Human adipose tissue morphology and function

Relation to insulin sensitivity and glucose tolerance with focus on pregnancy and women with previous gestational diabetes mellitus

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Stay hungry. Stay foolish.

-Steve Jobs

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ABSTRACT

Obesity is a global health problem and affects women of reproductive age. During pregnancy, obesity increases the risk for gestational diabetes mellitus (GDM), in turn predisposing for type 2 diabetes (T2D). Not only the amount and distribution of adipose tissue (AT) but also the AT morphology and function are of importance in pathogenesis of metabolic disease related to obesity. The aims of this thesis were 1) to compare subcutaneous (SC) and visceral AT regarding release of adipokines, implicated in insulin resistance/inflammation, using an in vitro system reflecting the release in vivo, and 2) to characterize AT morphology and function in normal weight (NW) and obese pregnant women in trimester 1 and 3, and in women with previous GDM, and identify AT-related factors associated with insulin resistance and impaired glucose metabolism.

AT biopsies were obtained from 1) patients undergoing surgery at Sahlgrenska University Hospital, and 2) women in the Pregnancy Obesity Nutrition and Child Health (PONCH) study. AT adipokine release and immune cell/blood vessel density, and adipocyte size/number and lipolytic activity were analyzed. Women were examined regarding insulin resistance (HOMA-IR), glucose tolerance, body composition and blood chemistry.

Chemerin, cytokines, and dipeptidyl peptidase 4 were more abundantly released from visceral than SC AT; adiponectin release was higher from the SC depot. During pregnancy, NW women accumulated fat in existing adipocytes (which became larger) and adiponectin levels were reduced. Obese women had signs of adipocyte recruitment and maintained adiponectin levels. Fat mass and the proportion of very large adipocytes were associated with HOMA-IR in trimester 3. In women with previous GDM, follow-up body mass index (BMI) was the best discriminator of normal vs impaired glucose metabolism, and waist-to-height ratio and adipocyte volume were associated with HOMA-IR.

To conclude, adipokines implicated in metabolic dysfunction are released from AT in a depot-dependent manner. AT mass/morphology contribute to gestational insulin resistance. During pregnancy, AT morphology appears to change oppositely in NW and obese women, possibly protecting obese women against even more severe insulin resistance. To prevent T2D, BMI and abdominal fat accumulation should be controlled in women after GDM.

Keywords: Adipose tissue, adipocyte, adipokines, insulin resistance, pregnancy, gestational diabetes mellitus, type 2 diabetes mellitus

SAMMANFATTNING PÅ SVENSKA

Bilden av fettväven som ett passivt organ för lagring av energi har ändrats drastiskt. Idag anses fettväven vara ett dynamiskt och viktigt organ, involverat i många biologiska processer i kroppen. Fettväven producerar och frisätter bl. a. ett stort antal bioaktiva ämnen, adipokiner, som påverkar aptitreglering, inflammation och insulinkänslighet. Förekomst av övervikt och fetma har ökat världen över och i fetmaepidemins spår följer metabola sjukdomar som diabetes typ 2 och hjärt-kärlsjukdom. Även kvinnor i reproduktiv ålder har blivit fetare. Fetma under graviditet kan leda till komplikationer som graviditetsdiabetes vilket i sin tur innebär en ökad risk för kvinnan att senare insjukna i diabetes typ 2. Fettvävens roll i utveckling av följdsjukdomar till fetma är inte helt klarlagd men det står klart att inte bara mängden fett, och dess placering på kroppen, utan även dess morfologi och funktion är av betydelse.

Syftet med denna avhandling var 1) att jämföra subkutan (under huden) och visceral (inne i bukhålan) fettväv med avseende på frisättning av några adipokiner, relaterade till fetma och metabola störningar, med hjälp av ett in vitro system som återspeglar frisättningen in vivo, och 2) att studera fettvävens morfologi och funktion hos normalviktiga och feta kvinnor longitudinellt under graviditet, och hos kvinnor som tidigare utvecklat graviditetsdiabetes, samt att identifiera fettvävsrelaterade faktorer med betydelse för utveckling av insulinresistens och försämrad glukosmetabolism.

Fettvävsbiopsier samlades från 1) patienter som opererades vid Sahlgrenska Universitetsjukhuset, och 2) kvinnor i den så kallade PONCH (Pregnancy Obesity Nutrition and Child Health)-studien. Fettvävens frisättning av adipokiner och innehåll av immunceller och blodkärl, samt fettcellernas storlek/antal och lipolytiska aktivitet analyserades. Kvinnorna undersöktes avseende insulinresistens (HOMA-IR), glukostolerans, kroppssammansättning och blodkemi.

Adipokiner såsom chemerin, cytokiner och dipeptidylpeptidas 4 frisattes i större omfattning från den viscerala fettväven medan frisättning av adiponectin var högst i subkutan fettväv. Under graviditet fylldes fett på i redan befintliga fettceller hos normalviktiga kvinnor (fettcellerna blev i snitt större) och deras adiponectin-nivåer sjönk. I jämförelse med den normalviktiga gruppen visade feta kvinnor tecken till nyrekrytering av fettceller (fettcellerna blev i snitt mindre) och deras adiponectin-nivåer bibehölls. Fettmassa och andelen mycket stora fettceller var associerade till HOMA-IR sent i graviditeten. Hos kvinnor med tidigare GDM var kroppsmasseindex (BMI) vid uppföljningen den faktor som var starkast associerad till försämrad glukosmetabolism, medan kvinnornas midja-längd-kvot och fettcellsvolym var starkast associerade till HOMA-IR.

Sammantaget visar dessa studier att flera adipokiner som är relaterade till fetma och metabola störningar har en depå-beroende frisättning från människans fettväv. Den viscerala fettvävens frisättningsmönster är mer ogynnsamt ur metabol synvinkel. Fettvävens massa och morfologi bidrar till den fysiologiskt förhöjda insulinresistens som uppstår under graviditet. Under graviditet tycks fettvävens morfologi förändras på motsatt sätt hos normalviktiga och feta kvinnor – andelen små fettceller ökar hos feta kvinnor medan andelen stora fettceller ökar hos normalviktiga kvinnor. Rekrytering av fettceller under graviditet kan, hypotetiskt, skydda feta kvinnor från än mer uttalad insulinresistens. För att förebygga diabetes typ 2 bör kvinnor efter GDM få information om sin förhöjda diabetesrisk och, vid behov, stöd för viktnedgång.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Svensson H, Odén B, Edén S, and Lönn M
Adiponectin, chemerin, cytokines and dipeptidyl peptidase 4 are released from human adipose tissue in a depot-dependent manner: an in vitro system including human serum albumin
BMC Endocrine Disorders. 2014; 14:7

II. Svensson H, Wetterling L, Odén B, Odén A, Jennische E, Edén S, Holmäng A, and Lönn M
Body fat mass and the proportion of very large adipocytes in pregnant women are associated with gestational insulin resistance
Submitted manuscript

III. Svensson H, Wetterling L, Odén A, Jennische E, Edén S, Holmäng A and, Lönn M
BMI, waist-to-height ratio and adipocyte volume are associated with impaired glucose metabolism and insulin resistance in women with previous gestational diabetes: A 6-year follow-up study
Submitted manuscript

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ABBREVIATIONS

AFABP	Adipocyte fatty acid-binding protein
BMI	Body mass index
BSA	Bovine serum albumin
CRP	C-reactive protein
DPP4	Dipeptidyl peptidase 4
ELISA	Enzyme-linked immunosorbent assay
GDM	Gestational diabetes mellitus
GLUT	Glucose transporter
HbA1c	Glycated hemoglobin
HOMA-IR	Homeostasis model assessment of insulin resistance
HSA	Human serum albumin
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IGM	Impaired glucose metabolism
IL	Interleukin
mRNA	Messenger ribonucleic acid
OGTT	Oral glucose tolerance test
SD	Standard deviation
SEM	Standard error of the mean
T2D	Type 2 diabetes

TNF	Tumor necrosis factor
WHO	World Health Organization
WHR	Waist-to-hip ratio

1 BACKGROUND

Adipose tissue is an organ for storage of energy and has the ability to expand in response to overnutrition and release lipids in response to energy deficit. However, adipose tissue is far more than a caloric reservoir, it is also a key component in maintaining metabolic homeostasis and is involved in a wide array of biological processes throughout the body. It has been proposed that the lifestyle of the modern human is detrimental for our health. Easy access to energy-rich food, combined with a sedentary lifestyle has led to obesity becoming the most common nutritional disorder worldwide. In its trail comes increasing numbers of people suffering from co-morbidities associated with obesity, such as diabetes, cardiovascular disease and certain cancers. The World Health Organization (WHO) calls this global increase in prevalence of overweight and obesity "globesity".

1.1 Obesity

In recent years the prevalence of obesity has been said to reach epidemic proportions and the WHO has made it one of their global targets to halt the rise in diabetes and obesity. Overweight and obesity is defined by body mass index (BMI), calculated by dividing body weight (in kilograms) by the square of body height (in meters). Having a BMI equal to or more than 25 kg/m^2 is defined as overweight and BMI of 30 kg/m^2 or more is defined as obesity. Obesity has more than doubled since 1980 and the most recent data from WHO (2014) shows that rates of obesity and overweight have continued to increase in all countries around the world. In 2014, 39% of adults were overweight and 13% were obese worldwide. In Sweden both overweight and obesity have increased between 2010 and 2014 according to data from WHO, Fig 1(1).

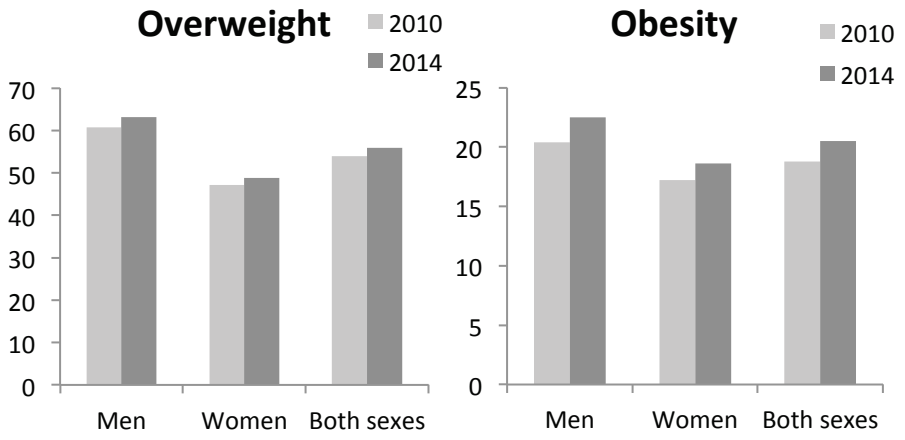

Figure 1. Prevalence of overweight and obesity in men and women in Sweden 2010 and 2014. Data from WHO global status report on non-communicable diseases 2014.

Overweight and obesity are associated with a profound health burden and have been identified as major risk factors for developing co-morbidities including hypertension, type 2 diabetes mellitus (T2D), cardiovascular disease and certain cancers (2, 3). Also, in parallel to increasing numbers of obesity, there is an increase in the prevalence of T2D, although somewhat delayed, and the majority of people with T2D are overweight or obese (4).

1.2 Obesity in pregnancy

As obesity has increased in the adult population at large, it has also increased in women of reproductive age. More women than ever before are entering pregnancy overweight or obese and maternal obesity is becoming an increasing public health issue (5). Obesity during pregnancy is associated with adverse outcomes for the mother as well for the child during pregnancy, at delivery and after delivery. Also, obesity negatively affects fertility (6).

During pregnancy, maternal complications related to obesity include increased risk for developing hypertension and pre-eclampsia (7, 8), thromboembolism (9) and gestational diabetes mellitus (GDM) (7, 9, 10). Several reviews have shown an association between obesity and the risk of developing GDM and obese women are at least four times more likely to develop GDM compared to normal weight pregnant women. The risk is even higher with severe obesity (11, 12).

Maternal obesity in pregnancy is also associated with increased risk for complications during parturition. The rate of instrumental deliveries and caesarian section is higher in obese mothers (7, 8). This increased risk is likely multifactorial and may be due to sub-optimal uterine contractions resulting in prolonged and ineffective labor or fat deposition in the pelvic area resulting in a narrow birth canal. In addition to this, maternal obesity increases the likelihood of giving birth to a child, large for gestational age, that may further aggravate the situation (13).

It has been suggested that increased glucose concentrations in maternal obesity or GDM lead to increased fetal growth (14, 15) and macrosomic babies (7, 9, 16) with increased risk for neonatal hypoglycemia and need for neonatal intensive care after birth (16, 17).

1.3 Insulin resistance and diabetes mellitus

In the recent decades there has been an increase in the prevalence of T2D globally and in low and middle-income countries the incidence rate of T2D is particularly high (1). Overweight, obesity and lack of physical activity are factors that have been attributed to drive the increase in T2D (18).

Diabetes is a chronic disease, resulting from insufficient insulin production by the pancreas, ineffective response to insulin stimulation in target tissues or both. Type 1 diabetes mellitus results from insufficient insulin production and requires lifelong substitution with insulin whereas T2D, which accounts for 90% of all cases of diabetes, is largely due to physical inactivity and excess body weight, although there are genetic components (19). T2D is characterized by ineffective use of insulin in the liver, skeletal muscles and adipose tissue. The insulin-producing β-cells of the pancreas compensate for the increased resistance to the actions of insulin by producing more insulin, leading to increased circulating insulin levels as well as progressively increasing glucose levels (20). Glucose levels in T2D are maintained by lifestyle changes, drugs that improve insulin sensitivity, lower glucose production by the liver, or stimulate the pancreas to produce more insulin. Eventually, the β-cells fail to maintain the high circulating insulin levels required and insulin must be substituted as in type 1 diabetes. Poor glycemic control accelerates development of complications associated with diabetes including cardiovascular disease, retinopathy, nephropathy and neuropathy.

1.3.1 Gestational diabetes mellitus

The prevalence of women diagnosed with diabetes during pregnancy has increased globally (21, 22). There are ethnical differences in incidence and there is no consensus regarding criteria used for diagnosis resulting in 1-18% prevalence in GDM worldwide (23). Beside ethnicity and obesity as risk factors for GDM, also GDM in previous pregnancies, advancing maternal age, and low socioeconomic status have been identified as risk factors for developing GDM (24, 25). Although the pathologic glucose resistance in GDM usually resolves following parturition, a pregnancy complicated by GDM predicts a high risk for developing T2D later in life (26, 27). Progression rate from GDM to T2D varies due to the length of the follow-up and diagnostic criteria, but as reviewed by Bellamy et al, there is at least a seven-fold increased risk for progression to T2D in women with previous GDM (27).

1.3.2 Diagnostic criteria

Diagnostic criteria for diabetes is based on circulating glucose levels, random in non-fasted state, in fasted state or during a 75g oral glucose tolerance test (OGTT), see Table 1. With the use of fasting plasma glucose and/or OGTT, the prediabetic states *impaired fasting glucose* (IFG) and *impaired glucose tolerance* (IGT) may be detected. IFG is thought to develop due to reduced hepatic insulin sensitivity combined with a stationary β-cell dysfunction, whereas in IGT insulin sensitivity is decreased in peripheral tissues and there is a progressive loss of β-cell function. Hepatic insulin sensitivity may not be affected in this case. In combined forms of IFG and IGT there are defects in both peripheral and hepatic insulin sensitivity (28).

Table 1. WHO criteria for diagnosis of diabetes, IGT and IFG

Normal	
Fasting plasma glucose	<6.1 mmol/l
and	and
OGTT 2-h plasma glucose	<7.8 mmol/l
Diabetes	
Fasting plasma glucose	≥7.0 mmol/l
or	or
OGTT 2-h plasma glucose	≥11.1 mmol/l
Impaired Glucose Tolerance (IGT)	
Fasting plasma glucose	<7.0 mmol/l
and	and
OGTT 2-h plasma glucose	≥7.8 and <11.1 mmol/l
Impaired Fasting Glucose (IFG)	
Fasting plasma glucose	≥6.1 mmol/l and ≤6.9 mmol/l
and (if measured)	and
2-h plasma glucose	<7.8 mmol/l

1.4 Adipose tissue

White adipose tissue is comprised of two principal components; tightly packed spherical adipocytes (29) and the stromal vascular fraction consisting mainly of preadipocytes, immune cells and fibroblasts. Endothelial cells and pericytes are also present, forming a network of capillaries and lymphatic vessels (30). Together adipocytes and cells of the stromal vascular fraction produce an extracellular matrix to maintain the structural and functional integrity of the tissue (31).

Adipose tissue was for long regarded as an inert and passive organ with the sole purposes to store energy and serve as insulation. Beginning with the discovery that leptin is secreted from adipocytes in 1994 this view has changed dramatically. Nowadays adipose tissue is considered to be an important and active organ for maintenance of systemic homeostasis through an intricate network of auto-, para- and endocrine crosstalk to other tissues and organs (32). Since the discovery of leptin, a large number of bioactive molecules, produced and secreted from adipose tissue have been identified, collectively named adipokines and still, novel adipokines and their roles in health and disease are proposed (33-35).

Although obesity and fat mass expansion is closely linked to adverse metabolic consequences, not all obese individuals develop insulin resistance and subsequently T2D. The concepts of the *metabolically healthy obese* and the opposite, the *metabolically obese, normal weight individual,* suggests that not only the quantity, but rather the quality and localization of the fat are important factors in the pathogenesis of co-morbidities connected to obesity (36, 37).

1.4.1 Adipose tissue in pregnancy

During pregnancy adipose tissue must expand rapidly to meet the needs of the growing fetus and to prepare for lactation. Maternal body fat mass increases from the first trimester and throughout pregnancy (38) and in healthy pregnancy most of the fat is stored subcutaneously on the trunk (39). Obesity during pregnancy further increases the progressive insulin resistance seen in pregnancy (40) and it has been proposed that inability of adipose tissue to expand properly during pregnancy is associated with development of GDM (41). Also, changes in lipolytic activity occurs during pregnancy in order to promote fat accumulation early in pregnancy, and to enhance fat mobilization late in pregnancy (42).

1.4.2 Regional adipose tissue distribution

The distribution of white adipose tissue may largely be divided into two compartments or depots; subcutaneous adipose tissue and visceral adipose tissue. Visceral adipose tissue is mainly located in the abdominal cavity and mediastinum, surrounding the inner organs, while subcutaneous adipose tissue forms a layer under the skin. Visceral adipose tissue can be further divided into depots depending on localization with omental fat superficially surrounding the intestines, mesenteric fat located more deeply around the intestines and retroperitoneal fat surrounding the kidneys. There are also smaller amounts of fat located in the mediastinum, surrounding the heart and larger vessels (29, 43). In pathological states fat may also accumulate in tissues and organs other than adipose tissue, thus forming ectopic fat. This can be seen in the liver, pancreas and skeletal muscle, leading to impaired function of the tissue or organ (44).

Central and peripheral adiposity

Vague postulated in the mid 20th century that obesity is not a homogenous condition and that regional distribution of adipose tissue is of importance in understanding metabolic disturbances linked to obesity (45). Since then several studies have shown that central adiposity, where fat is mainly located abdominally (apple-shape or android adiposity) is associated with higher risk for development of insulin resistance, T2D and cardio-vascular disease as opposed to having a more peripheral distribution of adipose tissue, in the lower body (pear-shaped or gynoid adiposity) which may be protective against T2D (46-51).

To assess visceral adiposity directly, imaging techniques such as computed tomography or magnetic resonance imaging must be used, as anthropometric measurements are unable to distinguish subcutaneous from visceral abdominal fat. However, calculating waist-to-hip ratio (WHR) from waist- and hip circumferences can be used as an index of abdominal fat accumulation. WHR has been shown to be a predictor of T2D (48, 52). Waist-to-height ratio is another surrogate measure of abdominal adiposity that has been suggested to be a better predictor of T2D than WHR or waist circumference alone (53-55).

1.4.3 Adipose tissue depot-specific differences

There are phenotypic differences between various adipose tissue depots. In recent years the interest of the physiology and the role of visceral adipose tissue in obesity-related pathology has increased. Visceral adipose tissue is more metabolically active than subcutaneous adipose tissue and the basal

lipolytic rate is higher in adipocytes isolated from omental and mesenteric adipose tissue compared to subcutaneous adipose tissue (56, 57). Catecholamine-induced lipolytic activity is stronger while the anti-lipolytic effect of insulin is weaker in visceral fat compared to subcutaneous fat (57-59).

Visceral adipose tissue has been proposed to have a specific secretory profile, with higher expression and secretion of pro-inflammatory cytokines and lower secretion of anti-inflammatory adipokines (60, 61). Visceral adipose tissue is drained by the portal venous system and is thus directly connected to the liver. In the portal hypothesis, it has been suggested that due to the high lipid turnover and secretory profile of visceral adipose tissue in combination with the vascular anatomy, having excess visceral fat results in increased concentrations of free fatty acids and pro-inflammatory cytokines in the portal circulation. This in turn, can impair insulin response in the liver (62, 63) and increase hepatic production of lipoproteins and glucose, promoting dyslipidemia, glucose intolerance and hyperinsulinemia (64). Infusion of free fatty acids in humans induced both peripheral and hepatic insulin resistance, supporting this theory (65, 66).

1.5 Adipocytes

The majority of the volume of mature white adipocytes is occupied by a centrally positioned, unilocular lipid droplet. The sparse cytoplasm, cup-shaped nucleus and other organelles are displaced to the periphery of the cell (67). The adipocyte is remarkably flexible in terms of energy storage. Adipocyte size varies depending on the balance between mobilization (lipolysis) of triglycerides to fatty acids released to fuel peripheral tissues upon metabolic demand, and uptake of fatty acids, esterification and storage of triglyceride in response to insulin stimulation, during positive energy balance.

The volume of adipocytes can vary several thousand-fold, in relation to the amount of lipid stored in the cell. The size of adipocytes within the adipose tissue is an important factor of the overall function of the tissue (68). Increased adipocyte size has been associated with insulin resistance, T2D and dyslipidemia (50, 68-70) and large abdominal adipocytes have been identified as being an independent predictor of T2D (55, 71).

1.5.1 Functional differences between small and large adipocytes

Enlarged adipocytes have higher glucose metabolism and increased lipid mobilization than smaller cells and in vitro studies have shown that large adipocytes are less sensitive to the stimulating actions of insulin on glucose metabolism (72-74). Following insulin stimulation of adipocytes, small adipocytes doubled the amount of GLUT4 glucose transporter in the plasma membrane, whereas in large adipocytes the amount of GLUT4 was unchanged (74). Increasing cell size alters adipokine expression and release-patterns of adipocytes with increased release of pro-inflammatory cytokines and leptin in large adipocytes (75, 76) while release of the insulin-sensitizing adipokine adiponectin is negatively associated with adipocyte size (77, 78), together contributing to a pro-inflammatory environment in the tissue as well as systemically. Also, large adipocytes have higher release of immune cell chemoattractant substances (75). In reduction of adipocyte size during weight loss, adipokine release patterns are restored (79-81), macrophage density is reduced (82) and whole-body insulin sensitivity is improved (81).

1.5.2 Adipose tissue expansion

In excess caloric intake, preexisting adipocytes can increase in volume (hypertrophy) or new adipocytes can be generated from preadipocytes (hyperplasia), or a combination of both (83) coupled with remodeling of the extra cellular matrix.

Adipocyte number is said to remain rather constant in adults suggesting primarily hypertrophy to occur in adults, but adipocyte turnover occurs at all ages, both in lean and obese subjects (84). Short term overfeeding in humans has been shown to lead to increased adipocyte size in lean subjects (85, 86) whereas obese subjects showed increased number of adipocytes with weight gain (50). Recruitment of new adipocytes has also been found to occur when women with large adipocytes were put on a high calorie diet but this was not seen in men, regardless of baseline adipocyte size (86). Recruitment of adipocytes has also been reported during pregnancy in normal weight women when gluteal adipose tissue was studied (87). It is possible that body fat mass expansion is associated with an initial increase in adipocyte size but when adipocytes reach a storage threshold, further expansion require an increase in the number of adipocytes, but this remains to be elucidated (88). Expansion and remodeling of adipose tissue is necessary for preventing ectopic deposition of fat in other tissues and organs leading to development of insulin resistance (89, 90).

1.6 Stromal vascular fraction

The stromal vascular fraction of adipose tissue consists mainly of preadipocytes, fibroblasts, endothelial cells, blood and lymphatic vessels and infiltrating immune cells (30). A majority of proinflammatory cytokines released from adipose tissue originates from the non-fat cells, (91). Infiltration and accumulation of immune cells in adipose tissue during tissue-expansion has been suggested to connect obesity to the chronic low-grade inflammation seen in obesity, insulin resistance and T2D.

1.6.1 Macrophages

In 2003 Weisberg et al (92) and Xu et al (93) studied gene expression-patterns in lean and obese mice and found differences in expression of genes related to macrophages. Morphologic studies confirmed an increased number of macrophages in adipose tissue of obese mice. Since then, adipose tissue accumulation of macrophages in obesity has also been shown in humans (92), however results are not as robust in humans as in rodents (82, 94, 95). Macrophage-accumulation in human obesity is more pronounced in visceral than subcutaneous adipose tissue (95, 96). Also, adipose tissue of lean individuals contains tissue resident macrophages, however there are phenotypic differences between the macrophages seen in lean and obese adipose tissue. In lean individuals, macrophages are mainly involved in tissue repair and have been identified as M2, or alternatively activated macrophages. In obese adipose tissue on the other hand, recruited macrophages are involved in inflammatory processes, secrete pro-inflammatory cytokines, and have been identified as M1 or classically activated macrophages (97). Macrophages can impact adipose tissue function by secretion of TNF-α, which in turn acts locally in the tissue inducing production of proinflammatory cytokines in adipocytes, leading to dysregulated basal lipolysis. The fatty acids released from adipocytes can activate macrophages via toll like receptors and thus, this viscous circle of cellular cross-talk aggravates and maintains the chronic low-grade inflammation (98). Weight loss decreases macrophage density and improves glucose sensitivity (82) and the remaining macrophages undergo a phenotypic shift with reduced expression of inflammatory genes and induced expression of the anti-inflammatory cytokine IL-10 (82, 99).

1.6.2 Mast cells

The functional role of mast cells in allergy is well known, however, mast cells may also have a role in development and maintenance of chronic inflammation (100). Increased mast cell density in adipose tissue has been

reported in diet induced obese mice (101, 102) and human subcutaneous adipose tissue of obese individuals (101). Infiltration of mast cells in adipose tissue has been proposed to precede macrophage infiltration, supported by the finding that mice, deficient in mature mast cells gained less weight and showed less macrophage infiltration when put on a high fat diet (103). Also, pharmacological inhibition of mast cell degranulation in mice showed similar results (101). Structurally, human adipose tissue mast cells have been located preferentially in fibrous areas of the tissue and there is a positive correlation between mast cell density and macrophage density in adipose tissue (104).

1.6.3 Vascularization of adipose tissue

A dense network of capillaries provides delivery of oxygen and nutrients to the tissue but also provides a route for secreted adipokines to reach other tissues and organs (105). Subcutaneous adipose tissue is more densely vascularized than visceral adipose tissue (106). During positive energy balance and adipose tissue expansion, angiogenesis is enhanced to provide adequate nutritional supply (107) but in obesity and insulin resistance decreased capillary density has been observed parallel to an increase in the number of larger vessels in adipose tissue (108).

In adipose tissue expansion, the interstitial oxygen tension decreases as adipocytes become enlarged, creating areas of local hypoxia (33). If neovascularization fails or is not rapid enough, increased hypoxia and cell death occurs, leading to an influx of macrophages and other immune cells, which in turn increases the inflammatory state of the tissue (109).

1.7 Adipokines

In the early 1990's Zhang et al identified leptin as a secreted product from adipose tissue and this discovery led to a paradigm-shift in obesity research (110). Around the same time of the discovery of leptin, Hotamisligil et al reported increased mRNA expression of TNF-α in obese adipose tissue, which was identified as a negative regulator of insulin signaling and proposing obesity to be a state of chronic low-grade inflammation (111). These findings provided a foundation for the subsequent discovery of many other adipokines and established adipose tissue as a dynamic and active secretory organ.

Adipokines, secreted from adipose tissue, are involved in a wide spectrum of biological processes, including regulation of energy homeostasis (satiety, appetite, lipid- and carbohydrate metabolism), adipocyte proliferation and

differentiation, inflammation, angiogenesis and regulation of coagulation and vascular function (33, 112, 113). Adipokines can act locally in adipose tissue (auto- and paracrine manners), but can also exert their effect in distant organs and tissues through the systemic circulation (endocrine manner). Some adipokines are adipocyte specific, such as leptin and adiponectin, while others, like proinflammatory cytokines, to a higher degree are secreted from the non-fat cells in adipose tissue (114). In obesity, dysregulation of pro- and anti-inflammatory cytokines released from adipose tissue drive the chronic low-grade systemic inflammation thereby contributing to development of metabolic and cardiovascular disorders (30, 115).

Adipokines are secreted through different mechanisms. Adiponectin and leptin are examples of adipokines secreted through the classical pathway, dependent of the endoplasmic reticulum and golgi apparatus. Other adipokines, like dipeptidyl peptidase 4 (DPP4) and TNF-α, are released from the adipocytes by proteolytic cleavage and shedding from the plasma membrane, releasing the soluble form of the adipokine. Release of adipokines may be constitutive, regulated or a combination of both. Further, the secretory mechanisms of several adipokines are still unknown (116).

1.7.1 Leptin

Leptin is mainly produced by adipocytes and is a multifunctional adipokine with widespread effects in the body, regulating food intake, energy expenditure and is also involved in angiogenesis and wound healing (117). Plasma levels of leptin increase with weight gain and decrease with weight loss (118). There is a positive linear correlation between circulating levels of leptin and total body fat mass and there is an increased release of leptin from large compared with small adipocytes (119). When leptin levels are decreased, appetite is increased and energy expenditure is lowered (120). However, the high leptin levels seen in obese subjects do not exert a suppressing effect on appetite due to resistance to the hormone (121). Leptin receptors are found in the hypothalamus, regulating metabolism, but receptors are expressed in several other tissues as well, and leptin has been shown to act in skeletal muscle to increase fatty acid oxidation (122).

Maternal concentrations of leptin increase during pregnancy, as do fat mass. However, there is evidence that the increase during pregnancy is not all due to the increased fat mass. Leptin levels start to increase early in pregnancy and rapidly decline after parturition (123). The human placenta express high amounts of leptin mRNA, and it has been suggested that increased levels of

leptin during pregnancy may facilitate mobilization of maternal fat stores, increasing lipid substrates to the growing fetus (124).

1.7.2 Adiponectin

Adiponectin is the most abundant adipokine, secreted exclusively from adipose tissue (125). Adiponectin has been shown to improve whole-body insulin sensitivity in models of diet-induced obesity (126). Adiponectin increase fatty acid oxidation and glucose uptake in skeletal muscle and adipose tissue by activation of AMP-activated protein kinase through adiponectin receptor 1 (127). Adiponectin also reduces glucose production by the liver, primarily by binding to adiponectin receptor 2 (128). It also has anti-inflammatory and anti-atherogenic properties (129). There is a strong negative correlation between plasma adiponectin concentration and fat mass in humans. Obesity reduces adiponectin levels, while weight loss has the opposite effect. Adiponectin levels are also decreased in T2D (127, 130). As reviewed by Briana et al (131), the influence of pregnancy on adiponectin levels is not clear. Some studies have shown decreased levels of adiponectin during pregnancy, parallel to the decrease in insulin sensitivity, while others have found no change during normal pregnancy. The latter is proposed as a protective mechanism against the decreased insulin sensitivity (131).

1.7.3 Cytokines

The increased fat mass in obesity has been linked to an increase in markers of inflammation, and TNF-α and IL-6 are the two best-studied cytokines in obesity. (132). However, studies have shown that this altered cytokine response is not primarily originating from adipocytes, but rather from the non fat cells of the stromal vascular fraction of adipose tissue, such as macrophages or leukocytes (92). TNF-α was the first cytokine to be suggested as a link between obesity, inflammation and insulin resistance, with higher expression in adipose tissue in obesity (111). TNF-α has been shown to impair insulin signaling in adipose tissue, and chronic treatment with TNF-α decreased insulin-stimulated glucose uptake in rat muscle (133, 134). Neutralization of TNF-α has been suggested as a therapeutic target for treating T2D, however, improvement in insulin sensitivity during therapy could not be seen (135).

As with TNF-α, most of the adipose tissue derived IL-6 originates from the stromal vascular fraction and approximately 1/3 of circulating levels is attributed to adipose tissue production (136, 137). Increased circulating levels of IL-6 are correlated with obesity and development of T2D (138).

Increased levels of TNF-α and IL-6 during pregnancy have mainly been attributed to placental production and maternal concentrations of these cytokines are increased in gestational diabetes mellitus (131, 139). However, knowledge of the contribution of adipose tissue in this respect is missing.

Besides TNF-α and IL-6, there are over 100 inflammatory cytokines identified with both pro- and anti-inflammatory properties but their role in obesity-related pathogenesis is largely missing (117).

1.7.4 Chemerin

Chemerin is a chemoattractant adipokine, highly expressed in mature adipocytes, liver and placenta (140). Chemerin has been proposed as a potential link between obesity and T2D (141), as serum levels of chemerin are increased in obesity and adipose tissue secretion from tissue explants is higher in obese subjects as compared to normal weight subjects (141, 142). It has been shown that chemerin is necessary for adipocyte differentiation and it has been proposed that enhanced expression of chemerin in enlarged adipocytes acts as a physiological stimuli to promote adipogenesis. Chemerin release from adipocytes is also increased by stimulation of TNF-α (142).

1.7.5 Dipeptidyl peptidase 4

DPP4 is an adipokine that has been implicated in the development of co-morbidities associated with obesity (143). Serum levels are increased in obesity and reduced following weight loss and there is a strong relationship between serum concentrations and adipocyte size (143). In mice lacking DPP4, insulin secretion was increased and glucose tolerance was improved (144), however the effects of DPP4 on β-cell function remains unclear (145).

1.7.6 Omentin

Omentin is proposed to be an adipokine with anti-inflammatory and insulin-sensitizing properties. It is a depot-specific adipokine, almost exclusively expressed in, and released from visceral adipose tissue (146). Omentin is expressed by the stromal vascular fraction of adipose tissue, rather than by the adipocytes and omentin gene expression and circulating levels are reduced in obesity (147). Circulating levels are negatively correlated to a number of metabolic risk factors, such as waist circumference, glucose intolerance and dyslipidemia (148). Omentin has been shown to stimulate glucose uptake in adipocytes cultured in vitro (146) and weight reduction in obese subjects increased circulating levels of omentin (149).

1.7.7 Adipocyte fatty acid-binding protein

Adipocyte fatty acid-binding protein (AFABP) is an adipokine that also has been described as being associated with development of insulin resistance, T2D and cardiovascular disease. Serum levels are increased in overweight and obese subjects and there is a positive correlation between AFABP levels and waist circumference (150). In a knockout model, mice lacking AFABP did not develop insulin resistance and diabetes, although weight gain was similar to control-animals (151). It has been suggested that high levels of circulating AFABP induce insulin resistance due to increased hepatic glucose production (152). Besides the association to T2D, increased circulating levels of AFABP has also been shown in gestational diabetes mellitus (153).

2 AIM

The overall aim of this thesis was to increase our knowledge of human adipose tissue morphology and function and its role in pathogenesis of metabolic disease with focus on GDM and T2D.

2.1 Specific aims

Paper I

- To determine the effect of commonly used bovine, and human, albumin preparations on cytokine release from adipose tissue in vitro.
- To establish a system for adipose tissue short-term incubation minimizing induction of cytokine release.
- To compare the release of adipokines, implicated in inflammation and insulin resistance, from subcutaneous and visceral adipose tissue.

Paper II

- To characterize adipose tissue morphology and function in normal weight and obese women longitudinally during pregnancy.
- To identify adipose tissue-related factors associated with gestational insulin resistance.

Paper III

- To characterize adipose tissue morphology and function, in relation to glucose metabolism, in women six years after gestational diabetes mellitus.
- To identify adipose tissue-related factors associated with impaired glucose metabolism and insulin resistance at follow-up

3 SUBJECTS AND METHODS

3.1 Subjects

3.1.1 Paper I

Adipose tissue biopsies were obtained from 6 men and 11 women undergoing elective abdominal surgery at Sahlgrenska University Hospital. To evaluate the effects of different albumin preparations on cytokine release from adipose tissue in vitro, subcutaneous adipose tissue from 6 patients was studied (abdominoplasty in 5, hysterectomy in 1). Paired subcutaneous and visceral adipose tissue biopsies were obtained from 11 patients (Roux-en-Y gastric by-pass in 9 and cholecystectomy in 2) to compare adipokine release from subcutaneous and visceral adipose tissue. Patients were recruited to the study at the time of admittance to the hospital prior to surgery together with collection of information on age, height, weight and medical history. No subjects with malignant disease were included.

3.1.2 Paper II

Normal weight and obese pregnant women in the *Pregnancy, Obesity, Nutrition and Child Health* study (PONCH) were included. The PONCH-study is a longitudinal study focused on health in normal weight and obese pregnant women and their children. The study scheduled three study visits at Sahlgrenska University Hospital during pregnancy (first visit in trimester 1, gestational weeks 8-12, second visit in trimester 2, weeks 24-26, and third visit in trimester 3, weeks 35-37). All three visits included blood- and urine sampling, determination of body composition, anthropometry and food- and physical activity questionnaires. The women and children were also followed postpartum. Inclusion criteria were BMI 18.5-24.9 or >30 kg/m^2 and >20 years of age. Exclusion criteria were non-European descent, vegan diet, diabetes, use of neuroleptic drugs and twin pregnancy. Women who consented to an optional adipose tissue biopsy at study visits in trimester 1 and 3 were eligible for this study. In total, 22 normal weight women and 11 obese women completed both study visits including adipose tissue sampling. Women were recruited through maternity care, by advertisement on a pregnancy-related website and by postings on public billboards. At inclusion, normal weight and obese women were randomized to either dietary counseling groups or control groups. Normal weight and obese control groups did not receive any dietary counseling in addition to the advice given

to all women at maternity care centers during pregnancy. The dietary counseling groups were given advise by a dietitian during pregnancy to increase adherence to the Nordic Nutrition Recommendations (154). The obese dietary counseling group was also given advice to reduce caloric intake 20% of their calculated need during pregnancy. Since the interventions had no significant effect on reported energy intake, body composition or any other variable, compared with corresponding control groups, the normal weight and obese groups were separately pooled.

3.1.3 Paper III

Participants were recruited from a larger cohort of women, all diagnosed with gestational diabetes mellitus in Gothenburg between 2005 and 2007. Between five to six years after index pregnancy the women were contacted by telephone and offered to participate in a follow-up study and those interested received further information by mail. Two study visits were scheduled in the follow-up study. The first visit included an OGTT, anthropometry and blood sampling. The second visit included an optional adipose tissue biopsy and determination of body composition. Women who consented to adipose tissue sampling were included in this study. Women who were pregnant at the time for follow-up were not eligible to participate, and further exclusion criteria were diagnosis of type 1 and type 2 diabetes or other endocrine diseases that affect adipose tissue, malignancies, use of neuroleptic drugs, and gastric bypass surgery between the index pregnancy and follow-up. In total, 39 women were included in the study.

3.2 Ethics

All participants received information about the studies and gave oral and written consent prior to inclusion. All studies were conducted in accordance with the principles of the Declaration of Helsinki and approved by the Regional Ethical Review Board in Gothenburg.

3.3 Methods

3.3.1 Adipose tissue sampling

In paper I surgical adipose tissue biopsies were obtained from participants at the beginning of the surgical procedure. Abdominal subcutaneous adipose tissue was sampled after the initial incision and visceral adipose tissue (greater omentum) was collected when the surgeon reached the peritoneal cavity. When subcutaneous adipose tissue was collected from patients

undergoing abdominoplasty, adipose tissue was collected when the larger piece of tissue had been removed from the donor. The tissue was immediately placed in Medium 199 (Invitrogen, Carlsbad, CA) and transported to the lab.

In papers II and III subcutaneous adipose tissue biopsies was obtained by needle aspiration. In the non-pregnant women in study III adipose tissue was sampled from the umbilical region, at a point one-third of the distance from the superior anterior iliac spine to the umbilicus. In pregnant women, especially late in gestation, a biopsy in this region is hard to acquire. The biopsy was therefore obtained more laterally on the abdomen, in the umbilico-lumbar region, at the level of the umbilicus. After superficial injection of local anesthetic, a needle attached to a syringe was inserted into the subcutaneous adipose tissue. A negative pressure was applied in the syringe and the needle was moved back and forth in the tissue allowing for numerous small shreds of adipose tissue to be collected in the syringe. The tissue was rinsed with saline, weighed and prepared according to the study protocols. All handling of tissue was performed under aseptic conditions.

3.3.2 Anthropometry and body composition

In paper I body weight and height was measured according to standard clinical procedure in the morning before surgery (paired biopsies of subcutaneous and visceral adipose tissue) or reported by the patients (subcutaneous adipose tissue).

In papers II and III waist- and hip circumferences, and height, were measured by standard protocols and body composition was determined using Bod Pod (Cosmed, Rome, Italy). The Bod Pod system is based on air displacement plethysmography and measures body volume and body mass (a calibrated scale connected to the system) so that total body density can be calculated (155). By using the equation of Siri (156), based on the two-component model and the densities of fat mass (0.900 kg/l) and fat free mass (1.100 kg/l), body fat mass and fat free mass can be estimated. The Bod Pod has been validated in a wide range of subjects, normal weight to obese, as well as different ethnic groups and in children (157). The use of air-displacement plethysmography has also been validated for use during pregnancy (158). However, as pregnancy progress the hydration of fat free mass increases, leading to a progressive over-estimation of fat mass with advancing gestational age if the density of fat free mass is not corrected for. By adjusting the density of fat free mass to compensate for the increased water content of the tissue this effect can be minimized (159).

3.3.3 Adipocyte size determination

Adipocyte size can be measured using a variety of methods and each method has its advantages and disadvantages. One commonly used technique is manual measurements of freshly isolated adipocytes in a suspension by using a microscope and an ocular with a calibrated scale. With this method about one to a few hundred cells are usually measured. The method is time consuming and there may be a selection-bias. Further, analysis must be performed immediately after collagenase digestion of the tissue to avoid changes in cell morphology (160). Adipocyte size can also be assessed using conventional histologic preparations for microscopic measurements. This method allows for more adipocytes to be analyzed, but adipocytes may be distorted during preparation of histological sections impairing interpretation (161). Another technique is automatic sizing of osmium tetroxide-fixed adipocytes allowing for a large number of cells to be analyzed (161, 162). However, this method involves toxic substances and may potentially fix and analyze cell debris from ruptured cells (88). Also, fixation of the cells with osmium tetroxide may cause cell-swelling (162).

Computerized analysis of photomicrographs of adipocytes, isolated by collagenase digestion, allows for a large number of adipocytes to be measured rapidly (163). Further, only image acquisition has to be performed on fresh cells and analysis may be carried out at a later stage. The digital images and results of the computerized analysis can be stored for future reference. Analysis of large numbers of adipocytes allows for more detailed size distribution curves and stratification of adipocytes in different size ranges. One possible limitation of methods using collagenase digestion is that potentially fragile large adipocytes may rupture during preparation. If breakage is profound, adipocyte size may be underestimated. By avoiding centrifugation of the cell suspension, using siliconized glassware, and adequate amounts of collagenase, cell breakage can probably be minimized. Cell breakage causes lipid droplets that must be manually excluded in the analysis. Lipid droplets are usually easy to distinguish from adipocytes in the images but if somewhat out of focus lipid droplets may be difficult to recognize, particularly if they are small.

In these studies adipocytes were isolated by collagenase digestion using, a water bath with gentle orbital shaking at 37°C. Undigested stroma was separated from the adipocytes by filtration through a nylon mesh. Adipocytes were allowed to float and the medium, including other cell types, was gently removed and replaced with fresh medium to wash the adipocytes free from collagenase and non-adipocytes. This procedure was performed three times.

The cell suspension was placed on a siliconized glass slide, immediately covered with a coverslip, and transferred to the microscope. By means of a computerized macro 12 visual fields (5X objective) were photographed. The same macro was used in analysis of the images (Leica QWin V3, Leica microsystems, Wetzlar, Germany). The area within contours meeting criteria for roundness and smoothness was determined and the corresponding diameter was calculated. Microspheres with a uniform diameter of 98 μm served as reference (Dynal, Invitrogen Corporation, Oslo, Norway). In paper I, mean adipocyte volume was calculated using Goldrick's formula ($\pi d [3(SD)^2 + d^2]/6$), where d is the mean adipocyte diameter and SD is the adipocyte diameter standard deviation (164). In papers II and III, mean adipocyte volume was determined as the average of all adipocyte volumes.

3.3.4 Basal lipolysis

Lipolysis is the process of hydrolysis of triglycerides in the adipocyte to free fatty acids and glycerol upon metabolic demand. The surface of the lipid droplet contains enzymes that respond to hormonal stimulation when fuel is needed. Three major lipases account for mobilization of triglycerides in the lipid droplet; adipose triglyceride lipase hydrolyzes the first fatty acid from the triglyceride, hormone-sensitive lipase hydrolyzes diacylglycerol to monoacylglycerol, and monoacylglycerol lipase removes the last free fatty acid, leaving the free fatty acids and one molecule of glycerol (165). In paper II and III basal lipolysis, i.e. the lipolytic activity measured without stimulating or inhibiting agents, was assessed by incubating adipocytes, isolated by collagenase digestion, in minimal essential medium for two hours. Following incubation, cells and media was centrifuged through silicone oil to remove cells, and glycerol released into the medium was taken as an index of basal lipolysis.

3.3.5 Immunohistochemistry

Immunohistochemistry was used to determine the density of macrophages (papers I-III), mast cells (papers II-III) and vessels (papers II-III) in adipose tissue. A part of each biopsy was placed in phosphate-buffered formalin and subsequently processed for immunohistochemical staining according to standard protocols including dehydration, embedding in paraffin and sectioning at 5 μm. Following rehydration, the slides were subjected to high temperature antigen retrieval, blocking of unspecific epitopes and incubation with primary antibodies over night. A secondary antibody and chromogen was added, followed by rapid dehydration and mounting. Glass slides were digitized prior to computerized analysis.

Quantification of immunoreactive cells in tissue is traditionally performed by manual counting of cells with positive signal in a slide using a microscope or a photomicrograph of the slide. In recent years, digital pathology has developed and software for automatic counting is available. In paper I macrophage density was determined manually on digitized tissue slides and the origin of the tissue unknown to the observer. To quantify macrophages, mast cells and vessels in paper II and III, the software Tissue Studio (version 3.6.1, Definiens, Munich, Germany) was used. In order to quantify immunoreactive signals using this software, a runtime had to be setup and tested to fit the properties of the tissue studied. The software has an intuitive self-learning feature, where the operator informs the software of all the different types of tissue in the slides, what to include in analysis and what to exclude. After self-learning, batch analysis can be performed. A challenge is the adipose tissue itself. During adipose tissue processing, lipid in the adipocytes is washed out leaving them empty. As a result, the tissue appears pale and the software may have difficulties differentiating tissue from background. Extra precautions and fine-tuning of settings had to be carried out prior to analysis. The number of mast cells and macrophages was normalized for surface area by the software. Adipocyte size could potentially affect the results why immune cell density was also expressed per 10^3 adipocytes using the adipocyte area distribution curve. However, the way of normalization did not influence the results.

In paper II, vessel density was expressed as the ratio of the positive signal area to the total specimen area. In paper III, vessel density was expressed as immunoreactive area per adipocyte.

3.3.6 In vitro release of adipokines

Adipokines may be quantified in a number of ways and each method has its advantages and disadvantages. The purpose of the analysis may be guiding in the choice of sample type and analytical method. Blood sampling followed by biochemical analysis is used extensively. An ELISA for example is relatively easy to perform and usually rather inexpensive. However, no conclusions can be drawn regarding the adipose tissue contribution to measured levels. Some adipokines, like leptin and adiponectin, are almost exclusively secreted from adipocytes while others, like cytokines, may originate from other organs, tissues and cells as well. Gene expression analysis is a method widely used to study mRNA levels of adipokines in specific cells or tissues. Expression analysis may for example be performed in whole adipose tissue, isolated adipocytes or the stromal vascular fraction. The analysis can be applied for a specific gene or large scale but mRNA

expression may not always reflect corresponding expression at the protein level or protein release. Subcutaneous microdialysis is a technique that may be used for determination of adipokine concentrations in the extra cellular space. A fluid is allowed to diffuse through the tissue in vivo and then collected for subsequent analysis of adipokine content. Microdialysis concentrations reflect the secretion of adipokines from the tissue but the technique is best suited for smaller studies as it is time consuming, both for the subject and the operator. Further, microdialysis can not be used to study adipokine release from visceral adipose tissue. Analysis of adipokines released in vitro from adipose tissue explants or isolated adipocytes is another commonly used technique. Using this approach, adipokine release specifically from adipose tissue or adipocytes is assessed, but the method is more invasive, compared with blood sampling, as an adipose tissue biopsy must be obtained. Also, in vitro "artifacts" have to be considered. In paper I, supplementation of incubation media with different albumin preparations was evaluated. Albumin is usually added to the incubation medium as a fatty acid acceptor. High concentrations of free fatty acids may otherwise exert toxic effects on the tissue during incubation. However, bovine serum albumin (BSA) has been reported to induce cytokine release from adipose tissue in vitro (166). This effect may be depending on endotoxin in the albumin preparations although cytokine induction has also been observed with endotoxin-free BSA (167). Species differences may thus underlie the reaction. As shown in paper I, human serum albumin did not induce cytokine release, as compared with no addition of albumin. Human serum albumin was therefore added to media used in papers I-III to study adipokine release in vitro. Adipose tissue explants of 5-15 mg each, in total about 250 mg, were placed in 10 ml Medium 199 (Invitrogen) supplemented with 1% HSA and incubated at 37°C for maximum 24 hours. In paper I, one incubation tube was prepared for each specific incubation period (2, 4, 6, 8, 24 hours), while in paper II and III, due to limited amount of tissue, the tissue was first incubated for four hours, medium was collected and replaced with fresh 10 ml of medium, and the incubation continued for another 20 hours. Media were stored at -80 °C until analysis.

3.3.7 Assessment of insulin sensitivity and glucose tolerance

There are several methods to estimate whole-body insulin sensitivity based on the relationship between glucose and insulin. The gold standard method is considered to be the euglycemic-hyperinsulinemic clamp during which the

amount of glucose metabolized per unit of body weight is measured during infusion of a predetermined amount if insulin. Plasma glucose levels are maintained by simultaneous infusion of glucose. However, this technique is time consuming and invasive why several surrogate measures for insulin sensitivity has been adopted. One widely used index for insulin sensitivity, based on the feedback loop of fasting insulin and fasting glucose, is the homeostasis model assessment of insulin resistance (HOMA-IR) (paper II and III). HOMA-IR is calculated by multiplying fasting insulin (mU/liter) and fasting glucose (mmol/liter), divided by 22.5 (168). HOMA-IR correlates well with insulin sensitivity as determined with the euglycemic hyperinsulinemic clamp technique, evaluated in the general population (R=0.88, P<0.0001)(168) as well as during pregnancy (R=0.73, P<0.0001) (169).

OGTT is widely used as a diagnostic tool for T2D and GDM. Glucose levels are measured in the fasted state followed by ingestion of a predefined amount of glucose (in Sweden most often a solution containing 75g glucose). Two hours after ingestion of the glucose load a new measurement of plasma glucose is made and the response to the glucose load can be interpreted. Based on the levels of fasting glucose and 2 h glucose levels NGT, IFG, IGT and T2D are defined. To assess glucose tolerance at follow-up in paper III all participants underwent a 75g OGTT and based on the results of fasting and/or 2 h plasma glucose women were classified as NGT, IFG, IGT or T2D.

3.3.8 Biochemical assays

All plasma/serum/medium concentrations of analytes were determined after an overnight fast. In paper I a multiplex immunoassay, based on electro-chemiluminiscens, was used to simultaneously analyze concentrations of nine cytokines in conditioned media (Human pro-Inflammatory 9-Plex Ultra-Sensitive Kit, Meso Scale Discovery, Gaithersburg, MD). In papers I-III enzyme-linked immunosorbent assays (ELISA) was used to measure concentrations of adipokines in serum and conditioned media. In principle, ELISA is a technique used to detect and quantify a biological substance by first binding it to a capture antibody at the surface of the well and then adding a detection antibody that binds to another site of the molecule of interest. This antibody is linked to an enzyme, which in turn is incubated in the presence of substrate to produce a measurable product, indicated by a color change that is in proportion to the concentration of the molecule analyzed. Results are often obtained through reading the absorbance of the sample and comparing it to a series of samples with known concentrations, thus making a standard curve. All blood and medium samples were analyzed

at the Clinical Chemistry Laboratory, Sahlgrenska University Hospital, accredited in accordance with the International Standard ISO 15189:2007, with the exception of HbA1c in paper III, which was analyzed using a point of care analyzer.

3.3.9 Statistical analyses

Statistical analyses were performed using SPSS statistical software version 18 for Microsoft Windows (paper I) and version 21 for MacOS X (papers II and III) (SPSS inc, Chicago IL). Results are expressed as mean ± standard error of the mean (SEM) in paper I, and mean ± standard deviation (SD) in paper II and III. $P<0.05$ was considered significant.

Paper I

Values were logarithm-transformed to stabilize variances. To evaluate the effect of different albumin preparations on cytokine release from adipose tissue, using four different media at five time points, three-way analysis of variance was used, with "subject" as fixed factor, followed by paired t-tests and Bonferroni correction. The influence of supplementation with 0.1% albumin was assessed by two-way analysis of variance. A similar approach as above was used to assess differences in cytokine release from subcutaneous and visceral adipose tissue over time using a three-way analysis of variance, but instead of different media there were two different depots. Differences in adiponectin release in obese and non-obese subjects were evaluated using a split-plot design with subjects as main plots (obese/non-obese) and incubation times as subplots. The split plot design originates in agricultural science where the yield using different combinations of seeds and fertilizers was evaluated, hence the name split plot. T-test was used to compare macrophage density and adipocyte size in subcutaneous and visceral adipose tissue, and Pearson correlation was used to evaluate the relationship between adipocyte size and adiponectin release.

Paper II

Comparisons between trimester 1 and 3 within groups were made using the related-samples Wilcoxon signed-rank test. Comparisons of groups early and late in gestation, as well as comparison of change between groups during pregnancy were assessed using Mann-Whitney U test. Spearman's rank-correlation test was used to evaluate associations between adipocyte size early in pregnancy and the change in the proportion of adipocytes <100 μm during pregnancy, and between the proportion of adipocytes >150 μm and circulating adipokines in trimester 3. Variables, significantly correlated to HOMA-IR as assessed by Spearman's rank-correlation test, were introduced

as independent variables in multivariable linear regression to identify determinants of insulin sensitivity early and late in gestation.

Paper III

Women with NGT and IGM were compared using Mann-Whitney U test for continuous variables. Fishers exact test was used to compare variables with two categories and chi-2 test was used to compare categorical variables with more than two categories. Multivariable linear regression was used to identify determinants of HOMA-IR. Independent variables were selected by Spearman correlation as in paper II. By using multivariable logistic regression and dichotomizing the women as NGT or IGM the probability of having impaired glucose metabolism could be determined. Adipose tissue-related variables were included as independent variables in this analysis.

4 RESULTS AND DISCUSSION

4.1 Paper I

In this study our aim was to develop an in vitro system reflecting the release of adipokines, including cytokines, from human adipose tissue in vivo. The system was subsequently used to compare the release of a number of adipokines, implicated in insulin resistance and inflammation, from subcutaneous and visceral human adipose tissue.

4.1.1 In vitro effects of albumin

Subcutaneous adipose tissue was incubated for 2, 4, 6, 8 and 24 hours in media supplemented with 1% BSA Fraction V, 1% BSA Essential Fatty Acid Free, 1% HSA, or no albumin. Medium concentrations of nine cytokines were measured using a multiplex immunoassay. Over 24 hours of incubation, levels of cytokines were higher in both media containing BSA than in media with HSA or no albumin. Also, supplementation of media with 0.1% BSA induced cytokine release. Release of adiponectin was not affected by BSA.

Higher levels of endotoxin in media supplemented with BSA may, at least partly, explain the increased release of cytokines. However, the stimulatory effect of BSA on cytokine release has also been observed in the absence of endotoxin contamination suggesting other immunomodulatory effects of BSA (167). Despite marginally higher endotoxin levels in medium with HSA as compared with medium without albumin, cytokine concentrations were similar in medium with HSA and medium without albumin. Thus, HSA did not influence the release of cytokines.

In a short-term, dilute system like this, with a high ratio of media to tissue, supplementation of the medium with albumin may not be necessary. In our final protocol, 1% HSA was added to the medium as a precaution due to the expected beneficial effects of albumin in stabilizing medium pH and binding free fatty acids released from the tissue during incubation. High concentrations of free fatty acids may exert toxic effects on the tissue (170). Thus, we conclude that BSA should be avoided in media for human cell/tissue incubation since it seems to have intrinsic effects on cytokine release.

4.1.2 The in vitro system

Since adiponectin is an adipocyte-specific adipokine, circulating levels of adiponectin reflect release from adipose tissue. Previous studies have reported decreased circulating adiponectin levels in obesity (171-173). In order to study if this difference between obese and non-obese adipose tissue was reflected in vitro, using our system, subcutaneous adipose tissue from five obese and five non-obese subjects was incubated according to our final protocol for 2, 4, 6, 8, and 24 hours. Adiponectin levels in conditioned media were analyzed by ELISA. Adiponectin release was higher in adipose tissue from non-obese subjects than from obese subjects in line with previous findings (171-173). There was also a negative correlation between adiponectin release from subcutaneous adipose tissue in vitro and adipocyte size at 4 h ($r= -0.85$, $P<0.05$, $n=7$) and 24 h ($r= -0.84$, $P<0.05$, $n=7$), also consistent with previous studies on circulating levels of adiponectin (77, 78). Therefore, in this respect the system seems to reflect the in vivo situation.

In line with the approach above, we chose to study omentin release in vitro since the expression of omentin mRNA has been reported to be markedly lower in subcutaneous than in visceral adipose tissue (146, 174). Subcutaneous and visceral adipose tissue from 11 subjects were incubated according to our standard protocol. Omentin was almost exclusively released from visceral adipose tissue, again supporting the conclusion that the release of adipokines in our system reflect in vivo release.

During the 24-hour incubation period, increasing amounts of adipokines accumulated in the medium and release was linear for 2-6 h. Previously, pieces of adipose tissue have been incubated under similar conditions, for several days, with maintained viability i. e. morphology and hormonal responsiveness (175). However, all in vitro systems have limitations. The tissue is subjected to different stressors during handling and incubation. Also, adipokine release in vivo is regulated through different mechanisms (116). Measured concentrations in vitro may be due to passive leakage, consecutive release, or both, and may also be stimulated or inhibited by different factors.

Taken together, under the conditions used adipokines accumulate in the medium over 24 h. The system reflects differences in adipose tissue adipokine release observed in vivo, depending on BMI and adipocyte size, as well as differences depending on gene expression levels. The results suggest that the system is suitable for further analyses of the adipose tissue secretome and differences between depots.

4.1.3 Depot-dependent release of adipokines

Paired subcutaneous and visceral adipose tissue biopsies were obtained from 11 subjects and incubated as described above using 1% HSA. Cytokines were analyzed in conditioned media by multiplex immunoassay. All nine cytokines, except IL-6, were more abundantly released from visceral than subcutaneous adipose tissue.

Adiponectin, chemerin, IL-6 and DPP4, all implicated in insulin resistance (30, 127, 142, 143), were also analyzed by ELISA in media from paired biopsies of subcutaneous and visceral adipose tissue. Adiponectin release was higher in subcutaneous adipose tissue. Previous studies regarding depot-dependent release of adiponectin are inconsistent, reporting higher release from subcutaneous adipose tissue explants (176, 177) and a tendency of higher secretion from omental than subcutaneous adipocytes (178). Gene expression analysis has shown lower expression of adiponectin mRNA in visceral vs subcutaneous fat, both in normal weight and obese subjects, consistent with our findings (179, 180).

Release of IL-6 was similar between the two adipose tissue depots. Previous reports have demonstrated higher secretion of IL-6 from visceral adipose tissue (91, 181, 182). Release of cytokines from adipose tissue is mainly due to cells of the stromal vascular fraction (91, 181, 182). In this study, visceral adipose tissue had a higher macrophage density consistent with more abundant release of IL-6 from the visceral depot in numerical values. A limited number of observations and large variations between subjects may explain the discrepancies.

Both chemerin and DPP4 were more abundantly released in visceral adipose tissue. Depot-dependent release of chemerin has not previously been investigated, but in obese subjects circulating levels were correlated with BMI and measures of central adiposity (WHR, waist circumference and visceral adipose tissue mass) suggesting a link between chemerin levels and visceral adipose tissue (183). The expression of chemerin mRNA was also higher in the omentum compared with subcutaneous adipose tissue in patients with T2D (184). Serum levels of DPP4 are increased in obesity and DPP4 has been proposed to be a marker of the metabolic syndrome and to impair insulin sensitivity (143). Previous studies, using western blot, showed higher DPP4 in visceral than subcutaneous adipose tissue (143), consistent with our finding.

In conclusion, this study shows that there are functional differences between subcutaneous and visceral adipose tissue, in terms of adipokine release, consistent with clinical observations. The developed in vitro system, including HSA, may be used to further explore the link between regional fat accumulation and metabolic disease.

4.2 Paper II

During pregnancy a physiologically normal insulin resistance is established (185). In this study our aim was to characterize adipose tissue morphology and function longitudinally during pregnancy and identify adipose tissue-related factors associated with gestational insulin resistance. Twenty two normal weight and 11 obese women were recruited early in pregnancy and consented to undergo adipose tissue sampling in trimester one and three together with assessment of body composition and blood sampling. At inclusion, subjects were randomized to either control or intervention groups with the main purpose to evaluate the effect of dietary counseling including energy restriction in obese pregnant women. However, as there were no differences between control and intervention groups regarding reported energy intake, body composition or metabolic status in trimester one or during pregnancy, the control and intervention groups were pooled.

4.2.1 Early pregnancy characteristics in normal weight and obese women

The mean BMI was 22.3 kg/m^2 (range 19.1-23.9) in the normal weight group and 35.3 kg/m^2 (range 30.4-41.8) in the obese group. Obese women had higher fat mass, circulating leptin, AFABP, and CRP and HOMA-IR, while circulating levels of adiponectin was higher in normal weight women early in pregnancy.

As expected, obese women had more adipocytes and larger mean adipocyte volume, reflected in a lower proportion of small adipocytes (50-100 μm in diameter) and a higher proportion of large (100-150 μm in diameter) and very large adipocytes (>150 μm in diameter). Basal lipolytic activity was higher in the obese group. Release of IL-6, TNF-α, and adiponectin in vitro, and adipose tissue immune cell and vessel densities were similar in the groups early in pregnancy.

4.2.2 Changes during pregnancy in normal weight and obese women

Normal weight, but not obese women increased in fat mass during pregnancy although there was a borderline significant increase in fat mass also in the obese group (P=0.050). Weight gain, and fat mass gain were similar when the groups were compared. Previous studies on changes of body composition during pregnancy are inconsistent; reporting lower (186), similar (187) or higher (188) accumulation of fat in obese women compared to normal weight women. Fat mass gain during pregnancy is tightly correlated with gestational weight gain (186, 188), why reported differences in fat mass gain may be explained by differences in gestational weight gain. In the study reporting lower fat mass gain in obese women (186), only obese women gaining weight as recommended by the Institute of Medicine (lower weight gain for obese than for normal weight women) were included. There may also be methodological considerations since assessment of body composition during pregnancy is a challenge. As for example, estimates of body composition during pregnancy using four-component models (186, 188) or two-component models (187) are unable to distinguish maternal from fetal tissues. However, if the density of fat-free mass is adjusted according to gestational age, the two-component model can provide useful results of body composition also during pregnancy (158).

HOMA-IR increased similarly in both groups during pregnancy, however, all women remained normoglycemic. Also, lipolytic activity increased similarly during pregnancy to meet the needs of the growing fetus. Circulating AFABP, implicated in inflammation and a suggested marker of metabolic disease (189), was higher in obese women both early and late in gestation but increased similarly during pregnancy when groups were compared. Obese women had higher levels of CRP in trimester one, however during pregnancy normal weight women increased in CRP, and in trimester three there were no differences between the groups. This finding is in line with previous observations of increased inflammatory activity in overweight and obese women compared with normal weight women early in pregnancy while the inflammatory activity was similar in the groups late in pregnancy (190).

Expansion of adipose tissue can be achieved by enlargement (hypertrophy) of preexisting adipocytes or recruitment of new adipocytes from preadipocytes (hyperplasia) or a combination of both (83). Hypertrophy of adipocytes may lead to increased inflammation, dysfunctional adipose tissue and insulin resistance (191) while hyperplasia was suggested to be protective (76). During pregnancy, adipocyte size (diameter and volume) increased in

normal weight women, reflected in a decreased proportion of small cells and an increased proportion of large cells, indicating mainly enlargement of existing adipocytes as fat mass increased. Total adipocyte number was not influenced in either group during pregnancy. When groups were compared, mean adipocyte size decreased in the obese group during pregnancy. Also, the proportion of small cells increased and the proportion of large cells decreased, the opposite of what was seen in normal weight women. These findings suggest recruitment of adipocytes during pregnancy in the obese group (Fig 2). Adiponectin release, negatively correlated with adipocyte size (77, 78), was decreased in normal weight women during pregnancy, and comparison of the groups revealed a greater reduction in adiponectin release in normal weight women than in obese women, further supporting adipocyte recruitment in the obese group. It should be noted that there were no significant changes within the obese group regarding adipocyte size or number why conclusions regarding adipocyte recruitment in obese pregnant women must be drawn with caution. Adipocyte recruitment has been reported during pregnancy (87) and in non-pregnant women (86) and previous studies have also shown that impaired ability of adipose tissue to expand during pregnancy is associated with development of GDM (41). It may be speculated that potential recruitment of adipocytes in obese pregnant women with already large adipocytes is a mechanism protecting against ectopic fat deposition and even more severe insulin resistance.

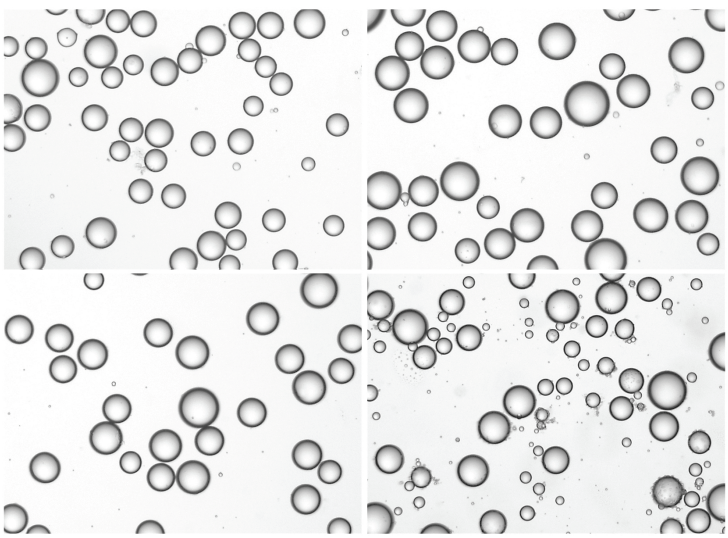

Figure 2. Images of isolated adipocytes illustrating enlargement in a normal weight woman gaining 5.5 kg fat (left panels), and recruitment in an obese woman gaining 6.9 kg fat (right panels) during pregnancy. Top images, trimester one and bottom images, trimester three, respectively.

4.2.3 Factors affecting insulin resistance during pregnancy

Stepwise multivariable linear regression analyses were performed with HOMA-IR in trimester one and three as dependent variables. Variables significantly correlated with HOMA-IR in the corresponding trimester were introduced as independent variables. A complete data set was available for 31 women in each trimester (data on lipolysis and HOMA-IR were missing in one woman each in trimester one; fat mass and HOMA-IR were missing in one woman each in trimester three). In trimester one, weight, basal lipolytic activity and circulating levels of leptin together explained 88.5% of the variance in HOMA and weight was the variable most strongly associated with insulin resistance. In trimester three body fat mass and the proportion of very large adipocytes (>150 μm) explained 75.1% of the variance in HOMA-IR and fat mass was the variable most strongly associated to insulin resistance late in gestation. This is in line with previous observations that fat mass has a significant impact on gestational insulin resistance (192). The results also suggest that the regulation of insulin sensitivity varies during pregnancy. It would have been interesting to include the insulin desensitizing placental hormones, mainly placental growth hormone, in the trimester 3 regression as well (193).

4.3 Paper III

It is well established that women with a history of GDM have an increased risk of T2D later in life (27). To study risk factors in this pathogenesis, all women who were diagnosed with GDM in Gothenburg between 2005 and 2007 were invited to participate in a follow-up study. At follow-up, the women underwent OGTT, blood sampling, and assessment of body composition. Women who consented to an optional adipose tissue biopsy were eligible for the present study. Our aim was to identify adipose tissue-related factors, associated with IGM and insulin resistance at follow-up, that may contribute to progression to T2D in this group of women. Characteristics related to the index pregnancy were collected from medical records.

Thirty nine women were included and based on the results of the OGTT, using the 1999 WHO criteria (20), NGT was identified in 31, IFG in one, IGT in three, combined IFG/IGT in two, and two women were identified with undiagnosed T2D. IFG, IGT and T2D were together denominated IGM.

4.3.1 Index pregnancy characteristics

Women who at follow-up had developed IGM weighed more and had higher BMI early in index pregnancy. Having high pre-gravid BMI has been reported as a risk factor for progression to T2D after GDM (194), in line with these results. Other factors that have been suggested to influence the risk for progression to T2D after GDM include age, family history of diabetes, parity, previous GDM, gestational weight gain, insulin treatment during pregnancy, early GDM diagnosis and ethnicity (195-199). However, no differences were seen between the NGT and the IGM groups regarding these variables in the present study. The limited number of subjects (8) in the IGM-group should be noted though.

4.3.2 Follow-up characteristics

At follow-up, women with IGM had higher BMI, weighed more, and had gained more weight since index pregnancy than women with NGT. These factors have previously been reported to be associated with progression to T2D after GDM (200, 201). Also, women with IGM had greater fat mass, reflected in higher levels of circulating leptin, and higher indices of abdominal fat distribution. As expected, the IGM-group had higher HOMA-IR and HbA1c consistent with the well established link between fat mass, especially abdominal fat, and T2D (46-50). Further, circulating AFABP was increased in women with IGM.

Total adipocyte number did not differ between the groups but women with IGM had larger adipocytes than women with NGT, reflected in a lower proportion of small cells and a higher proportion of large and very large cells in women with IGM. Larger fat cells were expected in the IGM group given the higher BMI and greater fat mass in these women. Enlarged adipocytes have been suggested to participate in development of metabolic disorders, however, the mechanisms are not completely understood. Large adipocytes have an increased production and release of inflammatory mediators, contributing to the low-grade inflammation as seen in obesity and metabolic disorders (76). Further, large adipocytes are less sensitive to the anti-lipolytic actions of insulin, leading to increased levels of circulating free fatty acids, and the limited capacity for further lipid storage may result in ectopic lipid storage (191). The proportions of large adipocytes were positively correlated with leptin, AFABP, CRP, and mast cell density and negatively with adiponectin, while the opposite was observed for the small population of adipocytes. Adipose tissue from women with IGM also had higher release of TNF-α in vitro and higher mast cell density, both implicated in development of metabolic disease (104, 202). Taken together, these findings confirm the

close connection between excess fat mass, central fat distribution, metabolic disturbances and signs of inflammation in adipose tissue.

4.3.3 Multivariable regressions

Stepwise multivariable logistic regression was performed to determine risk factors of belonging to the IGM group, while stepwise multivariable linear regression was performed to investigate determinants of insulin resistance at follow-up as determined by HOMA-IR.

Multivariable logistic regression

IGM was defined as the dichotomous dependent variable and all adipose tissue-related variables were introduced as independent variables. All 39 women were included in the final analysis. BMI at follow-up was associated with increased risk of belonging to the IGM group. Including the index pregnancy BMI did not change the outcome. As seen in Fig 3, the predicted probability of belonging to the IGM-group increased with increasing BMI in a non-linear fashion. The increased risk, per unit of increase in BMI, was lower in the lower BMI-range, whereas in the higher BMI-range, the probability of belonging to the IGM-group increased drastically with increasing BMI. By close monitoring of these women after parturition, especially the women in the high BMI-range, and by giving them support to limit weight gain, or loose weight, after pregnancy the women´s risk of developing IGM/T2D can be reduced.

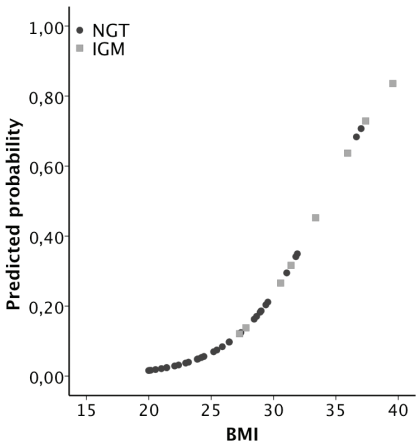

Figure 3. Multivariable logistic regression analysis with ±IGM as dichotomous variable showed increased predicted probability of belonging to the IGM group with increasing BMI.

Multivariable linear regression

HOMA-IR was defined as the dependent variable. Adipose tissue-related variables, correlated with HOMA-IR (Spearman rank correlation), were selected as independent variables. The final analysis included 38 participants (HOMA-IR could not be calculated in one woman) and revealed that waist-to-height ratio and adipocyte volume together explained 65.4% of the variation in HOMA-IR. The variable most strongly associated (highest correlation coefficient) with insulin resistance was waist-to-height ratio, an index of central fat accumulation.

Taken together, BMI and central fat accumulation at follow-up were identified as risk factors for T2D after GDM, in line with previous reports (200, 201, 203). In addition, adipocyte volume was identified, for the first time, as a risk factor for T2D in this group of women. BMI, waist-to-height ratio, and adipocyte volume are also established risk factors for development of T2D in the general population (52, 55, 204).

Adjustment of the differences in BMI between the NGT and IGM groups, and identification of potentially remaining differences regarding adipose tissue morphology and function, may reveal adipose tissue-related key components in the pathogenesis of T2D after GDM.

5 CONCLUSIONS AND FUTURE PERSPECTIVES

Several studies report that the release of adipokines from adipose tissue varies between depots, and that this depot-dependent release may explain the relation between regional fat accumulation and metabolic disorders. Incubation of adipose tissue pieces and analysis of the medium is one strategy to determine which adipokines are actually released from specific depots and to what extent. A prerequisite is that the in vitro conditions are selected with care, as in the present work, so that findings obtained in vitro reflect the in vivo situation as closely as possible. Using the developed system, excluding the commonly used component BSA in the medium, we found more abundant release of pro-inflammatory cytokines, chemerin and DPP4 from visceral than subcutaneous adipose tissue. The opposite was observed for the insulin sensitizing adipokine adiponectin. Our findings confirm and extend current knowledge regarding these adipokines. The system is suitable for further studies of the adipose tissue secretome and relative differences between the adipose tissue depots of the human body. New large-scale techniques for identification and quantification of proteins in solutions such as blood or medium provide exciting possibilities.

During pregnancy a physiologically normal insulin resistance is established and fat mass is usually gained. Therefore, pregnancy is a unique period to study development of insulin resistance and the potential role of adipose tissue-related factors in this process. We observed opposite changes in adipose tissue morphology in normal weight and obese women during pregnancy when the groups were compared. While normal weight women appeared to accumulate fat in pre-existing adipocytes (which became larger), the obese women had signs of adipocyte recruitment (resulting in a reduced mean fat cell size). Further, fat mass and the proportion of very large adipocytes were most strongly associated with insulin resistance late in pregnancy. These findings suggest that not only the placenta, but also the adipose tissue, contribute to gestational insulin resistance. The findings are also interesting given the established relationship between adipocyte size and metabolic dysfunction. Potential to recruit adipocytes during pregnancy may protect obese women against even more severe insulin resistance. The signs of adipocyte recruitment during pregnancy in obese women may be further explored using the conditioned media that have been stored. Studies of the capacity of the media to proliferate and/or differentiate preadipocytes in vitro may provide valuable information. Further, longitudinal determinations of adipose tissue morphology in women who develop GDM are missing. An

interesting focus for future studies is also to identify the potential key factor triggering adipocyte recruitment during pregnancy, and if the change in adipose tissue morphology during pregnancy is related to weight loss after pregnancy.

It is well known that women with previous GDM are at increased risk for T2D later in life. The exact mechanisms behind this risk are not known although previous studies have identified adipose tissue-related factors to be of importance e. g. high pre-pregnancy BMI and high follow-up BMI. We found that women who developed impaired glucose metabolism six years after GDM had higher BMI and greater fat mass as well as altered adipose tissue morphology and function compared with women who remained normal glucose tolerant. We also identified BMI, waist-to-height ratio (an index of central fat accumulation), and - for the first time in this group of women - adipocyte volume as risk factors for T2D. Adjustment of the differences in BMI between the NGT and IGM groups, and identification of potentially remaining differences regarding adipose tissue morphology and function, is a future approach to identify adipose tissue-related key components in the pathogenesis of T2D after GDM. A longitudinal study design with examinations and adipose tissue sampling both during GDM-pregnancy and at follow-up would be valuable. Further, health care providers should take initiative to close monitoring of these women after parturition, especially the women in the high BMI-range or with abdominal obesity. By giving these women support to loose weight after pregnancy, the risk of developing IGM/T2D can be reduced.

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