**Effect of pre-existing maternal obesity, gestational diabetes and adipokines on
the expression of genes involved in lipid metabolism in adipose tissue**

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ABSTRACT

Objective: To determine the effect of maternal obesity, gestational diabetes mellitus (GDM) and adipokines on the expression of genes involved in fatty acid uptake, transport, synthesis and metabolism.

30 **Materials/Methods:** Human subcutaneous and omental adipose tissue were obtained from lean, overweight and obese normal glucose tolerant (NGT) women and women with GDM. Quantitative RT-PCR (qRT-PCR) was performed to determine the level of expression. Adipose tissue explants were performed to determine the effect of the adipokines TNF α , IL-1 β and leptin on adipose tissue gene expression.

Results: Pre-existing maternal obesity and GDM are associated with decreased expression in genes involved in fatty acid uptake and intracellular transport (LPL, FATP2, FATP6, FABPpm and ASCL1), triacylglyceride (TAG) biosynthesis (MGAT1, MGAT2 and DGAT1), lipogenesis (FASN) and lipolysis (PNPLA2, HSL and MGLL). Decreased gene expression was also observed for the transcription factors involved in lipid metabolism (LXR α , PPAR α , PPAR δ , PPAR γ , RXR α and SREBP1c). On the other hand, the gene expression of the adipokines TNF α , IL-1 β and or leptin
40 were increased in adipose tissue from obese and GDM women. Functional *in vitro* studies revealed that these adipokines decreased the gene expression of LPL, FATP2, FATP6, ASCL1, PNPLA2, PPAR δ , PPAR γ and RXR α .

Conclusions: Pregnancies complicated by pre-existing maternal obesity and GDM are associated with abnormal adipose tissue lipid metabolism, which may play a role in the pathogenesis of these diseases.

Keywords: cytokines, fatty acid uptake, fatty acid transport, pregnancy, transcription factors

50 **List of Abbreviations:** GDM, gestational diabetes mellitus; NGT, normal glucose tolerant; qRT-PCR, quantitative RT-PCR; free fatty acid, FFA; FABP, fatty acid binding protein; plasma membrane fatty acid binding protein, FABPpm; FATP, fatty acid transport protein; ASCL1, acyl-CoA synthetase long-chain family member 1; LPL, lipoprotein lipase; FASN, fatty acid synthase; DGAT, diacylglycerol acyltransferases; MGAT, monoacylglycerol acyltransferases; PNPLA2, adipose triglyceride lipase; HSL, hormone-sensitive lipase; MGLL, monoglyceride lipase; LXR, liver X receptor; PPAR, proliferator activated receptor; RXR, retinoid X receptor; SREBP1c, sterol regulatory element binding protein; TAG, triacylglyceride; DAG, diacylglycerol; MAG, monoacylglycerol.

INTRODUCTION

White adipose tissue plays a central role in regulating lipid and energy homeostasis, by storing triacylglycerides (TAGs) or releasing free fatty acids (FFAs) in response to changes in energy demands [1]. During human pregnancy, a number of metabolic changes occur in maternal adipose tissue that is essential for fetal growth and development. Early gestation is associated with increased maternal fat stores and insulin sensitivity. However, the second half of human gestation is characterised by increased insulin resistance, which results in increases in maternal circulating glucose, TAG and FFA concentrations, allowing for greater substrate availability for fetal growth [2, 3]. In women with additional metabolic stress, such as those with gestational diabetes mellitus (GDM) and pre-existing obesity, there are alterations in adipose tissue glucose and lipid metabolism consistent with increased insulin resistance leading to increases in the circulating concentrations of fatty acids and lipids [4-7]. Of clinical significance, these infants are increased risk of later metabolic disease, including obesity, diabetes, cardiovascular disease, and certain cancers [8]. Thus, further understanding adipose tissue lipid metabolism is becoming increasingly important in the light of the rising incidence of diabetes and obesity during pregnancy [9, 10], and its associated disorders such as type 2 diabetes, dyslipidemia, and cardiovascular diseases later in life for both mother and offspring [11].

A number of genes are involved in adipocyte lipid metabolism [12-15]. Lipoprotein lipase (LPL) regulates the hydrolysis of circulating TAG into FFA. FFA entry into the adipocyte is the first step to lipid storage. Uptake of fatty acids into cells occurs via both passive diffusion and a protein-mediated mechanism. The most prominent and best characterised of these are three membrane-associated proteins, a 40 kDa plasma membrane fatty acid binding protein (FABPpm), FA translocase (FAT)/CD36, and 60 kDa fatty acid transport proteins (FATPs) also known as solute carrier proteins (SLC27A). Additionally, members of the fatty acid binding protein (FABP) family

are intracellular carriers for fatty acids which likely transport them to their intracellular sites of metabolism. The synthesis and accumulation of TAGs in adipose tissue occurs via a number of enzymes including fatty acid synthase (FASN), diacylglycerol acyltransferases (DGAT) and
 90 monoacylglycerol acyltransferases (MGATs). There are also a number of intracellular lipolytic enzymes that have an established function in the lipolytic breakdown of TAGs in adipose tissues, including adipose triglyceride lipase (ATGL or PNPLA2), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGLL).

Peroxisome proliferator activated receptor (PPAR), liver X receptor (LXR) and their heterodimeric partner, retinoid X receptor (RXR), and sterol regulatory element binding proteins (SREBP1c and SREBP2) have emerged as metabolic sensors for lipid metabolism in adipocytes [16-21]. A change in the activity or abundance of these transcription factors leads to major changes in intracellular as well as whole body lipid levels.

100 Defective FFA uptake, transport, oxidation and lipolysis are features of obesity, insulin resistance and type 2 diabetes [16, 22-24]. Despite the central physiological importance of these processes for maintaining normal glucose, lipid and energy homeostasis during pregnancy, the expression of genes involved in FFA metabolism is insufficiently characterised in adipose tissue in association with diabetes and obesity during human pregnancy. Thus, the aim of this study was to determine the effect of pre-existing maternal obesity and GDM on the expression levels of genes involved in fatty acid uptake and transport, synthesis and metabolism. Both subcutaneous and visceral adipose tissue will be examined as there is marked heterogeneity with regard to lipolysis regulation and fatty acid flux from these sites [25]. In addition, as adipokines are among the numerous effectors that control
 110 the lipid metabolism in various tissues [26-29], the effect of TNF α , IL- β and leptin on adipose tissue gene expression will also be assessed. This is of relevance as low grade inflammation and

increased levels of adipokines are key features of obese pregnancies and pregnancies complicated by diabetes [30-36].

ACCEPTED MANUSCRIPT

MATERIALS AND METHODS

Tissue Collection and Preparation

Approval for this study was obtained from the Mercy Hospital for Women's Research and Ethics Committee and informed consent was obtained from all participating subjects. Human subcutaneous adipose tissue (from the anterior abdominal wall) and omental adipose tissue were obtained from a total of 46 pregnant women (28 NGT and 18 GDM). Tissues were obtained within ten minutes of delivery and dissected fragments were thoroughly washed in ice-cold PBS to remove any blood, snap frozen in liquid nitrogen and stored at -80°C until required for RNA extraction. All tissues were obtained at the time of term Caesarean section before the onset of labour. Indications for Caesarean section included repeat Caesarean section or breech presentation. Women with any adverse underlying medical condition (i.e. including asthma, preeclampsia and pregestational diabetes) were excluded. Samples were collected from lean ($\text{BMI} < 24.9 \text{ kg/m}^2$), overweight (BMI between $25\text{--}29.9 \text{ kg/m}^2$) and obese ($\text{BMI} > 30 \text{ kg/m}^2$) subjects. The women were classified as lean, overweight or obese based on their BMI, calculated at their first antenatal visit at approximately 12 weeks gestation. Women with GDM were diagnosed according to the criteria of the Australasian Diabetes in Pregnancy Society (ADIPS) by either a fasting venous plasma glucose level of $\geq 5.5 \text{ mmol/l}$ glucose, and/or $\geq 8.0 \text{ mmol/l}$ glucose 2 h after a 75 g oral glucose load at approximately 26–28 weeks gestation. All women with GDM were prescribed insulin in addition to dietary management. All pregnant women were screened for GDM, and women participating in the NGT group had a negative screen. The relevant clinical details of the subjects are detailed in Table 1.

Adipose tissue explants

For these studies, omental adipose tissue ($n=6$ subjects) was obtained from lean NGT pregnant women, and tissue explants were performed as previously described [37–40]. Briefly, adipose tissues was finely diced and placed in DMEM at 37°C in a humidified atmosphere of 21% O_2 and

5% CO₂ for 1 h. Tissues were blotted dry on sterile filter paper and transferred to 24-well tissue culture plates (100 mg wet weight/well). The explants were incubated in 1 ml DMEM containing 100 U/ml penicillin G and 100 µg/ml streptomycin. Tissues were incubated in the absence or presence of 10 ng/ml TNFα, 5 ng/ml IL-1β and 50 ng/ml leptin. After 20 h incubation, tissue was collected, and RNA extracted and qRT-PCR performed as detailed below. Each treatment was performed from adipose tissues obtained from six subjects.

RNA extraction and qRT-PCR

150 Total RNA was extracted from approximately 100 mg of tissue using Tri Reagent according to manufacturer's instructions (Sigma-Aldrich, Saint Louis Missouri). RNA concentrations were quantified using a spectrophotometer (Smart Spec, Bio-Rad). RNA quality and integrity was determined via the A₂₆₀/A₂₈₀ ratio and agarose gels electrophoresis. One µg of RNA was converted to cDNA using the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, Carlsbad, California, USA) according to the manufacturer's instructions. The cDNA was diluted ten-fold and 2 µl of this was used to perform RT-PCR using Sensimix Plus SYBR green (Quantace, Alexandria, NSW, Australia) with 100 nM primer. All primers were purchased from Qiagen (Germantown, Maryland, USA). The RT-PCR was performed using an iQ5 Multicolour Real-Time PCR detection system (iCycler) from Bio-Rad Laboratories (Hercules, California, USA). The specificity of the product was assessed from melting curve analysis. RNA without reverse transcriptase during cDNA
160 synthesis as well as PCR reactions using water instead of template showed no amplification. Following baseline correction, the fluorescence threshold level was set during the geometric (exponential) phase of PCR amplification to generate the threshold cycle (C_T) value for each amplification curve. Average gene C_T values were normalised to the average actin RNA C_T values of the same cDNA sample. Variations in gene expression were calculated by the comparative C_T method. For Study 1, fold differences and expressed relative to control levels. For the explant studies (Study 2), the fold change was calculated relative to basal, which was set at 1.

Statistical Analysis

Statistics was performed on the normalised data unless otherwise specified. All statistical analyses were undertaken using GraphPad Prism (GraphPad Software, La Jolla, CA). Statistical differences between the three BMI groups (NGT lean, overweight and obese) were analysed using a one way ANOVA with LSD post test correction. For comparisons between NGT and GDM women, and between control and adipokine treatment, Student's *t* test was used to assess statistical significance between normally distributed data; otherwise, the nonparametric Mann-Whitney U (Wilcoxon) test was used. Statistical difference was indicated by a *P* value of less than 0.05. Data are expressed as mean $\pm$ standard error of the mean (SEM).

RESULTS

STUDY 1: Effect of pre-existing maternal obesity and GDM on lipid metabolism in adipose tissue

Participants

Lean vs. overweight vs. obese subjects: Demographic data of all participants involved in the investigation are summarised in Table 1A. There were no significant differences in maternal age, gestational age and fetal birth weight between any of the groups studied. As expected, maternal BMI, at both 12 weeks gestation and at term, were significantly different between lean versus overweight, lean versus obese, and overweight versus obese subjects. Fasting and one-hour plasma glucose concentrations at OGTT were significantly higher in the obese normal subjects compared with the lean subjects.

NGT vs. GDM subjects: Demographic data of all participants involved in the investigation are summarised in Table 1B. In this study, all samples were obtained from NGT and GDM women who were overweight or obese. There were no significant differences in maternal age, maternal BMI, gestational age and fetal birth weight between any of the groups studied. Fasting, one-hour and two-hour plasma glucose concentrations at OGTT were significantly greater in women with GDM compared with normal pregnant women.

Fatty acid uptake and intracellular transport

Compared to lean subjects, LPL mRNA expression in obese subjects was significantly lower in both omental and subcutaneous adipose tissue (Figure 1A) and in GDM compared to NGT subjects (Figure 2A). Additionally, subcutaneous LPL gene expression was statistically lower in obese compared to overweight subjects (Figure 1A).

There was no effect of obesity or GDM on FATP1, FATP3, FATP4 and FAT/CD36 gene expression in both omental and subcutaneous adipose tissue (data not shown). Compared to lean, omental and subcutaneous FATP2 (Figure 1B) and omental FATP6 (Figure 1C), where lower in overweight and/or obese subjects. Additionally, FATP2 expression was lower in obese compared to overweight women. In women with GDM, omental and subcutaneous FATP2 (Figure 2B) and omental FATP6 (Figure 2C) mRNA expression was lower compared to NGT women. Of note, FATP6 was undetectable in subcutaneous adipose tissue.

Data for FABP1, 4 and 7 are not presented as they were not detected in all samples of omental and subcutaneous adipose tissue. There was no effect of obesity and GDM on FABP3 mRNA expression in both omental and subcutaneous adipose tissue (data not shown). In omental adipose tissue, FABPpm mRNA levels were not different between lean (Figure 1D), overweight and obese women, or NGT and GDM women (Figure 2D). On the other hand, subcutaneous adipose tissue levels of FABPpm were lower in obese women compared to both lean and overweight women (Figure 1D), and in women with GDM compared to women with NGT (Figure 2D).

In omental and subcutaneous adipose tissue, ACSL1 mRNA expression was lower in obese subjects compared to overweight and/or lean subjects (Figure 1E). ACSL1 mRNA expression was also lower in GDM compared to NGT subjects (Figure 2E) for both tissues.

TAG biosynthesis

Omental MGAT1 (Figure 1F), omental MGAT 2 (Figure 1G) and subcutaneous DGAT1 (Figure 1H) mRNA levels were significantly lower in obese women when compared to overweight and lean women. No changes between the three BMI groups were observed for subcutaneous adipose tissue MGAT1 and MGAT2 and for omental DGAT1. GDM was associated with lower subcutaneous MGAT1 (Figure 2F) and omental and subcutaneous DGAT1 levels (Figure 2G).

230 MGAT2 levels in both tissues were similar between NGT and GDM women. DGAT2 was not detected in either tissue (data not shown).

Lipogenesis

There was no effect of increasing BMI on FASN mRNA expression in adipose tissue (data not shown). However, FASN gene expression was significantly reduced in both omental and subcutaneous adipose tissue from women with GDM compared to NGT women (Figure 2H).

Lipolysis

240 Compared to lean subjects, PNPLA2 mRNA expression in obese subjects was significantly lower in both omental and subcutaneous adipose tissue (Figure 1I). For both omental and subcutaneous adipose tissue, there was no effect of BMI on HSL and MGLL expression (data not shown). Subcutaneous adipose tissue PNPLA2 (Figure 1I), HSL (Figure 1J) and MGLL (Figure 1K) gene expression was significantly lower in obese compared to lean and overweight subjects. PNPLA2 (Figure 2I), HSL (Figure 2J) and MGLL (Figure 2K) mRNA expression was significantly lower in omental and subcutaneous adipose tissue from women with GDM compared to NGT women.

Transcription factor gene expression

250 Reduced LxR α mRNA expression was observed in overweight and obese omental adipose tissue when compared to lean (Figure 3A). Moreover, in subcutaneous adipose tissue, there was significantly less LxR α mRNA expression in obese compared to lean subjects (Figure 3A). PPAR α (Figure 3B), PPAR δ (Figure 3C) and PPAR γ (Figure 3D) mRNA levels were lower in both omental and subcutaneous adipose tissue from obese compared to lean women. Additionally, in subcutaneous adipose tissue, their expression was lower in obese compared to overweight women. Compared to lean and overweight subjects, RXR α (Figure 3E) and SREBP1c (Figure 3F) mRNA expression in obese subjects was significantly lower in both omental adipose tissue.

LXR α (Figure 4A), PPAR δ (Figure 4B), PPAR γ (Figure 4C) and RXR α (Figure 4D) were significantly lower in subcutaneous but not omental adipose tissue, from GDM compared to NGT women. There was no effect of GDM on PPAR α and SREBP1c gene expression in both tissues (data not shown).

STUDY 2: Effect of adipokines on lipid metabolism in pregnant adipose tissue

The final aim of this study was to determine of adipocytokines, which are increased in insulin resistant states, on lipid metabolism on pregnant adipose tissue. Due to sample size limitations, only omental adipose tissue was used these studies. Firstly, qRT-PCR was performed to determine the profile of TNF α , IL-1 β and leptin in adipose tissue from lean versus obese women, and NGT versus GDM women. As shown in Figure 5A, when compared to lean pregnant women, adipose tissue IL-1 β and leptin gene expression was significantly higher in obese women. TNF- α levels were also higher, however, these just failed to reach significance. In adipose tissue obtained from women with GDM, only leptin mRNA expression was significantly higher compared to NGT women (Figure 5B).

Tissue explants were then performed to determine the effect of TNF α , IL-1 β and leptin on gene involved in lipid metabolism. Eight genes were selected for further analysis in this study, and the data are depicted in Figure 6. LPL, FATP2, FATP6 and ASCL1, PNPLA2, PPAR δ , PPAR γ and RXR α gene expression was significantly decreased by treatment with IL-1 β . Similar results were obtained for TNF α and leptin; however, significance was not achieved for FATP2 by treatment with leptin and FATP6 by TNF α treatment.

280 **DISCUSSION**

These studies extend previous work, demonstrating that insulin resistance [4, 5] is accompanied by defects in the uptake, synthesis and breakdown of lipids in adipose tissue of women with pre-existing maternal obesity and GDM. By examining well-matched control subjects in each case, contrasting gene expression patterns in adipose tissue of obese and GDM subjects were clearly demonstrate. Omental and subcutaneous adipose tissue obtained from obese pregnant women and women with GDM at the time of term Caesarean section demonstrated decreased lipid related genes for lipid uptake, storage, synthesis and mobilisation. Similar decreases were also observed in the presence of the adipokines TNF α , IL-1 β and leptin.

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LPL regulates lipid storage; it enables tissues to uptake the fatty acids released from circulating TAG. Under basal conditions, LPL levels are increased so as fatty acids are stored in adipose tissues. However, in situations where TAG-storage mobilisation is required, such as diabetes, LPL activity decreases [41]. Similar trends have been observed during gestation. It is thought that in early pregnancy, adipose tissue LPL activity is high which enables the storage of fatty acids for use later in pregnancy, when the fetal demand is greater. In contrast, during late gestation, maternal hyperinsulinemia and insulin resistance is associated with lower LPL expression; an adaptation that is required to meet the needs of the growing fetus [42]. Indeed, in rodents, adipose tissue LPL activity and mRNA expression is reduced during late gestation [43]. In obese pregnant women or
300 women with GDM, however, peripheral insulin resistance is even more pronounced [5] leading to increases in the circulating concentrations of fatty acids and lipids [2]. In this study, LPL expression was lower in obese pregnant women and women with GDM. This is consistent with others showing decreased adipose tissue LPL expression in GDM subjects [4].

In addition to LPL, intracellular lipid levels are also determined by a regulated import/export facilitating the transport of fatty acids across the cell membrane system. Proteins implicated in fatty acid uptake and intracellular transport includes FAT/CD36, FATPs, ACSL family members and plasma membrane FAPBs. In support of previous studies whereby the mRNA levels of FATP2 are lower in adipose tissue of insulin-resistant subjects [44], in this study, FATP2 and FATP6
 310 expression was lower in adipose tissue of obese pregnant women and women with GDM. There was no difference in the gene expression of any of the FABP suggesting that the intracellular trafficking of fatty acids is not different between the groups. The ACSL enzymes catalyse the activation of FA to their CoA derivatives, with ACSL1 contributing to 80% of total ACSL activity in adipose tissue [45, 46]. Acyl-CoAs have multiple fates including mitochondrial β -oxidation, incorporation into phospholipids, cholesteryl esters, and TAGs, and activation of transcription factors. ACSL1 also interacts with FATPs to facilitate fatty acid uptake [46], and ACSL1 knockdown stimulates expression of lipogenic genes [47]. In keeping with the data reported in this study, previous studies have reported lower levels of ACSL1 in adipose tissue of obese rats fed a high fat diet [48]. Collectively, these data suggests the shunting of fatty acids away from adipose tissue where they are
 320 stored as fat and towards placenta where they would be oxidised as fuel or transported to fetus and used for fetal growth and development. This may contribute to the dyslipidemia and increased fat deposition observed in infants of women with GDM and pre-existing maternal obesity [8, 49].

The de novo synthesis of long-chain saturated fatty acids is catalysed by FASN. FASN activity measurements and FASN mRNA levels are frequently used as markers of de novo lipogenesis, and changes in FASN activity are primarily due to alterations in the transcription rate of the FASN gene. Indeed, FASN gene expression is dysregulated in some pathological conditions such as obesity. **FASN mRNA** expression is decreased in the subcutaneous adipose tissue of obese compared to lean individuals [50-54] despite this group having increased hepatic lipogenesis
 330 compared with lean subjects [51]. FASN levels have also been shown to correlate inversely with

markers of insulin resistance including glucose, glucose, HOMA-IR and HbA1c [53, 55]. In this study, although there was no effect of obesity on FASN gene expression, GDM was associated with decreased FASN gene expression in both omental and subcutaneous adipose tissue. Indeed, previous studies in adipose tissue have shown that FASN mRNA expression is lower in insulin resistant subjects [55], whereas its expression is increased by insulin in cultured human adipocytes [56].

MGAT and DGAT catalyse two consecutive steps of enzyme reactions in the synthesis of TAGs; MGATs catalyse the synthesis of diacylglycerol (DAG) from monoacylglycerol (MAG) and DGATs convert DAG to TAG [55]. In this study, maternal obesity and GDM were associated with lower MGAT1, MAGT2 and or DAGT1 levels. Other studies have reported similar findings. For example, DGAT1 expression is strongly correlated with insulin sensitivity [54, 55] and its expression is lower in subcutaneous adipose tissue of subjects with IGT compared to NGT subjects [55]. In addition, fat cell lipogenesis in vitro has been shown to correlate strongly and positively with insulin sensitivity, both basally and after insulin stimulation [57]. Collectively, the downregulation of lipogenic gene expression observed in this study may be a physiological adaptation to help prevent uncontrolled lipid storage.

PNPLA2 and HSL are the two main lipases, responsible for 95% of the lipolysis in adipose tissue [58, 59]. The enzymatic breakdown of TAG is initiated by PNPLA2 and leads to the formation of DAG that are in turn hydrolysed by HSL leading to the formation of MAG and fatty acid. The final step of lipolysis is the hydrolysis of MAGs by MGLL, leading to the release of glycerol and fatty acid. Reduction of adipose tissue HSL expression and activity has been observed in obesity [60-62] and in subjects with type 2 diabetes [63]. ATGL protein expression is also reduced in subcutaneous adipose tissue of obese subjects [64]. Furthermore, fasting insulin levels and the degree of insulin resistance are negatively correlated with ATGL and HSL expression [65]. The data presented in this

study, of reduced HSL, MGLL and PNPLA2 expression in adipose tissue from women with GDM and/or obese pregnant women, are in concordance with these studies. It is possible that this reduced expression of lipolytic gene expression is a compensatory mechanism to prevent excessive fatty acid outflow from adipose tissue and or to prevent worsening of the insulin-resistant state.

SREBP1, PPAR, RXR and LXR are key transcription factors that play an important role in adipogenesis, insulin sensitivity, and fatty acid homeostasis [18, 66-68]. PPARs are the most widely studied, and their activation induces the expression of genes involved in fatty acid uptake, mobilisation and metabolism in adipose tissue [69-71]. In subcutaneous adipose tissue of obese pregnant women with GDM, PPAR γ mRNA expression is decreased compared to pregnant controls [4]. The data presented in the present study extend these findings, demonstrating the expression of LXR α , PPAR α , PPAR γ , PPAR δ , RXR α and SREBP1 are significantly lower in adipose tissue from obese pregnant women and or women with GDM.

The pro-inflammatory adipokines TNF α , IL-1 β and leptin are increased with advancing gestation [72-74], contributing to the insulin resistance evident during this time. Indeed, and in keeping with previous studies in non-pregnant tissues [75-77], my previous studies show that IL-1 β decreases glucose uptake in omental adipose tissue from pregnant women (unpublished). There is a wealth of data on adipokines regulating lipid metabolism in non-pregnant tissues [78-80]. For example, in adipose tissue, TNF α , IL-1 β and leptin decrease FATP and FAT/CD36 mRNA levels, and fatty acid uptake and de novo fatty acid synthesis [28, 29, 66, 78-81]. Similarly, in this study, TNF α , IL-1 β and leptin decreased the expression of genes associated with fatty acid uptake, storage and lipolysis. Thus, it is possible, that in both obese and GDM pregnancies, increased expression of adipokines in adipose tissue may contribute to the defects in insulin signaling and lipid metabolism associated with these conditions.

This study has a number of limitations which might restrict the interpretation of the results. First, GDM mothers were treated with insulin to lower their blood sugar levels. This could influence the results as previous studies from my laboratory have shown differences in components of the insulin signaling pathway in adipose tissue between controls and insulin-treated GDM subjects but also between diet-controlled and insulin-managed GDM pregnancies [5]. Second, due to limited sample size, neither protein expression levels nor lipolysis or flux of fatty acids directly was evaluated. Relative gene expression does not necessarily imply that the protein will be translated or functional; thus, future studies should measure both protein levels and activity of lipid metabolism genes. Of note, however, the majority of published studies, performed in non-pregnant tissues, have also only evaluated gene levels [4, 44, 61, 64, 71, 82]. Thirdly, all samples were collected at the time of term Caesarean section. Thus, whether the observed effects in gene expression are due to metabolic changes during pregnancy or present only during the time tissue sampling occurred is not known. Further to this, samples were collected after women fasted for at least 6 hours; it known that these genes are dynamically modulated during eating cycles [83-85]. Lastly, these results do not take into account the effect of gestational weight gain or fat mass. Nevertheless, this is the first study performed in humans which has identified gene expression changes, induced by GDM, in both omental and subcutaneous adipose tissue.

It is of note, that the obese women in this study had increased fasting and one hour glucose concentrations. Although the obese group does not meet clinical criteria for GDM, from a physiologic and mechanistic perspective it is feasible that the results comparing adipose tissue gene expression in lean versus obese are confounded by the differences in glucose homeostasis between these groups.

In conclusion, this study demonstrated the presence of abnormal expression of genes involve in lipid metabolism in both omental and subcutaneous adipose tissue from pregnant women with

GDM and pre-existing maternal obesity with insulin resistance. These studies are an extension of
410 further studies in human adipose tissue, where decreased expression of critical elements of insulin-
stimulated glucose uptake [4, 5] and of lipogenic pathway [4] are demonstrated in adipose tissue of
obese pregnant women and or women with GDM. Decreased expression of genes involved in
glucose [4, 5] and fatty acid uptake may be a mechanisms whereby FFAs and TAGs are directed
towards the placenta. Indeed, infants of women with pre-existing maternal obesity and GDM often
display dyslipidemia and have increased fat deposition even when they are average weight for
gestational age [8]. At the same time, decreased synthesis and mobilisation of FFA from adipose
tissue of obese and GDM pregnancies may be an adaptation to limit excess nutrient supply. The
molecular mechanisms responsible for the abnormalities are not known. However, both maternal
obesity and GDM are associated with increased expression of adipokines, which could potentially
420 be very important in the overall insulin resistance and excess substrate supply that drives maternal-
fetal energy transfer and increased neonatal adiposity.

DECLARATION OF INTEREST, FUNDING, AND ACKNOWLEDGEMENTS**Declaration of interest**

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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FIGURE LEGENDS**Figure 1. Effect of maternal obesity on genes involved in lipid metabolism in adipose tissue.**

Omental and subcutaneous adipose tissue was obtained from lean (n=10 subjects), overweight (n=8 subjects) and obese (n=10 subjects) pregnant women at the time of term Caesarean section. (A) LPL, (B) FATP2, (C) FATP6, (D) FABPpm, (E) ASCL1, (F) MGAT1, (G) MGAT2, (H) DGAT1, (I) PNPLA2, (J) HSL and (K) MGLL mRNA expression was normalised to actin mRNA expression. Fold change was calculated relative to lean, and data is displayed as mean $\pm$ SEM. * $P < 0.05$ vs. lean mRNA expression; # $P < 0.05$ vs. overweight mRNA expression (one way ANOVA). ND, not detected.

Figure 2. Effect of GDM on genes involved in lipid metabolism in adipose tissue.

Omental and subcutaneous adipose tissue was obtained from NGT (n=18 subjects) and GDM (n=18 subjects) pregnant women at the time of term Caesarean section. (A) LPL, (B) FATP2, (C) FATP6, (D) FABPpm, (E) ASCL1, (F) MGAT1, (G) DGAT1, (H) FASN, (I) PNPLA2, (J) HSL and (K) MGLL mRNA expression was normalised to actin mRNA expression. Fold change was calculated relative to NGT, and data is displayed as mean $\pm$ SEM. * $P < 0.05$ vs. NGT mRNA expression (t-test). ND, not detected.

Figure 3. Effect of maternal obesity on gene expression of transcription factors involved in lipid metabolism in adipose tissue.

Omental and subcutaneous adipose tissue was obtained from lean (n=10 subjects), overweight (n=8 subjects) and obese (n=10 subjects) pregnant women at the time of term Caesarean section. (A) LxR α , (B) PPAR α , (C) PPAR δ , (D) PPAR γ , (E) RxR α and (F)

SREBP1c mRNA expression was normalised to actin mRNA expression. Fold change was calculated relative to lean, and data is displayed as mean $\pm$ SEM. * $P < 0.05$ vs. lean mRNA expression; # $P < 0.05$ vs. overweight mRNA expression (one way ANOVA).

Figure 4. Effect of GDM on gene expression of transcription factors involved in lipid metabolism in adipose tissue.

Omental and subcutaneous adipose tissue was obtained from NGT (n=18 subjects) and GDM (n=18 subjects) pregnant women at the time of term Caesarean section. (A) LxR β , (B) PPAR δ , (C) PPAR γ and (D) RxR α mRNA expression was normalised to actin mRNA expression. Fold change was calculated relative to NGT, and data is displayed as mean $\pm$ SEM. * $P < 0.05$ vs. NGT mRNA expression (t-test).

Figure 5. Effect of maternal obesity and GDM on adipokine gene expression in omental adipose tissue.

(A) Omental and subcutaneous adipose tissue was obtained from lean (n=10 subjects) and obese (n=10 subjects) pregnant women at the time of term Caesarean section. TNF α , IL- β and leptin mRNA expression was normalised to actin mRNA expression. Fold change was calculated relative to lean, and data is displayed as mean $\pm$ SEM. * $P < 0.05$ vs. NGT mRNA expression (t-test). (B) Omental adipose tissue was obtained from NGT (n=18 subjects) and GDM (n=18 subjects) pregnant women at the time of term Caesarean section. TNF α , IL- β and leptin mRNA expression was normalised to actin mRNA expression. Fold change was calculated relative to NGT, and data is displayed as mean $\pm$ SEM. * $P < 0.05$ vs. NGT mRNA expression (t-test).

Figure 6. Effect of adipocytokines on genes involved in lipid metabolism in adipose tissue.

Human omental adipose tissue was incubated in the absence or presence of 10 ng/ml TNF α , 5 ng/ml IL-1 β or 20 ng/ml leptin for 20 h (n=6 subjects). (A) LPL, (B) FATP2, (C) FATP6, (D) ASCL1, (E) PNPLA2, (F) PPAR δ , (G) PPAR γ and (H) RXR α mRNA expression was normalised to actin mRNA expression. Fold change was calculated relative to basal, and data is displayed as mean $\pm$ SEM. * $P < 0.05$ vs. basal (paired-sample comparison).

Table 1A. Characteristics of the study group

	NGT Lean (n=10)	NGT Overweight (n=8)	NGT Obese (n=10)
Maternal age (years)	30.8 ± 1.7	33.4 ± 1.5	34.5 ± 0.9
Maternal BMI at 12 wks (kg/m ²)	21.8 ± 0.5 [§]	27.5 ± 0.5 ^{*§}	36.2 ± 1.3 [*]
Maternal BMI at delivery (kg/m ²)	26.1 ± 0.5 [§]	31.0 ± 0.6 ^{*§}	42.5 ± 1.7 [*]
Gestational age at birth (weeks)	38.7 ± 0.2	39.2 ± 0.3	38.7 ± 0.2
Fetal birth weight (g)	3308 ± 127	3471 ± 168	3714 ± 148
Fasting plasma glucose (mmol/l)	4.2 ± 0.1 [§]	4.5 ± 0.1	4.8 ± 0.1 [*]
1 hour plasma glucose (mmol/l)	6.2 ± 0.5 [§]	7.0 ± 0.5	8.1 ± 0.4 [*]
2 hour plasma glucose (mmol/l)	5.8 ± 0.4	5.8 ± 0.5	6.6 ± 0.3

Values represent mean ± SEM

* $P < 0.05$ vs. lean (one way ANOVA)

§ $P < 0.05$ vs. obese (one way ANOVA)

Table 1B. Characteristics of the study group

	NGT (n=18)	GDM (n=18)
Maternal age (years)	34.0 ± 0.8	33.4 ± 1.0
Maternal BMI at 12 wks (kg/m ²)	32.3 ± 1.3	34.1 ± 1.3
Maternal BMI at delivery (kg/m ²)	37.1 ± 1.7	36.4 ± 1.0
Gestational age at birth (weeks)	38.9 ± 0.2	38.4 ± 0.4
Fetal birth weight (g)	3606 ± 112	3340 ± 140
Fasting plasma glucose (mmol/l)	4.7 ± 0.1	5.2 ± 0.1*
1 hour plasma glucose (mmol/l)	7.6 ± 0.3	10.8 ± 0.5*
2 hour plasma glucose (mmol/l)	6.3 ± 0.3	9.2 ± 0.4*

Values represent mean ± SEM

* $P < 0.05$ vs. NGT (t-test)

FIGURE 1

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FIGURE 5

FIGURE 6

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