Meas 20:141-51.

Kantartzis K, Peter A, Machicao F, Machann J, Wagner S, Konigsrainer I, Konigsrainer A, Schick F, Fritsche A, Haring HU, et al. 2009. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. Diabetes 58(11):2616-23.

Karakelides H, Irving BA, Short KR, O'Brien P, Nair KS. 2010. Age, obesity, and sex effects on insulin sensitivity and skeletal muscle mitochondrial function. Diabetes 59(1):89.

Karlsson HK, Hallsten K, Bjornholm M, Tsuchida H, Chibalin AV, Virtanen KA, Heinonen OJ, Lonnqvist F, Nuutila P, Zierath JR. 2005. Effects of metformin and rosiglitazone treatment on insulin signaling and glucose uptake in patients with newly diagnosed type 2 diabetes: A randomized controlled study. Diabetes 54(5):1459-67.

Karpe F, Dickmann JR, Frayn KN. 2011. Fatty acids, obesity, and insulin resistance: Time for a reevaluation. Diabetes 60(10):2441-9.

Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. 2000. Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 85(7):2402-10.

Katz J and Tayek JA. 1998. Gluconeogenesis and the cori cycle in 12-, 20-, and 40-h-fasted humans. Am J Physiol 275(3 Pt 1):E537-42.

Kellett GL and Helliwell PA. 2000. The diffusive component of intestinal glucose absorption

is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane. Biochem J 350 Pt 1:155-62.

Kelley D, Mitrakou A, Marsh H, Schwenk F, Benn J, Sonnenberg G, Arcangeli M, Aoki T, Sorensen J, Berger M. 1988. Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load. J Clin Invest 81(5):1563-71.

Kelley DE and Mandarino LJ. 1990. Hyperglycemia normalizes insulin-stimulated skeletal muscle glucose oxidation and storage in noninsulin-dependent diabetes mellitus. J Clin Invest 86(6):1999-2007.

Kelley DE, Williams KV, Price JC, Goodpaster B. 1999. Determination of the lumped constant for [18F] fluorodeoxyglucose in human skeletal muscle. J Nucl Med 40(11):1798-804.

Keramida G, Dizdarevic S, Bush J, Peters AM. 2015. Quantification of tumour (18) F-FDG uptake: Normalise to blood glucose or scale to liver uptake? Eur Radiol 25(9):2701-8.

Keyes JW,Jr. 1995. SUV: Standard uptake or silly useless value? J Nucl Med 36(10):1836-9.

Kim SG, Kim DM, Woo JT, Jang HC, Chung CH, Ko KS, Park JH, Park YS, Kim SJ, Choi DS. 2014. Efficacy and safety of lobeglitazone monotherapy in patients with type 2 diabetes mellitus over 24-weeks: A multicenter, randomized, double-blind, parallel-group, placebo controlled trial. PLoS One 9(4):e92843.

Köbberling J and Tillil H. 1982. Empirical risk figures for first-degree relatives of noninsulin dependent diabetics. In: The genetics of diabetes mellitus. Köbberling J and Tattersall R, editors. London: Academic Press. p. 201-9.

Kodama K, Tojjar D, Yamada S, Toda K, Patel CJ, Butte AJ. 2013. Ethnic differences in the relationship between insulin sensitivity and insulin response: A systematic review and meta-analysis. Diabetes Care 36(6):1789-96.

Koffert JP, Mikkola K, Virtanen KA, Andersson AD, Faxius L, Hallsten K, Heglind M, Guiducci L, Pham T, Silvola JMU, et al. 2017. Metformin treatment significantly enhances intestinal glucose uptake in patients with type 2 diabetes: Results from a randomized clinical trial. Diabetes Res Clin Pract 131:208-16.

Kooijman S, Wang Y, Parlevliet ET, Boon MR, Edelschaap D, Snaterse G, Pijl H, Romijn JA, Rensen PC. 2015. Central GLP-1 receptor signalling accelerates plasma clearance of triacylglycerol and glucose by activating brown adipose tissue in mice. Diabetologia 58(11):2637-46.

Koopmans SJ, Mandarino L, DeFronzo RA. 1998. Time course of insulin action on tissue-specific intracellular glucose metabolism in normal rats. Am J Physiol 274(4 Pt 1):E642-50.

Korenblat KM, Fabbrini E, Mohammed BS, Klein S. 2008. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. Gastroenterology 134(5):1369-75.

Kotronen A, Johansson LE, Johansson LM, Roos C, Westerbacka J, Hamsten A, Bergholm R, Arkkila P, Arola J, Kiviluoto T, et al. 2009. A common variant in PNPLA3, which encodes adiponutrin, is associated with liver fat content in humans. Diabetologia 52(6):1056-60.

Kotronen A, Juurinen L, Tiikkainen M, Vehkavaara S, Yki–Järvinen H. 2008. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. Gastroenterology 135(1):122-30.

Kowalski GM, Moore SM, Hamley S, Selathurai A, Bruce CR. 2017. The effect of ingested glucose dose on the suppression of endogenous glucose production in humans. Diabetes 66(9):2400.

Krssak M, Brehm A, Bernroider E, Anderwald C, Nowotny P, Dalla Man C, Cobelli C, Cline GW, Shulman GI, Waldhausl W, et al. 2004. Alterations in postprandial hepatic glycogen metabolism in type 2 diabetes. Diabetes 53(12):3048-56.

Kullberg J, Hedström A, Brandberg J, Strand R, Johansson L, Bergström G, Ahlström H. 2017. Automated analysis of liver fat, muscle and adipose tissue distribution from CT suitable for large-scale studies. Scientific Reports 7(1):10425.

Kuusisto J, Lempiainen P, Mykkanen L, Laakso M. 2001. Insulin resistance syndrome predicts coronary heart disease events in elderly type 2 diabetic men. Diabetes Care 24(9):1629-33.

Laakso M. 2015. Is insulin resistance a feature of or a primary risk factor for cardiovascular disease? Curr Diab Rep 15(12):105,015-0684-4.

Laakso M, Edelman SV, Brechtel G, Baron AD. 1990. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. J Clin Invest 85(6):1844-52.

Laaksonen DE, Lakka TA, Lakka H-, Nyyssönen K, Rissanen T, Niskanen LK, Salonen JT.

2002. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. Diabetic Med 19(6):456-64.

Lafontan M. 2014. Adipose tissue and adipocyte dysregulation. Diabetes Metab 40(1):16-28.

Lalia AZ and Lanza IR. 2016. Insulin-sensitizing effects of omega-3 fatty acids: Lost in translation? Nutrients 8(6):329. doi:10.3390/nu8060329.

Lallukka S and Yki-Järvinen H. 2016. Non-alcoholic fatty liver disease and risk of type 2 diabetes. Best Pract Res Clin Endocrinol Metab 30(3):385-95.

Landau BR, Wahren J, Chandramouli V, Schumann WC, Ekberg K, Kalhan SC. 1996. Contributions of gluconeogenesis to glucose production in the fasted state. J Clin Invest 98(2):378-85.

Langlais PR, Mandarino LJ, Garvey WT. 2015. Mechanisms of insulin signal transduction. In: International textbook of diabetes mellitus. Defronzo RA, Ferrannini E, Zimmet P, et al, editors. 4th ed. Chichester, West Sussex, UK: John Wiley & Sons, Ltd. p. 161-92.

Lanza IR, Short DK, Short KR, Raghavakaimal S, Basu R, Joyner MJ, McConnell JP, Nair KS. 2008. Endurance exercise as a countermeasure for aging. Diabetes 57(11):2933-42.

Larsson M, Vorrsjö E, Talmud P, Lookene A, Olivecrona G. 2013. Apolipoproteins C-I and C-III inhibit lipoprotein lipase activity by displacement of the enzyme from lipid droplets. The Journal of Biological Chemistry 288(47):33997-4008.

Latva-Rasku A, Honka MJ, Stancakova A, Koistinen HA, Kuusisto J, Guan L, Manning AK, Stringham H, Gloyn AL, Lindgren CM, et al. 2018. A partial loss-of-function variant in AKT2 is associated with reduced insulin-mediated glucose uptake in multiple insulin-sensitive tissues: A genotype-based callback positron emission tomography study. Diabetes 67(2):334-42.

Laurencikiene J, Skurk T, Kulyté A, Hedén P, Åström G, Sjölin E, Rydén M, Hauner H, Arner P. 2011. Regulation of lipolysis in small and large fat cells of the same subject. The Journal of Clinical Endocrinology & Metabolism 96(12):E2045-9.

Lautamäki R, Airaksinen KE, Seppänen M, Toikka J, Luotolahti M, Ball E, Borra R, Härkonen R, Iozzo P, Stewart M, et al. 2005. Rosiglitazone improves myocardial glucose uptake in patients with type 2 diabetes and coronary artery disease: A 16-week randomized, double-blind, placebo-controlled study. Diabetes 54(0012-1797; 9):2787-94.

Lee C and Li X. 2018. Platelet-derived growth factor-C and -D in the cardiovascular system and diseases. Mol Aspects Med 62:12-21.

Lee M, Saver JL, Liao H, Lin C, Ovbiagele B. 2017. Pioglitazone for secondary stroke prevention. Stroke 48(2):388.

Lee M, Martin H, Firpo MA, Demerath EW. 2011. Inverse association between adiposity and telomere length: The fels longitudinal study. Am J Human Biol 23(1):100-6.

Lee YH, Kim JH, Kim SR, Jin HY, Rhee EJ, Cho YM, Lee BW. 2017. Lobeglitazone, a novel thiazolidinedione, improves non-alcoholic fatty liver disease in type 2 diabetes: Its efficacy and predictive factors related to responsiveness. J Korean Med Sci 32(1):60-9.

Leenders M, Verdijk LB, van der Hoeven L, Adam JJ, van Kranenburg J, Nilwik R, van Loon LJ. 2013. Patients with type 2 diabetes show a greater decline in muscle mass, muscle strength, and functional capacity with aging. J Am Med Dir Assoc 14(8):585-92.

Leto D and Saltiel AR. 2012. Regulation of glucose transport by insulin: Traffic control of GLUT4. Nature Reviews Molecular Cell Biology 13:383.

Leung N, Sakaue T, Carpentier A, Uffelman K, Giacca A, Lewis GF. 2004. Prolonged increase of plasma non-esterified fatty acids fully abolishes the stimulatory effect of 24 hours of moderate hyperglycaemia on insulin sensitivity and pancreatic beta-cell function in obese men. Diabetologia 47(2):204-13.

Lewis GF, Vranic M, Harley P, Giacca A. 1997. Fatty acids mediate the acute extrahepatic effects of insulin on hepatic glucose production in humans. Diabetes 46(7):1111-9.

Li G, et al. 2008. The long-term effect of lifestyle interventions to prevent diabetes in the china da qing diabetes prevention study: A 20-year follow-up study. The Lancet 371(9626):1783-9.

Li JZ, Huang Y, Karaman R, Ivanova PT, Brown HA, Roddy T, Castro-Perez J, Cohen JC, Hobbs HH. 2012. Chronic overexpression of PNPLA3I148M in mouse liver causes hepatic steatosis. J Clin Invest 122(11):4130-44.

Li S, Brown MS, Goldstein JL. 2010. Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. Proc Natl Acad Sci U S A 107(8):3441-6.

Li Z, Yi CX, Katiraei S, Kooijman S, Zhou E, Chung CK, Gao Y, van den Heuvel JK, Meijer OC, Berbee JFP, et al. 2018. Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit. Gut 67(7):1269-79.

Liao H, Saver JL, Wu Y, Chen T, Lee M, Ovbiagele B. 2017. Pioglitazone and cardiovascular outcomes in patients with insulin resistance, pre-diabetes and type 2 diabetes: A systematic review and meta-analysis. BMJ Open 7(1).

Loke YK, Kwok CS, Singh S. 2011. Comparative cardiovascular effects of thiazolidinediones: Systematic review and meta-analysis of observational studies. Bmj 342.

Lönnroth P, Jansson PA, Smith U. 1987. A microdialysis method allowing characterization of intercellular water space in humans. American Journal of Physiology-Endocrinology and Metabolism 253(2):E228-31.

Lotta LA, Scott RA, Sharp SJ, Burgess S, Luan J, Tillin T, Schmidt AF, Imamura F, Stewart ID, Perry JR, et al. 2016. Genetic predisposition to an impaired metabolism of the branched-chain amino acids and risk of type 2 diabetes: A mendelian randomisation analysis. PLoS Med 13(11):e1002179.

Lundsgaard AM and Kiens B. 2014. Gender differences in skeletal muscle substrate metabolism - molecular mechanisms and insulin sensitivity. Front Endocrinol (Lausanne) 5:195.

Luo Z, Fujio Y, Kureishi Y, Rudic RD, Daumerie G, Fulton D, Sessa WC, Walsh K. 2000. Acute modulation of endothelial akt/PKB activity alters nitric oxide-dependent vasomotor activity in vivo. J Clin Invest 106(4):493-9.

Mackey RH, Mora S, Bertoni AG, Wassel CL, Carnethon MR, Sibley CT, Goff DC,Jr. 2015. Lipoprotein particles and incident type 2 diabetes in the multi-ethnic study of atherosclerosis. Diabetes Care 38(4):628-36.

MacLean PS, Vadlamudi S, MacDonald KG, Pories WJ, Houmard JA, Barakat HA. 2000. Impact of insulin resistance on lipoprotein subpopulation distribution in lean and morbidly obese nondiabetic women. Metabolism 49(3):285-92.

Macotela Y, Boucher J, Tran TT, Kahn CR. 2009. Sex and depot differences in adipocyte insulin sensitivity and glucose metabolism. Diabetes 58(4):803.

Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI. 1992. Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. J Clin Invest 90(4):1323-7.

Mahendran Y, Cederberg H, Vangipurapu J, Kangas AJ, Soininen P, Kuusisto J, Uusitupa M, Ala-Korpela M, Laakso M. 2013. Glycerol and fatty acids in serum predict the development of hyperglycemia and type 2 diabetes in finnish men. Diabetes Care 36(11):3732-8.

Mahendran Y, Jonsson A, Have CT, Allin KH, Witte DR, Jorgensen ME, Grarup N, Pedersen O, Kilpelainen TO, Hansen T. 2017. Genetic evidence of a causal effect of insulin resistance on branched-chain amino acid levels. Diabetologia 60(5):873-8.

Majithia AR, Flannick J, Shahinian P, Guo M, Bray MA, Fontanillas P, Gabriel SB, GoT2D Consortium, NHGRI JHS/FHS Allelic Spectrum Project, SIGMA T2D Consortium, et al. 2014. Rare variants in PPARG with decreased activity in adipocyte differentiation are associated with increased risk of type 2 diabetes. Proc Natl Acad Sci U S A 111(36):13127-32.

Makimura H, Stanley TL, Suresh C, De Sousa-Coelho AL, Frontera WR, Syu S, Braun LR, Looby SE, Feldpausch MN, Torriani M, et al. 2016. Metabolic effects of long-term reduction in free fatty acids with acipimox in obesity: A randomized trial. J Clin Endocrinol Metab 101(3):1123-33.

Mäkinen J, Hannukainen JC, Karmi A, Immonen HM, Soinio M, Nelimarkka L, Savisto N, Helmio M, Ovaska J, Salminen P, et al. 2015. Obesity-associated intestinal insulin resistance is ameliorated after bariatric surgery. Diabetologia 58(5):1055-62.

Mandarino LJ, Consoli A, Kelley DE, Reilly JJ, Nurjhan N. 1990. Fasting hyperglycemia normalizes oxidative and nonoxidative pathways of insulin-stimulated glucose metabolism in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 71(6):1544-51.

Manning A, Highland HM, Gasser J, Sim X, Tukiainen T, Fontanillas P, Grarup N, Rivas MA, Mahajan A, Locke AE, et al. 2017. A low-frequency inactivating AKT2 variant enriched in the finnish population is associated with fasting insulin levels and type 2 diabetes risk. Diabetes 66(7):2019-32.

Manning AK, Hivert M, Scott RA, Grimsby JL, Bouatia-Naji N, Chen H, Rybin D, Liu C, Bielak LF, Prokopenko I, et al. 2012. A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. Nat Genet 44:659.

Mannucci E., Monami M., Lamanna C., Gensini GF, Marchionni N. 2008. Pioglitazone and cardiovascular risk. A comprehensive meta-analysis of randomized clinical trials.

Diabetes, Obesity and Metabolism 10(12):1221-38.

Mannucci E, Monami M, Di Bari M, Lamanna C, Gori F, Gensini GF, Marchionni N. 2010. Cardiac safety profile of rosiglitazone: A comprehensive meta-analysis of randomized clinical trials. Int J Cardiol 143(2):135-40.

Mardinoglu A, Kampf C, Asplund A, Fagerberg L, Hallström BM, Edlund K, Blüher M, Pontén F, Uhlen M, Nielsen J. 2014. Defining the human adipose tissue proteome to reveal metabolic alterations in obesity. J Proteome Res 13(11):5106-19.

Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. 2001. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 24(3):539-48.

Mårin P, Högh-Kristiansen I, Jansson S, Krotkiewski M, Holm G, Björntorp P. 1992. Uptake of glucose carbon in muscle glycogen and adipose tissue triglycerides in vivo in humans. Am J Physiol 263(3 Pt 1):E473-80.

Marinangeli CPF and Jones PJH. 2011. Whole and fractionated yellow pea flours reduce fasting insulin and insulin resistance in hypercholesterolaemic and overweight human subjects. Br J Nutr 105(1):110-7.

Marventano S, Vetrani C, Vitale M, Godos J, Riccardi G, Grosso G. 2017. Whole grain intake and glycaemic control in healthy subjects: A systematic review and meta-analysis of randomized controlled trials. Nutrients 9(7):769. doi:10.3390/nu9070769.

Massa ML, Gagliardino JJ, Francini F. 2011. Liver glucokinase: An overview on the regulatory mechanisms of its activity. IUBMB Life 63(1):1-6.

Massollo M, Marini C, Brignone M, Emionite L, Salani B, Riondato M, Capitanio S, Fiz F, Democrito A, Amaro A, et al. 2013. Metformin temporal and localized effects on gut glucose metabolism assessed using 18F-FDG PET in mice. J Nucl Med 54(2):259-66.

Mather K, Laakso M, Edelman S, Hook G, Baron A. 2000. Evidence for physiological coupling of insulin-mediated glucose metabolism and limb blood flow. Am J Physiol Endocrinol Metab 279(6):E1264-70.

Mather KA, Jorm AF, Parslow RA, Christensen H. 2011. Is telomere length a biomarker of aging? A review. The Journals of Gerontology: Series A 66A(2):202-13.

Matsuda M and DeFronzo RA. 1999. Insulin sensitivity indices obtained from oral glucose tolerance testing: Comparison with the euglycemic insulin clamp. Diabetes Care 22(9):1462-70.

Matthews C, Gorenne I, Scott S, Figg N, Kirkpatrick P, Ritchie A, Goddard M, Bennett M. 2006. Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: Effects of telomerase and oxidative stress. Circ Res 99(2):156-64.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. 1985. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28(7):412-9.

Medina-Gomez G, Gray SL, Yetukuri L, Shimomura K, Virtue S, Campbell M, Curtis RK, Jimenez-Linan M, Blount M, Yeo GS, et al. 2007. PPAR gamma 2 prevents lipotoxicity by controlling adipose tissue expandability and peripheral lipid metabolism. PLoS Genet 3(1553-7404; 4):e64.

Medina-Gomez G, Virtue S, Lelliott C, Boiani R, Campbell M, Christodoulides C, Perrin C, Jimenez-Linan M, Blount M, Dixon J, et al. 2005. The link between nutritional status and insulin sensitivity is dependent on the adipocyte-specific peroxisome proliferator-activated receptor-gamma2 isoform. Diabetes 54(0012-1797; 6):1706-16.

Meek SE, Nair KS, Jensen MD. 1999. Insulin regulation of regional free fatty acid metabolism. Diabetes 48(1):10-4.

Meex RC, Schrauwen-Hinderling VB, Moonen-Kornips E, Schaart G, Mensink M, Phielix E, van de Weijer T, Sels JP, Schrauwen P, Hesselink MK. 2010. Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. Diabetes 59(3):572-9.

Mehta MB, Shewale SV, Sequeira RN, Millar JS, Hand NJ, Rader DJ. 2017. Hepatic protein phosphatase 1 regulatory subunit 3B (Ppp1r3b) promotes hepatic glycogen synthesis and thereby regulates fasting energy homeostasis. J Biol Chem 292(25):10444-54.

Merovci A, Solis-Herrera C, Daniele G, Eldor R, Fiorentino TV, Tripathy D, Xiong J, Perez Z, Norton L, Abdul-Ghani MA, et al. 2014. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. J Clin Invest 124(2):509-14.

Mesarwi O, Polak J, Jun J, Polotsky VY. 2013. Sleep disorders and the development of insulin resistance and obesity. Endocrinol Metab Clin North Am 42(3):617-34.

Metges CC, Petzke KJ, El-Khoury AE, Henneman L, Grant I, Bedri S, Regan MM, Fuller MF, Young VR. 1999. Incorporation of urea

and ammonia nitrogen into ileal and fecal microbial proteins and plasma free amino acids in normal men and ileostomates. Am J Clin Nutr 70(6):1046-58.

Meugnier E, Faraj M, Rome S, Beauregard G, Michaut A, Pelloux V, Chiasson JL, Laville M, Clement K, Vidal H, et al. 2007. Acute hyperglycemia induces a global downregulation of gene expression in adipose tissue and skeletal muscle of healthy subjects. Diabetes 56(4):992-9.

Mimura A, Kageyama S, Maruyama M, Ikeda Y, Isogai Y. 1994. Insulin sensitivity test using a somatostatin analogue, octreotide (sandostatin). Horm Metab Res 26(4):184-7.

Mitrou P, Boutati E, Lambadiari V, Maratou E, Papakonstantinou A, Komesidou V, Sidossis L, Tountas N, Katsilambros N, Economopoulos T, et al. 2009. Rates of glucose uptake in adipose tissue and muscle in vivo after a mixed meal in women with morbid obesity. J Clin Endocrinol Metab 94(8):2958-61.

Moore MC, Coate KC, Winnick JJ, An Z, Cherrington AD. 2012. Regulation of hepatic glucose uptake and storage in vivo. Adv Nutr 3(3):286-94.

Mora S, Otvos JD, Rosenson RS, Pradhan A, Buring JE, Ridker PM. 2010. Lipoprotein particle size and concentration by nuclear magnetic resonance and incident type 2 diabetes in women. Diabetes 59(5):1153-60.

Morley JE, Baumgartner RN, Roubenoff R, Mayer J, Nair KS. 2001. Sarcopenia. J Lab Clin Med 137(4):231-43.

Morley JE, Vellas B, van Kan GA, Anker SD, Bauer JM, Bernabei R, Cesari M, Chumlea WC, Doehner W, Evans J, et al. 2013. Frailty consensus: A call to action. J Am Med Dir Assoc 14(6):392-7.

Mudaliar S, Henry RR, Boden G, Smith S, Chalamandaris A, Duchesne D, Iqbal N, List J. 2014. Changes in insulin sensitivity and insulin secretion with the sodium glucose cotransporter 2 inhibitor dapagliflozin. Diabetes Technology & Therapeutics 16(3):137-44.

Muller YL, Bogardus C, Beamer BA, Shuldiner AR, Baier LJ. 2003. A functional variant in the peroxisome proliferator-activated receptor gamma2 promoter is associated with predictors of obesity and type 2 diabetes in pima indians. Diabetes 52(0012-1797; 7):1864-71.

Muniyappa R, Iantorno M, Quon MJ. 2008. An integrated view of insulin resistance and endothelial dysfunction. Endocrinol Metab Clin North Am 37(3):685-711.

Nagel SA, Keuper M, Zagotta I, Enlund E, Ruperez AI, Debatin K, Wabitsch M, Fischer-Posovszky P. 2014. Up-regulation of bcl-2 during adipogenesis mediates apoptosis resistance in human adipocytes. Mol Cell Endocrinol 382(1):368-76.

Nakae J, Kitamura T, Silver DL, Accili D. 2001. The forkhead transcription factor Foxo1 (fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. J Clin Invest 108(9):1359-67.

Natali A, Gastaldelli A, Camastra S, Sironi AM, Toschi E, Masoni A, Ferrannini E, Mari A. 2000. Dose-response characteristics of insulin action on glucose metabolism: A non-steady-state approach. Am J Physiol Endocrinol Metab 278(5):E794-801.

NatCen Social Research and UCL. 2017. Health Survey for England 2016. Surrey, UK: Health and Social Care Information Centre. NHS Digital Available from: https://digital.nhs.uk/data-and-information/publications/statistical/health-survey-for-england/health-survey-for-england-2016.

Nellemann B, Gormsen LC, Sorensen LP, Christiansen JS, Nielsen S. 2012. Impaired insulin-mediated antilipolysis and lactate release in adipose tissue of upper-body obese women. Obesity (Silver Spring) 20(1):57-64.

Nielsen S, Guo Z, Johnson CM, Hensrud DD, Jensen MD. 2004. Splanchnic lipolysis in human obesity. J Clin Invest 113(11):1582-8.

Nieman DC, Carlson KA, Brandstater ME, Naegele RT, Blankenship JW. 1987. Running endurance in 27-h-fasted humans. J Appl Physiol (1985) 63(6):2502-9.

Nissen SE, Nicholls SJ, Wolski K, Nesto R, Kupfer S, Perez A, Jure H, De Larochelliere R, Staniloae CS, Mavromatis K, et al. 2008. Comparison of pioglitazone vs glimepiride on progression of coronary atherosclerosis in patients with type 2 diabetes: The PERISCOPE randomized controlled trial. Jama 299(13):1561-73.

Nissen SE and Wolski K. 2007. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 356(24):2457-71.

Nuttall FQ, Ngo A, Gannon MC. 2008. Regulation of hepatic glucose production and the role of gluconeogenesis in humans: Is the rate of gluconeogenesis constant? Diabetes Metab Res Rev 24(6):438-58.

Nuutila P, Knuuti MJ, Mäki M, Laine H, Ruotsalainen U, Teräs M, Haaparanta M, Solin O, Yki-Järvinen H. 1995. Gender and insulin

sensitivity in the heart and in skeletal muscles. studies using positron emission tomography. Diabetes 44(0012-1797; 1):31-6.

Nuutila P, Koivisto VA, Knuuti J, Ruotsalainen U, Teräs M, Haaparanta M, Bergman J, Solin O, Voipio-Pulkki LM, Wegelius U. 1992. Glucose-free fatty acid cycle operates in human heart and skeletal muscle in vivo. J Clin Invest 89(6):1767-74.

Oberbach A, Bossenz Y, Lehmann S, Niebauer J, Adams V, Paschke R, Schön MR, Blüher M, Punkt K. 2006. Altered fiber distribution and fiber-specific glycolytic and oxidative enzyme activity in skeletal muscle of patients with type 2 diabetes. Diabetes Care 29(4):895.

Oh K, Lee DS, Kim WK, Han BS, Lee SC, Bae K. 2016. Metabolic adaptation in obesity and type II diabetes: Myokines, adipokines and hepatokines. International Journal of Molecular Sciences 18(1):8.

Oikonen V. Multiple Time Graphical Analysis (*MTGA*) [Internet]: Turku PET Centre, University of Turku; c2018a [cited 2018 09/04]. Available from: http://www.turkupetcentre.net/petanalysis/model_mtga.html .

Oikonen V. Fractional uptake rate (FUR) [Internet]: Turku PET Centre, University of Turku; c2018b [cited 2018 10/14]. Available from: http://www.turkupetcentre.net/petanalysis/model_fur.html .

O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, et al. 2016. Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44(D1):D733-45.

Oliveira HC and de Faria EC. 2011. Cholesteryl ester transfer protein: The controversial relation to atherosclerosis and emerging new biological roles. IUBMB Life 63(4):248-57.

Olivieri F, Lorenzi M, Antonicelli R, Testa R, Sirolla C, Cardelli M, Mariotti S, Marchegiani F, Marra M, Spazzafumo L, et al. 2009. Leukocyte telomere shortening in elderly Type2DM patients with previous myocardial infarction. Atherosclerosis 206(2):588-93.

Orava J, Nuutila P, Noponen T, Parkkola R, Viljanen T, Enerbäck S, Rissanen A, Pietiläinen KH, Virtanen KA. 2013. Blunted metabolic responses to cold and insulin stimulation in brown adipose tissue of obese humans. Obesity (Silver Spring) 21(11):2279-87.

Otsu N. 1979. A threshold selection method from gray-level histograms. IEEE Trans Syst Man Cybern 9(1):62-6.

Otten J, Ahren B, Olsson T. 2014. Surrogate measures of insulin sensitivity vs the hyperinsulinaemic-euglycaemic clamp: A meta-analysis. Diabetologia 57(9):1781-8.

Pacini G and Mari A. 2007. Assessment of insulin sensitivity from steady-state and dynamic tests. In: Clinical diabetes research: Methods and techniques. Roden M, editor. Chichester, West Sussex, England: John Wiley & Sons, Ltd. p. 27-42.

Pacini G and Bergman RN. 1986. MINMOD: A computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput Methods Programs Biomed 23(2):113-22.

Pagliassotti MJ, Holste LC, Moore MC, Neal DW, Cherrington AD. 1996. Comparison of the time courses of insulin and the portal signal on hepatic glucose and glycogen metabolism in the conscious dog. J Clin Invest 97(1):81-91.

Palmisano BT, Le TD, Zhu L, Lee YK, Stafford JM. 2016. Cholesteryl ester transfer protein alters liver and plasma triglyceride metabolism through two liver networks in female mice. J Lipid Res 57(8):1541-51.

Park SW, Goodpaster BH, Lee JS, Kuller LH, Boudreau R, de Rekeneire N, Harris TB, Kritchevsky S, Tylavsky FA, Nevitt M, et al. 2009. Excessive loss of skeletal muscle mass in older adults with type 2 diabetes. Diabetes Care 32(11):1993.

Patlak CS and Blasberg RG. 1985a. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. generalizations. J Cereb Blood Flow Metab 5(4):584-90.

Patlak CS and Blasberg RG. 1985b. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. generalizations. J Cereb Blood Flow Metab 5(0271-678; 4):584-90.

Pauta M, Rotllan N, Fernandez-Hernando A, Langhi C, Ribera J, Lu M, Boix L, Bruix J, Jimenez W, Suarez Y, et al. 2016. Akt-mediated foxo1 inhibition is required for liver regeneration. Hepatology 63(5):1660-74.

Pei D, Jones CN, Bhargava R, Chen YD, Reaven GM. 1994. Evaluation of octreotide to assess insulin-mediated glucose disposal by the insulin suppression test. Diabetologia 37(8):843-5.

Peltoniemi P, Lönnroth P, Laine H, Oikonen V, Tolvanen T, Grönroos T, Strindberg L, Knuuti J, Nuutila P. 2000. Lumped constant for [(18)F]fluorodeoxyglucose in skel-

etal muscles of obese and nonobese humans. Am J Physiol Endocrinol Metab 279(5):E1122-30.

Pereira MA, Jacobs DR,Jr, Pins JJ, Raatz SK, Gross MD, Slavin JL, Seaquist ER. 2002. Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults. Am J Clin Nutr 75(5):848-55.

Pereira S, Marliss EB, Morais JA, Chevalier S, Gougeon R. 2008. Insulin resistance of protein metabolism in type 2 diabetes. Diabetes 57(1):56-63.

Perez-Tasigchana RF, Leon-Munoz LM, Lopez-Garcia E, Gutierrez-Fisac JL, Laclaustra M, Rodriguez-Artalejo F, Guallar-Castillon P. 2017. Metabolic syndrome and insulin resistance are associated with frailty in older adults: A prospective cohort study. Age Ageing 46(5):807-12.

Perry RJ, Camporez JP, Kursawe R, Titchenell PM, Zhang D, Perry CJ, Jurczak MJ, Abudukadier A, Han MS, Zhang XM, et al. 2015. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. Cell 160(4):745-58.

Perseghin G, Caumo A, Caloni M, Testolin G, Luzi L. 2001. Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. J Clin Endocrinol Metab 86(10):4776-81.

Peterli R, Wolnerhanssen BK, Peters T, Vetter D, Kroll D, Borbely Y, Schultes B, Beglinger C, Drewe J, Schiesser M, et al. 2018. Effect of laparoscopic sleeve gastrectomy vs laparoscopic roux-en-Y gastric bypass on weight loss in patients with morbid obesity: The SM-BOSS randomized clinical trial. Jama 319(3):255-65.

Petersen MC and Shulman GI. 2018. Mechanisms of insulin action and insulin resistance. Physiol Rev 98(4):2133-223.

Phelps ME, Huang SC, Hoffman EJ, Selin C, Sokoloff L, Kuhl DE. 1979. Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18)2-fluoro-2-deoxy-D-glucose: Validation of method. Ann Neurol 6(5):371-88.

Pierce M., Keen H., Bradley C. 1995. Risk of diabetes in offspring of parents with non-insulin-dependent diabetes. Diabetic Med 12(1):6-13.

Popineau L, Morzyglod L, Carre N, Cauzac M, Bossard P, Prip-Buus C, Lenoir V, Ragazzon B, Fauveau V, Robert L, et al. 2016. Novel Grb14-mediated cross talk between insulin and p62/Nrf2 pathways regulates liver lipogenesis and selective insulin resistance. Mol Cell Biol 36(16):2168-81.

Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, Griffiths JR, Chung YL, Schulze A. 2008. SREBP activity is regulated by mTORC1 and contributes to akt-dependent cell growth. Cell Metab 8(3):224-36.

Poy MN, Yang Y, Rezaei K, Fernström MA, Lee AD, Kido Y, Erickson SK, Najjar SM. 2002. CEACAM1 regulates insulin clearance in liver. Nat Genet 30:270.

Prager R, Wallace P, Olefsky JM. 1986. In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects. J Clin Invest 78(2):472-81.

Puhakainen I, Koivisto VA, Yki-Järvinen H. 1992. Lipolysis and gluconeogenesis from glycerol are increased in patients with non-insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 75(3):789-94.

Quistorff B, Secher NH, Van Lieshout JJ. 2008. Lactate fuels the human brain during exercise. Faseb J 22(10):3443-9.

R Core Team. 2017. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Radziuk J and Pye S. 2002. Quantitation of basal endogenous glucose production in type II diabetes: Importance of the volume of distribution. Diabetologia 45(8):1053-84.

Ragolia L and Begum N. 1998. Protein phosphatase-1 and insulin action. Mol Cell Biochem 182(1-2):49-58.

Raitakari M, Nuutila P, Ruotsalainen U, Laine H, Teräs M, Iida H, Mäkimattila S, Utriainen T, Oikonen V, Sipila H, et al. 1996. Evidence for dissociation of insulin stimulation of blood flow and glucose uptake in human skeletal muscle: Studies using [15O]H2O, [18F]fluoro-2-deoxy-D-glucose, and positron emission tomography. Diabetes 45(11):1471-7.

Rakipovski G, Rolin B, Nohr J, Klewe I, Frederiksen KS, Augustin R, Hecksher-Sorensen J, Ingvorsen C, Polex-Wolf J, Knudsen LB. 2018. The GLP-1 analogs liraglutide and semaglutide reduce atherosclerosis in ApoE(-/-) and LDLr(-/-) mice by a mechanism that includes inflammatory pathways. JACC Basic Transl Sci 3(6):844-57.

Rao RS, Yanagisawa R, Kini S. 2012. Insulin resistance and bariatric surgery. Obes Rev 13(4):316-28.

Rasmussen BB, Fujita S, Wolfe RR, Mittendorfer B, Roy M, Rowe VL, Volpi E. 2006. Insulin

resistance of muscle protein metabolism in aging. Faseb J 20(6):768-9.

Reaven GM. 2011. Insulin resistance: The link between obesity and cardiovascular disease. Med Clin North Am 95(5):875-92.

Reaven GM, Hollenbeck C, Jeng CY, Wu MS, Chen YD. 1988. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. Diabetes 37(8):1020-4.

Rebrin K, Steil GM, Mittelman SD, Bergman RN. 1996. Causal linkage between insulin suppression of lipolysis and suppression of liver glucose output in dogs. J Clin Invest 98(3):741-9.

Rena G, Hardie DG, Pearson ER. 2017. The mechanisms of action of metformin. Diabetologia 60(9):1577-85.

Reynolds A, et al. 2019. Carbohydrate quality and human health: A series of systematic reviews and meta-analyses. The Lancet .

Ribaric G, Buchwald JN, McGlennon TW. 2014. Diabetes and weight in comparative studies of bariatric surgery vs conventional medical therapy: A systematic review and meta-analysis. Obes Surg 24(3):437-55.

Rizza RA, Mandarino LJ, Gerich JE. 1981. Dose-response characteristics for effects of insulin on production and utilization of glucose in man. Am J Physiol 240(6):E630-9.

Rizza RA, Mandarino LJ, Genest J, Baker BA, Gerich JE. 1985. Production of insulin resistance by hyperinsulinaemia in man. Diabetologia 28(2):70-5.

Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. 2005. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. Am J Clin Nutr 82(3):559-67.

Robertson MD, Wright JW, Loizon E, Debard C, Vidal H, Shojaee-Moradie F, Russell-Jones D, Umpleby AM. 2012. Insulin-sensitizing effects on muscle and adipose tissue after dietary fiber intake in men and women with metabolic syndrome. The Journal of Clinical Endocrinology & Metabolism 97(9):3326-32.

Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez J, Müller M. 2011. pROC: An open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 12(1):77.

Robinson C, Tamborlane WV, Maggs DG, Enoksson S, Sherwin RS, Silver D, Shulman GI, Caprio S. 1998. Effect of insulin on glycerol production in obese adolescents. Am J Physiol 274(4 Pt 1):E737-43.

Rocha N, Payne F, Huang-Doran I, Sleigh A, Fawcett K, Adams C, Stears A, Saudek V, O'Rahilly S, Barroso I, et al. 2017. The metabolic syndrome- associated small G protein ARL15 plays a role in adipocyte differentiation and adiponectin secretion. Scientific Reports 7(1):17593.

Röder PV, Geillinger KE, Zietek TS, Thorens B, Koepsell H, Daniel H. 2014. The role of SGLT1 and GLUT2 in intestinal glucose transport and sensing. Plos One 9(2):e89977.

Romeo S, Sentinelli F, Dash S, Yeo GS, Savage DB, Leonetti F, Capoccia D, Incani M, Maglio C, Iacovino M, et al. 2010. Morbid obesity exposes the association between PNPLA3 I148M (rs738409) and indices of hepatic injury in individuals of european descent. Int J Obes (Lond) 34(1):190-4.

Rönnemaa T, Rönnemaa EM, Puukka P, Pyörälä K, Laakso M. 1996. Smoking is independently associated with high plasma insulin levels in nondiabetic men. Diabetes Care 19(11):1229-32.

Rooyackers O, Thorell A, Nygren J, Ljungqvist O. 2004. Microdialysis methods for measuring human metabolism. Curr Opin Clin Nutr Metab Care 7(5):515-21.

Ross S, Gerstein HC, Eikelboom J, Anand SS, Yusuf S, Pare G. 2015. Mendelian randomization analysis supports the causal role of dysglycaemia and diabetes in the risk of coronary artery disease. Eur Heart J 36(23):1454-62.

Rothman DL, Magnusson I, Katz LD, Shulman RG, Shulman GI. 1991. Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with 13C NMR. Science 254(5031):573-6.

Rowe JW, Minaker KL, Pallotta JA, Flier JS. 1983. Characterization of the insulin resistance of aging. J Clin Invest 71(6):1581-7.

Ruotsalainen U, Raitakari M, Nuutila P, Oikonen V, Sipilä H, Teräs M, Knuuti MJ, Bloomfield PM, Iida H. 1997. Quantitative blood flow measurement of skeletal muscle using oxygen-15-water and PET. J Nucl Med 38(2):314-9.

Rupprecht LE, Kreisler AD, Spierling SR, de Guglielmo G, Kallupi M, George O, Donny EC, Zorrilla EP, Sved AF. 2018. Self-administered nicotine increases fat metabolism and suppresses weight gain in male rats. Psychopharmacology (Berl) 235(4):1131-40.

Saad MF, Steil GM, Kades WW, Ayad MF, Elsewafy WA, Boyadjian R, Jinagouda SD, Bergman RN. 1997. Differences between the tolbutamide-boosted and the insulin-

modified minimal model protocols. Diabetes 46(7):1167-71.

Sala-Rabanal Monica, Chiara G, Hirayama BA, Vladimir K, Jie L, Barrio JR, Wright EM. 2018. Intestinal absorption of glucose in mice as determined by positron emission tomography. J Physiol 596(13):2473-89.

Salminen P, Helmiö M, Ovaska J, Juuti A, Leivonen M, Peromaa-Haavisto P, Hurme S, Soinio M, Nuutila P, Victorzon M. 2018. Effect of laparoscopic sleeve gastrectomy vs laparoscopic roux-en-Y gastric bypass on weight loss at 5 years among patients with morbid obesity: The SLEEVEPASS randomized clinical TrialFive-year outcomes of laparoscopic sleeve gastrectomy vs gastric bypass for morbid ObesityFive-year outcomes of laparoscopic sleeve gastrectomy vs gastric bypass for morbid obesity. Jama 319(3):241-54.

Salpea KD and Humphries SE. 2010. Telomere length in atherosclerosis and diabetes. Atherosclerosis 209(1):35-8.

Samuel V and Shulman G. 2012. Mechanisms for insulin resistance: Common threads and missing links. Cell 148(5):852-71.

Sanders FWB and Griffin JL. 2016. De novo lipogenesis in the liver in health and disease: More than just a shunting yard for glucose. Biol Rev Camb Philos Soc 91(2):452-68.

Sandqvist M, Strindberg L, Schmelz M, Lönnroth P, Jansson P-. 2011. Impaired delivery of insulin to adipose tissue and skeletal muscle in obese women with postprandial hyperglycemia. The Journal of Clinical Endocrinology & Metabolism 96(8):E1320-4.

Sayer AA, Syddall H, Martin H, Patel H, Baylis D, Cooper C. 2008. The developmental origins of sarcopenia. J Nutr Health Aging 12(7):427-32.

Schmitt CC, Aranias T, Viel T, Chateau D, Le Gall M, Waligora-Dupriet AJ, Melchior C, Rouxel O, Kapel N, Gourcerol G, et al. 2017. Intestinal invalidation of the glucose transporter GLUT2 delays tissue distribution of glucose and reveals an unexpected role in gut homeostasis. Mol Metab 6(1):61-72.

Schönfeld P and Reiser G. 2013. Why does brain metabolism not favor burning of fatty acids to provide energy? - reflections on disadvantages of the use of free fatty acids as fuel for brain. J Cereb Blood Flow Metab 33(10):1493-9.

Scott RA, Lagou V, Welch RP, Wheeler E, Montasser ME, Luan J, Mägi R, Strawbridge RJ, Rehnberg E, Gustafsson S, et al. 2012. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. Nat Genet 44:991.

Seino Y, Fukushima M, Yabe D. 2010. GIP and GLP-1, the two incretin hormones: Similarities and differences. J Diabetes Investig 1(1-2):8-23.

Seki T, Hosaka K, Lim S, Fischer C, Honek J, Yang Y, Andersson P, Nakamura M, Näslund E, Ylä-Herttuala S, et al. 2016. Endothelial PDGF-CC regulates angiogenesis-dependent thermogenesis in beige fat. Nature Communications 7:12152.

Semple RK, Chatterjee VK, O'Rahilly S. 2006. PPAR gamma and human metabolic disease. J Clin Invest 116(0021-9738; 3):581-9.

Serra V, Grune T, Sitte N, Saretzki G, von Zglinicki T. 2000. Telomere length as a marker of oxidative stress in primary human fibroblast cultures. Ann N Y Acad Sci 908:327-30.

Sesti G. 2006. Pathophysiology of insulin resistance. Best Practice & Research Clinical Endocrinology & Metabolism 20(4):665-79.

Shan Z, Ma H, Xie M, Yan P, Guo Y, Bao W, Rong Y, Jackson CL, Hu FB, Liu L. 2015. Sleep duration and risk of type 2 diabetes: A meta-analysis of prospective studies. Diabetes Care 38(3):529.

Shanik MH, Xu Y, Škrha J, Dankner R, Zick Y, Roth J. 2008. Insulin resistance and hyperinsulinemia. Diabetes Care 31:S262.

She P, Van Horn C, Reid T, Hutson SM, Cooney RN, Lynch CJ. 2007. Obesity-related elevations in plasma leucine are associated with alterations in enzymes involved in branched-chain amino acid metabolism. Am J Physiol Endocrinol Metab 293(6):E1552-63.

Shen SW, Reaven GM, Farquhar JW. 1970. Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. J Clin Invest 49(12):2151-60.

Sheng B, Truong K, Spitler H, Zhang L, Tong X, Chen L. 2017. The long-term effects of bariatric surgery on type 2 diabetes remission, microvascular and macrovascular complications, and mortality: A systematic review and meta-analysis. Obesity Surg 27(10):2724-32.

Shrayyef MZ and Gerich JE. 2010. Normal glucose homeostasis. In: Principles of diabetes mellitus. Poretsky L, editor. 2nd ed. New York, NY, United States: Springer US. p. 19-35.

Sjöström L, Peltonen M, Jacobson P, Ahlin S, Andersson-Assarsson J, Anveden A, Bouchard C, Carlsson B, Karason K, Lonroth

H, et al. 2014. Association of bariatric surgery with long-term remission of type 2 diabetes and with microvascular and macrovascular complications. Jama 311(22):2297-304.

Small KS, Todorčević M, Civelek M, El-Sayed Moustafa JS, Wang X, Simon MM, Fernandez-Tajes J, Mahajan A, Horikoshi M, Hugill A, et al. 2018. Regulatory variants at KLF14 influence type 2 diabetes risk via a female-specific effect on adipocyte size and body composition. Nat Genet 50(4):572-80.

Soininen P, Kangas AJ, Wurtz P, Suna T, Ala-Korpela M. 2015. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circ Cardiovasc Genet 8(1):192-206.

Soininen P, Kangas AJ, Würtz P, Tukiainen T, Tynkkynen T, Laatikainen R, Järvelin MR, Kähönen M, Lehtimäki T, Viikari J, et al. 2009. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. Analyst 134(9):1781-5.

Solinas G and Becattini B. 2017. JNK at the crossroad of obesity, insulin resistance, and cell stress response. Mol Metab 6(2):174-84.

Solomon TP, Knudsen SH, Karstoft K, Winding K, Holst JJ, Pedersen BK. 2012. Examining the effects of hyperglycemia on pancreatic endocrine function in humans: Evidence for in vivo glucotoxicity. J Clin Endocrinol Metab 97(12):4682-91.

Sprague JE and Arbeláez AM. 2011. Glucose counterregulatory responses to hypoglycemia. Pediatr Endocrinol Rev 9(1):463-75.

Srikanthan P and Karlamangla AS. 2011. Relative muscle mass is inversely associated with insulin resistance and prediabetes. findings from the third national health and nutrition examination survey. J Clin Endocrinol Metab 96(9):2898-903.

Srisawat K, Shepherd SO, Lisboa PJ, Burniston JG. 2017. A systematic review and meta-analysis of proteomics literature on the response of human skeletal muscle to obesity/type 2 diabetes mellitus (T2DM) versus exercise training. Proteomes 5(4):10.3390/proteomes5040030.

Stančáková A, Javorský M, Kuulasmaa T, Haffner SM, Kuusisto J, Laakso M. 2009. Changes in insulin sensitivity and insulin release in relation to glycemia and glucose tolerance in 6,414 finnish men. Diabetes 58(5):1212-21.

Stancakova A and Laakso M. 2016. Genetics of type 2 diabetes. Endocr Dev 31:203-20.

Stanford KI and Goodyear LJ. 2014. Exercise and type 2 diabetes: Molecular mechanisms regulating glucose uptake in skeletal muscle. Adv Physiol Educ 38(4):308-14.

Stender S, Smagris E, Lauridsen BK, Kofoed KF, Nordestgaard B, Tybjærg-Hansen A, Pennacchio LA, Dickel DE, Cohen JC, Hobbs HH. 2018. Relationship between genetic variation at PPP1R3B and levels of liver glycogen and triglyceride. Hepatology 67(6):2182-95.

Stern SE, Williams K, Ferrannini E, DeFronzo RA, Bogardus C, Stern MP. 2005. Identification of individuals with insulin resistance using routine clinical measurements. Diabetes 54(2):333-9.

Stolic M, Russell A, Hutley L, Fielding G, Hay J, MacDonald G, Whitehead J, Prins J. 2002. Glucose uptake and insulin action in human adipose tissue--influence of BMI, anatomical depot and body fat distribution. Int J Obes Relat Metab Disord 26(1):17-23.

Stuart CA, McCurry MP, Marino A, South MA, Howell MEA, Layne AS, Ramsey MW, Stone MH. 2013. Slow-twitch fiber proportion in skeletal muscle correlates with insulin responsiveness. The Journal of Clinical Endocrinology & Metabolism 98(5):2027-36.

Stumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Järvinen H, Van Haeften T, Renn W, Gerich J. 2000. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care 23(3):295-301.

Sun L, Xie C, Wang G, Wu Y, Wu Q, Wang X, Liu J, Deng Y, Xia J, Chen B, et al. 2018. Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. Nat Med 24(12):1919-29.

Swerdlow DI, Preiss D, Kuchenbaecker KB, Holmes MV, Engmann JE, Shah T, Sofat R, Stender S, Johnson PC, Scott RA, et al. 2015. HMG-coenzyme A reductase inhibition, type 2 diabetes, and bodyweight: Evidence from genetic analysis and randomised trials. Lancet 385(9965):351-61.

Tabák A,G., Jokela M, Akbaraly TN, Brunner EJ, Kivimäki M, Witte DR. 2009. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: An analysis from the whitehall II study. The Lancet 373(9682):2215-21.

Tao W, Rong Z, Xiaojing M, Shiyun W, Zhen H, Yeping H, Bo X, Yangyang L, Hong Z, Feng J, et al. 2018. Causal association of overall obesity and abdominal obesity with type 2 diabetes: A mendelian randomization analysis. Obesity 26(5):934-42.

Taskinen M and Borén J. 2015. New insights into the pathophysiology of dyslipidemia in type 2 diabetes. Atherosclerosis 239(2):483-95.

Tchobroutsky G, Kopf A, Eschwege E, Assan R. 1973. Serial postprandial plasma insulin levels in 117 subjects with and without diabetes. Diabetes 22(11):825.

ter Horst K,W., Schene MR, Holman R, Romijn JA, Serlie MJ. 2016. Effect of fructose consumption on insulin sensitivity in nondiabetic subjects: A systematic review and meta-analysis of diet-intervention trials. Am J Clin Nutr 104(6):1562-76.

Ter Horst KW, Gilijamse PW, de Weijer BA, Kilicarslan M, Ackermans MT, Nederveen AJ, Nieuwdorp M, Romijn JA, Serlie MJ. 2015. Sexual dimorphism in hepatic, adipose tissue, and peripheral tissue insulin sensitivity in obese humans. Front Endocrinol (Lausanne) 6:182.

Teusink B, Voshol PJ, Dahlmans VE, Rensen PC, Pijl H, Romijn JA, Havekes LM. 2003. Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. Diabetes 52(3):614-20.

The Human Protein Atlas project. The Human Protein Atlas [Internet]; c2018 [cited 2018 07/16]. Available from: v18.proteinatlas.org/ .

Thie JA. 1995. Clarification of a fractional uptake concept. J Nucl Med 36(4):711-2.

Tibshirani R. 1996. Regression shrinkage and selection via the lasso. J R Stat Soc Ser B-Methodol 58(1):267-88.

Tillil H, Shapiro ET, Miller MA, Karrison T, Frank BH, Galloway JA, Rubenstein AH, Polonsky KS. 1988. Dose-dependent effects of oral and intravenous glucose on insulin secretion and clearance in normal humans. American Journal of Physiology-Endocrinology and Metabolism 254(3):E349-57.

Tinahones FJ, Coín Aragüez L, Murri M, Oliva Olivera W, Mayas Torres MD, Barbarroja N, Gomez Huelgas R, Malagón M,M., El Bekay R. 2012. Caspase induction and BCL2 inhibition in human adipose tissue: A potential relationship with insulin signaling alteration. Diabetes Care 36(3):513-21.

Titchenell PM, Quinn WJ, Lu M, Chu Q, Lu W, Li C, Chen H, Monks BR, Chen J, Rabinowitz JD, et al. 2016. Hepatocyte insulin signaling is required for feeding-induced lipogenesis but dispensable for the suppression of glucose production. Cell Metab 23(6):1154-66.

Tobin V, Le Gall M, Fioramonti X, Stolarczyk E, Blazquez AG, Klein C, Prigent M, Serradas P, Cuif MH, Magnan C, et al. 2008. Insulin internalizes GLUT2 in the enterocytes of healthy but not insulin-resistant mice. Diabetes 57(3):555-62.

Tönjes A, Scholz M, Loeffler M, Stumvoll M. 2006. Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma with pre-diabetic phenotypes: Meta-analysis of 57 studies on nondiabetic individuals. Diabetes Care 29(0149-5992; 11):2489-97.

Torrallardona D, Harris CI, Fuller MF. 2003. Pigs' gastrointestinal microflora provide them with essential amino acids. J Nutr 133(4):1127-31.

Tschritter O, Fritsche A, Stefan N, Haap M, Thamer C, Bachmann O, Dahl D, Maerker E, Teigeler A, Machicao F, et al. 2003. Increased insulin clearance in peroxisome proliferator-activated receptor gamma2 Pro12Ala. Metabolism 52(0026-0495; 6):778-83.

Tuomilehto J and Schwarz PEH. 2016. Preventing diabetes: Early versus late preventive interventions. Diabetes Care 39:S115.

Tura A, Chemello G, Szendroedi J, Gobl C, Faerch K, Vrbikova J, Pacini G, Ferrannini E, Roden M. 2018. Prediction of clamp-derived insulin sensitivity from the oral glucose insulin sensitivity index. Diabetologia 61(5):1135-41.

Tuulari JJ, Karlsson HK, Hirvonen J, Hannukainen JC, Bucci M, Helmio M, Ovaska J, Soinio M, Salminen P, Savisto N, et al. 2013. Weight loss after bariatric surgery reverses insulin-induced increases in brain glucose metabolism of the morbidly obese. Diabetes 62(8):2747-51.

U Din M, Saari T, Raiko J, Kudomi N, Maurer SF, Lahesmaa M, Fromme T, Amri E, Klingenspor M, Solin O, et al. 2018. Postprandial oxidative metabolism of human brown fat indicates thermogenesis. Cell Metabolism 28(2):207,216.e3.

Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, et al. 2015. Tissue-based map of the human proteome. Science 347(6220).

Uziel O, Singer JA, Danicek V, Sahar G, Berkov E, Luchansky M, Fraser A, Ram R, Lahav M. 2007. Telomere dynamics in arteries and mononuclear cells of diabetic patients: Effect of diabetes and of glycemic control. Exp Gerontol 42(10):971-8.

Vaag A, Damsbo P, Hother-Nielsen O, Beck-Nielsen H. 1992. Hyperglycaemia compensates for the defects in insulin-mediated glucose metabolism and in the activation of glycogen synthase in the skeletal muscle of patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 35(1):80-8.

van den Berg R, Kooijman S, Noordam R, Ramkisoensing A, Abreu-Vieira G, Tambyrajah LL, Dijk W, Ruppert P, Mol IM, Kramar B, et al. 2018. A diurnal rhythm in brown adipose tissue causes rapid clearance and combustion of plasma lipids at wakening. Cell Rep 22(13):3521-33.

van den Hoff J, Oehme L, Schramm G, Maus J, Lougovski A, Petr J, Beuthien-Baumann B, Hofheinz F. 2013. The PET-derived tumor-to-blood standard uptake ratio (SUR) is superior to tumor SUV as a surrogate parameter of the metabolic rate of FDG. EJNMMI Res 3(1):77,219X-3-77.

van der Berg JD, Stehouwer CD, Bosma H, van der Velde JH, Willems PJ, Savelberg HH, Schram MT, Sep SJ, van der Kallen CJ, Henry RM, et al. 2016. Associations of total amount and patterns of sedentary behaviour with type 2 diabetes and the metabolic syndrome: The maastricht study. Diabetologia 59(4):709-18.

van Hall G. 2010. Lactate kinetics in human tissues at rest and during exercise. Acta Physiol (Oxf) 199(4):499-508.

Vänttinen M, Nuutila P, Pihlajamäki J, Hällsten K, Virtanen KA, Lautamäki R, Peltoniemi P, Kemppainen J, Takala T, Viljanen AP, et al. 2005. The effect of the Ala12 allele of the peroxisome proliferator-activated receptor-gamma2 gene on skeletal muscle glucose uptake depends on obesity: A positron emission tomography study. J Clin Endocrinol Metab 90(7):4249-54.

Varghese RT, Dalla Man C, Sharma A, Viegas I, Barosa C, Marques C, Shah M, Miles JM, Rizza RA, Jones JG, et al. 2016. Mechanisms underlying the pathogenesis of isolated impaired glucose tolerance in humans. The Journal of Clinical Endocrinology & Metabolism :jc.2016-1998.

Vella A and Rizza RA. 2009. Application of isotopic techniques using constant specific activity or enrichment to the study of carbohydrate metabolism. Diabetes 58(10):2168-74.

Vella A, Shah P, Basu R, Basu A, Holst JJ, Rizza RA. 2000. Effect of glucagon-like peptide 1(7-36) amide on glucose effectiveness and insulin action in people with type 2 diabetes. Diabetes 49(4):611.

Vendelbo MH, Clasen BFF, Treebak JT, Møller L, Krusenstjerna-Hafstrøm T, Madsen M, Nielsen TS, Stødkilde-Jørgensen H, Pedersen SB, Jørgensen JOL, et al. 2012. Insulin resistance after a 72-h fast is associated with impaired AS160 phosphorylation and accumulation of lipid and glycogen in human skeletal muscle. Am J Physiol Endocrinol Metab 302(2):E190-200.

Verhulst S, Dalgård C, Labat C, Kark JD, Kimura M, Christensen K, Toupance S, Aviv A, Kyvik KO, Benetos A. 2016. A short leucocyte telomere length is associated with development of insulin resistance. Diabetologia 59(6):1258-65.

Veronese N, Stubbs B, Fontana L, Trevisan C, Bolzetta F, De Rui M, Sartori L, Musacchio E, Zambon S, Maggi S, et al. 2016. Frailty is associated with an increased risk of incident type 2 diabetes in the elderly. J Am Med Dir Assoc 17(10):902-7.

Vessby B, Aro A, Skarfors E, Berglund L, Salminen I, Lithell H. 1994. The risk to develop NIDDM is related to the fatty acid composition of the serum cholesterol esters. Diabetes 43(11):1353-7.

Viljanen AP, Lautamäki R, Järvisalo M, Parkkola R, Huupponen R, Lehtimäki T, Rönnemaa T, Raitakari OT, Iozzo P, Nuutila P. 2009. Effects of weight loss on visceral and abdominal subcutaneous adipose tissue blood-flow and insulin-mediated glucose uptake in healthy obese subjects. Ann Med 41(2):152-60.

Viljanen AP, Virtanen KA, Järvisalo MJ, Hällsten K, Parkkola R, Rönnemaa T, Lönnqvist F, Iozzo P, Ferrannini E, Nuutila P. 2005. Rosiglitazone treatment increases subcutaneous adipose tissue glucose uptake in parallel with perfusion in patients with type 2 diabetes: A double-blind, randomized study with metformin. J Clin Endocrinol Metab 90(12):6523-8.

Viljanen AP, Iozzo P, Borra R, Kankaanpää M, Karmi A, Lautamäki R, Järvisalo M, Parkkola R, Rönnemaa T, Guiducci L, et al. 2009. Effect of weight loss on liver free fatty acid uptake and hepatic insulin resistance. J Clin Endocrinol Metab 94(1):50-5.

Vind BF, Birk JB, Vienberg SG, Andersen B, Beck-Nielsen H, Wojtaszewski JF, Hojlund K. 2012. Hyperglycaemia normalises insulin action on glucose metabolism but not the impaired activation of AKT and glycogen synthase in the skeletal muscle of patients with type 2 diabetes. Diabetologia 55(5):1435-45.

Virtanen KA, Peltoniemi P, Marjamäki P, Asola M, Strindberg L, Parkkola R, Huupponen

R, Knuuti J, Lönnroth P, Nuutila P. 2001. Human adipose tissue glucose uptake determined using [(18)F]-fluoro-deoxy-glucose ([(18)F]FDG) and PET in combination with microdialysis. Diabetologia 44(0012-186; 12):2171-9.

Virtanen KA, Hällsten K, Parkkola R, Janatuinen T, Lönnqvist F, Viljanen T, Rönnemaa T, Knuuti J, Huupponen R, Lönnroth P, et al. 2003. Differential effects of rosiglitazone and metformin on adipose tissue distribution and glucose uptake in type 2 diabetic subjects. Diabetes 52(2):283-90.

Virtanen KA, Lönnroth P, Parkkola R, Peltoniemi P, Asola M, Viljanen T, Tolvanen T, Knuuti J, Rönnemaa T, Huupponen R, et al. 2002. Glucose uptake and perfusion in subcutaneous and visceral adipose tissue during insulin stimulation in nonobese and obese humans. J Clin Endocrinol Metab 87(8):3902-10.

Vitola BE, Deivanayagam S, Stein RI, Mohammed BS, Magkos F, Kirk EP, Klein S. 2009. Weight loss reduces liver fat and improves hepatic and skeletal muscle insulin sensitivity in obese adolescents. Obesity 17(9):1744-8.

Vovk U, Pernus F, Likar B. 2006. Intensity inhomogeneity correction of multispectral MR images. Neuroimage 32(1):54-61.

Walford GA, Gustafsson S, Rybin D, Stančáková A, Chen H, Liu C, Hong J, Jensen RA, Rice K, Morris AP, et al. 2016. Genome-wide association study of the modified stumvoll insulin sensitivity index identifies *BCL2* and *FAM19A2* as novel insulin sensitivity loci. Diabetes 65(10):3200.

Wan M, Leavens KF, Saleh D, Easton RM, Guertin DA, Peterson TR, Kaestner KH, Sabatini DM, Birnbaum MJ. 2011. Postprandial hepatic lipid metabolism requires signaling through Akt2 independent of the transcription factors FoxA2, FoxO1, and SREBP1c. Cell Metab 14(4):516-27.

Wan M, Leavens KF, Hunter RW, Koren S, von Wilamowitz-Moellendorff A, Lu M, Satapati S, Chu Q, Sakamoto K, Burgess SC, et al. 2013. A noncanonical, GSK3-independent pathway controls postprandial hepatic glycogen deposition. Cell Metab 18(1):99-105.

Wang H and Peng DQ. 2011. New insights into the mechanism of low high-density lipoprotein cholesterol in obesity. Lipids Health Dis 10:176,511X-10-176.

Wang J, Stancakova A, Soininen P, Kangas AJ, Paananen J, Kuusisto J, Ala-Korpela M, Laakso M. 2012. Lipoprotein subclass profiles in individuals with varying degrees of glucose tolerance: A population-based study of 9399 finnish men. J Intern Med 272(6):562-72.

Wang L, Folsom AR, Zheng ZJ, Pankow JS, Eckfeldt JH, ARIC Study Investigators. 2003. Plasma fatty acid composition and incidence of diabetes in middle-aged adults: The atherosclerosis risk in communities (ARIC) study. Am J Clin Nutr 78(1):91-8.

Wang Q, Holmes MV, Davey Smith G, Ala-Korpela M. 2017. Genetic support for a causal role of insulin resistance on circulating branched-chain amino acids and inflammation. Diabetes Care .

Wang T, Ma X, Tang T, Jin L, Peng D, Zhang R, Chen M, Yan J, Wang S, Yan D, et al. 2016. Overall and central obesity with insulin sensitivity and secretion in a han chinese population: A mendelian randomization analysis. Int J Obes 40:1736.

Wang Y, Parlevliet ET, Geerling JJ, van der Tuin SJ, Zhang H, Bieghs V, Jawad AH, Shiri-Sverdlov R, Bot I, de Jager SC, et al. 2014. Exendin-4 decreases liver inflammation and atherosclerosis development simultaneously by reducing macrophage infiltration. Br J Pharmacol 171(3):723-34.

Wang Y, van dT, Tjeerdema N, van Dam AD, Rensen SS, Hendrikx T, Berbée JFP, Atanasovska B, Fu J, Hoekstra M, et al. 2015. Plasma cholesteryl ester transfer protein is predominantly derived from kupffer cells. Hepatology 62(6):1710-22.

Wardhana DA, Ikeda K, Barinda AJ, Nugroho DB, Qurania KR, Yagi K, Miyata K, Oike Y, Hirata KI, Emoto N. 2018. Family with sequence similarity 13, member A modulates adipocyte insulin signaling and preserves systemic metabolic homeostasis. Proc Natl Acad Sci U S A 115(7):1529-34.

Warnes GR, Bolker B, Bonebakker L, Gentleman R, Liaw WHA, Lumley T, Maechler M, Magnusson A, Moeller S, Schwartz M, et al. 2016. Gplots: Various R programming tools for plotting data. R package version 3.0.1. CRAN.

Wasenius NS, Simonen M, Penttinen L, Salonen MK, Sandboge S, Eriksson JG. 2018. Effect of maternal weight during pregnancy on offspring muscle strength response to resistance training in late adulthood. Adv Med Sci 63(2):353-8.

Wasserman DH. 2009. Four grams of glucose. Am J Physiol Endocrinol Metab 296(1):E11-21.

Way KL, Hackett DA, Baker MK, Johnson NA. 2016. The effect of regular exercise on insulin sensitivity in type 2 diabetes mellitus: A

systematic review and meta-analysis. Diabetes Metab J 40(4):253-71.

Wefers J, van Moorsel D, Hansen J, Connell NJ, Havekes B, Hoeks J, van ML, Duez H, Phielix E, Kalsbeek A, et al. 2018. Circadian misalignment induces fatty acid metabolism gene profiles and compromises insulin sensitivity in human skeletal muscle. Proc Natl Acad Sci USA 115(30):7789.

Weickert MO, Möhlig M, Schöfl C, Arafat AM, Otto B, Viehoff H, Koebnick C, Kohl A, Spranger J, Pfeiffer AFH. 2006. Cereal fiber improves whole-body insulin sensitivity in overweight and obese women. Diabetes Care 29(4):775.

WHO and IDF Technical Advisory Group. 2006. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: Report of a WHO/IDF consultation. Geneva, Switzerland: World Health Organization.

Wiberg B, Sundström J, Zethelius B, Lind L. 2009. Insulin sensitivity measured by the euglycaemic insulin clamp and proinsulin levels as predictors of stroke in elderly men. Diabetologia 52(1):90-6.

Wilcox R. 2011. Introduction to robust estimation and hypothesis testing. 3rd ed. Cambridge, Massachusetts, USA: Academic Press.

Willemsen G, Ward KJ, Bell CG, Christensen K, Bowden J, Dalgard C, Harris JR, Kaprio J, Lyle R, Magnusson PK, et al. 2015. The concordance and heritability of type 2 diabetes in 34,166 twin pairs from international twin registers: The discordant twin (DISCOTWIN) consortium. Twin Res Hum Genet 18(6):762-71.

World Health Organization. Cardiovascular diseases (CVDs) [Internet]; c2018a [cited 2018 09/25]. Available from: http://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) .

World Health Organization. Obesity and overweight [Internet]; c2018b [cited 2018 03/13]. Available from: http://www.who.int/mediacentre/factsheets/fs311/en/ .

Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Manneras-Holm L, Stahlman M, Olsson LM, Serino M, Planas-Felix M, et al. 2017. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nat Med 23(7):850-8.

Wu Z, Lou Y, Jin W, Liu Y, Lu L, Lu G. 2012. The Pro12Ala polymorphism in the peroxisome proliferator-activated receptor gamma-2 gene (PPARγ2) is associated with increased risk of coronary artery disease: A meta-analysis. PLoS One 7(12):e53105. doi:10.1371/journal.pone.0053105.

Yang T and Soodvilai S. 2008. Renal and vascular mechanisms of thiazolidinedione-induced fluid retention. PPAR Research 2008:943614.

Yang YJ, Youn JH, Bergman RN. 1987. Modified protocols improve insulin sensitivity estimation using the minimal model. Am J Physiol 253(6 Pt 1):E595-602.

Yecies JL, Zhang HH, Menon S, Liu S, Yecies D, Lipovsky AI, Gorgun C, Kwiatkowski DJ, Hotamisligil GS, Lee CH, et al. 2011. Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and independent pathways. Cell Metab 14(1):21-32.

Yki-Järvinen H and Koivisto VA. 1983. Effects of body composition on insulin sensitivity. Diabetes 32(10):965-9.

Yu Q, Chen Y, Xu C. 2017. Statins and new-onset diabetes mellitus: LDL receptor may provide a key link. Frontiers in Pharmacology 8:372; 372-372.

Yu Z, Han S, Zhu J, Sun X, Ji C, Guo X. 2013. Pre-pregnancy body mass index in relation to infant birth weight and offspring overweight/obesity: A systematic review and meta-analysis. PLoS One 8(4):e61627.

Zaccagna F, Grist JT, Deen SS, Woitek R, Lechermann LM, McLean MA, Basu B, Gallagher FA. 2018. Hyperpolarized carbon-13 magnetic resonance spectroscopic imaging: A clinical tool for studying tumour metabolism. Br J Radiol 91(1085):20170688.

Zander M, et al. 2002. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: A parallel-group study. The Lancet 359(9309):824-30.

Zethelius B, Lithell H, Hales CN, Berne C. 2005. Insulin sensitivity, proinsulin and insulin as predictors of coronary heart disease. A population-based 10-year, follow-up study in 70-year old men using the euglycaemic insulin clamp. Diabetologia 48(5):862-7.

Zhang F, Tong Y, Su N, Li Y, Tang L, Huang L, Tong N. 2015. Weight loss effect of glucagon-like peptide-1 mimetics on obese/overweight adults without diabetes: A systematic review and meta-analysis of randomized controlled trials. J Diabetes 7(3):329-39.

Zhang M, Hu T, Zhang S, Zhou L. 2015. Associations of different adipose tissue depots

with insulin resistance: A systematic review and meta-analysis of observational studies. Sci Rep 5:18495.

Zhao J, Wang M, Deng W, Zhong D, Jiang Y, Liao Y, Chen B, Zhang X. 2017. ADP-ribosylation factor-like GTPase 15 enhances insulin-induced AKT phosphorylation in the IR/IRS1/AKT pathway by interacting with ASAP2 and regulating PDPK1 activity. Biochem Biophys Res Commun 486(4):865-71.

Zhao J, Zhu Y, Lin J, Matsuguchi T, Blackburn E, Zhang Y, Cole SA, Best LG, Lee ET, Howard BV. 2014. Short leukocyte telomere length predicts risk of diabetes in american indians: The strong heart family study. Diabetes 63(1):354-62.

Zhao L, Zhang F, Ding X, Wu G, Lam YY, Wang X, Fu H, Xue X, Lu C, Ma J, et al. 2018. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. Science 359(6380):1151.

Zhou Y, Llaurado G, Orešič M, Hyötyläinen T, Orho-Melander M, Yki-Järvinen H. 2015. Circulating triacylglycerol signatures and insulin sensitivity in NAFLD associated with the E167K variant in TM6SF2. J Hepatol 62(3):657-63.

Zhu L, Luu T, Emfinger CH, Parks BA, Shi J, Trefts E, Zeng F, Kuklenyik Z, Harris RC, Wasserman DH, et al. 2018. CETP inhibition improves HDL function but leads to fatty liver and insulin resistance in CETP-expressing transgenic mice on a high-fat diet. Diabetes 67(12):2494-506.

Zierler KL. 1961. Theory of the use of arteriovenous concentration differences for measuring metabolism in steady and non-steady states. J Clin Invest 40(12):2111-25.

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