UNIVERSITY OF TURKU

EFFECT OF BARIATRIC SURGERY ON LIVER METABOLISM

Studies using positron emission tomography

Heidi Immonen

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ABSTRACT

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EFFECT OF BARIATRIC SURGERY ON LIVER METABOLISM
Studies using positron emission tomography

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Obesity is a significant and increasing health challenge. Obesity is strongly associated with the incidence of type 2 diabetes (T2D). In obese individuals, liver insulin resistance is a major factor in the development and pathophysiology of T2D. Currently, bariatric surgery is the most effective therapy for morbidly obese patients. The aim of this thesis was to study the effects of surgery-induced weight loss on glucose and lipid metabolism in the liver, and to understand the beneficial effects of bariatric surgery on fatty liver, insulin resistance and T2D. Multimodality imaging by positron emission tomography (PET) combined with magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) were utilized to study brain glucose uptake (BGU) and hepatic glucose uptake (HGU), hepatic fatty acid uptake, liver blood flow and liver fat content in morbidly obese patients (n=46) with and without T2D before and six-months after surgery (either sleeve gastrectomy (SG) or Roux-en-Y gastric bypass (RYGB)). The results were then compared to healthy subjects.

Preoperatively, insulin-stimulated HGU was lower in obese patients before surgery as compared to the non-obese controls. HGU increased by 30-40% after surgery as compared to the preoperative situation. Postoperatively, liver fat and endogenous glucose production (EGP) were comparable to lean controls. Before surgery, the liver fatty acid uptake was increased in obese subjects as compared to lean controls and associated with body adiposity. Liver fatty acid uptake decreased after surgery but was still high compared to controls. Portal blood flow (per volume of tissue) was increased after surgery, but as the liver volume decreased along with the surgery-induced weight loss, the whole organ blood flow was unchanged. A positive association between BGU and EGP during insulin stimulation was found in obese subjects and the association persisted after surgery. High BGU at baseline predicted a smaller improvement in fasting plasma glucose at a 2 and 3 year follow-up.

The results of this study suggest that bariatric surgery, either SG and RYGB, is effective in improving hepatic glucose metabolism. The persistence of high fatty acid uptake, despite a normal fat content in the liver, suggests a change in the use of fatty acids from storage to oxidation after surgery. Accelerated portal blood flow may relate to improved liver metabolism after surgery. Moreover, these findings suggest the presence of a brain-liver axis in morbidly obese individuals. This axis might contribute to further deterioration of glucose homeostasis.

Keywords: fatty liver, hepatic glucose uptake, endogenous glucose production, liver fatty acid uptake, liver blood flow, bariatric surgery, positron emission tomography

TIIVISTELMÄ

Heidi Immonen

LIHAVUUSLEIKKAUKSEN VAIKUTUS MAKSAN AINEENVAIHDUNTAAN
Tutkimuksia positroniemissiotomografiaa käyttäen

Turun yliopisto, Lääketieteellinen tiedekunta, Sisätautioppi, Turun kliininen tohtoriohjelma, Valtakunnallinen PET-keskus, Turun yliopisto ja Turun yliopistollinen keskussairaala

Lihavuus on merkittävä ja paheneva terveysongelma. Tyypin 2 diabeteksen (T2D) ilmaantuvuus on yhteydessä lihavuuteen. Maksan insuliiniresistenssi vaikuttaa ratkaisevasti T2D:n kehittymiseen lihavilla henkilöillä. Lihavuusleikkaus on nykyisin tehokkain lihavuuden hoitokeino. Väitöskirjan tarkoitus oli tutkia lihavuusleikkauksen jälkeisen painonlaskun vaikutusta maksan glukoosi- ja rasva-aineenvaihduntaan ja ymmärtää lihavuusleikkauksen vaikutusta rasvamaksaan, insuliiniresistenssiin ja T2D:een. Positroniemissiotomografia (PET), magneettiresonanssi (MRI) ja magneettispektroskopia (MRS) -kuvantamisen avulla tutkittiin aivojen ja maksan glukoosin soluunottoa, maksan rasvahappojen soluunottoa, maksan verenkiertoa ja maksan kokoa ja rasvapitoisuutta lihavilla ennen lihavuusleikkausta ja kuusi kuukautta leikkauksen (joko mahalaukun kavennusleikkaus tai mahalaukun ohitusleikkaus) jälkeen. Tutkimuksessa käytettiin terveitä, normaalipainoisia verrokkiryhmänä.

Ennen leikkausta maksan glukoosin soluunottokyky oli heikentynyt lihavilla ja se parani 30-40%:lla lihavuusleikkauksen jälkeen leikkausta edeltäneeseen tilanteeseen verrattuna. Leikkauksen jälkeen maksan rasvapitoisuus ja maksan glukoosin tuotanto vähenivät terveiden, normaalipainoisten verrokkien tasolle. Maksan rasvahappojen soluunotto oli lisääntynyt lihavilla ennen leikkausta ja se oli yhteydessä kehon rasvapitoisuuteen. Leikkauksen jälkeen rasvahappojen soluunotto väheni jonkin verran, mutta ei normaalistunut. Maksan porttilaskimon verenvirtaus (ml/min/ml) lisääntyi lihavuusleikkauksen jälkeen, mutta koska maksan koko pieneni leikkauksen jälkeen ei koko elimen verenvirtaus muuttunut. Lihavilla aivojen ja maksan glukoosin soluunotto olivat yhteydessä toisiinsa, ja yhteys säilyi lihavuusleikkauksen jälkeen. Leikkausta edeltänyt lisääntynyt aivojen glukoosin soluunotto ennusti vähäisempää paastoglukoosin laskua 2 ja 3 vuoden kuluttua leikkauksesta.

Tutkimuksen perusteella lihavuusleikkaus parantaa tehokkaasti maksan aineenvaihduntaa. Vaikka rasvamaksa paranee leikkauksen jälkeen, pysyy maksan rasvahappojen soluunotto lisääntyneenä, mikä viittaa siihen, että rasvahapot käytetään energian tuotantoon sen sijaan että ne varastoitaisiin. Lisääntynyt porttilaskimon verenvirtaus saattaa olla yhteydessä parantuneeseen maksan aineenvaihduntaan leikkauksen jälkeen. Lisäksi tulokset viittaavat aivo-maksa -akselin olemassaoloon. Sillä voi olla merkitystä elimistön glukoosiaineenvaihdunnan heikkenemisessä.

Avainsanat: rasvamaksa, maksan glukoosin soluunotto, endogeeninen glukoosin tuotanto, maksan rasvahappojen soluunotto, maksan verenvirtaus, lihavuusleikkaus, positroniemissiotomografia

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ABBREVIATIONS

ALT	Alanine transferase
ATP	Adenosine triphosphate
BGU	Brain glucose uptake
BMI	Body-mass index
EASL	European Association for the Study of the Liver
EGP	Endogenous glucose production
FDR	False discovery rate
FFA	Free fatty acid
^{18}F-FDG	2-[^{18}F]fluoro-2-deoxy-*D*-glucose
^{18}F-FTHA	14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid
FUR	Fractional uptake rates
FXR	Farnesoid X receptor
GGT	Gamma-glutamyltransferase
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
HbA_{1c}	Glycosylated haemoglobin
HDL	High density lipoprotein
HGU	Hepatic glucose uptake
HOMA-IR	Homeostasis model assessment of insulin resistance
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
LC	Lumped constant
LFC	Liver fat content
MHO	Metabolically healthy obesity
MRS	Magnetic resonance spectroscopy
MUO	Metabolically unhealthy obesity
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
OGIS	Oral glucose insulin sensitivity
OGTT	Oral glucose tolerance test

^{15}O-H_2O	^{15}O-water
PET	Positron emission tomography
PYY	Peptide YY
ROI	Region-of-interest
ROS	Reactive oxygen species
RYGB	Roux-en-Y gastric bypass
SD	Standard deviation
SG	Sleeve gastrectomy
SOS	Swedish Obese Subjects
SPM	Statistical parametric mapping
T2D	Type 2 diabetes
TG	Triglyceride
TNF-α	Tumor necrosis factor alpha
UAG	Unacylated ghrelin
US	Ultrasound
VLCD	Very low calorie diet
VLDL	Very low density lipoprotein

LIST OF ORIGINAL PUBLICATIONS

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

I. Immonen, H., Hannukainen, J.C., Iozzo, P., Soinio, M., Salminen, P., Saunavaara, V., Borra, R., Parkkola, R., Mari, A., Lehtimaki, T., Pham, T., Laine, J., Karja, V., Pihlajamaki, J., Nelimarkka, L. & Nuutila, P. 2014, "Effect of bariatric surgery on liver glucose metabolism in morbidly obese diabetic and non-diabetic patients", Journal of Hepatology, vol. 60, no. 2, pp. 377-383.

II. Immonen, H., Hannukainen, J.C., Kudomi, N., Pihlajamaki, J., Saunavaara, V., Laine, J., Salminen, P., Lehtimaki, T., Pham, T., Iozzo, P. & Nuutila, P. 2018, "Increased Liver Fatty Acid Uptake Is Partly Reversed and Liver Fat Content Normalized After Bariatric Surgery", Diabetes Care, vol. 41, no. 2, pp. 368-371.

III. Rebelos, E., Immonen, H., Bucci, M., Hannukainen, J.C., Nummenmaa, L., Honka, M.J., Soinio, M., Salminen, P., Ferrannini, E., Iozzo, P. & Nuutila, P. 2019, "Brain glucose uptake is associated with endogenous glucose production in obese patients before and after bariatric surgery and predicts metabolic outcome at follow-up", Diabetes, Obesity & Metabolism, vol. 21, no. 2, pp. 218-226.

1 INTRODUCTION

Obesity is a significant and increasing public health challenge. Together with the global obesity epidemic, incidence of morbid obesity (body mass index, BMI ≥40 kg/m^2) has increased progressively during the past decades (Lahti-Koski M et al., 2010, Swinburn et al., 2011, Flegal et al., 2016). Obesity results in increased risk of having other diseases such as type 2 diabetes (T2D), fatty liver, high blood pressure, metabolic syndrome, coronary heart disease, stroke, obstructive sleep apnoea, gout, gallstones, knee arthrosis and cancer (postmenopausal breast cancer, cervical cancer, colon cancer and kidney cancer) (Guh et al., 2009). The prevalence of obesity (BMI ≥30 kg/m^2) in the US in 2013-2014 was 35% among men and 40% among women. The corresponding prevalence of morbid obesity (BMI ≥40 kg/m^2) was 5.5% among men and 9.9% among women (Flegal et al., 2016). In Finland 26.1% of men and 27.5% of women are obese (BMI ≥30 kg/m^2) (Koponen et al., 2018).

Obesity is strongly parallel to the incidence of T2D (Vazquez et al., 2007, Guh et al., 2009). Insulin resistance in the liver is a key factor in the development of T2D in obese individuals. The liver has a major role in controlling systemic glucose and lipid fluxes. T2D and non-alcoholic fatty liver disease (NAFLD) are associated with the impairment of the hepatic control of these fluxes (Jones, 2016).

The insufficient suppression of hepatic glucose production in both the fasting and postprandial conditions contributes to elevated blood glucose levels. More than 75% of the endogenous glucose production (EGP) takes place in the liver (Cherrington, 1997). As one-third of the ingested glucose is taken up by the liver, decreased insulin-induced hepatic glucose uptake contributes to postprandial hyperglycemia in T2D (Moore et al., 2012). Ectopic fat accumulation within non-adipose tissue such as the liver is associated with insulin resistance. Ectopic adipose tissue depots release free fatty acids (FFAs) and cytokines that interfere with insulin signaling.

Insulin action on the brain is disturbed in obesity and T2D (Kullmann et al., 2016). The effects of insulin on the human brain are not completely understood. Moreover, it is of interest to understand whether insulin action on the brain can affect peripheral glucose metabolism, specifically hepatic glucose production.

At present, based on the achieved amount of weight loss with favorable effects on co-morbidities (Sjoström et al., 2004) and the prevention of T2D (Carlsson et al., 2012), guidelines recommend bariatric surgery as the most effective therapy for morbidly obese patients. Surgery is considered a treatment option in Finland, as well as worldwide, for patients with a BMI ≥40 kg/m^2, or ≥35 kg/m^2 if there is

significant obesity-related co-morbidity. Given that bariatric surgery has a remarkable weight-lowering effect, it is of interest to study liver metabolism before and after surgery.

The aim of this thesis was to study the effects of surgery-induced weight loss on liver glucose and lipid metabolism, and to understand the beneficial effects of bariatric surgery on fatty liver, insulin resistance and T2D. In the last section, the associations between brain and liver glucose metabolism and the changes in this brain-liver connection after surgery were investigated.

2 REVIEW OF LITERATURE

2.1 Obesity and liver metabolism

2.1.1 *Liver glucose metabolism*

Obesity is characterized by hepatic insulin resistance resulting from blunted insulin action on the liver. Hepatic insulin resistance has a major role in the development of T2D. In T2D, the excessive rate of hepatic glucose production is a key factor responsible for the elevated fasting plasma glucose level (DeFronzo, 1988). Low hepatic postprandial glucose uptake contributes to hyperglycemia in patients with T2D (Iozzo et al., 2003). Hepatic steatosis is common in T2D, and it is related to decreased hepatic glucose uptake (HGU) (Borra et al., 2008) and impaired inhibition of endogenous glucose production (Seppälä-Lindroos et al., 2002).

Liver glucose uptake

In fasting conditions, ~50% of the glucose uptake occurs in the brain (DeFronzo, 1987); the rest of the glucose uptake takes place mainly in the skeletal muscle, liver and kidneys (Cherrington, 1997). Hepatic glucose uptake (HGU) is primarily determined by hepatic glucokinase activity. Glucokinase facilitates the phosphorylation of glucose to glucose-6-phosphate and acts as a glucose sensor in β-cells, the enteroendocrine cells of the gut, the hypothalamus, and the liver (Matschinsky et al., 2006). In maturity-onset diabetes of the young type 2 (MODY2), there is a mutation of the glucokinase gene, which results in reduced enzyme activity and mild fasting hyperglycemia (Fajans et el., 2001).

In a postprandial state, the liver has a significant role in disposing of ingested glucose. After an oral intake of glucose ~1/3 is taken up by the liver, ~1/3 by muscle and fat, and ~1/3 by non-insulin dependent tissues (Cherrington, 1997). Thus, the liver plays an important role in determining the extent of postprandial hyperglycemia. After a meal, elevated insulin and glucose concentrations increase HGU (Cherrington, 1997, Moore et al., 2012). Biochemical mechanisms are the translocation of glucokinase within the hepatocyte and activation of liver glycogen synthase. Glucose delivered by the portal vein as compared to peripheral delivery enhances HGU (Moore et al., 2003). Insulin, a peptide hormone secreted from the β-cells in the pancreas, promotes absorption of glucose from the blood into the liver. In the liver, glucose is converted into either glycogen via glycogenesis or triglycerides (TGs) via lipogenesis. Insulin enters the liver via the portal vein. Of the insulin entering the liver ~60% is extracted by binding to insulin receptors in

hepatocytes. The liver is exposed to insulin levels, which are about 3-fold higher than in non-hepatic tissues (Rojas and Schwartz, 2014).

T2D is associated with failure to increase HGU and suppress glucose output (DeFronzo et al., 1992). Excessive food intake impairs the ability of the liver to suppress its glucose production (Brons et al., 2009, Clore et al., 1995) and increase glucose uptake (Coate et al., 2015). Moreover, a high intake of fructose impairs HGU and storage of glycogen in the liver along with fall in glucokinase activity (Moore et al., 2012).

Liver glucose production

The fasting glucose concentration reflects the basal EGP. The renal contribution to the appearance of glucose in individuals that have fasted overnight is in debate (estimates range from 5% to 23%) (Cherrington, 1997). EGP is controlled by blood glucose concentration, insulin and glucagon. When glucose concentration rises, glucose production falls (Cherrington, 1997). Insulin binds to hepatic insulin receptors, which activates insulin signaling pathways in the liver (Edgerton et al., 2005). The indirect effects of insulin include a reduction of glucagon (Ito et al., 1995), inhibition of lipolysis in fat (Sindelar et al., 1997) and decreased protein catabolism in muscle, which results in a reduction of gluconeogenic precursors entering the liver. Glucagon acts on hepatocytes by releasing glucose from hepatic glycogen stores through glycogenolysis and by converting the lactate, glycerol, FFAs and amino acids into glucose.

Results from animal studies suggest that hypothalamic insulin signaling plays a significant role in the regulation of EGP (Obici et al., 2002). Furthermore, hormones such as epinephrine, cortisol, growth hormone and thyroid hormone affect glucose production. Hormonal signals can rapidly modify EGP to restore the plasma glucose concentration to its set point.

Disturbances in glucose production in obesity and T2D

Subjects with T2D show decreased postprandial hepatic glycogen synthesis and raised glucose output both in fasting and in postprandial state, mainly due to inappropriate high gluconeogenesis in the liver (Krssak et al., 2004). Gluconeogenesis from glycerol is enhanced in T2D as a consequence of accelerated lipolysis and increased hepatic conversion of glycerol to glucose (Puhakainen et al., 1992). Increased protein turnover and release of gluconeogenic amino acids in T2D contribute to enhanced EGP. In obese subjects without T2D glucose production is increased, but glycogenolysis is reduced resulting in a normal glucose output. However, in subjects with T2D, high glucagon level

contributes to inappropriately elevated glucose output from both gluconeogenesis and glycogenolysis (Gastaldelli et al., 2000).

Does metabolically healthy obesity exist?

The concept of metabolically healthy obesity (MHO) is in debate and no universally accepted criteria for MHO exist. However, in literature MHO is most often defined as having less than two criteria of metabolic syndrome (Stefan et al., 2017). The cutoff points for frequently used measures to define metabolic syndrome are TG concentration <1.7 mmol/l and not on drug treatment for elevated TG concentration, normal HDL cholesterol concentration (men ≥1.05 mmol/l, women ≥1.25 mmol/l and not on drug treatment to increase HDL cholesterol), normal blood pressure <135/85 mmHg and not on hypertensive drug treatment and normal fasting glucose <5.6 mmol/l and not on drug treatment for hyperglycemia (Grundy et al., 2005). Central obesity defined by high waist circumference may not be a useful parameter to stratify individuals with obesity as metabolically healthy or unhealthy, as most subjects with a BMI of ≥30 kg/m^2 are also centrally obese. MHO vs. metabolically unhealthy obesity (MUO) phenotype is related to differences in total fat mass and regional fat accumulation. Age, sex and total body fat content are the main predictors of body fat distribution, but also genetic factors play a significant role (Schleinitz et al, 2014). Visceral adipose tissue and ectopic fat accumulation, inflammation, impaired expansion of subcutaneous adipose tissue, impaired adipogenesis, hypertrophy and altered lipid metabolism of fat cells predispose to a MUO phenotype (Iacobini et al., 2019). Lack of insulin resistance might be the main characteristic of MHO (Stefan et al., 2013). Studies suggest that metabolically healthy obese are at higher risk of cardiovascular events than metabolically healthy normal weight individuals (Eckel et al., 2016; Lassale et al., 2018). However, the risk is lower than in metabolically unhealthy obese (Eckel et al., 2016).

2.1.2 *Central regulation of liver metabolism*

The brain was considered for a long time to be an insulin-insensitive organ. Studies from Turku PET centre and others have challenged this theory; Hirvonen and colleagues (2011) have shown that in individuals with impaired glucose tolerance, insulin stimulation enhances brain glucose uptake as compared to the fasting state. Insulin penetrates the blood brain barrier probably via a saturable receptor-mediated transport (Banks et al., 1997). Insulin receptors have been demonstrated to locate throughout the brain, with highest prevalence in the hypothalamus, cerebellum and cortex (Hopkins and Williams, 1997).

Mounting evidence suggests that insulin inhibits EGP not only via a direct effect on the liver, but also via a mechanism involving the brain. It has been shown that the hypothalamus, in particular the ventromedial nucleus, regulates hepatic glucose production (Shimazu et al., 2017). Obici and colleagues (2002) demonstrated that third-cerebral-ventricle administration of insulin-agonists suppressed the rate of glucose production in rats.

Recent data regarding the effect of central insulin administration on larger animals and humans have also supported an interrelation between the brain and liver. Ramnanan and colleagues (2011) have demonstrated that insulin administration into the head arteries increases hepatic glycogen synthesis and reduces mRNA expression of gluconeogenic enzymes; however hepatic glucose production was not suppressed. In humans, Dash and colleagues (2015) have showed that during a pancreatic clamp, intranasal insulin administration, in a dose that did not increase plasma insulin levels, suppressed EGP. Recently Heni et al. (2017) showed that intranasal insulin decreased EGP in lean but not in overweight subjects. Gancheva et al. (2015) demonstrated that intranasal insulin improves hepatic energy metabolism and reduces lipid storage in healthy individuals, and these effects are blunted in subjects with T2D.

The effect of insulin in the brain involves activation of insulin signaling, opening of neuronal ATP-sensitive potassium channels, signaling via hepatic efferents, phosphorylation of liver STAT3, and suppression of gluconeogenic gene expression resulting in reduced hepatic gluconeogenesis (Inoue et al., 2006, Obici et al., 2002, Pocai et al., 2005). The brain modulates pancreatic and liver function by both sympathetic and parasympathetic signals, and by the release of epinephrine from the adrenal medullae. Pocai and colleagues (2005) investigated the effects of hepatic vagotomy on the ability of a physiological rise in plasma insulin to inhibit glucose production in rodents. With the presence of the vagus nerve, the rise in insulin inhibited glucose production by 78%, while after vagotomy the rise in insulin decreased glucose production by 40%.

2.1.3 ***Liver lipid metabolism***

Adipose tissue – Liver axis

Liver takes up FFAs mobilized from adipose tissue through lipolysis. About 25% of the FFAs entering the liver are taken up (Iozzo et al., 2004). In hepatocytes, FFAs are either degraded by β-oxidation or re-esterificated to TGs and stored in the liver or released into the bloodstream in very-low-density lipoprotein (VLDL). Lipolysis has a major role in the regulation of liver FFA uptake. In subjects with

obesity and NAFLD approximately 59% of intrahepatic TGs originate from the circulating FFAs, 15% from a dietary supply and 26% from *de novo* lipogenesis (Donnelly et al., 2005). Imbalance between lipid accumulation (uptake, *de novo* lipogenesis) and disposal (oxidation, secretion) leads to hepatic steatosis. Accrual of lipids in the liver promotes the hepatic insulin resistance associated with obesity and T2D (Shulman, 2014).

Adipose tissue stores substrates and is one the factors modulating food intake and energy consumption by secreting adipokines. Obesity is associated with adipose tissue inflammation, overflow of FFAs into the circulation and abnormalities in adipose tissue endocrine function. Elevated plasma FFA level causes hepatic insulin resistance (Roden et al., 2000). Reduction in adipose tissue FFA release has been shown to greatly decrease liver FFA uptake, and lead to an enhanced liver and systemic insulin sensitivity (Rigazio et al., 2008).

Adipose tissue inflammation can induce hepatic insulin resistance. Studies in mice have shown that interleukin-6 (IL-6), which is a pro-inflammatory cytokine secreted by adipose tissue macrophages, inhibits the ability of insulin to suppress lipolysis in adipose tissue resulting in increased delivery of FFAs and glycerol to the liver. As a result, fatty acid β-oxidation is enhanced leading to elevated hepatic acetyl coenzyme A levels and increased conversion of pyruvate to glucose (Perry et al., 2015). Individuals with obesity and insulin resistance have increased concentrations of IL-6 in adipose tissue along with adipose tissue and hepatic insulin resistance (Perry et al., 2015).

Fatty acid oxidation

Elevated hepatic FFA uptake rate could be balanced by enhanced mitochondrial β-oxidation, which stimulates generation of adenosine triphosphate (ATP) and production of reactive oxygen species (ROS). Iozzo and colleagues (2010) have previously shown that in obese non-diabetic subjects, fatty acid oxidation rate is 2-fold higher than in lean controls. Increased fatty acid oxidation could lead to an overproduction ROS in obesity related liver disease.

De novo lipogenesis

Patients with NAFLD are reported to have 3-fold higher *de novo* lipogenesis than healthy controls (Lambert et al., 2014). As *de novo* lipogenesis can be stimulated both by insulin and by glucose, hyperinsulinemia and high fat and high carbohydrate diets contribute to the increased lipogenesis in subjects with NAFLD and obesity (Tilg et al., 2016). The effects of diets in liver fat are discussed in more detail in chapter *Treatment of NAFLD* page 21.

Export of very low density lipoprotein (VLDL)

Impaired export of VLDL cholesterol may contribute to an increased hepatic fatty acid pool in insulin-resistant subjects. Insulin normally decreases production of VLDL by inhibiting lipolysis in fat tissue, and directly by suppressing production of VLDL in the liver (Adiels et al., 2008). In insulin resistant obese subjects, insulin's ability to suppress lipolysis and production of TG rich VLDL particles from the liver is impaired. The increase in VLDL results in lowering of high density lipoprotein (HDL) cholesterol and the production of small, dense low density lipoprotein (LDL) particles (Tchernof and Despres, 2013). The combination of high TG, low HDL and small, dense LDL particles has been recognized as a major cardiovascular risk factor (Grundy, 1998).

Non-alcoholic fatty liver disease (NAFLD)

Epidemiology of NAFLD

NAFLD is one of the most common liver diseases globally (Ratziu et al., 2015). NAFLD is strongly related to obesity. In the DIONYSOS study cohort with 3000 subjects, aged 12–65 years, resident in two towns of northern Italy, 25% of normal weight (BMI 20-24.9 kg/m2), 67% of overweight (BMI 25-29.9 kg/m2) and 94% of obese (BMI ≥30 mg/m2) participants had NAFLD (diagnosed with ultrasonography) (Argo et al., 2009, Bellentani et al., 2004). Insulin resistance is an important pathophysiological factor in NAFLD (Tilg et al., 2016). A strong correlation between T2D and NAFLD exists: >70% of individuals with T2D also have NAFLD (Willams et al., 2011).

Diagnosis of NAFLD

In NAFLD steatosis is present in >5% of hepatocytes according to histological analysis or by a proton density fraction of >5.6% measured by magnetic resonance spectroscopy (^{1}H-MRS) or quantative fat/water selective magnetic resonance imaging (MRI) (European Association for the Study of the Liver (EASL), 2016). NAFLD includes two distinct conditions: NAFLD and non-alcoholic steatohepatitis (NASH). In order to make a diagnosis of NAFLD, the secondary causes such as increased alcohol consumption (≥30 g per day for men and ≥20 g per day for women), viral hepatitis, use of certain drugs including corticosteroid, methotrexate, amiodarone or tamoxifen medication need to be ruled out.

NASH is diagnosed by liver biopsy. The histological features of NASH include steatosis, ballooning and lobular inflammation (Kleiner and Brunt, 2012). The NAFLD Activity Score (NAS) scoring system was developed to be used in clinical trials for an evaluation of disease severity (Kleiner et al., 2005, Brunt et al., 2011).

The score is defined as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2); ranging from 0 to 8. Fibrosis is not included in the score and is not required to make a diagnosis of NASH however, it is often present in NASH.

NASH can progress to cirrhosis and end-stage liver disease. Fibrosis is the most important prognostic marker in NAFLD (Ekstedt et al., 2015). Non-invasive tools, such as transient elastography or biomarkers and fibrosis scores, can be useful for identification and monitoring of fibrosis (European Association for Study of Liver & Asociacion Latinoamericana para el Estudio del Higado, 2015). The NAFLD fibrosis score (NFS) and fibrosis 4 calculator (FIB-4), Enhanced Liver Fibrosis (ELF) and FibroTest® can be used to predict cardiovascular and liver-related and overall mortality (EASL, 2016). In advanced cases, a liver biopsy is recommended (EASL, 2016). NAFLD is the most common cause of hepatocellular carcinoma (Marengo et al., 2016).

Metabolically benign and malignant fatty liver

The amount of liver fat is related to insulin resistance (Häring, 2016). However, there is evidence of different phenotypes: metabolically benign and metabolically malignant fatty liver (Häring, 2016). A genetic susceptibility plays a role in the development of these two phenotypes. Many genetic variants of NAFLD have been identified (Romeo et al., 2008). The I148M variant of PNPLA3 gene, which encodes patatin-like phospholipase domain-containing protein 3 enzyme, is most strongly associated with high liver fat (Romeo et al., 2008). However, the subjects carrying this gene variant are not insulin resistant (Romeo et al., 2008). Depending on ethnicity, 20-50% of all subjects have this gene variant (Romeo et al., 2008). Another gene involved in the progression of a liver disease is TM6SF2 (Liu et al., 2014). Individuals with TM6SF2 E167K variant are at increased risk to develop a progressive NASH but are protected against cardiovascular disease (Dongiovanni et al, 2015). Liver-derived proteins, called hepatokines, produced by metabolically malignant fatty liver are different from those produced by metabolically benign fatty liver. Hepatokines are known to affect glucose and lipid metabolism. The first hepatokine that has been demonstrated to have a significant role in metabolic disorders is fetuin-A. The production of fetuin-A is high in liver steatosis and inflammation (Stefan and Häring, 2013).

Treatment of NAFLD

The key components in the treatment of NAFLD and NASH are lifestyle changes and diet. Relatively minor weight loss (5-10%) reduces intrahepatic fat and improve hepatic insulin resistance (Zelber-Sagi et al., 2011). It has been proposed that weight loss of 3-5% is associated with improved steatosis, weight loss of 5-

7% is needed to improve inflammation and subjects with weight loss of 7-10% may experience remission of NAFLD/NASH and regression of fibrosis (Hannah and Harrison, 2016). Guidelines recommend a deficit of 500-1000 kcal/day (Chalasani et al., 2012; AISF, 2017; EASL, 2016). Macronutrient composition may play a role in hepatic benefit of the diet. Low-carbohydrate diet seems to decrease liver fat content (LFC) more than a standard hypocaloric diet in a short term (Kirk et al., 2009; Browning et al., 2011). Studies comparing the effects of different isocaloric diets show that LFC decreases during the low fat–high carbohydrate diet and increases during the high fat–low carbohydrate diet (Westerbacka et al., 2005; van Herpen et al., 2011; Utzschneider et al., 2013). Saturated fat precipitates fat accumulation in the liver, while intake of polyunsaturated fat has beneficial effects in the liver (Blermo et al., 2012; Luukkonen et al., 2018; Rosqvist et al., 2014). Fructose appears to have an important role in inducing fatty liver and reducing sucrose and high fructose corn syrup intake may have a major benefit on NAFLD (Jensen et al., 2018). Coffee and tea consumption seem to have beneficial effects in NAFLD patients (Marventano et al., 2016). Both aerobic and resistance training help to reduce liver fat (EASL, 2016). The use of alcohol should be below the risk threshold, which is 30 g/day for men, and 20 g/day for women (EASL, 2016).

Currently, there are no drugs approved for treatment of NAFLD or NASH. Pioglitazone, a peroxisome proliferator activated receptor (PPAR) -γ agonist which is used in the treatment of T2D, has been shown to improve NASH, reduce liver fat, reduce alanine aminotransferase (ALT) and increase hepatic insulin sensitivity (Belfort et al., 2006, Aithal et al., 2008, Sanyal et al. 2010). PPAR -γ agonists promote fat redistribution from ectopic (including liver) to subcutaneous stores and their use is associated with increases in adiponectin level (Polyzos et al., 2019). The side effects of glitazones limits its use; weight gain (mean increase 2.0-3.5 kg), fluid retention, bone fractures in women and congestive heart failure. Selective PPAR- γ modulators (SPPARMs), such as INT131, have been developed to overcome the side effects of glitazones. INT131 and other SPPARMs are regarded as promising candidates in the treatment of NASH (Polyzos and Mantzoros, 2016).

Glucagon-like peptide-1 (GLP-1) agonists may be useful in patients with NASH. GLP-1 agonist liraglutide is associated with improvement in *de novo* lipogenesis, ß-oxidation, insulin resistance and with increased clearance of VLDL (Khan et al., 2018). In a phase 2 trial, liraglutide 1.8 mg daily for 48 weeks, led to a resolution of NASH in nine of 23 patients (39%). (Armstrong et al., 2016). Furthermore, treatment with exenatide, another GLP-1 agonist, is associated with favorable effects on liver steatosis and serum transaminases (Shao et al., 2014). The results from the SUSTAIN trials suggest that semaglutide is more effective than other

GLP-1 analogues in reducing HbA_{1c} in T2D patients (R Aroda et al., 2019). Based on these findings, a clinical trial on the effect of semaglutide in NASH is underway. Future research is needed to clarify whether the effects of GLP-1 agonists on NASH are independent of effects on weight loss and glycemic control.

Sodium glucose co-transporter 2 (SGLT2) inhibitors are new class of drugs exerting glucose lowering effect in T2D. The treatment with SGLT2 inhibitor empagliflozin has been associated with decrease in LFC (evaluated by MRI) and serum ALT (Kuchay et al., 2018). Also, the farnesoid X nuclear receptor (FXR) agonists are gaining attention as potential therapeutic agents in NASH. Activation of FXR results in decrease in bile acid synthesis and decline in insulin resistance, gluconeogenesis and *de novo* lipogenesis (Massafra and van Mil, 2018). FXR ligand, obeticholic acid have shown some promising results in the treatment of NASH, but its long-term benefits and safety need further clarification (Mudaliar et al., 2013; Neuschwander-Tetri et al., 2015). Treatment with vitamin E (800 IU/day) have shown improvement in liver steatosis and lobular inflammation (Sanyal et al., 2010).

Marked improvement in NAFLD has been observed after bariatric surgery (Aguilar-Olivos et al., 2016). Bariatric surgery is associated with improvement in insulin resistance, increase in adiponectin level and decrease in interleukin-8 (IL-8), c-reactive protein (CRP) and tumor necrosis factor alpha (TNF-α), which are factors associated with pathogenesis of NAFLD (Hafeez and Ahmed, 2012). Currently NAFLD or NASH is not an indication for bariatric surgery such as T2D or other comorbidities are. A recent meta-analysis reported that bariatric surgery resulted in resolution of hepatic steatosis in 66% of patients, inflammation in 50% and fibrosis in 40% (Lee et al., 2018). However, some patients developed new or worsened features of NAFLD, such as fibrosis in 12% of patients (Lee et al., 2018). Most often liver failure after bariatric surgery reported in the literature is related to malabsorptive bariatric techniques or an underlying advanced liver disease (Requarth et al., 1995). SG and standard RYGB are expected to be safer. The peri-operative risks of bariatric surgery are higher than usual in patients with cirrhosis, especially in individuals with decompensated cirrhosis (Jan et al., 2015). There are no guidelines on choice of procedure in NAFLD/NASH patients and the data on the comparative effects of bariatric surgery on NAFLD are scarce. Kalinowski and colleagues (2017) compared the effects of SG and RYGB on liver function tests in patients with NASH. They reported that patients undergoing RYGB were more susceptible to early transient deterioration of liver function (by measuring INR and albumin) than after SG, but liver function was normalized at 12 months follow-up. Caiazzo and colleagues (2014) reported that RYBG was superior to gastric banding in improving parameters of NAFLD.

2.1.4 *Liver blood flow*

Liver receives an input from both hepatic artery and hepatic portal vein. Venous blood containing nutrients is absorbed from the gastrointestinal track and drained into the liver by the vena portae. Arterial blood from the abdominal aorta is supplied to the liver via hepatic artery. Liver blood flow is an important factor in liver disease. Altered portal blood flow, measured with doppler ultrasonography (US), predicts worse outcome in alcoholic hepatitis (Duvoux et al., 2004). Liver blood flow is impaired in the fatty liver and responds to its resolution (Magalotti et al., 2004). As an access to the portal vein is not feasible in humans, *in vivo* studies on hepatic parenchymal perfusion are limited due to the highly invasive methodology required. Non-invasive measurements using positron emission tomography (PET) in combination with [^{15}O]water ([^{15}O]H_2O) can be used to measure liver parenchymal perfusion in humans (Slimani et al., 2008, Kudomi et al., 2009). Using [^{15}O]H_2O-PET Rijzewijk and colleagues (2010) found that hepatic parenchymal perfusion was decreased in T2D patients with increased liver fat but not in T2D patients with low liver fat. Portal perfusion might have a role in regulating liver metabolism (Slimani et al., 2008).

2.2 Methods to measure liver metabolism in humans

Hepatic glucose production has been measured by using an arterio-venous (A-V) balance technique. The net splanchnic or hepatic balance is calculated as the product of the A-V glucose concentration difference and hepatic blood flow (DeFronzo, 1987). Using the tracer method, a constant glucose tracer infusion is applied, and EGP in the basal state is derived as the ratio of the infusion rate to the activity of the tracer during steady-state (DeFronzo, 1987). When insulin and glucose are co-administered, the EGP is then calculated as the difference between the total rate of appearance and the rate of infusion of exogenous glucose (Radziuk et al., 1978, Gastaldelli et al., 1999). The PET tracer 2-[^{18}F]fluoro-2-deoxy-*D*-glucose ([^{18}F]FDG) is proven to be suitable in measuring EGP in fasting and during insulin stimulation (Iozzo et al., 2006). As compared with the A-V balance technique, the tracer method has the advantage of being non-invasive. However, the extrahepatic component of EGP is unaccounted for when using tracer methods. Because of their complexity, neither the A-V balance nor tracer methods are used in clinical practice.

It is difficult to quantify liver glucose and fatty uptakes in humans because of the invasiveness of the portal vein catheterization required. However, it has been

shown that parameters derived from PET imaging offer accurate quantification of HGU (Iozzo et al., 2007) and hepatic FFA uptake (Iozzo et al., 2010).

2.2.1 *Positron Emission Tomography (PET)*

PET in combination with specific tracer, is a valuable method for studying glucose metabolism, fatty acid uptake and blood perfusion. PET is a nuclear imaging tool, which provides functional and quantitative information of the biological processes *in vivo*. The use of PET imaging is based on the short-lived, positron-emitting radioactive isotopes incorporated into natural compounds that behave in a similar way to their physiological counterparts. Radionuclides are produced in cyclotrons or generators. Radioactive, positron emitting tracer is introduced into the body. Detection is based on the emitted positron combining with the local electron in the tissue. As a result, two high energy photons leave the tissue in the opposite directions. These co-incident photons are then detected by the PET scanner (Turkington, 2001). The computerized PET quantification is performed using the designated software either manually or automatically. The modelling is based on the tracer kinetics between the input and the tissue. The input can be obtained via frequent sampling of arterialized blood or directly from the PET image (Germano et al., 1992). The tissue function is derived from the PET image by placing the 3-dimensional volume-of-interest onto the tissue parenchyma, thus producing an averaged time-activity curve. PET scanners are usually combined with either computed tomography or MRI, to obtain the anatomical reference image (Turkington, 2001). The limitations of PET imaging are the exposure to ionizing radiation (effective dose given to study subjects is 2-10 mSv) (Stabin and Brill, 2008), spatial resolution limited to 4-6 mm (Spanoudaki and Ziegler, 2008) motion- and breath-holding -related problems and the duration and costs.

2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG)

The glucose analogue [^{18}F]FDG-PET is a PET tracer, which is transported across the cell membrane and accumulated intracellularly in proportion to its phosphorylation. [^{18}F]FDG is trapped in the cell in the form of 6-phosphate-fluoro-deoxyglucose because it is not metabolized through the glycolytic pathway. To quantify the HGU, a conversion with lumped constant (LC) is needed. The LC represents the ratio of the [^{18}F]FDG and glucose metabolic rates. The validation study to measure HGU in pigs showed that LC for [^{18}F]FDG in fasting and during hyperinsulinemia is 1.0 (Iozzo et al., 2007). The graphic analysis developed by Patlak and Blasberg can be used to determinate the liver's fractional [^{18}F]FDG uptake constant and the absolute glucose uptake (Patlak and Blasberg, 1985).

14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid ([^{18}F]FTHA)

Hepatic FFA uptake can be measured by the use of a palmitate analogue, [^{18}F]FTHA. [^{18}F]FTHA utilizes the same transportation protein and cellular handling proteins as natural fatty acids; it remains trapped in the mitochondria after β-oxidation (DeGrado et al., 1991). Modelling of FFA uptake is similar to [^{18}F]FDG. [^{18}F]FTHA is rapidly metabolized into label-carrying metabolites that do not take part to normal tissue trafficking and need to be corrected before analyzing.

[^{15}O]water ([^{15}O]H_2O)

^{15}O-water ([^{15}O]H_2O) is a chemically inert and freely diffusible tracer. It is regarded as the gold standard for evaluation of tissue perfusion. [^{15}O]H_2O has a short half-life (122 s) which enables its use in repeated measurements in dynamic studies. Kudomi and colleagues (2008) have shown that [^{15}O]H_2O can be used to estimate portal and hepatic artery blood perfusion. The input function for one-compartmental analysis can be obtained directly from a PET image (Germano et al., 1992).

2.3 Methods to measure liver fat content

A liver biopsy is a golden standard method for determining the liver fat content (LFC). However, obtaining a sample of liver is an invasive procedure with possible risk of bleeding, infection or biliary leakage, which make non-invasive methods more appealing.

A variety of imaging techniques can be used to assess LFC: transabdominal ultrasonography, computed tomography (CT), MRI and MRS.

2.3.1 *Transabdominal ultrasonography (US)*

US is the most common imaging method performed to examine liver and to evaluate liver fat. As intrahepatic triglyceride content increases the scattering of the US beam in a fatty liver looks brighter than in a normal liver. The benefits of US are a wide availability and low costs, but it has limited sensitivity and does not produce a reliable detection of steatosis at <20% (Saadeh et al., 2002) or BMI >40 kg/m^2 (Ryan et al., 2002).

2.3.2 *Computed Tomography (CT)*

CT is based on ionizing radiation and measures tissue density as a function of attenuation. CT has been widely used for assessing liver fat, but its main weakness is its incapability to detect low levels of liver fat and a dependence on ionizing radiation.

2.3.3 *Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS)*

Magnetic resonance (MR), and its application MRS are considered as the most sensitive imaging methods to evaluate LFC (Borra et al., 2009). Both MRI and MRS techniques utilize the spin properties of certain nuclei when brought into a magnetic field. For MRI, the proton nucleus is used to produce a detailed anatomical image based on the various water concentrations in different tissues. For MRS, these spin properties are used to determinate the concentration of specific metabolites in the analyzed tissue. MRI and MRS are more accurate than US or CT to detect low levels of liver fat. Although MRI and MRS have a superior performance, the use is limited because of the high costs and some contraindications such as a pacemaker implant and claustrophobia. Sometimes obese patients cannot fit comfortably inside an MRI scanner. Because of its complex implementation, MRS is mainly used for research purposes.

2.3.4 *Other imaging methods of the liver*

Only a few patients have progression of hepatic steatosis toward NASH or cirrhosis (Bhatia et al., 2012). Thus, measuring LFC alone is not specific enough to predict prognosis of liver disease. The important elements in liver disease progression are the occurrence of inflammation and fibrosis.

With the use of innovative MRS-based techniques, it is possible to distinguish the level of saturation of fatty acids inside tissue TGs (Iozzo, 2015). Lundbom et al. (2010) have demonstrated that liver fat is more saturated than subcutaneous or visceral fat. Further research is required to understand if the detection of lipid classes contributes to the evaluation of metabolic risk.

A variety of methods to assess liver stiffness are under evaluation, for example transient elastography (TE) and acoustic radiation force impulse imaging (ARFI). Contrast-enhanced ultrasound (CEUS) could be helpful in differentiating the subjects with steatohepatitis from those with simple steatosis (Iijima et al., 2007).

2.4 Obesity and brain glucose metabolism

The brain consumes much more energy than the other organs at rest and almost exclusively uses glucose for energy production (Howarth et al., 2012). Glucose provides the fuel for physiological brain function through the generation of ATP. During strenuous exercise or prolonged fasting glucose can be replaced by lactate or ketones (Mergenthaler et al., 2013). The capacity of the brain to store energy is limited and therefore its energy supply relies on exogenous nutrients.

Glucose is transported across the blood-brain barrier into extra-cellular fluid by glucose transporter protein 1 (GLUT1) (Dienel, 2012). From extracellular fluid glucose is transported via GLUT3 to neurons and via GLUT1 to astrocytes (Simpson et al., 2007) and phosphorylated to glucose-6-phosphate.

Recent PET studies with [^{18}F]FDG have shown that the brain is an insulin-sensitive organ. It has been demonstrated that brain glucose uptake is enhanced in insulin-resistant, obese individuals during hyperinsulinemia (Hirvonen et al., 2011), while no change is observed in lean controls. In a study by Tuulari and colleagues (2013), insulin-induced brain glucose uptake (BGU) was increased in morbidly obese individuals as compared to lean controls and reversed after bariatric surgery. Honkala and colleagues (2017) reported that short-term sprint interval training decreases BGU in insulin resistant subjects, while no change in brain fatty acid uptake was measured. These data are consistent in showing that insulin resistant individuals have enhanced insulin-stimulated BGU compared to healthy subjects. The molecular mechanisms of the increased BGU are currently unknown.

2.5 Methods to measure brain glucose metabolism in humans

Arteriovenous concentration differences can be used to evaluate the global cerebral metabolic rate from the disappearance of the metabolites from the circulation (Kety and Schmidt, 1948). This technique is highly invasive, which causes significant limitation in human studies. Modern metabolic and functional non-invasive neuroimaging techniques, including PET, functional MRI and MRS, have been a great improvement in studying brain metabolism.

2.5.1 *Positron Emission Tomography (PET)*

PET imaging is useful in studying brain glucose metabolism. [^{18}F]FDG is the most commonly used PET tracer in brain studies (Rooijackers et al., 2015). [^{18}F]FDG is taken up by the brain similarly to natural glucose and after it is phosphorylated, it

cannot be metabolized further, resulting in the accumulation of the tracer into the cell. [^{11}C]3-*O*-methyl-D-glucose (3-OMG) is a PET tracer that is not phosphorylated and can be used to study the brain glucose metabolism (Nakanishi et al., 1996). 3-OMG has a short half-life (~20 min) and its use requires complex preparation, which limit its use in a clinical setting.

2.5.2 *Magnetic Resonance Spectroscopy (MRS)*

MRS is a useful tool to quantify the large number of metabolites importantly for glucose metabolism. Carbon-13 (^{13}C) MRS can be used to assess brain glucose metabolism. The uptake and metabolism of [1-^{13}C]glucose is similar to a natural glucose (van de Ven et al., 2010). Another ^{13}C labeled tracer, ^{13}C-acetate, is metabolized spesifically in astroglia, and it can be used to separate astroglial metabolism from neuronal metabolism (Lebon et al., 2002). Brain energy metabolism can also be evaluated by using phosphorus-31 (^{31}P) MRS, which provides information of ATP production (Rooijackers et al., 2015).

2.6 Bariatric surgery

2.6.1 *Brief overview of the treatment of obesity*

Comprehensive lifestyle intervention is key for the treatment of a patient with obesity. The results from two large randomized clinical trials: Look AHEAD (Look AHEAD Reseach Group, 2014) and the Diabetes Prevention Program (Diabetes Prevention Program Research Group, 2009), support the efficacy of lifestyle intervention. A systematic review of 17 diets demonstrated that no one diet was superior for weight loss (Ryan and Heaner, 2014).

Increased physical activity is the key component of a comprehensive lifestyle intervention for obesity management. It seems that aerobic, resistance, high intensity and low intensity training provide similar effects on weight loss (Ross et al., 2015).

Pharmacotherapy for weight loss management is indicated when patient has a history of failure to achieve a clinically significant weight loss (>5% of total bodyweight) or to sustain the lost weight and BMI ≥27 kg/m^2 with one a more comorbidities or a BMI ≥30 kg/m^2 with or without associated comorbidities (Bray et al., 2016). Possible medications include phentermine, orlistat, lorcaserin,

phentermine/topiramate, naltrexone/bupropion and liraglutide. The combination of phentermine and topiramate (currently available only in the US) is associated with a greater mean weight loss than other available medications (-9.8% weight loss, full dose, >52 wk).

Anti-obesity drugs available in Finland are orlistat, naltrexone/bupropion and liraglutide. Orlistat is a pancreatic lipase inhibitor that blocks absorption of 30% one-third of ingested fat (Sjöström et al., 1998). It is one of the safest anti-obesity drugs leading to a moderate weight loss, but the gastrointestinal side-effects (steatorrhea) often limit its use (Bray et al., 2016). The combination of naltrexone/bupropion increases satiety and suppresses appetite. Naltrexone is an opioid receptor antagonist and bupropion a weak norepinephrine and dopamine reuptake inhibitor and nicotinic acetylcholine receptor antagonist. Naltrexone/buprobion can increase blood pressure, but no increased events were noted in the cardiovascular outcome trial (Nissen et al., 2016). Nausea is the most common side-effect (Pilitsi et al., 2019). Liraglutide is a GLP-1 analogue originally approved for the treatment of T2D at doses up to 1.8 mg daily subcutaneously. The optimal dose for obesity treatment is 3 mg daily. Liraglutide, in addition to enhancing insulin secretion and inhibiting glucagon secretion, slows gastric emptying and decreases appetite by acting on hypothalamus, limbic/reward system and cortex (van Can et al., 2014; Farr et al., 2016; Ten Culve et al., 2016). Nausea and gastrointestinal side effects are common and liraglutide is reported to have the highest probability of discontinuation due to side effects (Khera et al., 2016). Furthermore, other anti-diabetic medications are investigated as potential anti-obesity agents, such as GLP-1 receptor agonists exenatide and semaglutide, and SGLT2 inhibitors canagliflozin, dapagliflozin, empagliflozin and ertugliflozin. Overall, medications result in an average weight loss of 3-12% (Khera et al., 2016). Several agents, which many are referring to gastrointestinal hormones, are currently in the pipeline (Pilitsi et al., 2019).

2.6.2 *Surgical procedures*

Bariatric surgery is considered to be the most effective treatment for obesity. Swedish Obese Subjects (SOS) studies have showed that for many morbidly obese individuals, only bariatric surgery seems to be effective in reaching and maintaining a bodyweight in a normal or overweight range (Sjöström et al., 2012, Sjöström et al., 2007). Bariatric surgery results in considerable weight loss that is maintained long term. The SOS study demonstrated that obese patients who had surgery achieved a much greater mean body weight reduction (18%) compared with ~1% weight reduction in patients with standard medical treatment after 20

years of follow-up (Sjöström, 2013). In addition to being efficient in reducing weight, bariatric surgery improves quality of life (Salem et al., 2005), decreases obesity associated comorbidities and increases life expectancy (Buchwald et al., 2009). Bariatric surgery has been established to be beneficial in the remission of T2D. The STAMPEDE study showed that bariatric surgery (either RYGB or SG) was more effective than intensive medical therapy in decreasing or resolving hyperglycemia during the 5-year follow-up period (Schauer et al., 2017).

Generally accepted indications for bariatric surgery are: age 18 – 60 years and BMI ≥40 kg/m^2 or BMI 35–39.9 kg/m^2 with co-morbidities in which weight loss is expected to improve the disorder such as metabolic disorders, cardiorespiratory disease or severe joint disease (Fried et al., 2013). Recent International Diabetes Organizations guidelines recommend bariatric surgery also to be considered to treat T2D in patients with BMI 30.0–34.9 kg/m^2 and inadequately controlled hyperglycemia despite optimal medical treatment (Brito et al., 2017)

To be considered for bariatric surgery, patients should have failed to maintain long-term weight loss, despite comprehensive medical care (Fried et al., 2013). Contraindications for bariatric surgery are non-stabilized psychotic disorders, severe depression, personality and eating disorders (unless specifically advised by a psychiatrist), alcohol abuse and drug dependencies, diseases threatening life in the short term and patient´s inability to participate in a lifelong medical follow-up (Fried et al., 2013).

Sleeve gastrectomy and Roux-en-Y gastric bypass

Surgical procedures used to be classified as restrictive, malabsorptive or combined according to their influence on food ingestion. However, it is now known that most of the standard interventions cause weight loss and metabolic improvements by mechanism other than restriction and/or malabsorption and are referred to as metabolic operations. Numerous different operative techniques are performed. Standard bariatric procedures performed are adjustable gastric banding (AGB), sleeve gastrectomy (SG), Roux-en-Y gastric bypass (RYGB), biliopancreatic diversion (BPD) and BPD/duodenal switch (BPD-DS) (Fried et al., 2013). The most common procedures performed worldwide are SG (45.9%) and RYGB (39.6%) (Angrisani et al., 2017).

Laparoscopic RYGB is considered as the golden standard in bariatric surgery, and other bariatric procedures should be compared with RYGB. Laparoscopic SG was originally intended as a bridge procedure for high-risk superobese patients preceding the definitive bariatric procedure (Regan et al., 2003). However, the promising results of SG in weight loss and the resolution of comorbidities have encouraged SG being increasingly investigated as a stand-alone procedure for

morbid obesity (Bohdjalian et al., 2010, D'Hondt et al., 2011, Boza et al., 2012). SG is considered as less traumatic and easier and faster to perform when compared to RYGB (Helmiö et al., 2012). Advantages of SG include the preservation of endoscopic access to the upper gastrointestinal track, the lack of an intestinal anastomosis, which excludes the risk of internal herniation, normal intestinal absorption, and prevention of the dumping syndrome by pylorus preservation.

In RYGB, the stomach is divided generating a small gastric pouch (20-30 ml), which is then anastomosed with the mid-jejunum, creating the Roux limb (Smith et al., 2008). Ingested nutrients then bypass most of the stomach, duodenum and the proximal jejunum. The Roux limb varies in length from 75-150 cm (Smith et al., 2008) (Figure 1). The SG operation involves transection along the greater curvature creating a tube-like new stomach removing the fundus and body (Smith et al., 2008) (Figure 1).

RYGB appears be associated with greater weight loss than SG with fewer diabetes medication, but most of the studies are not powered sufficiently to detect differences between the two procedures (Li et al., 2016, Schauer et al., 2017, Peterli et al., 2018, Salminen et al., 2018, Zhang et al., 2014).

Complications of bariatric surgery

The overall 30-day morbidity rate in the SOS study for RYGB was 3.4% for serious complications and 8.3% for any complications (Stenberg et al., 2014). The most important perioperative complications are bleeding, gastrointestinal leak, wound complications and bowel obstruction (Nudel and Sanchez, 2019). The overall mortality rate is very low (0.04%) (Stenberg et al., 2014). SG is reported to be associated with fewer early complications than RYGB (Helmiö et al., 2012).

Long term complications of RYGB include abdominal pain, marginal ulceration (incidence 0.6-7.6%), internal hernia (incidence 1-10%), nutritional complications and dumping syndrome (Nudel and Sanchez, 2019). Early dumping is reported in 10-20% and late dumping in 5-10% of patients after RYGB (Nudel and Sanchez, 2019). SG patients seem to experience less early dumping than RYGB patients, but no differences for late dumping is reported (Emous et al., 2018). In the SLEEVEPASS study morbidity rate from 30 days after surgery until the 5-year follow-up for RYGB was 26% and for SG 19% (Salminen et al., 2018). Worsening or new onset of gastroesophageal reflux is reported after SG. In the SLEEVEPASS study 6% in the SG group underwent conversion to RYGB for severe reflux, and 11% needed daily proton pump inhibitors for reflux (Salminen et al., 2018). Important nutritional complications after bariatric surgery are B_{12}, iron, folate and vitamin D deficiencies (Bal et al., 2012). Life-long daily multi-vitamin and mineral supplementation is needed (Fried et al., 2013). Laboratory tests to evaluate the

metabolic and nutritional status should be carried out regularly (Fried et al., 2013). Bone mineral density decreases after bariatric surgery (Rodriquez-Carmona et al., 2014) and bariatric patients are at increased risk of fracture (Nakamura et al., 2014).

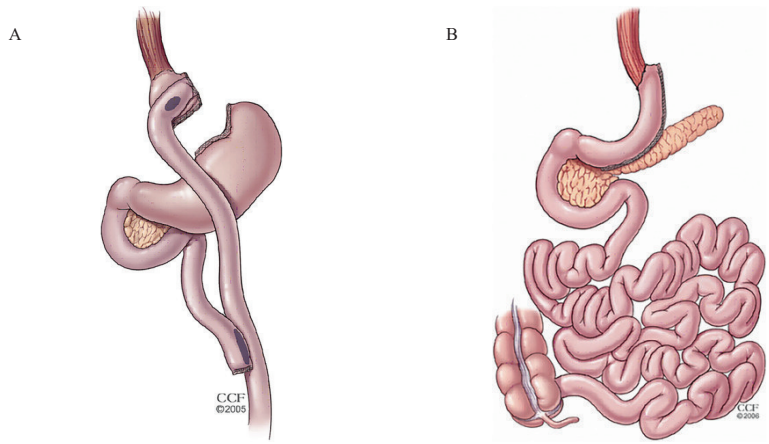

Figure 1. A: Roux-en-Y gastric bypass (RYGB): the stomach is separated into two compartments, leaving only the small upper chamber in the digestive continuity. Food passes from there to the proximal jejunum, bypassing most of the stomach, the duodenum and a small portion of jejunum. B: sleeve gastrectomy (SG): Most of the stomach is excised, leaving a narrow sleeve along the lesser curvature. Nutrients follow the normal route through the gastrointestinal track. Modified from American SFMABSCIC. Bariatric surgery procedures; 2018 (https://asmbs.org/patients/bariatric-surgery-procedures).

Changes in gastrointestinal hormones and other mechanisms of bariatric surgery

Although bariatric surgery is increasingly used in the treatment of morbid obesity, the underlying mechanism remain incompletely understood. It is likely that alterations in gastrointestinal hormones have a significant role in producing the beneficial effects of bariatric surgery. Decreased appetite and reduced neural responsiveness to food cues are reported after SG and RYGB (Pucci et al., 2018). Number of gastrointestinal hormones have been identified for their effects on appetite, including GLP-1, peptide YY (PYY), and ghrelin. The gut hormones GLP-1 and PYY are released following a meal (Murphy and Bloom, 2006). Both GLP-1 and PYY increase satiety and decrease food intake (Beckman et al., 2010).

Both hormones have an impact on glycemic regulation (Guida et al., 2017). GLP-1 secretion plays a key role in mediating the incretin effect. GLP-1 based medications are used in treatment of T2D and obesity. In contrast to GLP-1 and PYY, ghrelin, which is released from the stomach, stimulates appetite and energy intake (Murphy and Bloom 2006). Ghrelin is most active in its acylated form (Kojima et al., 1999). Unacylated ghrelin (UAG) is both a precursor to ghrelin and one of the split products (Vestergaard et al., 2010). It is proposed that UAG exerts metabolic effects partly by antagonizing ghrelin and it seems to play a role in the regulation of glucose metabolism (Delhanty and Lely, 2011; Cederberg et al., 2012).

RYGB and SG are associated with reduced ghrelin and increased postprandial PYY and GLP-1 concentrations (Pucci et al., 2018). Increases in GLP-1 and PYY are greater following RYGB. Cummings and colleagues (2002) showed that RYGB was associated with markedly suppressed total ghrelin levels, while a calorie-restricted diet led to an increase in ghrelin. SG results in a greater reduction in acylated ghrelin level than RYGB because of the removal of the fundus of the stomach where most of the cells that produce ghrelin are located (Yousseif et al., 2014). Other gut hormones, which are known to be involved in regulating the eating behavior like glucose-dependent insulinotropic polypeptide (GIP), oxyntomodulin, cholecystokinin (CKK), gastrin and neurotensin, have been investigated, but their role in mediating the effects of bariatric surgery are unclear. In additions to gut hormones, bile acids, gut microbiota and probably many yet unidentified mechanisms contribute to the effect of bariatric surgery (Pucci and Batterham, 2018).

3 AIMS OF THE STUDY

The hypotheses formulated for the studies reported in this thesis were the following: 1) Liver glucose metabolism is impaired in morbid obesity and particularly in obese patients with T2D and is improved after bariatric surgery; 2) Increased liver fatty acid uptake in morbid obesity is decreased after bariatric surgery; 3) Portal venous blood flow associates with liver metabolism; and 4) Increased brain glucose uptake in morbid obesity associates with endogenous glucose production. The specific aims were:

I. To investigate the effects of T2D and morbid obesity on liver insulin sensitivity (I).

II. To examine the changes in hepatic glucose uptake, endogenous glucose production and liver fat content and remission of T2D after surgery-induced weight loss and to evaluate whether the gain in hepatic insulin sensitivity after surgery differs between subjects with and without T2D (I).

III. To assess bariatric surgery-induced effects on hepatic fatty acid uptake and to evaluate if the changes in hepatic fatty acid uptake mediate the improvements in hepatic insulin sensitivity (II).

IV. To study the effects of bariatric surgery on portal venous and hepatic arterial blood flow with reference to metabolic changes (II).

V. To investigate whether brain glucose uptake is associated with liver glucose metabolism and whether the association is influenced after bariatric surgery (III).

4 SUBJECTS AND STUDY DESIGN

4.1 Study subject characteristics

The present study was initiated to explore liver metabolism in healthy controls, and obese individuals with and without T2D. The data from two projects were included: SLEEVEPASS (ClinicalTrial.gov NCT00793143) and SleevePET2 (ClinicalTrials.gov NCT01373892). The anthropometric characteristics of the study subjects at baseline (before surgery) are presented in Table 1.

Table 1. Baseline clinical characteristics

Study	Study group	N (M/F)	Bariatric procedure (RYGB/SG)	Age (years)	BMI (kg/m^2)	HbA_{1c} (%)	T2D (n)	IFG/ IGT n
I	ND	14 (1/13)	6/8	43 ± 9	44.1 ± 4.1	5.6 ± 0.3	0	5 (36%)
	T2D	9 (3/6)	7/2	53 ± 4	41.5 ± 2.5	6.5 ± 0.7	9	-
	C	10 (2/8)		47 ± 6	23.7 ± 1.8	5.7 ± 0.2	0	0
II	Obese	26 (0/26)	9/17	42 ± 10	41.5 ± 4.1	5.9 ± 0.6	9	7 (27%)
	C	15 (0/15)		45 ± 12	22.6 ± 2.8	5.6 ± 0.3	0	0

Data are show as mean ± SD. ND, non-diabetic obese subjects; T2D, type 2 diabetes obese subjects; C, healthy lean control subjects; M, male; F, female; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

For study I 25 morbidly obese patients were recruited. However, 2 patients did not proceed to surgery. Thus, 23 patients were included in study. Fourteen individuals did not have T2D, of whom five had impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). Nine individuals had T2D. IFG was defined by fasting plasma glucose level from 5.6 mmol/l to 6.9 mmol/l, and IGT by 2-hour glucose levels of 7.8 to 11.0 mmol/l on the 75g OGTT. T2D was defined by either the fasting plasma glucose ≥7 mmol/l or the 2-h plasma glucose value >11 mmol/l during the 75g OGTT, or HbA_{1c} ≥6.5% according to the American Diabetes Association recommendations (1997).

Four subjects were newly diagnosed with T2D and were treated with metformin started preoperatively. In the other five subjects, the median duration of T2D was

2.8 years. Patients with T2D were treated with oral hypoglycaemic medication (2 with metformin; 1 with metformin/sulphonylurea/gliptin; 1 with metformin /pioglitazone/ gliptin and 1 with metformin/pioglitazone). Insulin treatment was an exclusion criterion for the study. Thirteen (7 with T2D and 6 without T2D) subjects underwent RYGB and 10 (2 diabetic and 8 non-diabetic) patients SG. Ten healthy, lean age-matched controls were recruited.

Study II included 25 morbidly obese participants. For recruitment reasons, only women were included in the study.16 subjects did not have T2D, including 7 subjects with IFG or/and IGT. Nine subjects had T2D. Five subjects were treated with metformin alone, four with a combination of metformin and gliptin, and one subjects with gliptin alone. 17 subjects underwent SG and 9 subjects RYGB. 15 healthy, lean age-matched female controls were recruited.

Morbidly obese patients suitable for bariatric surgery were recruited to the study from an endocrinology outpatient clinic. Healthy, lean controls were recruited from a newspaper advertisement. The inclusion and exclusion criteria for the bariatric patients are shown in Table 2. Patients with insulin treated T2D were excluded to standardize [^{18}F]FDG-PET scanning and insulin clamp studies. The inclusion criteria for the healthy controls were an age 18-60 years, a BMI of 18-27 kg/m^2, a fasting and 2-hour plasma glucose in oral glucose tolerance test (OGTT) of less than 6.1 and 7.8 mmol/l respectively.

The experiments were performed after an overnight fast (12 hours), caffeine and nicotine were prohibited for 24 hours before the studies, alcohol use was prohibited for 72 hours before the studies, and a recommendation to avoid strenuous exercise for 48 hours before the studies was given. Anti-diabetic treatment was withheld 24 - 72 hours before the metabolic studies.

All subjects signed the informed consent prior to inclusion. The study protocol was reviewed and approved by the ethics committee of the Hospital District of Southwest Finland, and the study was conducted according to the principles of the Declaration of Helsinki.

Table 2. The inclusion and exclusion criteria for bariatric subjects

Inclusion criteria for bariatric subjects:
• BMI ≥40 kg/m^2 or ≥35 kg/m^2 with additional risk factor (T2D, hypertension, dyslipidemia, obstructive sleep apnea)
• Age 18-60 years
• History of non-successful carefully planned conservative treatments
Exclusion criteria for bariatric subjects:
• Insulin treatment
• Mental disorders (non-stabilized psychotic disorder, severe depression)
• Severe eating disorder
• Excessive use of alcohol (>30g/day for men and >20g/day for women)
• Poor compliance
• BMI >60 kg/m^2 or body weight >170 kg

4.2 Study designs

Subjects were screened before inclusion in the study. Clinical screening included their history, a physical examination, anthropometric measurements and fasting measurements of plasma glucose, insulin, glycosylated haemoglobin (HbA_{1c}), lipid profile, FFAs, CRP, cytokines and adipokines. At the visit, an OGTT was performed. During the OGTT, blood samples were taken before and at 30, 60, 90 and 120 min, for glucose, insulin and C-peptide.

MRI, MRS and PET studies were performed before at baseline before surgery. Studies were repeated six-months after surgery. The baseline studies were carried out before the patient started a four-week VLCD prior to surgery. MRI and MRS studies were performed after 2–3 hours of fasting, and the PET studies after overnight fasting. Lean control subjects were studied once. An outline of the study design for Study I is presented in Figure 2, and that of Study II in Figure 3.

4.2.1 *Design of study I*

For study I, the PET imaging was performed in a fasting state and during an euglycemic hyperinsulinemic clamp, on separate days less than two weeks apart (Figure 4). For PET studies, two venous catheters were inserted, one for injection of [^{18}F]FDG and infusions, and another for arterialised blood sampling. To arterialize the vein, an electrical heated cushion was placed around the arm for the length of the study. The euglycemic hyperinsulinemic clamp was performed as previously described (DeFronzo et al., 1979). The rate of insulin infusion was 1mU/kg/min (Actrapid; Novo Nordisk, Copenhagen, Denmark). During hyperinsulinemia, normoglycemia was maintained by a variable infusion rate of 20% glucose based on plasma glucose measurements taken every 5-10 min from arterialized blood. Blood samples were obtained at timed intervals in the fasting state and during the clamp, for the measurement of plasma glucose and insulin levels. 100 ± 10 min after beginning of the clamp, [^{18}F]FDG was injected intravenously over 15 s. The PET-scanning started from the brain (4 x 30 s, 3 x 60 s, 10 x 300 s frames), followed by the liver (5 x 180 s frames), abdomen (5 x 180 s frames) and legs (3 x 300 s frames).

4.2.2 *Design of study II*

In Study II PET imaging, a bolus of ^{15}O-labelled radiowater was injected, and was followed by a dynamic image acquisition (26 frames, 5 min 10 s) (Figure 5). 10 minutes after the radiowater injection, an [^{18}F]FTHA-bolus was given and dynamic imaging was again acquired. After 64–77 minutes, the liver was imaged (frames 5 x 180 s). During the imaging, blood was frequently drawn to measure plasma glucose, insulin, FFAs, and radioactivity.

In both Study I and II, whole body MRI scan (Gyroscan Intera CV Nova Dual, Philips, Amsterdam) was performed to obtain abdominal visceral and subcutaneous adipose tissue masses, liver volume, LFC and cardiac output. In obese subjects, the studies were repeated 6 months after the bariatric procedure. Obese subjects were followed up for 3 years after surgery. During the follow-up visits body weight, BMI, fasting plasma glucose and HbA_{1c} were recorded.

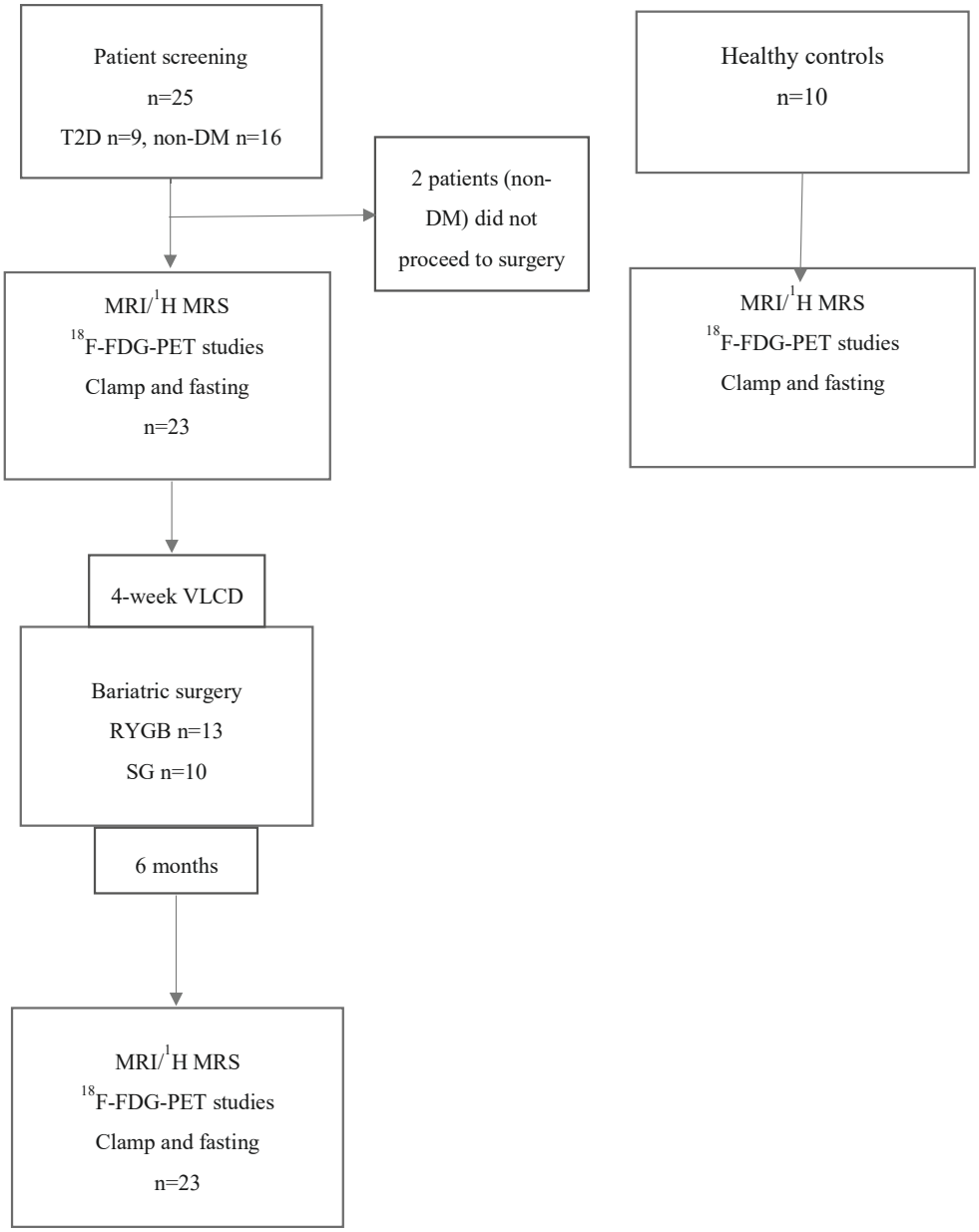

Figure 2. Flow chart for study I. The experiments were performed once for the healthy, lean controls and twice (before and after bariatric surgery) for obese patients. T2D, patients with type 2 diabetes; non-DM, non-diabetic obese patients; VLCD, very-low calorie diet; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy.

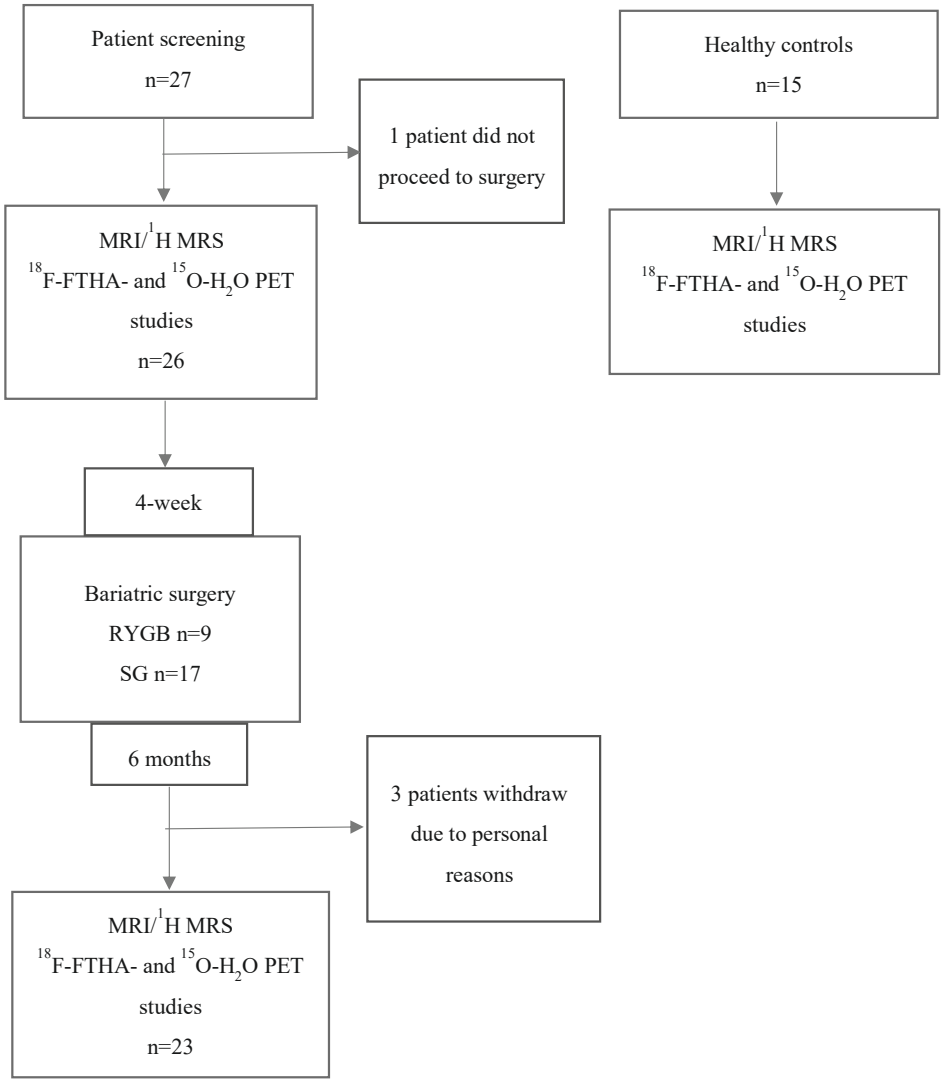

Figure 3. Flow chart for study II. The experiments were performed once for the healthy controls, and twice (before and six months after bariatric surgery) for obese patients. VLCD, very-low calorie diet; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy.

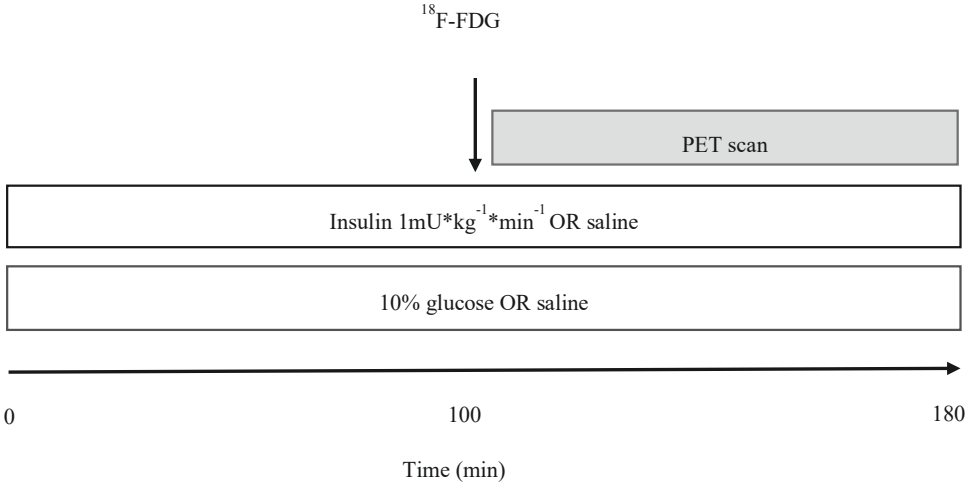

Figure 4. Diagram of the experimental protocol of Study I, SLEEVEPASS. Experiments were performed both in a fasting state and during an euglycemic hyperinsulinemic clamp, on separate days less than two weeks apart.

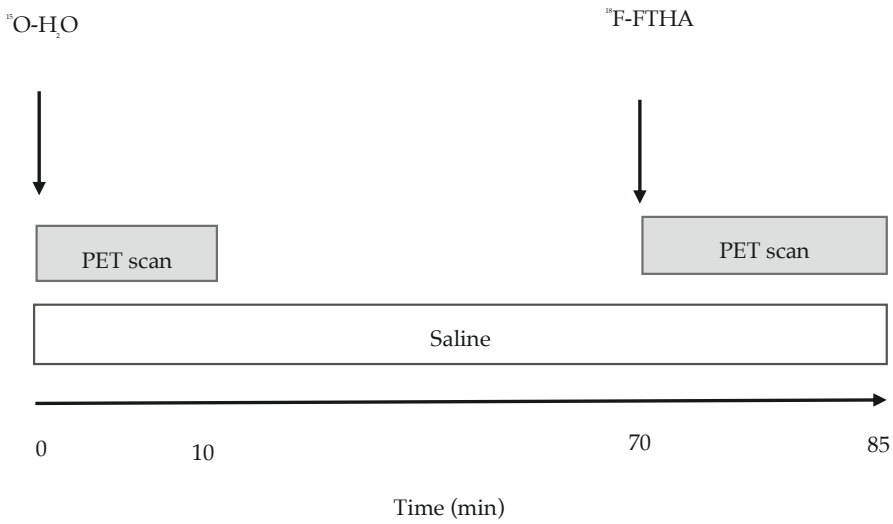

Figure 5. Diagram of the experimental protocol of Study II, SleevePET2. Experiments were performed in a fasting state.

5 MATERIALS AND METHODS

5.1 Surgical technique

All surgical procedures were performed by the same team at Turku University Hospital. For RYGB a gastric pouch (~20–40 ml) was created by dissecting along the lesser curvature using a Harmonic Scalpel™ (Ethicon Endo-Surgery, Cincinnati, OH, USA) and dividing the stomach horizontally and vertically with linear staplers using reinforced 3.5/45 and 3.5/60-mm cartridges with blue loads (Covidien, Mansfield, MA, USA). The biliopancreatic limb was measured by graspers at 50–80 cm. An antecolic end-to-side gastrojejunostomy was constructed as either a 25 mm circular stapler anastomosis (OrVil™, Covidien) or a 3.5/45 mm blue-load linear stapler anastomosis depending on the surgeon's preference. The omentum was not routinely transected. The jejunal opening in the circular stapler anastomosis was closed with a reinforced 3.5/45 mm blue-load linear stapler firing. The opening in the linear stapler anastomosis was closed with a running suture either manually or using EndoStitch™ (Covidien). The alimentary limb was measured by graspers at 150 cm and a side-to-side jejunojejunostomy was created by a linear stapler using a 2.5/60 mm cartridge with white load. The opening in the anastomosis was closed depending on the surgeon's preference with either a running suture or by a totally stapled technique using two reinforced 3.5/60 mm linear stapler firings with blue loads. The resected parts of tissue were extracted through one of the trocar sites by a plastic retrieval bag.

The SG was made narrow along a 33–35 Fr calibration bougie using linear staplers. The majority of the antrum was preserved as the resection was initiated 4–6 cm proximal to the pylorus. Two sequential 4.8/60 mm green-load firings for the antrum were used followed by approximately four sequential 3.5/60 mm blue-load firings with all the staple lines reinforced. The procedure was carried out either by first dividing the stomach or by first mobilizing the greater curvature upward until the angle of the His by dissection of the short gastric vessels using the Harmonic Scalpel™. The resected stomach was removed through one of the trocar sites by a plastic retrieval bag (EndoCatch™, Covidien).

5.2 PET imaging

5.2.1 *Production and characteristics of radiotracers (I-III)*

The glucose analogue [^{18}F]FDG ($T_{½}$ = 110 min) was produced with an automatic apparatus by a modified Hamacher method (Hamacher et al., 1986). [^{18}F]FDG is not further metabolized through the glycolytic pathway and remains trapped in tissue in proportion to its phosphorylation rate.

^{15}O-labelled radiowater ($T_{½}$ = 122 seconds) was synthesized using a low-energy deuteron accelerator Cyclone 3 (IBA International, Louvain-La-Neuve, Belgium) and the diffusion-membrane technique. Radiowater is a freely inert tracer that is diffusible between blood and tissues, and it is used to measure tissue perfusion.

The palmitate analogue, [^{18}F]FTHA ($T_{½}$ = 110 minutes) was synthesized by labelling 14(R,S)tosyloxy-6-thia-heptadecanoic acid with [^{18}F]fluoride and with HPLC (Takala et al., 2002). [^{18}F]FTHA is used to assess the tissue-specific consumption of fatty acids. After entering the cell, it goes through partial mitochondrial β-oxidation, and remains trapped (Iozzo et al., 2003).

5.2.2 *PET image acquisition and processing*

PET images were obtained using the PET scanner GE Advance (General Electric Medical Systems, Milwaukee, WI, U.S.A.) which has a transaxial resolution of 3.8 mm and a slice width of 4.2 mm in the center of the imaging field (DeGrado et al., 1994). The study subject was positioned in the scanner and transmission scan was performed to measure photon attention. After tracer administration, a dynamic PET scanning was carried out. All data were corrected for dead time, decay and photon attenuation and reconstructed in a 256 x 256 matrix. Blood samples to obtain plasma radioactivity were withdrawn once during each time frame and measured using an automatic gamma counter (Wizard 1480 3", Wallac, Turku Finland).

Quantification of liver metabolism (I, II)

Hepatic time-activity curves from [^{18}F]FDG, [^{18}F]FTHA and [^{15}O]H_2O images were obtained by manually drawing regions-of-interest in the liver. Carimas v.2.0.2 (www.pet.fi/carimas) was used to manually draw three-dimensional volumes of interest in the right lobe of the liver. For [^{18}F]FDG and [^{18}F]FTHA analyses, the input function was derived from arterialized blood radioactivity

samples obtained during scanning. The blood radioactivity curve was corrected for metabolites. The liver glucose uptake rate was obtained by multiplying influx constant (Ki) by the plasma glucose concentration and an LC of 1.0 (Iozzo et al., 2007). The fractional [^{18}F]FTHA uptake was multiplied by the mean plasma FFA concentration during the imaging period to obtain the liver FFA uptake rate. No LC was used (Iozzo et al., 2003).

Hepatic perfusion was calculated from the data derived from [^{15}O]H_2O study, by using a one-tissue compartment model (Slimani et al., 2008). An image-derived input function was obtained from the abdominal aorta as previously described (Germano et al., 1992).

Quantification of brain glucose metabolism (III)

The influx constant (Ki) was calculated for each voxel separately using the linear Gjedde-Patlak plot with an arterial plasma input function, with a linear phase start time of 20 min. Glucose uptake estimate of the BGU (µmol/100g/min) was then calculated at the voxel level as follows: BGUglu = Ki*Cp/LC, where Cp is the average plasma glucose concentration from the injection until the end of the brain scan, and the LC is the lumped constant for the brain (which was set at 0.65) (Wu et al., 2003). Brain density was set at 1.04 (Report of the task group on reference man, 1979). Summed PET images were normalized spatially to ligand-specific template in to the Montreal Neurological Institute (MNI) space (MNI International Consortium for Brain Mapping) using statistical parametric mapping (SPM12, www.fil.ion.ucl.ac.uk/spm/) running on Matlab for windows (version 9.1.0; Math Works, Natick, MA). Normalization parameters were subsequently applied to corresponding parametric glucose metabolism images. Parametric images were smoothed at 10 mm full width at half-maximum. Associations within grey or white matter were separately tested using masks obtained with a threshold of >0.3 and 0.6 on grey and white matter default segmentation images of SPM respectively.

5.3 Magnetic Resonance Imaging and Magnetic Resonance Spectroscopy (I, II)

An MRI was performed to obtain liver volume and visceral and subcutaneous adipose tissue masses. Axial T1-weighted dual fast field echo images (TE 2.3 and 4.6 ms, TR 120 ms, slice thickness 10 mm without gap), covering the whole-body area were acquired. A 1.5 T MR imager with flexible surface and body coils was used for MRS. For the LFC measurement, a single voxel is positioned in the liver outside the area of the great vessels. The voxel size was 20 mm x 20 mm x 30 mm.

To confirm similar voxel placement before and after the intervention, the voxel location was recorded in each patient. A PRESS 1H MRS sequence was performed with the following parameters: TR = 3000 ms, TE = 25 ms with data acquired during breath-hold intervals. Lipid ratio (peaks at 0.9 ppm and 1.3 ppm) to water was measured. Spectra analysis was done with LCModel (Version 6.3-0C) (Provencher et al., 1993). Data was corrected for T2 relaxation and the difference in molar concentrations of 1H nuclei in fat and water was accounted for (Thomsen et al., 1994, Szczepaniak et al., 1999). LFC was calculated as before (Thomsen et al., 1994). To measure liver volume, liver margins were manually outlined and volume was calculated by multiplying the measured surface areas of each slice by the slice thickness, as previously described (Lewis et al., 2006).

SliceOmatic software version 4.3 was used to calculate the abdominal subcutaneous fat volume and visceral adipose tissue volume (http://www.tomovision.com/products/sliceomatic.htm). The regions of interest were drawn semi-automatically using Morpho mode for subcutaneous fat and Region Growing mode for visceral fat. For converting the volumes into weight an adipose tissue density of 0.9196 g/ml was used.

Cardiac end-diastolic volume, end-systolic volume and ejection fraction were obtained from continuous short axis slices by using the balanced turbo field echo sequence. Imaging parameters included repetition time (TR) of 3.8 ms, echo time (TE) of 1.9 ms, and matrix of 256 × 256. Slice thickness was 8 mm with no gap between slices. Thirteen to 16 slices were required to cover the left and right ventricles completely from apex to atrium. Image analysis was done using Philips post-processing software (ViewForum R4.1; Philips Medical Systems). Cine loops were reviewed to detect end-diastolic and end-systolic frames. Epicardia and endocardia were manually outlined. Cardiac output was computed from ESV, EDV, and heart rate (Kankaanpaa et al., 2006)

5.4 Liver biopsies (I, II)

Liver tissue was obtained by a needle biopsy during the bariatric surgery and it was placed in formalin for histologic analyses. Liver biopsy specimens were evaluated for steatosis, inflammation and fibrosis according to a standardized histologic scoring system for NAFLD (Kleiner et al., 2005, Brunt et al., 2011). NAFLD Activity Score (NAS) was defined as previously described (Kleiner et al., 2005, Brunt et al., 2011). Histologic examination and scoring were performed by an experienced pathologist.

5.5 Calculations

5.5.1 *Whole-body glucose uptake, endogenous glucose production and insulin sensitivity (I, III)*

An insulin infusion of 1 mU/kg/min was administrated and a glucose infusion was initiated to maintain euglycemia (5.0 mmol/l). After reaching the steady state, the glucose infused was used to calculate the whole-body glucose uptake (M value) (DeFronzo et al., 1979).

[^{18}F]FDG kinetics were used to quantify EGP (Iozzo et al., 2006). The plasma clearance rate of [^{18}F]FDG (PCR_{FDG}) was derived by standard equations and corrected for urinary loss to obtain the metabolic clearance rate (MCR_{FDG}). MCR_{FDG} was multiplied by plasma glucose to estimate Ra (Ra_{FDG}): PCR_{FDG} ($mL \cdot min^{-1}$) = dose (kBq)/AUC_{FDG}($kBq \cdot mL^{-1} \cdot min$); MRC_{FDG} ($mL \cdot min^{-1}$) = PCR_{FDG} ($mL \cdot min^{-1}$) – kV ($mL \cdot min^{-1}$); Ra_{FDG}($\mu mol \cdot min^{-1}$) = Rd_{FDG} ($\mu mol \cdot min^{-1}$) = MCR_{FDG} ($mL \cdot min^{-1}$) × plasma glucose ($\mu mol \cdot mL^{-1}$), where k is the first-order kinetic constant describing urinary loss of [^{18}F]FDG and V is the apparent distribution volume of [^{18}F]FDG. V was calculated by multiexponential fitting of the radioactivity curves after 60 s of injection. AUC_{FDG} was computed by integration of the plasma [^{18}F]FDG radioactivity curves by the trapezoidal rule and extrapolation of the curve tails to infinity. Under fasting conditions, Ra equals EGP because no exogenous glucose is infused; during the clamp, EGP was computed by subtracting the glucose infusion rate from Ra.

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using plasma glucose and insulin concentrations: HOMA=(I X G)/22.5, where I is the fasting plasma insulin concentration (mU/L), and G is the fasting plasma glucose concentration (mmol/L). HOMA provides an index of insulin resistance rather than sensitivity. To measure insulin sensitivity, oral glucose insulin sensitivity (OGIS) was calculated from 2-hour OGTT-based data (Mari et al., 2001)

5.5.2 *Insulin clearance and ß-cell function (I, II)*

Insulin clearance from the OGTT was calculated as the mean insulin secretion divided by the mean insulin concentration during OGTT. Correspondingly, insulin clearance from the hyperinsulinemic euglycemic clamp was calculated as the insulin infusion divided by the steady-state insulin level during the clamp.

ß-cell function parameters were derived from mathematical modelling of plasma glucose, insulin and C-peptide concentrations, as measured during the frequently sampled OGTT, as described by Mari et al. (2002).

5.6 Biochemical, immunological and metabolite analyses

Plasma glucose concentrations were measured in the laboratory of the Turku PET Centre, in duplicate, using the glucose oxidase method (Analox GM7 or GM9 Analox Instruments Ltd., London, UK). Glycosylated haemoglobin was determinated/determined by HPLC (Variant II; Bio-Rad, Herculas, CA). Serum insulin was determined by time-resolved immunofluorometric assay (AutoDELFIA, PerkinElmer Life and Analytical Sciences). Serum FFAs were determined by photometric enzymatic assay (NEFA C, ACS-ACOD; Wako Chemicals GmbH, Neuss, Germany; and Modular P800, Roche Diagnostics GmbH). Serum high-sensitivity C-reactive protein was analyzed with the sandwich immunoassay method using an Innotrac Aio1 immunoanalyzer (Innotrac Diagnostics, Turku, Finland).

Serum samples were analyzed in duplicate by using Milliplex Human Serum Adipokine (Panel A) kit [cat.no: HADK1-61K-A containing IL-6 (interleukin-6), IL-8 (interleukin-8), TNFα (tumor necrosis factor alpha), MCP-1 (monocyte chemotactic protein-1), Leptin], Milliplex Human Serum Adipokine (Panel B) kit (cat.no: HADK2-61K-B adiponectin and resistin) and Milliplex Human Metabolic Hormone Panel kit [cat.no: HMH-34K containing active ghrelin and GIP (gastric inhibitory polypeptide) as recommended by the manufacturer (Millipore Corporation, USA). GLP-1 (glucagon-like peptide-1) and GLP-2 (glucagon-like peptide-2) concentration of lithium-heparin plasma samples were determined using a commercially available ELISA kit following the manufacturer's instructions (Human GLP-1 EIA kit, Cat. No.: YK160 and Human GLP-2 EIA kit, Cat. No.: YK141 Yanaihara Institute Inc., Japan). Optical density of samples was determined using a Multiscan Ascent spectrophotometer (Thermo Labsystems, Helsinki, Finland). Fasting metabolites quantification was performed with nuclear magnetic resonance spectroscopy (Soininen et al., 2015).

5.7 Statistical analysis

Data are shown as mean ± SD. Natural logarithmic transformation was applied for data that was not normally distributed. Differences between groups and paired data were addressed by Student *t* test, a two-tailed paired *t* test, Wilcoxon signed rank

test, Mann-Whitney *U* test. The effects of the group on FFA uptake and perfusion were assessed using two-way ANOVA. In the pairwise comparisons for metabolic data, a false discovery rate (FDR) correction was used to account for multiple testing. Pearson and Spearman (where appropriate) univariate correlation coefficients were calculated. Statistical calculations were performed using the SPSS version 20.0 and 22.0 for Windows (SPSS Inc., Chicago, IL). *P*-values <0.05 were considered significant.

For brain data, linear regressions were performed in SPM to evaluate the positive or negative correlations between the BGU (clusters in the images) and single regressors (EGP, inflammatory markers, metabolites, follow-up variables) while controlling for confounding factors (setting 0 in the contrast). In order to determine the scatterplots of the associations, mean BGU values from whole-brain region-of-interest (ROI)s for global effects or mean BGU values from only the voxels that were identified as being significant in SPM analyses, in which the effect was not global, were extracted. These values were then regressed against various parameters. These values visualized in the scatterplot data were used to support interpretation of the SPM analyses. All statistical inference was based on the whole-brain SPM analyses. Comparisons between the groups were performed in SPM with two-sample independent or paired *t* tests. The statistical threshold in the SPM analysis was set at a cluster level and corrected with FDR with p <0.05. For visualization purposes a threshold uncorrected at the voxel level with p<0.05 was used. Further statistical analyses were done using JMP version 13.0 (SAS Institute, Cary, NC, USA).

6 RESULTS

6.1 Bariatric surgery is effective in treatment of obesity and T2D (I, II)

The effects of bariatric surgery on clinical and biochemical characteristics are presented in Table 2. The table is a compilation of Study I and Study II. The mean weight loss at six months after bariatric surgery was 26.7 ± 8.0 kg. The weight loss after RYGB was greater than after SG (29.6 ± 7.3 kg vs. 24.1 ± 7.9 kg, p=0.018). The post-surgical BMI remained in the obese range (BMI 32.5 ± 4.2 kg/m^2). After surgery, insulin sensitivity indices HOMA-IR and 2h OGIS were no longer significantly different from healthy controls. Whole-body glucose uptake (M value), which was measured during the clamp in Study I, was doubled after surgery but was still lower when compared to lean controls. Twelve of the 18 subjects (67%) with preoperative T2D had remission after surgery (Figure 6). Remission was defined as achieving a glycemia level below the diabetic range in the absence of anti-hyperglycemic medications. Weight loss in patients without remission from diabetes (SG n=4, RYGB n=2) was not significantly different from patients with remission (23.8 ± 10.0 vs. 26.1 ± 7.1 kg). Table 3 presents the LFC, insulin resistance indices, insulin clearance rates and β-cell function in controls and separately in obese patients with and without T2D. The reductions in fasting plasma glucose, in HbA_{1c} and improvement in HOMA-IR were significantly different in subjects with preoperative T2D when compared to subjects without T2D.

Liver enzymes, ALT and gamma-glutamyltransferase (GGT) decreased postoperatively. The serum FFA concentration was higher in obese than in control subjects and was unchanged after surgery. Leptin concentration was halved postoperatively. Change in leptin concentration was related to a reduction in weight, whole-body fat content, visceral and subcutaneous adipose tissue masses (p<0.0001). CRP concentration was normalized, whereas no change in TNF-α, MCP-1, IL-8, resistin or adiponectin levels were measured. Fasting gut hormone concentrations were measured in Study I. No significant changes in GIP, GLP-1 and GLP-2 levels were found postoperatively (data not shown). After SG acylated ghrelin decreased (from 35 ± 30 pg/ml to 16 ± 6 pg/ml, p=0.057 vs. baseline), whereas a minimal rise after RYGB was measured (from 42 ± 38 pg/ml to 46 ± 51 pg/ml, p=0.004 vs. baseline), but the postoperative change in ghrelin levels between the operations was not significant (p=0.101).

Insulin secretion and clearance

Baseline ß-cell glucose sensitivity was low in patients with T2D and it was almost doubled postoperatively, whereas in patients without T2D the ß-cell glucose sensitivity was not different from controls. The baseline ß-cell glucose sensitivity was higher in subject with post-surgery remission from diabetes (49 ± 44 pmol/min/m²/mM) than in those without the remission (39 ± 19 pmol/min/m²/mM) but the difference was not statistically significant. In subjects with preoperative T2D, total insulin secretion during post-surgery OGTT increased significantly, while no change in non-diabetic group was measured. Insulin clearance was enhanced after surgery (Table 2 and 3).

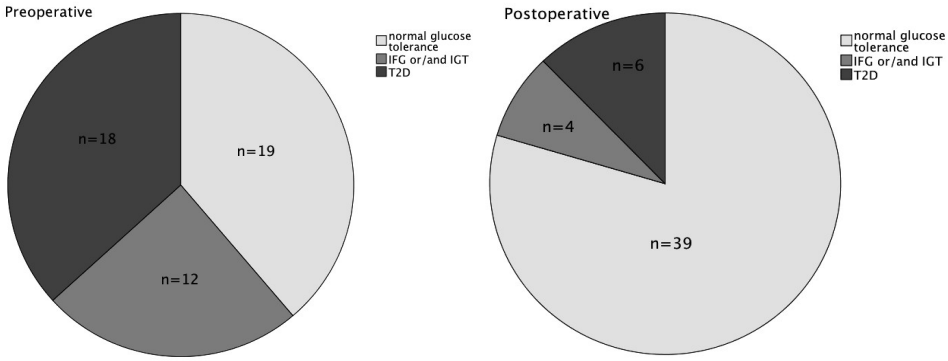

Figure 6. Glycemic control pre- and postoperatively in bariatric patients

6.2 Bariatric surgery leads to a resolution of fatty liver (I, II)

The average LFC in the obese group was higher than in the control group at baseline (8.3 ± 6.8 vs. 1.7 ± 1.4%, p <0.001) and was normalized after surgery (2.0 ± 1.7%) (Figure 7). Twenty-three (50%) of the obese study participants had liver steatosis (LFC ≥5%) preoperatively and 2 (4%) postoperatively. HOMA-IR correlated significantly with LFC at baseline (r=0.449, p<0.0001), but not postoperatively. The liver volume decreased by 20% but was not normalized (Figure 7). Liver biopsy data is shown in Table 3. Four bariatric patients had possible/borderline steatohepatitis at the time of the surgery. None had significant fibrosis or cirrhosis.

Table 2. Characteristics of the Study I and II groups

Variable	Controls (n=25)	Obese (n=46)		
		Presurgery	Postsurgery	P-value (post- vs. presurgery)
Antropometrics				
Body weight (kg)	64.8 ± 7.7	117.1 ± 13.5*	90.0 ± 13.5*	<0.0001
Weight change (kg)			-26.7 ± 8.0	<0.0001
BMI (kg/m^2)	23.0 ± 2.5	42.2 ± 3.9*	32.5 ± 4.2*	<0.0001
Body fat (%)	31 ± 7	49 ± 6*	42 ± 5*	<0.0001
Abdominal SAT (dm^3)	4.6 ± 1.6	18.9 ± 5.2*	12.0 ± 4.3*	<0.0001
VAT (dm^3)	1.2 ± 0.8	4.2 ± 1.6*	2.3 ± 1.1*	<0.0001
Biochemical data				
Fasting glucose (mmol/l)	5.4 ± 0.5	6.3 ± 1.2*	5.2 ± 0.6	<0.0001
2-h glucose (mmol/l)	5.6 ± 1.2	8.7 ± 3.2*	5.8 ± 2.8	<0.0001
Fasting insulin (mU/L)	5.8 ± 3.7	16.1 ± 11.7*	7.5 ± 4.7	<0.0001
Fasting FFA (mmol/l)	0.489 ± 0.204	0.653 ± 0.227*	0.630 ± 0.243*	0.737
HbA_{1c} (%)	5.6 ± 0.3	5.9 ± 0.7*	5.5 ± 0.4	<0.0001
ALT (U/L)	20 ± 10	31 ± 18*	21 ± 18	0.006
ALP (U/L)	50 ± 15	64 ± 18*	65 ± 19*	0.562
GGT (U/L)	26 ± 32	30 ± 15	20 ± 23	0.012
Cholesterol (mmol/l)	4.7 ± 0.9	4.3 ± 0.8	4.2 ± 0.7*	0.448
LDL-C (mmol/l)	2.5 ± 0.7	2.5 ± 0.7	2.3 ± 0.7	0.135
HDL-C (mmol/l)	1.9 ± 0.4	1.2 ± 0.2*	1.4 ± 0.3*	<0.0001
TG (mmol/l)	0.7 ± 0.3	1.3 ± 0.5*	1.0 ± 0.4*	0.001
Leptin (ng/ml)	9.3 ± 6.8	47.1 ± 6.8*	23.2 ± 16.0*	<0.0001
TNF-α (pg/ml)	4.3 ± 2.6	5.9 ± 3.4*	5.8 ± 3.3*	0.396
MCP-1 (pg/ml)	239 ± 119	305 ± 164	293 ± 126	0.110
Adiponectin (μg/ml)	20.2 ± 11.8	20.9 ± 47.8	20.8 ± 12.7	0.905
Resistin (ng/ml)	14.0 ± 4.6	17.6 ± 5.5*	17.7 ± 5.7*	0.617
IL-8 (pg/ml)	4.9 ± 2.0	6.1 ± 2.9	7.0 ± 6.1	0.298
IL-6 (pg/ml)$^{\#}$	1.9 ± 1.6	3.0 ± 1.5	2.1 ± 0.9	0.015
CRP (mg/l)	0.9 ± 0.9	4.5 ± 4.1*	1.5 ± 1.6	<0.0001
Insulin resistance indices				
Whole-body glucose uptake (μmol/kg/min)$^{\#}$	40.3 ± 9.5	12.6 ± 5.8*	23.3 ± 8.1*	<0.0001
HOMA-IR	1.4 ± 0.9	4.9 ± 5.6*	1.9 ± 1.3	0.002
2h OGIS	427 ± 58	327 ± 47*	417 ± 61	<0.0001
Insulin clearance in OGTT	1.94 ± 0.70	1.45 ± 0.52*	2.00 ± 0.92	<0.0001

Data are shown as mean ± SD. The table is a compilation of two data collections (SLEEVEPASS and SleevePET2). *P<0.05 vs. controls, $^{\#}$only SLEEEVEPASS study. BMI, body mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; T2D, type 2 diabetes; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; FFA, free fatty acids; TG, triglycerides; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TNF-α, tumornecrosis factor-alpha; MCP-1, monocyte chemotactic protein-1; IL-8, interleukin 8; C-reactive protein; HOMA-IR, homeostatic model assessment for insulin resistance; OGIS, oral glucose insulin sensitivity; OGTT, oral glucose tolerance test

Table 3. Liver fat content, insulin resistance indices, insulin clearance rates and β-cell function in controls and in obese patients with and without preoperative T2D

Value	Controls (n=25)	Obese ND (n=28)			Obese T2D (n=18)		
		Pre-surgery	Post-surgery	P-value	Pre-surgery	Post-surgery	P-value
Body weight (kg)	64.8 ± 7.7	118.3 ± 14.4*	90.3 ± 13.4*	<0.0001	115.0 ± 11.8*	89.7 ± 13.9*	<0.0001
Weight change (kg)			-27.5 ± 8.0	<0.0001		-25.3 ± 7.9	<0.0001
BMI (kg/m^2)	23.0 ± 2.5	43.0 ± 3.9	32.9 ± 4.0	<0.0001	40.8 ± 3.8	31.8 ± 4.5	<0.0001
Abdominal SAT (dm^3)	4.6 ± 1.6	20.1 ± 5.5*	12.5 ± 4.5*	<0.0001	17.2 ± 4.3*	11.3 ± 4.0*	<0.0001
VAT (dm^3)	1.2 ± 0.8	4.1 ± 1.7*	2.1 ± 0.9*	<0.0001	4.3 ± 1.3*	2.7 ± 1.3*	<0.0001
Liver fat content (%)	1.7 ± 1.4	7.8 ± 6.5	1.9 ± 1.8	<0.0001	9.2 ± 7.4	2.2 ±1.6	0.004
Whole-body glucose uptake (µmol/kg/min)#	40.3±9.5	13.0±6.0*	24.8±8.5*	<0.001	11.9±5.8*	20.9±7.3*	0.021
Fasting glucose (mmol/l)	5.6 ± 1.2	5.7 ± 0.5	5.0 ± 0.4	<0.0001	7.3 ± 1.4*†	5.5 ± 0.6†	<0.0001
Fasting insulin (mU/L)	5.8 ± 3.7	14.0 ± 9.2*	6.3 ± 2.8	<0.0001	19.8 ± 14.6*	9.3 ± 6.3*†	0.003
HbA_{1c} (%)	5.6 ± 0.3	5.6 ± 0.4	5.4 ± 0.4	<0.0001	6.5 ± 0.7*†	5.7 ± 0.4†	<0.0001
Fasting FFA (mmol/l)	0.489 ± 0.204	0.656 ± 0.243*	0.609 ± 0.242*	0.560	0.645 ± 0.120*	0.671 ± 0.250*	0.838
HOMA-IR	1.4 ± 0.9	3.4 ± 2.2*	1.5 ± 0.7	0.001	7.1 ± 7.8*†	2.5 ± 1.7*†	0.025
2 h OGIS	427 ± 58	341 ± 44*	437 ± 56	<0.0001	301 ± 43*†	379 ± 52*†	<0.0001
Insulin clearance in clamp (L/min/m²)#	1.55 ± 0.27	1.01 ± 0.33*	1.24 ± 0.34*	0.007	1.13 ± 0.31*	1.35 ± 0.39	0.011
Insulin clearance in OGTT (L/min/m²)	1.94 ± 0.70	1.42 ± 0.62*	1.97 ± 1.02	0.003	1.42 ± 0.50*	1.95 ± 0.69	0.001
Fasting insulin secretion rate (pmol/min/m²)	75 ± 22	138 ± 51*	86 ± 19*	<0.0001	145 ± 62*	108 ± 36*†	0.085
Total insulin output (nmol/m²)	40.5 ± 12.7	52.4 ± 13.6*	50.9 ± 14.8*	0.647	47.2 ± 11.1	54.9 ± 19.2*	0.020
ß-cell glucose sensitivity (pmol/min/m²/mM)	127 ± 45	137.6 ± 99.3	136.1 ± 67.7	0.630	45.3 ± 35.9*†	78.8 ± 35.6*†	<0.0001

Data are shown as mean ± SD. ND=subjects with normal or impaired glucose tolerance, T2D=subjects with type 2 diabetes. The table is a compilation of two data collections (SLEEVEPASS and SleevePET2). The P column represents 6 months post-surgery vs. pre-surgery, *P<0.05 vs. controls, †P<0.05 vs. obese ND, #only SLEEEVEPASS study. BMI, body mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; HOMA-IR, homeostatic model assessment for insulin resistance; OGIS, oral glucose insulin sensitivity; OGTT, oral glucose tolerance test.

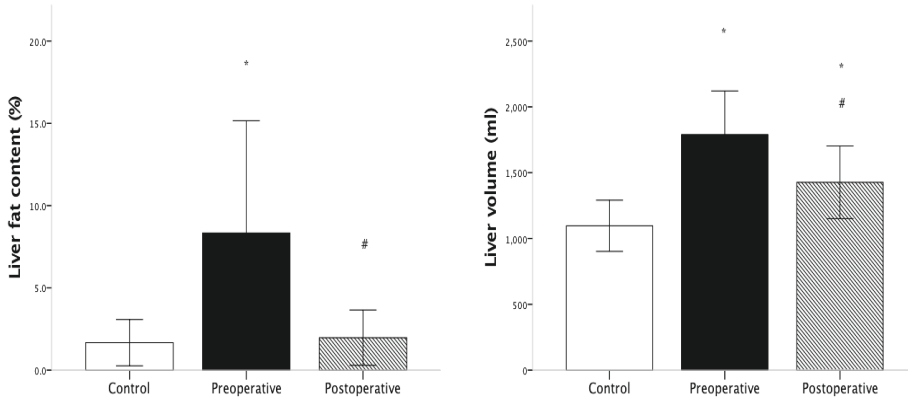

Figure 7. Liver fat content and volume. Data are expressed as mean (SD). *p<0.05 vs. control group and #p<0.05 vs. preoperative. Modified from Immonen et al. 2014 (I).

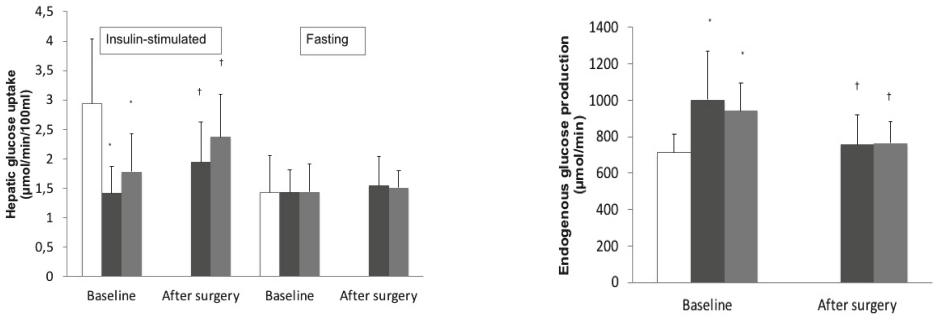

Figure 8. Hepatic glucose uptake measured during the euglycemic hyperinsulinemic clamp and in fasting and endogenous glucose production in fasting at baseline and after surgery. White bar, lean control subjects; dark grey bar, subjects with type 2 diabetes; light grey bar, non-diabetic subjects. Data are mean (SD). *p<0.05 vs. control group and †p<0.05 vs. baseline (preoperative). Modified from Immonen et al. 2014 (I).

Table 3. Liver histology in obese patients. Liver biopsy specimens were taken during the bariatric procedure.

Item	Definition	Score	Obese patients (n)
Steatosis grade	<5%	0	25/48* (52.1%)
	5%-33%	1	15 (31.3%)
	>33%-66%	2	7 (14.6%)
	>66%	3	1 (2.1%)
Fibrosis stage	None	0	32 (68.1%)
	Perisinusoidal or periportal	1	14 (29.8%)
	Perisinusoidal and portal/periportal	2	1 (2.1%)
	Bridging fibrosis	3	0 (0%)
	Cirrhosis	4	0 (0%)
Lobular inflammation	No foci	0	42 (89.4%)
	<2 foci	1	5 (10.6%)
	2-4 foci	2	0 (0%)
	>4 foci	3	0 (0%)
Ballooning	None	0	18 (38.3%)
	Few balloon cells	1	20 (42.6%)
	Many cells	2	9 (19.1%)
Diagnostic classification	Not steatohepatitis	0	43 (91.5%)
	Possible/borderline	1	4 (8.5%)

*One patient did not have a liver biopsy for histological analysis

6.3 Surgery-induced weight loss alleviates liver insulin resistance (I)

Insulin-stimulated HGU in obese subjects was significantly decreased compared to lean control subjects at baseline. At six-months follow-up, HGU was improved by 33% in patients without diabetes and by 36% in patients with T2D compared to

baseline (Figure 8). Fasting HGU was indifferent in the groups already at baseline (Figure 8).

EGP was elevated at baseline when compared to controls and decreased by 19% in non-diabetic patients after surgery, while the respective decrease in T2D patients was 25% (Figure 8). Consequently, at six-months follow-up, no significant differences between lean control and obese patients in EGP were measured.

In obese subjects, insulin-stimulated HGU correlated both pre- and postoperatively with whole-body glucose uptake (PRE: r=0.54, p=0.007; POST: r=0.52, p=0.011) and with visceral fat mass (PRE: r=-0.56, p=0.007; POST: r=-0.55, p=0.015). The correlations between HGU and whole-body glucose uptake and EGP at baseline are presented in Figure 9. No significant associations between NAFLD activity score (NAS) and HGU or M value were found. Postoperative reductions in LFC and liver volume were related to improvement in insulin-stimulated HGU (r=0.46, p=0.049 and r=0.54, p=0.012, respectively). No significant differences in whole-body glucose uptake, EGP or HGU were found when comparing the different surgical procedures.

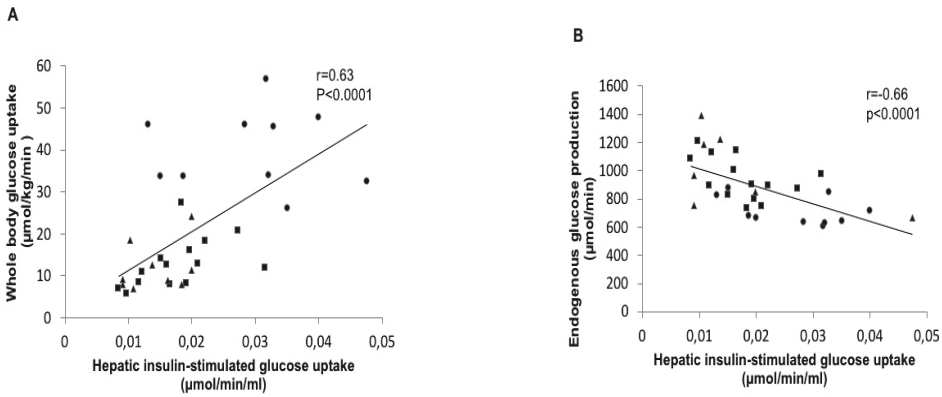

Figure 9. Relationship between insulin-stimulated hepatic glucose uptake and whole-body glucose uptake (A) and endogenous glucose production (B) in pooled analysis of control subjects (circles), obese type 2 diabetic (triangles) and obese non-diabetic (squares) subjects at baseline. Modified from Immonen et al. 2014 (I).

6.4 Increased liver fatty acid uptake is partly reversed after bariatric surgery (II)

Obese subjects had significantly higher hepatic fatty acid uptake when compared to control subjects both before and after surgery (Figure 10). Whole liver FFA uptake decreased by 20% after surgery (p=0.03) (Figure 10), but when expressed per 100 ml of tissue, no statistically significant changes were measured (p=0.29) (Figure 10). Postoperative changes in liver FFA uptake between SG and RYGB were not significantly different. When obese patients were divided into two groups with a LFC below (n=13) or above (n=11) 5%, no difference in liver FFA uptake was measured. Since fractional uptake rates (FUR) of FTHA were similar in both control and obese groups (18.0 ± 3.9 1/min vs. 18.2 ± 3.7 1/min, p=NS), the differences in FA uptake values were derived from the higher concentration of circulation FFAs in obese participants. No significant change in FUR or in plasma FFA level was measured after surgery. However, due to reduction in liver volume, the whole liver uptake of FFAs was decreased after surgery. SG and RYGB resulted in a similar effect on liver FFA uptake.

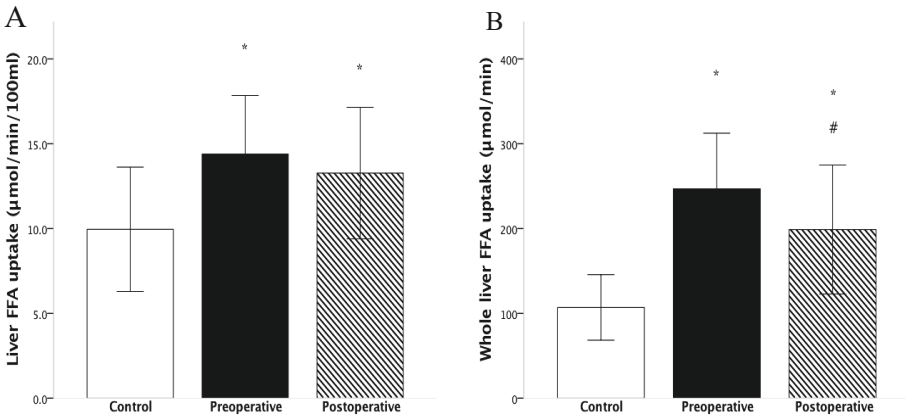

Figure 10. Liver FFA uptake per volume of tissue (A) and whole liver FFA uptake (B) in control subjects and in obese before and after surgery. Data are mean (SD). *p<0.05 vs. control, and #p<0.05 vs. preoperative. Modified from Immonen et al. 2018 (II).

At baseline, in pooled data, whole liver FFA uptake was associated with TGs (r=0.450, p=0.005), HDL cholesterol (r=0.501, p<0.0001), HOMA-IR (r=0.345, p=0.031), ALT (r=0.419, p=0.009), CRP (Figure 11) and with body adiposity:

visceral adipose tissue mass (Figure 11), BMI (r=0.736, p<0.0001), whole body fat content (r=0.656, p<0.0001), abdominal subcutaneous adipose tissue mass (r=0.731, p<0.001) and leptin (r=0.682, p<0.0001). The relationship between LFC and liver FFA uptake was not statistically significant. No association between NAFLD activity score (NAS) and liver FFA uptake was observed.

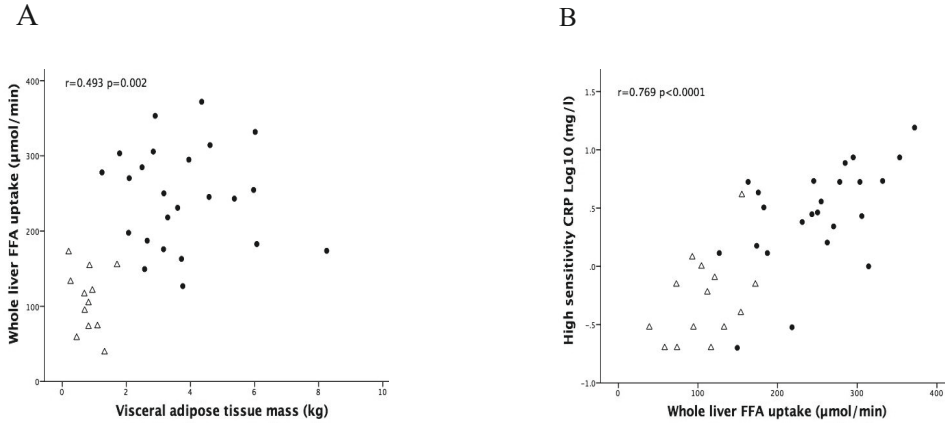

Figure 11. Correlations between visceral adipose tissue mass and whole liver FFA uptake (A) and whole liver FFA uptake and CRP (B) in pooled analysis of control subjects (white triangles) and obese (black circles) at baseline.

6.5 Portal venous blood flow and liver fatty acid uptake are inversely related (II)

Portal vein blood flow was not significantly different between lean and obese participants at baseline but was enhanced by 14% postoperatively (Figure 12). Liver arterial blood flow was increased in obese subjects before surgery and did not change significantly after surgery (Figure 12). Cardiac output significantly decreased after surgery (from 6.9 ± 1.5 to 6.1 ± 1.3 l/min, p=0.03 vs. preoperative).

Portal vein blood flow in obese subjects preoperatively was inversely correlated with liver FFA uptake (Figure 13). Postoperatively, portal blood flow correlated inversely with visceral fat mass (r=-0.619, p=0.004), but not with liver FFA uptake (r=0.254, p=0.266). Greater weight loss was related to a greater postoperative increase in portal venous blood flow.

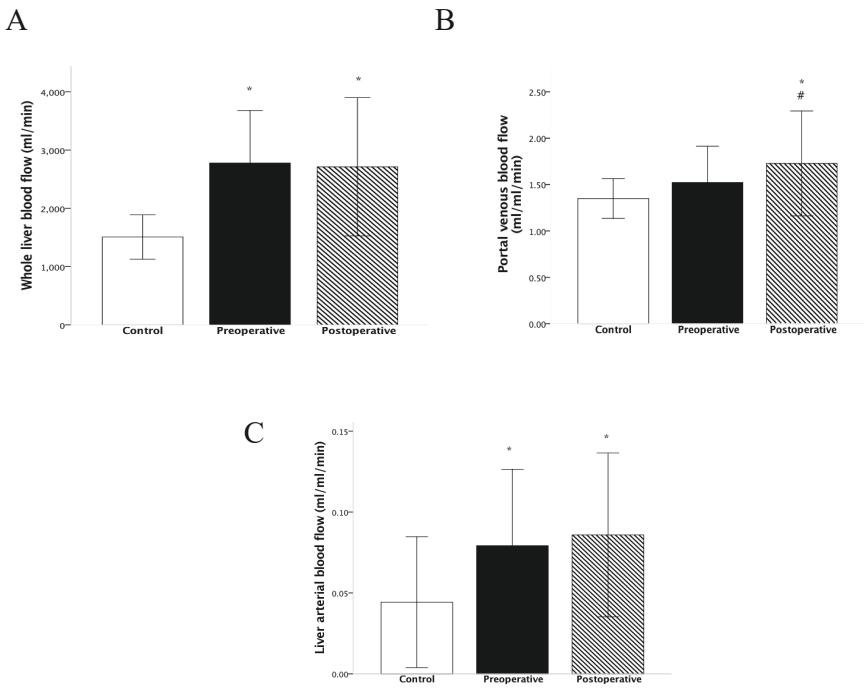

Figure 12. Whole liver (A), portal venous (B) and arterial blood flow (C) in control subjects and in obese subjects before and after surgery. Data are mean (SD). *p<0.05 vs. control and #p<0.05 vs. preoperative. Modified from Immonen et al. 2018 (II).

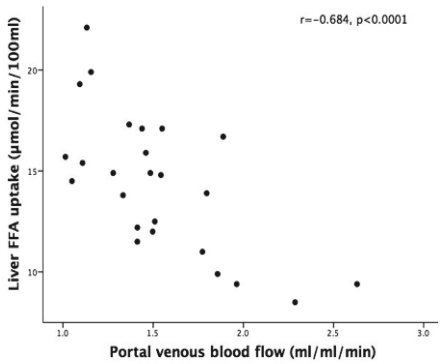

Figure 13. Correlation between portal venous blood flow and liver FFA uptake in obese subjects at baseline.

6.6 Brain glucose metabolism associates with liver glucose production (III)

At baseline before surgery, insulin-stimulated BGU was ~10% higher in obese subjects as compared to control subjects. Although the insulin infusion rate during the clamp was adjusted for body surface area, plasma insulin levels in steady-state were higher in obese subjects due to lower insulin clearance (Figure 15). Different plasma insulin levels between obese subjects and controls did not account for the difference in BGU. Insulin suppressed EGP was higher in obese subjects as compared to controls. A significant positive association between insulin-stimulated BGU and EGP was found in the obese subjects (p=0.003) (Figure 14), but not in controls. The association between insulin-stimulated BGU and EGP remained significant after accounting for BMI, age, or insulin levels (p=0.002, p=0.005, and p=0.003 respectively). In contrast to hyperinsulinemia, in a fasting state, no associations between EGP and brain glucose uptake were found.

IL-6 and CRP were positively correlated with insulin-stimulated BGU (r=0.52, p=0.006 at the cluster level for IL-6 and r=0.52, p=0.007 at the whole brain level for CRP). Additionally, correlation analysis showed positive correlations between insulin-stimulated brain ROI and the aromatic amino acid phenylalanine and the branch-chained amino acid leucine. The correlations between insulin-stimulated BGU and phenylalanine and leucine persisted significant also after accounting for whole-body glucose uptake (r=0.68, p=0.01 and r=0.67, p=0.01, respectively).

Postoperatively insulin-stimulated BGU was decreased ~5% (p=0.03 vs. baseline) but was still higher than in controls. Insulin-suppressed EGP was showed a slight, non-significant decrease after surgery and was no longer different from the value in controls. As before surgery, insulin-stimulated BGU associated positively with EGP (p=0.01) and the association persisted after correcting for age and BMI (p=0.01) (Figure 14). No effect of surgical procedure on the association between EGP and BGU was found. The reductions in EGP and BGU were not interrelated. No association between plasma IL-6 or CRP levels and insulin-stimulated BGU after surgery was found.

Patients were followed up to 2 to 3 years. They remained weight-stable (BMI 32.1 [4.1] and 32.1 [3.8] kg/m^2, respectively). A higher insulin-stimulated BGU before surgery was associated with smaller decrease of fasting plasma glucose at the 2-year follow-up (Figure 16). This association persisted after correcting for baseline BMI, and M-value. In 13 study subjects who had complete follow-up data for up to 3-years, baseline insulin-stimulated BGU continued to predict a smaller decrement of fasting plasma glucose levels (p=0.006). No association was found

between insulin-stimulated BGU and follow-up BMI or HbA_{1c} at the 2 or 3-year follow-up.

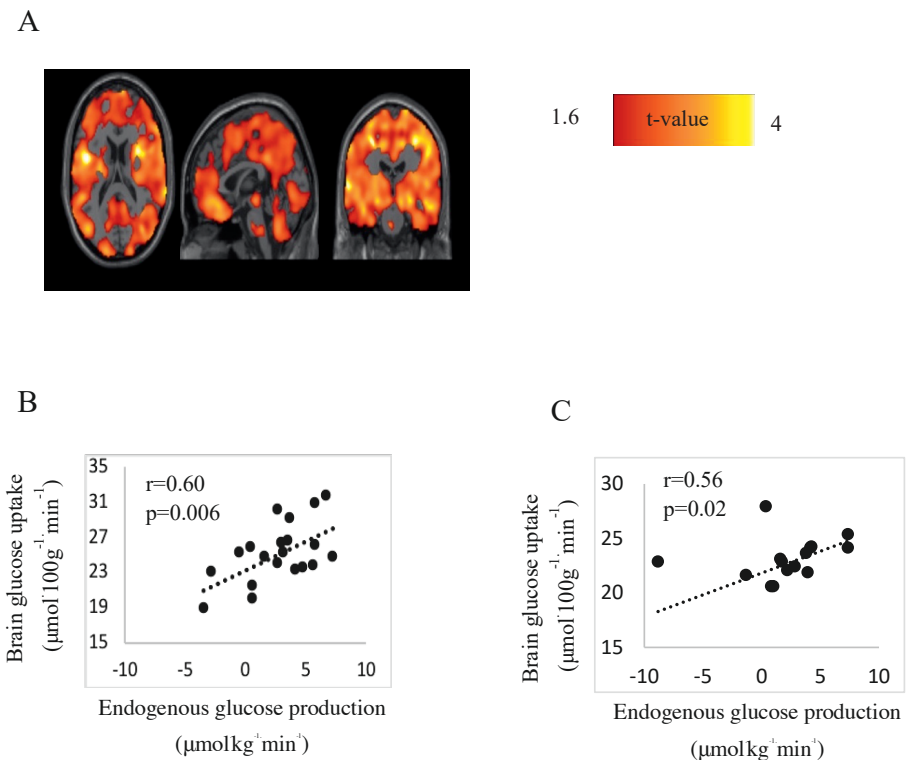

Figure 14. SPM images of the association between insulin-stimulated brain glucose uptake (BGU) and insulin-suppressed endogenous glucose production (EGP) in morbidly obese subjects before bariatric surgery (A). $p<0.05$ cluster level FDR corrected. The marked brain areas show where the correlation between BGU and EGP was statistically significant. Higher t-values denote stronger correlation between BGU and EGP. The scatterplot of the association between BGU and EGP before bariatric surgery (B) and after surgery (C). The data of BGU presented are extracted as a global region of interest from SPM for visualization. Modified from Rebelos et al. 2018 (III).

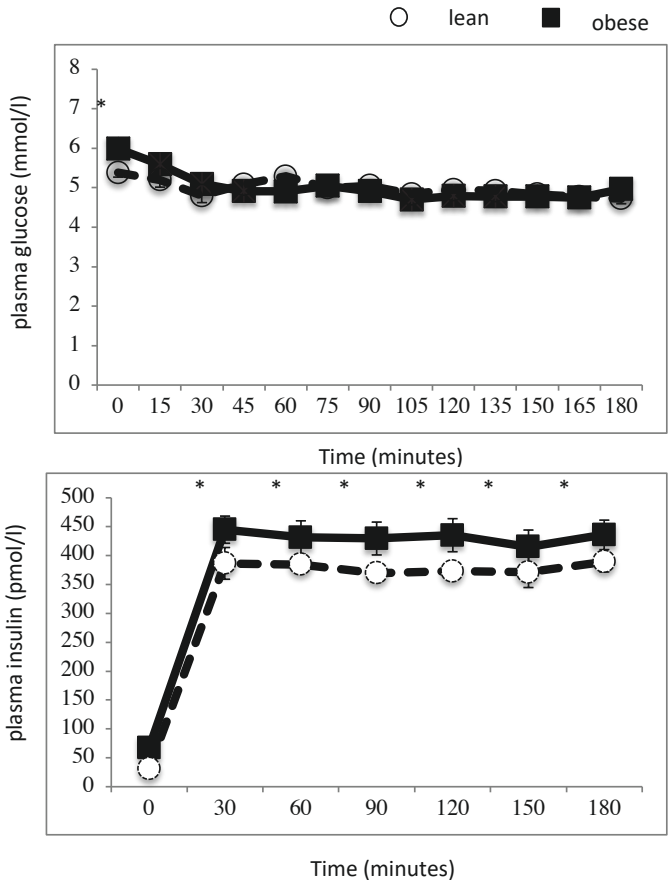

Figure 15. Time course of blood glucose and insulin during the hyperinsulinemic euglycemic clamp in the two groups. *p<0.05. Modified from Rebelos et al. 2018 (III).

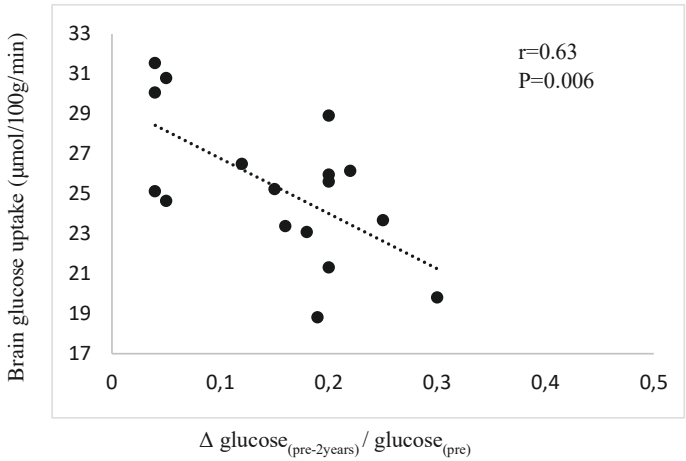

Figure 16. Association between insulin-stimulated brain glucose uptake and change in plasma glucose at the 2 year follow-up. Modified from Rebelos et al. 2018 (III).

7 DISCUSSION

The current study provided novel data on the mechanisms of improved hepatic insulin sensitivity following surgery-induced weight loss. An important finding in Study 1 was the postoperative increase in insulin-stimulated HGU along with normalization of EGP and liver fat content. Study II demonstrated that hepatic FFA uptake is increased in morbid obesity and is partly reversed after surgery. Furthermore, Study II provided novel data on the changes in liver blood flow in obesity and after surgery-induced weight loss. Finally, the brain-liver axis was explored *in vivo* using PET. The novel human data suggests that there is an association between EGP and brain glucose uptake in morbidly obese subjects. This association persisted after bariatric surgery when patients had achieved significant weight loss but were still obese.

7.1 Effects of bariatric surgery on body anthropometry and biochemistry (I, II)

As expected, bariatric surgery led to a marked weight loss in six months. The amount of weight loss was greater after RYGB than after SG. These results are consistent with the literature, which shows that RYGB is associated with greater weight loss than SG, but most of the studies do not have sufficient statistical power to detect differences between the two procedures (Li et al., 2016, Schauer et al., 2017, Peterli et al., 2018, Salminen et al., 2018, Zhang et al., 2014). Despite a difference in weight loss, no significant differences in metabolic effects between the procedures were found. Surgery-induced weight loss resulted in a marked improvement in whole-body insulin sensitivity as measured by insulin-stimulated glucose disposal (M value) during the hyperinsulinemic euglycemic clamp (Study I), HOMA-IR and 2 h OGIS (Study I and II). The hyperinsulinemic euglycemic clamp, first described by DeFronzo and colleagues in 1979, remains the golden standard for quantifying insulin sensitivity *in vivo* in humans (DeFronzo et al., 1979). The clamp technique is complex to undertake and has prompted the use of alternative methods, such as HOMA-IR and OGIS index. The HOMA-IR index is a simple, commonly used method, determined from results on fasting samples and therefore provides an estimation of hepatic insulin resistance (Matthews et al., 1985). The OGIS index provides an estimate of insulin sensitivity calculated using a model-derived formula from the OGTT glucose and insulin concentration (Mari et al., 2001). OGIS has proven to have a strong correlation with the hyperinsulinemic euglycemic clamp (Mari et al., 2005). The Matsuda index of insulin sensitivity in another OGTT derived index, which has shown a good correlation with the insulin clamp results (Matsuda and DeFronzo, 1999). The

Matsuda index seems to be useful for identification of insulin resistance (Stancakova et al., 2009; Lorenzo et al; 2015). In the present study, in patients with T2D before surgery, 12 out of 18 were in remission six months after surgery. Furthermore, the parameters of β-cell function were significantly improved, especially in subjects with T2D.

Insulin clearance was enhanced after surgery. ~80% of endogenous and ~50% of intravenously infused insulin is cleared by the liver (Ferrannini et al., 1983). Insulin clearance is decreased in obese (Meistas et al., 1983) and increases with weight loss (Viljanen et al., 2009). Decreases in insulin clearance contribute to hyperinsulinemia in individuals with T2D (Kotronen et al., 2009). Results from the current study show that the fasting insulin concentration was normalized after surgery, as a result of enhanced insulin clearance and decreased basal insulin secretion.

Even though marked metabolic improvements occurred postoperatively, changes in circulating cytokine and adipokine levels were small. The circulating CRP level normalized after surgery. The levels of circulating leptin were decreased along with the reduction in fat mass, as previously reported (Viljanen et al., 2009). Increases in adiponectin have been reported to associate with improved hepatic insulin sensitive measured with HOMA-IR after weight loss (Lin et al., 2007). However, in the current study, no significant changes in circulating adiponectin levels were measured.

Both RYGB and SG lead to a marked augmentation of the gastrointestinal hormone response to a meal (Batterham and Cummings, 2016). Unfortunately, gut hormone levels were measured only in a fasting state in the current study. The current study found that the fasting acylated ghrelin level decreased after SG, but not after RYGB. This finding was expected because the stomach produces the majority of ghrelin and is in agreement with other studies (Ramon et al., 2012; Peterli et al., 2012). According to a ghrelin hypothesis, the compromised secretion of ghrelin contributes to the anorexic and antihyperglycemic effects of bariatric surgery (Thaler and Cummings, 2009).

7.2 Effect of bariatric surgery on liver glucose metabolism (I)

In Study I, the focus was on the hepatic insulin sensitivity, and it was distinguished into the endogenous glucose production and to the hepatic glucose uptake. HGU, EGP and LFC were studied in diabetic and non-diabetic subjects before and after bariatric surgery. HGU was measured both in fasting and during the euglycemic hyperinsulinemic clamp. Preoperatively both obese subgroups (patients with and

without T2D) had significant insulin resistance at the whole-body level and in the liver. Insulin-stimulated HGU was reduced and fasting EGP increased in both groups when compared to controls. At six-months follow-up, both in non-diabetic and diabetic obese groups insulin-stimulated HGU was increased by 30–40%, the EGP and liver fat normalized and the liver volume decreased but was not normalized to the lean control levels. The post-surgery changes were not significantly different between non-diabetic and diabetic groups. Fasting HGU was already similar among the groups at baseline and did not change after surgery. A previous study by Iozzo and colleagues (2003) demonstrated that insulin-stimulated HGU is reduced in individuals with T2D. The current study showed that insulin-stimulated HGU was also impaired in non-diabetic, morbidly obese individuals. Viljanen and colleagues (2009) reported that 6-weeks of VLCD, in obese, non-diabetic patients, resulted in no change in insulin-stimulated HGU. The reasons for the differences in findings might be that the subjects in the present study were more obese at baseline, and the weight loss was much greater than after VLCD (baseline BMI was 43.1 ± 3.7 kg/m^2 vs. 33.7 ± 4.4kg/m^2 and weight loss 27.8 ± 7.6 kg vs. 11.2 ± 2.9 kg, respectively). In the previous study, the mean HGU (2.3 μmol/min/100ml) was similar to the postoperative HGU in the current study in non-diabetic obese subjects (2.4 μmol/min/100ml) for the same BMI.

The changes, from baseline to follow-up, in LFC and liver volume were significantly associated with the change in HGU. Furthermore, HGU correlated negatively with the amount of visceral fat. These results support the negative role of FFAs, which are derived from adipose tissue and from hepatic TGs, in the regulation of HGU by insulin. This explanation is consistent with the findings in a previous PET study, which showed that a three-fold elevation of circulating FFAs led to a 25% reduction in insulin-stimulated HGU in healthy subjects (Iozzo et al., 2004).

As well as the post-surgery increase in insulin-stimulated HGU, EGP showed a considerable improvement after surgery. The plasma fasting insulin concentration was normalized, indicating that the reduction in EGP had led to an improvement in liver insulin resistance. Rapid improvements in T2D occur after RYGB (Pories et al., 1995). Dunn and colleagues (2012) reported that liver insulin sensitivity, which was calculated as the inverse product of EGP and fasting plasma insulin level, was improved already one month after RYGB, while no change in peripheral insulin sensitivity was found. The finding might be explained by the caloric restriction, as a two-day caloric restriction (VLCD; 450 kcal/day) alone has been reported to decrease basal EGP without altering whole-body insulin sensitivity (Jazet et al., 2005). Jackness and colleagues (2013) compared RYGB patients with patients consuming a VLCL (500 kcal/day) and did frequently sampled intravenous glucose tolerance tests before and 3 weeks after the surgery. They

found that RYGB and VLCD resulted in similar improvements in insulin sensitivity and β-cell function in the short term. Camastra and colleagues (2011) reported that basal EGP was decreased in proportion to weight loss one year after RYGB. In the current study EGP was related to BMI and M at baseline, but not at follow-up, also the changes were not related (data not shown). However, HGU and EGP were interrelated and insulin-stimulated HGU in obese subjects correlated with peripheral insulin sensitivity both before and six months after surgery. The reason for some discrepancies in the results may be the length of follow-up; patients are more weight-stabilized one year after the surgery than six months after.

In spite of the fact that marked weight loss occurred, patients were still obese after surgery (mean BMI 32.5 ± 4.2 kg/m^2). The liver volume decreased by 20% with weight loss but was not normalized. This finding is consistent with the general organomegaly of obesity (Ferrannini et al., 1997). HGU is expressed as μmol per min by ml of liver volume. The larger liver size in obesity may compensate for the reduced glucose uptake.

Overall, it seems that the gain in liver insulin sensitivity has an important role in early remission in T2D (Dunn et al., 2012) and greater weight loss is needed to achieve improvement in peripheral insulin sensitivity after bariatric surgery (Campos et al., 2010, Batterham and Cummings, 2016). The current study showed that six months postoperatively, both hepatic and peripheral insulin sensitivity are greatly improved.

Both obese groups (subjects with and without T2D) showed marked insulin resistance at the whole-body level and in the liver, and the postoperative changes were comparable between the groups. Subjects with T2D had a significant impairment in preoperative ß-cell glucose sensitivity when compared to lean controls and subjects without T2D. After surgery, ß-cell glucose sensitivity was largely recovered in subjects with a preoperative diagnosis of T2D. ß-cell glucose sensitivity predicts remission of T2D after surgery (Nannipieri et al., 2011). The findings of the current study are consistent with this, though the differences did not reach significance. In the study of Dunn and colleagues (2012) preoperative and postoperative hepatic glucose production was lower in subjects with remission of diabetes than in subjects who remained diabetic after surgery. The findings of the present study do not support the previous findings, but this might be explained by the smaller sample size.

The impact of bariatric surgery on liver metabolism may be procedure dependent. However, no significant differences between the two procedures in terms of HGU, EGP or whole-body insulin sensitive, neither in hepatic FFA uptake or blood perfusion was found. Although the design of the study allowed for the comparison

of SG and RYGB, the power of the present study is a limiting factor and possibly interferes with the data interpretation.

7.3 Bariatric surgery and liver lipid metabolism (II)

With respect to the first research question, insulin-stimulated HGU was impaired in obese subjects and improved after bariatric surgery. The results from the previous studies reinforce the negative role of FFAs in the regulation of HGU (Iozzo et al., 2004). Study II was set out to explore whether there are changes in hepatic FFA uptake after bariatric surgery and are these changes are related to a resolution of fatty liver.

The results of the study demonstrated that liver fatty acid uptake in the fasting state is increased in morbid obesity. Liver FFA uptake, expressed per ml of tissue, was ~40% higher, and the whole liver uptake ~130% higher in obese when compared to lean subjects. After surgery the liver TG content was normalized and liver fatty acid uptake was reduced, but not normalized to controls (~30% higher per ml of tissue and ~90% higher depot uptake). This is consistent with previous observations, which showed that in obese non-diabetic subjects, liver FFA uptake (per ml of tissue) was reduced by 26% after a 6-week period of VLCD (Viljanen et al., 2009). The persistence of high FFA uptake as compared to controls, together with the pronounced reduction in intrahepatic TG content, suggests that after surgery, FFAs may be directed into oxidation instead of being stored in the liver.

It has been demonstrated that increased physical activity decreases liver FFA uptake and that FFA uptake is associated with the whole-body fat percentage (Hannukainen et al., 2011). In line with the above-mentioned study, the results here showed that liver FFA uptake was related to body fat stores: whole body fat percent, and the amount of subcutaneous and visceral fat. Enlarged body fat stores are accompanied by an increase in FFA release. In a fasting state, plasma FFAs result almost solely from adipose tissue lipolysis. Rigazio and colleagues (2008) showed that reduction in adipose tissue FFA release, provoked by the use of acipimox, decreased liver FFA uptake and enhanced liver insulin sensitivity, but no change in LFC was found. It is also reported, that after an overnight fast, intrahepatic TG are mainly originated from the FFAs released from the adipose tissue (Donnelly et al., 2005). In the present study, no association between adiposity and liver FFA uptake was measured after surgery. As the fasting insulin concentration was normalized postoperatively, the inhibition of lipolysis was downgraded and the serum FFA level, despite reduction in fat mass, was unaffected. Cumulatively, the present data suggest that liver FFA uptake is promoted by expanded body fat masses and high circulating FFA levels.

When interpreting the results, it should be taken into account that peripheral FFA concentrations were used, and that level of circulating FFAs in the portal vein might be higher than the peripheral FFA level. Iozzo and colleagues (2010) reported that obese patients have higher visceral adipose tissue fatty acid contribution compared to lean subjects. The difference in FFAs, which appear in the portal vs. systemic circulation, was significant in obese individuals, and less obvious in the lean controls. Consequently, when using peripheral FFA levels, the rate of liver FFA uptake may be more severely underestimated when visceral fat masses are enlarged.

Obesity is often associated with hepatic steatosis. The present study showed that surgery-induced weight loss had a dramatic effect on hepatic fat; LFC was normalized when measured six months after surgery. While, increased liver FFA uptake is likely to play a role in intrahepatic TG accumulation, FFA uptake and LFC were not related. Besides increased FFA uptake, other factors, such as increased *de novo* lipogenesis, reduced lipoprotein secretion and impaired fatty acid oxidation, might be part of the cause of TG accumulation and the progression of NAFLD. Increased liver FFA uptake could enhance lipid oxidation. Earlier PET studies with [^{11}C]palmitate have demonstrated that obese subjects have a 2-fold increase in hepatic lipid oxidation (Iozzo et al., 2010). Increased hepatic oxidation may produce ROS and promote the progression from steatosis to NASH.

FFAs cross the hepatocyte membrane by a combination of facilitated transport and diffusion (Berk et al., 1999). Liver FFA uptake and extraction are enhanced under conditions of increased hepatic fatty acid oxidation, such as caloric restriction. Fasting promotes lipolysis in adipose tissue, resulting in release of FFAs, which are converted into ketone bodies in hepatic mitochondria thought β-oxidation and ketogenesis. Glucose and ketone bodies produced in the liver provide the essential metabolic fuels for extrahepatic tissues during starvation and exercise. According, the data from the food records in the present study (data not shown), the study participants were energy deficit postoperatively. [^{18}F]FTHA is trapped intracellularly in proportion to its uptake, reflecting both fatty acid storage and oxidation rates (Iozzo et al., 2003), therefore it was not possible to directly measure hepatic fatty acid oxidation rate with the tracer used in the present study. The decrease in insulin level, as seen postoperatively, could be expected to decrease intrahepatic fat by diverting FFA flux from lipogenesis to oxidation.

Interestingly, CRP, an acute phase reactant that reflects low-grade systemic inflammation and is produced by liver, was related to liver FFA uptake. In Study II, only two obese subjects had possible NASH based on liver biopsy specimens, therefore it was not possible to evaluate further the potential role of increased FFA uptake in NASH. The findings in liver biopsies may be to some extend limited by

the fact that histological analyses were performed from biopsies taken during the surgery after a 4-week VLCD. Some of the pathological changes may have already been improved after the preoperative VLCD.

7.4 Liver blood flow in obesity and after bariatric surgery (II)

Measuring liver FFA uptake rate is important in understanding the mechanisms of metabolic changes after bariatric surgery. Uptake of FFAs by hepatocytes depends on the supply of FFAs to liver and on the capacity of the cells for FFA uptake. Data from literature on hepatic blood flow with reference to liver metabolism was limited. In Study II, surgery-induced changes in hepatic blood flow in conjunction with FFA uptake, were assessed. This study provides novel data on changes in liver blood flow in obesity and after surgery-induced weight loss. Perfusion incorporates both micro- and macrocirculatory contributions. Arterial blood flow is much lower than portal venous blood flow, as also shown in the present study. The arterial perfusion reported here is lower than in a validation study, in which the agreement with doppler ultrasonography and [^{15}O]H_2O-PET was good for total and portal liver perfusion, but poor for arterial perfusion (Slimani et al., 2008). Some of the discrepancies between the studies might also be attributed to the heterogeneity of arterial perfusion. The present study showed that portal blood flow per volume of tissue increases postoperatively, but as the liver volume decreases along with surgery-induced weight loss, the whole organ blood flow is unchanged. The cause for the increase in portal vein blood flow is unknown but it may be related to postoperative changes in vasoactive substances, such as incretin hormones. It is of note that cardiac output decreases after surgery and does not explain the changes in hepatic blood flow. Honka and colleagues (2018) studied portal venous blood flow in response to a mixed-meal in morbidly obese patients with T2D before bariatric surgery (either SG or RYGB) and early after surgery using [^{15}O]H_2O-PET. They found that the portal vein flow response to a meal was enhanced postoperatively without significant differences between SG and RYGB. These findings suggest that post-surgery enhancement in splanchnic blood flow results in improved nutrient absorption and post-prandial glucose tolerance and is one of the mechanisms of improved glycemic control after bariatric surgery. One interesting finding in the present study was that portal vein blood flow and hepatic FFA uptake were inversely related. The data here suggest that liver FFA uptake is suppressed at higher portal blood flow rates. One possible explanation is that the postoperative increase in hepatic perfusion limits the amount of FFA uptake and exposure in the liver by increasing substrate washout from the liver circulation.

7.5 Association between brain glucose uptake and liver glucose metabolism (III)

In recent years, there has been a growing interest in exploring the interrelation between the insulin action on the brain and hepatic glucose metabolism. Whereas the evidence from preclinical data is continuously increasing (Obici et al., 2002; Meek et al., 2013; German et al., 2011), human data is still limited. The aim in this study was to test whether the brain affects endogenous (mainly hepatic) glucose production *in vivo* in humans. The study demonstrated that insulin-suppressed EGP and insulin-stimulated BGU were associated in morbidly obese subjects. The association persisted six months after bariatric surgery when patients had achieved a significant weight loss. In contrast to the findings in the morbidly obese, in healthy lean controls BGU and EGP were not related.

The hypothalamus is a critical brain area for the control of body weight and energy homeostasis (Hochberg and Hochberg, 2010). Heni and colleagues (2017) showed that intranasal insulin application decreased EGP in lean but not in overweight subjects and that the hypothalamus and the striatum were involved in this process. Unfortunately, the spatial resolution of the PET scanner used in the present study (6-8 mm) did not permit the detection of as small a region as the hypothalamus. The data from the current study suggest that many areas of the brain are involved in the relation between brain glucose metabolism and hepatic glucose production.

The markers of systemic inflammation, IL-6 and CRP, were positively correlated with insulin-stimulated BGU. It is shown that a high-fat diet and obesity are associated with neuroinflammation (Thaler et al., 2012). Zimmer and colleagues (2017) demonstrated using PET imaging that the brain [^{18}F]FDG uptake is driven by astrocytes. This finding is in line with the astrocyte-neuron lactate shuttle (ANLS) model. According to the ANLS, astrocytes take up glucose, convert it to lactate and supply lactate to the neurons (Pellerin and Magistretti, 1994), but this concept is in debate (Diaz-Garcia et al., 2017). However, taken together, a possible interpretation of the data is that the high BGU, is at least in some extend, due to obesity-induced astrocyte proliferation. An obesity-induced astrocyte proliferation could explain an association between brain and EGP (Parsons and Hirasawa, 2010).

Insulin-stimulated BGU predicted the metabolic outcome after bariatric surgery. Even after adjusting for BMI and whole-body insulin sensitivity, insulin-stimulated BGU at baseline was associated with a smaller decrease in fasting glucose levels at follow-up. Impaired β-cell glucose sensing and decreased whole-body insulin sensitivity are known predictors of deteriorating glucose tolerance in non-diabetic subjects (Walker et al., 2005). In the present study the insulin-

stimulated BGU at baseline did not predict worse HbA_{1c} levels or weight regain at the 2 or 3-year follow-up. High BGU could predict early metabolic deteriorations that are primarily linked to increased EGP leading to fasting glycemia, as fasting glucose concentration reflects basal EGP. In order to further test the hypothesis that high insulin-stimulated BGU is a predictor of a poorer future metabolic outcome, metabolite data was analyzed. A positive association was found between BGU and known predictors of increased risk of T2D such as the branched-chain amino acid leucine and the aromatic amino acid phenylalanine (Guasch-Ferre et al., 2016).

7.6 Strengths, limitations of the study and future aspects

In the current study, liver metabolism and the brain-liver axis were explored using the state of art methodology for the study of *in vivo* human metabolism: hyperinsulinemic euglycemic clamp, quantitative PET imaging, MRS and liver biopsies. The first question in this study sought to determine the mechanisms of increased liver insulin sensitivity and the remission of T2D after surgery and to evaluate whether the gain in hepatic insulin sensitivity differs between obese subjects with and without T2D. Several factors, such as sex, age, genetics, BMI, fat distribution, glycemic status, duration of T2D, medications, impact of preoperative diet, stimulus used (oral or intravenous), oral nutrient stimuli, method used to assess glycemic response and time after operation, have to be taken account when evaluating the results from studies examining the effects of bariatric surgery. The subjects with T2D included in the current study had mild to moderate diabetes as considered by the degree of metabolic control (mean HbA_{1c} 6.5 ± 0.7%) and intensity of anti-diabetic treatment (only oral hypoglycemic agents). Insulin treated T2D patients were excluded. GLP-1 analogues were at the time the study was conducted. The results may not be directly generalized to patients with poorly controlled or more advanced disease. In addition, the group of subjects without diabetes was heterogeneous. There were subjects with IFG and/or IGT in this group. When comparing the liver metabolism in subjects with normal glucose tolerance, impaired glucose tolerance and T2D, the patients with impaired glucose tolerance seem to have a liver glucose metabolism behavior between the T2D and normal glucose tolerant group (data not shown). Moreover, only female subjects were recruited in Study II and only 16% of the study subjects in Study I were men. To apply the findings to a larger population, more men should be included.

It is possible that bariatric surgery itself may have a specific effect on HGU, given that the gastrointestinal tract is drained directly into the liver. HGU is known to be dependent on the glucose load and insulin concentration. GLP-1 has been shown

to increase HGU independent of insulin or glucagon secretion in canines (Dardevet et al., 2005). Exenatide (a long-acting mimetic of the GLP-1) stimulates HGU during the hyperglycemic hyperinsulinemic clamp (Zheng et al., 2009). RYGB and SG enhance postprandial incretin stimulation (Leferrere et al., 2009; Batterham and Cummings, 2016). Thus, postprandial changes in HGU may be greater than those measured during the euglycemic hyperinsulinemic clamp in the current study setting.

A genetic predisposition contributes to a metabolic phenotype. For instance, a number of genetic variants of NAFLD have been identified (Romeo et al., 2008). The best characterized genetic association is with *PNPLA3*. Carriers with *PNPLA3* I 148M have a higher LFC and increased risk of NASH, but no insulin resistance is observed (Romeo et al., 2008). The outcome of the bariatric surgery may vary according to the patient's genotype and therefore it could have been useful to genotype the study subjects included in the current study to evaluate the individual response.

Although BMI, age or plasma insulin levels did not explain the association between insulin-stimulated BGU and EGP, it was not possible to exclude all peripheral factors. Plasma glucagon levels were not measured, though it has been shown that under hyperinsulinemic euglycemia obese subjects have higher glucagon levels as compared to lean subjects (Weiss et al., 2011). The method used to calculate EGP produced some negative values. This method has been validated against the gold standard use of D-[6,6-^{2}H]glucose (Iozzo et al., 2006). It was interpreted that these negatives values during the euglycemic hyperinsulinemic clamp represent the highest suppression of EGP.

As expected, bariatric patients were still obese postoperatively and the control group was not matched for obesity. Therefore, it is not possible to evaluate whether the improvement in liver metabolism and changes in liver perfusion can be considered as an effect of bariatric surgery or weight loss itself.

Some obese patients were uncomfortable in the MRI scanner. Due to this limitation or technical issues some scans were not available for analysis: five MRS scan preoperatively and 10 scans postoperatively to measure LFC and two MRI scans preoperatively and five scans postoperatively to determine liver volume and adipose tissue masses. Technical issues with tracer production caused one preoperative fasting [^{18}F]FDG-PET scan to be canceled. Another preoperative fasting [^{18}F]FDG-PET scan needed to be cancelled because of the patient's severe hyperglycemia. One [^{18}F]FTHA-PET scan of a control subject and four postoperative scans in obese subjects were not available for analyses because of technical problems. Furthermore, four postoperative [^{15}O]H_2O-PET scans could

not be analyzed due technical issues. Due to technical issues, brain scans were only available for 16 obese subjects.

Overall, the studies in this thesis provide new information about the key regulatory pathways of glucose and fatty acids. The research on the effects of bariatric surgery help in an understanding of T2D. Future studies are needed to better understand how brain metabolic abnormalities associate or precede obesity, insulin resistance and T2D. Also, further research is required to establish the long-term therapeutic efficiency of bariatric surgery.

8 SUMMARY AND CONCLUSIONS

The present work was designed to determine liver glucose metabolism, fatty acid uptake and blood perfusion, and the association between brain and liver glucose metabolism in morbidly obese subjects and the effects of bariatric surgery on the former by using multimodal imaging, especially PET.

The results of the present study have led to the following conclusions:

I

Insulin-stimulated hepatic glucose uptake is reduced in morbidly obese subjects. Bariatric surgery leads to a significant improvement in hepatic glucose uptake and normalization in endogenous glucose production when measured six months after surgery. These metabolic effects are accompanied by the resolution of the fatty liver. Overall, the improvement in liver insulin sensitivity in subjects with T2D was quite similar to subjects without T2D. The findings of the study suggest that both sleeve gastrectomy and Roux-en-Y gastric bypass are effective in reducing liver insulin resistance.

II

Morbid obesity is characterized by increased liver fatty acid uptake. Elevated liver fatty acid uptake in obesity could be explained by increased body fat stores. Enhanced liver fatty acid uptake is only partly reversed by surgery-induced weight loss. The findings of the study suggest that, in a postoperative state, fatty acids are used for oxidation to provide energy instead of being accumulated in the liver. Furthermore, the results of the current study suggest that liver fatty acid uptake is promoted by enlarged body adiposity and high circulating fatty acid levels and is suppressed by higher portal blood flow rates.

III

Insulin-stimulated brain glucose uptake is increased in obese as compared to lean subjects and is positively associated with endogenous glucose production, markers of systemic inflammation, and levels of branched-chain and aromatic amino acids. Overall, these results suggest that the enhanced brain glucose uptake in obese subjects might not only be an epiphenomenon, but also a part of the pathophysiology that leads to further deterioration in metabolic diseases.

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REFERENCES

"Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus", 1997, *Diabetes care,* vol. 20, no. 7, pp. 1183-1197.

"Report of the task group on reference man", 1979, *Annals of the ICRP,* vol. 3, no. 1-4, pp. iii-6453(79)90123-4.

Adams, L.A., Lymp, J.F., St Sauver, J., Sanderson, S.O., Lindor, K.D., Feldstein, A. & Angulo, P. 2005, "The natural history of nonalcoholic fatty liver disease: a population-based cohort study", *Gastroenterology,* vol. 129, no. 1, pp. 113-121.

Adiels, M., Olofsson, S.O., Taskinen, M.R. & Boren, J. 2008, "Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome", *Arteriosclerosis, Thrombosis, and Vascular Biology,* vol. 28, no. 7, pp. 1225-1236.

Aguilar-Olivos, N.E., Almeda-Valdes, P., Aguilar-Salinas, C.A., Uribe, M. & Mendez-Sanchez, N. 2016, "The role of bariatric surgery in the management of nonalcoholic fatty liver disease and metabolic syndrome", *Metabolism: clinical and experimental,* vol. 65, no. 8, pp. 1196-1207.

Aithal, G.P., Thomas, J.A., Kaye, P.V., Lawson, A., Ryder, S.D., Spendlove, I., Austin, A.S., Freeman,

J.G., Morgan, L. & Webber, J. 2008, "Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis", *Gastroenterology,* vol. 135, no. 4, pp. 1176-1184.

Alatrach, M., Agyin, C., Adams, J., DeFronzo, R.A. & Abdul-Ghani, M.A. 2017, "Decreased basal hepatic glucose uptake in impaired fasting glucose", *Diabetologia,* vol. 60, no. 7, pp. 1325-1332.

Angrisani, L., Santonicola, A., Iovino, P., Vitiello, A., Zundel, N., Buchwald, H. & Scopinaro, N. 2017, "Bariatric Surgery and Endoluminal Procedures: IFSO Worldwide Survey 2014", *Obesity Surgery,* vol. 27, no. 9, pp. 2279-2289.

Anstee, Q.M., Targher, G. & Day, C.P. 2013, "Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis", *Nature reviews.Gastroenterology & hepatology,* vol. 10, no. 6, pp. 330-344.

Argo, C.K. & Caldwell, S.H. 2009, "Epidemiology and natural history of non-alcoholic steatohepatitis", *Clinics in liver disease,* vol. 13, no. 4, pp. 511-531.

Armstrong, M.J., Gaunt, P., Aithal, G.P., Barton, D., Hull, D., Parker, R., Hazlehurst, J.M., Guo, K., LEAN trial team, Abouda, G., Aldersley, M.A., Stocken, D., Gough, S.C., Tomlinson, J.W.,

Brown, R.M., Hubscher, S.G. & Newsome, P.N. 2016, "Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study", *Lancet (London, England),* vol. 387, no. 10019, pp. 679-690.

Bal, B.S., Finelli, F.C., Shope, T.R. & Koch, T.R. 2012, "Nutritional deficiencies after bariatric surgery", *Nature reviews.Endocrinology,* vol. 8, no. 9, pp. 544-556.

Banks, W.A., Jaspan, J.B. & Kastin, A.J. 1997, "Selective, physiological transport of insulin across the blood-brain barrier: novel demonstration by species-specific radioimmunoassays", *Peptides,* vol. 18, no. 8, pp. 1257-1262.

Batterham, R.L. & Cummings, D.E. 2016, "Mechanisms of Diabetes Improvement Following Bariatric/Metabolic Surgery", *Diabetes care,* vol. 39, no. 6, pp. 893-901.

Belfort, R., Harrison, S.A., Brown, K., Darland, C., Finch, J., Hardies, J., Balas, B., Gastaldelli, A., Tio, F., Pulcini, J., Berria, R., Ma, J.Z., Dwivedi, S., Havranek, R., Fincke, C., DeFronzo, R., Bannayan, G.A., Schenker, S. & Cusi, K. 2006, "A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis", *The New England journal of medicine,* vol. 355, no. 22, pp. 2297-2307.

Bellentani, S., Bedogni, G., Miglioli, L. & Tiribelli, C. 2004, "The epidemiology of fatty liver", *European journal of gastroenterology & hepatology,* vol. 16, no. 11, pp. 1087-1093.

Berk, P.D. & Stump, D.D. 1999, "Mechanisms of cellular uptake of long chain free fatty acids", *Molecular and cellular biochemistry,* vol. 192, no. 1-2, pp. 17-31.

Berthoud, H.R. 2011, "Metabolic and hedonic drives in the neural control of appetite: who is the boss?", *Current opinion in neurobiology,* vol. 21, no. 6, pp. 888-896.

Bjermo, H., Iggman, D., Kullberg, J., Dahlman, I., Johansson, L., Persson, L., Berglund, J., Pulkki, K., Basu, S., Uusitupa, M., Rudling, M., Arner, P., Cederholm, T., Ahlstrom, H. & Riserus, U. 2012, "Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial", *The American Journal of Clinical Nutrition,* vol. 95, no. 5, pp. 1003-1012.

Bohdjalian, A., Langer, F.B., Shakeri-Leidenmuhler, S., Gfrerer, L., Ludvik, B., Zacherl, J. & Prager, G. 2010, "Sleeve gastrectomy as sole and definitive bariatric procedure: 5-year results for weight loss and ghrelin", *Obesity Surgery,* vol. 20, no. 5, pp. 535-540.

Borai, A., Livingstone, C. & Ferns, G.A. 2007, "The biochemical assessment of insulin resistance", *Annals of Clinical Biochemistry,* vol. 44, no. Pt 4, pp. 324-342.

Borra, R.J., Salo, S., Dean, K., Lautamaki, 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,* vol. 250, no. 1, pp. 130-136.

Boza, C., Salinas, J., Salgado, N., Perez, G., Raddatz, A., Funke, R., Pimentel, F. & Ibanez, L. 2012, "Laparoscopic sleeve gastrectomy as a stand-alone procedure for morbid obesity: report of 1,000 cases and 3-year follow-up", *Obesity Surgery,* vol. 22, no. 6, pp. 866-871.

Brito, J.P., Montori, V.M. & Davis, A.M. 2017, "Metabolic Surgery in the Treatment Algorithm for Type 2 Diabetes: A Joint Statement by International Diabetes Organizations", *Jama,* vol. 317, no. 6, pp. 635-636.

Brons, C., Jensen, C.B., Storgaard, H., Hiscock, N.J., White, A., Appel, J.S., Jacobsen, S., Nilsson, E., Larsen, C.M., Astrup, A., Quistorff, B. & Vaag, A. 2009, "Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men", *The Journal of physiology,* vol. 587, no. Pt 10, pp. 2387-2397.

Browning, J.D., Baker, J.A., Rogers, T., Davis, J., Satapati, S. & Burgess, S.C. 2011, "Short-term weight loss and hepatic triglyceride reduction: evidence of a metabolic advantage with dietary carbohydrate restriction", *The American Journal of Clinical Nutrition,* vol. 93, no. 5, pp. 1048-1052.

Brunt, E.M., Kleiner, D.E., Wilson, L.A., Belt, P., Neuschwander-Tetri, B.A. & NASH Clinical Research Network (CRN) 2011, "Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings", *Hepatology (Baltimore, Md.),* vol. 53, no. 3, pp. 810-820.

Buchwald, H., Estok, R., Fahrbach, K., Banel, D., Jensen, M.D., Pories, W.J., Bantle, J.P. & Sledge, I. 2009, "Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis", *The American Journal of Medicine,* vol. 122, no. 3, pp. 248-256.e5.

Caiazzo, R., Lassailly, G., Leteurtre, E., Baud, G., Verkindt, H., Raverdy, V., Buob, D., Pigeyre, M., Mathurin, P. & Pattou, F. 2014, "Roux-en-Y gastric bypass versus adjustable gastric banding to reduce nonalcoholic fatty liver disease: a 5-year controlled longitudinal study", *Annals of Surgery,* vol. 260, no. 5, pp. 893-8; discussion 898-9.

Camastra, S., Gastaldelli, A., Mari, A., Bonuccelli, S., Scartabelli, G., Frascerra, S., Baldi, S., Nannipieri, M., Rebelos, E., Anselmino, M., Muscelli, E. & Ferrannini, E. 2011, "Early and longer term effects of gastric bypass surgery on tissue-specific insulin sensitivity and beta cell function in morbidly obese patients with and without type 2 diabetes", *Diabetologia,* .

Campos, G.M., Rabl, C., Peeva, S., Ciovica, R., Rao, M., Schwarz, J.M., Havel, P., Schambelan, M. & Mulligan, K. 2010, "Improvement in peripheral glucose uptake after gastric bypass surgery is observed only after substantial weight loss has occurred and correlates with the magnitude of weight lost", *Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract,* vol. 14, no. 1, pp. 15-23.

Carlsson, L.M., Peltonen, M., Ahlin, S., Anveden, A., Bouchard, C., Carlsson, B., Jacobson, P., Lonroth, H., Maglio, C., Naslund, I., Pirazzi, C., Romeo, S., Sjoholm, K., Sjostrom, E., Wedel, H., Svensson, P.A. & Sjostrom, L. 2012, "Bariatric surgery and prevention of type 2 diabetes in Swedish obese subjects", *The New England journal of medicine,* vol. 367, no. 8, pp. 695-704.

Cederberg, H., Koivisto, V.M., Jokelainen, J., Surcel, H.M., Keinanen-Kiukaanniemi, S. & Rajala, U. 2012, "Unacylated ghrelin is associated with changes in insulin sensitivity and lipid profile during an exercise intervention", *Clinical endocrinology,* vol. 76, no. 1, pp. 39-45.

Cederberg, H., Rajala, U., Koivisto, V.M., Jokelainen, J., Surcel, H.M., Keinanen-Kiukaanniemi, S. & Laakso, M. 2011, "Unacylated ghrelin is associated with changes in body composition and body fat distribution during long-term exercise intervention", *European journal of endocrinology,* vol. 165, no. 2, pp. 243-248.

Chalasani, N., Younossi, Z., Lavine, J.E., Diehl, A.M., Brunt, E.M., Cusi, K., Charlton, M., Sanyal, A.J., American Gastroenterological Association, American Association for the Study of Liver Diseases & American College of Gastroenterologyh 2012, "The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology", *Gastroenterology,* vol. 142, no. 7, pp. 1592-1609.

Cherrington, A.D., Moore, M.C., Sindelar, D.K. & Edgerton, D.S. 2007, "Insulin action on the liver in vivo", *Biochemical Society transactions,* vol. 35, no. Pt 5, pp. 1171-1174.

Clore, J.N., Helm, S.T. & Blackard, W.G. 1995, "Loss of hepatic autoregulation after carbohydrate overfeeding in normal man", *The Journal of clinical investigation,* vol. 96, no. 4, pp. 1967-1972.

Coate, K.C., Kraft, G., Shiota, M., Smith, M.S., Farmer, B., Neal, D.W., Williams, P., Cherrington, A.D. & Moore, M.C. 2015, "Chronic overeating impairs hepatic glucose uptake and disposition", *American journal of physiology.Endocrinology and metabolism,* vol. 308, no. 10, pp. E860-7.

Cummings, D.E., Weigle, D.S., Frayo, R.S., Breen, P.A., Ma, M.K., Dellinger, E.P. & Purnell, J.Q. 2002, "Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery", *The New England journal of medicine,* vol. 346, no. 21, pp. 1623-1630.

Dardevet, D., Moore, M.C., DiCostanzo, C.A., Farmer, B., Neal, D.W., Snead, W., Lautz, M. & Cherrington, A.D. 2005, "Insulin secretion-independent effects of GLP-1 on canine liver glucose metabolism do not involve portal vein GLP-1 receptors", *American journal of physiology.Gastrointestinal and liver physiology,* vol. 289, no. 5, pp. G806-14.

Dash, S., Xiao, C., Morgantini, C., Koulajian, K. & Lewis, G.F. 2015, "Intranasal insulin suppresses endogenous glucose production in humans compared with placebo in the presence of similar venous insulin concentrations", *Diabetes,* vol. 64, no. 3, pp. 766-774.

de Almeida, S.R., Rocha, P.R., Sanches, M.D., Leite, V.H., da Silva, R.A., Diniz, M.T., Diniz Mde, F. & Rocha, A.L. 2006, "Roux-en-Y gastric bypass improves the nonalcoholic steatohepatitis (NASH) of morbid obesity", *Obesity Surgery,* vol. 16, no. 3, pp. 270-278.

de Alwis, N.M. & Day, C.P. 2008, "Non-alcoholic fatty liver disease: the mist gradually clears", *Journal of hepatology,* vol. 48 Suppl 1, pp. S104-12.

DeFronzo, R.A. 1992, "Pathogenesis of type 2 (non-insulin dependent) diabetes mellitus: a balanced overview", *Diabetologia,* vol. 35, no. 4, pp. 389-397.

DeFronzo, R.A. 1988, "Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM", *Diabetes,* vol. 37, no. 6, pp. 667-687.

DeFronzo, R.A. 1987, "Use of the splanchnic/hepatic balance technique in the study of glucose metabolism", *Bailliere's Clinical Endocrinology and Metabolism,* vol. 1, no. 4, pp. 837-862.

DeFronzo, R.A., Bonadonna, R.C. & Ferrannini, E. 1992, "Pathogenesis of NIDDM. A balanced overview", *Diabetes care,* vol. 15, no. 3, pp. 318-368.

DeFronzo, R.A., Ferrannini, E. & Simonson, D.C. 1989, "Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake", *Metabolism: clinical and experimental,* vol. 38, no. 4, pp. 387-395.

DeFronzo, R.A., Tobin, J.D. & Andres, R. 1979, "Glucose clamp technique: a method for quantifying insulin secretion and resistance", *The American Journal of Physiology,* vol. 237, no. 3, pp. E214-23.

DeGrado, T.R., Coenen, H.H. & Stocklin, G. 1991, "14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid (FTHA): evaluation in mouse of a new probe of myocardial utilization of long chain fatty acids", *Journal of nuclear medicine : official publication, Society of Nuclear Medicine,* vol. 32, no. 10, pp. 1888-1896.

Delhanty, P.J. & van der Lely, A.J. 2011, "Ghrelin and glucose homeostasis", *Peptides,* vol. 32, no. 11, pp. 2309-2318.

Detre, J.A. & Wang, J. 2002, "Technical aspects and utility of fMRI using BOLD and ASL", *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology,* vol. 113, no. 5, pp. 621-634.

D'Hondt, M., Vanneste, S., Pottel, H., Devriendt, D., Van Rooy, F. & Vansteenkiste, F. 2011, "Laparoscopic sleeve gastrectomy as a single-stage procedure for the treatment of morbid obesity

and the resulting quality of life, resolution of comorbidities, food tolerance, and 6-year weight loss", *Surgical endoscopy,* vol. 25, no. 8, pp. 2498-2504.

Diabetes Prevention Program Research Group, Knowler, W.C., Fowler, S.E., Hamman, R.F., Christophi, C.A., Hoffman, H.J., Brenneman, A.T., Brown-Friday, J.O., Goldberg, R., Venditti, E. & Nathan, D.M. 2009, "10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study", *Lancet (London, England),* vol. 374, no. 9702, pp. 1677-1686.

Diaz-Garcia, C.M., Mongeon, R., Lahmann, C., Koveal, D., Zucker, H. & Yellen, G. 2017, "Neuronal Stimulation Triggers Neuronal Glycolysis and Not Lactate Uptake", *Cell metabolism,* vol. 26, no. 2, pp. 361-374.e4.

Dienel, G.A. 2012, "Fueling and imaging brain activation", *ASN neuro,* vol. 4, no. 5, pp. 10.1042/AN20120021.

Dongiovanni, P., Petta, S., Maglio, C., Fracanzani, A.L., Pipitone, R., Mozzi, E., Motta, B.M., Kaminska, D., Rametta, R., Grimaudo, S., Pelusi, S., Montalcini, T., Alisi, A., Maggioni, M., Karja, V., Boren, J., Kakela, P., Di Marco, V., Xing, C., Nobili, V., Dallapiccola, B., Craxi, A., Pihlajamaki, J., Fargion, S., Sjostrom, L., Carlsson, L.M., Romeo, S. & Valenti, L. 2015, "Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease", *Hepatology (Baltimore, Md.),* vol. 61, no. 2, pp. 506-514.

Donnelly, K.L., Smith, C.I., Schwarzenberg, S.J., Jessurun, J., Boldt, M.D. & Parks, E.J. 2005, "Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease", *The Journal of clinical investigation,* vol. 115, no. 5, pp. 1343-1351.

Dunn, J.P., Abumrad, N.N., Breitman, I., Marks-Shulman, P.A., Flynn, C.R., Jabbour, K., Feurer, I.D. & Tamboli, R.A. 2012, "Hepatic and peripheral insulin sensitivity and diabetes remission at 1 month after Roux-en-Y gastric bypass surgery in patients randomized to omentectomy", *Diabetes care,* vol. 35, no. 1, pp. 137-142.

Duvoux, C., Radier, C., Roudot-Thoraval, F., Maille, F., Anglade, M.C., Van Nhieu, J.T., Rosa, I., Hospitel, S., Abd-Alsamad, I., Sitruk, V., Seror, O., Ziol, M., Blondon, H., Dhumeaux, D. & Richardet, J.P. 2004, "Low-grade steatosis and major changes in portal flow as new prognostic factors in steroid-treated alcoholic hepatitis", *Hepatology (Baltimore, Md.),* vol. 40, no. 6, pp. 1370-1378.

Ekstedt, M., Hagstrom, H., Nasr, P., Fredrikson, M., Stal, P., Kechagias, S. & Hultcrantz, R. 2015, "Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up", *Hepatology (Baltimore, Md.),* vol. 61, no. 5, pp. 1547-1554.

Emous, M., Wolffenbuttel, B.H.R., van Dijk, G., Totte, E. & van Beek, A.P. 2018, "Long-term self-reported symptom prevalence of early and late dumping in a patient population after sleeve

gastrectomy, primary, and revisional gastric bypass surgery", *Surgery for obesity and related diseases : official journal of the American Society for Bariatric Surgery,* vol. 14, no. 8, pp. 1173-1181.

European Association for Study of Liver & Asociacion Latinoamericana para el Estudio del Higado 2015, "EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis", *Journal of hepatology,* vol. 63, no. 1, pp. 237-264.

European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) & European Association for the Study of Obesity (EASO) 2016, "EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease", *Journal of hepatology,* vol. 64, no. 6, pp. 1388-1402.

Fajans, S.S., Bell, G.I. & Polonsky, K.S. 2001, "Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young", *The New England journal of medicine,* vol. 345, no. 13, pp. 971-980.

Farr, O.M., Tsoukas, M.A., Triantafyllou, G., Dincer, F., Filippaios, A., Ko, B.J. & Mantzoros, C.S. 2016, "Short-term administration of the GLP-1 analog liraglutide decreases circulating leptin and increases GIP levels and these changes are associated with alterations in CNS responses to food cues: A randomized, placebo-controlled, crossover study", *Metabolism: clinical and experimental,* vol. 65, no. 7, pp. 945-953.

Ferrannini, E., Natali, A., Bell, P., Cavallo-Perin, P., Lalic, N. & Mingrone, G. 1997, "Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR)", *The Journal of clinical investigation,* vol. 100, no. 5, pp. 1166-1173.

Ferrannini, E., Wahren, J., Faber, O.K., Felig, P., Binder, C. & DeFronzo, R.A. 1983, "Splanchnic and renal metabolism of insulin in human subjects: a dose-response study", *The American Journal of Physiology,* vol. 244, no. 6, pp. E517-27.

Flegal, K.M., Kruszon-Moran, D., Carroll, M.D., Fryar, C.D. & Ogden, C.L. 2016, "Trends in Obesity Among Adults in the United States, 2005 to 2014", *Jama,* vol. 315, no. 21, pp. 2284-2291.

Frank, S., Wilms, B., Veit, R., Ernst, B., Thurnheer, M., Kullmann, S., Fritsche, A., Birbaumer, N., Preissl, H. & Schultes, B. 2014, "Altered brain activity in severely obese women may recover after Roux-en Y gastric bypass surgery", *International journal of obesity (2005),* vol. 38, no. 3, pp. 341-348.

Fried, M., Yumuk, V., Oppert, J.M., Scopinaro, N., Torres, A.J., Weiner, R., Yashkov, Y., Fruhbeck, G., European Association for the Study of Obesity & International Federation for the Surgery of Obesity - European Chapter 2013, "Interdisciplinary European Guidelines on metabolic and bariatric surgery", *Obesity facts,* vol. 6, no. 5, pp. 449-468.

Gancheva, S., Koliaki, C., Bierwagen, A., Nowotny, P., Heni, M., Fritsche, A., Haring, H.U., Szendroedi, J. & Roden, M. 2015, "Effects of intranasal insulin on hepatic fat accumulation and energy metabolism in humans", *Diabetes,* vol. 64, no. 6, pp. 1966-1975.

Gastaldelli, A., Coggan, A.R. & Wolfe, R.R. 1999, "Assessment of methods for improving tracer estimation of non-steady-state rate of appearance", *Journal of applied physiology (Bethesda, Md.: 1985),* vol. 87, no. 5, pp. 1813-1822.

German, J.P., Thaler, J.P., Wisse, B.E., Oh-I, S., Sarruf, D.A., Matsen, M.E., Fischer, J.D., Taborsky, G.J.,Jr, Schwartz, M.W. & Morton, G.J. 2011, "Leptin activates a novel CNS mechanism for insulin-independent normalization of severe diabetic hyperglycemia", *Endocrinology,* vol. 152, no. 2, pp. 394-404.

Germano, G., Chen, B.C., Huang, S.C., Gambhir, S.S., Hoffman, E.J. & Phelps, M.E. 1992, "Use of the abdominal aorta for arterial input function determination in hepatic and renal PET studies", *Journal of nuclear medicine : official publication, Society of Nuclear Medicine,* vol. 33, no. 4, pp. 613-620.

Grundy, S.M. 1998, "Hypertriglyceridemia, atherogenic dyslipidemia, and the metabolic syndrome", *The American Journal of Cardiology,* vol. 81, no. 4A, pp. 18B-25B.

Grundy, S.M., Cleeman, J.I., Daniels, S.R., Donato, K.A., Eckel, R.H., Franklin, B.A., Gordon, D.J., Krauss, R.M., Savage, P.J., Smith, S.C.,Jr, Spertus, J.A. & Fernando, C. 2005, "Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement: Executive Summary", *Critical pathways in cardiology,* vol. 4, no. 4, pp. 198-203.

Guasch-Ferre, M., Hruby, A., Toledo, E., Clish, C.B., Martinez-Gonzalez, M.A., Salas-Salvado, J. & Hu, F.B. 2016, "Metabolomics in Prediabetes and Diabetes: A Systematic Review and Meta-analysis", *Diabetes care,* vol. 39, no. 5, pp. 833-846.

Guh, D.P., Zhang, W., Bansback, N., Amarsi, Z., Birmingham, C.L. & Anis, A.H. 2009, "The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis", *BMC public health,* vol. 9, pp. 88-2458-9-88.

Guida, C., Stephen, S., Guitton, R. & Ramracheya, R.D. 2017, "The Role of PYY in Pancreatic Islet Physiology and Surgical Control of Diabetes", *Trends in endocrinology and metabolism: TEM,* vol. 28, no. 8, pp. 626-636.

Hafeez, S. & Ahmed, M.H. 2013, "Bariatric surgery as potential treatment for nonalcoholic fatty liver disease: a future treatment by choice or by chance?", *Journal of obesity,* vol. 2013, pp. 839275.

Hamacher, K., Coenen, H.H. & Stocklin, G. 1986, "Efficient stereospecific synthesis of no-carrier-added 2-[18F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution", *Journal of nuclear medicine : official publication, Society of Nuclear Medicine,* vol. 27, no. 2, pp. 235-238.

Hannah, W.N.,Jr & Harrison, S.A. 2016, "Effect of Weight Loss, Diet, Exercise, and Bariatric Surgery on Nonalcoholic Fatty Liver Disease", *Clinics in liver disease,* vol. 20, no. 2, pp. 339-350.

Hannukainen, J.C., Borra, R., Linderborg, K., Kallio, H., Kiss, J., Lepomaki, V., Kalliokoski, K.K., Kujala, U.M., Kaprio, J., Heinonen, O.J., Komu, M., Parkkola, R., Ahotupa, M., Lehtimaki, T., Huupponen, R., Iozzo, P. & Nuutila, P. 2011, "Liver and pancreatic fat content and metabolism in healthy monozygotic twins with discordant physical activity", *Journal of hepatology,* vol. 54, no. 3, pp. 545-552.

Hegarty, B.D., Turner, N., Cooney, G.J. & Kraegen, E.W. 2009, "Insulin resistance and fuel homeostasis: the role of AMP-activated protein kinase", *Acta physiologica (Oxford, England),* vol. 196, no. 1, pp. 129-145.

Hellerstein, M.K., Schwarz, J.M. & Neese, R.A. 1996, "Regulation of hepatic de novo lipogenesis in humans", *Annual Review of Nutrition,* vol. 16, pp. 523-557.

Helmio, M., Victorzon, M., Ovaska, J., Leivonen, M., Juuti, A., Jaser, N., Peromaa, P., Tolonen, P., Hurme, S. & Salminen, P. 2012, "SLEEVEPASS: a randomized prospective multicenter study comparing laparoscopic sleeve gastrectomy and gastric bypass in the treatment of morbid obesity: preliminary results", *Surgical endoscopy,* vol. 26, no. 9, pp. 2521-2526.

Helmio, M., Victorzon, M., Ovaska, J., Leivonen, M., Juuti, A., Jaser, N., Peromaa, P., Tolonen, P., Hurme, S. & Salminen, P. 2012, "SLEEVEPASS: a randomized prospective multicenter study comparing laparoscopic sleeve gastrectomy and gastric bypass in the treatment of morbid obesity: preliminary results", *Surgical endoscopy,* vol. 26, no. 9, pp. 2521-2526.

Heni, M., Wagner, R., Kullmann, S., Gancheva, S., Roden, M., Peter, A., Stefan, N., Preissl, H., Haring, H.U. & Fritsche, A. 2017, "Hypothalamic and Striatal Insulin Action Suppresses Endogenous Glucose Production and May Stimulate Glucose Uptake During Hyperinsulinemia in Lean but Not in Overweight Men", *Diabetes,* vol. 66, no. 7, pp. 1797-1806.

Hirvonen, J., Virtanen, K.A., Nummenmaa, L., Hannukainen, J.C., Honka, M.J., Bucci, M., Nesterov, S.V., Parkkola, R., Rinne, J., Iozzo, P. & Nuutila, P. 2011, "Effects of insulin on brain glucose metabolism in impaired glucose tolerance", *Diabetes,* vol. 60, no. 2, pp. 443-447.

Hochberg, I. & Hochberg, Z. 2010, "Expanding the definition of hypothalamic obesity", *Obesity reviews : an official journal of the International Association for the Study of Obesity,* vol. 11, no. 10, pp. 709-721.

Honka, H., Koffert, J., Kauhanen, S., Kudomi, N., Hurme, S., Mari, A., Lindqvist, A., Wierup, N., Parkkola, R., Groop, L. & Nuutila, P. 2018, "Liver blood dynamics after bariatric surgery: the effects of mixed-meal test and incretin infusions", *Endocrine connections,* vol. 7, no. 7, pp. 888-896.

Honka, H., Koffert, J., Kauhanen, S., Teuho, J., Hurme, S., Mari, A., Lindqvist, A., Wierup, N., Groop, L. & Nuutila, P. 2017, "Bariatric Surgery Enhances Splanchnic Vascular Responses in Patients With Type 2 Diabetes", *Diabetes,* vol. 66, no. 4, pp. 880-885.

Hopkins, D.F. & Williams, G. 1997, "Insulin receptors are widely distributed in human brain and bind human and porcine insulin with equal affinity", *Diabetic medicine : a journal of the British Diabetic Association,* vol. 14, no. 12, pp. 1044-1050.

Howarth, C., Gleeson, P. & Attwell, D. 2012, "Updated energy budgets for neural computation in the neocortex and cerebellum", *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism,* vol. 32, no. 7, pp. 1222-1232.

Iacobini, C., Pugliese, G., Blasetti Fantauzzi, C., Federici, M. & Menini, S. 2018, "Metabolically healthy versus metabolically unhealthy obesity", *Metabolism: clinical and experimental,* .

Iijima, H., Moriyasu, F., Tsuchiya, K., Suzuki, S., Yoshida, M., Shimizu, M., Sasaki, S., Nishiguchi, S. & Maeyama, S. 2007, "Decrease in accumulation of ultrasound contrast microbubbles in non-alcoholic steatohepatitis", *Hepatology research : the official journal of the Japan Society of Hepatology,* vol. 37, no. 9, pp. 722-730.

Inoue, H., Ogawa, W., Asakawa, A., Okamoto, Y., Nishizawa, A., Matsumoto, M., Teshigawara, K., Matsuki, Y., Watanabe, E., Hiramatsu, R., Notohara, K., Katayose, K., Okamura, H., Kahn, C.R., Noda, T., Takeda, K., Akira, S., Inui, A. & Kasuga, M. 2006, "Role of hepatic STAT3 in brain-insulin action on hepatic glucose production", *Cell metabolism,* vol. 3, no. 4, pp. 267-275.

Iozzo, P., Bucci, M., Roivainen, A., Nagren, K., Jarvisalo, M.J., Kiss, J., Guiducci, L., Fielding, B., Naum, A.G., Borra, R., Virtanen, K., Savunen, T., Salvadori, P.A., Ferrannini, E., Knuuti, J. & Nuutila, P. 2010, "Fatty acid metabolism in the liver, measured by positron emission tomography, is increased in obese individuals", *Gastroenterology,* vol. 139, no. 3, pp. 846-56, 856.e1-6.

Iozzo, P., Gastaldelli, A., Jarvisalo, M.J., Kiss, J., Borra, R., Buzzigoli, E., Viljanen, A., Naum, G., Viljanen, T., Oikonen, V., Knuuti, J., Savunen, T., Salvadori, P.A., Ferrannini, E. & Nuutila, P. 2006, "18F-FDG assessment of glucose disposal and production rates during fasting and insulin stimulation: a validation study", *Journal of nuclear medicine : official publication, Society of Nuclear Medicine,* vol. 47, no. 6, pp. 1016-1022.

Iozzo, P., Hallsten, K., Oikonen, V., Virtanen, K.A., Kemppainen, J., Solin, O., Ferrannini, E., Knuuti, J. & Nuutila, P. 2003, "Insulin-mediated hepatic glucose uptake is impaired in type 2 diabetes: evidence for a relationship with glycemic control", *The Journal of clinical endocrinology and metabolism,* vol. 88, no. 5, pp. 2055-2060.

Iozzo, P., Jarvisalo, M.J., Kiss, J., Borra, R., Naum, G.A., Viljanen, A., Viljanen, T., Gastaldelli, A., Buzzigoli, E., Guiducci, L., Barsotti, E., Savunen, T., Knuuti, J., Haaparanta-Solin, M., Ferrannini, E. & Nuutila, P. 2007, "Quantification of liver glucose metabolism by positron emission tomography: validation study in pigs", *Gastroenterology,* vol. 132, no. 2, pp. 531-542.

Iozzo, P., Lautamaki, R., Geisler, F., Virtanen, K.A., Oikonen, V., Haaparanta, M., Yki-Jarvinen, H., Ferrannini, E., Knuuti, J. & Nuutila, P. 2004, "Non-esterified fatty acids impair insulin-mediated glucose uptake and disposition in the liver", *Diabetologia,* vol. 47, no. 7, pp. 1149-1156.

Iozzo, P., Turpeinen, A.K., Takala, T., Oikonen, V., Solin, O., Ferrannini, E., Nuutila, P. & Knuuti, J. 2003, "Liver uptake of free fatty acids in vivo in humans as determined with 14(R, S)-[18F]fluoro-6-thia-heptadecanoic acid and PET", *European journal of nuclear medicine and molecular imaging,* vol. 30, no. 8, pp. 1160-1164.

Italian Association for the Study of the Liver (AISF) 2017, "AISF position paper on nonalcoholic fatty liver disease (NAFLD): Updates and future directions", *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver,* vol. 49, no. 5, pp. 471-483.

Ito, K., Maruyama, H., Hirose, H., Kido, K., Koyama, K., Kataoka, K. & Saruta, T. 1995, "Exogenous insulin dose-dependently suppresses glucopenia-induced glucagon secretion from perfused rat pancreas", *Metabolism: clinical and experimental,* vol. 44, no. 3, pp. 358-362.

Jackness, C., Karmally, W., Febres, G., Conwell, I.M., Ahmed, L., Bessler, M., McMahon, D.J. & Korner, J. 2013, "Very low-calorie diet mimics the early beneficial effect of Roux-en-Y gastric bypass on insulin sensitivity and beta-cell Function in type 2 diabetic patients", *Diabetes,* vol. 62, no. 9, pp. 3027-3032.

Jan, A., Narwaria, M. & Mahawar, K.K. 2015, "A Systematic Review of Bariatric Surgery in Patients with Liver Cirrhosis", *Obesity Surgery,* vol. 25, no. 8, pp. 1518-1526.

Jazet, I.M., Pijl, H., Frolich, M., Romijn, J.A. & Meinders, A.E. 2005, "Two days of a very low calorie diet reduces endogenous glucose production in obese type 2 diabetic patients despite the withdrawal of blood glucose-lowering therapies including insulin", *Metabolism: clinical and experimental,* vol. 54, no. 6, pp. 705-712.

Jensen, T., Abdelmalek, M.F., Sullivan, S., Nadeau, K.J., Green, M., Roncal, C., Nakagawa, T., Kuwabara, M., Sato, Y., Kang, D.H., Tolan, D.R., Sanchez-Lozada, L.G., Rosen, H.R., Lanaspa,

M.A., Diehl, A.M. & Johnson, R.J. 2018, "Fructose and sugar: A major mediator of non-alcoholic fatty liver disease", *Journal of hepatology,* vol. 68, no. 5, pp. 1063-1075.

Jones, J.G. 2016, "Hepatic glucose and lipid metabolism", *Diabetologia,* vol. 59, no. 6, pp. 1098-1103.

Kalinowski, P., Paluszkiewicz, R., Ziarkiewicz-Wroblewska, B., Wroblewski, T., Remiszewski, P., Grodzicki, M. & Krawczyk, M. 2017, "Liver Function in Patients With Nonalcoholic Fatty Liver Disease Randomized to Roux-en-Y Gastric Bypass Versus Sleeve Gastrectomy: A Secondary Analysis of a Randomized Clinical Trial", *Annals of Surgery,* vol. 266, no. 5, pp. 738-745.

Kankaanpaa, M., Lehto, H.R., Parkka, J.P., Komu, M., Viljanen, A., Ferrannini, E., Knuuti, J., Nuutila, P., Parkkola, R. & Iozzo, P. 2006, "Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels", *The Journal of clinical endocrinology and metabolism,* vol. 91, no. 11, pp. 4689-4695.

Kantartzis, K., Thamer, C., Peter, A., Machann, J., Schick, F., Schraml, C., Konigsrainer, A., Konigsrainer, I., Krober, S., Niess, A., Fritsche, A., Haring, H.U. & Stefan, N. 2009, "High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease", *Gut,* vol. 58, no. 9, pp. 1281-1288.

Karamanakos, S.N., Vagenas, K., Kalfarentzos, F. & Alexandrides, T.K. 2008, "Weight loss, appetite suppression, and changes in fasting and postprandial ghrelin and peptide-YY levels after Roux-en-Y gastric bypass and sleeve gastrectomy: a prospective, double blind study", *Annals of Surgery,* vol. 247, no. 3, pp. 401-407.

Karmi, A., Iozzo, P., Viljanen, A., Hirvonen, J., Fielding, B.A., Virtanen, K., Oikonen, V., Kemppainen, J., Viljanen, T., Guiducci, L., Haaparanta-Solin, M., Nagren, K., Solin, O. & Nuutila, P. 2010, "Increased brain fatty acid uptake in metabolic syndrome", *Diabetes,* vol. 59, no. 9, pp. 2171-2177.

Kety, S.S. & Schmidt, C.F. 1948, "The Nitrous Oxide Method for the Quantitative Determination of Cerebral Blood Flow in Man: Theory, Procedure and Normal Values", *The Journal of clinical investigation,* vol. 27, no. 4, pp. 476-483.

Khan, R., Bril, F., Cusi, K. & Newsome, P.N. 2018, "Modulation of Insulin Resistance in NAFLD", *Hepatology (Baltimore, Md.),* .

Khera, R., Murad, M.H., Chandar, A.K., Dulai, P.S., Wang, Z., Prokop, L.J., Loomba, R., Camilleri, M. & Singh, S. 2016, "Association of Pharmacological Treatments for Obesity With Weight Loss and Adverse Events: A Systematic Review and Meta-analysis", *Jama,* vol. 315, no. 22, pp. 2424-2434.

Kirk, E., Reeds, D.N., Finck, B.N., Mayurranjan, S.M., Patterson, B.W. & Klein, S. 2009, "Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction", *Gastroenterology,* vol. 136, no. 5, pp. 1552-1560.

Klein, S., Mittendorfer, B., Eagon, J.C., Patterson, B., Grant, L., Feirt, N., Seki, E., Brenner, D., Korenblat, K. & McCrea, J. 2006, "Gastric bypass surgery improves metabolic and hepatic abnormalities associated with nonalcoholic fatty liver disease", *Gastroenterology,* vol. 130, no. 6, pp. 1564-1572.

Kleiner, D.E., Brunt, E.M., Van Natta, M., Behling, C., Contos, M.J., Cummings, O.W., Ferrell, L.D., Liu, Y.C., Torbenson, M.S., Unalp-Arida, A., Yeh, M., McCullough, A.J., Sanyal, A.J. & Nonalcoholic Steatohepatitis Clinical Research Network 2005, "Design and validation of a histological scoring system for nonalcoholic fatty liver disease", *Hepatology (Baltimore, Md.),* vol. 41, no. 6, pp. 1313-1321.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. & Kangawa, K. 1999, "Ghrelin is a growth-hormone-releasing acylated peptide from stomach", *Nature,* vol. 402, no. 6762, pp. 656-660.

Kotronen, A., Juurinen, L., Tiikkainen, M., Vehkavaara, S. & Yki-Jarvinen, H. 2008, "Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes", *Gastroenterology,* vol. 135, no. 1, pp. 122-130.

Kroemer, N.B., Krebs, L., Kobiella, A., Grimm, O., Vollstadt-Klein, S., Wolfensteller, U., Kling, R., Bidlingmaier, M., Zimmermann, U.S. & Smolka, M.N. 2013, "(Still) longing for food: insulin reactivity modulates response to food pictures", *Human brain mapping,* vol. 34, no. 10, pp. 2367-2380.

Krssak, M., Brehm, A., Bernroider, E., Anderwald, C., Nowotny, P., Dalla Man, C., Cobelli, C., Cline, G.W., Shulman, G.I., Waldhausl, W. & Roden, M. 2004, "Alterations in postprandial hepatic glycogen metabolism in type 2 diabetes", *Diabetes,* vol. 53, no. 12, pp. 3048-3056.

Kuchay, M.S., Krishan, S., Mishra, S.K., Farooqui, K.J., Singh, M.K., Wasir, J.S., Bansal, B., Kaur, P., Jevalikar, G., Gill, H.K., Choudhary, N.S. & Mithal, A. 2018, "Effect of Empagliflozin on Liver Fat in Patients With Type 2 Diabetes and Nonalcoholic Fatty Liver Disease: A Randomized Controlled Trial (E-LIFT Trial)", *Diabetes care,* vol. 41, no. 8, pp. 1801-1808.

Kudomi, N., Slimani, L., Jarvisalo, M.J., Kiss, J., Lautamaki, R., Naum, G.A., Savunen, T., Knuuti, J., Iida, H., Nuutila, P. & Iozzo, P. 2008, "Non-invasive estimation of hepatic blood perfusion from H2 15O PET images using tissue-derived arterial and portal input functions", *European journal of nuclear medicine and molecular imaging,* vol. 35, no. 10, pp. 1899-1911.

Laferrere, B., Teixeira, J., McGinty, J., Tran, H., Egger, J.R., Colarusso, A., Kovack, B., Bawa, B., Koshy, N., Lee, H., Yapp, K. & Olivan, B. 2008, "Effect of weight loss by gastric bypass surgery versus hypocaloric diet on glucose and incretin levels in patients with type 2 diabetes", *The Journal of clinical endocrinology and metabolism,* vol. 93, no. 7, pp. 2479-2485.

Lahti-Koski, M., Seppanen-Nuijten, E., Mannisto, S., Harkanen, T., Rissanen, H., Knekt, P., Rissanen, A. & Heliovaara, M. 2010, "Twenty-year changes in the prevalence of obesity among Finnish adults", *Obesity reviews : an official journal of the International Association for the Study of Obesity,* vol. 11, no. 3, pp. 171-176.

Lambert, J.E., Ramos-Roman, M.A., Browning, J.D. & Parks, E.J. 2014, "Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease", *Gastroenterology,* vol. 146, no. 3, pp. 726-735.

Lassale, C., Tzoulaki, I., Moons, K.G.M., Sweeting, M., Boer, J., Johnson, L., Huerta, J.M., Agnoli, C., Freisling, H., Weiderpass, E., Wennberg, P., van der, A.D.L., Arriola, L., Benetou, V., Boeing, H., Bonnet, F., Colorado-Yohar, S.M., Engstrom, G., Eriksen, A.K., Ferrari, P., Grioni, S., Johansson, M., Kaaks, R., Katsoulis, M., Katzke, V., Key, T.J., Matullo, G., Melander, O., Molina-Portillo, E., Moreno-Iribas, C., Norberg, M., Overvad, K., Panico, S., Quiros, J.R., Saieva, C., Skeie, G., Steffen, A., Stepien, M., Tjonneland, A., Trichopoulou, A., Tumino, R., van der Schouw, Y.T., Verschuren, W.M.M., Langenberg, C., Di Angelantonio, E., Riboli, E., Wareham, N.J., Danesh, J. & Butterworth, A.S. 2018, "Separate and combined associations of obesity and metabolic health with coronary heart disease: a pan-European case-cohort analysis", *European heart journal,* vol. 39, no. 5, pp. 397-406.

Lebon, V., Petersen, K.F., Cline, G.W., Shen, J., Mason, G.F., Dufour, S., Behar, K.L., Shulman, G.I. & Rothman, D.L. 2002, "Astroglial contribution to brain energy metabolism in humans revealed by 13C nuclear magnetic resonance spectroscopy: elucidation of the dominant pathway for neurotransmitter glutamate repletion and measurement of astrocytic oxidative metabolism", *The Journal of neuroscience : the official journal of the Society for Neuroscience,* vol. 22, no. 5, pp. 1523-1531.

Lee, Y., Doumouras, A.G., Yu, J., Brar, K., Banfield, L., Gmora, S., Anvari, M. & Hong, D. 2018, "Complete Resolution of Nonalcoholic Fatty Liver Disease After Bariatric Surgery: A Systematic Review and Meta-Analysis", *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association,* .

Lewis, M.C., Phillips, M.L., Slavotinek, J.P., Kow, L., Thompson, C.H. & Toouli, J. 2006, "Change in liver size and fat content after treatment with Optifast very low calorie diet", *Obesity Surgery,* vol. 16, no. 6, pp. 697-701.

Li, J., Lai, D. & Wu, D. 2016, "Laparoscopic Roux-en-Y Gastric Bypass Versus Laparoscopic Sleeve Gastrectomy to Treat Morbid Obesity-Related Comorbidities: a Systematic Review and Meta-analysis", *Obesity Surgery,* vol. 26, no. 2, pp. 429-442.

Liistro, T., Guiducci, L., Burchielli, S., Panetta, D., Belcari, N., Pardini, S., Del Guerra, A., Salvadori, P.A. & Iozzo, P. 2010, "Brain glucose overexposure and lack of acute metabolic flexibility in obesity and type 2 diabetes: a PET-[18F]FDG study in Zucker and ZDF rats", *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism,* vol. 30, no. 5, pp. 895-899.

Lim, E.L., Hollingsworth, K.G., Aribisala, B.S., Chen, M.J., Mathers, J.C. & Taylor, R. 2011, "Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol", *Diabetologia,* vol. 54, no. 10, pp. 2506-2514.

Lin, E., Phillips, L.S., Ziegler, T.R., Schmotzer, B., Wu, K., Gu, L.H., Khaitan, L., Lynch, S.A., Torres, W.E., Smith, C.D. & Gletsu-Miller, N. 2007, "Increases in adiponectin predict improved liver, but not peripheral, insulin sensitivity in severely obese women during weight loss", *Diabetes,* vol. 56, no. 3, pp. 735-742.

Linder, K., Schleger, F., Ketterer, C., Fritsche, L., Kiefer-Schmidt, I., Hennige, A., Haring, H.U., Preissl, H. & Fritsche, A. 2014, "Maternal insulin sensitivity is associated with oral glucose-induced changes in fetal brain activity", *Diabetologia,* vol. 57, no. 6, pp. 1192-1198.

Linder, K., Schleger, F., Kiefer-Schmidt, I., Fritsche, L., Kummel, S., Heni, M., Weiss, M., Haring, H.U., Preissl, H. & Fritsche, A. 2015, "Gestational Diabetes Impairs Human Fetal Postprandial Brain Activity", *The Journal of clinical endocrinology and metabolism,* vol. 100, no. 11, pp. 4029-4036.

Liu, Y.L., Reeves, H.L., Burt, A.D., Tiniakos, D., McPherson, S., Leathart, J.B., Allison, M.E., Alexander, G.J., Piguet, A.C., Anty, R., Donaldson, P., Aithal, G.P., Francque, S., Van Gaal, L., Clement, K., Ratziu, V., Dufour, J.F., Day, C.P., Daly, A.K. & Anstee, Q.M. 2014, "TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease", *Nature communications,* vol. 5, pp. 4309.

Look AHEAD Research Group 2014, "Eight-year weight losses with an intensive lifestyle intervention: the look AHEAD study", *Obesity (Silver Spring, Md.),* vol. 22, no. 1, pp. 5-13.

Lorenzo, C., Haffner, S.M., Stancakova, A., Kuusisto, J. & Laakso, M. 2015, "Fasting and OGTT-derived measures of insulin resistance as compared with the euglycemic-hyperinsulinemic clamp in nondiabetic Finnish offspring of type 2 diabetic individuals", *The Journal of clinical endocrinology and metabolism,* vol. 100, no. 2, pp. 544-550.

Lotta, L.A., Gulati, P., Day, F.R., Payne, F., Ongen, H., van de Bunt, M., Gaulton, K.J., Eicher, J.D., Sharp, S.J., Luan, J., De Lucia Rolfe, E., Stewart, I.D., Wheeler, E., Willems, S.M., Adams,

C., Yaghootkar, H., EPIC-InterAct Consortium, Cambridge FPLD1 Consortium, Forouhi, N.G., Khaw, K.T., Johnson, A.D., Semple, R.K., Frayling, T., Perry, J.R., Dermitzakis, E., McCarthy, M.I., Barroso, I., Wareham, N.J., Savage, D.B., Langenberg, C., O'Rahilly, S. & Scott, R.A. 2017, "Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance", *Nature genetics,* vol. 49, no. 1, pp. 17-26.

Lundbom, J., Hakkarainen, A., Soderlund, S., Westerbacka, J., Lundbom, N. & Taskinen, M.R. 2011, "Long-TE 1H MRS suggests that liver fat is more saturated than subcutaneous and visceral fat", *NMR in biomedicine,* vol. 24, no. 3, pp. 238-245.

Luukkonen, P.K., Sadevirta, S., Zhou, Y., Kayser, B., Ali, A., Ahonen, L., Lallukka, S., Pelloux, V., Gaggini, M., Jian, C., Hakkarainen, A., Lundbom, N., Gylling, H., Salonen, A., Oresic, M., Hyotylainen, T., Orho-Melander, M., Rissanen, A., Gastaldelli, A., Clement, K., Hodson, L. & Yki-Jarvinen, H. 2018, "Saturated Fat Is More Metabolically Harmful for the Human Liver Than Unsaturated Fat or Simple Sugars", *Diabetes care,* vol. 41, no. 8, pp. 1732-1739.

Magalotti, D., Marchesini, G., Ramilli, S., Berzigotti, A., Bianchi, G. & Zoli, M. 2004, "Splanchnic haemodynamics in non-alcoholic fatty liver disease: effect of a dietary/pharmacological treatment. A pilot study", *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver,* vol. 36, no. 6, pp. 406-411.

Marengo, A., Rosso, C. & Bugianesi, E. 2016, "Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis", *Annual Review of Medicine,* vol. 67, pp. 103-117.

Mari, A., Pacini, G., Brazzale, A.R. & Ahren, B. 2005, "Comparative evaluation of simple insulin sensitivity methods based on the oral glucose tolerance test", *Diabetologia,* vol. 48, no. 4, pp. 748-751.

Mari, A., Pacini, G., Murphy, E., Ludvik, B. & Nolan, J.J. 2001, "A model-based method for assessing insulin sensitivity from the oral glucose tolerance test", *Diabetes care,* vol. 24, no. 3, pp. 539-548.

Mari, A., Schmitz, O., Gastaldelli, A., Oestergaard, T., Nyholm, B. & Ferrannini, E. 2002, "Meal and oral glucose tests for assessment of beta -cell function: modeling analysis in normal subjects", *American journal of physiology.Endocrinology and metabolism,* vol. 283, no. 6, pp. E1159-66.

Marventano, S., Salomone, F., Godos, J., Pluchinotta, F., Del Rio, D., Mistretta, A. & Grosso, G. 2016, "Coffee and tea consumption in relation with non-alcoholic fatty liver and metabolic syndrome: A systematic review and meta-analysis of observational studies", *Clinical nutrition (Edinburgh, Scotland),* vol. 35, no. 6, pp. 1269-1281.

Massafra, V. & van Mil, S.W.C. 2018, "Farnesoid X receptor: A "homeostat" for hepatic nutrient metabolism", *Biochimica et biophysica acta.Molecular basis of disease,* vol. 1864, no. 1, pp. 45-59.

Matschinsky, F.M., Magnuson, M.A., Zelent, D., Jetton, T.L., Doliba, N., Han, Y., Taub, R. & Grimsby, J. 2006, "The network of glucokinase-expressing cells in glucose homeostasis and the potential of glucokinase activators for diabetes therapy", *Diabetes,* vol. 55, no. 1, pp. 1-12.

Matsuda, M. & DeFronzo, R.A. 1999, "Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp", *Diabetes care,* vol. 22, no. 9, pp. 1462-1470.

Matsuda, M., Liu, Y., Mahankali, S., Pu, Y., Mahankali, A., Wang, J., DeFronzo, R.A., Fox, P.T. & Gao, J.H. 1999, "Altered hypothalamic function in response to glucose ingestion in obese humans", *Diabetes,* vol. 48, no. 9, pp. 1801-1806.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F. & Turner, R.C. 1985, "Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man", *Diabetologia,* vol. 28, no. 7, pp. 412-419.

McCullough, A.J. 2004, "The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease", *Clinics in liver disease,* vol. 8, no. 3, pp. 521-33, viii.

Meek, T.H., Wisse, B.E., Thaler, J.P., Guyenet, S.J., Matsen, M.E., Fischer, J.D., Taborsky, G.J.,Jr, Schwartz, M.W. & Morton, G.J. 2013, "BDNF action in the brain attenuates diabetic hyperglycemia via insulin-independent inhibition of hepatic glucose production", *Diabetes,* vol. 62, no. 5, pp. 1512-1518.

Meistas, M.T., Margolis, S. & Kowarski, A.A. 1983, "Hyperinsulinemia of obesity is due to decreased clearance of insulin", *The American Journal of Physiology,* vol. 245, no. 2, pp. E155-9.

Mergenthaler, P., Lindauer, U., Dienel, G.A. & Meisel, A. 2013, "Sugar for the brain: the role of glucose in physiological and pathological brain function", *Trends in neurosciences,* vol. 36, no. 10, pp. 587-597.

Mingrone, G., Panunzi, S., De Gaetano, A., Guidone, C., Iaconelli, A., Nanni, G., Castagneto, M., Bornstein, S. & Rubino, F. 2015, "Bariatric-metabolic surgery versus conventional medical treatment in obese patients with type 2 diabetes: 5 year follow-up of an open-label, single-centre, randomised controlled trial", *Lancet (London, England),* vol. 386, no. 9997, pp. 964-973.

Moore, M.C., Cherrington, A.D. & Wasserman, D.H. 2003, "Regulation of hepatic and peripheral glucose disposal", *Best practice & research.Clinical endocrinology & metabolism,* vol. 17, no. 3, pp. 343-364.

Moore, M.C., Coate, K.C., Winnick, J.J., An, Z. & Cherrington, A.D. 2012, "Regulation of hepatic glucose uptake and storage in vivo", *Advances in nutrition (Bethesda, Md.),* vol. 3, no. 3, pp. 286-294.

Moore, M.C., Satake, S., Lautz, M., Soleimanpour, S.A., Neal, D.W., Smith, M. & Cherrington, A.D. 2004, "Nonesterified fatty acids and hepatic glucose metabolism in the conscious dog", *Diabetes,* vol. 53, no. 1, pp. 32-40.

Mottin, C.C., Moretto, M., Padoin, A.V., Swarowsky, A.M., Toneto, M.G., Glock, L. & Repetto, G. 2004, "The role of ultrasound in the diagnosis of hepatic steatosis in morbidly obese patients", *Obesity Surgery,* vol. 14, no. 5, pp. 635-637.

Mudaliar, S., Henry, R.R., Sanyal, A.J., Morrow, L., Marschall, H.U., Kipnes, M., Adorini, L., Sciacca, C.I., Clopton, P., Castelloe, E., Dillon, P., Pruzanski, M. & Shapiro, D. 2013, "Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease", *Gastroenterology,* vol. 145, no. 3, pp. 574-82.e1.

Murphy, K.G. & Bloom, S.R. 2006, "Gut hormones and the regulation of energy homeostasis", *Nature,* vol. 444, no. 7121, pp. 854-859.

Nakamura, K.M., Haglind, E.G., Clowes, J.A., Achenbach, S.J., Atkinson, E.J., Melton, L.J.,3rd & Kennel, K.A. 2014, "Fracture risk following bariatric surgery: a population-based study", *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA,* vol. 25, no. 1, pp. 151-158.

Nakanishi, H., Cruz, N.F., Adachi, K., Sokoloff, L. & Dienel, G.A. 1996, "Influence of glucose supply and demand on determination of brain glucose content with labeled methylglucose", *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism,* vol. 16, no. 3, pp. 439-449.

Nannipieri, M., Mari, A., Anselmino, M., Baldi, S., Barsotti, E., Guarino, D., Camastra, S., Bellini, R., Berta, R.D. & Ferrannini, E. 2011, "The role of beta-cell function and insulin sensitivity in the remission of type 2 diabetes after gastric bypass surgery", *The Journal of clinical endocrinology and metabolism,* vol. 96, no. 9, pp. E1372-9.

Neuschwander-Tetri, B.A., Loomba, R., Sanyal, A.J., Lavine, J.E., Van Natta, M.L., Abdelmalek, M.F., Chalasani, N., Dasarathy, S., Diehl, A.M., Hameed, B., Kowdley, K.V., McCullough, A., Terrault, N., Clark, J.M., Tonascia, J., Brunt, E.M., Kleiner, D.E., Doo, E. & NASH Clinical Research Network 2015, "Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial", *Lancet (London, England),* vol. 385, no. 9972, pp. 956-965.

Nissen, S.E., Wolski, K.E., Prcela, L., Wadden, T., Buse, J.B., Bakris, G., Perez, A. & Smith, S.R. 2016, "Effect of Naltrexone-Bupropion on Major Adverse Cardiovascular Events in Overweight and Obese Patients With Cardiovascular Risk Factors: A Randomized Clinical Trial", *Jama,* vol. 315, no. 10, pp. 990-1004.

Nudel, J. & Sanchez, V.M. 2018, "Surgical management of obesity", *Metabolism: clinical and experimental,* .

Nummenmaa, L., Hirvonen, J., Hannukainen, J.C., Immonen, H., Lindroos, M.M., Salminen, P. & Nuutila, P. 2012, "Dorsal striatum and its limbic connectivity mediate abnormal anticipatory reward processing in obesity", *PloS one,* vol. 7, no. 2, pp. e31089.

Obici, S., Zhang, B.B., Karkanias, G. & Rossetti, L. 2002, "Hypothalamic insulin signaling is required for inhibition of glucose production", *Nature medicine,* vol. 8, no. 12, pp. 1376-1382.

Obici, S., Zhang, B.B., Karkanias, G. & Rossetti, L. 2002, "Hypothalamic insulin signaling is required for inhibition of glucose production", *Nature medicine,* vol. 8, no. 12, pp. 1376-1382.

Parsons, M.P. & Hirasawa, M. 2010, "ATP-sensitive potassium channel-mediated lactate effect on orexin neurons: implications for brain energetics during arousal", *The Journal of neuroscience : the official journal of the Society for Neuroscience,* vol. 30, no. 24, pp. 8061-8070.

Patlak, C.S. & Blasberg, R.G. 1985, "Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations", *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism,* vol. 5, no. 4, pp. 584-590.

Pekny, M. & Nilsson, M. 2005, "Astrocyte activation and reactive gliosis", *Glia,* vol. 50, no. 4, pp. 427-434.

Pellerin, L. & Magistretti, P.J. 2012, "Sweet sixteen for ANLS", *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism,* vol. 32, no. 7, pp. 1152-1166.

Pellerin, L. & Magistretti, P.J. 1994, "Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 91, no. 22, pp. 10625-10629.

Peterli, R., Steinert, R.E., Woelnerhanssen, B., Peters, T., Christoffel-Courtin, C., Gass, M., Kern, B., von Fluee, M. & Beglinger, C. 2012, "Metabolic and hormonal changes after laparoscopic Roux-en-Y gastric bypass and sleeve gastrectomy: a randomized, prospective trial", *Obesity Surgery,* vol. 22, no. 5, pp. 740-748.

Pilitsi, E., Farr, O.M., Polyzos, S.A., Perakakis, N., Nolen-Doerr, E., Papathanasiou, A.E. & Mantzoros, C.S. 2018, "Pharmacotherapy of obesity: Available medications and drugs under investigation", *Metabolism: clinical and experimental,* .

Pocai, A., Lam, T.K., Gutierrez-Juarez, R., Obici, S., Schwartz, G.J., Bryan, J., Aguilar-Bryan, L. & Rossetti, L. 2005, "Hypothalamic K(ATP) channels control hepatic glucose production", *Nature,* vol. 434, no. 7036, pp. 1026-1031.

Pocai, A., Lam, T.K., Obici, S., Gutierrez-Juarez, R., Muse, E.D., Arduini, A. & Rossetti, L. 2006, "Restoration of hypothalamic lipid sensing normalizes energy and glucose homeostasis in overfed rats", *The Journal of clinical investigation,* vol. 116, no. 4, pp. 1081-1091.

Polyzos, S.A., Kountouras, J. & Mantzoros, C.S. 2019, "Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics", *Metabolism: clinical and experimental,* vol. 92, pp. 82-97.

Polyzos, S.A. & Mantzoros, C.S. 2016, "Adiponectin as a target for the treatment of nonalcoholic steatohepatitis with thiazolidinediones: A systematic review", *Metabolism: clinical and experimental,* vol. 65, no. 9, pp. 1297-1306.

Pories, W.J., Swanson, M.S., MacDonald, K.G., Long, S.B., Morris, P.G., Brown, B.M., Barakat, H.A., deRamon, R.A., Israel, G. & Dolezal, J.M. 1995, "Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus", *Annals of Surgery,* vol. 222, no. 3, pp. 339-50; discussion 350-2.

Provencher, S.W. 1993, "Estimation of metabolite concentrations from localized in vivo proton NMR spectra", *Magnetic resonance in medicine,* vol. 30, no. 6, pp. 672-679.

Pucci, A. & Batterham, R.L. 2018, "Mechanisms underlying the weight loss effects of RYGB and SG: similar, yet different", *Journal of endocrinological investigation,* .

Puhakainen, I., Koivisto, V.A. & Yki-Jarvinen, H. 1992, "Lipolysis and gluconeogenesis from glycerol are increased in patients with noninsulin-dependent diabetes mellitus", *The Journal of clinical endocrinology and metabolism,* vol. 75, no. 3, pp. 789-794.

R Aroda, V., Ahmann, A., Cariou, B., Chow, F., Davies, M.J., Jodar, E., Mehta, R., Woo, V. & Lingvay, I. 2019, "Comparative efficacy, safety, and cardiovascular outcomes with once-weekly subcutaneous semaglutide in the treatment of type 2 diabetes: Insights from the SUSTAIN 1-7 trials", *Diabetes & metabolism,* .

Radziuk, J., Norwich, K.H. & Vranic, M. 1978, "Experimental validation of measurements of glucose turnover in nonsteady state", *The American Journal of Physiology,* vol. 234, no. 1, pp. E84-93.

Ramnanan, C.J., Saraswathi, V., Smith, M.S., Donahue, E.P., Farmer, B., Farmer, T.D., Neal, D., Williams, P.E., Lautz, M., Mari, A., Cherrington, A.D. & Edgerton, D.S. 2011, "Brain insulin action augments hepatic glycogen synthesis without suppressing glucose production or gluconeogenesis in dogs", *The Journal of clinical investigation,* vol. 121, no. 9, pp. 3713-3723.

Ramon, J.M., Salvans, S., Crous, X., Puig, S., Goday, A., Benaiges, D., Trillo, L., Pera, M. & Grande, L. 2012, "Effect of Roux-en-Y Gastric Bypass vs Sleeve Gastrectomy on Glucose and Gut Hormones: a Prospective Randomised Trial", *Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract, .*

Regan, J.P., Inabnet, W.B., Gagner, M. & Pomp, A. 2003, "Early experience with two-stage laparoscopic Roux-en-Y gastric bypass as an alternative in the super-super obese patient", *Obesity Surgery,* vol. 13, no. 6, pp. 861-864.

Requarth, J.A., Burchard, K.W., Colacchio, T.A., Stukel, T.A., Mott, L.A., Greenberg, E.R. & Weismann, R.E. 1995, "Long-term morbidity following jejunoileal bypass. The continuing potential need for surgical reversal", *Archives of surgery (Chicago, Ill.: 1960),* vol. 130, no. 3, pp. 318-325.

Rigazio, S., Lehto, H.R., Tuunanen, H., Nagren, K., Kankaanpaa, M., Simi, C., Borra, R., Naum, A.G., Parkkola, R., Knuuti, J., Nuutila, P. & Iozzo, P. 2008, "The lowering of hepatic fatty acid uptake improves liver function and insulin sensitivity without affecting hepatic fat content in humans", *American journal of physiology.Endocrinology and metabolism,* vol. 295, no. 2, pp. E413-9.

Rijzewijk, L.J., van der Meer, R.W., Lubberink, M., Lamb, H.J., Romijn, J.A., de Roos, A., Twisk, J.W., Heine, R.J., Lammertsma, A.A., Smit, J.W. & Diamant, M. 2010, "Liver fat content in type 2 diabetes: relationship with hepatic perfusion and substrate metabolism", *Diabetes,* vol. 59, no. 11, pp. 2747-2754.

Roden, M. & Bernroider, E. 2003, "Hepatic glucose metabolism in humans--its role in health and disease", *Best practice & research.Clinical endocrinology & metabolism,* vol. 17, no. 3, pp. 365-383.

Roden, M., Stingl, H., Chandramouli, V., Schumann, W.C., Hofer, A., Landau, B.R., Nowotny, P., Waldhausl, W. & Shulman, G.I. 2000, "Effects of free fatty acid elevation on postabsorptive endogenous glucose production and gluconeogenesis in humans", *Diabetes,* vol. 49, no. 5, pp. 701-707.

Rodriguez-Carmona, Y., Lopez-Alavez, F.J., Gonzalez-Garay, A.G., Solis-Galicia, C., Melendez, G. & Serralde-Zuniga, A.E. 2014, "Bone mineral density after bariatric surgery. A systematic review", *International journal of surgery (London, England),* vol. 12, no. 9, pp. 976-982.

Rojas, J.M. & Schwartz, M.W. 2014, "Control of hepatic glucose metabolism by islet and brain", *Diabetes, obesity & metabolism,* vol. 16 Suppl 1, pp. 33-40.

Romeo, S., Kozlitina, J., Xing, C., Pertsemlidis, A., Cox, D., Pennacchio, L.A., Boerwinkle, E., Cohen, J.C. & Hobbs, H.H. 2008, "Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease", *Nature genetics,* vol. 40, no. 12, pp. 1461-1465.

Rooijackers, H.M., Wiegers, E.C., Tack, C.J., van der Graaf, M. & de Galan, B.E. 2016, "Brain glucose metabolism during hypoglycemia in type 1 diabetes: insights from functional and metabolic neuroimaging studies", *Cellular and molecular life sciences : CMLS,* vol. 73, no. 4, pp. 705-722.

Rosqvist, F., Iggman, D., Kullberg, J., Cedernaes, J., Johansson, H.E., Larsson, A., Johansson, L., Ahlstrom, H., Arner, P., Dahlman, I. & Riserus, U. 2014, "Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans", *Diabetes,* vol. 63, no. 7, pp. 2356-2368.

Ross, R., Hudson, R., Stotz, P.J. & Lam, M. 2015, "Effects of exercise amount and intensity on abdominal obesity and glucose tolerance in obese adults: a randomized trial", *Annals of Internal Medicine,* vol. 162, no. 5, pp. 325-334.

Ryan, C.K., Johnson, L.A., Germin, B.I. & Marcos, A. 2002, "One hundred consecutive hepatic biopsies in the workup of living donors for right lobe liver transplantation", *Liver Transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society,* vol. 8, no. 12, pp. 1114-1122.

Ryan, D. & Heaner, M. 2014, "Guidelines (2013) for managing overweight and obesity in adults. Preface to the full report", *Obesity (Silver Spring, Md.),* vol. 22 Suppl 2, pp. S1-3.

Saadeh, S., Younossi, Z.M., Remer, E.M., Gramlich, T., Ong, J.P., Hurley, M., Mullen, K.D., Cooper, J.N. & Sheridan, M.J. 2002, "The utility of radiological imaging in nonalcoholic fatty liver disease", *Gastroenterology,* vol. 123, no. 3, pp. 745-750.

Salem, L., Jensen, C.C. & Flum, D.R. 2005, "Are bariatric surgical outcomes worth their cost? A systematic review", *Journal of the American College of Surgeons,* vol. 200, no. 2, pp. 270-278.

Salminen, P., Helmio, 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 Trial", *Jama,* vol. 319, no. 3, pp. 241-254.

Sanyal, A.J., Chalasani, N., Kowdley, K.V., McCullough, A., Diehl, A.M., Bass, N.M., Neuschwander-Tetri, B.A., Lavine, J.E., Tonascia, J., Unalp, A., Van Natta, M., Clark, J., Brunt, E.M., Kleiner, D.E., Hoofnagle, J.H., Robuck, P.R. & NASH CRN 2010, "Pioglitazone, vitamin

E, or placebo for nonalcoholic steatohepatitis", *The New England journal of medicine,* vol. 362, no. 18, pp. 1675-1685.

Schauer, P.R., Bhatt, D.L., Kirwan, J.P., Wolski, K., Aminian, A., Brethauer, S.A., Navaneethan, S.D., Singh, R.P., Pothier, C.E., Nissen, S.E., Kashyap, S.R. & STAMPEDE Investigators 2017, "Bariatric Surgery versus Intensive Medical Therapy for Diabetes - 5-Year Outcomes", *The New England journal of medicine,* vol. 376, no. 7, pp. 641-651.

Schleinitz, D., Bottcher, Y., Bluher, M. & Kovacs, P. 2014, "The genetics of fat distribution", *Diabetologia,* vol. 57, no. 7, pp. 1276-1286.

Seppala-Lindroos, A., Vehkavaara, S., Hakkinen, A.M., Goto, T., Westerbacka, J., Sovijarvi, A., Halavaara, J. & Yki-Jarvinen, H. 2002, "Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men", *The Journal of clinical endocrinology and metabolism,* vol. 87, no. 7, pp. 3023-3028.

Shao, N., Kuang, H.Y., Hao, M., Gao, X.Y., Lin, W.J. & Zou, W. 2014, "Benefits of exenatide on obesity and non-alcoholic fatty liver disease with elevated liver enzymes in patients with type 2 diabetes", *Diabetes/metabolism research and reviews,* vol. 30, no. 6, pp. 521-529.

Shimazu, T. & Minokoshi, Y. 2017, "Systemic Glucoregulation by Glucose-Sensing Neurons in the Ventromedial Hypothalamic Nucleus (VMH)", *Journal of the Endocrine Society,* vol. 1, no. 5, pp. 449-459.

Shulman, G.I. 2014, "Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease", *The New England journal of medicine,* vol. 371, no. 23, pp. 2237-2238.

Simpson, I.A., Carruthers, A. & Vannucci, S.J. 2007, "Supply and demand in cerebral energy metabolism: the role of nutrient transporters", *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism,* vol. 27, no. 11, pp. 1766-1791.

Sindelar, D.K., Chu, C.A., Rohlie, M., Neal, D.W., Swift, L.L. & Cherrington, A.D. 1997, "The role of fatty acids in mediating the effects of peripheral insulin on hepatic glucose production in the conscious dog", *Diabetes,* vol. 46, no. 2, pp. 187-196.

Sjostrom, L. 2013, "Review of the key results from the Swedish Obese Subjects (SOS) trial - a prospective controlled intervention study of bariatric surgery", *Journal of internal medicine,* vol. 273, no. 3, pp. 219-234.

Sjostrom, L., Lindroos, A.K., Peltonen, M., Torgerson, J., Bouchard, C., Carlsson, B., Dahlgren, S., Larsson, B., Narbro, K., Sjostrom, C.D., Sullivan, M., Wedel, H. & Swedish Obese Subjects

Study Scientific Group 2004, "Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery", *The New England journal of medicine,* vol. 351, no. 26, pp. 2683-2693.

Sjostrom, L., Narbro, K., Sjostrom, C.D., Karason, K., Larsson, B., Wedel, H., Lystig, T., Sullivan, M., Bouchard, C., Carlsson, B., Bengtsson, C., Dahlgren, S., Gummesson, A., Jacobson, P., Karlsson, J., Lindroos, A.K., Lonroth, H., Naslund, I., Olbers, T., Stenlof, K., Torgerson, J., Agren, G., Carlsson, L.M. & Swedish Obese Subjects Study 2007, "Effects of bariatric surgery on mortality in Swedish obese subjects", *The New England journal of medicine,* vol. 357, no. 8, pp. 741-752.

Sjostrom, L., Peltonen, M., Jacobson, P., Sjostrom, C.D., Karason, K., Wedel, H., Ahlin, S., Anveden, A., Bengtsson, C., Bergmark, G., Bouchard, C., Carlsson, B., Dahlgren, S., Karlsson, J., Lindroos, A.K., Lonroth, H., Narbro, K., Naslund, I., Olbers, T., Svensson, P.A. & Carlsson, L.M. 2012, "Bariatric surgery and long-term cardiovascular events", *Jama,* vol. 307, no. 1, pp. 56-65.

Sjostrom, L., Rissanen, A., Andersen, T., Boldrin, M., Golay, A., Koppeschaar, H.P. & Krempf, M. 1998, "Randomised placebo-controlled trial of orlistat for weight loss and prevention of weight regain in obese patients. European Multicentre Orlistat Study Group", *Lancet (London, England),* vol. 352, no. 9123, pp. 167-172.

Slimani, L., Kudomi, N., Oikonen, V., Jarvisalo, M., Kiss, J., Naum, A., Borra, R., Viljanen, A., Sipila, H., Ferrannini, E., Savunen, T., Nuutila, P. & Iozzo, P. 2008, "Quantification of liver perfusion with [(15)O]H(2)O-PET and its relationship with glucose metabolism and substrate levels", *Journal of hepatology,* vol. 48, no. 6, pp. 974-982.

Smeets, P.A., de Graaf, C., Stafleu, A., van Osch, M.J. & van der Grond, J. 2005, "Functional MRI of human hypothalamic responses following glucose ingestion", *NeuroImage,* vol. 24, no. 2, pp. 363-368.

Smith, B.R., Schauer, P. & Nguyen, N.T. 2008, "Surgical approaches to the treatment of obesity: bariatric surgery", *Endocrinology and metabolism clinics of North America,* vol. 37, no. 4, pp. 943-964.

Sofroniew, M.V. 2009, "Molecular dissection of reactive astrogliosis and glial scar formation", *Trends in neurosciences,* vol. 32, no. 12, pp. 638-647.

Soininen, P., Kangas, A.J., Wurtz, P., Suna, T. & Ala-Korpela, M. 2015, "Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics", *Circulation.Cardiovascular genetics,* vol. 8, no. 1, pp. 192-206.

Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M.H., Patlak, C.S., Pettigrew, K.D., Sakurada, O. & Shinohara, M. 1977, "The [14C]deoxyglucose method for the measurement of

local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat", *Journal of neurochemistry,* vol. 28, no. 5, pp. 897-916.

Spanoudaki, V.C. & Ziegler, S.I. 2008, "PET & SPECT instrumentation", *Handbook of Experimental Pharmacology,* vol. (185 Pt 1):53-74. doi, no. 185 Pt 1, pp. 53-74.

Stabin, M.G. & Brill, A.B. 2008, "Radiation dosimetry and exposure in nuclear medicine", *Seminars in nuclear medicine,* vol. 38, no. 5, pp. 306-307.

Stefan, N. & Haring, H.U. 2013, "The role of hepatokines in metabolism", *Nature reviews.Endocrinology,* vol. 9, no. 3, pp. 144-152.

Stefan, N., Haring, H.U., Hu, F.B. & Schulze, M.B. 2013, "Metabolically healthy obesity: epidemiology, mechanisms, and clinical implications", *The lancet.Diabetes & endocrinology,* vol. 1, no. 2, pp. 152-162.

Stefan, N., Staiger, H., Wagner, R., Machann, J., Schick, F., Haring, H.U. & Fritsche, A. 2015, "A high-risk phenotype associates with reduced improvement in glycaemia during a lifestyle intervention in prediabetes", *Diabetologia,* vol. 58, no. 12, pp. 2877-2884.

Stenberg, E., Szabo, E., Agren, G., Naslund, E., Boman, L., Bylund, A., Hedenbro, J., Laurenius, A., Lundegardh, G., Lonroth, H., Moller, P., Sundbom, M., Ottosson, J., Naslund, I. & Scandinavian Obesity Surgery Registry Study Group 2014, "Early complications after laparoscopic gastric bypass surgery: results from the Scandinavian Obesity Surgery Registry", *Annals of Surgery,* vol. 260, no. 6, pp. 1040-1047.

Stumvoll, M. & Jacob, S. 1999, "Multiple sites of insulin resistance: muscle, liver and adipose tissue", *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association,* vol. 107, no. 2, pp. 107-110.

Swinburn, B.A., Sacks, G., Hall, K.D., McPherson, K., Finegood, D.T., Moodie, M.L. & Gortmaker, S.L. 2011, "The global obesity pandemic: shaped by global drivers and local environments", *Lancet (London, England),* vol. 378, no. 9793, pp. 804-814.

Szczepaniak, L.S., Babcock, E.E., Schick, F., Dobbins, R.L., Garg, A., Burns, D.K., McGarry, J.D. & Stein, D.T. 1999, "Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo", *The American Journal of Physiology,* vol. 276, no. 5 Pt 1, pp. E977-89.

T Kelly 2008, "Global burden of obesity in 2005 and projections to 2030", *International journal of obesity,* vol. 32, no. 9, pp. 1431.

Takala, T.O., Nuutila, P., Pulkki, K., Oikonen, V., Gronroos, T., Savunen, T., Vahasilta, T., Luotolahti, M., Kallajoki, M., Bergman, J., Forsback, S. & Knuuti, J. 2002, "14(R,S)-[18F]Fluoro-6-thia-heptadecanoic acid as a tracer of free fatty acid uptake and oxidation in

myocardium and skeletal muscle", *European journal of nuclear medicine and molecular imaging,* vol. 29, no. 12, pp. 1617-1622.

Tchernof, A. & Despres, J.P. 2013, "Pathophysiology of human visceral obesity: an update", *Physiological Reviews,* vol. 93, no. 1, pp. 359-404.

Ten Kulve, J.S., Veltman, D.J., van Bloemendaal, L., Barkhof, F., Drent, M.L., Diamant, M. & IJzerman, R.G. 2016, "Liraglutide Reduces CNS Activation in Response to Visual Food Cues Only After Short-term Treatment in Patients With Type 2 Diabetes", *Diabetes care,* vol. 39, no. 2, pp. 214-221.

Thaler, J.P. & Cummings, D.E. 2009, "Minireview: Hormonal and metabolic mechanisms of diabetes remission after gastrointestinal surgery", *Endocrinology,* vol. 150, no. 6, pp. 2518-2525.

Thaler, J.P., Yi, C.X., Schur, E.A., Guyenet, S.J., Hwang, B.H., Dietrich, M.O., Zhao, X., Sarruf, D.A., Izgur, V., Maravilla, K.R., Nguyen, H.T., Fischer, J.D., Matsen, M.E., Wisse, B.E., Morton, G.J., Horvath, T.L., Baskin, D.G., Tschop, M.H. & Schwartz, M.W. 2012, "Obesity is associated with hypothalamic injury in rodents and humans", *The Journal of clinical investigation,* vol. 122, no. 1, pp. 153-162.

Thomsen, C., Becker, U., Winkler, K., Christoffersen, P., Jensen, M. & Henriksen, O. 1994, "Quantification of liver fat using magnetic resonance spectroscopy", *Magnetic resonance imaging,* vol. 12, no. 3, pp. 487-495.

Tilg, H., Moschen, A.R. & Roden, M. 2017, "NAFLD and diabetes mellitus", *Nature reviews.Gastroenterology & hepatology,* vol. 14, no. 1, pp. 32-42.

Turkington, T.G. 2001, "Introduction to PET instrumentation", *Journal of nuclear medicine technology,* vol. 29, no. 1, pp. 4-11.

Tuulari, J.J., Karlsson, H.K., Hirvonen, J., Hannukainen, J.C., Bucci, M., Helmio, M., Ovaska, J., Soinio, M., Salminen, P., Savisto, N., Nummenmaa, L. & Nuutila, P. 2013, "Weight loss after bariatric surgery reverses insulin-induced increases in brain glucose metabolism of the morbidly obese", *Diabetes,* vol. 62, no. 8, pp. 2747-2751.

Utzschneider, K.M., Bayer-Carter, J.L., Arbuckle, M.D., Tidwell, J.M., Richards, T.L. & Craft, S. 2013, "Beneficial effect of a weight-stable, low-fat/low-saturated fat/low-glycaemic index diet to reduce liver fat in older subjects", *The British journal of nutrition,* vol. 109, no. 6, pp. 1096-1104.

van Can, J., Sloth, B., Jensen, C.B., Flint, A., Blaak, E.E. & Saris, W.H. 2014, "Effects of the once-daily GLP-1 analog liraglutide on gastric emptying, glycemic parameters, appetite and energy metabolism in obese, non-diabetic adults", *International journal of obesity (2005),* vol. 38, no. 6, pp. 784-793.

van de Ven, K.C., van der Graaf, M., Tack, C.J., Klomp, D.W., Heerschap, A. & de Galan, B.E. 2010, "Optimized [1-(13)C]glucose infusion protocol for 13C magnetic resonance spectroscopy at 3T of human brain glucose metabolism under euglycemic and hypoglycemic conditions", *Journal of neuroscience methods,* vol. 186, no. 1, pp. 68-71.

van Herpen, N.A., Schrauwen-Hinderling, V.B., Schaart, G., Mensink, R.P. & Schrauwen, P. 2011, "Three weeks on a high-fat diet increases intrahepatic lipid accumulation and decreases metabolic flexibility in healthy overweight men", *The Journal of clinical endocrinology and metabolism,* vol. 96, no. 4, pp. E691-5.

Vargas, V., Allende, H., Lecube, A., Salcedo, M.T., Baena-Fustegueras, J.A., Fort, J.M., Rivero, J., Ferrer, R., Catalan, R., Pardina, E., Ramon Y Cajal, S., Guardia, J. & Peinado-Onsurbe, J. 2012, "Surgically induced weight loss by gastric bypass improves non alcoholic fatty liver disease in morbid obese patients", *World journal of hepatology,* vol. 4, no. 12, pp. 382-388.

Vazquez, G., Duval, S., Jacobs, D.R.,Jr & Silventoinen, K. 2007, "Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a meta-analysis", *Epidemiologic reviews,* vol. 29, pp. 115-128.

Vestergaard, E.T., Jessen, N., Moller, N. & Jorgensen, J.O. 2017, "Acyl Ghrelin Induces Insulin Resistance Independently of GH, Cortisol, and Free Fatty Acids", *Scientific reports,* vol. 7, pp. 42706.

Vidarsdottir, S., Smeets, P.A., Eichelsheim, D.L., van Osch, M.J., Viergever, M.A., Romijn, J.A., van der Grond, J. & Pijl, H. 2007, "Glucose ingestion fails to inhibit hypothalamic neuronal activity in patients with type 2 diabetes", *Diabetes,* vol. 56, no. 10, pp. 2547-2550.

Viljanen, A.P., Iozzo, P., Borra, R., Kankaanpaa, M., Karmi, A., Lautamaki, R., Jarvisalo, M., Parkkola, R., Ronnemaa, T., Guiducci, L., Lehtimaki, T., Raitakari, O.T., Mari, A. & Nuutila, P. 2009, "Effect of weight loss on liver free fatty acid uptake and hepatic insulin resistance", *The Journal of clinical endocrinology and metabolism,* vol. 94, no. 1, pp. 50-55.

Walker, M., Mari, A., Jayapaul, M.K., Bennett, S.M. & Ferrannini, E. 2005, "Impaired beta cell glucose sensitivity and whole-body insulin sensitivity as predictors of hyperglycaemia in non-diabetic subjects", *Diabetologia,* vol. 48, no. 12, pp. 2470-2476.

Weiss, R., D'Adamo, E., Santoro, N., Hershkop, K. & Caprio, S. 2011, "Basal alpha-cell up-regulation in obese insulin-resistant adolescents", *The Journal of clinical endocrinology and metabolism,* vol. 96, no. 1, pp. 91-97.

Westerbacka, J., Lammi, K., Hakkinen, A.M., Rissanen, A., Salminen, I., Aro, A. & Yki-Jarvinen, H. 2005, "Dietary fat content modifies liver fat in overweight nondiabetic subjects", *The Journal of clinical endocrinology and metabolism,* vol. 90, no. 5, pp. 2804-2809.

Williams, C.D., Stengel, J., Asike, M.I., Torres, D.M., Shaw, J., Contreras, M., Landt, C.L. & Harrison, S.A. 2011, "Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study", *Gastroenterology,* vol. 140, no. 1, pp. 124-131.

Wu, H.M., Bergsneider, M., Glenn, T.C., Yeh, E., Hovda, D.A., Phelps, M.E. & Huang, S.C. 2003, "Measurement of the global lumped constant for 2-deoxy-2-[18F]fluoro-D-glucose in normal human brain using [15O]water and 2-deoxy-2-[18F]fluoro-D-glucose positron emission tomography imaging. A method with validation based on multiple methodologies", *Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging,* vol. 5, no. 1, pp. 32-41.

Yki-Jarvinen, H. 2015, "Nutritional Modulation of Non-Alcoholic Fatty Liver Disease and Insulin Resistance", *Nutrients,* vol. 7, no. 11, pp. 9127-9138.

Yousseif, A., Emmanuel, J., Karra, E., Millet, Q., Elkalaawy, M., Jenkinson, A.D., Hashemi, M., Adamo, M., Finer, N., Fiennes, A.G., Withers, D.J. & Batterham, R.L. 2014, "Differential effects of laparoscopic sleeve gastrectomy and laparoscopic gastric bypass on appetite, circulating acyl-ghrelin, peptide YY3-36 and active GLP-1 levels in non-diabetic humans", *Obesity Surgery,* vol. 24, no. 2, pp. 241-252.

Zelber-Sagi, S., Ratziu, V. & Oren, R. 2011, "Nutrition and physical activity in NAFLD: an overview of the epidemiological evidence", *World journal of gastroenterology,* vol. 17, no. 29, pp. 3377-3389.

Zhang, Y., Zhao, H., Cao, Z., Sun, X., Zhang, C., Cai, W., Liu, R., Hu, S. & Qin, M. 2014, "A randomized clinical trial of laparoscopic Roux-en-Y gastric bypass and sleeve gastrectomy for the treatment of morbid obesity in China: a 5-year outcome", *Obesity Surgery,* vol. 24, no. 10, pp. 1617-1624.

Zheng, D., Ionut, V., Mooradian, V., Stefanovski, D. & Bergman, R.N. 2009, "Exenatide sensitizes insulin-mediated whole-body glucose disposal and promotes uptake of exogenous glucose by the liver", *Diabetes,* vol. 58, no. 2, pp. 352-359.

Zimmer, E.R., Parent, M.J., Souza, D.G., Leuzy, A., Lecrux, C., Kim, H.I., Gauthier, S., Pellerin, L., Hamel, E. & Rosa-Neto, P. 2017, "(18)F]FDG PET signal is driven by astroglial glutamate transport", *Nature neuroscience,* vol. 20, no. 3, pp. 393-395.

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