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SIGNIFICANCE OF ADIPOSE TISSUE CHARACTERISTICS FOR DEVELOPMENT OF METABOLIC COMPLICATIONS IN OBESITY

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Significance of adipose tissue characteristics for development of metabolic complications in obesity

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Till Mamma och Pappa

MAGNA SENTENTIA NON FACILE DESERITUR,
ETIAMSI FALSA EST.

ABSTRACT

Background: Obesity is closely related to development of insulin resistance and dyslipidemia. Intrinsic properties of adipose tissue are also of great importance for obesity related comorbidity. The aim of this thesis was to gain further knowledge of adipose depot specific effects of how fat cell size and lipolysis, as well as removal of a large portion of the visceral fat depot, affect metabolic risk.

Methods: All subjects were from a cohort of 81 obese women undergoing gastric bypass operation. Study I and study III were cross-sectional studies using baseline data, whereas study II and IV were longitudinal studies which also included an examination two years post-surgery. Insulin sensitivity was evaluated with hyperinsulinemic euglycemic clamp. Subcutaneous and visceral fat biopsies were obtained to determine mean fat cell size and lipolysis.

Results: Study I showed that subcutaneous fat cell size correlated with insulin sensitivity ($r= -0.40$ $p= 0.004$) and visceral fat cell size correlated with dyslipidemia ($r=0.32$-0.38 $p= 0.0006$-0.003). Subjects with combined hyperplasia (many small fat cells) in both subcutaneous and visceral fat depots had a favorable metabolic profile compared to subjects with combined hypertrophy (few but large fat cells) ($p= 0.0001$-0.02).

Study II focused on changes in fat cell size and insulin sensitivity following weight reduction induced by bariatric surgery in obese women. Changes in subcutaneous fat cell size correlated with improved insulin sensitivity independently of changes in subcutaneous fat mass ($r=0.32$ $p= 0.04$).

Study III investigated depot specific relations between lipolysis and cardiovascular risk factors. Visceral but not subcutaneous fat cell lipolysis correlated with several cardiovascular risk factors including insulin resistance, high plasma triglycerides and blood pressure ($r= 0.28$-0.44 $p= 0.0001$-0.02). Visceral fat cell lipolysis was also increased in subjects with metabolic syndrome ($F= 8.3$ $p= 0.005$).

Study IV, designed as a randomized double blind controlled trial, investigated the 2-year effects of omentectomy in conjunction with gastric bypass operation on insulin sensitivity and the lipid profile. Eighty-one subjects were included in the study and 62 were re-examined two years post-surgery. Omentectomy did not give any additional positive metabolic effects, neither in the primary outcome measure insulin sensitivity ($p= 0.54$), nor the secondary outcome measures such as lipid status or weight reduction ($p= 0.17$-0.98).

Conclusion: The studies in this thesis highlight that intrinsic factors of adipose tissue, such as fat cell size and lipolysis, independently of fat mass, are important for metabolic complications in obesity. A mere removal of a substantial part of the visceral fat depot does not enhance improved metabolic outcome after gastric bypass operation, suggesting that a metabolic change in adipose tissue and changes in fat cell size are important to achieve positive effects of fat mass reduction.

LIST OF SCIENTIFIC PAPERS

I. Hoffstedt J, Arner E, Wahrenberg H, **Andersson DP**, Qvisth V, Lofgren P, Ryden M, Thorne A, Wiren M, Palmer M, Thorell A, Toft E, Arner P: Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity. Diabetologia 2010;53:2496-2503

II. **Andersson DP**, Eriksson Hogling D, Thorell A, Toft E, Qvisth V, Naslund E, Thorne A, Wiren M, Lofgren P, Hoffstedt J, Dahlman I, Mejhert N, Ryden M, Arner E, Arner P: Changes in Subcutaneous Fat Cell Volume and Insulin Sensitivity After Weight Loss. Diabetes Care 2014;37:1831-1836

III. **Andersson DP**, Lofgren P, Thorell A, Arner P, Hoffstedt J: Visceral fat cell lipolysis and cardiovascular risk factors in obesity. Horm Metab Res 2011;43:809-815

IV. **Andersson DP**, Thorell A, Lofgren P, Wiren M, Toft E, Qvisth V, Riserus U, Berglund L, Naslund E, Bringman S, Thorne A, Arner P, Hoffstedt J: Omentectomy in addition to gastric bypass surgery and influence on insulin sensitivity: A randomized double blind controlled trial. Clin Nutr 2014; [Epub ahead of print]

CONTENTS

LIST OF ABBREVIATIONS

5´-AMP	5´ Adenosine monophosphate
ANP/BNP	Atrial /brain natriuretric peptide
A/B NPR	Atrial /brain natriuretric peptide receptor
AC	Adenylate cyclase
AMP/GMP	Adenosine/guanosine monophosphate
Apo A1/B	Apolipoprotein A1/B
AR	Adrenoreceptor
ATGL	Adipose triglyceride lipase
ATP/GTP	Adenosine/guanosine triphosphate
BMI	Body mass index
cAMP/cGMP	Cyclic adenosine/guanosine monophosphate
CCL-2	Chemokine ligand 2
DEXA	Dual-energy X-ray absorptiometry
DG	Diacylglycerol
ESAT	Estimated subcutaneous adipose tissue in the android region
EVAT	Estimated visceral adipose tissue in the android region
FCV	Fat cell volume
FFA	Free fatty acids
GLP-1	Glucagon like peptide -1
Gs/Gi	Guanine nucleotide binding protein (stimulatory/inhibitory)
GC	Guanylate cyclase
HDL	High density lipoprotein
HOMA-IR	Homeostasis model assessment of insulin resistance
HSL	Hormone sensitive lipase
IGF-1	Insulin like growth factor 1
IR	Insulin receptor
IRS	Insulin receptor substrate
LDL	Low density lipoprotein
MCP-1	Monocyte chemoattractant protein 1
MG	Monoacylglycerol

MGL	Monoacylglycerol lipase
mRNA	Messenger ribonucleic acid
PDE3	Phosphodiesterase 3
PKA/G	Protein kinase A/G
PPi	Pyrophosphate
RYGB	Roux en Y gastric bypass operation
TG	Triglycerides
TNFα	Tumor necrosis factor alpha
VLDL	Very low density lipoprotein

1 INTRODUCTION

1.1 OBESITY AND THE METABOLIC SYNDROME

Obesity is a major risk factor for development of type 2 diabetes, dyslipidemia and hypertension which in turn can lead to cardiovascular disease. Approximately 35 % of adults in the United States were considered obese (body mass index, BMI $\geq$ 30 kg/m^2) in 2012[1] and although less common in Sweden (14%),[2] obesity related comorbidities constitute a large and growing problem in Swedish healthcare.[3] However, as many as 30% of obese individuals do not develop obesity related complications.[4] Body fat distribution as well as altered adipose tissue function such as low grade inflammation and impaired lipolysis seem to play a role in pathogenesis.[4]

Already in 1956 Jean Vague acknowledged the importance of body fat distribution in development of metabolic complications.[5] An accumulation of fat in the upper body, more commonly seen in men and therefore called android fat mass, was more strongly associated with diabetes and arteriosclerosis compared to the more benign lower body fat accumulation often seen in women (gynoid fat). Upper body fat mass has two main depots, fat under the skin (subcutaneous adipose tissue) and fat around the intestines (visceral adipose tissue). Studies have shown that visceral adipose tissue is, independently of total fat mass, an additional risk factor for hypertension, insulin resistance and type 2 diabetes as well as cardiovascular disease.[6-8] The visceral fat depot consists of the greater omentum, mesenteric-, perirenal- and gonadal fat whereof the greater omentum is the largest part. The omentum is the only visceral depot that can be removed without medical risk.

The term “metabolic syndrome” is used to describe a metabolic state characterized by obesity together with several obesity related complications. The exact definition of what constitutes the metabolic syndrome has changed slightly over the years but the cornerstones today are central obesity, dyslipidemia, hyperglycemia and hypertension according to the International Diabetes Federation.[9] The more risk factors a person have the higher the risk of developing cardiovascular disease.

Several factors have been identified explaining why different fat depots exhibit different associations with cardiovascular risk factors. The anatomical proximity of visceral adipose tissue to the liver is such a factor. Björntorp forged the expression “portal theory” in 1990 when he described that the location of visceral adipose tissue, draining secreted factors into the portal vein leading directly to the liver, has an impact on liver induced insulin resistance.[10] Regional differences in the ability of adipose tissue to store free fatty acids as triglycerides (lipogenesis), variations in the ability to break down triglycerides into free fatty acids and glycerol (lipolysis), adipokine secretion, inflammation or differences in fat cell size are some adipose characteristics that also can be part of the explanation.

1.2 GASTRIC BYPASS OPERATION

Treating obesity has been proven difficult, and even though weight reduction can be achieved by exercise and/or changes in diet, the best validated method to accomplish a substantial sustained weight reduction is bariatric surgery.[11-14] Roux en Y gastric bypass operation (RYGB) is the most common method of bariatric surgery in Sweden. RYGB has been shown to improve metabolic control and even lead to remission of type 2 diabetes,[13, 15, 16] and reduced cardiovascular events.[17]

During a RYGB, the ventricle and about 120 cm of the smaller intestine is bypassed and a small gastric pouch is created distal to the esophagus and reconnected to the small intestine (Figure 1), resulting in restrictive and malabsorptive effects.

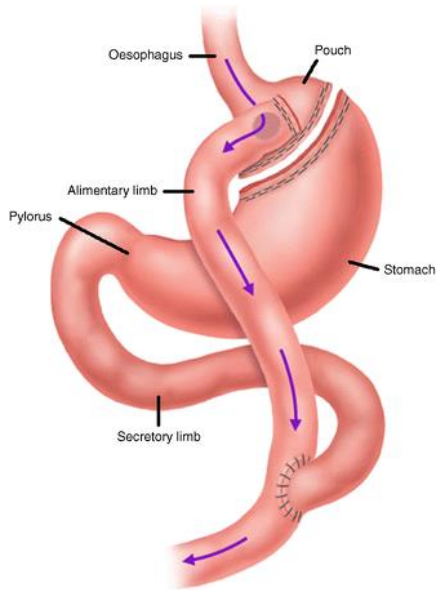


Figure 1.

Gastric bypass operation. Source: Dirksen et al Diabetologia[18].

In addition, it has been shown that RYGB also alters the hormonal response following a meal intake. For example, RYGB leads to increased levels of GLP-1 and peptide YY and decreased levels of ghrelin. These alterations interestingly seem to affect both satiety and hunger as well as food preference.[19-21] In addition to this, RYGB leads to increased hepatic and muscle insulin sensitivity and increased insulin secretion independently of weight reduction, probably due to hormonal changes.[22, 23] RYGB is more effective for weight reduction than ventricular restriction such as gastric banding (a different kind of bariatric surgery).[24]

1.3 INSULIN

Insulin is a hormone with strong anabolic effects that is produced in the β-cells on the isles of Langerhans of the pancreas. Several factors that stimulate secretion of insulin are depicted in Figure 2 such as increasing plasma glucose, rises in amino acid levels and several gastrointestinal hormones (incretins). In the liver, insulin stimulates glycogen production and inhibits glycogenolysis and release of glucose into the blood stream.

Insulin also has effects on fatty acid metabolism by increasing lipoprotein lipase activity which leads to increased uptake of free fatty acids (FFA) in adipocytes and in turn increased triglyceride synthesis. It is also a potent anti-lipolytic hormone by inhibiting the effect of hormone sensitive lipase, the rate limiting enzyme of triglyceride catabolism (Figure 2). Insulin inhibits glucose output from the liver and stimulates glucose uptake, primarily in muscle but also in adipose tissue. FFA in plasma inhibits the insulin mediated uptake of glucose into muscles and also phosphorylation of glucose that in turn leads to reduction of glycogen synthesis and glucose oxidation.[25, 26]

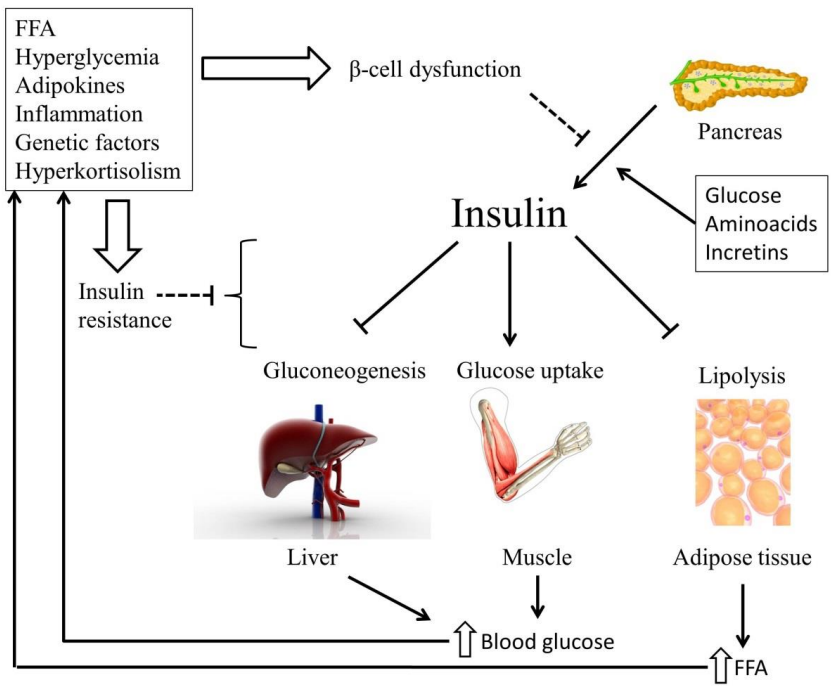


Figure 2.

Function of insulin and pathology behind insulin resistance and insufficient insulin secretion. FFA = free fatty acids, IGF-1 = insulin-like growth factor 1. GLP-1 → = stimulation ⊣ = inhibition ⇧= increase

Glucose and insulin levels in the body are tightly regulated to maintain homeostasis. In the fasting state, insulin secreted from the β-cells regulates glucose release from glycogen levels in the liver so it is equal to glucose uptake in peripheral tissues thereby maintaining the same blood glucose levels.

Hyperglycemia occurs if the β-cells are unable to secrete sufficient amounts of insulin which can manifest itself as impaired fasting glucose and/or impaired glucose tolerance following a meal. These two conditions often precede the development of type 2 diabetes.[27]

The metabolic answer following insulin stimulation in a subject or a target tissue is a measure of insulin sensitivity and the term insulin resistance can be used if the metabolic answer is inadequately low. Insulin resistance can occur in both the liver and/or peripheral tissues such as muscle and fat. Different factors that influence and cause insulin resistance as well as β-cell dysfunction are listed in Figure 2.

1.3.1 Insulin sensitivity measurements

Insulin sensitivity *in vivo* can be assessed in several ways that highlight different aspects of insulin sensitivity. In this thesis we have used an indirect method, homeostasis model assessment of insulin resistance (HOMA-IR), and a direct method, euglycemic hyperinsulinemic clamp to determine insulin sensitivity. Although important, we have not evaluated the β-cell function in this thesis.

1.3.1.1 Hyperinsulinemic euglycemic clamp

Hyperinsulinemic euglycemic clamp is considered to be the gold standard technique to evaluate insulin sensitivity. During a hyperinsulinemic clamp a fixed insulin infusion (based on weight or body surface area) induces a hyperinsulinemic state. The blood glucose levels are then maintained (clamped) at a euglycemic level (4.5-5.5 mmol/l) with an exogenous glucose infusion. The rate of exogenous glucose infusion is then equal to glucose disposal rate, i.e. insulin sensitivity. An insulin sensitive subject will require higher rates of glucose infusion compared to a subject who is insulin resistant. During the induced hyperinsulinemia, almost all infused glucose is taken up by skeletal muscle (80-90%) and therefore the hyperinsulinemic euglycemic clamp reflects primarily skeletal muscle insulin sensitivity.[28] It is important that the insulin infusion is sufficiently high to suppress hepatic glucose production for a correct assessment of muscle insulin sensitivity (if hepatic glucose production is not suppressed the "true" glucose disposal rate will be higher than the obtained value).

1.3.1.2 Homeostasis model assessment of insulin resistance

Homeostasis model assessment of insulin resistance (HOMA-IR) is a mathematical model developed by Matthews and colleagues to assess insulin resistance and β-cell function.[29] The model uses fasting state plasma insulin and plasma glucose to determine insulin resistance. As discussed above, in the fasting state, glucose and insulin levels are highly dependent on hepatic glucose production. Therefore, HOMA-IR reflects hepatic insulin resistance rather

than the skeletal muscle insulin sensitivity measured with hyperinsulinemic euglycemic clamp.

1.4 ADIPOSE TISSUE

Adipose tissue is composed mainly of adipocytes and these are surrounded by the stroma vascular fraction that comprises a large variety of cells (approximately 50% of all the tissue cells) such as different pre-cursor cells, immune cells, vascular cells and fibroblasts together with extra cellular proteins.[30]

1.4.1 Fat cell size

In 1968 Hirsch and Gallian described a method for isolation and determination of human fat cell size and many elegant studies were performed on adipose tissue in the 1960ies and 1970ies.[31] Fat cell size was found to correlate with obesity and serum insulin.[32, 33] The term hypertrophy was starting to be used to describe adipose tissue with large and few fat cells whereas the term hyperplasia was used to describe an adipose tissue characterized by many but small fat cells. Björntorp and Sjöström showed that in normal weight subjects fat cell size increases with increasing body weight, and in subjects with a higher amount of body fat there is both an increase in fat cell size and number.[33] Many of the older studies were small and did not describe human visceral adipose tissue. The determination of total fat cell number also demands an estimation of fat mass, which was previously hard to perform.

In the last decades, new methods have been developed for determination of total fat mass and fat mass in different depots. The realization that adipocytes are hormonally active have led to new studies on the impact of fat cell size. In 2008, our group showed that about 10% of adipocytes are renewed annually and in the same publication we also confirmed a curve-linear relationship between fat mass and fat cell size.[34] This curve could be used to get a numerical value, which we called morphology value, illustrating to what extent a subjects adipose tissue is hypertrophic (positive value) or hyperplastic (negative value). Our group later showed that the renewal of fat cells in subjects with hypertrophic adipose tissue had 70% lower turnover (in absolute numbers) than in subjects with hyperplastic adipose tissue and that hypertrophy is linked to a disadvantageous metabolic profile.[35] Adipose tissue morphology has been shown to correlate positively with tumor necrosis factor α (TNFα), a potent inducer of inflammation and also involved in metabolic dysregulation in adipose tissue.[36]

1.4.2 Fat cell metabolism

The ability to degrade triglycerides (lipolysis) and re-esterify free fatty acids to triglycerides are important functions of adipose tissue. Both functions occur at the same time and depending on the nutritional status, one of these functions prevails over the other resulting in release of FFA from the adipocyte or storage of FFA as triglycerides in lipid droplets inside the adipocyte.[37, 38]

1.4.2.1 Triglyceride synthesis in adipocytes - lipogenesis

Fatty acids are transported in the blood as triglycerides contained in different lipoproteins. Following a meal dietary lipids absorbed from the gut are transported in chylomicrons. Endogenous lipids are transported to various locations in the form of very low density lipoproteins (VLDL) produced by the liver. The various lipoproteins reach the adipocyte through the blood stream. Adipocytes secrete the enzyme lipoprotein lipase, which binds to the endothelium in the capillaries and hydrolyses triglycerides from the lipoproteins into free fatty acids and glycerol. This enables the FFA to be transported into the adipocyte where they together with glucose serves as a substrate in the process of re-esterification in to TG subsequently stored in the adipocyte. Both lipoprotein lipase enzyme activity and glucose uptake are stimulated by insulin.

1.4.2.2 Breakdown of triglycerides in adipocytes - lipolysis

During lipolysis, each triglyceride molecule is broken down in a stepwise fashion into three fatty acids and one glycerol molecule (Figure 3). The first rate limiting step in lipolysis is the conversion of triglycerides to diacylglycerol (DG) and one FFA molecule. This reaction is catalyzed by adipose triglyceride lipase (ATGL), also called desnutrin, and to a less extent hormone sensitive lipase (HSL). ATGL seems to have larger impact on basal lipolysis whereas HSL is important under both basal and stimulated conditions.[39, 40] HSL is also responsible for the second rate limiting step in lipolysis, conversion of DG to monoacylglycerol (MG). Monoacylglycerol lipase is responsible for the last non-rate limiting step of hydrolyzing MG to FFA and glycerol. Some of the FFA are re-esterified into new TG,[38] whereas glycerol is released from the adipocyte and later taken up by the liver. The liver, in contrast to adipose tissue, contains glycerol kinase that converts glycerol to glycerol-phosphate which can be used for adipogenesis or as fuel.

1.4.2.2.1 Lipolysis regulation

Lipolysis is strongly regulated by hormones, whereof only relatively few have an active role in lipolysis regulation in adults, of which the most important are catecholamines, insulin and atrial /brain natriuretric peptides (ANP/BNP). Thyroid stimulating hormone stimulates lipolysis in infants. There are a number of other hormones that stimulate lipolysis or enhance the lipolytic effects of catecholamines, for example growth hormone, interleukin 6, tumor necrosis factor α, thyroid hormones, glucocorticoids, estrogen, glucagon, parathyroid hormone and cholecystokinin in rodents. Insulin is the most important inhibitor of lipolysis but adenosine, prostaglandins, neuropeptide Y and peptide YY also have some anti lipolytic effects in humans.

Catecholamines exhibit their effect in adipose tissue through four different receptors. The β1, β2 and β3 adrenoreceptors stimulate lipolysis via their connection to stimulatory guanine nucleotide binding protein that activates adenylate cyclase which leads to increased rates of cyclic AMP (cAMP) (Figure 3). cAMP activates protein kinase A which in turn activates HSL through phosphorylation. The fourth catecholamine receptor, the α2 receptor, is

connected to an inhibitory guanine nucleotide binding protein and decrease cAMP-levels, HSL activity and lipolysis. The net effect of catecholamines on lipolysis is stimulatory but the rate of α2- and β adrenoreceptors expressed on the cell surface determines to what extent.

Insulin is a powerful inhibitor of lipolysis and binds to the insulin receptor. This activates the insulin receptor substrate which in a cascade reaction involving phosphodiesterase 3 leads to decreased levels of cAMP and decreased HSL activity.

The atrial /brain natriuretic peptides (ANP/BNP) also stimulate lipolysis but by another pathway that leads to increased levels of cyclic guanosine monophosphate which in turn activates protein kinase G and HSL (Figure 3).

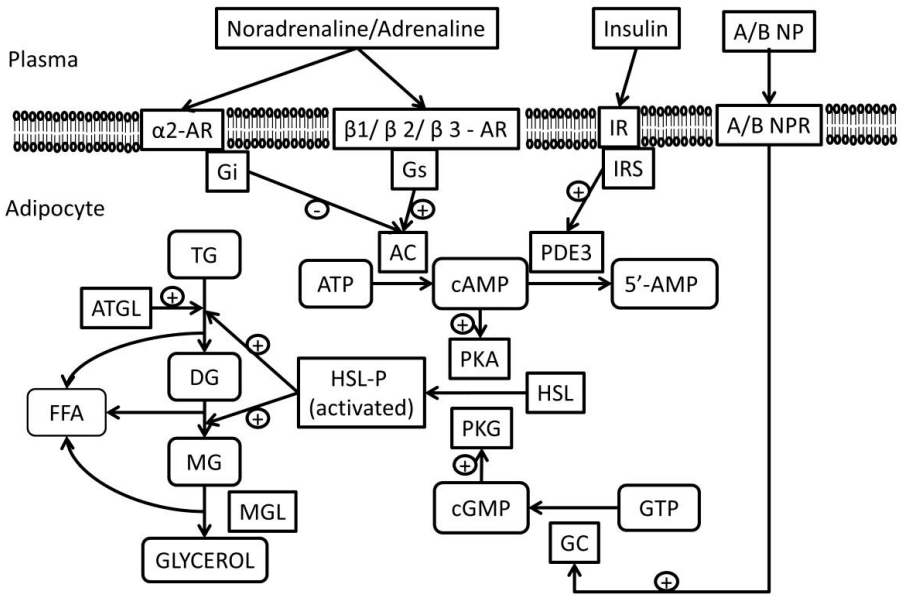


Figure 3.

Overview of important regulatory mechanisms of fat cell lipolysis. TG = Triglycerides, DG = Diacylglycerol MG = Monoacylglycerol, MGL = Monoacylglycerol lipase, FFA = Free fatty acids, HSL = Hormone sensitive lipase, HSL-P = Phosphorlyated HSL, ATGL= Adipose triglyceride lipase, ATP = Adenosine triphosphate, AC = Adenylate cyclase, cAMP = Cyclic adenosine monophosphate, PDE3 = Phosphodiesterase 3, 5´-AMP = 5´Adenosine monophosphate, PKA= Protein kinase A, A/B NP = Atrial /brain natriuretic peptide, A/B NPR = Atrial /brain natriuretic peptide receptor, GTP = Guanosine triphosphate, GC = Guanylate cyclase, cGMP = Cyclic guanosine monophosphate, PKG= Protein kinase G, IR = insulin receptor, IRS = Insulin receptor substrate, Gs/Gi = guanine nucleotide binding protein (stimulatory/inhibitory), AR = Adreno receptor.

1.4.2.2.2 Lipolysis in different fat depots and different metabolic conditions

The fat cell lipolysis rate is different in different fat depots. There are also differences between the correlation between fat mass in different fat depots and metabolic complications. Changes in basal or stimulated lipolysis rate may explain some of the fat depots specific

correlations with metabolic disease and this has been studied extensively in different settings. Visceral fat cells show a higher lipolytic answer to catecholamine induced lipolysis than subcutaneous fat cells probably due to variations in the rate of β- and α2-adrenoreceptor mediated glycerol release.[40] The anti lipolytic effects of insulin is more pronounced in subcutaneous than visceral adipose tissue due to differences in insulin receptor affinity.[41] Subcutaneous adipose tissue in the upper body has higher lipolytic response to catecholamines compared to lower body subcutaneous adipose tissue.[42]

In obesity, basal lipolysis is elevated in all examined regions. Whether the antilipolytic effect of insulin is altered in obesity is unclear, as reviewed.[41] The answer to stimulation of lipolysis seems to be different in different fat depots in obesity. One study showed that stimulated subcutaneous lipolysis is blunted,[37] whereas another study showed increased stimulated lipolysis in visceral fat cells in obesity.[43]

1.4.3 Adipose tissue protein production

Over the past few decades it has become evident that adipose tissue secretes a large number of proteins (adipokines) that exert both local paracrine/autocrine functions as well as systemic endocrine effects. These adipokines are involved in different processes such as appetite control, inflammation, cell proliferation, adipogenesis, glucose metabolism and vascularization.[30] In this thesis, mRNA levels of tumor necrosis factor α (TNFα), interleukin 6 (IL-6) and monocyte chemotactic protein 1 (MCP-1) were analyzed and a brief description of these hormones will follow below.

1.4.3.1 Tumor necrosis factor alpha

As the name implies macrophage derived tumor necrosis factor α was originally found to cause necrosis in tumor cells.[44] Later TNFα was linked to weight loss in cancer cachexia[45]. In 1993, Hotamisligil and colleagues showed that in obese rats and rats with diabetes, there was an increased expression of TNFα in fat cells.[46] Furthermore, neutralization of TNFα lead to increased peripheral glucose uptake in response to insulin.[46] Increased levels of TNFα has also been found in obese humans with hyperinsulinemia.[47] Subsequent investigations have shown that most of the TNFα production in adipose tissue is not derived from adipocytes but from infiltrating macrophages.[48] The TNFα from adipose tissue is not released into the circulation but instead exerts multiple para/autocrine effects in adipose tissue, as reviewed.[48] These functions include impairing insulin action by decreased expression of glucose transporters, down regulation of IRS function and expression. TNFα also stimulates lipolysis by effects on HSL and perilipin. Perilipin is a protein involved in storage of lipids in lipid droplets inside the adipocyte. TNFα release from adipocytes correlates negatively with insulin induced lipogenesis in human adipose tissue.[49]

1.4.3.2 Interleukin 6

Interleukin 6 is a pro-inflammatory cytokine produced by both adipocytes and stroma vascular fraction cells in adipose tissue. It correlates positively with adiposity and weight reduction leads to reduced levels of IL-6 as well as improved insulin sensitivity.[30, 50] IL-6 induces an increase of C-reactive protein, a marker of inflammation. Both C-reactive protein and IL-6 are associated with development of type 2 diabetes.[51] Induced hyperinsulinemia increases levels of plasma IL-6 which may be a mechanism that could contribute to the low-grade inflammation seen in obesity.[52] IL-6 is also produced in skeletal muscle during exercise and a recent review suggest that, in contrast to adipose tissue derived IL-6, IL-6 from the muscle actually have beneficial effects on metabolism by increasing insulin sensitivity, partially through increasing levels of GLP-1.[53]

1.4.3.3 Monocyte chemoattractant protein 1

Monocyte chemoattractant protein 1 (MCP-1), also called chemokine ligand 2 (CCL-2) increases in obesity and type 2 diabetes and the expression of MCP-1 is increased in omental compared to subcutaneous adipose tissue, as reviewed.[54] MCP-1 contributes to macrophage accumulation in adipose tissue. Obese homozygous MCP-1 knockout mice exhibit reduced insulin resistance and reduced hepatic steatosis.[55] Acute administration of MCP-1in circulating blood leads to insulin resistance but not macrophage infiltration into adipose tissue in rats, whereas an infusion of MCP-1 over a long time also results in macrophage infiltration in adipose tissue.[56] This indicates that MCP-1 might have other effects on insulin resistance irrespective of macrophage infiltration and inflammation. Endothelial cells from human subjects with diabetes that were treated with high levels of glucose resulted in 40-70% increased release of MCP-1 which may be a partial explanation how hyperglycemia leads to increased inflammation.[57]

2 AIMS

Obesity and visceral fat accumulation are strong risk factors for the development of type 2 diabetes and cardiovascular disease. Other factors than fat accumulation *per se* are also important for the development of obesity related complications. This thesis aims to study depot specific properties of fat cell size and lipolysis in relation to cardiovascular risk factors. In addition, the aim was to evaluate the potential metabolic effects of removing a large portion of visceral adipose tissue.

2.1 STUDY-SPECIFIC AIMS

I. To investigate whether fat cell size is differently linked to the metabolic phenotype in subcutaneous and visceral adipose tissue. A second aim of this study was to elucidate whether co-existing subcutaneous and visceral hyperplasia, compared to hypertrophy, correlates with a more benign metabolic phenotype.

II. To investigate whether changes in subcutaneous fat cell volume correlates more strongly than fat mass changes with improvements in insulin resistance following weight reduction.

III. To investigate whether lipolysis in subcutaneous and visceral adipocytes display depot specific correlations with cardiovascular risk factors and the metabolic syndrome.

IV. To investigate whether removal of the greater omentum in conjunction with gastric bypass operation results in enhanced improvement of insulin sensitivity and cardiovascular risk factors compared to gastric bypass operation alone.

3 MATERIAL AND METHODS

3.1 SUBJECTS

The subjects in this thesis were recruited from four different surgical centers in Stockholm (Ersta Hospital, Karolinska University Hospital Huddinge, Södertälje Hospital and Danderyds Hospital) after they had been accepted for gastric bypass operation. To be eligible for inclusion, the subjects had to be female, between 18-70 years old and have a BMI $\geq$ 40 kg/m^2. Subjects could also be included with a BMI $\geq$ 35 kg/m^2 if they had concomitant obesity associated complications such as type 2 diabetes or hypertension. Exclusion criteria were treatment with warfarin, insulin or severe ongoing psychiatric disease. All subjects were part of a randomized clinical trial (study IV) and were randomized to either gastric bypass operation alone or gastric bypass operation in conjunction with omentectomy. They were re-investigated two years postoperatively. Figure 4 shows a flowchart for inclusion and exclusion of patients to study IV.

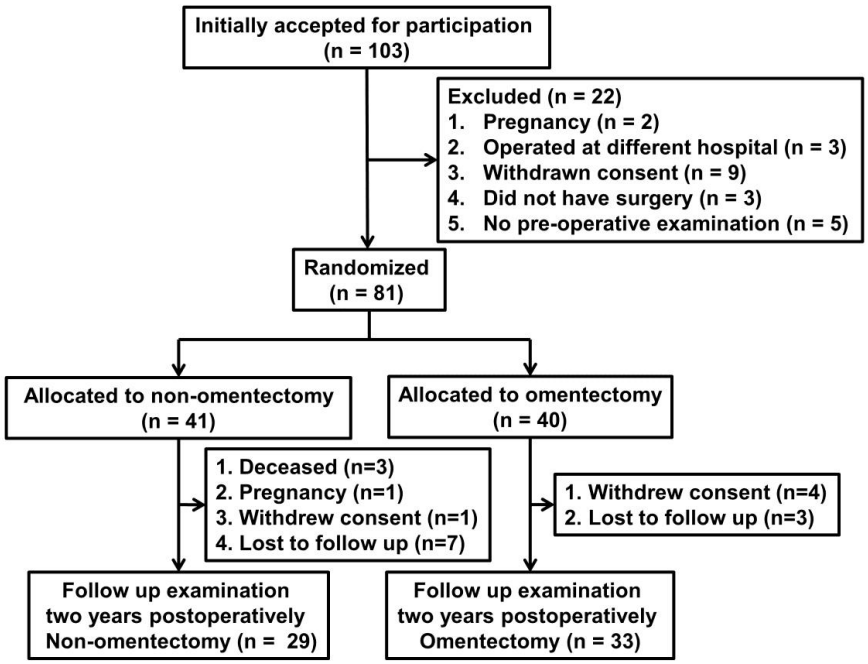


Figure 4.

Flow chart of included subjects in Study IV.

The number of subjects included in each study is presented in Table 1. Study I and III were cross-sectional studies and analyzed data that had been collected pre-operatively. Study II and IV were longitudinal studies with data both from preoperative examinations and from a follow up examination two years post-operatively when the subjects had been weight stable for at least three months. Sixty-two subjects were reexamined post-operatively. The clinical

characteristics of the subjects are presented in Table 2. In study II an additional 62 healthy subjects with a large inter-individual variation in body mass index (20-62 kg/m^2) were included to obtain a reliable relationship between fat mass and fat cell volume.

Table 1.

Number of subjects included in each study and reason for exclusion.

	Before surgery	After surgery	Comments
Study I	80		One subject excluded due to missing data on both subcutaneous and visceral fat cell size.
Study II	62	62	Only subjects that had participated in both pre- and post-operative examinations were included. In addition, 62 healthy women with a large inter-individual variation in BMI were included to obtain a standard curve for adipose tissue morphology.
Study III	73		Seven patients were excluded since they had a fasting plasma glucose ≥ 7.0 mmol/l. One subject excluded due to missing data on both subcutaneous and visceral fat cell lipolysis.
Study IV	81	62	

3.2 RANDOMIZATION

Randomization was performed in blocks of 20 patients. At the beginning of the gastric bypass operation, an opaque envelope was opened that contained information of allocation to gastric bypass operation alone (non-omentectomy group) or gastric bypass plus omentectomy (omentectomy group). Researchers that participated in collection of data and subjects were blinded to group allocation.

Table 2.

Clinical characteristics of the cohort. Values are mean ± SD.

	Before surgery	After surgery
Age (years)	42±9	45±9
Body mass index (kg/m^2)	44±5	29±4
Waist circumference (cm)	132±11	97±12
Total body fat (kg)	62±11	30±11
Estimated visceral adipose tissue in the android region (kg)	2.4±0.8	0.7±0.4
Estimated subcutaneous adipose tissue in the android region (kg)	4.0±1.0	1.8±0.9
Plasma glucose (mmol/l)	5.5±1.2	4.9±0.6
Plasma insulin (mU/l)	16.5±8.6	4.8±1.9
HOMA-IR (mmol/l x mU/l)	4.1±2.5	1.0±0.4
Glucose disposal rate (mg glucose/kg body weight/min)	3.8±1.4	6.6±1.5

3.3 ETHICAL CONSIDERATIONS

The studies were performed in accordance with the ethical standards of the Declaration of Helsinki and approved by the local ethics committee in Stockholm. Signed informed consent was obtained from all subjects prior to inclusion.

3.4 CLINICAL EXAMINATIONS AND PROCEDURES

Patients were examined at the laboratory up to three weeks before operation. At that time, body weight had been stable for at least six months. The subjects came to the laboratory after an overnight fast. Weight was measured with a digital scale (TANITA TBF-305) to the nearest 0.5 kg. Waist circumference was measured (at the midpoint between the iliac crest and the lowest rib with a non-stretchable tape measure) and height was measured to the nearest 0.5 cm. Blood pressure was measured in the right arm with a cuff of the appropriate size with a fully automatic device (Omron M10-IT, Omron Health Care, Hoofddorp, The Netherlands). Blood samples were taken for subsequent analyses of fasting insulin, glucose, cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, FFA, apolipoprotein A1 (Apo A1), apolipoprotein B (apo B), TNF-α, C-reactive protein, fibrinogen, MCP-1 and

IL-6. Low density lipoprotein (LDL) cholesterol was estimated with the Friedewald formula as follows[58]

$$\text{LDL cholesterol} = \text{Cholesterol} - \text{High density cholesterol} - 0{,}45 \text{ x Triglycerides}$$

HOMA-IR was calculated from the formula developed by Matthews and used to evaluate hepatic insulin resistance.[29]

HOMA-IR = fasting blood glucose (mmol/l) x fasting plasma insulin (mU/l) / 22.5.

The subjects were instructed to live their life as usual after their visit to the laboratory and were not put on low-calorie diet prior to the operation. The body weight was essentially the same at the time of baseline examination at the laboratory and at the time of operation.

Approximately 24 months post-surgery, when then subjects had been weight-stable for at least 3 months, thy were reexamined according to the same protocol mentioned above.

3.4.1 Definition of the metabolic syndrome

The guidelines from the International Diabetes Federation was used for definition of the metabolic syndrome, i.e. waist circumference ≥ 80 cm plus any 2 of the following; Systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg, raised fasting plasma triglycerides > 1.7 mmol/l, fasting HDL cholesterol < 1.29 mmol/l or raised fasting plasma glucose ≥ 5.6 mmol/l.[9]

3.4.2 Measurement of body composition by Dual-energy x-ray absorptiometry

Body composition (total-, android-, gynoid fat mass and android/gynoid fat ratio) was measured with dual-energy x-ray absorptiometry (DEXA) using a GE-Lunar iDXA with the software enCORE (version 14.10.022) from GE Healthcare, Madison, WI, USA.[59] In addition, using the CoreScan feature, the software estimated the amount of subcutaneous adipose tissue in the android region (ESAT) and then calculated the amount of visceral adipose tissue in the android region (EVAT). Figure 5 shows a schematic view of measurement of fat mass in the android region (defined inferiorly at the pelvis cut line, superiorly by 20% of the distance between neck cut and the pelvis, laterally at the arm cut lines) and the software estimation of ESAT. The following formula is used in the CoreScan feature: EVAT = total body fat in the android region – ESAT. Estimated EVAT using the formula shows a very high concordance (r^2=0.96) with measurement of visceral adipose tissue using computed tomography, which is the gold standard method.[60] Since both android fat mass and EVAT are valid measures ESAT could be calculated as total android fat minus EVAT.

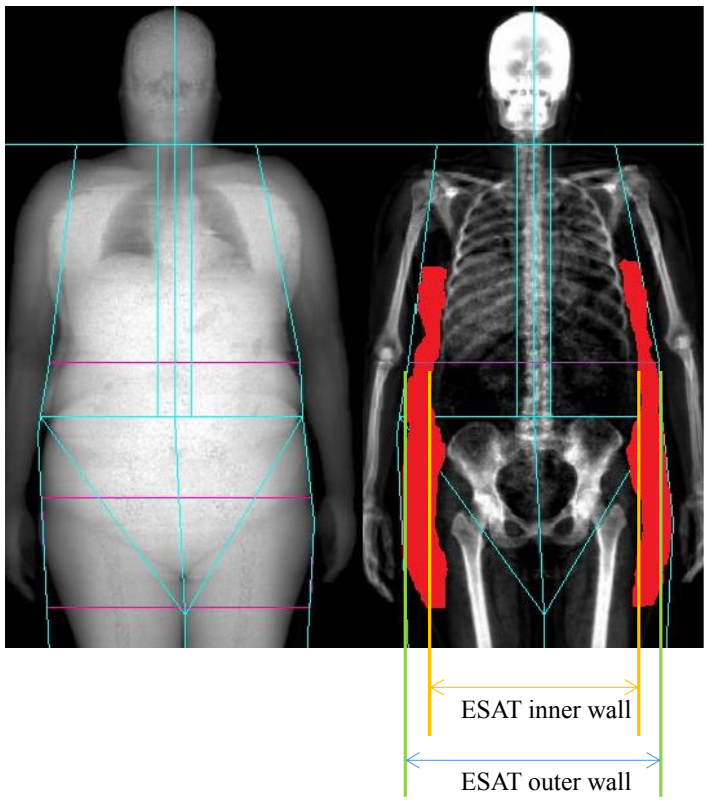


Figure 5.

Dual-energy x-ray absorptiometry used to measure android and gynoid fat mass (left) and estimation of subcutantoues adipose tissue (ESAT) that is used to estimate visceral adipose tissue (EVAT) (right).

3.4.3 Hyperinsulinemic euglycemic clamp

We used hyperinsulinemic euglycemic clamp to measure whole body insulin sensitivity *in vivo*. Two catheters were inserted, one into a forearm vein for infusions and a second catheter into a vein on the dorsal side of the hand used for blood sampling. To obtain arterialised blood, the hand was kept in a heating box (Figure 6, MTA, Karolinska University Hospital, Stockholm, Sweden) with a temperature of 63ºC during the clamp.[61] All infusions during the clamp were given in the forearm vein. Body area was calculated from a formula developed by Du Bois.[62] At the beginning of the clamp a bolus dose of insulin (1.6 U/m^2 body surface area; Actrapid, Novo Nordisk, Copenhagen, Denmark) was given. The bolus injection was followed by a continuous infusion of insulin (0.12 U/m^2 body surface area/minute) suspended in 82 ml sodium chloride (9 mg/ml) together with 2 ml (200 g/l) human albumin (1.7 mg/minute Albunorm, Octapharma, Stockholm, Sweden) and 16 ml potassium cloride (67 µmol/minute) with an infusion rate of 25 ml/h for 120 minutes (Figure 6). Blood glucose was

measured in duplicates every fifth minute (Hemocue, Ängelholm, Sweden) and euglycemia (4.5 to 5.5 mmol/l) was maintained through a variable intravenous infusion of glucose (200 mg/ml). The last sixty minutes of the clamp was considered to be steady state and the infusion rate of glucose during that time period was used for calculation of whole-body insulin sensitivity (expressed as glucose disposal rate with the unit mg glucose/kg body weight/minute). Ninety-five percent of the blood glucose values during the last sixty minutes of the clamp were within euglycemic level. The average blood glucose levels during steady state were 5.05±0.19 mmol/l at baseline and 5.10±0.18 mmol/l at follow up. The average insulin levels at steady state during clamp were 243±77 mU/l at baseline and 169±42 mU/l at follow up.

Figure 6.

Hyperinsulinemic euglycemic clamp and the heating box used to obtain arterialized blood.

3.4.4 Gastric bypass operation and omentectomy

In the past decade, bariatric surgery has become a common method to treat obesity and Roux en Y gastric bypass operation is the most prevalent method. Nowadays, almost all RYGB are performed with laparoscopic technique, but at the time this study was performed, both open and laparoscopic techniques were used in clinical practice. Open surgery was needed in order to remove the entire major omentum. As study IV was a double blinded study, all subjects underwent open surgery. An upper midline incision was made and a small gastric pouch (20-30 ml) was created with the help of linear staples. Linear or circular staples were used for gastro-enteroanastomosis and linear staples were used for creation of the entero-enteroanastomosis. The alimentary limb was approximately 120 cm and the bilio-pancreatic

limb about 75 cm, respectively. In subjects allocated to omentectomy, the entire major omentum was resected.

3.5 FAT CELL EXAMINATIONS

3.5.1 Adipose tissue biopsies

A subcutaneous injection of 25 ml lidocaine 10 mg/ml (without adrenalin), approximately 15-30 cm laterally of the umbilicus, was used for local anesthesia. Under sterile conditions a small incision was made to help the aspiration needle to penetrate the skin. Using a syringe with negative pressure generated by a 10 ml syringe, subcutaneous fat was aspirated and subsequently rinsed in sodium chloride over a plastic filter (Sefar Nitex 06-210/33, Bigman AB, Sweden) (Figure 7). Coagulated blood was manually removed with the help of a spatula.

At the beginning of surgery, a visceral fat biopsy from the greater omentum was obtained and then handled in the same way as the subcutaneous fat biopsy.

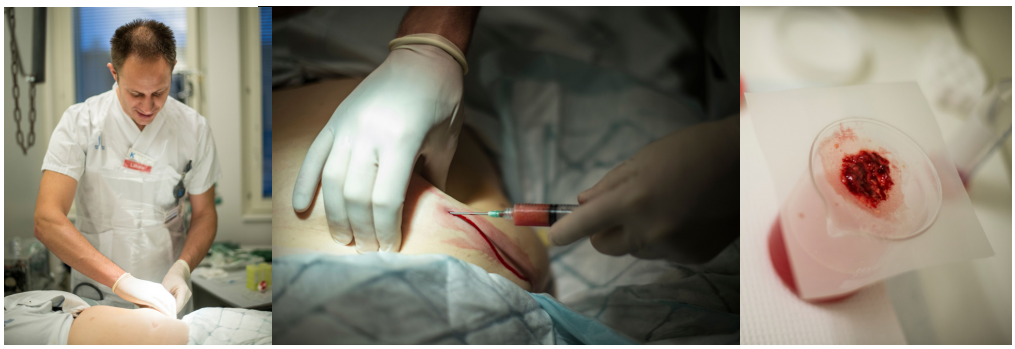


Figure 7.

Abdominal subcutaneous fat biopsy and aspirated adipose tissue.

3.5.2 Isolation of fat cells and determination of fat cell size and number

The fat biopsies were transported in sodium chloride to the lipid laboratory where they were once again rinsed with sodium chloride over a plastic filter (Sefar Nitex 06-210/33, Bigman AB, Sweden). The fat was washed several times with a washing buffer (Krebs Ringer Phosphate buffer with 1% bovine serum albumin) and subsequently incubated in 37ºC water bath under gentle shaking in a washing buffer with 0.5 mg/ml collagenase and 4% bovine serum albumin for 60 minutes. The adipocytes were then washed again (Krebs Ringer Phosphate buffer with 0.1% bovine serum albumin) and filtered thrice through a nylon filter.

A droplet of cell suspension was placed on a microscope slide and covered with a cover-slide. The diameter of 100 cells was determined manually using a ruler fitted in the ocular of the microscope (unpublished data from our laboratory shows no difference in average diameter whether 100 or 300 cells are measured). With the formula described below, the diameter of the fat cells was used to calculate fat cell volume.

$$V = \left(\frac{\pi \cdot d^3}{6}\right) (\mu m^3)$$

V = cell volume d = diameter (µm)

Although the diameter of fat cells is normally distributed, its cube is skewed and as previously discussed,[31, 63] the arithmetic mean of d^3 cannot be used to calculate mean fat cell volume. Instead the average fat cell volume is approximated by a computer program using the formula

$$V_{mean} = \left[\frac{\pi}{6}\right] X \left[3\sigma^2 \times \overline{d} + \overline{d}^3\right]$$

where $\overline{d}$ is the mean diameter and σ is the standard deviation of the diameter.

The average density of triolein (triglycerides) is 0.915 g/cm^3 and this was multiplied with the fat cell volume to obtain mean fat cell weight. The fat mass was then divided by the mean fat cell weight to get fat cell number (total or in different depots).

3.5.3 Determination of fat cell morphology

Previous publications from our research group have shown that the relationship between fat cell volume and fat mass is curve-linear.[34, 35] The relationship can be described with the following formula

$$V = \frac{(a \cdot m)}{(1 + b \cdot m)}$$

where V = mean cell volume (pl) and m = fat mass (kg). The variables a (pl/kg) and b (pl/kg) are obtained by fitting the formula to subject data. The difference between the actual measured fat cell volume and the expected fat cell volume from the mean curve fit is an indication of the adipose morphology. A positive value indicates hypertrophy, whereas a negative value is indicative of hyperplasia (example in Figure 8). In study I the morphology was determined both with variables a and b from previous publications.[34, 35] Total fat mass assessed with DEXA was used in study I. Separate curves were used for abdominal subcutaneous and visceral adipose tissue as fat cell size is different in the two compartments. In study II, new standard curves were created for subjects before and after weight reduction and estimated subcutaneous adipose tissue was used as a measure of fat mass.

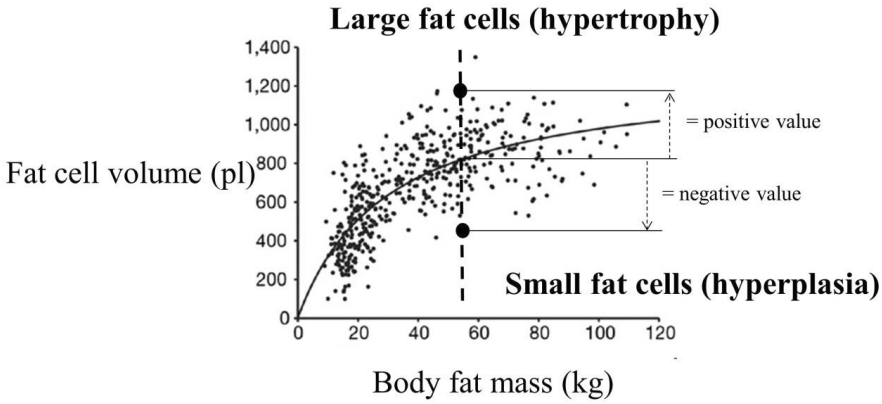


Figure 8.

Determination of adipose tissue morphology value. A fat cell volume that is larger than expected fat cell volume from the cure fit model (hypertrophy) renders a positive morphology value whereas a small mean fat cell volume below the curve fit model (hyperplasia) gives a negative morphology value.[34]

3.5.4 Lipolysis experiments

Isolated fat cells that were diluted in Krebs-Ringer phosphate buffer (pH 7.4) with bovine serum albumin (20 mg/ml), glucose (1 mg/ml) and ascorbic acid (0,1 mg/ml) were incubated in duplicate with different concentrations of noradrenaline (10^{-12} to 10^{-4} mol/l) for 2 hours in a shaking bath at 37ºC. Noradrenaline, a non-selective α- and β-adreno receptor agonist, was the lipolytic agent of choice since it is an important endogenous agent. After incubation the samples were put on ice to stop lipolysis. A fat cell free aliquot was removed and used for measurement of glycerol release with a very sensitive bioluminescent method as described by Hellmer and colleagues.[64] As mentioned before, during lipolysis, each triglyceride molecule is broken down into three fatty acids and one glycerol molecule. Fat cells do not contain glycerol kinase and therefore are unable to reuse glycerol, making glycerol a good marker for lipolysis. The luminometer measures light emitted in the following reactions

$$ATP + glycerol \xrightarrow{Glycerokinase} glycerol - 1 - phosphate + ADP$$

$$ATP + luciferin + O_2 \xrightarrow{Luciferase} Oxyluciferin + AMP + PPi + CO_2 + light$$

Luciferase and glycerokinase compete for the use of ATP and a decreased light indicates higher lipolytic activity. The glycerol concentration was calculated with a computer program.

4 RESULTS

All results included in this thesis are presented and discussed in detail in each individual article. A summary of the most important findings will be presented below.

4.1 REGIONAL IMPACT OF ADIPOSE TISSUE MORPHOLOGY ON THE METABOLIC PROFILE IN MORBID OBESITY (STUDY I)

Study I aimed to investigate whether fat cell size is linked to metabolic and inflammatory markers differently in subcutaneous and visceral adipose tissue in obese women. Fat cell volume (FCV) in the subcutaneous depot (FCV= 989±174 pl) were larger as compared to the average visceral fat cell volume (FCV= 631±184 pl; p< 0.0001). As expected, subcutaneous fat cell size correlated significantly with insulin sensitivity measured by hyperinsulinemic euglycemic clamp (r= -0.40 p= 0.004 Figure 9), fasting insulin (p= 0.0001) and fasting glucose (p= 0.0009). Visceral fat cell size also had an inverse relationship to insulin sensitivity (r= -0.29 p= 0.01 Figure 9) but not as strongly as subcutaneous fat cell size. There was also a positive correlation between visceral fat cell size and TG, cholesterol (Figure 9) and LDL cholesterol (r= 0.32-0.38 p= 0.0006-0.003). Fat cell size in neither the subcutaneous nor the visceral fat depot correlated significantly with mRNA levels of TNFα, MCP-1 or IL-6 in subcutaneous or visceral adipose tissue (p= 0.21-0.70). In multiple regression analysis with subcutaneous and visceral fat cell size as independent variables, subcutaneous fat cell volume related significantly with insulin, glucose and glucose disposal rate (p= 0.002-0.005). On the other hand, visceral fat cell volume correlated with plasma cholesterol, LDL cholesterol, TG and apo B (p= 0.002-0.015). This indicates that fat cell volume has depot specific impact on metabolic parameters, where large subcutaneous fat cells correlate with insulin resistance and large visceral fat cells correlate with dyslipidemia.

Study I also aimed to investigate whether hyperplasia in both visceral and subcutaneous regions correlated with a more benign metabolic profile than hypertrophy in both regions. There were no significant differences between the combined hyperplasia and combined hypertrophy group regarding, age, BMI, waist circumference or body fat percent. On the other hand, significant and potentially clinically relevant differences were seen between the combined hyperplasia and combined hypertrophy subjects when looking at glucose disposal during clamp (4.8 and 3.3 mg glucose / kg body weight/ min respectively), HOMA-IR (2.2 and 5.3 mmol/l x mU/l respectively), cholesterol (4.4 and 5.1 mmol/l respectively), LDL (3.0 and 3.7 mmol/l respectively) and TG (1.2 and 1.9 respectively) (p= <0.0001-0.02).

The finding that combined adipose hyperplasia, in comparison to combined hypertrophy, correlates with insulin sensitivity and a more beneficial lipid profile may have important clinical impact because it puts adipose morphology phenotype, independently of adipose mass, as an important metabolic regulator.

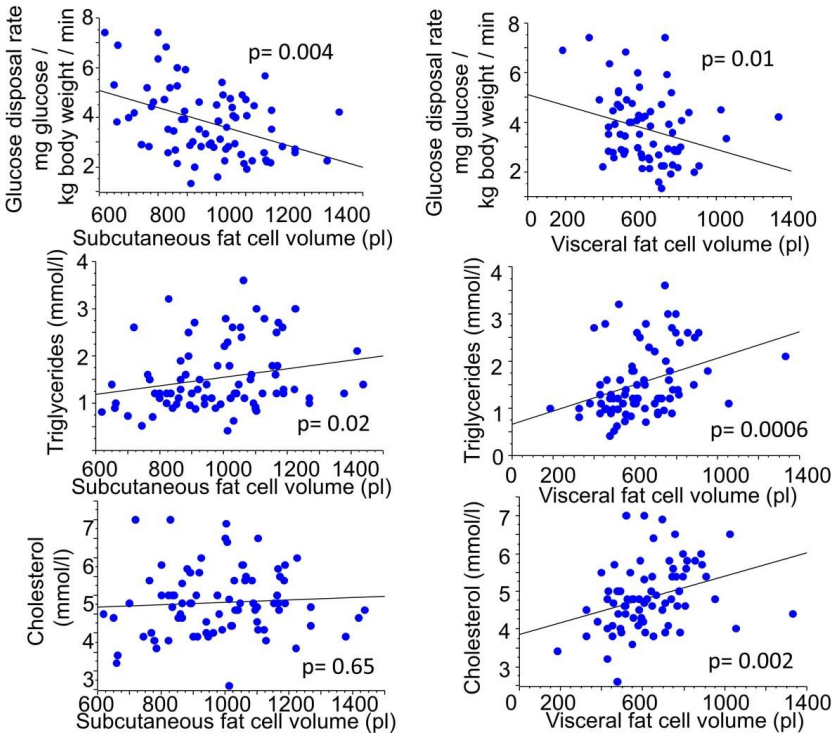


Figure 9.

Subcutaneous fat cell volume (left) and visceral fat cell volume (right) and its correlation to glucose disposal rate measured by hyperinsulinemic euglycemic clamp (top), triglycerides (middle) and cholesterol (bottom).

4.2 CHANGES IN SUBCUTANEOUS FAT CELL VOLUME AND INSULIN SENSITIVITY AFTER WEIGHT LOSS (STUDY II)

After our findings in study I, we were eager to further analyze the clinical impact of fat cell size. In study II we investigated whether changes in subcutaneous fat cell volume in response to bariatric surgery correlate with improvements in metabolic risk factor profile following weight reduction. The 62 subjects who had undergone both the baseline and a follow-up examination two years post-operatively were included in the study. Noteworthy, the cohort consisted of subjects who had undergone either regular RYGB (n=29) or RYGB in conjunction with omentectomy (n=33) (Figure 3). However, there were no significant clinical differences in the results between these groups (data not shown). As expected, all measured clinical parameters improved following RYGB and subsequent weight reduction ($p< 0.0001$ for all measured variables). Subcutaneous fat cell size decreased ($p< 0.0001$) but subcutaneous fat cell number was not statistically different following weight reduction ($p= 0.39$) which is in concordance with previous studies.[34, 65]

The most important result in study II was that changes in insulin sensitivity (measured by hyperinsulinemic euglycemic clamp) correlated with changes in subcutaneous fat cell size ($\rho= 0.38$ $p= 0.0057$) but not changes in estimated subcutaneous fat mass ($\rho= 0.11$ $p= 0.31$) or changes in total fat mass ($\rho= 0.26$ $p= 0.06$). This was further analyzed with multiple

regression with changes in insulin sensitivity as dependent variable and changes in subcutaneous fat cell size as one independent variable (r= 0.32 p= 0.04 and r= 0.33 p= 0.07 respectively) and changes in subcutaneous (p=0.99) or total fat mass (p=0.85) as an additional independent variable. Changes in subcutaneous fat cell size seems to correlate much stronger with changes in whole body insulin sensitivity than changes in fat mass even though the correlation was only of borderline significance when corrected for changes in total fat mass.

4.3 VISCERAL FAT CELL LIPOLYSIS AND CARDIOVASCULAR RISK FACTORS IN OBESITY (STUDY III)

Study III was designed as a cross-sectional study that investigated whether lipolysis in subcutaneous and visceral adipocytes display depot specific correlations with cardiovascular risk factors in obesity. The study included 73 obese women (seven women from the cohort in study IV had been excluded due to fasting plasma glucose > 7.0 mmol/l and one subject due to missing data on both subcutaneous and visceral fat cell lipolysis). The correlation between noradrenaline stimulated maximal lipolysis and clinical parameters was determined with simple regression analysis. There was a significant correlation between visceral fat cell lipolysis and plasma insulin, HOMA-IR, glucose disposal rate, cholesterol, LDL cholesterol, TG, and apo B (r= 0.28-0.44 p= 0.0001-0.02). However, visceral fat cell lipolysis did not correlate with fasting glucose, HDL cholesterol or apo A1 (r=0.02-0.16 p= 0.18-0.90). The positive correlations remained significant after adjustment for total body fat %, abdominal- or hip body fat percent by multiple regression analysis. In contrast, there were no significant relations between maximal subcutaneous fat cell lipolysis and any of the above mentioned parameters (r= 0.001-0.23 p= 0.055-0.99). To further illustrate the impact of depot specific adipocyte lipolysis, subjects were divided into quartiles depending on their lipolysis rate. Clinical parameters in the quartiles with the highest and lowest lipolysis rate were then compared. As expected, no significant differences in clinical parameters were seen between the lowest and highest quartile of lipolysis in subcutaneous fat cells. There were no significant differences in anthropometric measurements between the highest and lowest quartile of visceral fat cell lipolysis (p= 0.50-0.99). On the other hand, subjects in the highest quartile of visceral fat cell lipolysis had, compared to the quartile with the lowest visceral fat cell lipolysis rate, a more unfavorable metabolic profile. For example, plasma insulin was 22.4 mU/l compared to 11.4 mU/l (p= 0.0004), plasma glucose was 5.54 mmol/l compared to 5.16 mmol/l (p=0.03), glucose disposal rate 3.23 mg/glucose/kg/min compared to 4.45 mg glucose/kg/min (p= 0.0098), cholesterol 5.32 mmol/l compared to 4.68 mmol/l (p=0.016) and TG 1.93 mmol/l compared to 1.25 mmol/l (p= 0.003).

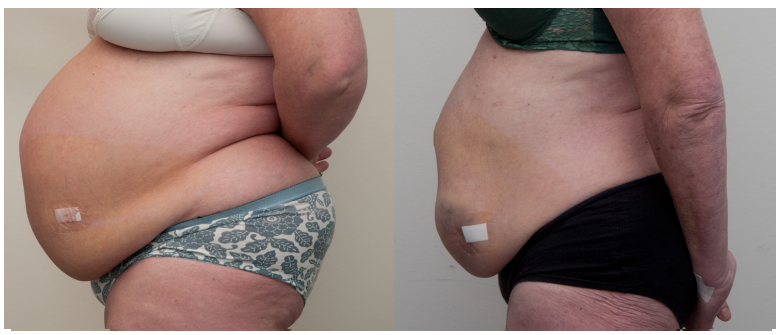
We subsequently divided the subjects, based on whether they fulfilled the criteria for the metabolic syndrome according to the International Diabetes Federation as described,[9] and then analyzed the differences in adipocyte lipolysis between subjects with or without the metabolic syndrome. Subjects with the metabolic syndrome had a 40% higher visceral fat cell

lipolysis rate than subjects without the metabolic syndrome. No differences were seen in subcutaneous maximal fat cell lipolysis rate.

To conclude, visceral, as opposed to subcutaneous, fat cell lipolysis rate correlates with insulin resistance, dyslipidemia and hypertension. Subjects with the metabolic syndrome have higher visceral fat cell lipolysis rate than subjects without the metabolic syndrome.

4.4 OMENTECTOMY IN ADDITION TO GASTRIC BYPASS SURGERY AND INFLUENCE ON INSULIN SENSITIVITY: A RANDOMIZED DOUBLE BLIND CONTROLLED TRIAL (STUDY IV)

Of 81 included subjects, 62 were reexamined two years post-surgery and a flowchart for the study subjects is presented in Figure 4. At baseline, the non-omentectomy and omentectomy group were very similar in all measured parameters such as weight, age, BMI, body fat percent, blood lipids and insulin sensitivity measured by both HOMA-IR and hyperinsulinemic euglycemic clamp. The average weight of the removed omentum was 552 ± 227g. As expected, there was a substantial reduction of mean body weight from 117 kg to 79 kg following RYGB (Figure 10 and Figure 11) and all measured parameters improved significantly.



Figur 10.

Example of weight reduction following gastric bypass operation, before operation (left) and after operation (right). The bandaid indicates the location of the subcutaneous fat biopsy.

However, there was no difference between the groups in the primary outcome measure, insulin sensitivity measured by hyperinsulinemic euglycemic clamp, either at baseline (3.9 ± 1.2 to 6.7 ±1.6 mg glucose/kg body weight/minute in the non-omentectomy group vs. 3.6 ± 2.6 to 6.6 ± 1.5 mg glucose /kg body weight/minute in the omentectomy group), follow up (6.7 ± 1.6 mg glucose/kg body weight/ min in the non-omentectomy group vs. 6.6 ± 1.5 mg glucose/kg body weight/min in the omentectomy group).

In fact, no statistically significant differences between improvement in any measured parameters were seen when comparing the non-omentectomy and omentectomy group (p= 0.17-0.98), besides a lower reduction of triglycerides in the omentectomy group (TG 1.5 to 0.8 mmol/l in the non-omentectomy group and 1.5 to 1.0 mmol/l in the omentectomy group p = 0.019). We considered the differences of reduction of TG a random finding which did not remain significant after a Bonferroni p-value correction.

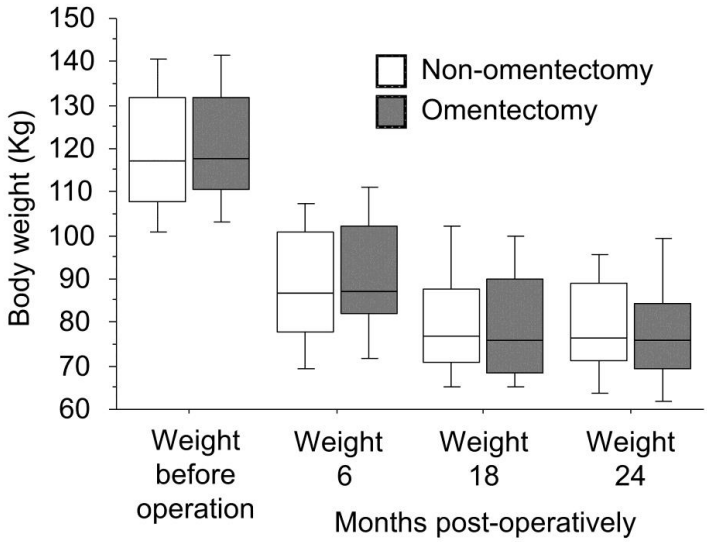


Figure 11.

Weight development following gastric bypass operation.

5 DISCUSSION

In the following chapter, a general discussion about the most important findings in the studies, along with some methodological considerations, will be presented.

It has been known for several decades that enlarged subcutaneous fat cells are associated with hyperinsulinemia,[32] and as expected, study I confirmed that large subcutaneous fat cell size correlated with both fasting hyperinsulinemia and decreased glucose disposal rate (more strongly than visceral fat cell size). On the other hand, visceral fat cell size, as opposed to subcutaneous fat cell size, correlated with dyslipidemia. This suggests that fat cell size has depot specific effects on the cardiovascular risk factor profile. Furthermore, study I showed that combined hyperplasia in both the subcutaneous and visceral adipose depot, compared to combined hypertrophy, was related to a healthier metabolic profile with higher insulin sensitivity and lower plasma lipid levels. Thus, fat cell cellularity has an impact on the metabolic risk factor profile. This has later been confirmed in a study on monozygotic twins with dis- concordant body weight where subjects with hyperplastic obesity (hyperplasia was defined as more fat cells in the obese than lean twin) had a more benign metabolic profile compared to subjects with hypertrophic obesity (hypertrophy defined as less fat cells than lean twin).[66]

Study II found that reduction of subcutaneous fat cell size actually correlates stronger than reduction of subcutaneous fat mass with improved in insulin sensitivity following weight reduction. The results from study I and study II suggest that subcutaneous fat cell size, independently of fat mass, is an important intrinsic property of subcutaneous adipose tissue that correlates with insulin resistance. The underlying mechanisms behind the impact of fat cell size on the metabolic profile are still unknown but some potential explanations will be discussed below.

Increasing obesity (which also leads to increased fat cell size), shifts the adipokine secretion pattern towards a pro-inflammatory profile.[30] Macrophages in adipose tissue change their expression of surface markers towards a more pro-inflammatory pattern (from M2 to M1-expression of macrophages) which results in insulin resistance, as reviewed.[30] On the other hand, in study I, we did not see any correlation between fat cell size and mRNA levels of inflammatory markers such as TNF-α, MCP-1 and IL-6, suggesting a less important role of fat cell size for inflammation in obesity. However, in lean subjects our group has observed a linear relationship between TNF-α levels, and cellularity levels, with subjects with hyperplastic adipose tissue having lower TNF-α levels than subjects with hypertrophic adipose tissue.[36]

In addition to this, enlargement of adipocytes can, at least in mice, lead to impaired angiogenesis and hypoxia.[67] Hypoxia has in turn been found to alter adipokine secretion and reduce insulin sensitivity, as reviewed.[68] If we further speculate, adipocyte turnover and remodeling of adipose tissue might play a role how fat cell size changes have an impact on insulin sensitivity. Adipocyte turnover during the study period of two years is approximately

20%.[34] In study II, we saw that the curve of mean fat cell volume and subcutaneous adipose tissue differed significantly before and after weight reduction (p= 0.03). The new adipocytes, developed during a period of caloric restriction and weight reduction, may have different size and properties than the adipocytes they replace, resulting in improvement of the metabolic profile.

Study III investigated adipocyte lipolysis rate in different depots and found that visceral fat cell lipolysis rate, in contrast to subcutaneous fat cell lipolysis rate, correlated with cardiovascular risk factors. In subjects with the metabolic syndrome visceral, but not subcutaneous fat cell lipolysis, was elevated by 40% compared to subjects not fulfilling the criteria for the metabolic syndrome. These results highlight visceral fat cell lipolysis as a possible partial explanation why visceral fat accumulation is associated with cardiovascular risk factors. The potential effect of enhanced FFA release from visceral adipose tissue to the liver is further mentioned below in the discussion about differences between HOMA-IR and hyperinsulinemic euglycemic clamp.

Study IV aimed to investigate the potential additional positive effects of removing the greater omentum in conjunction with RYGB compared to RYGB alone. When study IV was planned in 2003, only one pilot study had previously been published that investigated the potential positive effects of omentectomy (in conjunction with gastric banding, a kind of bariatric surgery that is no longer widely used).[69] The previous study showed a potential enhanced improvement in oral glucose tolerance and increased weight reduction in the subjects who had undergone omentectomy. Therefore a larger randomized controlled trial was planned to determine if omentectomy in conjunction with gastric bypass surgery really had additional positive effects. Due to various reasons, the study did not start until 2006 and ended in 2011. During that time, other groups had already performed and published several clinical trials studying omentectomy in conjunction with RYGB.[70-75] Therefore, the novelty of the present study was not very high when it was published. Nevertheless, our study was larger than the earlier ones, had a fairly long follow up time, and used the gold standard method to determine insulin sensitivity which gave it some impact. The short-term studies with a follow-up of only 1-3 months showed some inconsistencies regarding the potential effect of omentectomy. Hepatic but not peripheral insulin sensitivity was improved by omentectomy one month post-operatively in one study.[71] Another short-term study concluded that omentectomy added to favorable changes in glucose homeostasis although the improvement was only significant within the omentectomy group and not significantly different from the control group.[72] On the other hand, a third short-term study with a follow up of 1-3 months post-operatively did not see any effect of omentectomy.[73] Furthermore, four studies with relatively long follow-up periods of 12-24 months did not find any enhanced improvement of insulin sensitivity following gastric bypass in conjunction with omentectomy.[70, 74-76]

The results in study IV concur with results from the other published studies, with a follow up time of at least one year,[70-75] that removal of the greater omentum in conjunction with RYGB does not have any additional beneficiary effects on insulin sensitivity or weight reduction.

The weight of the removed omentum is only about 2% of the total weight reduction achieved by RYGB. Thus, it is possible that the positive effects of omentectomy are clouded by the massive overall weight reduction. However, Fabbrini and colleagues studied 7 obese subjects with type 2 diabetes who underwent isolated omentectomy and they did not see any improvement in insulin sensitivity after 3 months.[70]

Interestingly, Klein and colleagues investigated surgical removal of abdominal subcutaneous adipose tissue by liposuction and did not see any effect on fat cell size or improvement of insulin sensitivity or the lipid profile either at 3 months[77] or 4 years post-surgery.[78] These results suggest that positive metabolic effects of weight reduction are likely closely associated with a different caloric input/output and/or a change in fat cell size rather than the weight loss *per se*. Most subjects that have undergone substantial weight reduction will eventually regain some weight and effects on the metabolic profile as well as fat cell size would of course be very interesting to study.

Adipose tissue may also have positive effects by acting as a "buffer" for the influx of dietary fat and prevent ectopic lipid storage in liver and muscle which have been shown to lead to impaired insulin signaling, as reviewed.[79] Obese and lean subjects have approximately the same plasma levels of FFA, while TG, insulin and glucose levels are higher in obese subjects.[80] Subcutaneous adipose tissue in obese compared to lean is characterized by a decreased uptake,[80] and lipolysis followed by oxidation is also decreased.[81] It has been suggested that a high storage but low triglyceride removal promotes fat tissue accumulation whereas reduced triglyceride storage capacity and TG removal promotes dyslipidemia.[81] Surgical removal of visceral or subcutaneous adipose tissue may result in even more decreased capacity in the obese subjects to store and mobilize FFA which could lead to increased levels of FFA and ectopic fat storage. In addition, subjects that undergo surgical removal of adipose tissue do not necessarily change their food intake and may continue to have the same habits that once led to their obesity. This may also contribute to the persistent metabolic profile despite a decrease in body fat mass.

5.1 STRENGTHS AND LIMITATIONS

5.1.1 Study design

The different study designs in this thesis (study I and III are cross sectional observational studies, study II a longitudinal cohort study and study IV a randomized double blind controlled trial) have an impact on what conclusions can be made from each study. Cross-sectional observational studies have a relatively lower value of evidence and cannot determine causality, whereas longitudinal studies and randomized interventional studies in particular provide a high level of evidence.

Both study I and study III show a correlation between metabolic risk factors and fat cell size and lipolysis respectively. Longitudinal studies involving weight changes and visceral adipose tissue changes would be of interest. Unfortunately, as mentioned before, they are difficult to perform due to the limited accessibility of visceral adipose tissue. Study II, a

longitudinal study, further strengthens the results seen in study I, namely that subcutaneous fat cell size could have an impact on insulin sensitivity.

The design of study IV offers several strengths. Study IV was designed as a randomized double blinded controlled trial, *i.e.* all subjects and staff who participated in data collection were blinded for group allocation. The study population size was determined with a power calculation based on the primary outcome measure. Both the primary and secondary outcome measures were predetermined.

5.1.2 Selection bias and non-completers

All patients in the presented studies had visited a physician for referral to a surgical unit where they had been accepted for gastric bypass operation. The fact that they had sought medical care for their overweight may indicate that they were more concerned about their health than obese subjects in general. Subjects who were treated with insulin, warfarin or had a severe ongoing psychiatric disorder were excluded in these studies which could have led to a more healthy study population than a random sample of obese women in general. This might partially explain the low cholesterol levels before operation (4.9±0.9 mmol/l), when compared to women in a population based study in Sweden.[82] All factors mentioned above might have resulted in a selection bias.

Comparisons in study IV were made with per protocol analyses instead of intention to treat analysis since the gastric bypass operation leads to large differences in all parameters between the examinations before and after surgery. The subjects that for some reason did not complete the study had a higher baseline BMI (46.1 kg/m^2 compared to 42.9 kg/m^2 p= 0.013) compared to subjects that completed the study protocol which could have affected the results. However, there was no statistical difference between the number of non-completers in the study groups.

5.1.3 Gender

More obese women than men are trying to lose weight,[83] and approximately 75% of the patients that undergo gastric bypass operation are women.[84] We therefore chose to include only women since it would have been difficult to include enough male subjects to get power in study IV. This affects the generalizability of the results.

We have not taken into account whether the subjects were pre- or postmenopausal, even though it has been shown that post-menopausal women have an increased risk of developing the metabolic syndrome independently of age.[85] In fact, obese men have a more disturbed metabolic profile than obese pre-menopausal women but this risk reduction in women is abolished after menopause, as discussed.[86] In addition, we did not take into account which part of the menstrual cycle the premenopausal women were. However, it has been shown that the menstrual cycle has no effect on insulin sensitivity.[87]

5.1.4 Exercise

Weight reduction induced by exercise and changes in diet leads to a decrease in subcutaneous fat cell size, where the decrease preferentially is seen in the abdominal depot over the gluteal depot.[88] Exercise seems to augment the effects of catecholamines and insulin on adipose tissue, as reviewed.[89] We cannot exclude that the subjects´ degree of physical activity might have had an effect on our results as we did not adjust for that.

5.1.5 Nicotine use

Smoking induces release of noradrenaline and adrenaline and lead to increased levels of glycerol.[90] In humans, using microdialysis technique in adipose tissue, systemically administered nicotine has been shown to induce lipolysis, probably by inducing release of catecholamines that stimulate β-adrenoreceptors, and also activating lipolysis through a nicotinic receptor.[91] This indicates a clear effect of nicotine on lipolysis. However, in another study using microdialysis for *in situ* measurements of glycerol release from adipose tissue following smoking, no increased glycerol levels could be detected.[92] We did not take smoking habits into account when we analyzed our results in study III and cannot exclude that this might have had an impact on our results.

5.1.6 Methodological considerations

5.1.6.1 Hyperinsulinemic euglycemic clamp

Even though the hyperinsulinemic euglycemic clamp is considered to be the gold standard for measurement of whole body insulin sensitivity there are some methodical considerations to be made to optimize the accuracy of the procedure. The cohort that we studied constituted of both severely obese but also lean subjects after weight reduction. To measure whole body glucose disposal during the clamp, hepatic glucose production needs to be suppressed, otherwise less glucose is needed to maintain euglycemia and the glucose disposal rate will become falsely low. Traditionally an insulin infusion rate of 40 mU / m^2 per minute or 1mU/kg per minute is used during a hyperinsulinemic euglycemic clamp. However, based on previous experiences with our own obese subjects and a study by Prager *et al*,[93] we concluded that this concentration would be inadequate in our severely obese population to suppress hepatic glucose output before weight reduction. Instead, we decided to use an insulin concentration of 120 mU/m^2 which had been proved to be sufficient for our population.[93] We used the body surface area instead of body weight for insulin infusion rate since the infusion rate of insulin per kilo becomes very high in obese subjects. However, the achieved insulin levels in our studies are sufficient to inhibit glucose release from the liver.[93]

We had significantly higher serum insulin levels during our clamp in the obese subjects compared to subjects after weight reduction and this would have been even more pronounced if we had adjusted the insulin infusion rate for body weight instead of body surface area. The higher insulin levels before bariatric surgery might be due to decreased clearance/degradation of insulin in obesity, as reviewed.[94]

One way of solving the problem of hepatic glucose production when measuring whole body insulin sensitivity with hyperinsulinemic euglycemic clamp without using our high insulin concentrations could have been to use radiolabeled glucose in the infusion. Unfortunately we did not have that opportunity at our lab.

5.1.6.2 Differences between hyperinsulinemic euglycemic clamp and HOMA-IR

In study II, changes in fat cell size correlated with changes in whole body glucose disposal rate. However, changes in fat cell size (or changes in fat mass in different depots) did not correlate with changes in fasting insulin, fasting glucose or HOMA-IR (p= 0.15-0.52). The reason behind the differences in correlation between subcutaneous fat cell size, whole body insulin resistance and hepatic insulin sensitivity is unclear, but FFA might be involved. Free fatty acids have been shown to inhibit glucose disposal during hyper- and euglycemic hyperinsulinemia.[26, 95, 96] Free fatty acids also induce insulin resistance in muscle by reduction of glucose transport and phosphorylation of glucose.[25] Nielsen and colleagues showed that upper body subcutaneous adipose tissue is the main contributor of systemic FFA (~70%).[97] Visceral adipose tissue contribution to hepatic FFA delivery has been shown to be about 5-10% in lean, 20-30% in obese and in some individuals up to 50%.[97] However, our study group had a higher BMI than the obese in the study of Nielsen and colleagues,[97] hence our cohort may have an even larger contribution of hepatic FFA from visceral adipose tissue. Differences in origin of free fatty acids delivered to the liver and muscle may offer a partial explanation to the discordant correlations of changes in subcutaneous fat cell size, hepatic- and whole body insulin sensitivity.

5.1.6.3 Isolation of fat cells

The abdominal subcutaneous fat biopsy is obtained by needle aspiration whereas the visceral fat biopsy is obtained by surgical excision. These methods do not result in any differences when examining adipose tissue characteristics according to a recent study.[98]

Some investigations have found two distinct pools of fat cell sizes in the same subject whereof one pool consists of very small fat cells.[99, 100] Surprisingly, a pool of very small fat cells have been found in subjects with adipose inflammation and insulin resistance[99, 101] These very small fat cells, with a diameter of 20-50 µm might be missed with the method we used to isolate fat cells.[102] We can therefore not exclude that this pool of very small fat cells, if it exists, could have had an impact on our results. However, the very small "fat cells" have only been found by one group of investigators and their existence has been questioned. The very small fat cells could very well be lipid droplets produced as artefacts during preparation of fat cells, or a release from large fat cells undergoing cell death.

5.1.6.4 Expression of lipolysis rate

In study III, we choose to express lipolysis rate as µmol glycerol released/10^7 cells/ 2 hours. However, there is no consensus how to express glycerol release from fat cells as a measure of

lipolysis. Both glycerol release /weight unit, glycerol/number of cells, or as a ratio of hormone stimulated/baseline lipolysis have been used previously.

5.1.6.5 Measurement of body fat mass by DEXA

DEXA has been used to evaluate body fat mass for some decades. It is relatively accurate compared with the absolute method (i.e. under water weighing), widely accessible and only exposes the subject to a minimal amount of radiation. As mentioned before, the accuracy of DEXA (with the Corescan feature) to estimate visceral adipose tissue in the android region is very high (r^2=0.96).[60] EVAT has also been shown to correlate with the size of the removed omentum in this cohort (r= 0.48 p= 0.0038).[103] From the formula used by the DEXA software we calculated ESAT as total android fat mass minus EVAT. When we used this calculation we presumed that the values of EVAT and android fat mass were valid.

5.1.6.6 Effects of omentectomy in study II

Study II included subjects from study IV and these subjects had undergone RYGB with or without removal of the greater omentum. All analyses done in study II were also performed separately on the omentectomy group and no significant clinical differences in results were seen between the omentectomy group and the whole group.

6 CONCLUSION

This thesis aimed to study two intrinsic adipose tissue characteristics, fat cell size and lipolysis, and their depot specific correlations with insulin resistance and dyslipidemia. In addition, the aim was to evaluate the potential positive metabolic effects of removing a large portion of visceral adipose tissue.

Study I showed that in obese women, fat cell size correlated with the metabolic phenotype. The influence seems to be region specific. Large visceral fat cell size is more strongly correlated with dyslipidemia whereas large subcutaneous fat cell size correlates with insulin resistance. Obese women with hyperplasia in both regions have a more beneficial metabolic profile than those with combined hypertrophy. However, no causal relationships can be proved in this cross sectional study.

Study II showed that a decrease in subcutaneous fat cell size rather than a decrease in fat mass *per se* is associated with improved insulin sensitivity after marked weight reduction. This prospective study strengthens the hypothesis that changes in intrinsic adipose tissue characteristics are more important for metabolic improvement following weight loss than decrease in fat mass *per se*.

Study III evaluated the relative roles of subcutaneous and visceral fat cell lipolysis for cardiovascular risk factors in obesity. Visceral but not subcutaneous adipocyte triglyceride mobilization correlated with dyslipidemia, insulin resistance and estimated liver fat as well as the metabolic syndrome.

Study IV investigated the potential additional positive metabolic effects of performing gastric bypass operation in conjunction with omentectomy compared to traditional gastric bypass operation. No such additional positive metabolic effects were seen on insulin resistance, blood lipids or weight reduction.

In summary, the studies in this thesis highlight potential depot specific correlations between fat cell size, lipolysis and cardiovascular risk factor profile. Furthermore, changes in subcutaneous fat cell size, independently of a decrease in subcutaneous fat mass, correlate with improvement of insulin resistance following weight loss. A mere removal of a substantial part of the visceral fat depot does not enhance improved metabolic outcome after gastric bypass operation. The results in this thesis show that adipose characteristics are important for metabolic complications in obesity. Furthermore, it strengthens the hypothesis that intrinsic changes in adipose tissue are important for positive effects associated with weight reduction.

7 FUTURE PERSPECTIVES

The results in this thesis generated some new interesting research questions that we will try to answer in the future.

Can fat cell size be used as a prognostic factor for improvement of metabolic factors following bariatric surgery?

The indication for bariatric surgery today is based on BMI both for obese subjects[104] and subjects with type 2 diabetes.[105, 106] However, baseline BMI does not predict the effect of bariatric surgery in terms of remission of diabetes, mortality, cardiovascular events or cancer according to the Swedish obesity study, as reviewed.[24] Another study has shown that preoperative BMI does not predict improvement of hyperglycemia.[13] Therefore it has been suggested that the indications should be modified and more importance should be given to metabolic variables and less to BMI.[24] We currently investigate in a longitudinal study if subcutaneous fat cell can be used as a predictor of improved insulin sensitivity following weight reduction. The caveat with fat cell size as a predictor is that it demands a fat biopsy which is not convenient to use in a daily clinical setting. However, our group recently identified a transcription factor, early B cell factor (EBF-1) that is produced in adipocytes with reduced expression in adipose hypertrophy.[107] EBF-1 is also negatively associated with lipolysis and down regulated after stimulation with TNFα *in vitro*.[107] Although it is much too early to label EBF-1 as a prognostic factor it illustrates that there are signal substances produced in adipose tissue that in the future may be used in clinical practice as risk factor markers.

What happens with fat cell size, insulin sensitivity and lipid status following weight regain?

Subjects that decrease in weight actually have smaller fat cells than subjects with the same BMI that have been weight stable over time.[108] However, as mentioned before, it is very difficult to maintain a weight reduced state over time,[109] and subjects that lose weight tend to regain at least some weight.[24, 110] It would be very interesting to see what happens with fat cell size and the metabolic profile following weight regain.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Fetma är en känd riskfaktor för utveckling av insulinresistens och blodfettsrubbningar som i sin tur kan leda till hjärtinfarkt och stroke. Fettvävens egenskaper har också visat sig ha betydelse då dessa komplikationer uppkommer.

Syftet med denna avhandling var trefaldigt. Dels att studera sambandet mellan fettcellernas storlek och insulinresistens och blodfettsrubbningar (studie I och studie II). Dessutom att studera fettcellernas förmåga att bryta ner fett (lipolys) och dess eventuella koppling till insulinresistens och blodfettsrubbningar (studie III). Vi undersökte vidare om det fanns skillnader i sambandet mellan dessa funktioner beroende på om fettet satt under huden (subkutant fett) eller kring tarmarna (visceralt fett) (studie I och studie III). Slutligen studerade vi om borttagande av en stor del av fettet kring tarmarna (omentektomi) i samband med gastric bypassoperation gav positiva metabola effekter jämfört med endast gastric bypassoperation (studie IV).

Alla försökspersoner i avhandlingen ingick i en grupp av 81 kvinnor som genomgick gastric bypassoperation för att gå ner i vikt. Vid gastric bypassoperation kopplas matstrupen direkt till tunntarmen så att maten aldrig passerar magsäcken. I studie I och studie III användes resultat från en undersökning som gjordes före operationen, medan studie II och studie IV även inkluderade en undersökning 2 år efter gastric bypassoperationen.

Fettmängden i kroppen och på olika lokalisationer mättes med hjälp av bentäthetsmätare (DEXA, Dual-energy x-ray absorptiometry, som mäter mängden fett som ett bifynd). För att mäta insulinkänsligheten gavs försökspersonerna en förutbestämd mängd insulin. Sedan mättes hur mycket sockerlösning patienten behövde få för att upprätthålla en normal blodsockernivå (hyperinsulinemisk euglykemisk clamp). Mängden tillfört socker till blodet användes som ett mått på insulinkänslighet. Blodfetter mättes. Fettvävsprov togs både från fettet under huden samt det inre bukfettet. Storleken på fettcellerna bestämdes och även fettcellernas förmåga att bryta ner lagrat fett (lipolys) mättes.

Studie I visade att större fettceller i underhudsfettet korrelerar med en ökad insulinresistens, vilket kan leda till utveckling av typ 2 diabetes. Större fettceller i fettet kring tarmarna korrelerar med både ökad insulinresistens och högre blodfetter. Personer med små fettceller i båda regionerna har mindre risk för typ 2 diabetes och blodfettsrubbningar än personer med stora fettceller oberoende av fettmängd.

Studie II visade att förändringar i fettcellsstorlek i samband med viktnedgång korrelerar med förbättrad insulinkänslighet. Förändringen i subkutan fettcellstorlek var viktigare för förbättrad insulinkänslighet än minskningen av mängden fett.

Studie III visade att förmågan att bryta ner fettsyror i fettet runt tarmarna korrelerade med ökad insulinresistens och högre blodfetter (kolesterol, triglycerider, LDL kolesterol). Något sådant samband fanns inte i underhudsfettet.

Studie IV var konstruerad som en randomiserad dubbel blind studie, där patienterna lottades till gastric bypassoperation med eller utan borttagande av omentfettet. Det vill säga, varken patienterna eller de som genomförde undersökningarna var medvetna om vilken grupp patienterna tillhörde. Studien visade att borttagande av en stor del av det inre bukfettet inte ger några förstärkta positiva effekter avseende förbättrad insulinresistens eller förbättrade blodfetter.

Sammanfattningsvis visar studierna i avhandlingen att egenskaper i fettväven, såsom cellstorlek och lipolys, har olika påverkan på insulinresistens och blodfetter beroende på var fettet är lokaliserat (under huden eller vid tarmarna). Dessutom verkar förändringar i fettcellernas storlek i underhudsfettet, oberoende av förändringar i fettmängd, vara viktiga för förbättrad insulinkänslighet efter viktnedgång. Att kirurgiskt ta bort en stor del av fettet kring tarmarna i samband med överviktskirurgi verkar inte ha några positiva effekter jämfört med endast överviktskirurgi. Resultaten i avhandlingen talar för att fettvävens egenskaper, såsom fettcellsstorlek och lipolysförmåga, oberoende av fettmängd, är viktiga för utveckling av metabola komplikationer. Dessutom verkar en minskning av fettcellernas storlek vara en viktig förutsättning för positiva effekter på insulinkänslighet och blodfetter vid viktnedgång.

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