UNIVERSITY OF TURKU

THE EFFECTS OF BARIATRIC SURGERY ON ADIPOSE TISSUE ENERGY METABOLISM IN TYPE 2 DIABETES

Prince Dadson

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To Austin and Alvin

ABSTRACT

Prince Dadson

Effect of Bariatric Surgery on Adipose Tissue Energy Metabolism in Type 2 Diabetes

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

Obesity-related metabolic disturbances typically occur when the available energy exceeds the storage capacity of white adipose tissue (WAT), which can lead to ectopic lipid deposition, insulin resistance and type 2 diabetes. The re-discovery of the existence of energy consuming brown adipose tissue (BAT) in adult humans has ignited interest in finding novel approaches for BAT activation to be used for the treatment of obesity and diabetes. Currently, bariatric surgery procedures provide marked and sustained weight loss, and remission of diabetes in some severely obese patients. Energy metabolism in adipose tissue in the presence of obesity and diabetes, and the changes that occur after weight loss induced by surgery are largely understudied. The present work hypothesized that the WAT and BAT energy metabolism are different between obese and non-obese subjects, and also between obese with and without diabetes subgroups. Bariatric surgery improves adipose energy metabolism in severely obese patients independent of diabetes status.

Here, severely obese patients, with a mean BMI of 40 kg/m², roughly half with diabetes, and age-matched lean metabolically healthy controls, were studied with PET-CT and MRI to measure adipose tissue energy (glucose and fatty acid) metabolism, and blood flow distribution. The regional distribution of WAT; and the browning of supraclavicular BAT were also studied. White adipocyte size and numbers in biopsies were measured. Obese patients were studied before and at 6 months after surgery, and normal weight healthy controls studied once.

When the obese patients were treated as a combined group, their blood flow distribution, glucose and fatty acid metabolism rates in WAT were significantly impaired compared to nonobese healthy control subjects. Blood flow, fatty acid or glucose uptake rates when expressed per expanded depot mass were higher in the obese group compared to controls. Fatty acid uptake per adipocyte was higher only for diabetic patients of the obese group compared to the controls. Excessive accumulation of WAT was a limiting factor with regards BAT fatty acid metabolism, or then the browning of adipose tissue in the supraclavicular depot. After surgery, adipose glucose uptake rates increased independent of diabetes status. Fatty acid and blood flow rates decreased significantly when expressed per depot size, and per adipocyte. However, the postsurgery depot glucose uptake rates were similar to values in the presurgery state. BAT lipid metabolism increased significantly with weight loss, and values were associated with decreased adiposity along with improved whole body insulin sensitivity.

The current study highlights the negative impact of severe obesity and diabetes on adipose tissue energy metabolism, which appears to be partly due to expanded fat mass mediated by adipocyte hypertrophy or hyperplasia. Weight reduction with bariatric surgery improves adipose energy metabolism, and consequently the overall metabolic health in obese individuals regardless of the presence of diabetes.

Keywords: obesity, type 2 diabetes, positron emission tomography, white and brown adipose tissue, energy metabolism, glucose, fatty acid, blood flow, bariatric surgery

TIIVISTELMÄ

Prince Dadson

Lihavuusleikkausen vaikutus rasvakudoksen energia-aineenvaihduntaan tyypin 2 diabeteksessa

Turun yliopisto, Lääketieteellinen tiedekunta, Kliininen fysiologia ja isotooppilääketiede, Turun kliininen tohtoriohjelma, PET-keskus, Turku, Suomi.
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Lihavuuteen liittyvät aineenvaihduntahäiriöt tapahtyvat tyypillisesti, kun saatavilla oleva energiamäärä ylittää valkoisen rasvakudoksen varastointikyvyn. Tätä seuraa rasvan kertyminen muualle elimistöön, insuliiniresistenssi ja tyypin 2 diabetes. Energiaa kuluttavan ruskean rasvakudoksen löytyminen aikuisilta ihmisiltä on synnyttänyt kiinnostuksen löytää tapoja ruskean rasvan aktivointiin lihavuuden ja diabeteksen hoitamiseksi. Lihavuusleikkaus tarjoaa tehokkaan keinon painon pitkäaikaiseen alentamiseen ja diabeteksen lievittämiseen. Rasvakudoksen energia-aineenvaihduntaa lihavuudessa, diabeteksessa ja leikkauksen aikaansaaman painonpudotuksen jälkeen on tutkittu vähän. Tämän työn hypoteesi oli, että valkoisen ja ruskean rasvakudoksen energia-aineenvaihdunta ovat erilaisia lihavilla ja ei-lihavilla henkilöillä ja lihavilla diabetesta sairastavilla ja ei-diabeetikoilla. Lisäksi oletettiin että lihavuusleikkaus parantaa rasvakudoksen energia-aineenvaihduntaa diabetes-statuksesta riippumatta.

Tässä työssä tutkittiin rasvakudoksen energia-aineenvaihduntaa ja verenkiertoa vaikeasti lihavilla potilailla, joiden BMI oli 40 kg/m² ja noin puolella oli diabetes, ja samanikäisillä aineenvaihdunnan osalta terveiltä verrokeilta PET-TT- ja magneettikuvantamisella. Lisäksi tutkittiin valkoisen rasvakudoksen jakautumista elimistössä ja solisluun yläpuolisen ruskean rasvan esiintymistä. Valkoistern rasvasolujen kokoa ja määriä mitattiin kudosnäytteistä. Tutkimus suoritettiin lihavilla kahdesti, ennen lihavuusleikkausta ja kuusi kuukautta leikkauksen jälkeen. Verrokkihenkilöt tutkittiin yhden kerran.

Valkoisen rasvakudoksen verenkierto sekä glukoosi- ja rasva-aineenvaihdunta olivat merkitsevästi häiriintyneet lihavilla tutkittavilla verrattuna terveisiin kontrollihenkilöihin. Rasvakudoksen verenvirtaus sekä glukoosin- ja rasvahappojen otto oli lihavilla suurempi rasvakudosten kokonaismassoihin nähden. Rasvahappojen otto yksittäistä rasvasolua kohti oli suurempi lihavilla diabeetikoillta verrattuna terveisiin kontrollihenkilöihin. Rasvahappojen otto solisluun yläpuoliseen rasvakudokseen oli alhaisempi lihavilla tutkittavilla terveisiin verrattuna, mikä viittaa alhaisempaan ruskean rasvan suhteelliseen osuuteen valkoiseen rasvaan nähden. Leikkauksen jälkeen rasvakudosten glukoosinotto rasvakiloa kohden kasvoi sekä diabeetikoilla että ei-diabeetikoilla. Rasvahappojen otto ja verenvirtaus, mutta ei glukoosinotto koko kudoksia ja yksittäistä rasvasolua kohden aleni leikkauksen jälkeen. Ruskean rasvakudoksen rasva-aineenvaihdunta kasvoi merkitsevästi painonpudotuksen yhteydessä ja kudoksen rasva-aineeinvaihdunta liittyi vähentyneeseen lihavuuteen ja parantuneeseen koko kehon insuliiniherkkyyteen.

Työn tuloksissa korostuvat vaikean lihavuuden ja diabeteksen aiheuttamat haitat rasvakudoksen aineenvaihduntaan. Näiden haittojen taustalla on todennäköisesti sekä rasvakudosten kokonaismassojen että yksittäisten rasvasolujen koon kasvu. Lihavuusleikkauksella saavutettava painonlasku parantaa rasvakudoksen energia-aineenvaihduntaa ja siten aineenvaihdunnan toimintaa kokonaisuutena riippumatta siitä onko henkilöillä diabetes tai ei.

Avainsanat: lihavuus, tyypin 2 diabetes, positroniemissiotomografia, valkoinen ja ruskea rasvakudos, energia-aineenvaihdunta, glukoosi, rasvahappo, verenvirtaus, lihavuusleikkaus

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ABBREVIATIONS

^{15}O-H_2O	oxygen-15 labeled water
^{18}F-FDG	2-deoxy-2-(^{18}F) fluoro-D-glucose
^{18}F-FTHA	14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid
acyl-CoA	acetyl coenzyme A
AMP	adenosine monophosphate
ANG II	angiotensin II
AR	androgen receptor
ATBF	adipose tissue blood flow
ATP	adenosine triphosphate
BAT	brown adipose tissue
BMI	body mass index
BMP-7	bone morphogenetic protein
Brite	brown-in-white adipocytes
C/EBP	CCAAT-enhancer-binding proteins
CoA	coenzyme A
C/EBP-β	CCAAT/enhancer-binding protein beta
CRP	C-reactive protein
CT	computed tomography
DPP-4	dipeptidyl peptidase 4
ER	extraction ratio
FAT/CD36	fatty acid translocase/cluster of differentiation 36
FAU	fatty acid uptake
FDG-6-P	FDG-6-phosphate
FFA	free fatty acids
FFE	fast field echo
FGF21	fibroblast growth factor 21
Foxo1	forkhead box protein O1
FUR	fractional tracer uptake
GH	growth hormone
GIP	gastric inhibitory polypeptide
GLP-1	glucagon-like peptide-1
GLUT4	glucose transporter type 4
GOX	carbohydrate oxidation
GU	glucose uptake
$HbA1_C$	glycated hemaglobin
HU	Hounsfield unit
IGF1	insulin-like growth factor 1
IFP	infrapatellar fat pad
IMCL	intramyocellular lipids
IMTG	intramuscular triglyceride

*K*i	flux rate
LCFA	long chain fatty acids
LDL	low-density lipoprotein
LOX	lipid oxidation rate
MCP-1	monocyte chemoattractant protein 1
MRI	magnetic resonance imaging
Myf5	myogenic factor 5
NEFA	non-esterified fatty acid
ND	non-type 2 diabetics
NO	nitric oxide
OGTT	oral glucose tolerance test
Pax 7	paired box protein 7
PET	positron emission tomography
PGC-1α	receptor gamma coactivator 1 alpha
PPAR-γ	peroxisome proliferator-activated receptor gamma
PPARγ2	Peroxisome proliferator-activated receptor γ2
PRDM16	positive regulatory domain containing 16
PYY	peptide YY
REE	resting energy expenditure
ROIs	regions of interest
RYGB	roux-en-Y gastric bypass
SAT	subcutaneous adipose tissue
sBAT	supraclavicular brown adipose tissue
SG	sleeve gastrectomy
SGLT-2	sodium-glucose co-transporter-2
T1W	T1 weighted
T2D	type 2 diabetes
TAC	time activity curve
TAG	triacylglycerol
TNF-α	tumor necrosis factor alpha
TZD	thiazolidinedione
UCP1	uncoupling protein 1
VAT	visceral adipose tissue
VCO2	carbon dioxide output
VLCD	very low calorie diet
VLDL	very low density lipoprotein
VO2	oxygen uptake
WAT	white adipose tissue

LIST OF ORIGINAL PUBLICATIONS

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

I Dadson P, Landini L, Helmiö M, Hannukainen JC, Immonen H, Honka MJ, Bucci M, Savisto N, Soinio M, Salminen P, Parkkola R, Pihlajamäki J, Iozzo P, Ferrannini E, Nuutila P. Effect of Bariatric Surgery on Adipose Tissue Glucose Metabolism in Different Depots in Patients With or Without Type 2 Diabetes. Diabetes Care. 2016; 39(2):292-9.

II Dadson P, Ferrannini E, Landini L, Hannukainen JC, Kalliokoski KK, Vaittinen M, Honka H, Karlsson HK, Tuulari JJ, Soinio M, Salminen P, Parkkola R, Pihlajamäki J, Iozzo P, Nuutila P. Fatty acid uptake and blood flow in adipose tissue compartments of morbidly obese subjects with or without type 2 diabetes: effects of bariatric surgery. Am J Physiol Endocrinol Metab. 2017; 313(2):E175-E182.

III Hannukainen JC, Lautamäki R, Pärkkä J, Strandberg M, Saunavaara V, Hurme S, Soinio M, Dadson P, Virtanen KA, Grönroos T, Forsback S, Salminen P, Iozzo P, Nuutila P. Reversibility of myocardial metabolism and remodelling in morbidly obese patients 6 months after bariatric surgery. Diabetes Obes Metab. 2018 Apr; 20 (4):963-973

IV Dadson P, Hannukainen JC, Din MU, Lahesmaa M, Kalliokoski KK, Iozzo P, Pihlajamäki J, Karlsson HK, Parkkola R, Salminen P, Virtanen KA, Nuutila P. Brown adipose tissue lipid metabolism in morbid obesity: the effect of bariatric surgery-induced weight loss. Diabetes Obes Metab. 2018 May; 20 (5):1280-1288.

1 INTRODUCTION

Excessive deposition of white adipose tissue (WAT) through an increase in cell size (adipocyte hypertrophy), and cell numbers (adipocyte hyperplasia) characterizes the development of obesity (Spalding et al., 2008). The study of WAT biology has therefore attracted considerable research attention due to its pathophysiological role in the development of obesity-related metabolic abnormalities including type 2 diabetes (T2D) (World Health Organization, 2016). Body Mass Index [(BMI, expressed as weight in kilograms divided by the square of the height in meters (kg/m^2)] is used to classify underweight (less than 18.5 kg/m^2), normal weight (18.5 - 24.9 kg/m^2), overweight (25.0 - 29.9 kg/m^2), obese (30.0 - 34.9 kg/m^2) and severe obesity (35.0 - 39.9 kg/m^2). A BMI greater than or equal to 40.0 kg/m^2 (or greater than 36.0 kg/m^2, with an additional comorbid condition) is classified under morbid obesity (World Health Organization, 2016).

Generally, WAT is grouped on the basis of its anatomical location, cellular composition, molecular markers, physiological functions, and clinical or prognostic features. Anatomically, subcutaneous adipose tissue (SAT) WAT is located beneath the skin and is predominantly distributed in the back and anterior abdominal wall, gluteofemoral regions, with deposits in the facial regions (Bjorndal et al, 2011). The distribution and the depot size of SAT varies in the population on the basis of genetics, age and sensitivity to hormones and glucocorticoids (Bjorntorp, 1991). Functionally, SAT acts as a metabolic sink for the storage of excess free fatty acids (FFA) and glycerol as triglycerides in their adipocytes during periods of excess energy intake with limited energy expenditure (Seale & Lazar, 2009).

When the physiological storage capacity of SAT is reached, it is often compounded by the impaired proliferation and differentiation of adipocytes, and the storage of excess fatty acids are shifted to ectopic sites (Slawik & Vidal-Puig, 2007). Ectopic adipose tissue is defined according to their location and their potential systemic or localized metabolic effects (Britton & Fox, 2011). Deposition of fat in the mesenteric and omental regions, generally referred to as visceral adipose tissue (VAT), exert the most profound systemic effects (Britton & Fox, 2011). Increasing VAT volume is associated with the infiltration by macrophages and other immune cells that impair adipocyte function and this may contributing to the development of insulin resistance and diabetes (Amano et al., 2014). Ectopic deposition of fat in both intrahepatic (Fabbrini et al., 2009) and intramuscular (Muoio, 2012), adipose tissue are also profoundly associated with insulin resistance and systemic metabolic disorders. In contrast, adipose tissue with localized effects include tissue surrounding the heart (pericardial and epicardial), blood vessels (perivascular), coronary arteries (periarterial), which are found to be associated with incidence of cardiovascular events (Ding et al., 2009). Likewise, increased amounts of cardiomyocellular lipid accumulation have been observed in individuals with impaired glucose tolerance, and in those with overt diabetes

(McGavock et al., 2007). Ectopic adipose accumulation in the renal sinuses compress the renal vasculature, which subsequently leads to renal injury and hypertension (Irazabal & Eirin, 2016). Furthermore, large infrapatellar fat pads (IFP) are located at the anterior of the knee joints can be found adjacent to the quadriceps and prefemoral fat pad bodies (Gallagher, Tierney, Murray, & O'Brien, 2005). Cells in the IFP serve a localized source of pro-inflammatory adipokines and cytokines, fibroblast growth factors, vascular endothelial growth factor, and inflammatory mediators such fatty acids, nitric oxide and prostaglandins (Chuckpaiwong, Charles, Kraus, Guilak, & Nunley, 2010; Ushiyama, Chano, Inoue, & Matsusue, 2003). Apart from the presence of fat pads in the knee, fat pads are also located in the shoulder (Vahlensieck, 2000) and elbow (Awaya et al., 2001) joints of the body.

Brown adipose tissue (BAT) has long been known to be the main thermogenic tissue in newborn humans. However, in 2009, three independent research groups demonstrated that adult humans also have functional BAT, which is located in the cervical-supraclavicular region and which express an uncoupling protein 1 (UCP1) required for non-shivering thermogenesis (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). Research shows that in addition to WAT there are also beige/"brite" (brown-in-white) cells, which possess the potential for a reversible switch between energy storage and expenditure, a process called "browning". Under basal conditions beige cells perform energy storage role, they are however transformed to UCP1-expressing BAT-like cells upon cold exposure or β3-adrenergic stimulation (J. Wu, Cohen, & Spiegelman, 2013).

Imaging using Positron Emission Tomography (PET) provides a three-dimensional non-invasive approach for the quantification of metabolic activities of adipose tissue glucose (Virtanen et al., 2001), fatty acids (Hannukainen et al., 2010) and blood flow distribution (Virtanen et al., 2002) in WAT. PET imaging has also been used for the measurement of BAT metabolic activities (Orava et al., 2013; Virtanen et al., 2009). As evidence accumulates suggesting the link between morbid obesity and dysfunctional WAT (Hoffstedt et al., 2017) and BAT (Vijgen et al., 2012), bariatric surgery provides an efficacious method to achieve marked sustained weight loss, and improvements in T2D (Adams et al., 2012; Courcoulas et al., 2013; Salminen et al., 2018; Sjostrom et al., 2007). However, BAT and WAT-specific metabolism among the morbidly obese (some of whom expressed diabetes phenotype) and the changes that may occur after bariatric surgery has scarcely been studied. In the present series of studies, the PET imaging modality has been utilized for the assessment of WAT metabolism. Adipose tissue lipid content has been quantified from CT imaging and, and adipocyte size from biopsies. The goal was to assess the effect of surgery induced weight loss on adipose tissue metabolism and the association with improvements in overall metabolic health in morbidly obese patients.

2 REVIEW OF THE LITERATURE

2.1 Developmental origin of adipocytes

The primary roles of white adipocytes are energy storage, and the regulation of glucose and lipid metabolism, in addition to performing endocrine and paracrine functions (Sanchez-Gurmaches, Hung, & Guertin, 2016). Brown adipocytes have become a topical issue in metabolic research owing to its role in health and disease, and especially in regard to the possibility of harnessing its thermogenic properties for the treatment of obesity and diabetes. Therefore, understanding the developmental origins of adipocytes will provide the basis for the distribution patterns observed in human populations, particularly in obese and T2D populations. Moreover, the adipocyte origin could explain the metabolic profiles in different adipose tissue depots, and also identify precursor cells and the mechanisms, which prevent or promotes its expansion in excess or negative caloric balance. Studying the developmental origins could assist in re-engineering and manipulation of certain specific type of adipocytes (e.g. brown adipocytes) for anti-obesity and anti-diabetes therapeutic interventions.

2.1.1 White adipocytes

White adipocytes originate from adipocyte progenitor cells that arise from the mesenchymal stem cells located in the stromal vascular fraction (Schulz et al., 2011) (Figure 1). These progenitor cells are also derived from fat-tissue endothelial cells and subsequently from pericytes due of their close connections to blood vessels (Tang et al., 2008; Tran et al., 2012). The differentiation of adipocytes is regulated by transcription factors such as peroxisome proliferator-activated receptor gamma (PPARγ) and members of the CCAAT/enhancer binding protein (C/EBP) family (Rosen, Walkey, Puigserver, & Spiegelman, 2000). Specifically, the transcription factors peroxisome proliferator-activated receptor gamma-2 (PPARγ2) and C/EBPα play pivotal roles in adipocyte gene expression profiles (Rosen et al., 2000). Additional transcription factors such as forkhead box protein O1 (Foxo1), Sterol regulatory element-binding transcription factor 1c (SREBP-1c) known for their roles in adipogenesis have been reported (Nakae et al., 2003). A fully mature white adipocyte is spherical and contains and larger unilocular lipid droplet (Parlee, Lentz, Mori, & MacDougald, 2014) in shape best suited for its function of storing lipid in the form of triglycerides (Lin & Farmer, 2016). Most of the knowledge on the developmental origins of white adipocytes is derived from mouse models and cell line studies.

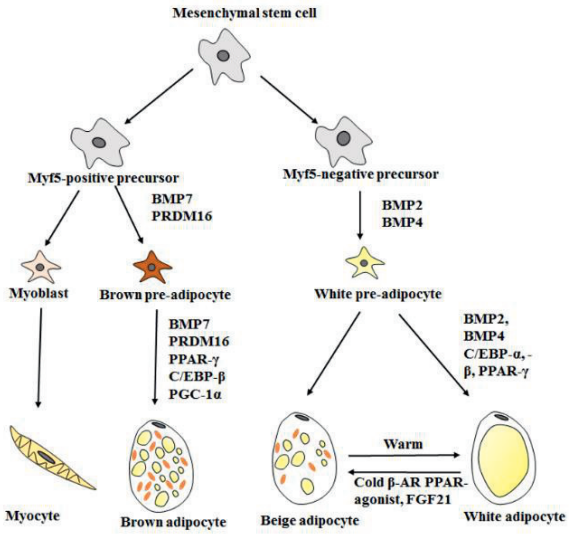

Figure 1. The cellular lineage showing the differentiation into brown, beige and white adipocytes (modified from mesenchyme stem cells; diagram from (Park, Kim, & Bae, 2014). Brown adipocyte share a common myogenic factor 5 (Myf5+) precursor with skeletal muscle and not with white adipocytes. The Myf5+ transforms into fully mature brown adipocytes by such factors as bone morphogenic protein 7 (BMP7), peroxisome proliferator-activated receptor-γ (PPAY-γ) and CCAAT/enhancer-binding proteins (C/EBPs) with transcriptional co-regulator PR domain-containing 16 (PRDM16) and peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC-1α). White adipocytes are transformed into beige adipocytes by cold exposure α β-adrenergic agonist or a PPAR-γ agonist, adrenergic receptor (AR), fibroblast growth factor 21 (FGF21).

2.1.2 Brown adipocytes

Brown adipocytes are derived from myogenic factor 5 (Myf5)-positive progenitor, which is a cellular lineage similar to that of skeletal muscle (Seale & Lazar, 2009) (Figure 1). In rodents, BAT is located in the interscapular, sub-scapular, cervical and along the trunk and neck regions (Walden et al., 2012). In mice, the distribution of BAT can also be found around aorta and kidneys (Fitzgibbons et al., 2011). The BAT found in human neonates protects against cold exposure through a thermogenic process, and as such its formation and differentiation take place before birth. Brown preadipocytes isolated from interscapular depot possess strikingly similar gene expression profiles, including my5f genes, to skeletal muscles than to cells isolated from perigonadal WAT depot (Timmons et al., 2007). Cell lineage studies show the PPAR-γ, receptor gamma coactivator 1 alpha (PGC-1α), CCAAT/enhancer-binding protein beta (C/EBP-β) and PR domain containing 16 (PRDM16) to be the primary transcription factors in the differentiation of brown adipocytes, which also mediate the regulation of the BAT thermogenic process by activating the expression of uncoupling protein 1 (UCP1) (Tanaka, Yoshida, Kishimoto, & Akira, 1997). UCP1 is an inner mitochondrial membrane protein that dissipates the proton gradient to

uncouple fuel oxidation from the synthesis of adenosine triphosphate (ATP) synthesis (Cannon & Nedergaard, 2004). The combination of the activities of PGC-1α, PPAR-γ and PPAR-α has been shown to regulate oxidative metabolism and mitochondrial biogenesis (Townsend & Tseng, 2012). Brown adipocytes have smaller diameters to those of WAT adipocytes (Rosenwald & Wolfrum, 2014), and are characterized by multilocular architecture, high mitochondrial density and increased expression UCP1. Brown adipocytes are usually located in depots that are highly innervated and vascularized (W. Wang & Seale, 2016).

2.1.3 Beige adipocytes

It has been known for a long time that UCP1-expressing adipocytes can be induced in animals (Cousin et al., 1992). Beige, also referred to as brite (brown-in-white) (Lazar, 2008; Petrovic et al., 2010; Schulz et al., 2011) are multilocular UCP1-expressing adipocytes with characteristics similar to classical brown adipocytes (Rosenwald & Wolfrum, 2014). The embryonic origin and formation of beige cells is still unclear. Two models have been proposed to explain the origins of beige cells: one model states that adipocytes arise from the trans-differentiation of matured preexisting white adipocytes, the second model states that beige adipocytes arise *de novo* from distinct precursor cells (W. Wang & Seale, 2016; J. Wu et al., 2012). In rodent studies, beige adipocytes arise predominantly from myf5-deficient lineage and are mainly located in the perigonadal and inguinal WAT, whereas beige adipocytes originating from myf5 positive lineage are found within the anterior and retroperitoneal WAT depots (Sanchez-Gurmaches et al., 2012; Seale et al., 2008). Ongoing discussions and debates have attempted to distinguish classical BAT from beige fat (Park et al., 2014; Sidossis & Kajimura, 2015) on the basis of their development origin, cell size features, and the expression of specific type of transcriptomes (Giralt & Villarroya, 2013). Moreover, the two cell types possess differential response to hormonal stimuli or genetic manipulation (Bostrom et al., 2012; Harms & Seale, 2013). Brown adipocytes minimally react to browning agents (Kalinovich, de Jong, Cannon, & Nedergaard, 2017). It has been speculated that classical brown and beige adipocytes are not independent cell types because both cell types express UCP1 and share similar β-adrenergic receptor or intracellular cyclic AMP-dependent pathway that regulate thermogenic gene expression (Mulya & Kirwan, 2016; W. Wang & Seale, 2016; J. Wu et al., 2013)

2.2 Adipose tissue distribution and metabolic complications of obesity

The metabolic characteristics that are exhibited by adipose tissue are based on the distribution (Arner, 2005), which varies considerably in the subject with respect to the physiological state, sex, family (first-degree relatives), age, hormonal changes or in response to certain therapeutic interventions (Tchkonia et al., 2010). Obesity is characterized by depot specific enlargement of adipose tissue through adipocyte hyperplasia, hypertrophy or a combination of both processes (Sun, Kusminski, & Scherer, 2011). In vitro and in vivo studies indicate that adipocyte hypertrophy is one of the primary events that underlie the decline in insulin sensitivity in obese and in lean subjects (Chandalia et al., 2007; Hoffstedt et al., 2010). In obese subjects, the adipose tissue is inundated with proinflammatory markers in varying degrees and phenotype abundance. Infiltration of macrophages into adipose tissue is a marked characteristic of the systemic metabolic derangements that are observed during obesity (Caer et al., 2017). Previous studies have shown that macrophages are the principal sources of biomolecules known to induce inflammation, fibrosis and insulin resistance (Permana, Menge, & Reaven, 2006). Macrophages and adipocytes within the adipose tissue interact in a paracrine fashion that leads to the release of proinflammatory adipokines such as tumor necrosis factor alpha (TNF-α), monocyte chemoattractant protein 1 (MCP-1), and a decreased production of anti-inflammatory adiponectin (Suganami, Nishida, & Ogawa, 2005). Macrophages are categorized into M1 phenotype known for its role in insulin resistance through the secretion of proinflammatory cytokines, and M2 macrophages known for their insulin sensitizing role through the release of interleukins (IL)-4 or IL-10 (Tateya, Kim, & Tamori, 2013). Elevation in levels of the proinflammatory macrophage-derived cytokines such as IL-6 and -8 have been observed in patients with expanded adipocytes (Chandalia et al., 2007). Various acute-phase proteins are known to underlie the development of low-grade inflammation, impaired glucose tolerance and diabetes. Notably, expressions of fibrinogen, orosomucoid, haptoglobin, and alpha-1-antitrypsin have been found to be strongly associated with the early onset of T2D (Schmidt et al., 1999). C-reactive protein (CRP), an acute phase protein of hepatic origin, is synthesized mainly in hepatocytes in response to adipocytes-derived proinflammatory cytokines (Pepys & Hirschfield, 2003). Elevated levels of CRP have been described in individuals with diabetes, it is however not clear if CRP is upregulated in individuals expressing both obesity and diabetes (Lu et al., 2010).

2.2.1 Subcutaneous adipose tissue

The SAT is distributed under the skin and constitute approximately 80 percent of total body fat. It is broadly classified into lower body fat (gluteal fat, subcutaneous femoral fat, and femoral intra- intermuscular fat), and upper-body subcutaneous fat (Tchkonia et al., 2010). The growth of SAT is not uniform. Under chronic excess caloric exposure, femoral SAT undergoes adipocyte hyperplasia, whereas abdominal SAT undergoes hypertrophy (Tchoukalova et al., 2008). On the whole, SAT appears to be immunologically, metabolically and mechanically protective because of its potential to expand outward without anatomic constraint (Goossens & Blaak, 2015). SAT is specialized to provide long-term energy storage, and also acts as a sink for potentially lipotoxic fatty acids (Tchkonia et al., 2013). It has been shown that the removal or loss of SAT through lipodystrophies or aging is heavily linked to low-grade systemic inflammation, lipotoxicity, and insulin resistance (Armstrong et al., 2014; Monteiro & Azevedo, 2010).

In the abdominal region, the Scapa's fascia separates the SAT into two depots: the areolar layer or the superficial region and the deep or the lamellar layer with different cell morphology and functional characteristics (Sbarbati et al., 2010). The superficial region is located below the skin with tightly packed small fat lobules between fibrous septae, arranged perpendicularly to the skin. The deep depot comprises large fat lobules that are loosely packed within widely spaced vertical and oblique fibrous septae. The superficial SAT is widespread in the general body surface, whereas the deep SAT is concentrated in the abdominal region, along the hips, and the internal surface of the upper third of the thigh, knees and the posterior surface of the arm (Wan et al., 2014). The quantity of deep subcutaneous fat correlates with the accumulation of visceral fat (Kelley, Thaete, Troost, Huwe, & Goodpaster, 2000).

2.2.2 Intra-abdominal adipose tissue

The anatomical locations of intra-abdominal adipose tissue VAT, are located within the omental and mesenteric depots. Functionally, the mesenteric depot fat is metabolically, and morphologically distinct from omental fat (Caserta et al., 2001). Based on the 'portal theory', increasing amounts of FFAs and pro-inflammatory cytokines released from omental VAT into the portal vein directly end up in the liver thus, the development of hepatic insulin resistance and liver steatosis (Bjorntorp, 1990a). The mesenteric fat is the first depot to encounter lipids as they travel within chylomicrons from the gut through lymphatics to join the circulation at the thoracic duct and vena cava, bypassing the liver (Tchkonia et al., 2013). Cellular composition, lipid synthesis and lipolysis and gene expression profiles are different between omental and mesenteric adipose tissue depots (Fried, Leibel, Edens, & Kral, 1993). Recent evidence suggests that mesenteric fat is an independent determinant of

cardio-metabolic complications in humans (Liu, Chan, Chan, Chan, & Chu, 2006). In adults with severe obesity, large omental depot adipocytes are more predictive of metabolic abnormalities than a larger abdominal SAT (Hoffstedt et al., 2010b). In general, visceral obesity in normal weight or moderately overweight individuals is strongly associated with insulin resistance, but in severe overall obesity, visceral obesity is a weak independent predictor (Stefan et al., 2008).

2.2.3 Ectopic lipid deposition

Ectopic fat is the accumulation of triglyceride droplets into cells of non-adipose tissues which more often than not, already contain small amounts of triglyceride (Lettner & Roden, 2008). The adverse metabolic effects of ectopic lipid deposition is not solely attributable to the excess of lipids per se, but also to the influx of toxic lipid metabolites into metabolically relevant structures such as the heart, liver, skeletal muscle, and kidneys (Snel et al., 2012). In addition, the tissue/organ-specific insulin resistance associated with lipid deposition is partly due to the insufficient production and release of cytokines and the decrease production and release of adipokines that are known for the regulation of immune cells, increased energy expenditure and insulin sensitizing properties (Lettner & Roden, 2008).

2.2.3.1 Mediastinal adipose tissue

Mediastinal adipose tissue is situated within the mediastinum, but outside the pericardium that encloses the heart, and accounts for a significant proportion of intra-thoracic adipose tissue (Rosito et al., 2008). Mediastinal fat depot has variously been referred to as comprising the intra-thoracic, extra-pericardial and pericardial adipose tissues (Sicari et al., 2011; Sironi et al., 2012). Epicardial adipose tissue is located between the outer wall of the myocardium and the visceral layer of the pericardium, whereas pericardial adipose tissue is located between the visceral and parietal pericardium (Iacobellis, Corradi, & Sharma, 2005). Ectopic triglyceride accumulation in the epicardium and pericardium is a risk factor for cardiovascular diseases (Rabkin, 2007). The epicardial perivascular adipocytes of obese individuals exhibit reduced adipocytic differentiation, decreased production of anti-inflammatory adiponectin, and increased secretion of proinflammatory IL-6, -8, and MCP-1 (Chatterjee et al., 2009; Fitzgibbons et al., 2011). Results from previous studies showed that the steady accumulation of epicardial adipose tissue is closely associated with metabolic syndrome, visceral adiposity, and insulin resistance and increased fasting glucose (Iacobellis, Barbaro, & Gerstein, 2008). Interestingly, other studies have suggested that it is the increased volume of mediastinal, and not epicardial adipose tissue that is associated

with plasma triglycerol and CRP levels (Sironi et al., 2012; Tadros et al., 2010). Both the historical (Heaton, 1972), and the current and emerging evidence (Cypess et al., 2009; Nedergaard, Bengtsson, & Cannon, 2007; Saito et al., 2009), suggest that mediastinal adipose tissue could contain cells with BAT-like characteristics. The mediastinal fat depot has been found to contain smaller multilocular lipid droplets that are rich in mitochondria, and exhibit more mRNA UCP-1 expression signature when compared to abdominal SAT (Cheung et al., 2013).

2.2.3.2 Skeletal muscle lipid storage

Lipid storage in the cytoplasm of skeletal muscles cells constitute the intramyocellular lipids (IMCL) (Kuhlmann et al., 2003). The IMCL is functionally different from intramuscular triglyceride (IMTG). IMCL are metabolically inert lipids that are contained in lipid droplets, and provides a potential substrate for skeletal muscle metabolism (Li, Xu, Zhang, Yi, & Cichello, 2015). Research shows that increased amounts of IMCL are associated with higher systemic fasting insulin and glucose, and total cholesterol levels, which is indicative its involvement in the development of insulin resistance and T2D (Goodpaster et al., 2003). Previous studies suggest a strong correlation between IMCL and insulin resistance in metabolically healthy, prediabetics or in diabetic individuals with or in the absence of obesity (Forouhi et al., 1999; Phillips et al., 1996). Individuals with T2Ds, increased amounts of IMCL along with VAT have been suggested as significant predisposition factors for the development of obesity-induced metabolic derangements (Bjorndal et al., 2011). Advances in imaging modalities such as CT-radiodensity (Aubrey et al., 2014), have made it possible for noninvasive quantification and separation of IMCL and IMTG (Perseghin, Petersen, & Shulman, 2003).

2.3 Adipose tissue metabolism in obesity and diabetes

2.3.1 Glucose metabolism in adipose tissue

Glucose is the predominant substrate for cellular metabolism in mammalian cells. The influx of glucose is facilitated by the glucose transporter protein (GLUT) as the GLUT molecules are evenly distributed along the plasma membrane. The over-expression of the glucose transporter isoform 4 (GLUT4) upon the administration of insulin is responsible for the elevated glucose uptake (GU) into the insulin sensitive adipose and by the skeletal muscle, and the associated clearance of glucose from the blood (Saltiel & Kahn, 2001). Research has demonstrated that the contribution of WAT to whole body glucose uptake is lower when it is compared to the GU in the skeletal muscle of metabolically healthy insulin

sensitive individuals (DeFronzo, Gunnarsson, Bjorkman, Olsson, & Wahren, 1985). Early in vivo imaging studies suggest that impaired skeletal muscle glucose uptake, rather than adipose tissue, is more representative of the whole-body insulin-resistant state (Virtanen et al., 2002). Experimental studies with skeletal muscle insulin receptor knockout mice expressed decreased skeletal muscle GU, but recorded substantially higher adipose GU (Bruning et al., 1998). Mice with defective adipose glucose transporter systems exhibited reduced whole-body glucose uptake, and increased endogenous glucose production (Abel et al., 2001). Human studies found that adipose tissue GU when expressed as per kilogram of fat tissue was lower in obese and T2D patients when compared with non-obese lean insulin sensitive individuals (Virtanen et al., 2001). BAT is regarded as potential target for the prevention of obesity and T2D due to its capacity to use blood glucose as substrate for non-shivering thermogenesis upon exposure to cold conditions (Harms & Seale, 2013; Schulz & Tseng, 2013). Several preclinical studies indicate that obese diabetic mice have impaired BAT activities and hence reduced capacity for non-shivering thermogenesis (Trayhurn, 1979; Yoshioka, Yoshida, Wakabayashi, Nishioka, & Kondo, 1989). In clinical settings, age (Yoneshiro et al., 2011), obesity (Saito et al., 2009), and the presence of diabetes (Ouellet et al., 2011) have been shown to be negatively correlated with thermogenic activities of BAT.

2.3.2 Fatty acids release and uptake in adipose tissue

Adipocytes form WAT, which are specialized cell types that are capable of accommodating massive amount of lipids in the form of triacylglycerol (TAG). During periods of positive energy balance WAT releases FFAs into plasma (lipolysis) for use by other tissue in times of nutritional stress (Thompson, Lobo, & Bernlohr, 2010). Plasma long chain fatty acids (LCFA), derived from the hydrolysis of triglycerides in chylomicrons or very low density lipoprotein, are transported by the cell membrane of adipocytes via transportation across the plasma membrane of adipocytes (Berk & Stump, 1999). Membrane receptors responsible for the transportation of FFAs are fatty acid translocase (e.g. FAT/CD36), long-chain fatty acyl coenzyme A (acyl-CoA) synthetases, and fatty acid transport proteins (FATP) (Karpe, Dickmann, & Frayn, 2011). Upper body WAT depots have efficient dietary fatty acid storage capacity than do lower body SAT depot in normal weighted individuals (Romanski, Nelson, & Jensen, 2000). Women store a substantial amount of their systemic FFA in the gluteal-femoral region compared to men (Geer & Shen, 2009; Koutsari, Ali, Mundi, & Jensen, 2011), potentially disposing of excess dietary fat (Votruba & Jensen, 2006). The major fatty acid constituent of the lipid deposition in femoral SAT has been shown to be palmitoleic acid ($C_{16}H_{30}O_2$) (Pinnick et al., 2012), which is known to improve metabolic markers that are impaired in obesity and diabetes (Nunes & Rafacho, 2017). Adipocytes in the BAT, LCFA serve as substrate for mitochondrial β-oxidation used for non-shivering thermogenesis (Bernlohr, Coe, & LiCata, 1999). Under negative energy balance, the release of FFA is mainly triggered by the stimulation of adipocytes with catecholamines,

which leads to the translocation of phosphorylated hormone sensitive lipases from the cytosol to the surface of lipid droplets (Holm, Osterlund, Laurell, & Contreras, 2000). Upper-body SAT accounts for the majority of the systemic FFAs and to a greater extent than lower-body SAT in both men and women under basal conditions (Jensen, 1995). Under similar conditions of fasting, femoral SAT contributes approximately one-fifth of plasma FFA release in normal weight adults (Jensen, 1995). Enlarged adipocytes possess a greater potential for lipolysis than small adipocytes, even when harvested from the same depot (Hoffstedt et al., 2010a). Fatty acid release without a corresponding uptake rate leads to lipotoxity, insulin resistance, which are hallmarks of T2D (Kelley, 2005).

2.3.3 Adipose tissue blood flow

The metabolic processes of adipose tissue require the appropriate fuel substrates such as glucose and fatty acids in addition to optimal blood flow (ATBF) (Green et al., 2008). Blood flow (Minokoshi et al., 2002) and metabolic products (Lewis, Uffelman, Szeto, Weller, & Steiner, 1995) provide the medium for the physiological interaction of adipocytes with other metabolically active tissues (Sotornik et al., 2012). Typically, adipose tissue is supplied with arterial blood and venous blood drains into the venous pool (Frayn & Karpe, 2014). The ATBF can vary under different physiological conditions it is lower in basal conditions compared to post oral glucose load, or after the ingestion or a mixed meal (Evans, Clark, & Frayn, 1999). Under basal conditions, changes in ATBF are mainly dependent on effect of insulin through its activation of vasoactive compounds - vasodilatory endothelial nitric oxide (NO) and α_2-adrenergic and angiotensin II (ANG II) for their vasoconstrictive roles. In the fed state ATBF is predominantly dependent on β-adrenergic stimulation (Sotornik et al., 2012). The distribution of ATBF is severely disturbed in obesity-induced insulin resistance and T2D (Summers et al., 1996; Thorand et al., 2006). Research shows that the oxidative stress that underlie obesity and adipose tissue inflammation, and insulin resistance severely affect the function of NO thereby, interfering with insulin-mediated vasodilation (Scherrer & Sartori, 1997). In obesity, the rates of adipocyte hypertrophy and expansion of adipose tissue mass far outpace the corresponding increase in capillary density and longer diffusion distances (Goossens et al., 2011), leading to insufficient blood supply to the adipocytes (Digirolamo & Esposito, 1975). Fasting ATBF is lower in obese individuals than in lean metabolically healthy individuals (Summers, Samra, Humphreys, Morris, & Frayn, 1996). The postprandial increment in abdominal and femoral adipose tissue blood flow (Romanski et al., 2000) is impaired in obesity (Summers et al., 1996). Both fasting and postprandial blood flow is negatively correlated with measures of adiposity such as BMI (Summers et al., 1996) and abdominal adipose tissue mass (J. Andersson et al., 2012). Individuals with prediabetes or overt diabetes or normal weighted first-degree relatives of individuals with T2D expressed impaired fasting or postprandial adipose tissue blood flow (Summers et al., 1996). Under cold induction, copious amounts

of glucose and lipids are used as substrates for non-shivering thermogenesis by the dissipation of mitochondrial proton gradient through UCP1. During this process, optimal blood flow is required for the delivery of oxygen and essential macronutrients and for the removal of metabolic products such as carbon dioxide and it also mediates the distribution of the generated warm blood into the systemic circulation (Cannon & Nedergaard, 2004). In obesity, blood flow in BAT is severely blunted (Orava et al., 2013).

Accumulating evidence from human studies indicates that basal or cold-induced BAT metabolism is severely hampered in obesity and diabetes (Orava et al., 2013). Animal studies involving the transplantation of BAT from healthy to high-fat diet-induced obese mice can reverse the obesity phenotype through the secretion of IL-6, and adiponectin (Zhu et al., 2014). These results highlight the role of BAT stimulated energy expenditure as a possible target for the treatment of obesity and metabolic derangement through the utilization of systemic lipid (Bartelt et al., 2011) and glucose as substrates for BAT thermogenesis through the activation of UCP1 (Chondronikola et al., 2014). Furthermore, obese mice exposed to cold conditions recorded significant decrement in body weight. Cold exposure is a known activator of BAT and the formation of beige adipocytes through the activation of the sympathetic nervous system to release noradrenaline that binds to β3-adrenergic receptor that in turn upregulates the expression of UCP1 in BAT (Murano, Barbatelli, Giordano, & Cinti, 2009).

2.4 Molecular imaging of adipose tissue

Molecular imaging is a noninvasive visualization, characterization, and the measurement of biological processes such as physiological and pathological process in a living organism. The measurements that are used are at the cellular and molecular parameters of the living organism (Z. Y. Chen et al., 2014). Currently, the principal molecular imaging modalities available for clinical studies include PET, CT, and MRI (James & Gambhir, 2012). High quality molecular images require instruments with high resolution and high sensitivity that are capable of linking the imaging signal with the molecular mechanisms (Luo, Zhang, Su, Cheng, & Shi, 2011). Typically, this requirement is achieved with PET-CT and EPT-MRI hybrid scanners. Through molecular imaging, therapeutic response and diagnoses of disease can be detected before changes are observed at the anatomical levels (Z. Y. Chen et al., 2014).

2.4.1 Functional metabolic studies with PET

Positron emission tomography is a quantitative tool that provides noninvasive acquisition of three-dimensional reconstruction of the distribution of the positron emitting radiotracers within living tissue (Bergmann, Herrero, Markham, Weinheimer, & Walsh, 1989). The first human adipose tissue validation studies were done in the Turku PET Centre (Virtanen et al., 2002). PET provides a platform to study the biochemical and physiological processes in the human body (Yamamoto, Thompson, Diksic, Meyer, & Feindel, 1984), and detect the presence of disease through observable changes in tissue metabolism (Gordon, Flanagan, & Dehdashti, 1997). The determination of the underlying biological process in tissue involves analyzing the inputs (delivery of materials from the blood), and measuring the tissue response (metabolic process) (Shoghi & Gropler, 2015).

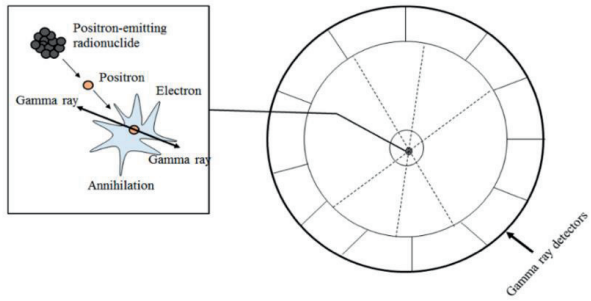

Figure 2. A schematic diagram of annihilation in positron emission tomography showing the principles of β^+ and e^- (low energy antimatter-matter annihilation) and the detection of the two resulting gamma photons at 511 KeV (Modified from (Cherry & Gambhir, 2001).

2.4.1.1 Glucose uptake studies with PET

Fluorine labeled FDG [2-deoxy-2-(^{18}F) fluoro-D-glucose] is a glucose analog that is most commonly used in PET imaging. The "deoxy" part denotes a break off of a hydroxyl group from the glucose molecule. The subsequent attachment of [^{18}F] tracer replaces the hydroxyl group (Ahmad Sarji, 2006). The radioactive FDG is transported across the cell membrane into the cells and where it phosphorylated intracellularly by the enzyme hexokinase to FDG-6-phosphate (FDG-6-P). FDG-6-P does not undergo further metabolism in the glycolysis pathway and therefore gets trapped in cells (e.g. skeletal muscle, adipose tissue) (Reinhardt et al., 1999). In a normal physiological state, FDG accumulation in cells is based on glucose uptake and phosphorylation. In quantitation of tissue glucose uptake, a correc-

tion factor known as the lumped constant is used to account for the differences in the metabolic rates between the physiological glucose and FDG (Peltoniemi et al., 2000; Virtanen et al., 2001). The measurement of the GU rate in human subcutaneous fat was validated at the Turku PET Centre in 2001 against Fick's principle (Virtanen et al., 2001). In 2009, three independent groups confirmed the existence of cold-induced metabolically active BAT in adult humans using FDG in combination with PET imaging, two of those expressed results using semi-quantitative units counts (van Marken Lichtenbelt et al., 2009); or standardized uptake value (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009).

2.4.1.1.1 Whole body glucose metabolism with the clamp technique

The clamp technique is the gold-standard method to assess insulin action on glucose utilization in human and animal studies. This procedure involves raising the plasma insulin levels and maintaining at a plateau level of approximately 100μU/ml by the continuous infusion of insulin into the peripheral vein. The plasma glucose concentration is maintained at a constant basal rate by means of variable glucose infusion (DeFronzo et al., 1979). Under conditions of euglycemic-hyperinsulinemia, the glucose infusion rate plus the release of residual endogenous glucose equals the amount of glucose taken up by all tissues into the body and thus a measure of tissue sensitivity (DeFronzo et al., 1979).

2.4.1.2 Measurement of adipose tissue fatty acid uptake

White adipose tissue fatty acid uptake have been previously measured with 14(R, S)-[18F] Fluoro-6-thia-heptadecanoic acid (^{18}F-FTHA) (Bucci et al., 2015; Hannukainen et al., 2010). ^{18}F-FTHA have also been used to quantify BAT NEFA uptake during acute cold exposure (Ouellet et al., 2012). Fluorine [^{18}F]-labeled FTHA is LCFA-palmitate analog designed to undergo metabolic trapping following incomplete mitochondrial β-oxidation pathway. FTHA is β-oxidized to 12-[^{18}F] fluoro-4-thia-2-pentadecanoyl-CoA and 12-[^{18}F] fluoro-4-thia-3-hydroxy-pentadecenoyl-CoA within the mitochondrion (DeGrado, Coenen, & Stocklin, 1991). Further β-oxidization of FTHA from the terminal end is blocked by the presence of the [^{18}F] fluorine atom at carbon-14 (Knust, Kupfernagel, & Stocklin, 1979) and the sulfur substitution at the sixth carbon of FTHA delays tissue clearance of the tracer (DeGrado et al., 1991). The net accumulation of radioactivity in tissue is the net

uptake rate of LCFA, which is the sum of the rates of esterification and oxidation (DeGrado et al., 1991).

2.4.1.3 Quantitation of adipose tissue blood flow distribution

Oxygen-15 labeled water ($[^{15}O]H_2O$) is a freely diffusible and metabolically inert tracer and hence a perfect candidate for the assessment of tissue specific blood flow expressed in ml (blood) ml^{-1} (tissue) min^{-1} (Hermansen et al., 1998). The positron emitting $[^{15}O]H_2O$ has several advantages for the measurement of tissue blood flow compared with tracers such as Nitrogen-13, ammonia or Rubidium-82 (Hermansen et al., 1998). The short half-life of oxygen-15 ($t_{1/2}$ = 2.1 mins) allows the rapid evaluation of tissue blood flow with a minimum of ionizing radiation exposure to the subjects regardless of the changes in tissue metabolism (Bergmann et al., 1989). The blood flow capacity in SAT and VAT of WAT depots have been previously studied with $[^{15}O]$ H_2O (Virtanen et al., 2002). Blood flow in the BAT of obese and lean subjects have been previously assessed using $[^{15}O]H_2O$ (Orava et al., 2013).

2.5 Quantification of adipose tissue volume

Measurement of the distribution of adipose tissue has previously been performed using many methods including the following: anthropometry, hydrodensitometry, air-displacement plethysmography, bioelectric impedance, and dual energy X-ray absorptiometry (Hu, Li, Nagy, Goran, & Nayak, 2011). A significant number of these methods measure body density of resistance, the values are then converted into mass using generalized equations (Jackson & Pollock, 1982). Most of these indirect methods cannot differentiate between subcutaneous and visceral, or intermuscular adipose tissue depots (Hu et al., 2011). Advances in imaging techniques such as MRI and CT, however, have made it possible for the absolute quantification and differentiation of specific adipose tissue depots such as visceral, subcutaneous and ectopic fat depots (Samara, Ventura, Alfadda, & Goran, 2012).

2.5.1 Computed tomography imaging

Computed tomography (CT) scans combine a series of a specialized X-ray images taken from different angles and uses computer processing to create a cross sectional images or slices test that produces non-cross-sectional images using X-rays and a computer (Allisy-

Roberts P, 2008). CT uses X-rays that are collimated to provide a fan-shaped beam that is passed through the body while an array of detectors is positioned on the opposite side of the subject to detect the transmitted radiation. The X-ray source and detectors assembly are rotated, as a single unit, around the subject thus covering a full 360º circle comprising a series of arcs. At each degree of rotation, the transmitted intensity is recorded for each detector, which provides information about the internal structures along the beam path. The basic anatomic image is similar to that obtained from using MRI, except it contains additional information about the tissue's true density at each pixel. This information coupled with the anatomical location of the pixel within the image can be used to identify it as adipose tissue, muscle, skin, visceral, or bone tissue. Earlier research shows that CT images can be used to separate the total adipose tissue mass into subcutaneous and visceral components, or the lean tissue into skeletal muscle and visceral fat or organ mass (Ellis, 2000).

2.5.2 Magnetic resonance imaging

Magnetic resonance imaging (MRI) involves subjecting the body to a strong magnetic field (orders of magnitude greater than the earth's fields), hydrogen protons (^{1}H) will attempt to align with or against the magnetic field (Bibb P, Eggbeer D, Paterson A., 2015). The frequency with which each element flips (relative to the direction of the constant magnetic field) is called the Larmor frequency. When radiofrequency energy, at the Larmor frequency, is applied perpendicular to the direction of the magnetic field, the nuclei will absorb this energy and change the alignment. When the radiofrequency is turned off, the nuclei will lose their alignment and release the stored energy (Ellis, 2000). MRI is a widely used as a validated method for the quantification of VAT and SAT without the potential for ionizing radiation as typical of CT imaging (Abate, Burns, Peshock, Garg, & Grundy, 1994). There are conflicting reports, and no standardized protocol for the quantification of the abdominal fat, since abdominal fat measurement by MRI at the site selected has high within-individual variations (Greenfield, Samaras, Chisholm, & Campbell, 2002). However, in image analysis, a single image, between the 4th and 5th lumbar vertebra, L4-L5, and close to the umbilicus has previously been selected as a more suitable representation of total abdominal SAT (Abate, Garg, Coleman, Grundy, & Peshock, 1997), and VAT (Shen et al., 2003) volumes, and have been used in the prediction of obesity-related metabolic complications. Yet, other studies adopted fat depots between the 12th thoracic and L1 vertebrae (T12-L1) (Kuk, Church, Blair, & Ross, 2006), or between the L1-L2 and L3-L4 as a more suitable surrogate for total intra-abdominal adipose tissue (Han, Kelly, Walsh, Greene, & Lean, 1997).

2.5.3 Adipose tissue triglyceride content

The intensity of CT image expressed as Hounsfield units is related to the efficiency with which X-rays are attenuated as they traverse the volume element (voxel) in the human body, and are represented by the picture element (pixels) in the CT image (Razi, Niknami, & Alavi Ghazani, 2014). CT-radiodensity serves a surrogate marker of triglyceride content in WAT (Shah et al., 2016) and BAT (Lubura et al., 2012).

2.6 Treatment of obesity and diabetes

The classification of obesity as a chronic medical condition (Allison et al., 2008) has prompted considerable action for weight management strategies that involve both non-surgical and surgical approaches (Boza et al., 2010; Kushner, 2014). Non-surgical interventions include dietary approaches, pharmaceutical therapy, and lifestyle changes (Boza et al., 2010). Should the conventional non-surgical approach of weight management fail, clinicians resort to surgery (bariatric surgery) for the treatment of severe obesity or patients presented with an obesity-related comorbidities such as T2D (Kushner, 2014).

2.6.1 The non-surgical approach

The non-surgical intervention with lifestyle intervention is mainly focused on making healthier dietary choices and participating in or increasing physical activities, all of which contribute at promoting negative energy balance in the overweight and or obese individual. Results from the Finnish Diabetes Prevention (Lindstrom et al., 2003) study involving a nonpharmacological approach of very-low-calorie dietary and increased physical activity interventions of individuals at higher risk of developing diabetes (impaired glucose tolerance) coupled with general dietary advice prevented or delayed the onset of diabetes (Lindstrom et al., 2003). The principal aim of diet-induced weight loss program is to achieve moderate weight loss (5% to 10% of the initial body weight) (Chaput et al., 2011). The main component of weight loss through dietary intervention is the overall reduction in the total caloric intake and not just the reductions in the macronutrient composition (carbohydrates, fat, and protein) in the diet (Sacks et al., 2001). The patient's metabolic profile and risk factors greatly influences the type of dietary prescription (Blumenthal et al., 2010; Elmer et al., 2006; Nordmann et al., 2011; Sacks et al., 2001). In a larger clinical trial involving moderately obese individuals who were randomly assigned into three groups of

low-fat and Mediterranean-diet with restricted calories, or low-carbohydrates with unrestricted calories, the degree of weight loss, improvement in lipids, inflammatory and glycemic profiles at six months after the intervention were similar among the various arms (Wood, Stefanick, Williams, & Haskell, 1991). In another large study, subjects that were followed after being randomly assigned to caloric-restricted, carbohydrate-restricted, and fat-restricted or macronutrient balanced diet recorded improvement in cardio-metabolic parameters independent of the assigned dietary type (Dansinger, Gleason, Griffith, Selker, & Schaefer, 2005). Moreover, both low-carb and low-fat diets have been found to achieved similar amounts of body weight loss at 12 months of follow-up (Gardner et al., 2018).

The optimal weight and the associated metabolic benefits can be achieved when, a reduction in caloric intake could be complemented by physical activities and exercise regiments. Physical activities can be defined as by everyday activities (light to moderate in intensity) such as walking, gardening, climbing of stairs, primarily involving bodily movement with the intention to increase energy expenditure. Exercise is a specific form of physical activities involving a well-planned, regimented, structured and repetitive bodily movement purposefully executed to enhance an aspect of physical fitness (Caspersen, Powell, & Christenson, 1985). Studies show that physical activities and structured exercise in improving cardio-metabolic functions in addition to weight loss (Swift, Johannsen, Lavie, Earnest, & Church, 2014). Exercise training programs have been shown to reduce cardiovascular risk factors including hyperglycemia, hypertriglyceridemia and improve levels of HDL (Katzmarzyk et al., 2003; Kelley & Goodpaster, 2001).

Pharmacotherapy is recommended either for individuals with a BMI greater than or equal to 30 kg/m^2, or for individuals with a BMI greater than or equal to 27 kg/m^2 and also have obesity-related comorbidities (Samaranayake, Ong, Leung, & Cheung, 2012). Medications for obesity fall under two broad categories, anorexants and gastrointestinal fat blockers. The anorexants or appetite suppressing medications target noradrenergic, dopaminergic, and serotonergic systems in the hypothalamus (Haddock, Poston, Dill, Foreyt, & Ericsson, 2002). The fat blockers are synthetic hydrogenated derivatives that inhibits pancreatic, gastric, and carboxylester lipase and phospholipase A_2, required for the synthesis of gastrointestinal dietary fat into fatty acids and monoacylglycerols (K. H. Lucas & Kaplan-Machlis, 2001). These drugs, therefore, block the digestion and absorption of roughly 30 percent of dietary fat (K. H. Lucas & Kaplan-Machlis, 2001).

The pharmacologic approach to glycemic treatment include the use of metformin, which increases liver glucose uptake and inhibit gluconeogenesis by activating adenosine monophosphate-activated protein kinase in the liver (Viollet et al., 2012). Metformin is also

known to increase indirectly peripheral insulin sensitivity, and whole-body insulin sensitivity while decreasing hepatic gluconeogenesis, which thereby delay the progression of T2D and associated complications (Viollet et al., 2012). Incretin-based therapies such the injectable glucagon-like peptide (GLP-1) receptor agonist (exenatide and liraglutide) stimulate the secretion of insulin and inhibit the production of glucagon output depending on the availability of glucose. The GLP-1 receptor agonist also slows down gastric emptying and decreases appetite, which causes weight loss. The usage of GLP-1 agonists has been associated with improved glycemic control, a reduction in levels of fasting glucose and $HbA1_C$ (Nauck & Meier, 2016). The use of dipeptidyl peptidase (DPP-4) inhibitors in the treatment of T2D is primarily to prolong the activities of the endogenously produced incretin hormones by preventing the inactivation of GLP-1 (Holst & Deacon, 1998). Treatment with DPP-4 inhibitors have been shown to decrease postprandial systemic triglyceride in T2D patients. Sodium-glucose co-transporter (SGLT)-2 inhibitors work by blocking the reabsorption of glucose thereby facilitating glucose excretion in urine (Riser Taylor & Harris, 2013). With the increasing excretion of glucose, the plasma level decreases leading to an improvement in all glycemic parameters (Riser Taylor & Harris, 2013). Treatments with SGLT-2 inhibitor empagliflozin has been shown to significantly decrease overall body fat, and the amounts of abdominal SAT and VAT depots (Ridderstrale et al., 2014). A randomized trial of diabetic patients at higher risk of cardiovascular events treated with an empagliflozin resulted in a significant reduction in cardiovascular mortality compared to individuals in a placebo group (Zinman et al., 2015). Another study found that a SGLT-2 inhibitor neither increased nor reduced cardiovascular events but decreased the cardiovascular mortality of risk of hospitalization due to heart failure (Wiviott et al., 2018). The SGLT-2 inhibitors are associated with modest weight loss and improved cardiovascular outcomes (Riser Taylor & Harris, 2013). The mechanism of action of sulfonylureas involve binding to sulfonylureas-specific receptors on the β-pancreatic cells, blocking the inflow of potassium through ATP-sensitive channel (Sola et al., 2015). The cell membrane depolarizes, which allows the in-flow of calcium into the cytosol, which leads to increased insulin production. Meglitinide binds to different sulfonylurea-specific receptor in pancreatic β-cells and hence has a weaker binding affinity and faster dissociation than sulfonylurea, which makes it easily to administer (Chaudhury et al., 2017). The mechanism of action of thiazolidinediones (TZDs) or glitazones including rosiglitazone and pioglitazone are mediated through the modulation of PPARs. Glitozones promote fatty acid uptake (FAU) and storage in adipose tissue thereby preventing ectopic lipid deposition and the associated tissue lipotoxicity. The use of TZD has been associated with reduced production of inflam-

matory cytokines, increased adiponectin levels leading to the preservation of β-cell integrity and function. The use of rosiglitazone has been associated with serious side effects such as myocardial infarction (Rizos, Elisaf, Mikhailidis, & Liberopoulos, 2009).

2.6.2 Bariatric surgical approach

Over the recent past decades bariatric surgery (Greek word *baros*, meaning weight) has been the most preferred method for achieving marked and sustained weight loss in severely obese patients (Rubino et al., 2014). Bariatric surgery procedures are performed laparoscopically and hence there is less pain, shorter hospitalization, fewer wound infections, lower incidence of incisional hernias and faster return to normal activities (Olbers, Lonroth, Fagevik-Olsen, & Lundell, 2003). To be considered as a surgical candidate, patients should have either a BMI greater than or equal to 40 kg/m^2 or a BMI greater than 35 kg/m^2 but associated with significant obesity-related comorbidities such as diabetes, dyslipidemia, hypertension and cardiopulmonary disease (Kushner, 2014). Previously considered as weight loss surgery, proceeding series of research indicated that the benefits of bariatric extend beyond weight loss per se and include drastic improvement in favorable glycemic and lipid parameters, and a reduction in overall mortality (Adams et al., 2007; Sjostrom et al., 2007). Bariatric surgery is also termed metabolic surgery which broadly describes surgical approach aimed at the treatment of metabolic derangements in addition to a reduction in weight. Bariatric surgery procedures are broadly categorized into restrictive (e.g. gastric banding, vertical banded gastroplasty and sleeve gastrectomy) (Karra, Yousseif, & Batterham, 2010), mal-absorption (e.g. jejuno-ileal bypass, duodenal-jejunal bypass and biliopancreatic diversion) (Organ, Kessler, & Lane, 1984), or a hybrid of both restrictive and malabsorption procedures, e.g. roux-en-Y (RYGB) gastric bypass (Buchwald et al., 2009; Pories et al., 1995). For purposes of this research, emphasis will be placed on the sleeve gastrectomy and gastric bypass, and most commonly performed surgical procedures (Angrisani et al., 2015; Miras & le Roux, 2013).

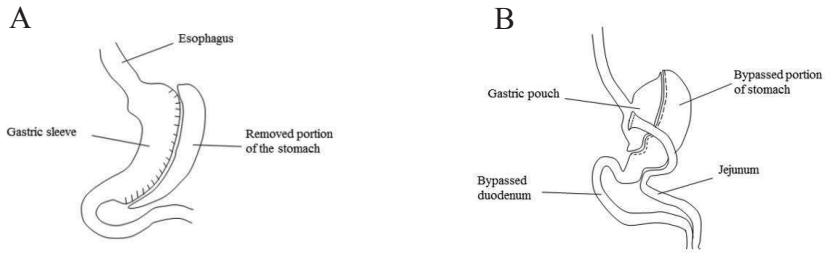

Figure 3. Laparoscopic sleeve gastrectomy [A] involves the removal of a larger portion of the stomach along the greater curvature leaving a "sleeve-like" portion; Roux-en-Y gastric bypass [B] involves the creation of small gastric pouch, the first part of the small intestine (the duodenum), food enters directly into the jejunum thus limiting the amount of calories absorbed (Modified from (Levine JW, Feng Z, Feng DP, Melvin WV., 2017).

2.6.2.1 Sleeve gastrectomy

Sleeve gastrectomy is a stand-alone laparoscopic procedure in which a significant portion of the stomach, along the greater curvature, is removed leaving a tabularized or "sleeve-like" stomach (Karra et al., 2010). This procedure is simpler to perform, has less malabsorption and reduced risk of ulceration because the procedure does not involve rerouting the intestinal tract (Karra et al., 2010). Patients feel less hungry, possibly owing to the removal of a large portion of stomach and less secretion of appetite stimulating hormones such as ghrelin (Zizzari, Longchamps, Epelbaum, & Bluet-Pajot, 2007). Gastric emptying is greatly enhanced after sleeve gastrectomy procedure (Braghetto et al., 2009). L-cells in the hindgut are more quickly exposed to the nutrients, which results in an increased postoperative secretion of incretin hormone including peptide YY (PYY) levels (Karra et al., 2010).

2.6.2.2 Gastric bypass surgery

Roux-en-Y gastric bypass is a surgical procedure for the treatment of severe obesity and related comorbidities. It is a gastric bypass procedure whereby a small gastric pouch is created by dividing the stomach with a stapler (Cummings, Overduin, & Foster-Schubert, 2004). The division of the stomach starts midway from the lesser curvature and runs parallel to it. Further division, rearrangement and anastomosis of both stomach pouches leads to a Y-configuration. Nutrients are directed from the small stomach pouch through the Roux limb (Cummings et al., 2004). Changes in the eating habits as reported by Post-

RYGB patients as a reduced appetite, reduced intake of energy-concentrated foods and the consumption of fewer calories, which could explain the more marked weight loss outcome compared to other bariatric surgery procedures (Cummings et al., 2004). Researchers have attributed the resolution of diabetes associated with RYGB to the "hindgut hypothesis" which postulates that the control of diabetes results from the increased delivery of incompletely digested nutrients through the altered or shorter gastrointestinal tract, which in turn leads to an overstimulation of enteroendocrine L-cells. The L-cells produce glucagon-like peptide-1 (GLP-1), Gastric inhibitory polypeptide (GIP) and PYY, which tend to enhance insulin secretion and insulin sensitivity (Melissas et al., 2013). In addition, both GLP-1 and PYY exert anorexigenic effect of and are implicated in the weight loss associated with bariatric surgery (Karra et al., 2010). Post-bariatric surgery resolution of diabetes may involve multi-organ interaction involving the brain, liver, pancreas, muscle and adipose tissue (Pok & Lee, 2014)

2.6.3 Bariatric surgery and white adipose tissue

The most noticeable effect of bariatric surgery is the substantial decrease in adipose tissue mass along with an improved systemic metabolism over the short term (Galanakis et al., 2015). Results from a meta-analysis shows that weight loss achieved by bariatric surgery persist over the long term (Golzarand, Toolabi, & Farid, 2017). However, a significant proportion of surgical patients experience long-term weight regain after surgery, with the greatest effects observed in superobese patients (Magro et al., 2008). Results on the effect of bariatric surgery on adipocyte size indicate that the SAT cells decrease in size after surgery eventually nearing values similar to normal weighted controls subjects (Cancello et al., 2013). The total number of subcutaneous adipocytes, remains unchanged after weight loss induced by bariatric surgery (D. P. Andersson et al., 2014). Marked reductions in adipocyte size has been shown to correlate with improvement in insulin sensitivity 2 years after gastric bypass surgery (Martinez, Tucker, Bailey, & LeCheminant, 2017). Improvement in diabetes risks was observed in post-RYGB patients with significantly reduced adipocyte size compared to patients with minimal or no improvements in their diabetes status (Ferrannini & Mingrone, 2009). The observable post-bariatric surgery change in adipocyte size contribute favorably to the improved adipose tissue function (Frikke-Schmidt, O'Rourke, Lumeng, Sandoval, & Seeley, 2016). The post-bariatric surgery reductions in obesity related comorbidities such as T2D and overall reduction in mortality is attributed

to the reduction in inflammation (Sams et al., 2016). Assessment of adipocyte size in bariatric surgery studies are primarily restricted to SAT since obtaining VAT sampling in humans is limited and considered unethical after surgery.

2.6.4 Bariatric surgery and brown adipose tissue

Plasticity of brown or beige fat is a dominant characteristic of brown or beige fat (Enerback, 2013; Gerhart-Hines et al., 2013; Kozak, Koza, Anunciado-Koza, Mendoza, & Newman, 2012). The underlying factors for the activation and reduction of brown fat activities occur bidirectionally throughout the life of an individual (Enerback, 2013; Kozak et al., 2012). Brown fat is involved in energy metabolism (Virtanen et al., 2009) and triglyceride clearance (Bartelt et al., 2011). However, the amount and/or activity of BAT in relation to whole body mass (expressed as BMI or percentage body fat) is substantially lower in obese compared to nonobese subjects (van Marken Lichtenbelt et al., 2009). Low brown fat activity could also be an adaptive trait of obesity (Vijgen et al., 2012), suggesting that as a result of the decrease insulation associated, brown fat recruitment will be increased after changes in body composition such as weight loss (Vijgen et al., 2012).

It has been previously shown in rodent studies that both SG and RYGB increased the volume and metabolic activities of BAT (Chen, Yang, Nie, Song, & Gu, 2018). A contributing factor attributed to the metabolic activities of BAT is circulating bile acids which have been shown to increase after SG and RYGB surgical procedures in both human and animal models (Kohli et al., 2013; Pournaras & le Roux, 2013). Bile acids increase overall energy expenditure (Pournaras & le Roux, 2013; Werling et al., 2013) possibly through the activation of BAT in humans (Broeders et al., 2015). Using ^{18}F-FDG-PET scans, Vijgen and associates demonstrated an increased brown/beige adipose tissue recruitment in obese adults one year after gastric banding surgical intervention, which were accompanied by a marked reduction in overall body weight (Vijgen et al., 2012). Earlier research showed that there is increase in brown and beige fat volumes and increased expression of brown fat biopsy-derived signature genes in the supraclavicular fat depot after gastric bypass surgery (Rachid et al., 2015). The underlying mechanisms for weight reduction-associated with activation or recruitment of brown/beige are the improvement in insulin action (C. P. Lucas et al., 1987; Ross, Freeman, Hudson, & Janssen, 2002; Umeda et al., 2011), adrenergic

stimulus (Champigny et al., 1991; Sidossis & Kajimura, 2015), stimulation of thyroid hormone (Doniach, 1975; Sidossis & Kajimura, 2015), and fibroblast growth factor 21 (FGF21) (Lee, Greenfield, Ho, & Fulham, 2010).

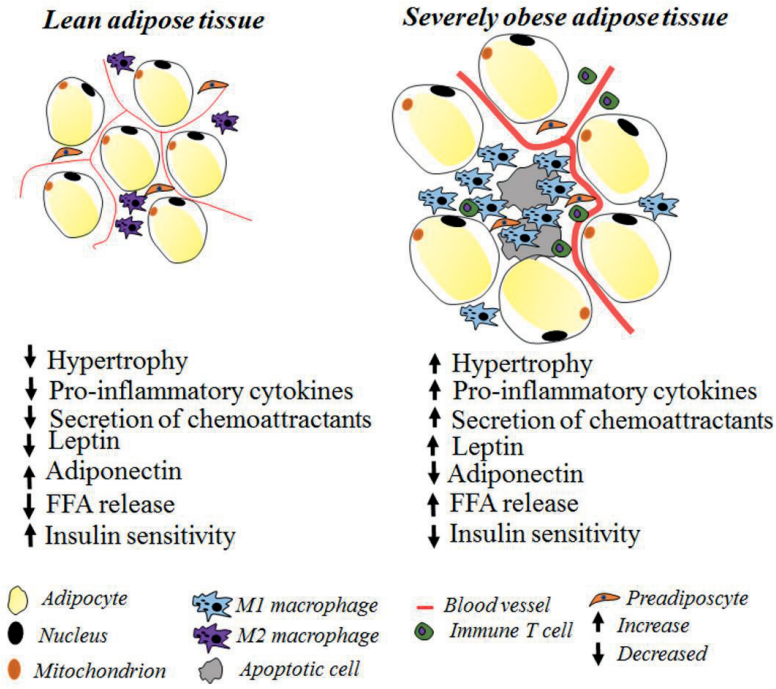

Figure 4. A summary schematic diagram of the metabolic dysregulation associated with the hypertrophied adipocytes (modified from (Harford, Reynolds, McGillicuddy, & Roche, 2011; McArdle, Finucane, Connaughton, McMorrow, & Roche, 2013). As individuals progress to obesity status, the adipocytes hypertrophy and continuous cell death occur potentiating the release of chemoattractants such as MCP-1. Pro-inflammatory mediators including IL-1, TNF-α and IL-6 are secreted by pre- and matured adipocytes, and coupled with the infiltration of immune cells results in the transition of macrophage polarization from M2 to M1 phenotype. Levels of adipocyte derived leptin increase while adiponectin decrease. Insulin signaling pathways and energy metabolism are severely disrupted with the increasing rate of lipolysis leading to insulin resistance. In lean insulin sensitive adipose tissue, the M2 macrophage phenotype predominates, fewer FFAs are released, and levels of adiponectin are significantly increased.

2.7 Summary review of the literature

White and brown adipose tissue have diametrically opposing morphological, physiological, metabolic and functional characteristics. Brown adipocytes originates from myogenic factor 5 (Myf5), and the white from non-My5f progenitor lineage. Functionally, white adipocytes are known to esterify fatty acids and store the products in the form of triglycerides. The enlargement of white fat through the increase in cell size (hypertrophy), and the proliferation of numbers (hyperplasia) at certain sites are the main characteristic features of obesity. An expansion of white fat in visceral and intrathoracic depots that is greater than subcutaneous fat depots is implicated in the development of deleterious metabolic outcome such as insulin resistance and T2D. Brown or brown-like adipocytes, are specialized for thermogenic energy expenditure, and therefore capable of regulating systemic metabolism, and preventing obesity and diabetes. Although BAT was initially considered to be absent in adults, recently published investigations have shown that adult humans possess functional BAT depot, which are symmetrically distributed in the supraclavicular region, and are known to have a role in energy homeostasis.

In obesity, the energy metabolic rates in regard to lipids and glucose of both brown and white fat are severely affected, thus contributing to the manifestation of the abysmal metabolic health observed in obese individuals. The PET imaging modality provides a noninvasive tool to study adipose tissue metabolism. Accessing the metabolic changes using the PET modality in adipose tissue of morbidly obese individuals with or without diabetes has not been thoroughly studied. Bariatric surgery is the preferred treatment for severe obesity. It achieves long lasting weight loss, restores glucose homeostasis, and improves lipid profiles, and ameliorate metabolic disorders including diabetes more than the conservative non-surgical intervention of exercise or dietary interventions. It remains unclear how bariatric surgery affects adipose tissue/adipocyte energy metabolism in severely obese individuals.

3 AIMS OF THE PRESENT STUDY

The purpose of this doctoral studies is to understand the status of adipose tissue energy metabolism in severe obesity, and also the changes in adipose tissue metabolism, which occur after weight loss induced by bariatric surgery. The study aims are contingent on the hypotheses that morbid obesity severely affects adipose tissue energy metabolism. Weight loss induced by surgery improves adipose tissue metabolism, which in turn, improves systemic metabolism and overall metabolic health in severely obese patients.

The specific objectives of the thesis are as follows:

1. To investigate the effect of severe obesity and diabetes on WAT glucose metabolism. Change in adipose tissue glucose metabolism after weight loss induced by surgery was also be studied (Study 1).

2. To determine the effect severe obesity and diabetes on WAT and adipocyte-specific fatty acid metabolism and blood flow distributions. Alterations in fatty acid uptake and blood flow distribution which occur after weight loss induced by surgery was also studied (Study II).

3. To study whether bariatric surgery induced weight alters intrathoracic adipose tissue FFA uptake in morbidly obese individuals (Study III).

4. To investigate the effect of severe obesity on supraclavicular BAT lipid metabolism. The effect of weight loss following bariatric surgery on brown adipose tissue lipid metabolism was also determined (Study IV).

The studies are hereafter referred to by their Roman numerals in this dissertation.

4 SUBJECTS AND STUDY DESIGNS

4.1 Study subjects (Studies I-IV)

The study participants for all four studies were 52 morbidly obese patients (5 males, 47 females; 20 with and 32 without diabetes (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003) whom were eligible for bariatric surgery and whom had been recruited from two larger prospective randomized control clinical trial studies [SleevePass (https://clinicaltrials.gov/ct2/show/NCT00793143)], and SleevePET2 https://clinicaltrials.gov/ct2/show/NCT01373892] comparing sleeve gastrectomy and Roux-en-Y gastric bypass for the treatment of morbid obesity (Salminen et al., 2018). Twenty-five (2 men and 23 women) age-matched with non-obese healthy volunteers recruited through local newspaper advertisement served as controls. The eligible criteria for the obese group included age between 18 to 60 years, a BMI greater or equal 40 kg/m² alternatively a BMI greater than 35 kg/m² but with an additional obesity-related high risk comorbidities (such as T2D or cardiovascular risk factors) and individuals for whom previous conservative attempt to achieve weight loss had failed. Patients were excluded from the study when they had one or more of the following: a BMI greater than 60 kg/m², a weight greater than 170 kg, a waist circumference more than 150 cm, suffered from eating or mental disorder, were chronic heavy drinkers, had diabetes (fasting glucose greater than 7 mmol/L) requiring insulin treatment, were experiencing severe ulcus disease, were pregnant, and had past exposed to a radiation dose in the past.

Inclusion criteria for the healthy controls subjects comprised age between 18 to 60 years, a BMI between 18.5 to 24.9/ kg/m², normal glycemic indices (a normal fasting glucose of 6.1 mmol/L and a 2-hour oral glucose tolerance test (OGTT) value less than 7.8 mmol/L). The control subjects were excluded from the study when blood pressure measurement exceeded 140/90 mmHg, when subjects experienced chronic ailments or diagnoses of psychopathologies, when there experienced debilitating injuries, when they were chronic alcohol users and had eating disorders or when they were pregnant. At the screening, medical history was taken, and physical examination, anthropometric, blood and oral glucose tolerance tests were performed after an overnight fast (12 hours).

The obese individuals underwent a preoperative four-week very low calorie diet (VLCD) (Optifast 800; Nestlé HealthCare Nutrition GmbH, Frankfurt, Germany) of 1906 kJ (800 kcal/day, including 70g of protein, 15g of fat and 100g of carbohydrates) including the recommended daily intake of various important vitamins, minerals and trace elements (Van Nieuwenhove et al., 2011). Twenty-two of the obese patients underwent sleeve gastrectomy and 27 Roux-en-Y gastric bypass surgery. Three participants did not proceed to surgery. The PET and MRI studies were repeated in the obese patients 6 months after the

bariatric surgical procedures. Of the 49 operated participants, 3 withdrew from the post-operational studies for personal reasons.

Table 1. Characteristics of studies

	Study I		Study II, IV		Study III	
	Obese	**Controls**	**Obese**	**Controls**	**Obese**	**Controls**
Subject N°	23	10	23	15	46	25
Age (years)	46.5 ± 9.0	47.3 ± 6.0	42.8 ± 9.6	44.9 ± 12.6	49.9 ± 9.5	45.8 ± 10.2
BMI (kg/m²)	43.1 ± 3.6	23.7 ± 1.8	41.2 ± 4.2	22.6 ± 2.8	42.1 ± 4.0	23.0 ± 2.5
Female/male	20/3	8/2	23/0	15/0	42/2	23/2
Diabetes (%)	64% (9/14)	0/10	77% (10/13)	0/15	64% (18/28)	0/25
PET tracer(s)	[^{18}F]FDG	[^{18}F]FDG	[^{18}F]FTHA, [^{15}O]H_2O	[^{18}F]FTHA, [^{15}O]H_2O	[^{18}F]FTHA, [^{18}F] FDG	[^{18}F]FTHA, [^{18}F] FDG
Surgery type						
SG	10	-	15	-	28	-
RYGB	13	-	8	-	18	-

A summary of subject characteristics under the different study types; Subject number, N°; Tracer type including 2-deoxy-2-(18F) fluoro-D-glucose, [^{18}F] FDG; 14(R, S)-[^{18}F] fluoro-6-thia-heptadecanoic acid (FTHA); oxygen- 15 radiolabeled water, [^{15}O]H_2O PET tracers; Study I (SleevePASS/NCT00793143); Study II, IV (NCT01373892/SleevePET); Study III (NCT00793143/Sleeve-PASS, NCT01373892/SleevePET).

4.1.1 The aims and designs of the different studies

4.1.1.1 Study I

Aims: To measure adipose tissue GU in morbidly obese patients with and without diabetes and compare it to the GU in nonobese healthy controls and also measure changes in GU that occur before after bariatric surgery.

Design and Methods: Adipose tissue GU in the thoracic and upper arm, abdominal and femoral regions was studied under fasting conditions and during euglycemic-hyperinsulinemic clamp using [^{18}F] FDG as the tracer. In addition, glucose uptake in skeletal muscle was also measured (Figure 4).

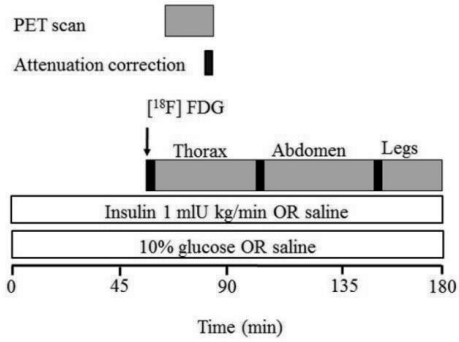

Figure 5. FDG PET studies were performed during fasting and during insulin stimulation (Clamp technique) with two-week interval. Obese patients were studied before and 6 months after surgery, non-obese controls were studied once, during the baseline.

4.1.1.2 Study II

Aims: To study basal adipose tissue fatty acid uptake and blood flow distribution in morbidly obese patients with and without diabetes, and compare the results to nonobese controls. Alterations in adipose tissue fatty acid uptake and blood flow in response to surgery are also studied.

Design and Methods: Fatty acid uptake and blood flow were respectively measured using [^{18}F] FTHA and [^{15}O] H_2O tracers under fasting conditions (Figure 5).

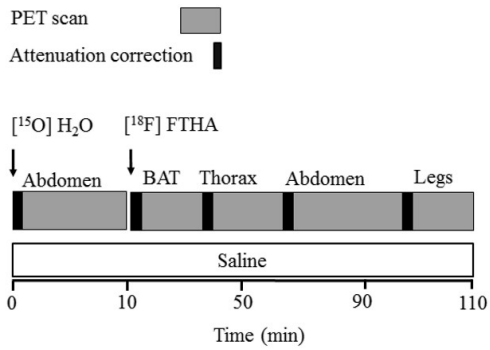

Figure 6. Quantification of adipose tissue blood flow distribution with [^{15}O] H_2O, fatty acid metabolism studies with [^{18}F] FTHA PET performed during fasting in 23 obese subjects studied before and 6 months after surgery, and 15 non-obese lean controls studied once.

4.1.1.3 Study III

Aims: Fasting free fatty acid in the intrathoracic, abdominal subcutaneous and visceral adipose tissues are studied in obese patients with and without diabetes, and the results compared with nonobese controls. Changes in tissue fatty acid uptake as a consequence of surgery-induced weight loss are measured.

Design and Methods: Fatty acid uptake was studied using [^{18}F] FTHA during fasting (Figure 5). Obese were studied before and after surgery and controls studied once during baseline.

4.1.1.4 Study IV

Aims: To study by comparing basal supraclavicular brown adipose tissue lipid metabolism between obese and controls. Changes in brown adipose tissue in obese in response to surgery was also measured.

Design and Methods: Supraclavicular adipose tissue fatty acid uptake was measured with [^{18}F] FTHA, and triglyceride content [assessed with CT-radiodensity in (Hounsfield's unit)]. Both measurements were performed under fasting conditions (Figure 5).

4.2 PET imaging (Studies I-IV)

4.2.1 Production of PET tracers

Oxygen-15 radiolabeled water [^{15}O] H_2O ($t_{1/2}$ = 122 seconds) was produced using the low-energy deuteron accelerator Cyclone 3 (IBA International) and the diffusion membrane technique as previously described (Powell & O'Neil, 2006) (Study II). The production of [^{18} F] FTHA ($t_{1/2}$ = 110 minutes) was performed as described previously (DeGrado et al., 1991) (Study II). The synthesis of [^{18}F] FDG is by electrophilic fluorination involving the use of the use of 3, 4, 6-tri-O-acetyl-D-glucal as precursor (Fowler & Ido, 2002).

4.3 Acquisition of PET (Studies I-IV)

PET imaging was performed after an overnight fast. Consumption of alcohol or caffeinated products was withheld for 24 hours before the metabolic PET studies. Subjects were advised to abstain from strenuous exercises for 48 hours. All antidiabetic and antihypertensive medication were withheld 72 hours before the onset of the PET studies.

4.3.1 Adipose tissue glucose uptake (Study I)

Adipose tissue GU studies were performed under basal conditions and during hyperinsulinemic-euglycemic clamp (DeFronzo, Tobin, & Andres, 1979) on separate days 14 days apart using the advance GE PET camera (General Electric Medica Systems, Milwaukee, WI) (Figure 4). A transmission scan of 5 min was performed with a pair of pin sources containing Germanium-68 before the emission scan to correct for the tissue attenuation of the gamma photons (Study I). The study participants lay in the supine position during the scanning process. Two catheters, one for each arm, were inserted into antecubital vein of the arm. One catheter was for the infusion of glucose and insulin, and also for administration of radiopharmaceutical tracers. The contralateral catheter was for arterialized blood sampling. During the clamp study, insulin was infused at a prime dose of 40 mU/m^2/min (Actrapid; Novo Nordisk, Copenhagen, Denmark). Simultaneously, plasma glucose was constantly collected and measured (every 5 minutes), and maintained at a basal level of (5.0 ± 0.5 mmol/L) with an infusion of 20% glucose during the study. After 90 ± 10 minutes when the study stage is achieved, a venous injection of a bolus (187 ± 9 MBq) of [^{18}F] FDG over a period of 15 seconds. Sixty minutes after injection, dynamic PET scanning of the myocardial/thoracic region started (5×180s frames), followed by the abdominal region at 80 mins (5×180s frames) and the femoral region at 100 mins (3×300s frames). Collection of blood samples occurred throughput the PET scanning process for analysis of plasma radioactivity with automatic γ counter (Wizard 1480; Wallac, Turku, Finland), glucose, insulin and FFA levels.

4.3.2 Adipose tissue fatty acid uptake and blood flow distribution (Studies II, IV)

Assessments of the uptake of LCFAs, and the blood flow distribution in adipose tissue were performed during fasting. The acquisition of PET images was performed using a hybrid PET/CT scanner Discovery and Discovery VCT (General Electric Medica Systems, Milwaukee, WI). Attenuation correction was carried out by means of transmission computed tomography before the PET transmission scans commenced (C. Wu, Gratama van Andel, Laverman, Boerman, & Beekman, 2013) (Figure 5). After an overnight fast, abdominal adipose tissue blood was assessed with an intravenous injection of ^{15}O-labeled tracer (554 ± 124 MBq) followed by a dynamic PET scan (26 frames, 310 seconds) (Kudomi et al., 2009). After 10 mins, there was an intravenous bolus injection of ^{18}F-FTHA (185 ± 46 MBq) after which the dynamic PET was resumed. The measurement of supraclavicular and myocardial (including the pericardial fat) fatty acid uptake started 68 ± 2.7 mins after [^{18}F] FTHA injection (185 ± 46 MBq) for 15 mins (5 frames × 180s). Dynamic PET acquisition continued in the abdominal region for 15 mins (5 frames × 180 secs), followed by a one-frame static scan of the femoral region (Honka et al., 2015). During the

entire scanning process, blood samples were frequently obtained to measure plasma glucose, insulin, and FFA concentrations, and serum radioactivity levels. Blood pressure measurements and the well-being of the subjects were constantly monitored.

4.4 Measurement of adipose tissue volume (Studies I-IV)

Body fat percentage was assessed by bioelectric impedance (Omron Model HBF400) as previously described (Immonen et al., 2014). MRI image acquisition was performed with 1.5 Tesla system (Intera, Philips Medical Systems, Best, the, Netherlands). Whole body T1W FFE images of the thoracic, abdominal, and femoral regions were obtained under basal conditions for the analysis of adipose tissue volume (Abate et al., 1997), and as an anatomical reference for PET images (Studies I and III). MRI was performed before the administration of the VLCD in obese patients, and the measurements were repeated 6 months after the bariatric surgical intervention (Studies I-IV). MRI exclusion criteria one or more of the following: weight in excess of 200 kg, the presence of pacemaker, inner ear implants or ferromagnetic objects in the body. SliceOmatic software (Tomovision, Magog, Canada) version 4.3 (downloadable at http://www.tomovision.com/index.html) was used for the quantification of adipose tissue volume (mm^3). The 'Region Growing' mode with the 'Grow 2D' and 'Paint' tools were used in adipose tissue volume (mm^3) in the humeral, thoracic, abdominal and femoral adipose tissue depots (Studies I-II). Abdominal adipose tissue was further divided into deep and superficial SAT, intraperitoneal and extraperitoneal VAT (Figure 7 A, B) (Study I). Volumes were converted to mass assuming a tissue density of 0.9196 kg/L (Abate et al., 1994) (Study I).

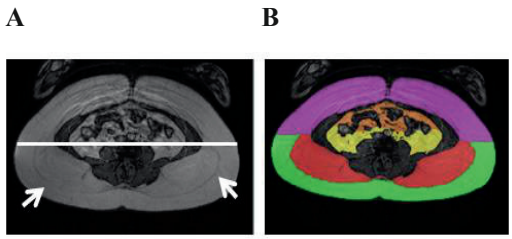

Figure 7. MRI of the subdivision of abdominal adipose tissue depot. On the trans-axial planes, abdominal SAT depots were divided into anterior and posterior regions as previously described [A, B] (He, Engelson, & Kotler, 2005; Ross et al., 2002) and the visceral adipose region was further divided into the intraperitoneal (brown) and extraperitoneal (yellow) regions with specific anatomical reference (Abate et al., 1994; Abate et al., 1997) [B]; the white arrows [A] indicate the Scapa's fascia separating the abdominal subcutaneous adipose tissue into deep (red) and superficial (green), and anterior regions (purple) [B].

4.5 PET image preparation and analysis (Studies I-IV)

PET images were reconstructed in a 256×256 matrix after correction for decay time, dead time, and photon attenuation.

4.5.1 Regions-of-interest for adipose tissue and skeletal muscle (I-IV)

PET images analyses were performed using Carimas v.2.9 (easily and freely downloadable at http://turkupetcentre.fi/). PET-MRI (Study I), PET-CT images (Studies II-IV) were co-registered using the normalized mutual information (Hill, Maurer, Studholme, Fitzpatrick, & Hawkes, 1998). To obtain the time activities curves (TAC) (expressed as average radioactivity per tissue volume as function of time), ROIs were manually drawn in the thoracic, abdominal, and femoral adipose tissue compartments while avoiding bone, muscle, and skin and in the latissimus dorsi skeletal muscles without intermuscular adipose tissue (Studies I-II). ROIs were drawn on the supraclavicular BAT taking into cognizance the underlying CT-radiodensity between -250 and -50 HU (Figure 6) (Study IV). The input functions, representing the delivery of tracer to tissue, were obtained by, i) analysis of blood samples obtained during the scanning process, and ii) from PET images by ROIs drawn in the mid-section of the abdominal aorta whilst avoiding the vascular wall (Bentourkia & Zaidi, 2007).

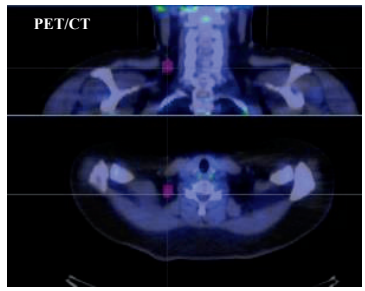

Figure 8. ROIs were symmetrically drawn in the supraclavicular fat depot taking into account the underlying CT-radiodensity (U Din et al., 2016).

4.5.2 Calculation of tissue-specific metabolism (Studies 1-IV)

In calculating the adipose tissue glucose and FAU rates, the flux rate constant (Ki) and fractional uptake rate (FUR) from late imaging, were obtained from the Patlak linearization graphical analysis (Patlak & Blasberg, 1985). The K_i or fractional tracer uptake (FUR) values were corrected for adipose tissue density (adipose, 0.9196 kg/L; skeletal muscle 1.2 kg/L) and multiplied by the systemic glucose level (GU, expressed as µmol/ L/min) and FFAs expressed as µmol/100g/min (FAU). In the measurement of GU, values were divided by the lumped constant of 1.14 for adipose tissue (Virtanen et al., 2001) and 1.2 for skeletal

muscle (Peltoniemi et al., 2000). For the calculation of FAU, metabolite correction was performed for the radioactivity curves on the assumption that any residual activity after 30 min is attributable to metabolites. Depot-specific GU and FAU (μmol/ min) rates were calculated by multiplying tissue-specific values by the size of the fat depot (in kg). Regional measurement of blood flow is expressed per unit mass of tissue [ml (blood)/min/g (tissue)], the concentration of [^{15}O] H_2O in a volume of interest measure at time t (C_{PET} (t)) is related to tracer radioactivity of vascular blood within the measured volume V_A, tissue C_T and arterial concentrations C_A as: C_{PET} (t) = C_T(t) + V_A × C_A(t). Regional blood flow (mL/100g/min) expressed per whole depot gives depot specific uptake (mL/min).

4.5.3 Indirect calorimetry (Study II)

Indirect calorimetry was performed on the PET scanning days. The procedure was performed once for the controls during the baseline measurements, and in the obese participants it was performed before the VLDL regimen, and at 6 months after the bariatric surgery procedures. The procedure (Merilainen, 1987), including the measurement of the gaseous exchanges was performed as previously described (Sherman, 1994). Carbohydrates as glucose (GOX) and lipid (LOX) oxidation rates were calculated and expressed as absolute amounts (g/min), and also normalized as resting energy expenditure (REE) which represent the mass of metabolically active tissue (Study II). An estimation of the whole body oxidative efficiency (or extraction ratio in percentage) was calculated by dividing GOX and LOX by substrate delivery, i.e., the product of cardiac output by the mean plasma glucose and FFA concentrations, respectively (Study II).

4.5.4 Biopsy procedure and histological analysis (Study II)

Subcutaneous fat biopsies were obtained from the periumbilical area under local anaesthesia (1% lidocaine) (Kolaczynski et al., 1994; Mutch et al., 2009). During surgery, visceral fat biopsies were obtained from the omentum (Petrus et al., 2015). The mean quantity of fat obtained was ~1-3 grams. All samples were washed in phosphate-buffered saline, immediately frozen in liquid nitrogen and thereafter stored at –80ºC. Digital images of hematoxylin-eosin stained slides of visceral and subcutaneous adipose tissue were scanned using the Panoramic slide scanner system (v1.15.4; 3DHISTECH, Budapest, Hungary). Diameter of adipocytes (adipocytes size expressed in μm) of approximately 100 completely visible cells (Hoffstedt et al., 2017) within the scanned areas were manually outlined using the ImageJ® (https://imagej.nih.gov/ij/index.html) analysis software. Values for the means adipocyte size were computed from the measured adipocytes. Mean adipocyte sizes were converted to volumes (μm³) assuming that the cells are spherical ($V = \frac{4}{3}\pi r^3$) (Tchoukalova et al., 2008). The estimated adipose cellularity (expressed as 10^9 cells) was calculated

by dividing the depot size (expressed in kg) by mean volume (μm³) corrected with tissue density (0.9196 kg/L). Tissue blood flow per adipocyte (nL/min/cell) was expressed as ratio of blood flow per fat depot size (μmol/min) divided by the estimated depot cell number ($\times 10^9$).

4.6 Ethical considerations (Studies I-IV)

All study participants signed a written informed consent after they had been briefed about the nature, purpose and potential risks of participating in the studies. The study protocols were approved by the local ethics committee of the Hospital District of Southwest Finland and were performed in compliance with the Declaration of Helsinki (Studies I-IV). Ethical approval certificates: ETMK: 6/180/2008; ETMK: 99/180/2010 (Studies I-IV).

4.7 Statistical analyses (Studies I-IV)

Data analyses were performed using Statistical Package for the Social Sciences (SPSS) Version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.), IL). Statistical significance was set at a p-value ($P < 0.05$). Continuous variables are expressed as mean ± SD. Normality of distribution was assessed using the Shapiro-Wilk test. Variables that were not normally distributed were log transformed before analysis. Pearson correlation analyses were performed to investigate the univariate associations between adipose tissue distribution and tissue-specific metabolism with metabolic and lipid variables. A two-way repeated-measures analysis of variance (ANOVA) was used to assess the interaction of T2D status with surgery.

5 RESULTS

5.1 Bariatric surgery, adiposity and metabolic characteristic (Studies 1-IV)

Baseline weight, body fat content was similar with respect to obese with and without diabetes (Table 1). Obese with T2D had significantly more deep (5.5 ± 2.0 vs. 3.9 ± 1.2 kg, P = 0.004, but not superficial (5.9 ± 2.2 vs. 4.7 ± 1.8 kg, P = 0.058) SAT depot size, intraperitoneal (2.6 ± 0.9 vs. 3.3 ± 1.5 kg, P = 0.046), and extraperitoneal (1.4 ± 0.7 vs. 2.0 ± 1.1 kg, P = 0.043) (Studies I-II), constituting the abdominal VAT (Table 2) were significantly higher in T2D compared to ND. Baseline upper arm SAT fat was not statistically different with respect to ND and T2D groups (1.0 ± 0.2 vs. 1.0 ± 0.3 kg, P = 0.87). There was no difference in the abdominal SAT (Table 1) and VAT (89.2 ± 13.8 vs. 94.2 ± 13.7μm, P = 0.26) cell sizes between obese ND and T2D groups. All depot specific adipose tissue mass, in addition to size were significantly higher in the obese group compared to normal weighted controls (Table 2). Deposition of fat in upper arms was significantly higher for all diabetes group compared to controls (0.5 ± 0.2 kg, all P < 0.001). Obese T2D expressed poorer glycemic and lipid indices compared to the obese ND group (Table 1) except for FFA and LDL cholesterol levels (Table 2). All lipid and glycemic parameters were higher in obese compared to lean controls (Table 2).

Four of the obese patients with newly diagnosed T2D were treated with metformin, and 5 patients with an average duration of T2D of 3 years were treated with combinations of oral glucose-lowering drugs (metformin in two patients and metformin/sulfonylurea/gliptin, metformin/pioglitazone/gliptin, and metformin/pioglitazone for each of the remaining three patients) (Study 1). In Study II, 9 of the 10 obese T2D patients were on either metformin or DPP-4 inhibitor or a combination of both and the remaining patient was on dietary regimen. All the anti-diabetes treatments were withheld 24–72 hours prior to the onset of metabolic studies (Studies I-IV).

Six months after surgery, the total body and vast majority of fat had decreased significantly from the scanned regions (Table 2). Upper arm fat decreased only in NDs (from 1.0 ± 0.2 to 0.7 ± 0.1 kg, P < 0.001). Subcutaneous adipocyte size decreased after surgery (Study II) (Table 1). As VAT biopsies could not be obtained after surgery, SAT could only be used for the analysis of the biopsy studies. Bariatric surgery decreased all measured lipid and metabolic characteristics except for FFA and LDL levels (Table 1).

Table 2. Metabolic characteristics of diabetic and nondiabetic study participants before and after bariatric surgery studies

		ND			T2D		
Anthropometrics	**Controls**	**Before**	**After**	***P value***	**Before**	**After**	***P value***
Weight (kg)	64.8 ± 7.7	118.6 ± 14.5*	91.2 ± 13.1*	<0.001	114 ± 12.6*	88.4 ± 14.2*	<0.001
BMI (kg/m²)	23.0 ± 2.5	42.7 ± 3.8*	32.9 ± 4.1*	<0.001	41.3 ± 4.3*	32.0 ± 4.4*	<0.001
Body fat (%)	31.1 ± 6.5	49.7 ± 4.8*	43.5±4.1*	<0.001	48.2 ± 7.1	40.9 ± 6.7*	<0.001
Adipose tissue distribution							
Upper arm SAT (kg)	0.5 ± 0.2	1.0 ± 0.3*	0.7 ± 0.1*	<0.001	1.0 ± 0.2*	0.7 ± 0.1*	0.007
Thoracic SAT (kg)	1.4 ± 0.6	5.0 ± 1.2*	3.3 ± 0.9*	<0.001	5.0 ± 1.3*	3.2 ± 1.1*	<0.001
Abdominal SAT (kg)	4.1 ± 1.1	20.4 ± 6.4*	11.9 ± 4.0*	<0.001	16.1 ± 3.7*†	10.6 ± 4.2*	<0.001
Femoral SAT (kg)	6.4 ± 2.3	16.0 ± 4.0*	11.5 ± 3.6*	<0.001	13.1 ± 4.0*†	8.8 ± 3.8*	<0.001
Abdominal VAT (kg)	1.4 ± 1.1	4.1 ± 1.5*	2.3 ± 1.2*	<0.001	5.3 ± 2.4*†	3.3 ± 2.3*	<0.001
SAT adipocyte size (µm)	89.6 ± 18.0	100.0 ± 10.7*	91.2 ± 11.5	0.017	104.4 ± 11.2*	89.1 ± 6.6	<0.001
Glycemic parameters							
Fasting glucose (mmol/l)	5.4 ± 0.5	5.7 ± 0.5*	5.1 ± 0.5	<0.001	7.2 ± 1.4*†	5.7 ± 0.8	<0.001
Fasting insulin (mU/l)	5.8 ± 3.6	12.4 ± 7.9	6.2 ± 2.9	<0.001	19.6 ± 14.2†	9.6 ± 6.1*	0.002
2-hour OGTT (mmol/l)	5.6 ± 1.2	7.0 ± 1.2*	5.0 ± 2.2	<0.001	11.5 ± 3.3*†	7.0 ± 3.2	<0.001
HbA1C (%)	5.6 ± 0.3	5.6 ± 0.4	5.4 ± 0.4	<0.001	6.5 ± 0.7*†	5.7 ± 0.4	<0.001
Matsuda Index	8.9 ± 5.7	3.9 ± 2.2*	5.8 ± 2.6*	0.002	2.3 ± 1.1*†	4.1 ± 2.1*	<0.001
HOMA-IR	1.4 ± 0.9	3.1 ± 2.0*	1.5 ± 0.8	<0.001	6.9 ± 7.6*†	2.4 ± 1.6*	0.013
ISI (mmol/l)	0.13 ± 0.01	0.06 ± 0.02*	0.11 ± 0.01	<0.001	0.05 ± 0.02*	0.09 ± 0.03	<0.001
Lipid parameters							
FFA levels (mmol/l)	0.5 ± 0.2	0.7 ± 0.2*	0.6 ± 0.2*	0.313	0.7 ± 0.2*	0.6 ± 0.2	0.082
Triglycerides (mmol/l)	0.7 ± 0.3	1.1 ± 0.4*	0.9 ± 0.3*	0.003	1.5 ± 0.4*†	1.2 ± 0.5*	0.046
HDL cholesterol (mmol/l)	1.9 ± 0.4	1.3 ± 0.3*	1.5 ± 0.3*	0.003	1.2 ± 0.2*†	1.4 ± 0.3*	0.005
LDL cholesterol (mmol/l)	2.5 ± 0.7	2.4 ± 0.5	2.2 ± 0.6	0.039	2.5 ± 0.9	2.4 ± 0.7	0.687

Data shown as mean ± SD; ND, nondiabetics; T2D, type 2 diabetics; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; OGTT, oral glucose tolerance test; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment for insulin resistance; ISI, insulin sensitivity index (Stumvoll et al., 2000); FFA, free fatty acids; HDL, high density cholesterol; LDL, low density cholesterol; *P* value, before vs. after surgery; **P* < 0.010 obese vs. controls; †*P* < 0.010 T2D vs. ND at baseline.

Table 3. Insulin-stimulated whole body, tissue-specific glucose uptake (GU) in diabetic and nondiabetic patients before and after bariatric surgery studies (Study I)

	Controls	ND		*P* value	T2D		
		Before	After		Before	After	*P* value
Tissue-specific GU (μmol/L/min)							
Extraperitoneal VAT	26.7 ± 11.7	9.2 ± 3.0*	15.5 ± 5.0*	<0.001	7.3 ± 1.7*	10.4 ± 3.5*	0.004
Intraperitoneal VAT	17.9 ± 7.5	11.0 ± 2.6*	15.2 ± 3.0	0.001	8.9 ± 2.1*†	11.6 ± 4.2*	0.047
Deep SAT	17.3 ± 9.1	7.1 ± 2.0*	13.1 ± 4.2	<0.001	5.5 ± 1.1*†	9.5 ± 5.3*	0.033
Superficial SAT	11.8 ± 5.5	6.6 ± 1.7*	10.0 ± 3.1	0.001	5.0 ± 1.3*†	8.2 ± 3.8	0.010
Abdominal SAT	14.0 ± 6.4	6.0 ± 1.4*	11.0 ± 3.5	<0.001	4.8 ± 1.0*†	8.2 ± 4.5*	0.032
Femoral SAT	10.2 ± 3.9	5.6 ± 1.2*	10.0 ± 4.4	0.001	4.4 ± 0.9*†	7.1 ± 4.5	0.117
Upper arm SAT	8.6 ± 4.4	7.1 ± 2.1	9.4 ± 3.4	0.042	6.4 ± 1.4	9.5 ± 4.3	0.053
Thoracic SAT	8.8 ± 4.4	7.6 ± 2.1	8.7 ± 3.6	0.350	6.7 ± 2.8	10 ± 4.1	0.060
Skeletal muscle	72.5 ± 26.2	24.7 ± 21.3*	51.2 ± 36.1	0.005	17.8 ± 8.9*†	35.7 ± 19.1*	0.121
M-value (μmol/min/kg)	40.3 ± 9.5	14.2 ± 6.5*	25.3 ± 8.5*	<0.001	10.1 ± 3.6*	20.2 ± 6.7*	0.010
Depot-specific GU (μmol/min)							
Extraperitoneal VAT	27.3 ± 14.6	17.7 ± 6.0*	19.9 ± 11.3	0.298	23.3 ± 9.8	22.5 ± 9.0	0.713
Intraperitoneal VAT	17.5 ± 5.8	33.5 ± 7.3*	25.9 ± 12.5	0.039	44.8 ±12.1*†	32 ± 12.3*	0.003
Deep SAT	23.6 ± 16.6	49.6 ± 23.4*	52.4 ± 25.8*	0.629	27.0 ± 2.2†	30.3±9.5	0.393
Superficial SAT	19.4 ± 13.5	48.6 ± 22.5*	43.8 ± 22.4*	0.317	32.9 ± 16.0	35.3 ± 20.9	0.462
Abdominal SAT	71.9 ± 53.4	148.1 ± 52.8*	160.3 ± 78.7*	0.504	92.7 ± 24.8†	104.4 ± 56.1	0.402
Femoral SAT	89.9 ± 54.5	106.7 ± 35.8	132.1 ± 49.5	0.136	74.1 ± 23.0†	82.7 ± 48.2	0.543
Upper arm	4.3 ± 3.7	7.4 ± 2.7*	7.4 ± 3.5	0.425	6.9 ± 2.3	7.5 ± 3.7	0.702
Thoracic SAT	12.9 ± 7.6	37.4 ± 11.4*	30.3 ± 12.1*	0.090	33.2 ± 12.9*	34.4 ± 13.2*	0.887

Data shown as mean ± SD; ND, nondiabetics; T2D, type 2 diabetics; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; M-value, a measure of whole body glucose disposal; *P* value, before vs. after surgery; *$P < 0.010$ obese vs. controls; †$P < 0.010$ T2D vs. ND at baseline.

Table 4. Glucose uptake in adipose tissue and skeletal muscle during fasting in diabetic and nondiabetic patients before and after bariatric surgery studies (Study I)

		ND			T2D		
	Controls	**Before**	**After**	***P* value**	**Before**	**After**	***P* value**
Tissue-specific GU (µmol/L/min)							
Extraperitoneal VAT	7.8 ± 2.2	5.4 ± 1.2*	7.1 ± 2.5	0.042	6.3 ± 1.3	6.5 ± 2.1	0.638
Intraperitoneal VAT	8.0 ± 1.4	8.4 ± 1.3	9.8 ± 2.5*	0.083	6.8 ± 1.6†	8.9 ± 2.9	0.011
Deep SAT	4.2 ± 1.6	4.1 ± 0.7	4.7 ± 1.1	0.045	4.8 ± 1.6	5.2 ± 1.4	0.531
Superficial SAT	4.5 ± 1.7	4.3 ± 1.1	5.6 ± 2.0	0.034	5.0 ± 2.0	5.7 ± 1.7	0.259
Abdominal SAT	3.9 ± 1.4	3.6 ± 0.7	4.2 ± 1.1	0.073	4.3 ± 1.4	4.8 ± 1.0	0.398
Femoral SAT	2.1 ± 0.4	3.1 ± 1.0	3.0 ± 0.8*	0.829	3.9 ± 1.1*	2.8 ± 1.2	0.146
Humeral SAT	5.0 ± 2.4	4.7 ± 1.3	6.6 ± 1.4	0.001	5.6 ± 2.1	7.4 ± 3.5	0.084
Thoracic SAT	5.8 ± 3.1	5.5 ± 1.8	7.0 ± 1.8	0.072	6.6 ± 1.8	6.5 ± 2.9	0.877
Skeletal muscle	7.7 ± 1.7	7.3 ± 2.0	7.5 ± 1.7	0.588	8.7 ± 1.6	9.4 ± 1.3	0.639
Depot-specific GU (µmol/min)							
Extraperitoneal VAT	8.6 ± 3.9	11.1 ± 4.9	8.9 ± 4.6	0.079	18.7 ± 7.0*†	12.4 ± 6.4	<0.001
Intraperitoneal VAT	9.5 ± 5.5	26.2 ± 6.5*	16.1 ± 5.9*	<0.001	32.4 ± 9.5*	21.0 ± 9.5*	0.002
Deep SAT	6.2 ± 4.3	27.7 ± 8.9*	17.6 ± 5.7*	<0.001	21.8 ± 5.3*	15.9 ± 5.1*	0.044
Superficial SAT	7.5 ± 4.3	32.5 ± 20.1*	23.1 ± 10.2*	0.061	31.6 ± 18.1*	21.8 ± 10.5*	0.083
Abdominal SAT	20.5 ± 14.2	88.5 ± 31.5*	57.8 ± 17.2*	0.001	81.5 ± 25.9*	56.5 ± 14.9*	0.037
Femoral SAT	15.8 ± 5.4	58.0 ± 25.0*	42.1 ± 17.4*	0.019	62.9 ± 30.7*	28.9 ± 14.9*	0.018
Humeral SAT	2.5 ± 2	5.3 ± 2.3*	5.3 ± 1.6*	0.711	6.1 ± 1.7*	5.6 ± 2.7*	0.667
Thoracic SAT	8.4 ± 5.5	28.4 ± 10.0*	25.8 ± 8.6*	0.168	33.1 ± 7.5*	19.6 ± 7.4*	0.021

Data shown as mean ± SD; ND, nondiabetics; T2D, type 2 diabetics; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; *P* value, before vs. after surgery; *$P < 0.010$ obese vs. controls; †$P < 0.010$ T2D vs. ND at baseline.

5.2 Insulin-stimulated adipose tissue glucose uptake in obesity and type 2 diabetes (Study 1)

Obese individuals had lower insulin-mediated GU in the whole body (M-value) and in skeletal muscle and all measure adipose tissue depots compared to lean controls (Table 2) but not different between the ND and T2D subjects at baseline (Table 2). Among the obese patients with T2D, GU by the intraperitoneal VAT, abdominal and femoral adipose tissue depot was higher compared to their ND counterparts (Table 2). The baseline percentage contribution of total VAT GU to whole body insulin-mediated glucose disposal rates were significantly higher in both T2D and ND groups compared to controls (Figure 7A). The contribution of total subcutaneous fat to whole body GU in both T2D and ND alike were significantly higher compared to controls (Figure 7B). Values in the visceral fat depot were statistically significant between the diabetes group (T2D vs. ND, P = 0.010) but not for the subcutaneous fat depot. Taking into account the area (kg) of the fat depots, obese patients recorded significantly higher depot-specific GU because of the expanded depot mass except for the extraperitoneal VAT, and femoral SAT compartments. Insulin-induced glucose uptake in all of the fat depots increased markedly after surgery and were comparable to lean controls except for humeral, superficial and thoracic SAT regions (Table 2). Glucose uptake in the extraperitoneal and skeletal muscle groups were lower compared to that of the lean controls. The substantial reduction in fat depot size as expressed as per fat depot volume, when coupled with increased glucose uptake, produced no significant difference compared with the presurgery values. However, the depot-specific GU in the metabolically active intraperitoneal region remained higher compared to controls for both the ND and T2D groups.

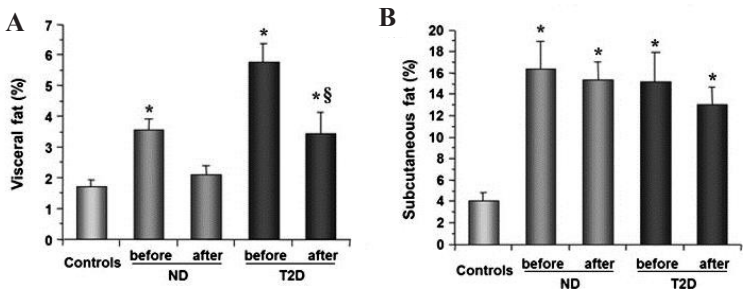

Figure 9. Percentage contribution of VAT depots [A] and SAT depots [B] to whole-body insulin-mediated GU in lean control subjects and in obese (ND) nondiabetic and with (T2D) type 2 diabetes patients before and 6 months after bariatric surgery. Data are mean ± SEM. **P* < 0.050 compared with control subjects; §*P* < 0.050 for the presurgery to post surgery comparison.

A dataset was obtained by pooling before- and after-surgery, which showed that insulin-mediated glucose uptake in visceral fat depot was non-lineally related to plasma glucose after a 2-hour OGTT (r = - 0.52, P < 0.001) (Figure 8A) and clamp fatty acids levels (Figure 8B) recorded at study states (r = - 0.58, P = 0.001).

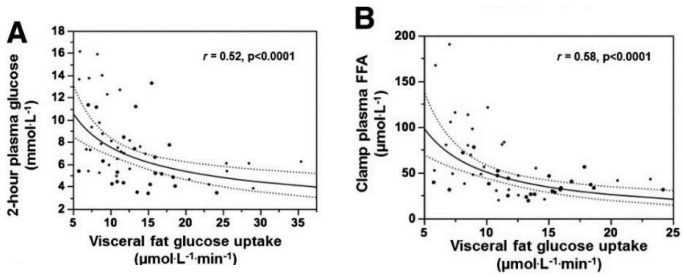

Figure 10. Nonlinear, reciprocal relationship between visceral fat depot GU and 2-hour plasma glucose concentrations (A) and steady-state plasma FFA on the clamp (B) in the pooled presurgery and postsurgery dataset. Lines are best fit with 95% CIs.

5.3 Fasting adipose tissue glucose uptake and the effect of surgery (Study 1)

Before surgery, obese T2D group expressed reduced intraperitoneal visceral GU compared to ND (Table 4). There were no statistically significant difference (NS) between the diabetes groups for the remainder of the studied fat depot and skeletal muscle (all NS). As a consequence of the fat mass expansion, fasting GU expressed per depot size were significantly higher in obese compared to lean subjects. Surgery did not significantly affect fasting GU of skeletal muscle or in any of the studied fat regions in obese subjects. However, taking into account the post-surgery decreased depot fat size, GU decreased across the studied adipose tissue regions except for humeral fat depot (Table 4).

5.4 Adipose tissue cellularity in obesity and T2D (Study II)

Abdominal SAT depot cellularity (i.e., number of cells per 100 g of fat tissue) was 30% reduced in the obese compared to the control group (Figure 9). Because SAT mass was expanded (Table 2), adipocyte number were similarly decreased in ND and T2D obese subjects as compared controls. In the obese, VAT adipocyte size did not differ between T2D and ND, and VAT adipocytes were smaller than SAT adipocytes (0.43 ± 0.27 vs. 0.61 ± 0.24 mm^3, $P < 0.001$, Table 5) for all the combined obese subjects. As a result, VAT depot cellularity was higher than SAT cellularity (254 ±164 vs. 195 ± 139 $.10^6$/100 g, $P < 0.001$).

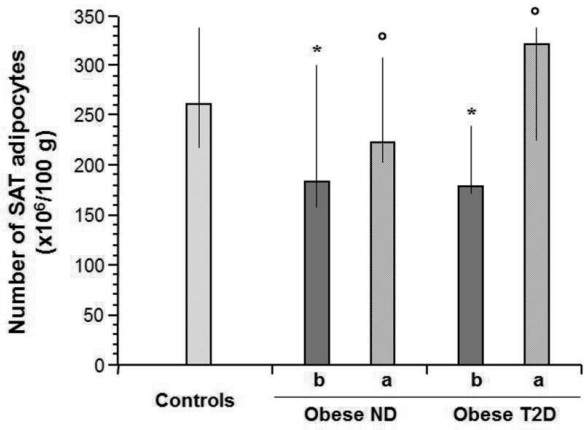

Figure 11. Abdominal subcutaneous (SAT) cellularity (the number of cells per 100 g of tissue) in /lean subjects/normal weight subjects/ (*controls*) and obese nondiabetic subjects (*ND*) or diabetic patients (*T2D*) before (*b*) and 6 months after (*a*) surgery. Data are median and 95% confidence intervals; * $P < 0.050$ obese *vs.* controls.

Table 5. Fatty acid uptake and blood flow in diabetic and nondiabetic patients before and after bariatric surgery (Studies II, III)

	Lean controls	ND		*P* value	T2D		*P* value
		Before	After		Before	After	
Adipocyte features							
SAT adipocyte volume (mm^3)	0.41 ± 0.17	0.57 ± 0.21*	0.44 ± 0.14*	0.178	0.61 ± 0.24*	0.38 ± 0.11*	0.003
SAT adipocyte N° ($x10^9$)	11.8 ± 4.2	38.0 ± 17.0*	29.0 ± 12.5*	0.173	28.8 ± 11.4*	29.5 ± 18.6*	0.333
VAT adipocyte volume (mm^3)	-	0.48 ± 0.24§	-	-	0.43 ± 0.27§	-	-
VAT adipocyte N° ($x10^9$)	-	12.6 ± 8.5§	-	-	10.9 ± 4.3§	-	-
Blood flow measurement							
SAT blood flow (ml/min/100g)	2.3 ± 0.4	1.5 ± 0.7*	1.5 ± 0.5*	0.991	1.6 ± 0.5*	1.6 ± 0.6*	0.848
VAT blood flow (ml/min/100g)	4.8 ± 1.6	3.7 ± 0.7*§	3.8 ± 1.0§	0.768	3.9 ± 1.2*§	4.1 ± 1.1§	0.601
SM blood flow (ml/min/100g)	3.3 ± 0.9	2.3 ± 0.5*	2.1 ± 0.4*	0.196	2.4 ± 0.6*	2.3 ± 0.7*	0.450
SAT depot blood flow (ml/min)	84.9 ± 31.2	274.8 ± 142.2*	160.2 ± 74.1*	0.021	222.5 ± 81.2*	150.4 ± 76.2*	0.004
VAT depot blood flow (ml/min)	37.7 ± 20.3§	124.6 ± 67.2*§	44.5 ± 32.2§	<0.001	140.5 ± 49.7*§	49.4 ± 25.3§	0.002
SAT blood flow (nL/min/cell)	8.4 ± 3.5	7.3 ± 3.9	6.5 ± 3.2	0.537	9.3 ± 5.0	6.2 ± 2.5	0.032
VAT blood flow (nL/min/cell)	-	16.4 ±10.7§	-	-	14.9 ± 8.2§	-	-
Fatty acid uptake (FAU)							
SAT FAU (µmol/min/100g)	0.28 ± 0.15	0.29 ± 0.09	0.33 ± 0.14	0.360	0.34 ± 0.15	0.33 ± 0.10	0.791
VAT FAU (µmol/min/100g)	0.57 ± 0.25	0.52 ± 0.21	0.59 ± 0.29	0.266	0.64 ± 0.19	0.64 ± 0.13	0.987
FEM. SAT (µmol/min/100g)	0.24 ± 0.06	0.31 ± 0.19	0.27 ± 0.13	0.481	0.34 ± 0.14*	0.28 ± 0.18	0.305
SM FAU (µmol/min/100g)	0.36 ± 0.16	0.37 ± 0.10	0.39 ± 0.15	0.571	0.51 ± 0.12*†	0.41 ± 0.15	0.099
SAT FAU (µmol/min)	9.1 ± 6.7	51.3 ± 17.6*	32.1 ± 10.2*	0.003	50.0 ± 22.9*	28.8 ± 13.0*	0.011
VAT FAU (µmol/min)	3.9 ± 2.0§	16.3 ± 8.2*	6.4 ± 4.5*§	<0.001	24.9 ± 14.0*§	8.3 ± 5.5§	0.001
FEM. SAT (µmol/min)	14.1 ± 7.1	43.5 ± 20.2*	31.4± 18.8*	0.127	39.5 ± 22.4*	18.7 ± 12.4	0.103
SAT FAU (fmol/min/cell)	0.99 ± 0.83	1.54 ± 0.64	1.39 ± 0.84	0.753	1.84 ± 1.34*	1.21 ± 0.59	0.093
VAT FAU (fmol/min/cell)	-	2.19 ± 1.70§	-	-	2.68 ± 2.40§	-	-

Data are means ± SD. SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; SM, skeletal muscle; FAU, fatty acid uptake; N°, depot adipocyte number (expressed $\times 10^9$); *$P < 0.050$ vs. controls; *P* value, before vs. after surgery; §$P < 0.050$ vs. the corresponding SAT value.

5.5 Adipose tissue blood flow (Study II)

Adipose tissue blood flow distribution was significantly lower in the combined obese group than in healthy control subjects for abdominal deep (2.4 ± 1.1 vs. 4.2 ± 1.7 mL/min/100g, P < 0.001) and superficial (1.3 ± 0.8 vs. 2.9 ± 1.8 mL/min/100g, P = 0.001), and in total SAT (Table 3). Intraperitoneal (4.3 ± 1.4 vs. 6.2 ± 2.2 mL/min /100g, P = 0.003) and extraperitoneal (4.2 ± 1.4 vs. 5.6 ± 1.9 mL/min /100g, P = 0.012) VAT were also statistically different. There was no significant difference in blood flow in all studied adipose tissue depot between obese T2D and ND (all P < 0.050, Table 4). There were no differences in blood flow expressed by per cell between obese diabetes groups compared to lean healthy controls. Surgery did not significantly change adipose tissue blood flow expressed per tissue or per cell (Table 4). However, expressed per depot volume (kg) blood flow decreased significantly in all the scanned adipose tissue depots (Table 4).

5.6 Adipose tissue fatty acids uptake (Study II, III, IV)

Before surgery, adipose tissue fatty acid uptake rate expressed per tissue was similar in subcutaneous and visceral fat depots between the combined obese group and lean controls or for the obese group with and without diabetes (Table 5). Uptake rate in the pericardial fat was significantly higher in the obese compared to controls (0.018 ± 0.011 vs. 0.0068 ± 0.0031 ± 0.0031 1/min, P = 0.044). There was no difference in the uptake rates with respect to the ND (0.016 ± 0.010 1/min) and T2D (0.021 ± 0.013 1/min) groups, P = 0.092. FAU rates in skeletal muscle were lower in diabetes and nondiabetes groups compared to control subjects (Table 5). Expressed per adipocyte, FAU was higher in abdominal VAT than in the SAT cells of the obese subjects (Table 4). There were no significant differences neither among the obese diabetes groups nor for the combined obese group and lean subjects in SAT or VAT depots post bariatric surgery (Table 5). Fatty acid uptake in the pericardial fat decreased for the combined obese groups 6 months after surgery (from 0.018 ± 0.011 to 0.011 ± 0.005 1/min, P = 0.005).

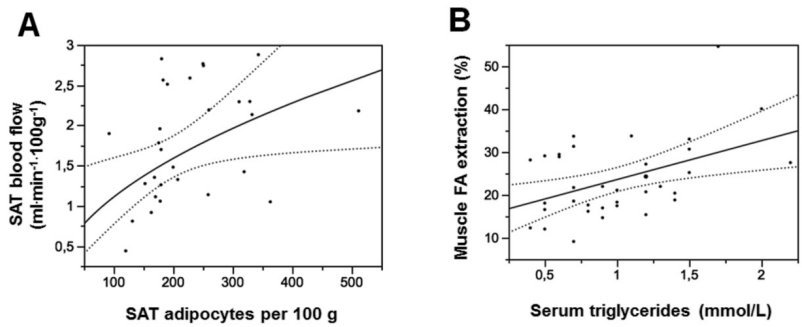

Figure 12. Relationship between subcutaneous adipose tissue (SAT) blood flow (per unit tissue mass) and SAT cellularity at baseline [A], and relationship between skeletal muscle fractional FFA extraction and serum triglycerides [B]. Lines are best fit and 95% confidence intervals.

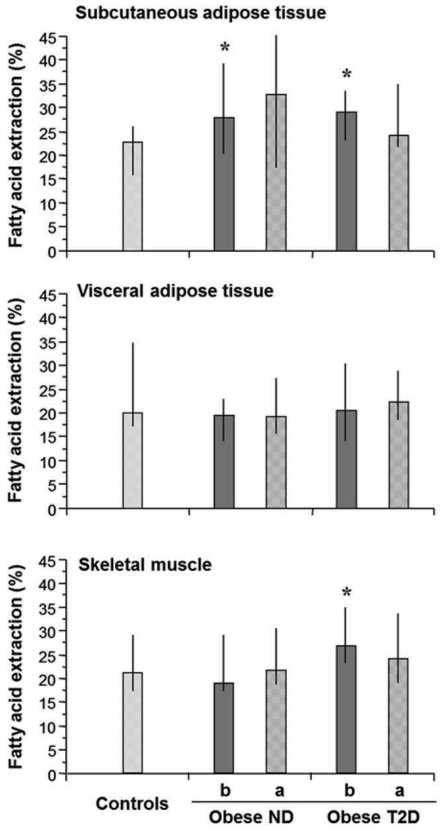

Figure 13. Fractional fatty acids uptake in lean subjects (*controls*) and obese nondiabetic (*ND*) or diabetic patients *(T2D)* before (*b*) and 6 months after (*a*) surgery. Data are median and 95% confidence intervals. * $P < 0.050$ obese *vs.* controls; ° $P < 0.050$ for the comparison of pre- and postsurgery values for the combined obese groups. Error bars are standard deviations.

5.7 BAT lipid metabolism in obesity (Study IV)

Supraclavicular BAT lipid metabolism sites were assessed on the basis of their triglyceride lipid content, fractional [^{18}F] FTHA uptake and NEFA uptake after normalization with systemic free fatty acid levels. In order to ascertain the paracrine effect of supraclavicular BAT, lipid metabolism of skeletal muscles in close proximity to (sternocleidomastoid, lavitor scapulae, deep cervical muscles), and distant from (pectorialis major and trapezius) were studied (Table 6).

5.7.1 BAT fatty acid metabolism and the effect of surgery (Study IV)

Fractional ^{18}F-FTHA tracer uptake from the supraclavicular fat depot was significantly decreased in the combined obese group compared with the normal weight controls (Table 6). There was no significant difference between supraclavicular uptake and neck subcutaneous fat in obese patients (Table 6). Skeletal muscle groups proximal to and distal from supraclavicular fat depot had higher FUR values compared to BAT values (Table 6). Upon normalizing with systemic NEFA levels (Table 1), BAT NEFA uptake rates were similar among the obese and controls (Table 6). Surgery-induced weight loss increased supraclavicular fat FUR by 46% and NEFA uptake by 40% (Table 6). Surgery did not significantly increase either supraclavicular BAT FUR nor NEFA uptake rates in the neck SAT nor in any of the skeletal muscle groups (Table 6).

Table 6. Tissue NEFA uptake and CT-radiodensity in obese patients before and after bariatric surgery studies (Study IV)

	Controls (n = 15)	**Obese patients (n = 23)**		***P* value**
		Before surgery	**After surgery**	
Fractional ^{18}F-FTHA uptake rate (1/min)				
Supraclavicular fat	0.0161 ± 0.0177	0.0055 ± 0.0035*	0.0074 ± 0.0035*	0.010
Neck SAT	0.0091 ± 0.0148	0.0047 ± 0.0018	0.0054 ± 0.0017	0.241
Skeletal muscles				
Close group	0.0115 ± 0.0022	0.0104 ± 0.0020†	0.0102 ± 0.0023*	0.755
Distant group	0.0083 ± 0.0017	0.0083 ± 0.0018†	0.0081 ± 0.0018	0.727
NEFA uptake rate (µmol/100g/min)				
Supraclavicular fat	0.57 ± 0.50	0.39 ± 0.27	0.50 ± 0.27	0.008
Neck SAT	0.47 ± 0.79	0.34 ± 0.17	0.38 ± 0.16†	0.517
Skeletal muscles				
Close group	0.62 ± 0.24	0.81 ± 0.24†	0.77 ± 0.27	0.538
Distant/Distal group	0.45 ± 0.16	0.64 ± 0.21*†	0.61 ± 0.23*	0.597
CT-based measures				
Radiodensity				
Supraclavicular fat (HU)	-82.4 ± 5.8	-101.2 ± 10.1*	-86.5±9.6	<0.001
sBAT (HU)	-67.9 ± 1.5	-68.9 ± 0.4*	-68.3±1.1	0.010
sWAT (HU)	-113.2 ± 7.6	-127.7 ± 10.4*	-115.5 ± 8.9	<0.001
Neck SAT (HU)	-73.2 ± 25.2	-91.6 ±16.5*†	-80.9 ± 15.8	0.017
Skeletal muscle				
Close/Proximal/ group (HU)	61.2 ± 6.2	57.6 ± 7.8	62.2 ± 8.2	0.066
Distant/Distal group (HU)	55.3 ± 8.6	45.2 ± 15.0*	53.3 ± 8.6*	0.045
Adipose tissue mass				
Supraclavicular fat (g)	111.1 ± 67.4	439.1 ± 141.4*	252.1 ± 95.1	<0.001
sBAT (g)	65.8 ± 31.3	183.9 ± 49.5*	138.9 ±39.3*	<0.001
sWAT (g)	39.1 ± 35.9	240.6 ± 108.1*	99.9 ± 64.5*	<0.001
sBAT (%)	64.5 ± 12.4	43.4 ± 8.4*	58.0 ±10.7	<0.001
sWAT (%)	29.3 ± 12.1	53.2 ± 11.2*	36.5 ±11.9	<0.001

Fractional and NEFA uptake rates, and CT radiodensity in Hounsfield units (HU) of the supraclavicular fat depot including the subcutaneous brown (sBAT) and white (sWAT) adipose tissue component, neck (from posterior cervical region) subcutaneous adipose tissue (SAT), and skeletal muscle proximal to (sternocleidomastoid, lavitor scapulae, deep cervical muscles) and distal (pectorialis major and trapezius) from the supraclavicular fat depot; sBAT (%) and sWAT (%), the ratio of sBAT(g) and sWAT (g) to whole supraclavicular depot mass (g) × 100%; Data presented as mean ± SD; *P <0.050 vs.* after surgery; **P < 0.050 vs.* control; †*P <0.050 vs.* whole supraclavicular fat (with ANOVA).

5.7.2 Triglyceride content in brown adipose tissue (Study IV)

Baseline supraclavicular CT- radiodensity (-250 to -50 HU) was significantly lower (towards the negative HU direction) in the obese patients compared to lean healthy controls (Table 5). A lower CT-radiodensity value is indicative increased supraclavicular triglyceride accumulation and a thus decreased BAT activity. Supraclavicular BAT recorded higher triglyceride content compared to SAT (Table 2). To test the extent of browning of supraclavicular fat, the amount of BAT (sBAT %) and WAT (sWAT %) within the supraclavicular fat were calculated (Table 5). Morbidly obese patients had decreased rates of browning and increased triglyceride content compared to normal weight healthy controls (Table 4). Surgery-induced weight loss significantly increased supraclavicular CT-radiodensity (toward the positive direction) (Table 5), suggesting a decreased supraclavicular triglyceride content. The amount of supraclavicular BAT increased after surgery. Correspondingly, sWAT decreased significantly after surgery (Table 6).

5.7.3 Triglyceride content, adiposity and insulin sensitivity (Study IV)

Post-surgery change (pre - post-surgery) were calculated to assess surgery-specific effects on BAT triglyceride content. Decrease in triglyceride content was significantly associated with change in whole body adiposity measure (assessed with BMI kg/m²) ($r = -0.72$, $P < 0.001$). In the same vein, decreases in triglyceride content were significantly associated with improvement in whole body insulin sensitivity measure (assessed by the insulin sensitivity index) ($r = 0.66$, $P = 0.001$). Improvement in percentage sBAT was significantly associated with decrease in BMI, and with increase whole body insulin sensitivity index (Figure 10).

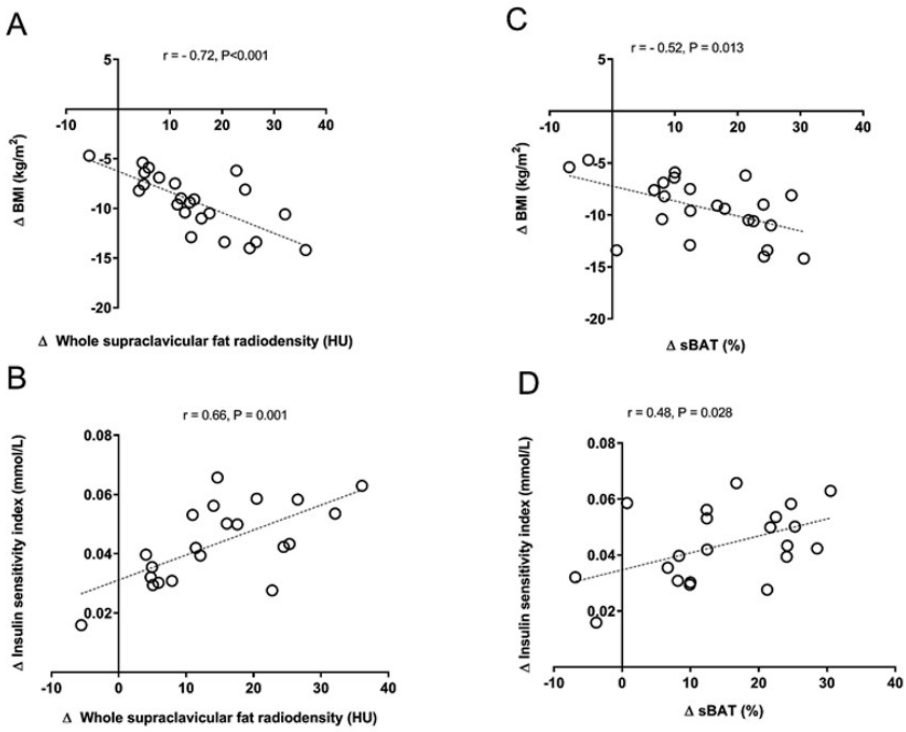

Figure 14. Pearson's correlation coefficient of the change (Δ = after -before surgery) in entire supraclavicular fat radiodensity (HU) with BMI (kg/m^2), insulin sensitivity index (mmol/L) and the proportion of brown fat (sBAT [%] = sBAT[g]/ entire supraclavicular fat mass[g] × 100%) with BMI (kg/m^2); and with insulin sensitivity index (mmol/L).

6 DISCUSSION

6.1 Hypertrophied adipocytes and uneven adipose tissue distribution underlie the development of morbid obesity

The current study series extensively assessed adipose tissue distribution patterns in the major metabolically significant depots of the body, which are the: upper arms, thoracic, abdominal and femoral regions. In addition, these studies addressed the controversy surrounding the acceptable measurement sites for abdominal SAT or VAT (i.e. T10-L1, L1-L2, or L4-L5) by taking the whole abdominal depot into account. A BMI-based classification of obesity is fraught with countless challenges including inter-racial/ethnic variations (Deurenberg, Yap, & van Staveren, 1998). Therefore, the real contribution of adiposity should be based on body composition, cellular morphology, body fat content and regional fat distribution (Poirier, 2007). Fat biopsy-derived histological analysis indicates that the SAT adipocyte mean size, volume and number were significantly higher in obese individuals regardless of diabetes status compared to lean controls (Table 3, Study II). However, no significant differences were observed in diabetes group with regards to SAT or VAT adipocyte size. In periods of chronic caloric exposure, adipose tissue expands through adipocyte hypertrophy or hyperplasia. Previous studies have suggested that hypertrophied adipocytes are closely related to metabolic derangements including insulin resistance (Fang, Guo, Zhou, Stahl, & Grams, 2015). Enlarged mean adipocyte size have been shown to be associated with insulin resistance and the occurrence of T2D independent of percentage body fat and BMI (Lonn, Mehlig, Bengtsson, & Lissner, 2010; Lundgren et al., 2007; Weyer, Foley, Bogardus, Tataranni, & Pratley, 2000). Hypertrophied adipocytes also associate with increased inflammation involving acute phase proteins, which include serum amyloid A, and which contributes to the cardiometabolic risk factors associated with obesity (Poitou et al., 2005; Poitou et al., 2006).

Baseline results from these studies indicate that obese patients with diabetes had increased amounts of deep SAT compared with their ND counterparts but had no increase in superficial SAT. The deep SAT is known to expand disproportionally with increasing obesity compared to the superficial SAT, and it correlates with cardiometabolic risk factors (Marinou et al., 2014), and liver pathologies (Tordjman et al., 2012), independent of other measures of adiposity. Deep SAT also over-expresses proinflammatory, lipogenic, lipolytic genes, and hence is a potent predictor of global insulin resistance (Marinou et al., 2014). In contrast, favorable metabolic genes including adiponectin are preferentially expressed in the superficial SAT (Kelley et al., 2000). In terms of the fatty acid composition, deep SAT expressed higher ratios of saturated to monounsaturated fatty acid compared to superficial SAT (Lundbom, Hakkarainen, Lundbom, & Taskinen, 2013).

To elucidate further the contribution of regional adipose tissue distribution in obesity induced metabolic complications, VAT was subdivided into intraperitoneal and extraperitoneal VAT depots as has been previously done in earlier studies (Abate et al., 1994; Bjorntorp, 1990b). The intraperitoneal VAT drains into the portal vein, whereas the extraperitoneal VAT empties into the inferior vena cava (Abate et al., 1994; Bjorntorp, 1990b). From the current results, obese T2D had increased amounts of intra- and extraperitoneal VAT compared to the ND subjects, which may be indicative of the known association between VAT accumulation in the development of T2D (Bray et al., 2008).

To examine further the significance of the major fat depots in relation to diabetes, the current series of studies found no statistically significant differences between upper arm and thoracic SAT with respect to T2D. Moreover, the severely obese individuals with diabetes at baseline had significantly lower femoral SAT compared to obese subjects without diabetes (Table 2). It has been shown that the increased deposition of gluteofemoral SAT in obese individuals may be associated with decreased prevalence of T2D (Aasen, Fagertun, & Halse, 2009). Contrary to findings pointing to the protective effect of gluteal-femoral adipose tissue in the etiology of T2Ds, in a cross-sectional study, no significant association was observed between increased femoral SAT size as assessed by CT and the incidence of T2D (Goodpaster et al., 2003). Previous studies have attributed the association between a measure of lower body adiposity such as WHR and glucose metabolism of T2D to increased waist circumference and also to a smaller gluteal-femoral circumference (Seidell, Perusse, Despres, & Bouchard, 2001; Snijder et al., 2005). A cross-sectional and a population based study have both reported a reverse association between gluteal-femoral adipose tissue distribution and systemic glycaemia, dyslipidemia and the prevalence of T2D (Snijder et al., 2005). From the anatomical standpoint, SAT can be broadly categorized into the truncal (abdominal plus thoracic), and peripheral regions including upper and lower extremities and upper arm and the gluteofemoral regions (Garg, 2004). Although SAT in both central and peripheral depot drains into the systemic circulation, these depots may play different metabolic roles in the development of insulin resistance and diabetes (Patel & Abate, 2013). Women generally tend to distribute SAT in the upper arm due to their sex hormones (Yamauchi, Kurihara, Yoshikawa, Taguchi, & Hashimoto, 2015).

6.1.1 Bariatric surgery decreases body fat and adipocyte size regardless of baseline diabetes status

A predominant feature of fat mass loss is a reduction in adipocyte hypertrophy. The current data shows that bariatric surgery decreased SAT adipocyte size in the obese independent of the baseline diabetes status (Table 2). Hypertrophied adipocytes are a known risk factor for T2D, and other metabolic abnormalities (Bays et al., 2008; Henninger, Eliasson, Jenndahl, & Hammarstedt, 2014; O'Connell et al., 2010). A previous study by (Cotillard et al., 2014) showed that obese individuals with fewer metabolic improvements or unresolved diabetes status after bariatric surgery expressed hypertrophied adipocytes.

At 6 months after the surgical procedures, all the MRI-derived adipose tissue volumes had decreased significantly in the obese patients regardless of their initial diabetes status (Table 1). Specifically, bariatric surgery induced a greater loss of VAT mass than SAT, but the amounts of loss of VAT and SAT were similar among individuals with and without T2D (Table 2). An observable and a reported effect of bariatric surgery is the marked and sustained decrement in fat mass along with marked improvement in overall metabolic health (Galanakis et al., 2015; Weiss et al., 2009). An earlier report indicates that (Gray et al., 1991) obese subjects with comparatively increased intra-abdominal fat at baseline tended to lose more fat from this depot during weight loss. Studies by Kim and colleagues (Kim et al., 2011), suggested that weight loss after bariatric surgery preferentially targets the VAT in T2D patients. Furthermore, mild weight loss following dietary changes have been shown to preferentially target VAT, whereas rapid weight loss following bariatric surgery proportionally targets both SAT and VAT compartments (Chaston & Dixon, 2008; Weiss et al., 2009). The

upper arm fat decreased significantly in obese ND subject, although T2D patients still maintained a significant amount of arm fat at 6 months after surgery. Upper arm fat accumulation is to some extent positively associated with the presence of diabetes independent of age, gender and percentage body fat (Miljkovic-Gacic et al., 2008). A study found no association between the volumes of upper arm fat and the risk of developing diabetes (Hara, Saikawa, Kurokawa, Sakata, & Yoshimatsu, 2004). Caloric restriction within a half-year period has been shown to significantly decrease anthropometrically derived upper arm fat in obese women (J. Wang, Laferrere, Thornton, Pierson, & Pi-Sunyer, 2002).

6.2 Obesity and/or diabetes impairs adipose tissue energy metabolism

6.2.1 White adipose tissue (Studies I-III)

Earlier human studies with [^{18}F] FDG PET and clamp techniques demonstrated decreased whole body GU and a significant impairment in abdominal SAT and VAT GU in obese individuals compared to nonobese subjects (Virtanen et al., 2002). The current studies utilized PET-clamp techniques to quantify adipose glucose metabolism in severely obese patients, and also examined alterations in adipose glucose uptake after bariatric surgery. A noticeable feature of obesity-induced diabetes is an impairment in insulin-stimulated GU in the abdominal and femoral adipose tissue compartments compared to nonobese controls (Table 3, Study I). In addition, whole body glucose utilization (M-value) measured during clamp in addition to skeletal muscle GU were significantly reduced in the obese compared to controls (Table 3, Study I). Whereas baseline M-value did not differ with respect to diabetes status, the T2D group demonstrated impaired skeletal muscle GU compared to the ND group (Table 3, Study I). In a previous study, it was demonstrated that abdominal SAT and omental adipose tissue from obese and overweight subjects are irresponsive to the action of insulin (Stolic et al., 2002). Insulin resistance is the hallmark of diabetes, and it is characterized by the inability of adipose tissue and skeletal muscle to take up systemic glucose, and also suppress endogenous glucose production with rising insulin levels (Walker et al., 2007). Indeed, reduced response of skeletal muscle to insulin is regarded as the primary defect in the development of diabetes (DeFronzo & Tripathy, 2009). Optimal glucose uptake in adipose tissue is driven by the action of the translocase GLUT4, for which the expression is down regulated in obese prediabetic and diabetic subjects (Ducluzeau et al., 2001). Ex vivo analysis of adipocyte from VAT of obese T2D indicates decreased capacity of GLUT4 for glucose transportation (Maianu, Keller, & Garvey, 2001).

Under basal conditions, there was no difference in adipose GU values with respect to diabetes. The GU values in the metabolically active extraperitoneal VAT was significantly lower in the pooled T2D and ND obese individuals compared to controls. The GU values were similar between the obese and controls in the remainder of the fat depot and in skeletal muscle (Table 4, Study I). Previous ex vivo studies obtained similar findings that indicates that basal GU in the mesenteric adipose tissue depot was significantly higher in lean controls compared to obese subjects (Stolic et al., 2002). Basal adipose tissue FAU, and blood flow distribution in all of the studied adipose tissue depots compared to lean control subjects (Table 5, Studies II-IV).

Under conditions of fasting or limited caloric exposure, the body switches from glucose to fatty acids utilization aided by increased adipocyte lipolysis. The current dataset demonstrated decreased adipose tissue FAU in the obese patients compared to lean controls (Table 5, Studies II, IV). There were no differences in FAU between obese T2D and ND (Table 5, Studies II, IV).

Regardless of the presence of diabetes, the expansion of SAT was due to both adipocyte hyperplasia, and hypertrophy (Figure 9). Consequently, adipose tissue blood flow was reduced when expressed per SAT mass, but similar to control values when expressed per cell, in obese T2D and in ND patients (Table 5, Study II). The nonlinear relationship between blood flow and cellularity was evident in the abdominal SAT depot in all subjects (Figure 10A), the fatty acid extraction was selectively increased in T2D (Figure 10B), which paralleled a similar increase in skeletal muscle FAU (Table 5, Study II). This was not the case, however, in abdominal VAT depots: although VAT depots were populated with smaller adipocytes than subcutaneous depots and supplied by a larger blood flow. Visceral fat did not show increased FAU, either per unit tissue mass or as a fractional extraction (in nondiabetic or in T2D subjects).

Both lipid and glucose oxidation rates (expressed as a fraction of systemic FFA delivery to tissues) were severely reduced in the obese compared to nonobese subjects (Figure 11). The decreased uptake and storage of fatty acids in obesity may also be attributed to the reduced adipocyte differentiation and expandability contributing to the increased systemic levels acids (Stinkens, Goossens, Jocken, & Blaak, 2015). Impairment in adipose tissue lipid uptake in the presence of obesity, insulin resistance and T2D may be partly attributed to the decreased activities of insulin-mediated lipoprotein lipases and decreased fatty acid translocase CD36 in adipocytes (Stinkens et al., 2015). When expressed per unit of tissue or per adipocyte, FAU was similar between obese and nonobese control subjects (Table 5, Study II). However, after taking into account the blood flow and the concentration of FFA, fatty acid fractional uptake was higher in the obese group compared to controls (Figure 11). In the case of skeletal muscle, there was decrease in blood flow per unit mass but increased fatty acid extraction only in the obese T2D patients (Figure 9). Metabolic flexibility, which is defined as the body's capacity to switch in-between fuel oxidation is based on the availability of the said fuel. The inability to respond to the changes in fuel availability is implicated in the ectopic accumulation of lipids in skeletal muscle, which leads to insulin resistance (Goodpaster & Sparks, 2017) a hallmark of T2D. On the basis the aforementioned evidence, it may imply that the higher skeletal muscle FAU obtained only in the obese with T2D compared to controls (Table 5, Study II) may underlie their current diseased state. Even under stimulated conditions, the ability to transition from fatty acid to glucose utilization is severely impaired in insulin resistant subjects and this is mainly attributed to the impaired of adipose and skeletal GU (Table 3, Study I).

6.2.2 Supraclavicular brown adipose tissue (Study IV)

Supraclavicular BAT uptake of systemic nonesterified fatty acid (NEFA) was reduced in the obese group compared to controls, similar to findings from an earlier study (Vijgen et al., 2011). The presence of diabetes, or lack thereof, did not produce statistically significant difference in the BAT NEFA uptake rate in the studied

obese women. Similar findings were reported in a previous study involving young men (Blondin et al., 2015). Supraclavicular BAT triglyceride content was higher in the obese compared to controls, and was inversely associated with the fractional [^{18}F] FTHA tracer uptake (Table 6, Study IV). These data indicate that a dense triglyceride accumulation may be contributing to the reduced NEFA metabolism associated with the obesity (Din et al., 2017). Supraclavicular BAT radiodensity assessed by CT (Din et al., 2017), or by proton magnetic resonance spectroscopy (Raiko et al., 2015), has been shown to correlate with markers of adiposity and unfavorable metabolic profiles (Koksharova et al., 2017). In adult humans, the supraclavicular fat depot is mixture of cells of brown (sBAT) and white (sWAT) (Study IV). The absolute quantity [sBAT (g)], and the proportion of brown fat [sBAT (%)] in the supraclavicular fat depot were lower in the obese group compared to the controls (Table 6, Study IV). The metabolic potential of brown fat is known to be negatively correlated with the presence of obesity and/or metabolic abnormalities (Cypess & Kahn, 2010). The increasing amounts and activity of BAT can potentially play a role in the treatment of obesity and obesity-related metabolic abnormalities through the utilization of glucose and fatty acid fuel for BAT substrate metabolism (Bartelt et al., 2011; Berbee et al., 2015).

6.3 Bariatric surgery enhances adipose tissue metabolism regardless of the presence of diabetes

The massive weight loss (~ 25 kg) at 6 months after bariatric surgery produced marked improvement in whole body glucose utilization in the T2D group, as well improved glucose tolerance (Table 2, Study I-IV). However, skeletal muscle insulin-mediated GU was impaired in the obese T2D group (Table 2, Study I). Intraperitoneal VAT GU remained subnormal, and femoral SAT insulin sensitivity was still impaired in the T2D group (Table 2, Study I). Impaired glucose metabolism in the VAT is a known risk factor for T2D (Abate et al., 1996). However, increased lipid accumulation and metabolism in femoral SAT is associated with favorable metabolic parameters known for their role in diabetes prevention (Azuma et al., 2007). It is important to reiterate that the postsurgery studies were conducted while patients were still losing weight. Therefore, whether insulin would have exerted further action in improved glucose utilization is still unknown. A marked reduction in overall and SAT adipose tissue nonetheless, SAT depot continued to make significant contribution to the overall insulin-mediated glucose disposal resulting in more efficient glucose utilization compared to nonobese controls (Figure 7). In the postsurgical state, expanded adipose tissue continues to protect from further increases in glycemia.

Subcutaneous adipose tissue cellularity increased, and total depot blood flow and FAU decreased in both SAT and VAT (Table 3). Weight loss induced by surgery had no effect of FAU expressed per tissue mass. However, pericardial fat FAU decreased after surgery and this was possibly attributable to the sensitivity of this depot to weight loss in addition to being an active depot for lipid metabolism. Both SAT blood flow and FAU when expressed per cell decreased (Table 5, Study II). In both SAT and skeletal muscle, fractional fatty acid extraction did not change but remained higher in the T2D patients compared to nonobese controls (Figure 3). The metabolic profiles of the diabetes group improved significantly after surgery to the same extent and similar to the nondiabetic obese, and none of the blood flow or FAU parameters changed differentially

between obese subjects with and without diabetes (Table 5, Study II). In periods of obesity and during weight loss induced by surgery, the strong connection between adipose tissue blood flow and adipocyte cellularity may have enhanced tissue FAU. The capacity of adipose tissue and skeletal muscle to extract FFA from the circulation was unchanged with weight loss induced by surgery at 6 months. The observed phenomenon may be due to systemic free fatty acid and triglyceride levels that remained elevated after surgery, as has been previously reported (Honka et al., 2015). Another possible explanation may be the fact that the release, rather than the uptake and storage of circulating FFAs is higher under basal conditions (Bucci et al., 2015). Furthermore, the postsurgery studies were performed while the obese patients were still losing weight and were thus in a catabolic state.

With the rapid weight loss at 6 month after surgery, adipose tissue blood flow expressed as mass within the scanned regions remained unchanged in the obese patients (Table 5, Study II). A previous study found slight but significant increase in the SAT blood flow in obese patients at 12 months after RYGB (Rossi et al., 2012). The current [^{15}O] H_2O-PET modality differs from the laser-doppler flowmetry methodology used in that study, however. Moreover, postsurgery patients in the current study had lost an average of 25 kg after 6 months as compared (Table 2) with 40 kg after 12 months of sustained weight loss in the study conducted by Rossi and colleagues. It is therefore tempting to expect that an even longer follow up in future studies could produce significant improvement in adipose tissue blood flow rates. Blood flow rates in SAT and VAT depot decreased significantly through adipose tissue depot as was previously observed in obese subjects following a calorie restriction-induced weight loss (Viljanen et al., 2009).

6.4 Bariatric surgery improves brown adipose tissue lipid metabolism

Marked weight reduction achieved through bariatric surgery produced increments in NEFA uptake, the amounts and proportions of BAT also increased, whereas a decrement in triglyceride content in the supraclavicular fat depot was measured (Table 6, Study IV). Postsurgery, NEFA uptake, sBAT% and triglyceride content values were comparable to values obtained in control subjects (Table 6, Study IV). The increases in the relative amounts of supraclavicular BAT may be responsible for the increased NEFA uptake possibly used as substrate for BAT thermogenesis (Blondin et al., 2017). A previous study on the effect of surgery on BAT suggest that the postsurgery increment in BAT NEFA uptake may be the result of upregulation BAT thermogenesis related gene expression (Hankir et al., 2015). A previous study also proposed that post-bariatric surgery increase in NEFA metabolism may be attributed to the release of intestinal hormones GLP-2 (le Roux et al., 2010), GLP-1 (Kooijman et al., 2015), or also an increase in FGF21 levels (Harris et al., 2017), which are known potential activators of BAT. Furthermore, the reduced density and thickness of the skin around the supraclavicular region results in decreased thermal insulation and skin temperature (Bartelt et al., 2011), which may have played a contributory role in the increased BAT lipid metabolism. The increase in the relative amounts of supraclavicular BAT after surgery induced weight loss is supported by a finding from a previous report (Vijgen et al., 2012). The current study was conducted under basal and thermoneutral

conditions, whereas the aforementioned study Vijgen and colleagues utilized cold-stimulated FDG-PET uptake. The relative increase in the browning in the supraclavicular depot may have resulted from the decreased triglyceride accumulation and the upregulation of genes involved in the browning of white fat (Seale & Lazar, 2009). The potential role of BAT in the improvement in whole metabolism was evidenced in the current study in which a 14% reduction in supraclavicular triglyceride content was positively associated with improvement in whole-body insulin sensitivity 6 months after surgery. These findings are in agreement with previous reports from studies on obese subjects (Raiko et al., 2015), and in subjects with T2D (Koksharova et al., 2017).

6.5 Strengths and limitations of the current study

The novelty in the current series of studies is the use of a multi-modality approach in the evaluation of the effects of bariatric surgery. Subjects were studied with novel metabolic PET imaging modality for the non-invasive measurements of tissue-specific energy metabolism (Studies I-IV). Hyperinsulinemic-euglycemic clamp, the gold standard technique for assessing whole body insulin sensitivity, was performed for the subjects to assess whole body and tissue-specific glucose utilization (Study I). MRI was used for the absolute quantification of the regional distribution of adipose tissue (Studies I-IV). CT-radiodensity has been validated (Lubura et al., 2012), and was used for tissue triglyceride content including supraclavicular brown fat (Study IV). Fat biopsies were obtained for histological assessment of adipocyte sizes (Study II).

The limitations of the present study must be recognized. VAT biopsies were taken during surgery but not as postoperative biopsies. The studied participants were predominantly women (Study I) or only women (Studies II, III, IV), and it would therefore be of interest to conduct similar studies in men. The postsurgery studies were performed when subjects were still losing weight. Longer follow-up is still needed to determine whether the observed adipose tissue-specific change in tissue metabolism remain stable over longer periods. The obese patients underwent one of two bariatric surgery procedures, i.e. sleeve and gastric bypass. In one study (Study IV) the participants were heterogeneous (i.e. obese participants with and without diabetes). The current series of studies consisted of small samples sizes, thus, non-significant differences were obtained with respect to the surgical techniques used. These differences may have been significant had larger sizes been used. Bioelectric impedance analysis may not be an accurate measure of body fat content in morbidly obese subjects. Assessment of BAT lipid metabolism was performed under thermoneutral and fasting conditions without cold stimulation.

6.6 Future prospects

Giant strides have been made with regards to the use of molecular PET imaging to understand metabolic mechanisms that underlie abnormalities such as obesity-induced T2D. The current study highlights the role of adiposity and tissue-specific energy metabolism in the development of diabetes among obese individuals. Alterations in these measured variables as a result of weight loss induced by surgery have also been highlighted. In spite of the benefits associated with surgery, the metabolic status of certain groups of patients remains unchanged after surgery. One area of interest is to study the differences between the cohorts of respondents versus non-respondents to surgery, particularly with respect to the adipose tissue biology. That said, particular attention should be paid to an integrated biomarker profiling comprising genomics, proteomic, and metabolomics (OMICS) to identify the mechanisms that underlie obesity-induced diabetes. It would also be interesting to apply OMICS techniques to research those obese individuals whose diabetes status remains unchanged and compare them with those free of diabetes after weight loss induced by bariatric surgery. Furthermore, the interpretation of OMICS data using systemic biology, advanced bioinformatics and advanced data processing techniques will provide clearer, and a more complete understanding of the root cause of obesity and diabetes.

7 SUMMARY AND CONCLUSIONS

The pathological expansion of WAT through adipocyte hypertrophy, and/or hyperplasia, is a widely known predisposing factor for insulin resistance and T2D. The regional variations in adipose tissue distribution, and metabolic activities, play demonstrable roles in the development of obesity-related metabolic complications. The re-discovery of functional thermogenic BAT in human adults potentially offer a non-conservative and non-surgical approach to the treatment of obesity and diabetes. However, the metabolic functioning of both white and brown fat are greatly hampered in severely obese persons. Treatment strategies for severe obesity may involve a combination of lifestyle modifications, including nutritional and exercise regimen, and pharmacotherapy evaluation. In the event that these conservative approaches are unsuccessful, bariatric surgery is known to achieve long-term weight reduction effect, and results in the remission of T2D in the majority of the obese individuals. Until recently, few studies had attempted to provide tangible explanation for role of adipose tissue metabolism in the pathophysiology of diabetes. Therefore, the present study was conducted to examine regional variation in adipose tissue energy metabolism with PET, MRI and CT imaging modalities, along with fat biopsy in morbidly obese patients (some of whom expressed the diabetes phenotype) and in metabolically healthy nonobese controls subjects. The obese patients were followed-up at 6 months after bariatric surgery to assess the impact of weight loss on adipose tissue metabolism. Initially, the obese were characterized by dysfunctional lipid and glycemic profiles, and they were insulin resistant compared to healthy normal weight controls. As a consequence of their increased total and regional adipose tissue mass, the morbidly obese patients had many more fat cells than individuals of normal weight. Obese patients with diabetes expressed fat depot-specific differences in the metabolically active abdominal adipose tissue depot. Obese patients with diabetes also expressed a blunted response to insulin in glucose uptake in a majority of the studied fat depots compared to the nondiabetic obese group. This particular finding affirms that tissue specific insulin resistance is a major pathophysiological underpinning of obesity-induced diabetes. Long chain fatty acid uptake and blood flow distribution in adipose tissue were lower in these obese patients compared to nonobese healthy controls. The extent, and the rate of fatty acid metabolism of BAT were lower in the obese patients. This is partly attributed to the increased deposition of triglyceride in the supraclavicular fat depot as a consequence of the obesity. Bariatric surgery induced weight loss decreased adiposity measures, along with increased adipocyte cellularity. Bariatric surgery also enhanced the effects of insulin on adipose glucose metabolism regardless of the presence of diabetes. Adipose blood flow distribution, and fatty acid metabolism expressed per depot size, or per adipocyte decreased significantly with weight loss. Adipose blood flow coupled with adipocyte cellularity enhance fatty acid uptake. With respect to brown fat, weight loss decreased the triglyceride content, thus triglyceride was possibly utilized as substrate for enhanced brown fat fatty acid metabolism in the supraclavicular depot. Postsurgery change in that quantity of brown fat triglyceride content, coupled with the increased recruitment of brown adipocytes, may have contributed to the overall favorable insulin sensitivity increase observed in those obese subjects. Taken together, molecular PET imaging offers a novel approach for studying the effects of obesity and also the effects of diabetes on adipose tissue metabolism. The effects of bariatric surgery transcend the loss of adipose tissue mass, but also improvements in tissue metabolic profiles in obese individuals without or with diabetes

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