Fatty acid metabolism and adipocyte function in healthy and gestational diabetes mellitus pregnancy

Amaal Faraj Alrehaili

MBBS MSc

Submitted in fulfilment of the requirement for the degree of Doctor of Philosophy

Institute of Cardiovascular & Medical Sciences
College of Medical, Veterinary & Life Sciences
University of Glasgow

March 2020

Abstract

Gestational diabetes mellitus (GDM) is defined as a glucose intolerance of varying severity with first recognition during pregnancy. The prevalence of GDM is increasing worldwide, largely attributable to the dramatic rise in maternal obesity, resulting in several maternal and fetal complications. The maternal metabolic adaptation during pregnancy is challenged by maternal obesity, resulting in β cell dysfunction and exaggerated insulin resistance in women with GDM. However, the exact cellular mechanisms involved in the development of GDM are not yet completely understood. Increasing evidence from clinical and experimental studies has suggested that adipose tissue dysfunction could be one of the underlying mechanisms for the metabolic abnormalities observed in women with GDM. However, most of the published literature on this topic has been focused on plasma measures of adipocyte-derived adipokines and how they are linked to insulin resistance. Functional measures of adipocytes, such as adipocyte size, lipolysis, insulin sensitivity and direct measurement of adipocyte secretory function, have not been widely studied in GDM. Failure to efficiently increase the adipocyte cell number (hyperplasia) rather than increase size (hypertrophy) in order to store excess free fatty acids (FFA), and the subsequent failure to suppress adipocyte lipolysis when FFA demands are low, is believed to be a key mechanism in the development of type 2 diabetes in the non-pregnant. Similarly, this could be a major candidate pathway for pregnancy complicated by GDM, leading to the observed higher plasma FFA and higher plasma pro-inflammatory cytokine concentrations, which may result from exaggerated adipocyte insulin resistance and inflammation. Therefore, the hypothesis tested in this thesis was that GDM results from defective expansion of SAT adipocytes, resulting in adipocyte hypertrophy. Subsequently, there is increased adipocyte lipolysis and inflammatory adipokine secretion.

The aim of this thesis was, firstly, to explore the epidemiological evidence for the role of maternal obesity in the development of GDM and other maternal and fetal complications, specifically in the highly diverse local Greater Glasgow and Clyde population. Data from the Scottish Morbidity Record 2 (SMR02) and the Scottish Care Information – Diabetes Mellitus (SCI-diabetes) databases for pregnant women between 2010 and 2015 was combined, and the prevalence of maternal obesity and GDM established among 38,178 births. The associated risks for several adverse pregnancy outcomes among women with maternal obesity and GDM in the Greater

Glasgow and Clyde population were calculated. It was found that in the local population maternal obesity and GDM were at higher prevalence (22.3% and 2.2%, respectively) compared to recent historical studies, and are associated with an increase in the incidence of a range of adverse pregnancy outcomes. Furthermore, the resulting adverse maternal and fetal complications of both first trimester maternal obesity and GDM were likely to be a considerable burden on clinical resources. Assessment of the long-term implications of maternal and fetal complications secondary to GDM and maternal obesity was not included, and a prospective follow-up analysis of this cohort is recommended.

The second aim of this thesis was to determine the evidence for the role of failure of adipocyte expansion in the development of underlying metabolic abnormalities in women with GDM. Several functional measures of adipocyte expansion were assessed, including adipocyte size, lipolytic function, adipokine secretion, and the expression of genes involved in adipocyte physiology and biochemistry. Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) biopsies from non-labouring women with GDM (n=22) and healthy (n=22) BMI-matched controls, undergoing elective caesarean section, were collected. Maternal blood was collected prior to delivery and maternal phenotyping was carried out by the assessment of plasma glucose, insulin, plasma lipids (triglyceride, cholesterol, glycerol and non-esterified fatty acids), pregnancy hormones (estradiol and progesterone), liver enzymes (gamma-glutamyl transferase, alanine aminotransferase and aspartate aminotransferase), plasma inflammatory cytokines and oxidised low-density lipoprotein (oxLDL), a biomarker of lipotoxicity. Maternal BMI at booking was recorded from the patient notes, along with other demographic information. Fetal weight and sex were recorded after the baby was delivered. Adipocyte isolation and sizing were carried out. The *ex vivo* lipolytic activity (basal and β adrenergic- stimulated lipolysis, and insulin suppression of lipolysis) and adipokine production (basal and lipopolysaccharide stimulated conditions) were assessed in isolated adipocytes. The adipocyte expression of genes involved in adipocyte differentiation, lipid storage, lipid and glucose metabolism and angiogenesis were also performed by RT-qPCR.

In GDM, VAT adipocytes had higher mean adipocyte diameter ((control) 62.9[3.8] vs (GDM) 75.5[11.6] μm, p=0.004) and volume ((control) 0.00007[0.00000002] vs (GDM) 0.00012[0.0000005] mm^3, p=0.003) compared to controls. VAT adipocytes

from women with GDM had higher basal lipolysis compared to controls (0.02[0.02] vs 0.07[0.07] glycerol mmol/hr/ug of DNA, p=0.001). The fat cell insulin sensitivity index (FCISI), a measure of adipocyte insulin suppression of lipolysis, was six times lower in the VAT adipocytes of women with GDM, but failed to reach statistical significance ((control) 62[204] vs (GDM) 35[52] FCISI, p=0.61). In contrast, there were no differences observed in SAT adipocytes with regard to adipocyte size, lipolysis and FCISI between the two groups. Hypertrophic expansion of VAT adipocytes may be one of the contributors to the increased basal lipolysis rate seen in this depot. This will favour portal release of fatty acids, and could be an important factor in triggering metabolic abnormalities associated with liver fat accumulation in obese pregnant women. Thus, dysfunctional VAT adipocytes may represent an important event contributing to the emergence of metabolic dysfunction in women with GDM.

The study of isolated VAT adipocyte adipokine secretion in basal or activated (LPS-stimulated) conditions in a subset of healthy and GDM women did not show any differences in VAT adipocyte adipokine release in GDM compared to controls. However, these results should be interpreted with caution, because of the small sample size. Therefore, further tissue collection is recommended in order to increase the power of the study. Further investigation of macrophage and other immune cell contribution to adipose tissue inflammation in women with GDM is warranted. Isolated adipocyte gene expression analysis in both SAT and VAT showed that women with GDM had significantly higher insulin receptor (INSR) expression in both SAT (9.0(3.1) vs 11.7(3.7) *INSR* percentage expression relative to *PPIA*, p= 0.031) and VAT (10.8(3.6) vs 15.8(5.6) *INSR* percentage expression relative to *PPIA*, p= 0.022) compared to controls. There were no differences in the expression of other genes involved in adipocyte differentiation, lipid storage, lipid and glucose metabolism and angiogenesis in isolated SAT and VAT adipocytes for women with GDM compared to controls.

The final aim was to assess the role, in early pregnancy, of very low-density lipoprotein (VLDL) as a potential maternal plasma carrier of the extremely important long chain polyunsaturated fatty acid (LC-PUFA) docosahexaenoic acid (DHA), required by the fetus for neuronal development. During pregnancy, there is a three-fold increase in VLDL synthesis by the liver. The liver is the primary site for *de novo* LC-PUFA synthesis, which is shown to be increased in early pregnancy; therefore,

VLDL could be the carrier of DHA at early pregnancy. Infants born to mothers with GDM had lower DHA levels, and were shown to have lower cognitive performance, partly attributed to lower placental transfer of DHA in GDM. However, the underlying mechanism for defective DHA metabolism and transport in GDM pregnancy is not fully understood. Using an archival plasma collection of women undergoing frozen embryo transfer (FET), fasting blood samples were collected at approximately day 10 following the last menstrual period (LMP) (pre-luteinizing hormone (LH) surge), and on days 18, 29 and 45 post-LH surge from women who were successful in getting pregnant (n= 27). VLDL FA composition was assessed by gas chromatography in women who were successful in getting pregnant and women who were not. There was no evidence for VLDL being the main carrier of DHA at the critical time of neuronal tube closure, as DHA concentration in VLDL and VLDL DHA enrichment was unchanged over time. A reduction in VLDL particle number (measured by apo-B concentration) by 18 days post-LH surge was observed ($p<0.001$), which was then recovered to pre-LH surge level by 45 days post-LH surge. It is likely that DHA is carried by other lipoproteins such as HDL, as is observed in the non-pregnant population. Further investigation of the main lipoprotein carrier for DHA during pregnancy, and the study of its metabolism and transport in GDM pregnancy, is needed.

In conclusion, GDM and maternal obesity are associated with increased risk of adverse pregnancy outcomes. Effective intervention strategies are required for weight control prior to pregnancy. The data presented in this thesis provides evidence that in GDM, VAT adipocytes expand in a hypertrophic manner, and have increased basal lipolysis. Hypertrophic expansion of VAT adipocytes may be linked to systemic insulin resistance. Thus, defective VAT adipocyte expansion might have a role in the underlying pathophysiology of GDM. Further studies on the role of adipocyte differentiation and ectopic fat storage in normal and complicated human pregnancy are warranted. DHA is not transported by VLDL in early pregnancy. Further investigation of the main carrier of DHA during pregnancy, and the study of DHA metabolism in pregnancies complicated by GDM, is needed.

Table of Contents

List of Tables

List of Figures

List of publications

Published abstracts

Alrehaili, A., Freeman, D., Lindsay, R., Increased risk of gestational diabetes, caesarean delivery and large for gestational age infants among overweight and obese women in Greater Glasgow and Clyde “Presentation Abstracts”, *International Journal of Obesity* 8 (2018):14-60.

Alrehaili, A., Anderson, M., Hagan, M., Lindsay, R., and Freeman, D., In gestational diabetes mellitus, maternal third trimester visceral adipocytes are hypertrophic with enhanced basal lipolysis and isoproterenol stimulation of lipolysis “Obesity Facts”, *The European Journal of Obesity* (2019), vol. 12, suppl. 1.

Manuscript in process

Nicola Zamai, Colin H. Cortie, Eleanor M. Jarvie, Christopher C. Onyiaodike, Amaal Alrehaili, Monique Francois, Dilys J. Freeman and Barbara J. Meyer. In pregnancy, maternal high density lipoprotein is enriched in docosahexaenoic acid, but not arachidonic acid, and carries the largest fraction of these fatty acids in plasma

List of presentations

Oral presentations

In gestational diabetes mellitus, maternal third trimester visceral adipocytes are hypertrophic with enhanced basal lipolysis and isoproterenol stimulation of lipolysis. 26th European congress on obesity, Glasgow, May 2019

Increased adipocyte diameter and upregulated *CIDEC* expression in visceral adipocytes from mothers with gestational diabetes mellitus. Adipose tissue discussion group, Edinburgh, December 2018.

Adipose tissue function in gestational diabetes. 2nd Glasgow Pregnancy Research Symposium. Glasgow, February 2017.

Poster presentations

Increased risk of gestational diabetes, caesarean delivery and large for gestational age infants among overweight and obese women in Greater Glasgow and Clyde. 5th UK congress on obesity (UKCO), Newcastle, September 2018

Maternal VLDL composition in early pregnancy and its relationship to estradiol and progesterone. World Obesity federation, Belgium, Leuven, November 2017

This thesis is dedicated to the soul of my father, Mr. Faraj Abdullah Alrehaili, who sadly passed away one year before our dream comes true, from him I learned how to turn my pain into drive and made it to submission of this thesis.

To my father,

With gratitude for his inspiration, love and support.

Acknowledgement

It is with the upmost gratitude that I would like to acknowledge my supervisor Dr.Dilys Freeman and Dr.Robert Lindsay, for offering the exceptional guidance and support needed to compete this body of work. Without their invaluable advice, I would not have completed my thesis.

I would like to thank Prof. Barbra Myer and her team from Wollongong University in Australia for their assistance and support to conduct our collaborative research. I would like to thank Dr.Martin McBride for his help and guidance to carry the Ingenuity Pathway analysis. Dr.Lesely Graham generosity and assistance has been essential to carry out the multiplex analysis. Additionally, I would like to thank Mrs. Fiona Jordan for help and guidance to conduct several lab techniques and her unwavering support whenever needed. I would like to thank our research group members, including Fiona Jordan, Wan Noraini, Xuan Gao andOom Patamat, we have worked together for past few years and I have learned so much from each one of you.

I would like to present my sincere appreciation to the midwifes in recovery unit at QEUH for their cooperation and support when needed to carry out the patient recruitment of this study. Special thanks to pathology lab personnel at QEUH who supported me in using their lab facility to process and store the blood samples. Many thanks are deservedly due to all the study participants who made the project worth doing. Special thanks go to Maria Hagan, Melisa Anderson, Kirsten Mitchell, Fiona Currie and Scott McCoull, the lovely student participated in several parts of my project.

I would like to thank Taibah University and Saudi Arabian Ministry of Education, who funded this project.

Special thanks to my husband, Abdulmajeed Albalawi, who has taught me the importance of working and living passionately through example. His unwavering support has allowed us to share our successes and affords me the ability to fully devote myself to my scientific research. Thank you to my mother, sisters and brothers, who are never hesitant to listen and offer help and encouragement. My mother’s fortitude, dedication, and ingrained compassion inspire me to strive for the

incredible; for this I am most grateful. I would also like to thank my mother- and father- and sisters- in-law for their love and support, welcoming me into their family, and teaching me the true meaning of success. Lastly, I would like to express my gratitude to my lovely beautiful kids Battal, Bassam and the little one who did not show appearance yet, you are my biggest supporters and advocates to live this life truly with love and passion.

Author's Declaration

The contents of this thesis have not been submitted elsewhere for any other degree, diploma or professional qualification.

This thesis has been written by me, and unless otherwise acknowledged I have been responsible for epidemiological data analysis, patient recruitment and consent, adipose tissue and blood samples collection and processing, adipocyte function studies including adipocyte sizing, lipolysis, adipokine secretion using multiplex technique and adipocyte DNA extraction, plasma ELISA technique and VLDL isolation for FA metabolism in early pregnancy study.

I would therefore like to acknowledge the contributors below for their assistance in the laboratory techniques used. Plasma cholesterol, triglycerides, glucose, insulin, C-Reactive Protein, non-esterified fatty acids, Alanine Aminotransferase, Aspartate aminotransferase and Glutamyl transferase for plasma samples of adipocyte function in GDM participants and the analysis of VLDL lipoprotein fraction triglycerides, phospholipid, total cholesterol, free cholesterol, cholesteryl ester and apo-B for FA metabolism in early pregnancy study were performed by Josephine Cooney from the University of Glasgow.

Maria Hagan, Melisa Anderson, Fiona Currie, Scott McCoull contributed to the overall study of gene expression analysis for adipocyte function in GDM study.

Kirsten Mitchell conducted the phenformin and metformin dose response curve (this data is not presented in this thesis).

Fiona Jordan from institute of cardiovascular and medical sciences University of Glasgow carried out the fatty acid extraction for VLDL samples of FA metabolism in early pregnancy study.

Nicola Zamai form Wollongong University, Australia carried out gas chromatography for VLDL samples of FA metabolism in early pregnancy study.

Amaal Faraj Alrehaili, March 2020

List of Abbreviations

AC	Adenyl cyclase
ACS	acyl-CoA synthase
ADMSC	Adipose tissue mesenchymal stem cells
AMPK	AMP-protein kinase
ATGL	Adipose triglyceride lipase
BMI	Body mass index
cDNA	Complementary deoxyribonucleic acid
CETP	Cholesteryl ester transfer protein
CRP	C- reactive protein
DAG	Diacylglycerides
DBP	Diastolic blood pressure
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
ER	Endoplasmic reticulum
FA	Fatty acid
FABP	Fatty acid binding protein
FATP	Fatty acid transport protein
FCISI	Fat cell insulin sensitivity index
FFA	Free fatty acid
GDM	Gestational diabetes mellitus
GLUT4	Glucose transporter 4
HDL	High density lipoprotein

HSL	Hormone sensitive lipase
HTN	Hypertension
IADPSG	International Association of Diabetes and Pregnancy Study Groups
LC-PUFA	Long chain polyunsaturated fatty acid
LD	Lipid droplet
LDAP	Lipid droplet-associated proteins
LGA	Large for gestational age
LPL	Lipoprotein lipase
LPS	Lipopolysaccharide
MAG	Monoacylglyceride
NEFA	Non-esterified fatty acid
NICU	Neonatal intensive care unit
oxLDL	oxidised low density lipoprotein
PE	Pre-eclampsia
PKA	Protein kinase A
PLIN1	Perilipin 1
RNA	Ribonucleic acid
ROS	Reactive oxidative stress
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
sdLDL	Small dense low density lipoprotein
SVF	Stromal vascular fraction

T2DM	Type 2 diabetes mellitus
TAG	Triacylglycerides
TLR-4	Toll-like receptors 4
TNF-α	Tumour necrosis factor alpha
UPR	Unfolded protein response
VAT	Visceral adipose tissue
VEGF	Vascular endothelial growth factor
VLDL	Very low density lipoprotein
WAT	White adipose tissue
WHO	World health organization

Chapter 1 Introduction and Literature Review

1.1 Maternal obesity

Rapidly increasing rates of obesity worldwide represent a major health concern. Maternal obesity is a well-established risk factor for adverse pregnancy outcomes such as gestational diabetes (GDM), preeclampsia (PE), miscarriage and Caesarean section. Obesity can be classified by BMI into three classes, according to World Health Organization (WHO) criteria: obese class 1 (30–34.99 kg/m²), obese class 2 (35–39.99kg/m²) and obese class 3 (≥ 40 kg/m²) (World Health Organization, 2000). In the United Kingdom, there are no population-wide data that directly estimates maternal BMI just before pregnancy. In Scotland, a study examining maternal obesity using a national dataset from 1981 to 2012 reported a 21.1% prevalence of maternal obesity (Collier et al., 2017). Another observational study of the Scottish population for all singleton pregnancies between 2003 and 2010 reported a 19.6% prevalence of maternal obesity (Denison et al., 2014). In England in 2010, the incidence of first-trimester maternal obesity has doubled over the last 19 years since 2010 from 7.6% to 15.6% (Heslehurst et al., 2010a). Another study examined the trends of obesity among the Glasgow population in 1990 compared to 2002-2004 and found there was a significant increase in the proportion of women who were obese (BMI> 30 kg/m^2) at booking, from 9.4% to 18.9%. This trend of increasing maternal obesity is a warning sign, and this issue needs to be addressed in order to avoid serious implications for obstetric care.

Maternal obesity has adverse outcomes for both mother and baby, and it can lead to a repeated cycle of metabolic risk between generations (O'Reilly and Reynolds, 2013). Obese mothers are at higher risk of type 2 diabetes mellitus (T2DM) development later in life (Kim et al., 2002). The offspring of obese mothers are at higher risk of childhood obesity via programming effects, as reviewed by Freeman (2010). The increased prevalence of maternal obesity is associated with an observed increase of pre-existing conditions among mothers, such as T2DM and chronic hypertension (HTN), which could potentially increase fetal and maternal adverse outcomes. Pre-existing T2DM increases the risk of congenital malformation, pregnancy loss, fetal macrosomia (Inkster et al., 2006) and poor maternal outcomes, such as PE, caesarean delivery and retinopathy progression (Morrison et al., 2016a, Morrison et al., 2016b). Pre-existing chronic HTN increases

the risk of preeclampsia, caesarean section and preterm delivery (Chappell et al., 2008).

Pre-pregnancy, obese women are more likely to suffer from infertility than normal-weight women (Gesink Law et al., 2006). This can be related to ovulatory dysfunction due to increased insulin resistance (Chavarro et al., 2007). In early pregnancy, obese mothers had a 30% increased chance of having a miscarriage (Marchi et al., 2015). Maternal obesity is associated with increased risk of congenital malformations, such as neural tube defects and cardiovascular, orofacial and limb anomalies (Marchi et al., 2015).

From mid-pregnancy onwards, obese women are more likely to develop complications such as GDM and PE. Obese mothers have four- to nine-fold increased risk of developing GDM during pregnancy (Poston et al., 2016), and this risk is linearly related to obesity class (Kim et al., 2016). Both maternal obesity and GDM are associated with maternal and fetal adverse outcomes, such as large for gestational age babies (LGA), macrosomia, shoulder dystocia, caesarean delivery and NICU admission. In a study assessing pregnancy outcomes in GDM mothers by obesity class, mode of treatment and glycaemic control, pregnancy outcomes were found to be compromised, independent of all other factors (Yogev and Langer, 2008). Several studies highlighted the importance of gestational weight gain over pre-pregnancy BMI regarding the risk of LGA babies in GDM obese mothers (Santos et al., 2016b, Santos et al., 2016a).

PE is a hypertensive disorder during pregnancy. It is considered to be the leading cause of maternal and fetal morbidity and mortality, occurring in approximately 1–8% of pregnancies. Obesity is a risk factor for PE. An obese mother has 3–10 times increased risk of developing PE compared to normal pregnant women (Marchi et al., 2015). PE presents risk of convulsion, kidney failure, liver failure and death for the mother, and premature delivery and intra-uterine growth restriction (IUGR) for the baby. An increase in pre-pregnancy BMI is associated with a higher risk of developing PE. Compared with women with a BMI of 21, the risk of preeclampsia doubles at a BMI of 26 (OR 2.1), triples at a BMI of 30 (OR 2.9), and increases further with severe obesity (OR 3.5) (Bodnar et al., 2005). Furthermore, women with higher BMI were found to be at risk of developing severe PE (Stone, 1994).

1.2 Gestational diabetes – the clinical problem

GDM was defined by the WHO as ‘glucose intolerance resulting in hyperglycaemia of variable severity with first onset during pregnancy’. This definition applies even if this condition continues after pregnancy and does not exclude the possibility that glucose intolerance could be undiagnosed at the start of pregnancy. By contrast, the American Diabetes Association (ADA) recently redefined GDM as ‘diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes’ (American Diabetes Association, 2015). Therefore, women with glucose intolerance in the first trimester are classified as T2DM. The global prevalence of diabetes mellitus is increasing rapidly worldwide, affecting people at different stages in life. In 2010, an estimated 285 million people in the world had T2DM (Shaw et al., 2010). This number is expected to rise in 2030 to reach 439 million, making T2DM one of the most critical public health challenges, and a major burden on health care systems worldwide. GDM risk factors include family history of T2DM, ethnicity, maternal age, obesity, and past history of GDM and macrosomia. Gestational diabetes is a heterogeneous disorder and has adverse maternal and fetal consequences. The majority of β-cell dysfunction observed in GDM is due to a pathophysiology similar to that observed in T2DM. GDM prevalence figures are affected by the geographic population, GDM screening intensity, and the employed diagnostic criteria. A recent review of global estimates of GDM suggested that the Middle East and North Africa had the highest prevalence, with median estimates of 12.9%, whereas Europe had the lowest estimates of 5.8% (Zhu and Zhang, 2016). Recent analysis showed that the prevalence of GDM among the Scottish population was only 1.9%.

There are two common screening approaches in use: universal screening or selective screening at mid-pregnancy (24–28 weeks of gestation). Early screening is recommended for high-risk individuals in order to rule out pre-existing diabetes and prevent possible fetal and maternal complications. The Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study was conducted in response to the need for internationally agreed diagnostic criteria for GDM, based upon their predictive value for adverse pregnancy outcomes. Based on this observational study, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) (Metzger et al., 2010) proposed a universal screening method using a 75g glucose load test at 24–28 weeks of gestation. Increases in each of the three

values (fasting blood glucose ≥ 5.1, 1 hour ≥ 10.0, 2 hours ≥ 8.5 mmol/L) on the 75g, 2-hour oral glucose tolerance test were associated with graded increases in the likelihood of pregnancy outcomes, such as LGA, caesarean section, high fetal insulin levels and increased neonatal fat content. A diagnosis of GDM can be made if one result of blood glucose levels reaches one or more of the previously mentioned values. These new criteria resulted in an 18% increase in GDM prevalence. The widespread use of the IADSPG criteria would result in nearly 1 in 5 pregnant women being classified as GDM (Metzger et al., 2010). However, the implementation of these new criteria to diagnose GDM has not yet been proven to be effective in terms of cost-effectiveness. Recent studies (Welch, 2011, Chen et al., 2009) suggested that the use of a two-step approach (first a non-fasting 50g glucose challenge test, to be followed, if positive (7.8 mmol/L), by a fasting 100g oral glucose tolerance test (OGTT)) is more cost-effective. Another study (Werner et al., 2012) suggested that IADPSG criteria could be more effective if post-partum care and monitoring were included in the cost-effectiveness analysis. The IADPSG criteria were adopted by the WHO, the American Diabetes Association (ADA) (2011), and the International Federation of Gynaecology and Obstetrics (FIGO), but not by the American College of Obstetricians and Gynecologists (ACOG) (2013), or the National Institute for Health and Care Excellence (NICE) (Bilous, 2015) (Table 1-1). In the UK, NICE proposed another criterion based on health economic analysis, using a wide range of glucose thresholds, and published new clinical guidelines in 2015. The NICE analysis did not support the cost-effectiveness of IADPSG criteria (Bilous, 2015). In the NICE guidelines, GDM is diagnosed if: fasting blood glucose (FBG) ≥ 5.6 mmol/L, or 2-hour glucose after a 75g OGTT ≥ 7.8 mmol/L. The Scottish Intercollegiate Guidelines Network (SIGN), in their 2010 recommendations, advised screening with clinical risk factors for GDM at booking, with HBA1c or fasting glucose (The Scottish Intercollegiate Guidelines network, 2010). At 24–28 weeks of gestation, all high-risk women should undergo a 75-g OGTT with the IADPSG criteria used for diagnosis, and all low-risk women should undergo the fasting plasma glucose testing.

Table 1-1 Different international guidelines for gestational diabetes screening and diagnosis

Current criteria	IADPSG, 2010 (Metzger et al., 2010)	WHO, 2013	ADIPS, 2013 (Nankervis et al., 2014)	SIGN, 2010	NICE, 2015 (Thompson et al., 2013)	ACOG*, 2013 (Obstetricians and Gynecologists, 2013)
Initial screen	Universal	Universal	Universal	Risk factors	Risk factors	Universal
Biochemical test	75g OGTT	75g OGTT	75g OGTT	75g OGTT	75g OGTT	1- 50 g GCT≥ 7.8 or 7.5 2- 100 OGTT
Diagnostic threshold for GDM (mmol/L) fasting	≥ 5.1	≥ 5.1	≥ 5.1	≥ 5.1	≥5.6	5.3 or 5.8
1 hour	≥ 10	≥ 10	≥ 10	≥ 10	N/A	10 or 10.6
2 hours	≥ 8.5	≥ 8.5	≥ 8.5	≥ 8.5	≥7.8	8.6 or 9.2
3 hours	N/A	N/A	N/A	N/A	N/A	7.8 or 8.0
Required abnormal value	1	1	1	1	1	2

IADPSG – International Association of Pregnancy Study Group; WHO – World Health Organization; ADIPS – Australian Diabetes in Pregnancy Society; SIGN - Scottish Intercollegiate Guidelines Network; NICE – National Institute for Health and Clinical Excellence; ACOG – American College of Obstetricians and Gynecologists. ACOG endorsed either the National Diabetes Data Group (NDDG) or Coustan and Carpenter criteria.

There are several adverse outcomes associated with GDM. The many differences in the reported associations of GDM with adverse outcomes are due to the lack of universal agreement on GDM screening, diagnostic criteria, and the best methodology to screen or diagnose GDM. In GDM, there are increased risks of perinatal mortality, macrosomia, birth trauma, shoulder dystocia, and metabolic and cardiovascular complications in untreated GDM. A retrospective study suggested that unrecognised GDM is an independent risk factor for perinatal morbidity after controlling for confounders, and that dietary control of GDM decreases perinatal morbidity (Adams et al., 1998). A recent meta-analysis (Horvath et al., 2010), including two of the most important randomised controlled trials – the Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) (Crowther et al., 2005) and the Maternal Fetal Medicine Unit Network (MFMU) (Landon et al., 2009) – showed that the pregnancy outcomes improved by treating GDM are a reduction in macrosomia (OR 0.38), large for gestational age infants (LGA) (OR 0.48) and shoulder dystocia (OR 0.40).

The adverse consequences of GDM for mothers are mainly the development of T2DM post-pregnancy, preeclampsia and caesarean delivery. Studies have suggested that up to 50% of GDM mothers developed overt T2DM, with the highest occurrence rate in the first five years following delivery (Bellamy et al., 2009). Other factors such as glycaemic status during pregnancy, gestational weight gain, and obstetric complications such as PE are known to influence the future risk of diabetes (Kwak et al., 2013). A recent systematic review and meta-analysis of 95,750 women showed that the future risk of diabetes is mainly influenced by gestational glycaemic status, and that both hypertensive disorders in pregnancy and preterm delivery in GDM pregnancies were associated with future onset of T2DM (Rayanagoudar et al., 2016a, Rayanagoudar et al., 2016b). Therefore, postnatal counselling of women with GDM regarding the risk of future diabetes should be individualised. Catalano et al. (1991) suggested that 50% of GDM mothers remained insulin-resistant following delivery. This suggests that GDM diagnosis is a good opportunity for intervention and prevention of T2DM, and slowing its epidemic progress.

Fetal outcomes are improved after treating GDM. Adequate glycaemic control enhances perinatal outcomes in GDM (Crowther et al., 2005). This can be achieved by the use of oral hypoglycaemic drugs such as metformin, and/or by the use of insulin to manage maternal blood glucose levels. Metformin is a second-generation

biguanide that has been shown to be effective in T2DM. Metformin was found to provide adequate glycaemic control in GDM mothers, resulting in reduced weight gain and lower frequency of neonatal hypoglycaemia. In a large randomised controlled trial (RCT) (Rowan et al., 2008), there was no difference between metformin and insulin in the level of glycaemic control and pregnancy outcomes. The degree of glycaemic control has a key role regarding the degree to which pregnancy outcomes in GDM are improved (Langer et al., 1994).

The matter of which criteria are the most clinically useful and cost-effective remains controversial. Some studies have attempted to examine pregnancy complications in women fulfilling different criteria in order to address this. However, there are few studies that have assessed the differences between IADPSG and NICE criteria in the same population. It is important to note that the populations of these studies are usually populations treated under one or other criteria, making interpretation more challenging. For example, a retrospective study (Meek et al., 2015) assessed the neonatal and obstetric outcomes in 25,543 live singleton births from a single centre in Cambridge, using IADPSG and NICE 2015 criteria for GDM diagnosis; GDM prevalence was 4.13% (NICE) and 4.62% (IADPSG). This study showed that 167 women tested negative using NICE criteria, but positive using IADPSG criteria. These women had a higher risk of LGA OR 3.12 (95% CI 2.44–3.98), caesarean delivery OR 1.44 (95% CI 1.15–1.81) and polyhydramnios OR 6.90 (95% CI 3.94–12.08) compared to healthy pregnant women. This study concluded that the IADPSG criteria identify women at higher risk of GDM complications, and the number of cases needed to treat one case of LGA is 5.8. A similar study conducted at a centre in Croatia included 4,646 women (Djelmis et al., 2016); GDM prevalence was 23.1% (IADPSG) and 17.8% (NICE). Women with FBG (5.1–5.5 m.mol/l) had higher risk of LGA OR 3.7 (95% CI 2.0–4.6) and caesarean delivery OR 1.8 (95% CI 1.3–2.3) compared to the control group. These women were at greater risk of maternal and neonatal adverse outcomes, and were not diagnosed by NICE criteria. One issue must be addressed when comparing the results of the previous two studies. In the Cambridge study, NICE criteria were used to diagnose GDM, which means that the IADPSG-positive women were untreated. The Croatia study was analysed in the time period when IADPSG criteria were used to diagnose GDM, which means that IADPSG-positive women were treated. This could possibly account for the high caesarean section rates in the Cambridge study. However, it is

difficult to reach conclusions with regard to use of the IADPSG and NICE criteria when each study used different selection criteria.

Ethnicity has long been recognized as a risk factor for GDM development. Several ethnicities were recognized to have a higher risk for GDM such as Hispanic, African, Native American, South and East Asian (Berkowitz et al., 1992). It is recommended that women from high risk group get screened for GDM as soon as feasible and to repeat at 24-28 weeks of gestation (International Association of Diabetes and Pregnancy Study Groups, 2010). Therefore, the regional prevalence of GDM varies from 10.4% in Africa to 24.2% in South Asia (Ogurtsova et al., 2017). Furthermore, the prevalence of GDM among various ethnic group varies considerably. Sanchalika and Teresa (2015) showed in a large retrospective study that among South Asian women living in New Jersey, Bangladeshi women had highest rate of GDM followed by Indians, Sri Lankans and Pakistanis. There are also differences in GDM pregnancy outcomes between different ethnicities. It was reported previously that South Asian women had lower preterm birth and LGA rates than white women, but higher small for gestational age infants (Sanchalika and Teresa, 2015).

1.3 Maternal metabolic adaptation to pregnancy in healthy, obese and GDM pregnancy

1.3.1 Healthy pregnancy

In early pregnancy, there is an increase in maternal lipogenesis and fat accumulation, which was found to account for most of the conceptus-free weight gain (Herrera, 2002b). This was suggested to result from the development of hyperphagia and a relative increase in lipoprotein lipase in early pregnancy, which increases fat storage. There is increased insulin sensitivity in early pregnancy due to high estrogen levels. Insulin strongly inhibits lipolysis at adipose tissue with the suppression of hormone-sensitive lipase. The acquired adipose tissue is a source of fatty acid supply during late gestation, which is required to support rapid fetal growth (Herrera, 2002b). In a previous study, where maternal body composition was assessed by hydrodensitometry, there was a significant increase in fat mass in lean women with normal glucose tolerance during pregnancy (Catalano et al., 1998). This relationship was significant only for the period before conception up to early gestation, and not late pregnancy (Catalano et al., 1998). The mechanism underlying this observed relationship between insulin sensitivity and weight gain in

early pregnancy remains unknown. Healthy normal-weight women gain around 3.8 kg of fat during pregnancy, but there is high inter-individual variability.

At mid-gestation, a time of increasing fetal demand, the maternal body switches from anabolic lipogenesis to a catabolic lipolytic, insulin-resistant state (Figure 1-1). This is a physiological response to preserve glucose, as the primary fuel for fetal metabolism, by reducing maternal carbohydrate utilisation. Due to increased total energy expenditure at late gestation (Catalano et al., 1998), any reduction in maternal carbohydrate utilisation is substituted by fat utilisation, as an alternative source of energy. Consequently, lipid metabolism is markedly altered. The increased insulin resistance at late gestation increases lipolysis, which enhances the availability of free fatty acids (FFA) and glycerol (the main substrate for gluconeogenesis) for both maternal and fetal use (Huda et al., 2009). During healthy pregnancy there is an increase in plasma cholesterol and triglyceride (TAG) concentration by 20–25% and 200–400%, respectively (Huda et al., 2009). This increase in TAG was found to be due to a three-fold increase in very low-density lipoprotein (VLDL) synthesis from 14 weeks of gestation to the end of pregnancy. This increase in TAG incorporation into VLDL by the liver is augmented by the effect of estrogen. Insulin resistance decreases lipoprotein lipase activity, the enzyme responsible for VLDL clearance from the plasma and TAG uptake from VLDL by peripheral tissues. As a result, VLDL remains longer in the plasma, which increases LDL accumulation. This potentially can lead to the appearance of small dense low-density lipoproteins (sdLDL). Plasma LDL concentration increases during late pregnancy by 70%. At 28 weeks of gestation, HDL is also increased by 40% compared to 14-week gestation levels (Fahraeus et al., 1985). This is attributed to the effect of estrogen, which promotes ApoA-I production by the liver, thereby increasing HDL formation. Furthermore, accelerated transfer of TAG to lipoproteins of higher density is attributed to the long exposure to cholesteryl ester transfer protein (CETP). The increase in HDL is suggested to be a protective mechanism to counteract any detrimental effects of pregnancy hyperlipidaemia (Huda et al., 2009).

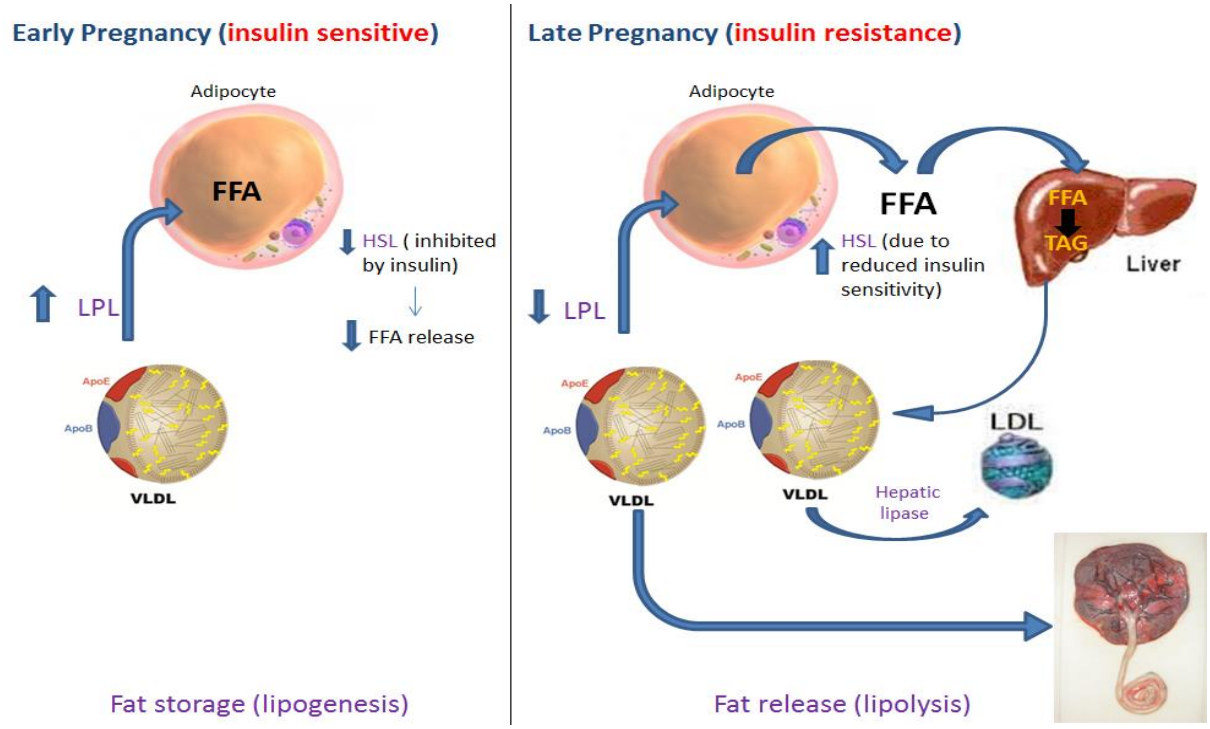


Figure 1-1 Maternal adipose tissue response to metabolic changes during early and late pregnancy.
In early pregnancy, increased insulin sensitivity promotes fat storage, via high LPL activity, and reduces lipolysis at adipocytes, via inhibition of HSL, promoting fat storage. In late pregnancy, due to the physiological insulin resistance of pregnancy, there is no insulin suppression of HSL, which accelerates lipolysis and VLDL formation, and consequently its conversion by hepatic lipase to LDL, and there is reduced LPL by insulin resistance. LPL: lipoprotein lipase, HSL: hormone-sensitive lipase.

There is evidence of oxidative stress during healthy pregnancy. Oxidative stress is defined as an imbalance between prooxidant mediators, such as free radicals and oxidised lipids, and antioxidant mechanisms, which include superoxide dismutases, glutathione peroxidases, catalase and peroxiredoxins, modulating enzyme systems which act as the first-line defence against ROS (Karihtala and Soini, 2007). Toescu et al. (2002) studied oxidative stress during healthy pregnancy and recruited non-pregnant women to form a control group. Plasma lipid hydroperoxides (LHP) were measured at first, second and third trimester, and 8 weeks postpartum. There was no significant difference in LHP between the two groups in early pregnancy, but LHP continued to increase in the second trimester, and reached abnormally high levels in the third trimester, a level comparable to that observed in diabetic patients with vascular disease. This increase was associated with the predominance of sdLDL subfractions (high LDL score). The LHP levels returned to baseline levels by 8 weeks postpartum. This data showed that during late pregnancy there is an increase in sdLDL particles and an increase in oxidative damage (Toescu et al., 2002). This could potentially harm the vascular endothelium and accelerate inflammatory processes.

Pregnancy is characterised by physiological systemic inflammation. During pregnancy, the balance between cell-mediated immunity (Th1) and humoral immunity (Th2) is strongly shifted towards the predominance of Th2 cytokines (IL-5, IL-10 and IL-13), which have a potentially protective role in pregnancy (Challis et al., 2009). Inflammatory processes towards late pregnancy alter this balance and lead to a progressive shift back towards Th1 cytokines (IL-2, IFN-gamma and lymphotoxin), which initiates and intensifies the inflammatory cytokine production involved in adverse pregnancy outcomes (Challis et al., 2009). In pregnancy there are increased levels of inflammatory markers, particularly in the third trimester, such as TNF-α, which may be major mediators for pregnancy-induced insulin resistance. TNF-α promotes IR by suppressing tyrosine phosphorylation of the insulin receptor and its substrate-insulin receptor substrate-1 (IRS-1) via inhibition of tyrosine kinase (Hotamisligil et al., 1994). Furthermore, it diminishes the expression of glucose transporter type 4 (GLUT 4) in adipose tissue and skeletal muscle. The pro-inflammatory activity of TNF-α in beta cells of the pancreas plays a key role in their apoptosis, contributing to T2DM development (Akash et al., 2018).

Healthy pregnancy is associated with enhanced vascular function. The vascular endothelium has a major role in maintaining vascular function despite the metabolic and inflammatory changes during pregnancy. The vascular endothelium is involved in the control of various functions such as inflammation, vascular tone, permeability, thrombosis, platelet and leukocyte adhesion and aggregation. Disturbances in endothelium function have been associated with pregnancy complications such as preeclampsia. Vascular function can be determined by both endothelial cell production of vasoactive substances and smooth muscle responsiveness – termed endothelium-dependent and endothelium-independent function, respectively – and they can be assessed *ex vivo* by the use of endothelium-dependent agonists (acetylcholine and bradykinin) and endothelium-independent agonists (sodium nitroprusside and nitro-glycerine). These vascular responses are often tested in different vascular beds, which makes it difficult to compare endothelial function assessed in different studies.

Enhanced endothelial function during healthy pregnancy was apparent from 10 weeks of gestation (Savvidou et al., 2000). There was an increase in endothelium-dependent function in lean pregnant mothers, which remained improved up to 4 months postpartum (Stewart et al., 2007b). This has been demonstrated in several

studies using flow-mediated dilatation (FMD). FMD is an *in vivo* assessment method used to assess macrovascular function, in which the brachial (most commonly), femoral or radial artery diameter changes in response to increased flow (shear stress) are measured using high resolution ultrasound. This is a non-invasive method dependent on nitric oxide (NO) release from endothelial cells to assess endothelium-dependent vascular function. Lack of standardisation and intra-observer variability are the major limitations of this technique. After 30 weeks of gestation, a fall in FMD was observed and negatively correlated with an increase in brachial artery resting diameter at this time point (Savvidou et al., 2000). A positive correlation between maternal plasma TAG and FMD suggested that enhanced vascular function in pregnancy is dependent on the hyperlipidaemia status (Saarelainen et al., 2006). Laser Doppler imaging/flowmetry (LDF) is a non-invasive *in vivo* method used to assess microvascular function by the measurement of cutaneous perfusion accompanied by iontophoresis of acetylcholine and sodium nitroprusside. The assessment of microvascular function during pregnancy using laser Doppler imaging shows improved endothelium-dependent and endothelium-independent vascular function (Stewart et al., 2007b). Microvascular function in isolated arteries can be assessed *in vitro* by wire myography. This method was found to have a strong correlation with other studies using brachial artery FMD, and is suggested to be more sensitive (Endemann and Schiffrin, 2004). Wire myography studies on pregnant women confirmed the improved FMD in small resistance arteries isolated from pregnant women compared to non-pregnant women (Cockell and Poston, 1997).

1.3.2 Obese pregnancy

Metabolic adaptation during pregnancy is exaggerated in obese mothers. There is a significant increase in fat mass in obese mothers during pregnancy, but interestingly this is to a lesser extent than that observed in lean mothers. This is most likely related to lower insulin sensitivity in early pregnancy in obese mothers (Okereke et al., 2004). Fat mass accretion is variable among obese women, ranging from 2 to 13.1 kg (Okereke et al., 2004). The progressive decline in insulin sensitivity during healthy pregnancy is magnified in obese mothers. Both central and peripheral insulin sensitivity are reduced in obese pregnant women (Catalano et al., 1992). Peripheral insulin sensitivity is 40% lower in obese mothers, leading to an increase

in pregnancy hyperinsulinemia in this population that could potentially lead to increased vascular inflammation and oxidative stress.

Due to reduced insulin suppression of HSL in late gestation and lower levels of LPL, the release of FFA from adipocytes increases, which leads to VLDL enrichment with TAG (Alvarez et al., 1996). Interestingly, this causes TAG enrichment in HDL and LDL particles that have low TAG content under non-pregnant conditions. CETP transfers TAG from VLDL particles to lipoproteins of higher density (LDL and HDL), and the transfer of cholesteryl esters (CE) from HDL to apolipoprotein B (apoB)-containing particles (VLDL and LDL) (Knopp et al., 1998). In the liver, hepatic lipase hydrolyses HDL-associated TAG, and also phospholipids, inducing the formation of smaller HDL particles that can be easily cleared by the kidney (Alvarez et al., 1996). Higher plasma LDL enriched in TAG can lead to the production of easily oxidised sdLDL particles, which leads to the production of oxidised LDL (oxLDL) particles (Griffin et al., 1994). OxLDL is highly atherogenic because of its reduced affinity for the native LDL receptor. OxLDL can be taken up by macrophages promoting the formation of foam cells and development of early atherosclerotic lesions (Clausen et al., 2001). This can initiate endothelial dysfunction and, ultimately, vascular occlusion. These pathways explain the development of metabolic syndrome features in obese pregnancy characterised by elevated plasma concentrations of TAG and VLDL and decreased plasma HDL concentration (Figure 1-2).

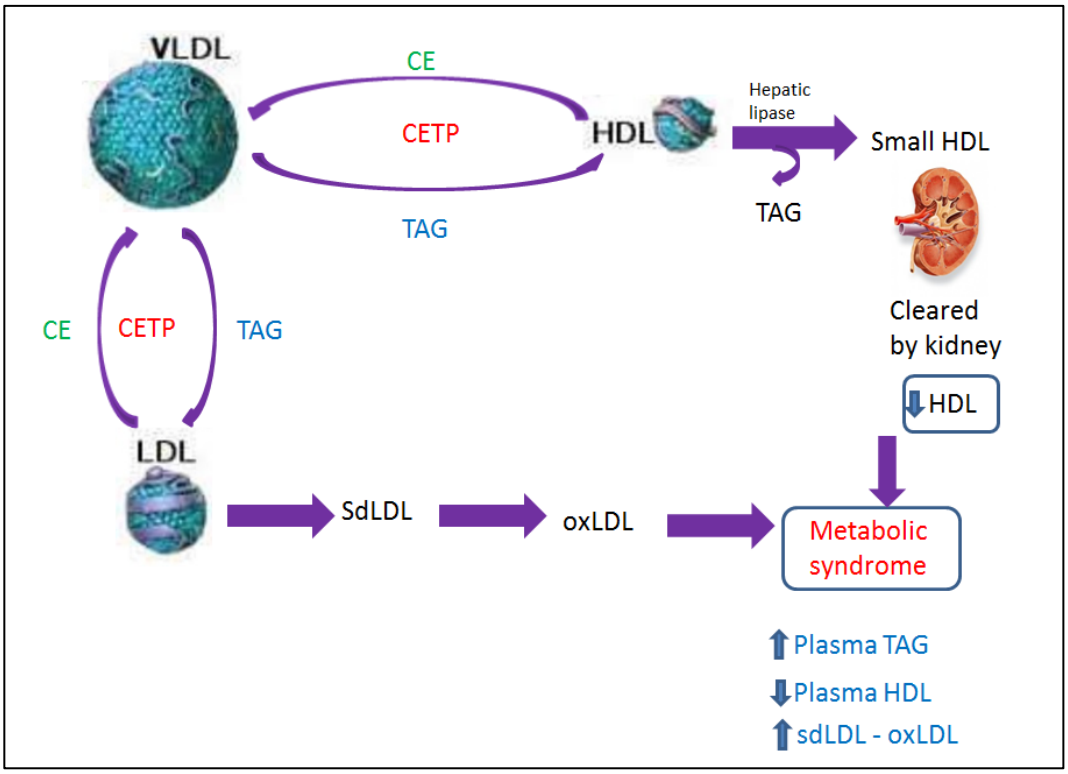


Figure 1-2: Changes in lipoprotein metabolism during obese pregnancy leads to development of metabolic syndrome features.
Due to VLDL enrichment with TAG in obese pregnancy, CETP transfers TAG from VLDL to LDL and HDL particles in exchange for CE. The hepatic lipase in the liver leads to more release of TAG from HDL particles, which shrink in size and can be easily cleared by the kidney. When LDL particles become smaller in size this leads to the production of sdLDL, which can be easily oxidised to oxLDL. This can lead to the development of metabolic syndrome features. CETP: Cholesterol Ester Transfer Protein, HDL: High density lipoprotein, LDL: Low-density lipoprotein, sdLDL: small density LDL, oxLDL: oxidised LDL, TAG: triglycerides, CE: cholesteryl ester.

An obese mother tends to enter pregnancy with pre-existing chronic inflammation and endothelial activation. Adiposity is a key factor implicated in the low-grade, chronic inflammation that is observed in obese mothers. In a study comparing obese and lean pregnant women's inflammatory markers, CRP demonstrated a strong relationship with overweight in pregnant women, as early as the tenth week of pregnancy (Kac et al., 2011). Obesity-driven inflammation could be implicated in the pathogenesis of adverse pregnancy outcomes such as preeclampsia. Circulating inflammatory factors could activate and damage endothelial cells in the maternal systemic circulation. Increased levels of inflammatory cytokines such as TNF-α can promote the activation of adhesion molecules (VCAM-1) via activation of transcription factor NF-Kβ (Zhou et al., 2007). This can increase monocyte adhesion to endothelial cells and the development of vascular inflammation. Furthermore, impaired release of the vasodilator nitric oxide and increased concentrations of the vasoconstrictor endothelin-1 in maternal obesity resulted in vascular dysfunction (Rao et al., 2014b, Rao et al., 2014a).

Laser Doppler imaging was used in a longitudinal study to assess microvascular vascular function in lean and obese women, and revealed that although there was a similar pattern of improvement in vascular function during pregnancy, obese mothers had significantly lower endothelium-dependent and -independent vascular function than lean women (Stewart et al., 2007b). In the postpartum period, endothelium-dependent function in obese mothers declined to first-trimester levels and failed to maintain the gestational improvement, whereas in lean women it remained at an enhanced level (Stewart et al., 2007b). In another study, myometrial arteries isolated from obese mothers (BMI>36kg/m²) showed reduced relaxation in response to bradykinin compared to vessels obtained from pregnant non-obese women (BMI<30 kg/m²) (Myers et al., 2006).

1.3.3 GDM pregnancy

Increased insulin resistance during late gestation in healthy women is accompanied by increased insulin production from β-cells to maintain euglycemia; if this does not occur then GDM can develop. GDM is a pregnancy metabolic complication resulting from increased insulin resistance during pregnancy. Glucose homeostasis is impaired in GDM. Inadequate β-cell compensation for insulin resistance has been found in women with GDM, suggesting that β-cell dysfunction is a common feature of GDM (Buchanan, 2001). Mechanisms that lead to β-cell failure in women with GDM are yet to be identified. The frequent occurrence of chronic insulin resistance in women who have or had GDM suggests that the propensity for β-cells to fail in the presence of insulin resistance may be a common feature of the disease (Prentki and Nolan, 2006). The same mechanism may be involved in the progression from GDM to T2DM and in the pathogenesis of T2DM in general. Other factors such as maternal and placental hormones, genetic predisposition, and suboptimal lifestyle might contribute to the development of GDM (Prentki and Nolan, 2006).

In a longitudinal study comparing body composition in lean, normal glucose tolerant (NGT) mothers and lean GDM mothers, there was a significant increase in weight in both groups over time, but no significant difference between the two groups (Catalano et al., 1998). However, GDM mothers had significantly smaller increases in body fat (1.3 kg, P=0.04) and percentage of fat (1.6%, P=0.02) than lean NGT mothers (2 kg and 3.6%, respectively) (Catalano et al., 1998). Total weight gain was 2.5 kg less than that of NGT women. This could be related to the decreased pre-

gravid insulin sensitivity in GDM women, which limited their ability to conserve energy expenditure and increase body fat (Okereke et al., 2004).

GDM mothers had higher concentrations of VLDL particles and a lower number of large HDL particles compared to healthy pregnant women controls (Mokkala et al., 2019). Interestingly, the study of Mokkala et al. (2019) found that the most predictive biomarker for GDM at early pregnancy was higher concentrations of small HDL particles. Other studies have reported that small HDL particles are associated with type 1 diabetes (Gourgari et al., 2018), inflammation (Phillips et al., 2018), and dyslipidaemias, probably due to impaired capacity to esterify cholesterol (Hernáez Camba et al., 2019). In healthy pregnancies, maternal glycemia before delivery correlates with neonatal weight and fat mass (Metzger et al., 2008). However, in women with well-controlled GDM, both maternal plasma TAG and NEFA, rather than maternal glucose, are positively correlated with neonatal weight and fat mass (Schaefer-Graf et al., 2011, Schaefer-Graf et al., 2008). This indicates that maternal dyslipidaemia in GDM may enhance the maternal fatty acid transfer to the fetus contributing to increased fetal adipose tissue mass and the consequent risk of developing macrosomia (Figure 1-3).

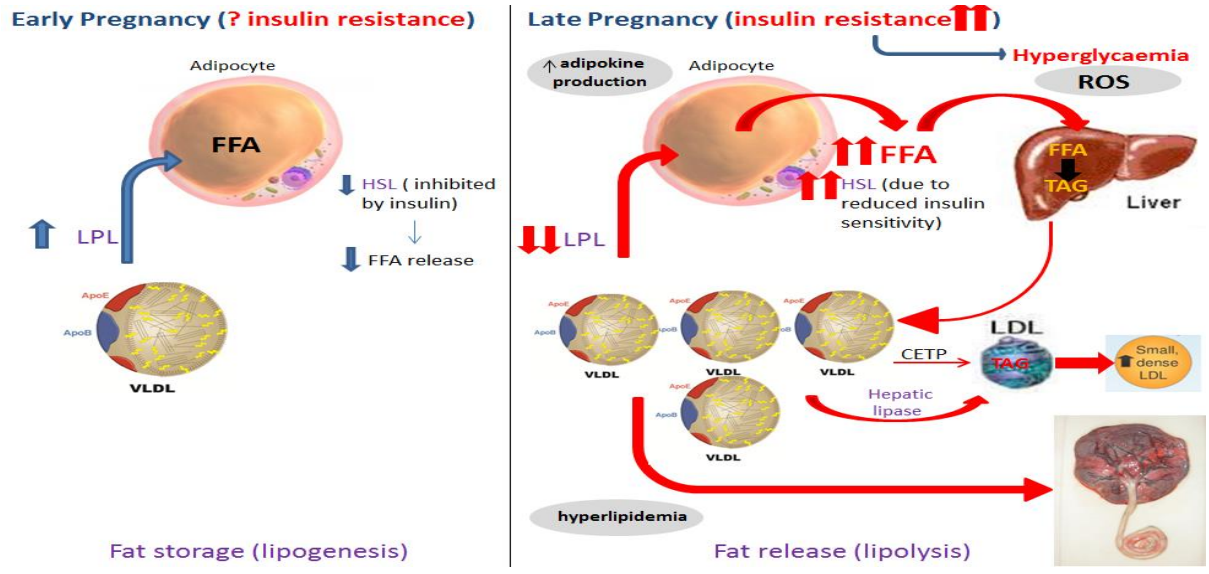


Figure 1-3: Maternal adipose tissue metabolism during early and late pregnancy in GDM. In GDM, women start pregnancy with some degree of insulin resistance due to pre-pregnancy obesity. At late gestation, there is a pronounced increase in insulin resistance due to failure of β-cell compensation with an increased insulin requirement during pregnancy leading to hyperglycaemia. There is pronounced production of VLDL carrying more TAG more than normal pregnancy (explained in figure 1-1), which probably contributes to fetal macrosomia via delivery of fatty acids to the placenta. In GDM, TAG-rich VLDL exchanges TAG and CE with HDL and leads to production of sdLDL, which is more easily oxidised and leads to the production of atherogenic oxLDL.

The literature for vascular function studies during pregnancy in GDM women is limited. With the use of wire myography, impaired endothelium-dependent microvascular function was indicated by impaired relaxation to acetylcholine in small arteries isolated from normotensive GDM mothers compared to normotensive non-diabetic pregnant women (Knock et al., 1997). The microvascular response in newly diagnosed GDM mothers (33 weeks of gestation) was assessed using LDF, and no significant difference was found between women with or without GDM during pregnancy (Pontes et al., 2015).

1.4 Docosahexaenoic acid (DHA)

There are two families of long chain polyunsaturated fatty acids (LC-PUFA) derived from two essential fatty acids (EFA), the n-6 and n-3 families. The main EFA of the n-6 family is linoleic acid (LA; 18:2 n-6), while that of the n-3 family is α-linolenic acid (18:3 n-3). LA and ALA cannot be synthesised in the human body and must be obtained from the diet. Both EFAs can undergo desaturation and elongation in humans to produce LC-PUFA with 20 or 22 carbon atoms (Figure 1-4). LA is endogenously converted to arachidonic acid (AA; C20:4 n-6), whereas ALA can be converted to eicosapentaenoic acid (EPA; C20:5 n-3 and DHA; C22:6 n-3) (Rustan and Drevon, 2001). However, excess of certain FA may affect the availability of others, and may lead to consequent adverse outcomes to the fetus (Herrera, 2002a). Humans can synthesise AA from its precursor, the essential fatty acid LA. The parallel synthetic pathways for AA and DHA share common enzymes, and delta-6 desaturase is the enzyme responsible for the initial desaturation of both LA and ALA (Burdge and Wootton, 2002). Given that human dietary intake of LA is eight times greater than that of ALA (Meyer, 2016), the synthesis of AA predominates. Women can produce more DHA from ALA than men, suggesting its potentially important role in female reproduction (Burdge and Wootton, 2002).

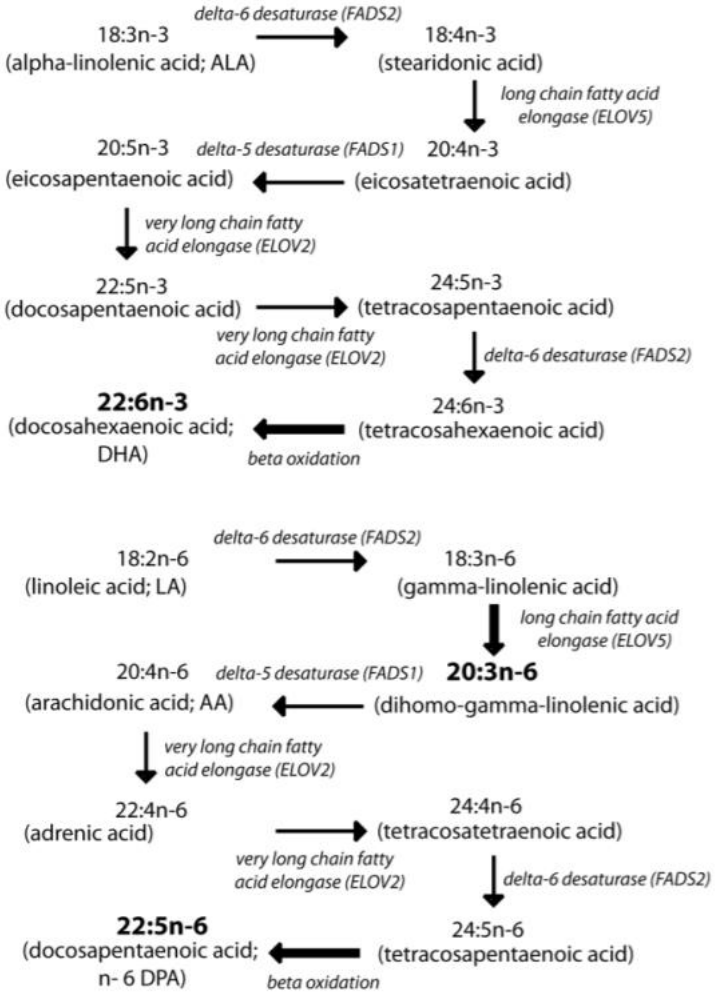


Figure 1-4 PUFA synthesis pathway.
Adapted from (Meyer and Freeman, 2017), journal permission is pending.

Fatty acids are needed by the fetus to control the fluidity and permeability of cell membranes, as a source of energy, and as precursors of bioactive compounds such as eicosanoids. Although all fatty acids can be used as energy sources, the LC-PUFAs are predominantly used for structural and metabolic functions (Herrera and Ortega-Senovilla, 2018). The LC-PUFAs, AA and DHA are important structural fatty acids in neural tissue. During pregnancy, there is increased accretion of maternal, placental and fetal tissues, and consequently the LC-PUFA requirements of pregnant women and their developing fetuses are high. The fetal requirements for AA and DHA are especially high during the third trimester, due to the rapid synthesis of brain tissue (Martinez, 1992). As the fetus may have a limited capacity to synthesise DHA, and requires essential FA to do it, the fetus depends primarily on placental transfer, and thus on the maternal supply of DHA (Innis, 1991).

DHA is the predominant LC-PUFA in phospholipids in the neural membranes of the cerebral cortex and in the photoreceptors of the retina (Guesnet and Alessandri, 2011). Both retinal function and learning ability become permanently impaired if the accumulation of DHA during intrauterine life is insufficient (Innis, 1991), indicating that the supply of DHA during pregnancy is critical for these functions. Of particular importance for fetal growth and development is AA, which is alone among the plasma fatty acids of premature infants in showing a significant linear positive correlation with birth weight (Koletzko and Braun, 1991). This growth-promoting effect of AA could be related to its function as a precursor of prostaglandins and other eicosanoids, or to its structural roles in membrane phospholipids.

It was previously demonstrated that maternal NEFA can cross the placenta by passive diffusion without prior modification or protein-mediated transport (Herrera, 2002a). Several studies have demonstrated placental selectivity of FA transfer and a strong preference of the placental plasma membrane binding sites for LC-PUFA was observed (Campbell et al., 1996). LC-PUFA storage in fetal adipose tissue provides an important source of LC-PUFA during the critical first months of postnatal life. The placenta has a pivotal role for the selective transport of DHA from maternal diet and body stores to the fetus. Several studies have associated various fatty acid transport and binding proteins (FATP) with the preferential DHA transfer, but the importance of the different lipolytic enzymes has also been shown. However, the exact mechanisms and the interaction of these factors remains elusive (Hanebutt et al., 2008).

As discussed previously, maternal obesity is characterised by greater FA flux from adipose tissue stores. The maternal FA flux affects the placental lipid uptake, but the role of placental tissue in moderating this transfer is yet to be clarified. In GDM compared to normoglycemic pregnancies, there was a higher accumulation of lipid droplets in placental tissue (Tewari et al., 2011). In addition, there was reduced mitochondrial FA oxidation by 20% in placentas from women with GDM compared to healthy controls, contributing to enhanced triglyceride content within the placenta. It is also important to consider that the functionality of the placenta may affect the fetal levels of key FA such as LC-PUFA. It was found that LPL activity is increased in placentas from GDM pregnancies, and positively correlated to TAG and NEFA in the offspring of mothers with GDM (Dubé et al., 2013). These changes could adversely affect the fetal metabolism and predispose the fetus to future metabolic

diseases. Thus, it is important to control dyslipidemia in pregnant women to avoid increased neonatal fat accretion and disturbed LC-PUFA transport to the fetus, which could have implications for the fetal neurodevelopment and the later risk of obesity.

1.5 Non-pregnant adipose tissue

Adipose tissue is a critical organ in maintaining energy homeostasis under conditions of excess nutritional intake by storing it in the form of TAG in the fed state (lipogenesis). In prolonged starvation, adipose tissue releases FFA to meet the body energy demands (lipolysis). These processes are controlled by insulin and epinephrine, which regulate lipogenic and lipolytic enzymes. There are two major types of adipose tissue in humans: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT comprises two major depots according to their anatomical distribution: subcutaneous adipose tissue (SAT) localised beneath the skin, and visceral adipose tissue (VAT) distributed in the abdominal cavity. The gluteofemoral depot is the predominant subcutaneous depot in humans, while the predominant visceral depot is the omental depot. Important differences in depot function exist between these categories, as discussed below in 1.5.3 and 1.5.4. Adipose tissue is a heterogeneous tissue containing several cell types in addition to adipocytes and preadipocytes, collectively called the stromal vascular fraction (SVF). The SVF includes endothelial cells, fibroblasts, histiocytes, adipose tissue-derived mesenchymal stem cells (ADMSC), immune cells and adipose tissue macrophages (ATM). The discovery that adipose tissue is capable of secreting various hormones and cytokines, named collectively as adipokines, changed our understanding of adipose tissue (Kershaw and Flier, 2004).

1.5.1 Adipose tissue lipid storage

Adipose tissue stores FA in the form of TAG in the lipid droplet. TAG is formed from three FA molecules esterified on the backbone of a glycerol molecule. FA stored in TAG can be released by lipase enzymes (as discussed in section 1.5.5) to oxidative organs according to energy demand (Braun and Severson, 1992). The FA stored in adipocyte lipid droplets originate from the diet and from *de novo* lipogenesis (synthesis of FA from glucose) in adipocytes or in the liver. In the post-prandial state, TAG from the diet is absorbed by enterocytes and delivered as chylomicrons in the

lymphatic ducts, which later enter the bloodstream. The synthesised TAG by the liver is transported in the blood by VLDL. Chylomicrons and VLDL are TAG-rich lipoproteins that are too large to cross the endothelium of the blood vasculature surrounding the adipocytes. LPL is an enzyme synthesised by adipocytes that is exported to the luminal surface of adipocytes to hydrolyse TAG in TAG-rich lipoproteins into FA (Braun and Severson, 1992). Once the FA is liberated from TAG-rich lipoproteins, it enters the adipocytes by several protein-mediated uptake mechanisms, including fatty acid transport protein (FATPpm), the human scavenger receptor CD36, and the plasma membrane fatty acid binding protein (FABP) (Hajri and Abumrad, 2002). Intracellularly, another family of FATPs transport the FA from the cytoplasm to various compartments (Hajri and Abumrad, 2002). FA must be activated into acyl-CoA to be esterified and stored in adipocyte lipid droplets, a process catalysed by acyl-CoA synthase (ACS) (Ellis et al., 2010). Once activated, FA can be stored in the form of TAG in adipocyte lipid droplets through the glycerol-3-phosphate pathway. This pathway starts with esterification of the first acyl-CoA on glycerol-3-phosphate molecule to form lysophosphatidic acid (Takeuchi and Reue, 2009). Then, the second acyl-CoA is esterified to form phosphatidic acid. The phosphatidic acid is then dephosphorylated to form diacylglycerol (DAG), and then the third acyl-CoA is added to synthesise one molecule of TAG (Takeuchi and Reue, 2009).

1.5.2 Adipocyte differentiation (adipogenesis)

Adipogenesis is the process whereby ADMSC restrict their fate to the adipogenic lineage and become triglyceride-filled mature adipocytes. There are two phases of adipogenesis. The determination phase involves commitment of ADMSC to the adipocyte lineage. This results in the conversion of the stem cell to a pre-adipocyte that exhibits no morphological changes, but has lost the potential to differentiate into other cell types. This commitment is then followed by the differentiation phase, where the pre-adipocyte develops the characteristics of a mature adipocyte, and acquires the machinery necessary for lipid transport and synthesis, insulin sensitivity, and the secretion of adipokines.

The nuclear hormone receptor PPARγ is characterised as the master regulator of adipogenesis. The expression of PPARγ is sufficient to induce adipocyte differentiation in fibroblasts (Tontonoz et al., 1994). No factor has been discovered

that promotes adipogenesis in the absence of PPARγ. These findings are consistent with the observation that most pro-adipogenic factors seem to function at least in part by activating PPARγ expression or activity. Adipogenesis promotes healthy adipose tissue expansion while limiting hypoxia, chronic inflammation and fibrosis (Kusminski et al., 2012). Evidence from mouse models suggests that improved metabolic health in animals can be induced by promoting the healthy expansion of fat mass by adipogenesis (Combs et al., 2004). These findings imply that obesity-associated metabolic disturbances are not due to adiposity *per se*, but probably are a result of an insufficient capacity of adipose tissue to expand by adipogensis.

1.5.3 Adipocyte hypertrophy vs hyperplasia

There are two modes of adipocyte expansion: adipocyte hyperplasia and adipocyte hypertrophy. An increased requirement to store fat stimulates the expansion of adipocyte numbers by increasing pre-adipocyte differentiation into mature adipocytes (adipogenesis) capable of storing excess TAG efficiently (Hoffstedt et al., 2010). This adipocyte hyperplastic expansion is associated with greater insulin sensitivity and normal glucose and lipid profile (Veilleux et al., 2011). Adipocyte hypertrophy is an alternative form of adipocyte expansion, where adipocytes are increased in size to contain the larger lipid droplet rather than increasing the number of adipocytes. Hypertrophic expansion of adipocytes is less metabolically favourable. Hypertrophic adipocytes have impaired cellular functions, restricting their ability to store TAG. Hypertrophic adipocytes are insulin-resistant, and any stored TAG is increasingly broken down and released as FA (Tchoukalova et al., 2010b). Adipocyte hypertrophy generates areas of tissue hypoxia, thus adipocytes become hypoxic and show necrotic changes, which leads to macrophage infiltration, inducing inflammatory cytokine production (Skurk et al., 2007). Dysfunctional hypertrophic adipocytes result in higher circulating plasma FA turnover and accumulation in ectopic sites and lipotoxicity, which has a deleterious effect on metabolic health. Adipocyte hypertrophic expansion has been associated with dyslipidaemia and insulin resistance (Kim et al., 2015). The exact cellular mechanisms involved in the metabolic disturbances demonstrated by larger adipocytes are not well understood.

1.5.4 Depot differences in adipocyte expansion

Considering that the main function of WAT is the storage of excess energy, SAT is considered to be the main storage site for TAG and a safer depot to store fat than VAT. In the SAT depot, fat mass increases predominantly through adipocyte hyperplasia, whereas hypertrophy is predominantly observed in VAT (Joe et al., 2009). The lower differentiation potential of VAT progenitors relative to SAT could potentially explain the preferential adipocyte hypertrophy expansion in the VAT depot. Increased adipocyte hyperplasia in SAT is thought to be a result of the greater number and activity of precursor cells (Tchoukalova et al., 2010a). Moreover, if there is a reduction in precursor cell division in SAT, i.e. SAT dysfunction, it compromises SAT expansion, leading to systemic insulin resistance and metabolic disturbance (Kim et al., 2014). Arner et al. (2013) provided evidence that in obese human subjects an increase in VAT mass involves an increase in VAT adipocyte number, as well as adipocyte size. Similarly, in mice, Jeffery et al. (2015) observed increased adipocyte number in VAT after 8 weeks of a high-fed diet. Taken together, these results suggest that there is increased SAT proliferative capacity under physiological conditions, whereas in obesity, VAT adipogenesis may occur in the context of SAT dysfunctional ability to expand.

1.5.5 Adipose tissue lipolytic function

During lipolysis, TAG is hydrolysed into diacylglyceride (DAG), monoacylglyceride (MAG) and glycerol, releasing one FA molecule at each step. Three lipases are involved in TAG hydrolysis: HSL, adipose tissue triglyceride lipase (ATGL) and monoacylglycerol lipase (MGL), respectively(Ribet et al., 2010). The hydrolysis process is followed by the transport of NEFA and glycerol to the bloodstream, and then on to other tissues. Glycerol is mainly transported to the liver for gluconeogenesis, while NEFA is transported to skeletal muscle, the heart, the liver, or re-esterified by adipocytes into TAG (Ribet et al., 2010).

The catecholamines are the primary mediators of adrenergic signalling in adipose tissue. The catecholamines can both stimulate and inhibit lipolysis depending on their relative affinity for different adrenergic receptor subtypes. Thus, stimulation of lipolysis requires the activation of β-adrenergic receptors on the surface of the adipocyte, while anti-lipolytic signals are transmitted by the α2-adrenergic receptors. Both α2- and β-adrenergic receptors belong to the G-protein-coupled receptor

family. The G-protein associated with α2-adrenergic receptors contain the inhibitory (Gi) subunit, while β-adrenergic receptors contain the stimulating (Gs) subunit (Lafontan and Berlan, 1993). The activation of the receptors causes the G-proteins to interact with adenylyl cyclase (AC), which is consequently inhibited by interaction with Gi and activated by interaction with Gs (Lafontan and Berlan, 1993). Activation of β-adrenergic receptors results in AC activation and conversion of ATP to cAMP, resulting in an increase in intracellular cAMP levels, which activates protein kinase A (PKA). Activated PKA phosphorylates the lipid droplet-associated protein perilipin 1 (PLIN1) (Greenberg et al., 1991) and cytoplasmic HSL (Garton et al., 1988). Phosphorylation of PLIN1 promotes the release of a potent co-activator of ATGL, which is the comparative gene identification-58 (CGI-58) (Granneman et al., 2009). This facilitates the activation of ATGL and initiates the stimulation of lipolysis. Furthermore, PKA-mediated phosphorylation of HSL causes a rapid activation and translocation of the lipase from the cytosol to the surface of the lipid droplets. The lipase docks with the phosphorylated PLIN1 and thereby gains access to its DAG substrate, which is being generated by ATGL (Figure 1-5) (Wang et al., 2009).

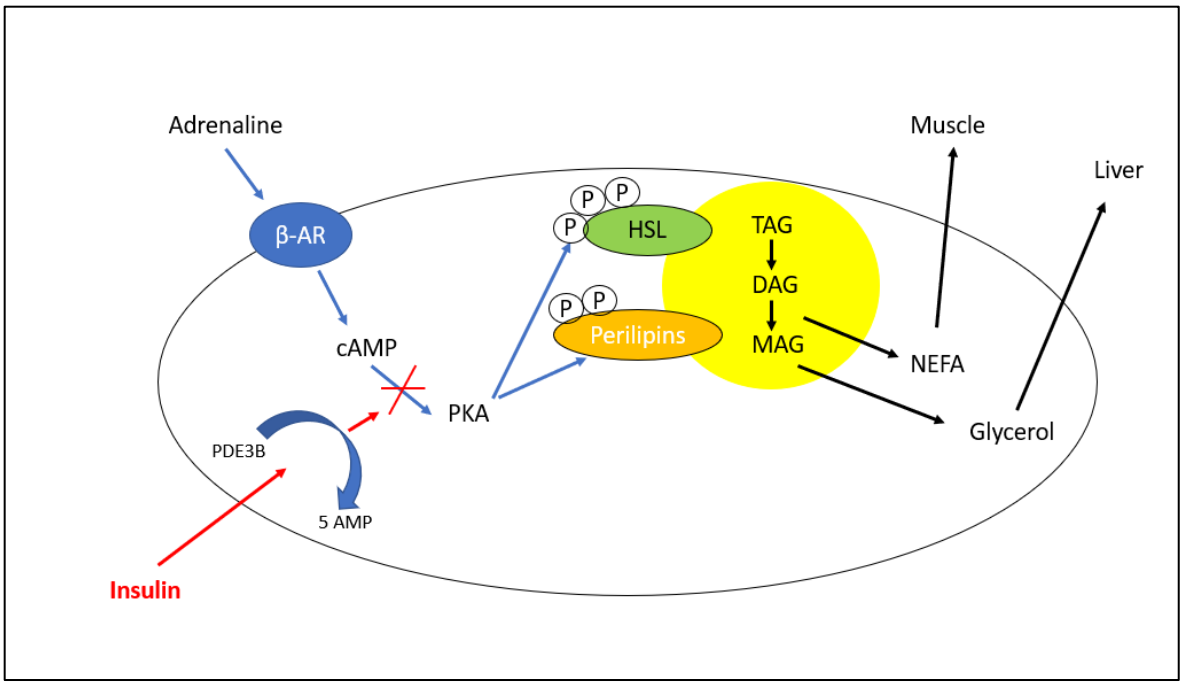


Figure 1-5 Hormonal regulation of adipocyte lipolysis
Adipocyte lipolysis is stimulated by activation of β adrenergic receptors which stimulates cAMP production and consequently activates PKA. PKA phosphorylates HSL and perilipins, stimulating the hydrolysis of TAG to MAG. This results in NEFA and glycerol production. Insulin suppresses adipocyte lipolysis by suppression of PKA production by stimulation of PDE3B and cAMP hydrolysis.

The observed depot-specific differences in catecholamine responsiveness can be explained by differences in the expression of plasma membrane lipolytic β

adrenergic receptors (β1 and β2) and α2-adrenergic anti-lipolytic receptors in adipocytes from different adipose tissue depots (Mauriege et al., 1987). It is understood that omental adipocytes have higher lipolytic activity compared to SAT adipocytes, due to reduced insulin action and lower availability of the anti-lipolytic alpha 2-adrenoreceotor in VAT adipocytes (Goldrick and McLoughlin, 1970). The converse is true for SAT, which shows less response to catecholamine-induced lipolysis, due to higher availability of the anti-lipolytic alpha 2-adrenoreceptors compared to VAT (Wahrenberg et al., 1989). Insulin has a major role in the inhibition of lipolysis in adipocytes (as explained above). In the SAT depot, there is increased adipocyte sensitivity to the insulin suppression of lipolysis (Jensen, 2008). VAT adipocyte lipolysis was shown to be more resistant to insulin suppression than SAT adipocytes in humans (Mauriege et al., 1987).

It is well established that increased adipocyte size contributes to higher basal lipolysis rate (Goldrick and McLoughlin, 1970). In a study that used cell fractionation by a flotation technique to separate large and small adipocytes from the same healthy individual, lipolytic activity of larger adipocytes was shown to be higher in unstimulated (basal), hormone-stimulated (adrenergic) and hormone-inhibited (insulin) conditions. Furthermore, there was higher protein expression of HSL, ATGL and perilipin in larger adipocytes compared to small adipocytes, independent of the donor characteristics (Laurencikiene et al., 2011). Therefore, enhanced lipolytic activity might provide the link between adipocyte hypertrophy and the associated metabolic disturbances, such as systemic insulin resistance.

1.5.6 Adipose tissue secretory function

Adipocytes, SVF and immune cells resident in adipose tissue are capable of producing several adipokines, contributing to the regulation of energy expenditure, insulin sensitivity, appetite control, adipogenesis, adipocyte function, and immune cell migration to adipose tissue (Schling and Löffler, 2018). These adipokines act via autocrine and paracrine signalling pathways. Changes in adipose tissue function and adipocyte size could promote changes in the secretory function, and subsequent secondary changes in the target organs of these adipokines (White and Ravussin, 2019). Adipose tissue expresses a variety of adipokines: pro-inflammatory adipokines (such as TNF-α, CRP, IL-1, IL-6 and IL-8) and adipose

tissue-specific adipokines (such as leptin, adiponectin, resistin and visfatin) (Fantuzzi, 2005).

Leptin is secreted from adipocytes during the fed state and acts on the hypothalamus to regulate energy balance and appetite control (Matarese et al., 2005). The plasma leptin levels correlate positively with the body fat mass and more leptin is released from hypertrophic adipocytes compared to smaller adipocytes (Lönnqvist et al., 1997). Adiponectin is the only adipokine considered to have insulin-sensitising ability. Adiponectin functions to promote peripheral insulin sensitivity, therefore, its reduction is associated with insulin resistance and hyperinsulinemia (Kubota et al., 2002). Adiponectin plasma level is reduced with increasing adiposity (Guenther et al., 2014). Furthermore, adiponectin is reported to elicit systemic anti-inflammatory effects (Ouchi and Walsh, 2007).The release of these adipokines (except adiponectin) by adipocytes, or the macrophages that infiltrate adipose tissue, leads to a chronic inflammatory state (Pessin and Kwon, 2013). With the development of adipose tissue inflammation, the pattern of adipokine secretion is significantly altered towards a pro-inflammatory and atherogenic profile.

The VAT depot has a higher secretory capacity than the SAT depot (Hocking et al., 2010). This difference is an intrinsic feature, due to VAT different cellular components compared to SAT. Differences in the secretory function of adipocyte depot is addressed in more detail in Chapter 5. Several components of human adipose tissue secretory function remain unknown, or their actions remain poorly understood due to the complexity of paracrine interactions between several cellular types in adipose tissue. The human adipose tissue secretory function is an area of research that has expanded rapidly.

1.5.7 The adipose tissue expandability hypothesis (the overspill hypothesis)

Adipose tissue dysfunction in terms of adipocyte size, number and lipolytic function may underlie obesity-associated metabolic abnormalities (Gustafson et al., 2009). In individuals with impaired SAT expandability, the so-called overspill hypothesis suggests that impaired SAT expandability initiates fat accumulation in VAT, primarily as an alternative site for fat storage to minimise the deleterious effect of systemic FA. This then contributes to later development of hepatic and peripheral insulin

resistance, due to increased FA flux from VAT and ectopic fat deposition in organs such as the liver, pancreas and heart (Drolet et al., 2008). Ectopic fat deposition can lead to lipotoxic effects such as impaired cellular signalling, dysfunction and possibly cellular death (Summers, 2006) (Figure 1-6). Moreover, the portal hypothesis suggests that the anatomical location of VAT could contribute to the associated metabolic risk, because visceral fat directly transports FA to the liver in the portal circulation without peripheral tissue uptake (Jensen, 2008), leaving the liver exposed to portal FA and the inflammatory cytokines released from VAT, contributing to insulin resistance.

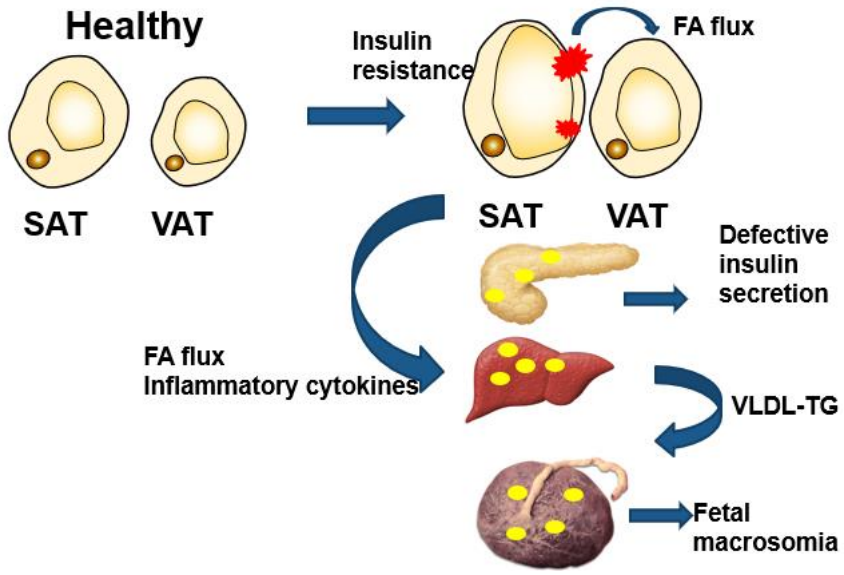


Figure 1-6 The consequences of adipose tissue limited expandability according to the overspill hypothesis.
Limited adipocyte ability to expand may lead to FA overflow from SAT to VAT, as an alternative site for fat storage. VAT is an active depot, with exaggerated lipolytic response. Thus, the direct liver exposure to portal FA and adipokines from VAT may enhance hepatic insulin resistance and ectopic fat deposition in the liver. This could enhance the liver secretion of VLDL particles rich in TAG, which could consequently enhance lipid deposition in non-adipose tissue organs with increasing insulin resistance, and might contribute to fetal macrosomia in pregnant women due to enhanced FA delivery to the fetus through the placenta.

1.5.8 Ectopic fat

Ectopic fat is defined as the storage of TAG in tissues other than adipose tissue that normally contain only small amounts of fat, such as the liver, skeletal muscle, the heart and the pancreas. Since adipocytes are the only cell specialised in fat storage, the reduced ability to store FA triggers lipotoxicity in other tissues (Weinberg, 2006). FA may enter deleterious pathways once it accumulates in non-adipose tissue. TAG may function as a storage site for long chain saturated FA and their metabolic flux

may favour the synthesis of complex lipids such as ceramides, DAG and CEs (Summers, 2006). The accumulation of complex lipids results in endoplasmic reticulum stress and insulin resistance, which affect organ function (Summers, 2006).

Lipid 'spillover' to non-adipose tissues may occur in response to over-accumulation of FA in adipocytes, exceeding the adipocyte hypertrophy and hyperplasia necessary to accommodate the increased influx of FA (Kusminski et al., 2009). While some non-adipose tissues have the compensatory capability to enhance FA oxidation in response to modest FA surplus, such as the liver (Mason, 1998) and skeletal muscle, other tissues such as pancreatic β-cells (Shimabukuro et al., 1998) and the myocardium (Szczepaniak et al., 2007) have limited FA oxidation capabilities. Ectopic fat accumulation in oxidative organs such as the liver and skeletal muscles results in enhanced systemic insulin resistance. Stefan et al. (2008) assessed total body fat, SAT and VAT with magnetic resonance tomography, and ectopic fat deposition in the liver and skeletal muscles with proton magnetic resonance spectroscopy in insulin-sensitive and insulin-resistant obese individuals. Although VAT mass did not differ across obese individuals, obese insulin-sensitive individuals had 54% less fat accumulation in the liver than the equally obese insulin-resistant group. These findings indicated the predominant role of fatty liver in the regulation of glucose and lipid metabolism, and the development of insulin resistance as a consequence for ectopic fat deposition in the liver. Furthermore, the lack of liver fat can be used as a key determinant of metabolically benign obesity and maintained insulin sensitivity in obese individuals.

Ectopic fat deposition in other non-adipose organs can be illustrated by the intrinsic differences between lipid droplet (LD) composition in adipocytes or other ectopic tissues. The lipid droplet-associated proteins (LDAP) are present only in adipocytes, not in ectopic sites of lipid accumulation (Brasaemle, 2007). These proteins, such as perilipin1, FSP27 (CIDEC) and caveolin-1, are abundantly present in adipocyte LD and function, primarily as regulators of lipid metabolism, by protecting the LD from degradation by cytosolic lipases (Brasaemle et al., 2000). Indeed, the absence of perilipin expression in animal models greatly increases basal FA release, suggesting its role in the control of lipase access to LD, in a manner that is responsive to the metabolic status of adipocytes (Tansey et al., 2001). Perilipin null mice are lean, and resistant to diet-induced obesity, due to accelerated adipose

tissue lipolysis (Tansey et al., 2001). Interestingly, perilipin null mice had glucose intolerance and peripheral insulin resistance, highlighting that lipid mobilisation from adipose tissue is critical in the control of glucose homeostasis (Tansey et al., 2004).

1.5.9 Non-pregnant adipose tissue in obesity

Adult human obesity is associated with an increase in hypertrophic adipocytes, which trigger metabolic disorders such as insulin resistance, metabolic syndrome and T2DM. There is a remarkable increase of fat mass in both SAT and VAT depots in obese women. Adipocyte hypertrophy due to excess TAG accumulation results in an imbalance between adipogenesis and lipolysis in adipocytes. The adipogenic capacity in human obesity remains a debatable subject (as discussed in section 1.5.4); however, adipose tissue lipolysis is an important process in determining circulating FA levels. The adipose tissue of obese individuals with enlarged adipocytes is less responsive to insulin, which reduces anti-lipolytic action in these cells (Salans et al., 1968). The reason for this diminished responsiveness to insulin is presently unknown. A reduction of insulin receptors (number, affinity and coupling) due to cell enlargement is one of the suggested reasons (Salans et al., 1968). Another explanation could be that the reduction in insulin responsiveness is a protective mechanism to control further cell enlargement, cell rupture and death. Adipocyte lipolytic activity also depends on the level of expression of adipocyte lipases. There is reduced ATGL activity and expression level in SAT for obese individuals. Moreover, the mRNA and protein levels of ATGL and HSL in SAT for insulin-resistant individuals are reduced compared to insulin-sensitive healthy individuals (Jocken et al., 2007). Variation in perilipin1 levels relative to adipocyte size may contribute to differences between basal lipolysis rate between fat depots (Sohn et al., 2018). Downregulated perilipin1 gene expression was observed in both the SAT and VAT samples of obese women compared to normal-weight women (Wang et al., 2003).

A plethora of deleterious reactive lipids accumulate in non-adipose tissues in holding ectopic fat in obesity, and these contribute to the pathophysiology of insulin resistance, liver steatosis, impaired myocardial function and β-cell failure (van der Zijl et al., 2011, Shulman, 2014). Ectopic fat is extremely toxic to tissues, because it will favour the synthesis of complex metabolites such as ceramides, diacylglycerol (DAG) and cholesteryl esters (Shulman, 2014). These metabolites antagonise

insulin-signalling pathways and lead to insulin resistance and disturbed glucose metabolism within the tissue (Shulman, 2014). Furthermore, this may contribute to chronic inflammation, oxidative stress, disturbed mitochondrial function and programmed cell death.

1.5.10 Other processes involved in adipose tissue dysfunction in obesity-related metabolic disorders

1.5.10.1 Adipose tissue inflammation

Dysregulated adipokine secretion is another feature of adipose tissue dysfunction which results in disturbed WAT homeostasis and dysregulated whole-body metabolism, such as in obesity (Divella et al., 2016). Adipocytes and SVF cells are capable of secreting pro-inflammatory factors. In obesity, there is altered adipose tissue cellularity (AT remodelling), secondary to adipocyte hypertrophy, cellular dysfunction and inflammation (Trayhurn, 2013). Farley et al. (2009) showed that maternal obesity is associated with macrophage infiltration in adipose tissue in response to hypertrophic necrotic adipocytes in a ‘crown-like structure’. The accumulation of CD 68(+) macrophages was documented in subcutaneous abdominal tissue in obese insulin-resistant women (Basu et al., 2011).

The pro-inflammatory classical macrophages are named M1, while M2 have the anti-inflammatory phenotype (Zeyda and Stulnig, 2007). In obesity there is a shift towards the pro-inflammatory phenotype M1, which is considered to be the major contributor to the development of adipose tissue inflammation (Weisberg et al., 2003). In contrast, M2 improves insulin sensitivity and suppresses the M1 macrophage function. Adipose tissue responds to inflammatory signals such as FA and lipopolysaccharide (LPS) with induction of the inflammatory response through the activation of TLR-4 and the associated intracellular signalling pathway NF-κB. Similarly, macrophages respond to adipose tissue hypoxia with the production of cytokines and inflammatory mediators which can lead to upregulation of TLR-4, thus participating in a paracrine loop between the two cell types. The activation of these receptors in adipocytes alters mediators in the insulin-signalling cascade and glucose uptake, which leads to insulin resistance (Song et al., 2006). In situations of induced adipose tissue hypoxia (to mimic obesity), there is decreased production of the glucose transporter (GLUT4), which reduces the insulin-stimulated glucose uptake (Trayhurn, 2013).

Adipokines may provide the link between the changes in adipose tissue function and the development of systemic insulin resistance, dyslipidaemia and T2DM (Blüher, 2013). The production of adipokines in obese women could induce systemic insulin resistance with the decreased production of adiponectin, or increased production of cytokines such as IL-6 (Engeli et al., 2003). Increased inflammatory adipokine production in obesity can activate various signalling pathways that interfere with insulin signalling in adipocytes. Adipocyte insulin resistance contributes to adipose tissue dysfunction and systemic insulin resistance, observed during obesity and T2DM.

1.5.10.2 ROS and endoplasmic reticulum (ER) stress

Adipose tissue inflammation is not the only pathophysiological mechanism involved in obesity-related adipose tissue dysfunction. With increasing insulin resistance in obese pregnancy, there are increased stressors for adipose tissue, such as ROS, endoplasmic reticulum stress and adipose tissue hypoxia, which induce local inflammation in adipose tissue (Trayhurn, 2013). During obesity, the cellular antioxidant mechanisms in adipocytes were reported to have reduced ability to restore protein oxidative damage. Oxidised proteins are toxic to the cells. Proteasomal dysfunction results in protein oxidation and the unfolded protein response (UPR), which are major mechanisms in the development of insulin resistance (Diaz-Ruiz et al., 2015). Proteasomal dysfunction leads to obesity-induced ER stress and insulin resistance in adipocytes through the activation of the c-Jun N-terminal Kinase (JNK) pathway (Otoda et al., 2013). ER stress is a condition characterised by the accumulation of misfolded proteins in the ER lumen, resulting from inflammation. ER stress is a well-known feature of insulin resistance and obesity (Yang et al., 2015).

1.5.10.3 Hypoxia and angiogenesis

Hypoxia is a well-known condition that increases the production of angiogenic factors. Adipocyte hypertrophic expansion resulting in cells of more than 100μm exceeds the maximum cellular oxygen diffusion distance and creates an area of hypoxia. This could lead to adipocyte necrosis, and probably adipose tissue infiltration by immune cells such as macrophages and disturbed adipokine secretion (Goossens, 2008). Indeed, diet-induced weight loss following diet-induced obesity

is associated with reduced inflammation and improved oxygenation of adipose tissue in mice (Ye et al., 2007).

Healthy adipose tissue expansion requires angiogenesis (Bouloumié et al., 2002). Adipose tissue plasticity requires constant growth and remodelling of adipose tissue blood vessels. The adipose tissue vasculature is important for adipose tissue metabolic and secretory function, as it facilitates interactions between the blood compartment and adipocytes. Adipose tissue angiogenesis is a complex process requiring balanced production of the extracellular matrix, endothelial cell proliferation and migration. Several angiogenic factors are produced by adipocytes and the SVF, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). VEGF gene expression is upregulated in adipocytes cultured in 1% oxygen (hypoxic condition) (Wang et al., 2007). Overweight and obese subjects have 44% lower capillary density and 58% lower VEGF compared to lean subjects. This might be due to lower PPARγ and higher collagen VI mRNA expression, which correlates with the lower adipose tissue oxygen partial pressure (Pasarica et al., 2009). Rupnick et al. (2002) showed that in conditions of impaired angiogenesis, there was inhibited adipose tissue accumulation. Gealekman et al. (2011) found a higher capillary density per adipocyte and higher angiogenic growth capacity in SAT compared to VAT. This supports the biological and metabolic variability between the two different WAT depots.

1.6 Adipose tissue in pregnancy

1.6.1 Adipose tissue adaptation to healthy pregnancy

Changes in adipose tissue metabolic and endocrine function are central to maintain whole-body energy homeostasis during pregnancy. In response to the metabolic changes during pregnancy (previously described in section 1.3.1), adipose tissue undergoes dynamic remodelling. This includes changes in AT mass, AT expansion and adipokine secretion.

During pregnancy, maternal body fat distribution moves from the lower body towards upper and central compartments, which are relatively resistant to insulin suppression of lipolysis (Stevens-Simon et al., 2001). Kinoshita and Itoh (2006) observed an increase in fat accumulation in VAT measured by ultrasound (US) in

lean healthy pregnant women towards the end of pregnancy. This observation is consistent with the observed hyperlipidaemic status during late pregnancy.

In a cohort of healthy non-obese women, recruited pre-gravid and followed in early (8–12 weeks of gestation) and late (36–38 weeks of gestation) pregnancy, gluteal fat samples were obtained, and Resi et al. (2012) observed that gluteal adipose tissue mass, adipocyte size and cell number significantly increased in late pregnancy compared with the pre-gravid measurements, but remained unchanged in early pregnancy. These results are consistent with a previous observation by Rebuffe-Scrive et al. (1985), who found higher LPL activity in the femoral region than in the abdominal region in pregnant women. This pattern favours triglyceride accumulation in the femoral adipocytes in pregnant women at early pregnancy. In contrast, lipolysis was considerably higher in the abdominal than in the femoral SAT adipocytes. Thus, it is possible that femoral adipose tissue may serve a specialised function in women, as an important source of energy supply during lactation. This is supported by the observation that women usually have a larger gluteal fat depot than men (Sjöström et al., 1972). Vesco et al. (2018b) assessed healthy pregnant women for ectopic fat deposition (intra-hepatic, intra-myocellular and extra-myocellular) in early and late pregnancy with magnetic resonance spectroscopy, and found no evidence of ectopic fat deposition. Moreover, whole-body insulin sensitivity was measured by insulin clamp, and was not related to ectopic fat or fat mass measured by BODPOD. Thus, Vesco et al. (2018b) suggested that non-body composition factors appear to be the primary determinant of gestation-related insulin resistance. In a second study, Vesco et al. (2018a) showed that gestational weight gain in healthy pregnant women is not related to ectopic fat deposition during pregnancy.

1.6.2 Adipose tissue in obese pregnancy

Maternal obesity has been the driver for the interest in adipose tissue biology in pregnancy. Pre-gestational obesity has been shown to have a significant influence on adipose tissue distribution during pregnancy (Ehrenberg et al., 2003). There was an increase in VAT accumulation measured by ultrasound in overweight/obese pregnant women compared to lean pregnant controls (Straughen et al., 2013). This finding suggests the possibility that the observed differences in VAT deposition across pregnancy are related to the different metabolic adaptations to pregnancy

seen in overweight and obese women compared with lean women (as discussed in section 1.3.2).

Adipocyte size and number in abdominal SAT was examined in a cohort of obese pregnant women, compared to normal pregnant women, during the first and third trimesters of pregnancy. The mean adipocyte size and the proportion of large adipocytes was higher in normal-weight women than obese women, and was accompanied by reduced adiponectin release (Svensson et al., 2016). In contrast, obese women had a higher proportion of small cells and lower proportion of large cells in the third trimester, suggestive of adipocyte recruitment, and maintained adiponectin levels. This study suggested that changes in adipose tissue cellularity during pregnancy in obese women help to protect against even more severe insulin resistance. Adipocyte lipolysis was increased in the third trimester compared to first-trimester levels in both the lean and obese groups (Svensson et al., 2016). The increase in lipolysis levels from first trimester to third trimester was slightly higher in the obese group, but did not reach statistical significance. Although VAT fat mass was reported to be increased in obese pregnant women during pregnancy, VAT lipolysis has not been examined to the best of our knowledge. Increased VAT FA flux could potentially provide the link to the metabolic disturbances observed in obese women during pregnancy, as suggested by the overspill hypothesis discussed in 1.5.7.

1.6.3 Adipose tissue and GDM pregnancy

In a cohort of women with GDM (lean and obese) and healthy pregnant (lean and obese) controls, body composition was measured longitudinally at preconception, first trimester and third trimester by hydrodensitometry and serial skin fold measurement. Healthy lean women were noted to have significantly more peripheral fat, whereas healthy obese women accumulated adipose tissue more centrally (Ehrenberg et al., 2003). This pattern of adipose tissue accumulation over the course of pregnancy may contribute to the insulin-resistant state of obesity. In contrast, there were no significant differences in the pre-gravid body composition measures between GDM and control subjects in either the lean group or obese group. This suggests that pre-gravid obesity is more influential in body fat distribution than glucose tolerance status. Women with a previous history of GDM, followed for five years after the index pregnancy, had a higher VAT content

compared to non-GDM women (Lekva et al., 2015). There was a strong association of higher VAT content, with low β-cell function, indicating that the VAT compartment may contribute to the metabolic disturbances in women with a history of GDM in the long-term (Lekva et al., 2015).

Rojas-Rodriguez et al. (2015) studied adipocyte size and capillary density in a small cohort of women with GDM (n=11), compared to healthy pregnant controls (n=3). In GDM, VAT adipocyte hypertrophy and reduced capillary density compared to controls was observed. This study suggested that VAT adipocyte hypertrophy is probably due to diminished hyperplastic potential in this depot. ADMSC were strongly associated with the AT capillary network, and a reduction in capillary density may underlie the diminished hyperplastic growth with subsequent VAT adipocyte hypertrophy (Rojas-Rodriguez et al., 2015). Functional studies of adipose tissue during GDM pregnancy are very limited, and to the best of our knowledge, adipocyte lipolysis in women with GDM has not been studied previously.

1.6.3.1 Ectopic fat in GDM

There is emerging evidence to suggest that ectopic fat deposition in the liver, skeletal muscle and pancreas could be one of the factors that causes IR in women with GDM. Non-alcoholic fatty liver disease (NAFLD) is characterised by the presence of fat within the liver in a person who does not drink significant amounts of alcohol (Angulo, 2002). A recent study examined the relationship between first-trimester NAFLD (measured by ultrasound) and subsequent development of GDM, and revealed that NAFLD is an independent risk factor for GDM development (Lee et al., 2019). However, the molecular mechanisms linking hepatic fat accumulation and GDM development remained elusive. It could be that maternal hyperinsulinemia in women with GDM derives hepatic *de novo* lipogenesis and inhibits VLDL-TAG secretion from the liver, leading to hepatic steatosis. Additionally, obese mothers with GDM may experience greater portal NEFA flux from VAT stores, which has been found to preferentially increase fat accumulation in women with GDM (as discussed in 1.6.3). These mechanisms are speculative and require further investigation. Liver fat accumulation during pregnancy is probably the link between GDM and future risk of T2DM. Bozkurt et al. (2012) found that fatty liver is associated with subclinical inflammation in women with a previous history of GDM (3–6 months), and demonstrated that the fatty liver index (FLI) is predictive of

T2DM. Recently, Mehmood et al. (2018) demonstrated that there is a higher prevalence of liver fat in women with a previous history of GDM, and it is associated with an increased risk of pre-diabetes/diabetes among them.

Vesco et al. (2019) assessed liver and muscle fat content in GDM using magnetic resonance spectroscopy (MRI). There was no increase in intra-hepatic, intra-myocellular or extra-myocellar lipid content in women with GDM compared to healthy pregnant controls. However, this cohort of women had not demonstrated an increase in VAT accumulation during pregnancy. In contrast, women with a previous history of GDM were found to have higher intra-myocellular fat content compared to women with normal glucose tolerance (Kautzky-Willer et al., 2003). Kautzky-Willer et al. (2003) suggested that intra-myocellular fat content could serve as an additional parameter of increased diabetes risk, because it identified women with a previous history of GDM, and those women who were diagnosed earlier in pregnancy with GDM. It was suggested that a reduction in the compensatory insulin release from pancreatic β cells in obese women during pregnancy could be related to ectopic fat deposition in β cells (Giacca et al., 2011). However, there are no primary studies to confirm this yet. A decrease in β cell function was found to be associated with VAT mass in women with a history of GDM five years after the index pregnancy (Lekva et al., 2015). This may suggest that FA flux from VAT stores is the underlying mechanism for ectopic fat in the pancreas in women with GDM.

1.7 Adipose tissue inflammation in healthy, obese and GDM pregnancy

Compared to non-pregnant women, healthy pregnancy is characterised by mild elevations in both serum pro-and anti-inflammatory cytokine levels (Curry et al., 2008). Christian and Porter (2014) examined the longitudinal changes in several plasma inflammatory markers during and after pregnancy; pregnancy was associated with higher levels of plasma IL-6 and TNF-α, but decreased CRP level, whereas obese women had higher CRP levels, and obesity predicted their plasma IL-6 levels. Serial gluteal fat samples were obtained from healthy non-obese women, sampled pre-gravid and in early (8–12 weeks of gestation) and late (36–38 weeks of gestation) pregnancy. Adipose tissue transcriptomic analysis revealed early pregnancy molecular changes in the TLR4/NF-κB signalling pathway with respect to inflammatory adipokine genes (Resi et al., 2012). Furthermore, increased

expression of macrophage markers such as CD68 and CD14 emphasised the recruitment of the immune network in both early and late pregnancy. This biphasic pattern suggests that there could be an early enhancement of immune pathways as an initial step in pregnancy, proceeding the development of insulin resistance, which peaked in late pregnancy. This hypothesis would suggest that the activation of inflammatory pathways could be necessary to induce gestational insulin resistance towards the third trimester. Adipose tissue cytokines were suggested to contribute to the low-grade inflammation status at the third trimester of pregnancy.

Maternal obesity is considered to be the primary driver of inflammation during pregnancy due to increased fat mass. An increase in TNF-α mRNA levels in the maternal SVF cells of obese women has been observed (Basu et al., 2011). Recently, the VAT depot in overweight and obese pregnant women was shown to be strongly associated with adipocyte hypertrophy and increased macrophage infiltration compared to SAT (Bravo-Flores et al., 2018). Macrophages recruited to adipose tissue might be a noticeable source of inflammatory cytokines in obese pregnancy (Challier et al., 2008). The activation of inflammatory pathways in adipocytes impairs triglyceride storage and increases the release of FFAs, an excess of which is known to induce insulin resistance in skeletal muscle and the liver in the non-pregnant population (Guilherme et al., 2008). Thus, adipose tissue inflammation appears to be a clinically important change that may occur in the adipose tissue of obese pregnant women who are metabolically challenged by pregnancy. Previous observations provide evidence for defective insulin signalling in the adipose tissue of obese pregnant NGT mothers, and adipose tissue inflammation is one of the suggested underlying mechanisms (Colomiere et al., 2009). TNF-α induction of IRS-1 serine phosphorylation links inflammation to disturbed insulin action. One leading hypothesis for the role of cytokines such as TNF-α, adiponectin and resistin is that they link inflammation to metabolic changes by modifying insulin sensitivity (Matarese and La Cava, 2004). The sequence of events that leads to adipose inflammation, and how those events are regulated, is still poorly understood.

Enhanced insulin resistance and inflammation are the main hallmarks of GDM. The macrophages infiltrating adipose tissue are laid out in a crown-like structure surrounding adipocytes in GDM (Cinti et al., 2005). It was previously identified that GDM is associated with increased macrophage infiltration in omental fat, rather than

SAT, compared to normal pregnancy (Harlev et al., 2014). In the same study, omental fat macrophages correlated positively with HOMA-IR, suggesting that low-grade inflammation could contribute to the development of insulin resistance in GDM. Several pro-inflammatory adipokines were found to be increased in adipose tissue samples of women with GDM compared to controls. An increase in IL-6 mRNA in the SAT of women with GDM has been reported previously (Kleiblova et al., 2010). Nucleotide-binding oligomerisation domain (NOD) is an intracellular molecule that recognises danger signals by initiating inflammation and the activation of NF-KB signalling. Lappas (2014b) observed increased NOD1 expression in the omental and subcutaneous fat of women with GDM compared to NGT women, suggesting its importance in the development of inflammation and IR in GDM. The pro-inflammatory cytokine IL-1β is cytokine that impairs insulin signalling in adipocytes. The inflammasome is a large protein complex that processes IL-1β to its active secreted form. Caspase-1 is a key factor in the inflammasome, activated under conditions of cellular stress, which leads to the maturation of IL-1β. A previous study confirmed that there is increased expression of caspase-1 and IL-1β in the adipose tissue of GDM women (Lappas, 2014a). This study highlighted the importance of caspase-1/IL-1β signalling in the development of insulin resistance in GDM by decreasing AMP-activated protein kinase (AMPK) activity in the adipose tissue of pregnant women.

1.8 Project hypothesis

The hypothesis tested in this thesis is that in women with GDM, SAT adipocytes have defective adipocyte expansion, resulting in adipocyte hypertrophy. Subsequently, there is increased lipolysis and inflammatory adipokine secretion.

1.8.1 Study aims and objectives

This thesis aimed to explore the epidemiological evidence for the role of maternal obesity in the development of GDM and other maternal and fetal complications, specifically in the highly diverse local Greater Glasgow and Clyde population. Additionally, this thesis aimed to compare SAT and VAT adipocyte size, adipocyte lipolytic function and adipokine secretion between GDM and healthy (BMI-matched) pregnant women. A final aim was to assess the role in early pregnancy of VLDL as a potential maternal plasma carrier of the extremely important long chain

polyunsaturated fatty acid docosahexaenoic acid (DHA), required by the fetus for neuronal development.

Objective 1 (Chapter 3)

To explore the epidemiological evidence that women with maternal obesity are at greater risk of GDM and maternal and fetal adverse outcomes in the Greater Glasgow and Clyde population, and to understand the clinical characteristics of the background population of the overall study.

Objective 2 (Chapter 4)

To compare adipocyte function in isolated SAT and VAT adipocytes of healthy and BMI-matched GDM women in the third trimester. The aspects of adipocyte function assessed were adipocyte size, adipocyte lipolytic function (basal and β adrenergic-stimulated lipolysis, and insulin suppression of lipolysis) and fat cell insulin sensitivity index (FCISI). To explore the relationship between maternal BMI, markers of maternal systemic insulin sensitivity and maternal plasma pregnancy hormone concentrations with SAT and VAT adipocyte size and lipolytic function.

Objective 3 (Chapter 5)

To compare adipocyte inflammation in basal or activated (LPS stimulated) conditions in isolated SAT and VAT adipocytes from healthy and BMI-matched GDM women in the third trimester. To assess plasma cytokine concentrations in both groups.

Objective 4 (Chapter 6)

To explore plasma lipoprotein VLDL fatty acid composition in healthy pregnant women prior to pregnancy and throughout early gestation.

Chapter 2 Materials and methods

2.1 Study participants

Ethical approval for adipose function in GDM study was obtained from the West of Scotland Research Ethics Committee and Research and Development, NHS Greater Glasgow and Clyde reference number 11/AL/0017 . All participants were fully informed of the study objectives and participation requirements by means of verbal communication and a written information sheet. Written consent was obtained from all participants.

2.1.1 Recruitment

Adipose tissue and blood samples were obtained from non-labouring healthy pregnant women at term (n=24) and non-labouring women with GDM (n=23) undergoing Caesarean section at the Queen Elizabeth University Hospital in Glasgow. The International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria were utilized locally at Queen Elizabeth University Hospital (QEUH) to establish the diagnosis of GDM (Metzger et al., 2010). According to IADPSG criteria, a diagnosis of GDM can be made if one result of blood glucose levels reaches one or more of the following values (fasting blood glucose ≥ 5.1, 1 hour≥ 10.0, 2 hours≥8.5 mmol/L) using a 75g glucose load test at 24-28 weeks of gestation. Treatment (diet, insulin, metformin or both insulin and metformin) was recorded for women with GDM. Women undergoing elective Caesarean section between 37+0 weeks to 39+6 weeks were eligible for this study. Women of all ages and ethnic groups were eligible while multiple pregnancies were excluded. Women were categorized according to the World Health Organization categorization of obesity to: normal weight (BMI=20-24.9 kg/m^2), overweight (BMI=25-29.9 kg/m^2) and obese (BMI ≥30kg/m^2) (Nuttall, 2015) using booking BMI. Participants were excluded if they had any history of medical conditions such as cardiovascular or metabolic disease and other adverse pregnancy outcomes such as preeclampsia or intrauterine growth restriction. The study participants were not on regular medication such as antiemetic drugs, aspirin, low molecular weight heparin, thyroxine or blood pressure medications.

The subject's demographic characteristics and delivery information were recorded from the patient's medical record. SAT samples were obtained by the obstetric

surgeon from the fat layer beneath the skin while VAT samples were obtained from greater omentum. The fresh adipose tissue was placed in warm buffer and quickly transported to the laboratory for adipocyte preparation.

2.1.2 Tissue collection

Maternal blood was collected prior to the Caesarean section by the anaesthetist in charge in a 20 ml syringe and handed in to the researcher. The blood then added to a selection of blood tubes with various additives including K_2 EDTA, clot activator, sodium citrate, lithium heparin and potassium oxalate/sodium fluoride. The blood tubes were transferred immediately to the pathology lab at QEUH for immediate centrifugation within approximately 20 minutes of blood collection. The blood tubes were centrifuged at 3000rpm for 15 minutes then the resultant plasma or serum were aliquoted into colour coded (to the appropriate additive) microtubes and stored at -80°C. The plasma obtained from the sodium citrate tube was transferred into Eppendorf tubes and were further centrifuged using microcentrifuge at 13000 rpm for 4 minutes to remove platelets. Then the supernatant was transferred in to microtubes with color-coded lids. The surgeon was briefed to collect subcutaneous adipose tissue (SAT) sample from the fat layer beneath the skin on entry of the abdominal cavity about the size of 50 pence coin (approximately 4-6 gm) and this was placed in a previously labelled collection tube containing warm collection buffer. After delivery of the baby and placenta, a sample of visceral adipose tissue (VAT) about the size of 50 pence coin was collected from greater omentum and placed immediately in a previously labelled collection tube with warm collection buffer. The adipose tissue was transported immediately to the laboratory by the researcher and processing was commenced immediately on arrival.

2.1.3 Power calculation

Power calculation for this study was carried out before commencing tissue collection. The primary outcome was adipocyte diameter (physical measure) while secondary outcomes were insulin suppression of lipolysis (functional measure), adipokine production by adipocytes (consequences of the previous changes). Adipocyte diameter has been studied recently in GDM (Rojas-Rodriguez et al., 2015), but all other outcomes have not been assessed in the population of women with GDM. A formal power calculation for the adipocyte diameter requires up to n= 7 per group for 80% power. We have no information about the effect size of the

insulin suppression of lipolysis in GDM but this was estimated using the effect size in preeclampsia. In preeclampsia a previous study by our group found a significant difference in n=14 per group (Huda et al., 2014). Our power calculations suggests that we might find half the insulin resistance that we previoulsy observed in PE and we need closer to 60 per group to observe the same level of insulin resistance (Table 2-1). Therefore, we aimed to collect a GDM group double the size of the PE group, that n=30 per group.

Table 2-1 Power calculation

	Control mean (SD)	Test mean (SD)	Standardized difference (control mean-test mean/ control mean)	Standardized SD (control SD/ control mean)	n per group for 80% power
Adipocyte diameter (Rojas-Rodriguez et al., 2015)	4.16 (1.38)	7.48 (2.98)	0.79	0.40	GDM 6/7
Insulin suppression of lipolysis (Huda et al., 2014)	65	52	PE= 0.8 ½ GDM=0.4	1.57	PE 62 GDM 123
Adipose activated macrophage content (Huda et al., 2017)	9 (10)	18 (36)	PE= 1 ½ GDM= 0.5	0.94	PE 15/20 GDM 57/76
Adipokine production by adipocytes (Huda et al., 2017)	31 (27)	62 (57)	PE= 1 ½ GDM= 0.5	0.92	PE= 15/19 GDM= 55/73

2.2 Adipocyte preparation

2.2.1 Buffers

The following physiological buffers were prepared using distilled water and chemicals from VWR UK, unless otherwise stated. With the exception of Krebs Ringer- HEPES (KRH) buffer, all buffers were made within 14 to 18 hours before

sample collection and kept at 4ºC overnight before collection, then warmed to 37°C on collection day. To confirm buffer stability using this approach, an experiment was conducted to compare buffer made fresh on the collection day and buffer made approximately 14 to 18 hours before sample collection using the same subcutaneous adipose tissue sample of healthy individuals (n=3). The experiment is detailed in section 2.4.5.

2.2.1.1 Krebs Ringer - HEPES (KRH) Buffer

KRH buffer consisted of 118mM NaCl (Merck, UK), 5mM $NaHCO_3$, 4.7mM KCl, 1.2mM KH_2PO_4, 1.2mM $MgSO_4.7H_2O$ (Sigma, UK) and 25mM HEPES (Sigma, UK). The buffer was adjusted to pH 7.4, autoclaved and stored at 4°C.

2.2.1.2 Wash buffer

Wash buffer was KRH buffer containing 2.5mM $CaCl_2$ and 151µM BSA, essentially FA free (Sigma-Aldrich, UK, cat.no. A6003).

2.2.1.3 Collection buffer

Collection buffer was wash buffer containing D-glucose 3mM (Fisher Scientific, UK).

2.2.1.4 Digestion buffer

Digestion buffer consisted of 4ml collection buffer per 1g of adipose tissue plus 2mg/ml collagenase (Type 1, Worthington biomedical corporation, UK, cat. no. L5004197)

2.2.2 Collagenase digestion of adipose tissue and isolation of adipocytes

If the adipose tissue samples were more than 6-8 g, a small piece of adipose tissue was flash frozen in liquid nitrogen and stored at -80°C until further use. The remaining unfrozen adipose tissue sample was placed in a petri dish with warm collection buffer. Scissors and forceps were used to remove any skin, visible blood vessels and fibrous tissue. The sample was dried quickly on filter paper and weighed. The weight of the tissue was used to determine the required volume of digestion buffer containing collagenase at 2mg/ml. The sample was then placed into

a 50ml centrifuge tube containing the digestion buffer and minced finely with scissors. Collagenase digestion occurred for 30mins at 37°C in a shaking water bath at 100 cycle per minute.

After collagenase digestion the adipose-buffer mixture was filtered first through a plastic tea strainer (600µm pore) to remove any large pieces of undigested tissue. The digestate was then passed through a 400µm pore mesh. The adipocytes were allowed to float to the top and the buffer underneath was aspirated away using a 20ml syringe and 18-gauge needle and replaced with fresh warm wash buffer of a similar volume. This process was repeated up to a maximum of four times, or until the buffer under the adipocytes was clear. The buffer was finally aspirated away as much as possible such that the adipocytes were suspended at approximately 90% cytocrit.

The experimental tubes (15ml centrifuge tubes) were prepared to contain 900µl warm (37°C) wash buffer. The adipocyte cell suspension (100µl) was added to the experimental tubes followed by all other reagents. All experimental conditions were carried out in duplicate. The tubes were placed in a shaking water bath at 37°C with gentle agitation of 91 cycles per minute for 2 hours. Remaining adipocytes were frozen in aliquots at -80°C for subsequent DNA quantification and RNA isolation.

2.2.3 Adipocyte DNA extraction

DNA was isolated from a known volume of adipocyte cellular suspension from each preparation that had been flash frozen in liquid nitrogen and then transferred to a -80°C freezer immediately after adipocyte isolation was carried out. Two aliquots of 100µl adipocytes were thawed at room temperature and DNA was extracted using a Nucleospin® DNA Lipid Tissue kit (Machery-Nagel, Germany) which utilizes Silica-membrane technology. Lipid-rich tissues can cause difficulties in DNA isolation by influencing the tissue disruption or changing the chemistry of the DNA isolation buffer. The Nucleospin® DNA Lipid Tissue kit combined enzymatic lysis and mechanical disruption of lipid-rich tissues with the Nucleospin® Bead Tubes which were used to obtain high DNA yields from adipocytes. Following the manufacturer's protocol, 200µl of thawed adipocyte suspension was transferred to bead tube D (3mm steel balls) and 100µl of elution buffer BE was added to prepare the sample for lysis. Cell lysis was achieved by adding 40µl of lysis buffer LT, 10 µl

of liquid proteinase K and agitating the tubes in the bead tube holder (Vortex mixer, VV3, VWR) for 20minutes at full speed. The tube was spun afterward to clear the lid at 13000rpm for 30 seconds in a bench microcentrifuge. To adjust DNA binding conditions, 600µl of lysis buffer LT was added, vortexed and centrifuged as above. DNA binding was achieved by transferring the clear liquid supernatant (approximately 500-600µl) to a spin column placed inside a collection tube and then centrifuged as above. The silica membrane was washed twice, by adding 500µl of wash buffer BW and then 500µl of wash buffer B5 followed by centrifugation with discard of the flow through at each wash. Further centrifugation took place to dry the silica membrane and remove the residual wash buffer. DNA elution was completed by placing the spin column in an autoclaved Eppendorf tube and 100µl of warm elution buffer BE at 70°C was added to the column followed by incubation at room temperature for one minute and the tube was then centrifuged as above. The resultant eluate was then reused in another elution step to improve DNA yields. The DNA was then transferred to a new autoclaved Eppendorf tube and left incubating overnight in the fridge. DNA quantification by Nanodrop was carried out the following day after resuspension. A 260/280 ratio of ~1.8 is generally accepted as “pure” for DNA.

2.2.4 Normalising NEFA, glycerol and inflammatory cytokines to total DNA content

Lipolysis rates as measured by NEFA and glycerol are influenced by adipocyte cellular density in each experimental condition. Therefore, DNA was extracted and quantified from a known volume of adipocytes cellular suspension. The adipocytes cell number is directly proportional to DNA content within the same volume. Lipolysis rates are expressed as per ug of DNA.

2.3 Adipocyte sizing

Equal volumes of unfixed fresh adipocyte cellular suspension (5µl) and KRH buffer (5µl) were mixed on a glass slide. The slide was placed under an Olympus BX50 microscope connected to a 3-CCD colour camera (JVC) and 7-9 digital images of the adipocytes were captured on different fields at x10 magnification (Figure 2-1). Computer visualisation of images was carried out using Image-Pro Plus 4.0 and the images were analysed with Adobe Photoshop version 7.0. The internal diameters of 100 adipocytes were measured manually using a graticule that was calibrated

such that one pixel = 78μm. The diameters of the cells were determined in μm by the following equation: μm=Y/X*100, given that Y=cell diameter in pixels and X=78 pixels. The volume of the cells in mm^3 was calculated using the formula:

$$(4/3) * 3.14159 * (1/8) * (d^3)/1000000000$$

Where d = adipocyte diameter in μm. One hundred adipocytes from the acquired images were randomly selected and their horizontal intracellular diameter was manually measured. Two blinded observers each measured 100 diameters for both GDM and BMI matched controls from which the mean diameter and volume of each adipocyte preparation was derived.

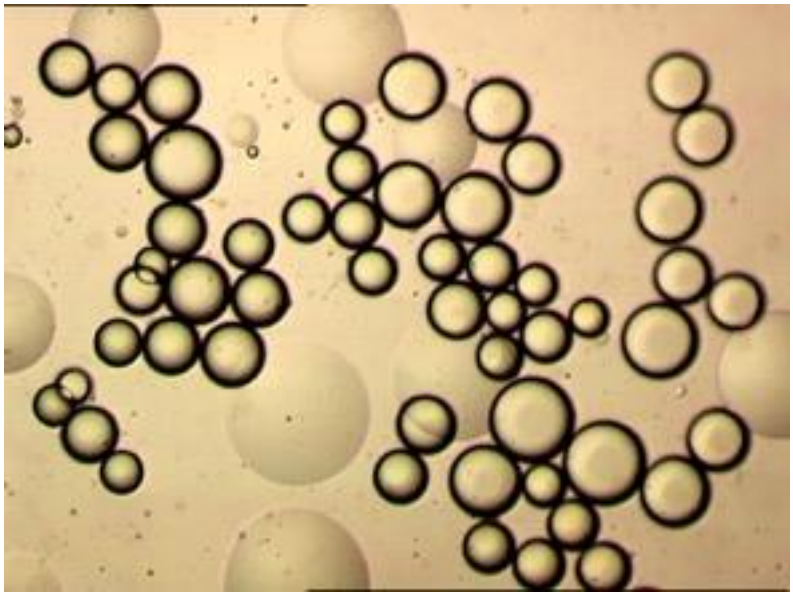


Figure 2-1 : Example of a digital image captured of an isolated adipocyte suspension

2.4 Lipolysis assay

2.4.1 Lipolysis assay conditions

2.4.1.1 Basal Lipolysis

Experimental tubes contained only of wash buffer and adipocytes.

2.4.1.2 Isoproterenol stimulation of basal lipolysis (isoproterenol only)

The non-specific beta-adrenergic agonist isoproterenol (Sigma, UK, cat. no. 16604) was dissolved in KRH buffer to obtain a stock of 2mM. This stock was diluted by a

factor of 100 to give 0.02mM. Isoproterenol (10µl of 0.02mM) was added to the experimental tubes to give a final isoproterenol concentration of 200nM.

2.4.1.3 Insulin inhibition of basal lipolysis (insulin only)

Insulin solution human (Sigma-Aldrich, UK, 19278 – 300 Units/ml) (1.76µl) was added to 3ml of KRH buffer to produce insulin stock (1000nM). Insulin stock solution (10µL of 1000nM) was added to the adipocyte cellular suspension (total volume of 1ml) to produce a final concentration of 10nM.

2.4.1.4 Insulin inhibition of stimulated lipolysis (isoproterenol + insulin)

Isoproterenol and insulin were prepared as above and 10µl of each were added to adipocyte cellular suspension. The final concentration was 200nM for isoproterenol and 10nM for insulin.

2.4.1.5 Lipopolysaccharide (LPS)

Sterile balanced salt solution (1ml of autoclaved KRH buffer) was added to 1mg lipopolysaccharide from Escherichia coli O55:B5 (Sigma Aldrich, UK, L6529) powder to produce LPS stock (1mg/ml) frozen in 25µl aliquots and kept at -80 until needed. On the day of experiment, 10µl of 1mg/ml LPS stock were added to culture vial contains 900µl of wash buffer and 100µl of adipocyte cellular suspension to obtain final concentration of 10µg/ml.

2.4.2 Timeline and dose response determination for lipolysis assay reagents

Time course of lipolysis experiment and dose response curve of isoproterenol, insulin and lipopolysaccharide (LPS) was determined previously (unpublished Shahzya Huda).

2.4.3 Assaying NEFA and glycerol as indicators of adipocyte net and total lipolysis respectively

Both NEFA and glycerol concentrations in the medium below the adipocytes were assayed as indicators of lipolysis. Once released from the adipocytes, NEFA can be taken up by the adipocytes and re-esterified to triglyceride a process referred to as recycling. Hence assaying NEFA gives a measure of **net** lipolysis. Glycerol,

however, once released from the adipocytes cannot be reutilised by the adipocytes due to the minimal expression of glycerol kinase. As such, assaying glycerol gives a measure of **total** lipolysis.

The medium below the adipocyte suspension was sampled at 2 hours for determination of NEFA and glycerol concentrations. Aliquots (5µl for NEFA and 10µl for glycerol) of the medium were added to individual wells of a 96 well plate. Each experimental condition was assayed for NEFA and glycerol in duplicate.

2.4.3.1 Measuring NEFA concentrations

NEFA concentrations were estimated using an enzymatic colorimetric NEFA assay kit (Wako, Alpha Laboratories, UK) using a protocol adapted for 96 well plate analysis. The kit provides substrates (coenzyme A and ATP) and enzymes (acyl-CoA synthetase) that convert NEFA in the medium to acyl-CoA. The Acyl-CoA is oxidised to produce hydrogen peroxide, which in the presence of peroxidase yields a violet pigment. NEFA concentration was obtained by measuring the absorbance of the pigment on a spectrophotometer (Multiscan EX, Thermo Electron Corporation) at 550nm. The values are compared to a NEFA standard curve. The standard curve was obtained by making serial dilutions of a NEFA standard (Wako, Alpha Laboratories, UK) to achieve a concentration curve ranging from 0.1-1mmol/L.

2.4.3.2 Measuring glycerol concentrations

Glycerol concentrations were determined by means of a colorimetric kit (Randox). The kit utilises a quinoneimine chromogen system whereby in the presence of glycerol kinase, peroxidase and glycerol phosphate oxidase, glycerol in the medium is converted to the product n-(4-antipyryl)-3-chloro-5-sulphonate-p-benzoquinoneimine (ACSB). Production of ACSB yields an orange pigment. Glycerol concentration was obtained by measuring the absorbance of the pigment on a spectrophotometer (Multiscan EX, Thermo Electron Corporation) at 520nm. The values are compared to a glycerol standard curve. The standard curve was obtained by making serial dilutions of a glycerol standard (Sigma, UK, G7793) to achieve a concentration curve ranging from 10-400µmol/L.

2.4.4 Calculation of percent inhibition, percent stimulation and fat cell insulin sensitivity index (FCISI)

The degree of lipolysis stimulation by isoproterenol was calculated as the percentage of release of NEFA or glycerol in the presence of isoprotenerol relative to basal lipolysis i.e. Percentage stimulation= ((lipolysis rate in presence of isoproterenol – basal lipolysis) / basal lipolysis)*100.

The degree of lipolysis suppression by insulin was calculated as the percentage of release of NEFA or glycerol in the presence of insulin relative to basal release i.e. ((basal lipolysis – lipolysis rate in the presence of insulin) / basal lipolysis)*100.

FCISI is a direct measure of adipocyte insulin sensitivity. It can be calculated as the percentage suppression of isoproterenol stimulated lipolysis by insulin measured either as NEFA or glycerol release i.e. FCISI= ((lipolysis rate in presence of isoproterenol – lipolysis rate in presence of isoproterenol and insulin) / (lipolysis rate in presence of isoproterenol – basal lipolysis))*100.

2.4.5 Buffer stability

For logistical reasons, preparation of buffer 14-18 hours in advance was advantageous due to the distance between the laboratory and QEUH hospital, while our standard protocol stated same day preparation. To ensure buffer stability when made the day before, an experiment was conducted to compare buffer made fresh on the day of sample collection and buffer made approximately 14 to 18 hours before sample collection using the same subcutaneous adipose tissue sample from healthy pregnant women. Subcutaneous adipose tissue samples (n=3) were cut into 2 pieces immediately after collection at the hospital in a petri dish using surgical scissors. One piece was placed in warm buffer made on the day of collection and the other one was placed in warm buffer made 14-18 hours before collection. The samples were then transferred immediately to the laboratory and adipocytes were isolated as described previously in section 2.2.2. Isoproterenol and insulin were prepared as above and added to adipocyte cellular suspension. Basal, isoproterenol stimulation of basal lipolysis, insulin inhibition of basal lipolysis and insulin inhibition of isoproterenol stimulated lipolysis conditions were carried out for each sample using buffer made on the day of collection or 14-18 hours before. After 2 hours incubation, the buffer below adipocytes were sampled for NEFA analysis to test if

there was an effect of earlier buffer preparation preparation on lipolysis. A two sample T test was carried out on NEFA lipolysis data corrected for DNA. There was no difference in basal lipolysis, isoproterenol stimulation of basal lipolysis, insulin inhibition of basal lipolysis and insulin inhibition of isoproterenol stimulated lipolysis between the two samples carried out using the two different buffer preparation approaches (Table 2-2).

Table 2-2 NEFA release in response to different experimental conditions using buffer prepared the day before collection (DB) and buffer prepared on the same day of collection (SD) (n=3)

Experimental condition	Buffer DB NEFA Mean (SD) (n=3)	Buffer SD NEFA Mean (SD) (n=3)	*P* value
Basal Lipolysis (mmol/l/ug of DNA)	1.11 (0.72)	1.36 (1.17)	0.78
Lipolysis rate in presence of 200nM isoproterenol (mmol/l/ug of DNA)	2.28 (0.63)	2.67 (0.79)	0.55
Lipolysis rate in presence of 10nM insulin (mmol/l/ug of DNA)	0.72 (0.25)	0.61 (0.19)	0.59
Lipolysis rate in presence of isoproterenol 200nM and 10nM insulin	1.98 (0.68)	2.11 (0.65)	0.81

2.5 Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was used to quantitate the mRNA expression after pre-amplifcation of cDNA of genes of interest in adipocytes.

2.5.1 Gene selection using Ingenuity Pathway Analysis (IPA)

An Ingenuity Pathway Analysis (QIAGEN) search was carried out by Maria Hagan, an intercalated medicine BSc student, for her BSc dissertation at the University of Glasgow, to identify genes of interest for quantitation. This tool which includes an extensive and up-to-date library of signalling and metabolic pathways, was used to systematically approach the selection of genes to investigate in terms of differential expression. Under “Genes and Chemicals”, the terms “insulin”, “progesterone” and “metformin” were searched individually, and the appropriate human gene or chemical was selected to create a “new pathway”. These search terms were

selected due to the role of the insulin pathway in the currently understood disease process of GDM, and progesterone as the prominent hormone involved in maternal metabolic adaptation to pregnancy. As many GDM patients are effectively treated with the drug metformin, the involvement of these pathways in understanding of the pathophysiology of the condition may be beneficial. By selecting the options “Build” and “Grow”, all molecules which are known to be directly or indirectly downstream of the gene or molecule investigated were added to the pathway. Using the option “Overlay”, a pre-existing dataset of genes expressed in adipose tissue was overlaid on the pathway, highlighting molecules which were both downstream of the gene of interest and expressed in adipose tissue. The three data sets (from “insulin”, “progesterone” and “metformin”) acquired from this analysis were compared manually in a Venn diagram to identify common genes expressed in these pathways in adipose tissue (Figure 2-2).

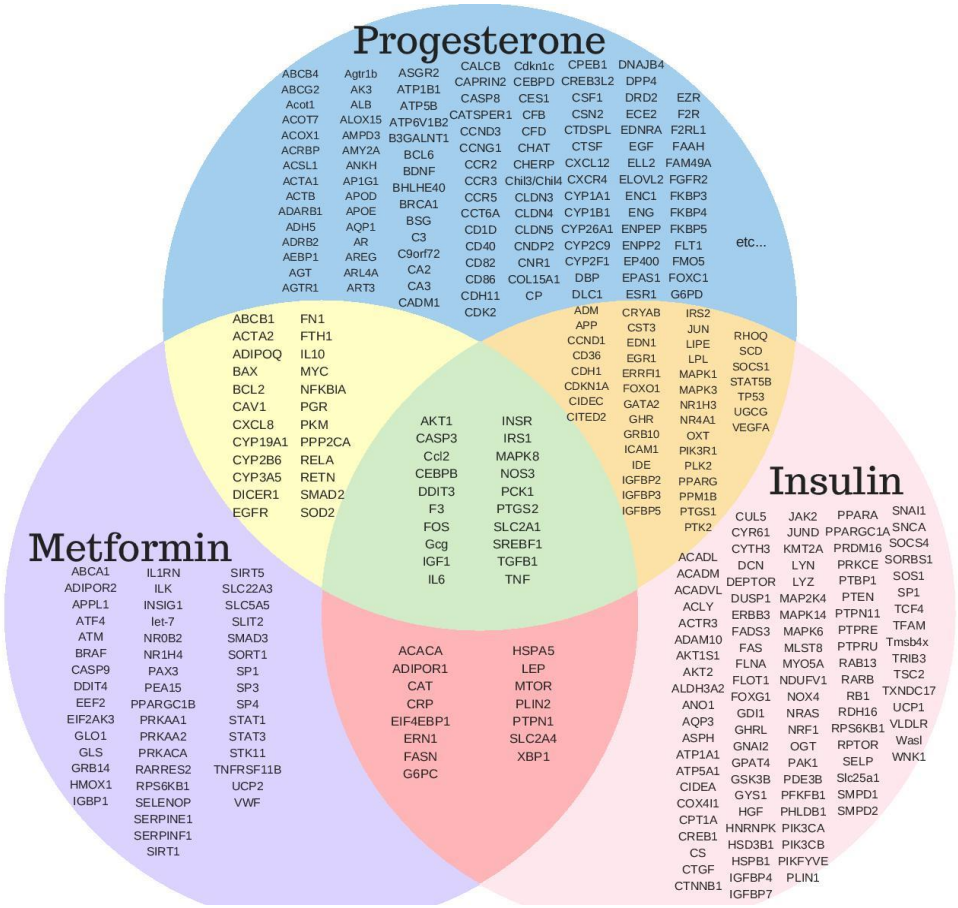


Figure 2-2 Venn diagram to identify common genes expressed in relation to Insulin, Metformin and progesterone pathways in adipose tissue using Ingenuity Pathway Analysis (QIAGEN).

2.5.2 Isolation of total RNA

The RNeasy Lipid Tissue Mini Kit (Qiagen) was used to isolate total RNA from frozen stored adipocytes, as per the manufacturer's instructions. Briefly, 100µl of isolated adipocytes were homogenised in 1ml Qiazol lysis reagent using a hand-held homogeniser (Omni UH Motor Battery Pack & Charger, Camlab). The homogenate was transferred to a 1.5ml centrifuge tube and incubated at room temperature for 5 minutes. Chloroform (200µl) was added to the homogenate and shaken vigorously for 15 seconds followed by incubation at room temperature for a further 3 minutes. The samples were subsequently centrifuged at 13000rpm for 15 minutes at 4°C using Beckman Coulter™ Microfuge 22R centrifuge. The upper aqueous phase (approximately 400µl) was transferred to a clean 1.5ml centrifuge tube, to which the same volume of 75% ethanol was added, and the sample was vortexed. The mixture was then transferred to RNeasy spin columns in batches and centrifuged for 15 seconds at 10,000rpm in the microcentrifuge. The flow-through was discarded and this step repeated until the entire sample was processed. The column was then washed twice with RPE buffer (500µl) (QIAGEN) and centrifuged at 10,000rpm for 15 seconds and two minutes respectively. The column membrane was dried by centrifugation at 10,000rpm for 1min. RNase-free water (30µl) was then added to the sample column prior to centrifugation at 10,000rpm for one minute. The concentration and quality of the eluted RNA was determined using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific). This produced a 260/280nm absorbance ratio and concentrations in ng/µl. A 260/280 ratio of ~2.0 is generally accepted as "pure" for RNA. The RNA was stored at -80°C until required.

2.5.3 DNase treatment of isolated RNA

A DNA-*free*™ kit (Ambion, cat. no.AM1906) was used to remove contaminating genomic DNA from the extracted RNA samples. Nuclease free water (6.5µl) was added to 15µl of RNA sample to make a final volume of 21.5µl. 10X DNase 1 buffer (2.5µl) was then added to the mixture and centrifuged at 13000rpm for 6 seconds at 4°C using Beckman Coulter™ Microfuge 22R centrifuge. The mixture was then incubated for 30 minutes at 37°C in an OMN-E thermalcycler. Following this, DNase Inactivation Reagent was vortexed before adding 3ml and the aliquots were mixed. They were incubated for a further 2 minutes at room temperature. The samples were mixed again to re- suspend the DNase Inactivation Reagent. It was then removed

by centrifuging the samples at 13,000rpm for 1 minute using the microcentrifuge. The transparent supernatant layer containing RNA was transferred to a new tube, leaving the DNase Inactivation Reagent at the bottom. DNA-free RNA was stored at -80°C.

2.5.4 cDNA synthesis

To make complementary DNA (cDNA), RNA was reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, cat. no. 4368813) in the presence of ThermoFisher SUPERase:In (cat. no. 2696) RNase. DNase-treated RNA samples and reagents were thawed and kept on ice for the duration of this procedure. The following volumes were added into a new Eppendorf tube to make 2x RT mastermix: 34µl of 10X RT buffer, 13.6µl of 25X dNTPs, 34µl of 10X random primers, 17µl of multiscribe reverse transcriptase (mRT), 17µl of SUPERase:In and 54.4µl of nuclease free water. An additional control “No RT mastermix” was also prepared using the same volumes of reagents but without mRT. These were mixed by vortexing. 2X RT mastermix (5µl) was added to 5µl of each sample. They were then briefly centrifuged followed by incubation at 25°C for 10 minutes. This was followed by incubation at 37°C for 120 minutes then 85°C for 5 seconds using the ThermoFisher Hybaid PCR Express Thermal Cycler. The samples were stored at -20°C.

2.5.5 Preamplification of cDNA and Test of Uniformity

Due to the very small quantity of RNA which can be isolated from adipocytes, a very small quantity of cDNA is obtained from reverse transcription. The TaqMan PreAmp Master Mix Kit (Applied Biosystems, cat. no. 4384266) was used to increase the quantity of the cDNA targets for gene expression analysis. The preamplification product was used for TaqMan Real-Time PCR. A pooled assay mix was prepared according to the manufacturer instructions containing the appropriate volume of Tris-EDTA (TE) PH 8 buffer (Ambion, cat. no. AM9849), primer probes of the genes to be investigated including Cyclin-dependant Kinase Inhibitor 1B *(CDKN1B)* the recommended endogenous uniformity reference gene and *PPIA* the endogenous control gene to be used in relative quantitation Real-Time PCR analysis. Pooled assay mix, TaqMan PreAmp Master Mix (2X), nuclease free water and the samples cDNA was added to a 96 well plate. The plate was centrifuged briefly in Jouan CR412 centrifuge, loaded into a thermocycler (StepOnePlus Real-Time PCR

System (Applied Biosystems)) and run for 10 preamplification cycles which was programmed to heat the samples at 95°C for 10 minutes followed by 10 cycles of 95°C for 15 seconds and 60°C for 4 minutes. The resulting preamplified cDNA was diluted with TE buffer in a 1:5 ratio and stored at -20°C.

It is vital that preamplification is uniform and this was tested for each of the TaqMan probes utilised, using a uniformity reaction plate to perform a relative quantitation run for a pre-amplified cDNA and a non-limited cDNA sample. This was carried out following the protocol published by ThermoFisher (ThermoFisher, 2012). Gene expressions of each gene were examined in duplicate non-pre-amplified cDNA samples and compared to duplicate pre-amplified samples using *CDKN1B* as a reference gene.

Calculated threshold cycle (C_T) values for the target genes and *CDKN1B* were obtained for different dilutions of pre-amplified and non-pre-amplified cDNA. $\Delta\Delta C_T$ values were used for analysis and obtained using the following calculations:

ΔC_T of sample = (Mean C_T of target gene – mean C_T of *CDKN1B*)

$\Delta\Delta C_T$ of sample = (ΔC_T pre-amplified cDNA - ΔC_T of non-pre-amplified cDNA)

The $\Delta\Delta C_T$ values for all the genes tested were within ±1.5 (Figure 2-3) and therefore preamplification was deemed successfully uniform. Applied Biosystems manufactured the gene probes was used (Table 2-3) and assured that >90% of the *CDKN1B* control is capable of amplifying uniformly (Applied Biosystems, 2009).

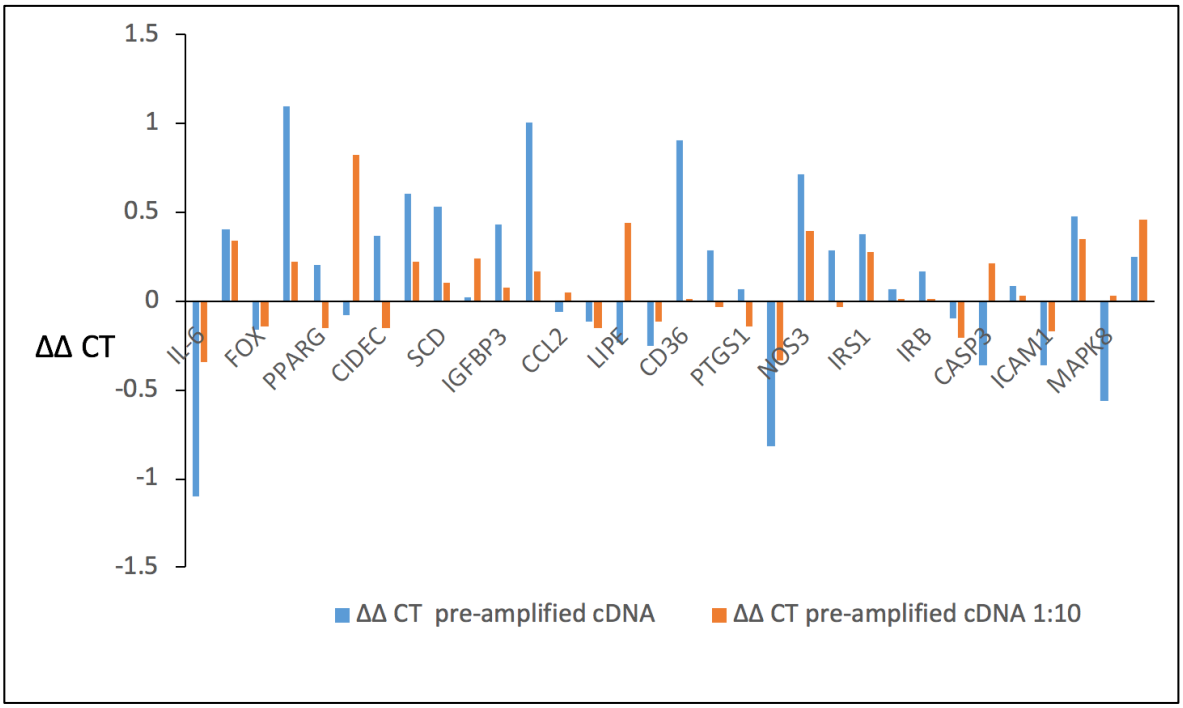


Figure 2-3 Preamplification test of uniformity results

Table 2-3 TaqMan gene expression assays

Gene symbol	TaqMan® gene expression assay	Assay ID	Source
PPIA	Peptidyl-Protyl Isomerase A	4333763T	Applied biosystems
FOXO1	Forkhead box protein 01	HS00231106_m1	Applied biosystems
CEBPB	CCAAT/Enhancer binding protein beta	HS00279023_S1	Applied biosystems
IGF1	Insulin-like growth factor 1	HS01547656_m1	Applied biosystems
FOS	Fos protein	HS99999140_m1	Applied biosystems
CCL2	C-C Motif Chemokine Ligand 2	HS00234140_m1	Applied biosystems
IGFBP5	Insulin-like growth factor- binding protein 5	HS00181213_m1	Applied biosystems
SCD	Stearoyl-CoA desaturase	HS01682761_m1	Applied biosystems
CD36	Platelet glycoprotein 4	HS00354519_m1	Applied biosystems
LIPE	Lipase E, Hormone Sensitive Type	HS00193510_m1	Applied biosystems
CASP3	Caspase 3	HS00234387_m1	Applied biosystems
MAPK8	Mitogen-activated protein kinase 8	HS01548508_m1	Applied biosystems
CIDEC	Cell death activator CIDE-3	HS01032998_m1	Applied biosystems
TNF	Tumour necrosis factor alpha	HS00174128_m1	Applied biosystems
PPARG	Peroxisome proliferator- activated receptor gamma	HS01115513_m1	Applied biosystems
SREBF1	Sterol regulatory element- binding protein 1	HS01088691_m1	Applied biosystems
SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	HS00892681_m1	Applied biosystems
LPL	Lipoprotein lipase	HS001173425_m1	Applied biosystems
PCK1	Phosphoenolpyruvate carboxykinase 1	HS00159918_m1	Applied biosystems
GCG	Glucagon	HS01031536_m1	Applied biosystems
TGFB1	Transforming growth factor beta 1	HS01031536_m1	Applied biosystems
ICAM1	Intercellular adhesion molecule 1	HS00164932_m1	Applied biosystems
VEGFA	Vascular endothelial growth factor A	HS00900055_m1	Applied biosystems
F3	Coagulation factor 3	HS01076029_m1	Applied biosystems
IGFBP2	Insulin-like growth factor- binding protein 2	HS01040719_m1	Applied biosystems
AKT1	RAC-alpha serine/threonine- protein kinase	HS00178289_m1	Applied biosystems
IRS2	Insulin receptor substrate 2	HS00275843_S1	Applied biosystems
INSR	Insulin receptor	Hs00961554_m1	Applied biosystems
IGFBP3	Insulin-like growth factor- binding protein 3	HS00181211_m1	Applied biosystems
IRS1	Insulin receptor substrate 1	HS00178563_m1	Applied biosystems
DDIT3	DNA Damage Inducible Transcript 3	HS01090850_m1	Applied biosystems
PTGS2	Prostaglandin-Endoperoxide Synthase 1	HS00153133_m1	Applied biosystems
PTGS1	Prostaglandin-Endoperoxide Synthase 2	HS00377726_m1	Applied biosystems
NOS3	Nitric Oxide Synthase 3	HS01574659_m1	Applied biosystems
IRA	Insulin receptor isoform A	1770698B8	Applied biosystems (custom made)
IRB	Insulin receptor isoform B	1771710A12	Applied biosystems (custom made)

2.5.6 Insulin receptors isoforms A (*IRA*) and B (*IRB*) probes

The mature human insulin receptor has two isoforms, *IRA* and *IRB*. They result from alternative splicing of the same primary transcript. *IRA* differs from *IRB* by exclusion of exon 11. A recent method to specifically measure *IRA* and *IRB* expression has been described by Huang and colleges (Huang et al., 2011). This method was based on TaqMan qRT-PCR. They designed qRT-PCR primer and probes for *IRA* and *IRB*. For the *IRA* assay, Haung and colleagues targeted the exon 10/12 junction region for the gene specific probe; the exon 10 coding region for the forward primer pairs; and the exon 12 coding regions for the reverse primer pairs. For *IRB* assay, they targeted the exon 11 interior coding region for the gene specific probe; exon 11/12 junction for the forward primer pairs; and exon 12 for the reverse primer pairs. All probes were designed to incorporate a minor groove binding moiety (MGB) and were labelled with a fluorescent dye (FAM) for detection and a non-fluorescent quencher. We used the sequences for *IRA* and *IRB* primer/probe published by Huang et al. (2011), and primer/probes were custom ordered from Applied Biosystems (Table 2-3).

The sequences for primers and probes were as follows:

for *IRA*, probe sequence is 5'-TCCCCAGGCCATCT-3'; forward primer sequence is 5'-TGAGGATTACCTGCACAACG-3'; reverse primer sequence is 5'-ACCGTCACATTCCCAACATC -3'.

For *IRB*: probe sequence is 5'-CCGAGGACCCTAGGC-3'; forward primer sequence is 5'-CGTCCCCAGAAAAACCTCTTC-3'; reverse primer sequence is 5'-GGACCTGCGTTTCCGAGAT-3'.

This assay was proven to be more sensitive and specific for *IRA* and *IRB* isoforms compared to the commercially available Applied Biosystems assays which had a strong cross reaction and could not measure insulin receptors isoforms accurately (Huang et al., 2011).

2.5.7 TaqMan RT-PCR

Target gene expression was quantified relative to an endogenous control gene, *PPIA* (Neville et al., 2011), using TaqMan probes. A PCR reaction mixture was

prepared for each target gene (12.5µl of TaqMan Universal PCR Mastermix, 1.25µl target assay probe mixture and 5 µl of nuclease-free water) and 18.75µl of this was added to 6.25µl of diluted pre-amplified cDNA sample for a final reaction volume of 25µl per well of a 96 well plate. *PPIA* endogenous controls and no-RT controls (using 6.25µl of water instead of cDNA) were carried out for all samples. Target gene expression of the pre-amplified cDNA samples were carried out in duplicate on 96-well plate and appropriate negative controls used. The plate was centrifuged briefly using Jouan CR412 centrifuge, loaded into a StepOnePlus Real-Time PCR System (Applied Biosystems) which completed a standard RT-PCR cycle i.e. 40 cycles of 50º°C for 2 minutes, 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds alternated with 60°C for 1 minute. This produced an amplification plot. Average Ct values between the duplicate samples were obtained using SDS Software version 2.3. Expression of target genes was normalized to the endogenous control gene PPIA – previously shown to be a stable control gene for adipose tissue (Neville et al., 2011).These were converted to percentage expression relative to *PPIA* using the ΔCT method (C_T value of target assay – C_T value of PPIA).The calculation $2^{-\Delta CT}$ was used to determine the fold difference in expression relative to control, and the expression of the target assay was then expressed as a percentage relative to *PPIA*. Percent gene expression relative to control has an inter-assay CV <13% in adipocytes for a wide variety of target genes when carried out using a commercial primer process from Applied Biosystems (unpublished data from our lab).

2.6 Adipocyte inflammation

2.6.1 LPS

Isolated adipocytes were incubated with LPS for 2 hours as outlined in section 2.4.1.5. The medium underneath the adipocytes were aliquoted into two 300µl and frozen at -20°C for later analysis of adipokines.

2.6.2 Multiplex bead arrays

2.6.2.1 Assay principle

For the quantification of cytokines and chemokines in biological samples, multiplex bead arrays are an efficient alternative to the traditional enzyme-linked

immunosorbent assays (ELISA). In a traditional ELISA only one cytokine at a time is studied. The total number of possible analytes studied can be limited by sample volume when using ELISA. Multiplex bead arrays allow simultaneous quantification of several analytes (up to 100) in the same sample, and generally no more than 50µl of sample is required for the entire panel.

The principle of the multiplex bead assays is similar to that of ELISA based methods except that capture antibodies are covalently coupled to the surface of beads instead of the bottom of a 96-well plate. The bead conjugates are distinguished from one another by the fluorescent intensity ratio of two different fluorescent dyes embedded on each bead. Sample incubation is similar to ELISA, as well as the detection steps, which uses biotinylated detection antibodies and a streptavidin-phycoerythrin (PE) conjugate reporter. Sample acquisition is performed using a specialised flow cytometer which performs a series of analyses similar to flow cytometric analysis of cell targets. Individual beads are first gated for the correct size, then the bead type is determined based on ratio of the two internal fluorescent dyes, and lastly the fluorescent intensity of the reporter is determined for each individual analyte. It correlates with the concentration of a given analyte in solution. A standard curve is generated to calculate the final analyte concentration.

In the current study, kits utilizing magnetic bead technology were used. This technology is compatible with the MAGPIX® xPONENT 4.2 system, which uses a magnetic plate to capture all of the beads simultaneously in a monolayer, while two spectrally distinct light-emitting diodes (LEDs) illuminate the beads. One LED identifies the analyte that is being detected and, the second LED determines the magnitude of the PE-derived signal. Each well is imaged with a CCD camera system to capture a digital image and determine the fluorescence intensity of each bead. Analytes measured for VAT adipocytes are detailed in Table 2-4.

Table 2-4 Analytes measured for VAT adipocytes cytokine release in basal and LPS stimulated condition

Pro-inflammatory	**Anti-inflammatory**
Interferon Gamma (IFN-gamma) Interleukin 1-*beta* (IL-1β) Interleukin 8 (IL-8) Monocyte chemoattractant protein-1 (MCP-1) Tumour necrosis factor alpha (TNF-α) (by ELISA)	Interleukin 10 (IL-10) Adiponectin (by ELISA)
Both pro- and anti-inflammatory	**Angiogenic factors**
Interleukin 6 (IL-6)	Placental growth factor (PIGF) Vascular endothelial growth factor (VEGF)

2.6.2.2 Preparation of reagents for multiplex bead array assays

Preparation of antibody-immobilized beads

Individual vials of beads were provided with the kits. Each antibody-bead vial was sonicated for 30 seconds then vortexed for 1 minute. From each antibody vial, 60 µl (150 µl for the PlGF singleplex kit) was added to the provided mixing bottle and the final volume was brought to 3 ml with bead diluent and the final mixture was vortexed.

Preparation of Quality Controls

Quality Control 1 (QC1) and Quality Control 2 (QC2) vials were reconstituted with 250 µl of deionized water which were mixed, vortexed and allowed to sit for 10 minutes. Then the controls were transferred to appropriately labelled microcentrifuge tubes.

Preparation of Wash Buffer

The 10X Wash Buffer was brought to room temperature and mixed to bring all salts into solution. Sixty ml of 10X Wash Buffer was diluted with 540 ml deionized water.

Preparation of Human Cytokine Standard

The Human Cytokine Standard was reconstituted with 250 µl deionized water to give 10,000 pg/ml concentration of standard for all analytes. The vial was mixed and vortexed several times and allowed to sit for 10 minutes for complete reconstitution. This was used as the 10,000 pg/ml standard (standard 6).

Preparation of Human Cytokine Working Standards

Fife polypropylene microfuge tubes were labelled Standard 5, Standard 4, Standard 3, Standard 2 and Standard 1. Two hundred µL of Assay Buffer was added to each of the fife tubes. Serial 1:5 dilutions were performed by adding 50µL of the Standard 6 to the Standard 5 tube, mixed well and transferred 50µL of the Standard 5 to the Standard 4 tube, mixed well and transferred 50 µL of the Standard 4 tube to the Standard 3 tube, mixed well and transferred 50 µL of the Standard 3 tube to the Standard 2 tube, mixed well and transferred 50 µl of the Standard 2 tube to the Standard 1 tube, mixed well. The 0 pg/ml standard (Background) was Assay Buffer. Standard concentrations for each cytokine are shown in Table 2-5.

Table 2-5 Standard concentration of MILLIPEX Map Human Cytokine/Chemokine Magnetic Bead Panel

Standard concentration (pg/ml)	**Volume to add**	**Volume of standard to add**
(Standard 6) 10,000	250 µl of deionized water	0
(Standard 5) 2000	200 µl of Assay Buffer	50 µl of 10,000 pg/ml
(Standard 4) 400	200 µl of Assay Buffer	50 µl of 2000 pg/ml
(Standard 3) 80	200 µl of Assay Buffer	50 µl of 400 pg/ml
(Standard 2) 16	200 µl of Assay Buffer	50 µl of 80 pg/ml
(Standard 1) 3.2	200 µl of Assay Buffer	50 µl of 16 pg/ml

Preparation of Human CVD panel 1 standard for PlGF measurement

The Human CVD panel 1 Standard was reconstituted with 250 µl deionized water to give 2000 pg/ml concentration of standard for all analytes. The vial was mixed and vortexed several times and allowed to sit for 10 minutes for complete reconstitution. This was used as the 2000 pg/ml standard (standard 7).

Preparation of Human CVD panel 1 working standard for PlGF measurement

Six polypropylene microfuge tubes were labelled as Standard 6, Standard 5, Standard 4, Standard 3, Standard 2 and Standard 1. Two hundred and fifty µL of Assay Buffer was added to each of the six tubes. Serial 1:3 dilutions were performed by adding 125 µL of the Stock Standard to the Standard 6 tube, mixed well and transferred 125 µL of the Standard 6 to the Standard 5 tube, mixed well and transferred 125 µL of the Standard 5 tube to the Standard 4 tube, mixed well and transferred 125 µL of the Standard 4 tube to the Standard 3 tube, mixed well and transferred 125 µL of the Standard 3 tube to the Standard 2 tube, mixed well and transfer 125 µL of the Standard 2 tube to the Standard 1 tube, mixed well. The 0 pg/mL standard (Background) was Assay Buffer. Standard concentrations for PlGF are shown in Table 2-6.

Table 2-6 Standard Concentration for MILLIPEX Map Human Cardiovascular Disease (CVD) Magnetic Bead Panel 1 (for PlGF measurement)

Standard concentration (pg/ml)	**Volume to add**	**Volume of standard to add**
(Standard 7) 2000	250 µl of deionized water	0
(Standard 6) 666.7	250 µl of Assay Buffer	125 µl of 2000 pg/ml
(Standard 5) 222.2	250 µl of Assay Buffer	125 µl of 666.7 pg/ml
(Standard 4) 74.1	250 µl of Assay Buffer	125 µl of 222.2 pg/ml
(Standard 3) 24.7	250 µl of Assay Buffer	125 µl of 74.1 pg/ml
(Standard 2) 8.2	250 µl of Assay Buffer	125 µl of 24.7 pg/ml
(Standard 1) 2.7	250 µl of Assay Buffer	125 µl of 8.2 pg/ml

2.6.2.3 Multiplex bead array assay procedure

The multiplex bead array assay procedure was carried out according to the manufacturer's protocol and was similar for both multiplex and singleplex assay used apart from some volume differences which are highlighted below.

Plate layout

The following plate layout was used for all assays (Figure 2-4). Each standard/quality control was run in duplicate. Samples were run in singlecate due to limited resources.

WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 Standard (Background)	Standard #4	QC-1 Control	Etc.								
B	0 Standard (Background)	Standard #4	QC-1 Control									
C	Standard #1	Standard #5	QC-2 Control									
D	Standard #1	Standard #5	QC-2 Control									
E	Standard #2	Standard #6	Sample 1									
F	Standard #2	Standard #6	Sample 1									
G	Standard #3	Standard #7	Sample 2									
H	Standard #3	Standard #7	Sample 2									

Figure 2-4 Example of plate layout for multiplex bead arrays

Multiplex procedure - day 1

Into each well of the plate, 200 µl of Wash Buffer (100 µl for PlGF singleplex) was added. The plate was sealed and mixed on a plate shaker for 10 minutes at room temperature (20-25°C). The Wash Buffer was decanted, and residual removed from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. The appropriate matrix for this experiment was the wash buffer used in our lipolysis experiment (see section 1.2.1) containing Krebs Ringer HEPES buffer -118 mM NaCl, 5mM $NaHCO_3$, 4.7 mM KCl, 1.2mM KH_2PO_4, 1.2mM $MgSO_4$, 25mM HEPES) and 2.5mM $CaCl_2$, 151mM BSA (Sigma, UK) at pH 7.4. This wash buffer was prepared fresh on the day of multiplex assay and 25 µl was added in to standard and QC wells. For each diluted standard or QC, 25 µl was added into the appropriate wells. The assay buffer was used for 0 pg/mL standard (background). Assay buffer (25 µl) of was added to the sample wells. The samples (25 µl) were added into the sample wells. The Premixed Beads (25 µl) were added to each well and the bottle was shaken intermittently during addition to avoid beads settling. The plate was sealed and wrapped in foil and incubated with agitation on a plate shaker overnight (16-18 hrs) at 4°C.

Multiplex procedure - day 2

The plate was placed on a handheld magnet (EMD Millipore Cat. no. 40-285) for 60 seconds to allow complete settling of magnetic beads. The well contents were removed by gently decanting the plate into an appropriate waste beaker and gently tapping on absorbent pads to remove residual liquid. The plate was washed 2 times with 200 µL of Wash Buffer (3 times for PlGF singleplex) by removing the plate from magnet, adding wash buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Detection Antibodies (25 µl) was added per well (50 µl for PlGF singleplex). The plate was sealed, covered with foil and incubated with agitation on a plate shaker for 1 hour at room temperature (20-25°C). By keeping the well content during this step, 25 µl of Streptavidin-Phycoerythrin (50 µl for PIGF singleplex) was added to each well containing the 25 µl of Detection Antibodies (50 µl for PIGF singleplex). The plate was sealed, covered with foil and incubated with agitation on a plate shaker for 30 minutes at room temperature (20-25°C). The well contents were gently removed, and the plate was washed 2 times as above (3 times for PlGF singleplex). Drive fluid (150 µl) was added to all wells and the beads were resuspended on a plate shaker for 5 minutes (100 µl for PIGF singleplex). The plate was run on MAGPIX® with xPONENT® software. The Median Fluorescent Intensity (MFI) data was analysed using a 5-parameter logistic method (standard curve) and used to calculate analyte concentrations in samples and controls.

2.6.2.4 Multiplex assay run

The Luminex® MAGPIX® instrument was calibrated with the MAGPIX® Calibration Kit (EMD Millipore, cat. no. 40-049) and performance verified with the MAGPIX® Performance Verification Kit (EMD Millipore, cat. no. 40-050). The start-up, daily maintenance and probe height adjustment procedures were performed on the day of running the assays on the MAGPIX® machine.

2.6.2.5 Generation of protocol using luminex xPONENT software

Each type of kit has its own optimal settings (e.g. number of beads to be acquired, bead size, sample volume etc). To generate a protocol all the information required is contained in the Milliplex kit. The information needed to generate a protocol included the Milliplex Protocol booklet, QC sample Certificate of Analysis, Kit Lot

and Expiry Details, Standard Lot and Expiry Details, and QC sample Lot and Expiry Details. The sample IDs and plate layout couls also be added. Once the protocol was saved it could be used to run the assays at any time. The bead region and the quality control ranges are shown in (Table 2-7 and Table 2-8).

Table 2-7 Antibody-Immobilized Magnetic Beads Region for MILLIPLEX® map kits

Analyte	Luminex® Magnetic Bead Region
IFN-gamma	25
IL-10	27
IL-1b	46
IL-6	57
IL-8	63
MCP-1	67
VEGF	78
PLGF	52

Table 2-8 The Quality Control ranges for MILLIPLEX® map kits

Analyte	QC level	Expected range	Units
IFN-gamma	Control 1	110- 229	pg/ml
	Control 2	575- 1167	
IL-10	Control 1	103- 215	pg/ml
	Control 2	515- 1070	
IL-1b	Control 1	108- 224	pg/ml
	Control 2	535- 1111	
IL-6	Control 1	124- 258	pg/ml
	Control 2	602- 1251	
IL-8	Control 1	108- 224	pg/ml
	Control 2	521- 1082	
MCP-1	Control 1	120- 249	pg/ml
	Control 2	589- 1224	
VEGF	Control 1	106- 221	pg/ml
	Control 2	538- 1117	
PlGF	Control 1	29- 60	pg/ml
	Control 2	177- 368	

2.6.2.6 Data collection and analysis

The Luminex xPONENT software was used to analyse the data. Standard curves and sample concentrations were calculated at the end of the acquisition step and were exported as a portable document file (.pdf). The exported file contained several read outs such as: median bead count per sample (not minus the background or corrected to standard curve), mean fluorescence intensity per sample (minus the background but not corrected to the standard curve), bead count for each sample

(minus the background), result pg/ml (normalised to standard curve and minus the background) and average MFI (normalised to standard curve and minus the background). Here “result” was used for analysis purposes. This is the result per sample minus the background and corrected to the standard curve. Following advice from Merck, bead counts ≤50 would be considered low and beads counts ≤35 would be considered void. Any samples with bead counts ≤35 were excluded.

The standard curves for each analyte were generally sigmoidal and each had their own formula for best fit line depending on the binding behaviour of each antibody type (best fit is determined by the software). Samples were checked for their location on the standard curve and most samples fell within the linear range of the curve, but some fell out with the lower plateau. Bead count for these samples were assessed and if ≤35, were discounted. All our analytes QCs fell within the range provided by the manufacturer. Intra-assay precision was generated by the manufacturer from the mean of the %CV from 16 reportable results across two different concentrations of cytokines in single assay as follows: IL-6 2%, IL-8 1.9%, IL-10 1.6%, 1L-1β 2.3%, MCP-1 1.5%, IFN-gamma 1.6%, VEGF 3.7% and PIGF <10%. Inter-assay precision was generated by the manufacturer from the mean of the %CV’s from four reportable results across two different concentrations of cytokines across six different experiments as follows: IL-6 18.3%, IL-8 3.5%, IL-10 16.8%, 1L-1β 6.7%, MCP-1 7.9%, IFN-gamma 12%, VEGF 10.4% and PIGF <20%.

2.7 Maternal blood phenotyping

All analysis was carried out using plasma collected in the fasted state. Cholesterol and triglycerides were determined by enzymatic colorimetric assay on a Roche Cobas C311 analyser (Roche/Hitachi Cobas c systems, United Kingdom). Glucose was measured by Glucose hexokinase/ Glucose 6 phosphate dehydrogenase assay on a Roche Cobas C311 analyser (Roche/Hitachi Cobas c systems, United Kingdom). Insulin was measured by sandwich electrochemiluminescence immunoassay “ECLIA” using a Roche 411 Immunology analyser (cat. no. 12017547122, Roche/Hitachi Cobas c systems, United Kingdom). CRP was measured by the immunoturbidimetric assay Cardiac C-Reactive Protein (Latex) High Sensitivity kit (Roche/Hitachi Cobas c systems, United Kingdom). Alanine Aminotransferase (ALT), Aspartate aminotransferase (AST), Glutamyl transferase (GGT) was measured using a Roche Cobas C311 analyser. NEFA in plasma was

measured by autoanalyzer. All tests listed above were carried out by Mrs. Josephine Cooney, in the Department of Metabolic Medicine, University of Glasgow.

Glycerol in plasma was measured by enzymatic colorimetric assay using a glycerol assay (cat. no. GY105, Randox, UK), glycerol standard (cat. no. G7793, Sigma) and glycerol control (cat. no. GY1369, Randox, UK) the plate was read on a microplate spectrophotometer at 520 nm. IL-6 (HS600C), TNF-α (HSTA00E) and adiponectin (DRP300) were measured by quantative sandwich ELISA assay (R&D laboratories, UK). Oxidized LDL (oxLDL) was measured by sandwich ELISA (cat. no. 10114301, Mercodia, Sweden).

2.7.1 Insulin resistance estimated by the homeostasis model assessment-estimated insulin resistance (HOMA-IR)

The HOMA-IR was developed by Matthews et al. (1985). HOMA-IR has been widely used for the estimation of insulin resistance. HOMA-IR has been the most frequently employed technique both in clinical practice and in epidemiological studies, due to the simplicity of its determination and calculation. HOMA-IR was calculated using the following formula (fasting plasma insulin in mU/l X fasting plasma glucose in mmol/l)/22.5 (Wallace et al., 2004). Low HOMA-IR values indicate high insulin sensitivity, whereas high HOMA-IR values indicate low insulin sensitivity (insulin resistance) (Matthews et al., 1985).

2.8 Early pregnancy study

2.8.1 Subjects

This study utilized plasma samples from an archival collection of women undergoing frozen embryo transfer (Meyer et al., 2016). This was a prospective, observational study conducted at Glasgow Royal Infirmary and was approved by the Local Research Ethics Committee (07/S0704/49). Plasma samples were collected from women undergoing frozen embryo transfer (FET) treatment for infertility and were recruited from the Assisted Conception Unit between October 2007 and April 2010 (Meyer et al., 2016). Samples were stored at -80ºC.

2.8.2 Study design

Women were eligible for the study if they had a regular menstrual cycle. Patients who had ovulation stimulation or induction were excluded. No progesterone supplementation or other hormonal supplements were given. All women were recommended to take 400 mg per day of folic acid in line with World Health Organization guidelines. Women were informed of the study when notifying the clinic nurse of their last menstrual period (LMP) date with a view to booking FET treatment. At day 10 after LMP, the women attended the Assisted Conception Unit to commence daily hormonal sampling to detect the luteinizing hormone (LH) surge in order to time embryo replacement. At this point women provided written informed consent and a basal blood sample. Embryo transfer was performed on day 3 post-LH surge. Information on patient demographics and fertility history was collected from patient notes. Patient height and weight data were collected at the pre-LH surge visit. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared. Fasting blood samples were collected at day 10 after LMP (pre-LH surge) and on days 18, 29, and 45 post-LH surge. Plasma was collected by low-speed centrifugation and frozen at -80°C within 2 hours.

2.8.2.1 Preparation of density solution

1.006 g/ml density solution was prepared by adding 11.4g NaCl, 0.1g EDTA, 1000ml distilled water (dH_2O) and 1ml N NaOH. The solution density was checked using a PAAR DMA 35 Density meter (cat. no. 84138, Paar Scientific Ltd).

2.8.2.2 Isolation of VLDL by sequential density ultracentrifugation

EDTA plasma (500 µL) was overlaid with 500 µl density solution (density (d) <1.006 g/mL) in 11*34mm Thick wall Polycarbonate Optima™ Ultracentrifuge tubes (cat. no. 343778, Beckman Instruments Inc., UK). The samples were then transferred to a TLA 120.2 rotor and the lid secured. Using a Optima™ TLX Table-Top Ultracentrifuge the samples were centrifuged at 100,000 rpm (224000g) at 23 ^{o}C for 2.5 hours with 4 deceleration.

Using a drawn-out glass pipette, the top 500 µL fraction was removed from each tube by placing the pipette on the edge of the meniscus to ensure removing the very top fraction. The isolated VLDL (500 µL) was removed and divided into two tubes;

both were stored at -20^{o}C. One was sent for lipoprotein composition analysis, and the other was used for FA extraction and later gas chromatography (GC) analysis.

2.8.2.3 Lipoprotein composition analysis

VLDL lipoprotein fraction triglycerides (TG), phospholipid (PL), total cholesterol (TC), free cholesterol (FC), cholesteryl ester (CE), were analysed by autoanalyzer and this was carried out by Mrs. Josephine Cooney, in department of Metabolic Medicine, University of Glasgow.

2.8.3 Fatty acid extraction

2.8.3.1 Preparation of reagents

Methanol: toluene (4:1), with containing C21 fatty acid internal standard and butylated hydroxytoluene (BHT) was prepared via the following steps. First, 0.02 g of Heneicosanoic acid (H-5149, Sigma) was measured and transferred to a glass bottle. Second, in the fume hood, 100 ml of toluene (BDH, 102846G) was measured and added to the bottle. Third, 400 ml of methanol (BDH, 101586B) were added and mixed with amagnetic stirrer. Finally, 0.05g of butylated hydroxy toluene (BHT) (B1378, Sigma) were added and mixed with the previous solution under the fume hood. Potassium carbonate (K_2CO_3) 10% w/v solution (101964H, BDH) was prepared by mixing 100g of K_2CO_3 with 1L of distilled water.

2.8.3.2 Procedure

Fatty acids were extracted as described previously (Lepage and Roy, 1986). In a fume hood, 2 mL of methanol: toluene 4:1 (w/v) + 0.01% BHT and 0.2 mL of 0.2 mg/mL 21:0 internal standard (Sigma-Aldrich, St. Louis, MO, USA) was added to 200 µL of VLDL in a Pyrex glass culture tube with a Teflon lined screw cap (Bibby Sterilin, Staffordshire). Then 200 µL of acetyl chloride (100055V, BDH) was added slowly while vortexing and sealed with Teflon tape (Z-104388, Sigma). The tubes were heated on a heating block (DB.3D, TECHNE DRI-block) for 1 hour at 100^{o}C in the fume hood. The tubes were then cooled in a water bath in a metal rack for 15 minutes, after which 3 mL of 10% K_2CO_3 w/v solution was added and the tubes vortexed. Then,100µl toluene were added and mixed. Tubes were centrifuged for 8min, at 3000 rpm at 5°C using Jouan CR412 refrigerated centrifuge. The upper

toluene phase was transferred to a GC vial (Supelco) using a glass Pasteur pipette. Tubes were stored at - 20°C until required for injection or transport.

After VLDL isolation and FA extraction was carried out by the researcher, samples were shipped to our collaborator at Wollongong University in Australia and Gas Chromatography analysis was carried out by Nicola Zamai.

2.8.4 Gas Chromatography (GC)

The VLDL fatty acids were analysed by flame-ionization gas chromatography (model GC-17A; Shimadzu, Rydalmere, NSW, Australia). A 50 m x 0.25 mm internal diameter capillary column was used. Oven temperature was initially set at 150^{o}C and rose to 170^{o}C at a rate of 10^{o}C/min, then to 200^{o}C at a rate of 2^{o}C/min, and finally to 211^{o}C at a rate of 1.3^{o}C/min. A 1 µL of sample was injected into the column, and individual fatty acids were identified by comparison with known fatty acid standards (Nu-Chek and Sigma, Sydney, NSW, Australia) and quantified by comparison with the 21:0 internal standard using Shimadzu analysis software (Class-VP 7.2.1 SP1).

2.9 Statistical analysis

Data distribution was assessed by Ryan Joiner test and was log or square root transformed where appropriate to achieve a normal distribution. Two sample T test was used for comparisons between groups (Control Vs GDM). Paired t test was used for comparison of SAT vs VAT. Data were expressed as mean and standard deviation. For non-parametric data, Mann-Whitney U test was used, and data were expressed as median and inter quartile range. To assess association between variables, Pearson's correlation coefficient was used, and results were expressed as r value and *P* value. A *P* value of <0.05 was considered significant. Multiple testing was carried out using *post hoc* Tukey-Kramer honest significant difference (HSD) test. More specific detailed statistical analysis is provided within the relevant chapters. Minitab version 18 was used for experimental data analysis.

Chapter 3 Pregnancy outcomes in women with underweight, overweight and obese BMI and gestational diabetes in Greater Glasgow and Clyde, 2010 to 2015: a retrospective cohort using linked national datasets

3.1 Introduction

The worldwide prevalence of obesity has increased markedly over the past few decades posing serious threat to public health. Obesity among women who are pregnant is of particular concern as it carries additional risk for the baby. Obesity during pregnancy is defined as body mass index (BMI) >30 kg/m^2. It affects between16 and 18% of the antenatal population as reported in two observational studies conducted between 2002 and 2004 in maternity units in Glasgow and north England, respectively (Kanagalingam et al., 2005, Heslehurst et al., 2010b). Maternal obesity has been recognised to affect maternal, fetal and neonatal outcomes. These include: preeclampsia (PE) (O'brien et al., 2003), gestational diabetes mellitus (GDM), operative delivery (Sebire et al., 2001b), fetal macrosomia, birth injury (Cedergren, 2004), stillbirth (Nohr et al., 2005) and neonatal intensive care unit (NICU) admission (Heslehurst et al., 2008). In addition to maternal obesity, being an overweight mother was also shown to increase the risk of several pregnancy complications such as GDM, caesarean delivery, fetal macrosomia (Abrams and Parker, 1988) and birth asphyxia related complications (Persson et al., 2014). At the other end of the spectrum, being underweight has long been recognised as presenting increased risk of low fetal birth weight and preterm delivery (Sebire et al., 2001a).

The incidence of gestational diabetes mellitus (GDM) is increasing worldwide, secondary to the epidemic rise in maternal obesity. GDM is a heterogeneous disorder with adverse maternal and fetal consequences. As discussed in the general introduction, metabolic complications during pregnancy such as maternal obesity, GDM and pre-eclampsia seems to be an early marker of disturbances in insulin sensitivity, lipid metabolism and endothelial function later in life. The development of pregnancy metabolic complication such as GDM and PE could predict the future risk of T2DM and CVD in the mother (Bilhartz et al., 2011). Research into the developmental origins of disease have suggested that intrauterine environment may

have significant influence on fetal growth and the later development of disease in the offspring (Barker, 1998). Childhood obesity poses a substantial risk of a future epidemic of CVD in early adulthood (Logue and Sattar, 2011). Thus, the higher prevalence of maternal obesity and GDM among women of reproductive age from high risk ethnicities or in socially deprived areas may have major health consequences across generations.

Given the fact that many factors associated with increased maternal and fetal morbidity and mortality are not amenable to intervention, evidence has suggested that maternal weight control before pregnancy might offer an opportunity for reduction of gestational adverse outcomes. Therefore, the purpose of the analysis in this chapter was to assess the impact of first-trimester high (and low) BMI on the subsequent development of clinical complications later in pregnancy, in a retrospectively evaluated cohort of women representing a real-world sample of the general population in Greater Glasgow and Clyde. Using the Scottish Morbidity Record 2 (SMR02), maternal and fetal information was obtained about obstetric events. To derive a more robust diabetes status diagnosis, the data from SMR02 was then merged with data from the Scottish Care Information – Diabetes Mellitus (SCI-diabetes) database using the patient ID held by the NHS Greater Glasgow & Clyde Safe Haven. This merge resulted in dataset containing no patient identifiable information and was provided to the researcher through a secure access portal. The study period was chosen from 2010- 2015 due to improved BMI recording compared to the period before and to avoid selective recording of women with higher BMI. Maternal BMI was examined as a categorical variable to provide better information about women at the extreme ends of the spectrum because BMI is not linearly associated with obstetric complications which tends to affect women with higher BMI (El-Chaar et al., 2013). The study subjects were mothers with different levels of socio-economic status, including women who lived in areas that were socially deprived.

3.2 Aims

1. To describe the characteristics of pregnant women with or without obesity and/or gestational diabetes.

2. To estimate the prevalence of obesity and gestational diabetes in pregnant women recorded within a single health board in the SMR02 and SCI-diabetes databases.

3. To examine the outcomes of pregnancy in women who are underweight, overweight or obese compared to normal weight women.

4. To examine the outcomes of pregnancy in women who develop GDM.

3.3 Specific research questions

1. What are the pregnancy outcomes and their frequency for women with gestational diabetes and those who are overweight or obese?

2. How do obesity and gestational diabetes affect the following key outcomes from SMR02: mode of delivery, outcome of pregnancy (live, stillbirth), birth weight adjusted for sex, gestational age at delivery and parity (z score for birth weight), maternal smoking, parity, APGAR score at 5 minutes, feeding intention at discharge.

3. How do outcomes compare depending on ethnicity, social deprivation (quintiles of the Scottish Index of Multiple Deprivation (SIMD)) and age?

3.4 Materials and methods

3.4.1 Data source

Approval for this study was obtained from NHS Greater Glasgow & Clyde Safe Haven GSH/16/DI/005. Anonymised data was retrieved retrospectively on all singleton deliveries in Greater Glasgow and Clyde, between 1 January 2010 and 1 January 2016, from the SMR02 held at the Information Services Division (ISD) of NHS Scotland. An SMR02 dataset collects episode-level data of discharge information every time a mother had an obstetric event, and includes information on mother and baby characteristics, such as maternal age, maternal weight and height, baby birth weight, gestational age, mode of delivery, and outcome of pregnancy. The SMR02 covers all obstetric events in Scotland. This dataset has been shown to include 98.5% of births in comparison with the number of births registered by National Records of Scotland. The register is subject to regular data quality assessment, the most recent quality assurance showed that all data items were 90% or more complete in comparison with the medical record/Scottish Women Held Maternity Record (SWHMR), therefore, the register is considered robust (Information Services Division Scotland, 2010).

Data on diabetes diagnoses was obtained from the SCI-diabetes dataset (McKnight et al., 2008). This dynamic clinical register was established in 2000, and currently collates demographic and clinical data for people with a diagnosis of diabetes in Scotland. From 2004 onwards, the coverage of the register was estimated to be 99.5% for people diagnosed with diabetes in Scotland (Anwar et al., 2011). Diabetes type was defined in this register using an algorithm which utilises age at diagnosis, prescriptions data, and clinically assigned type of diabetes (Anwar et al., 2011).

Information from SMR02 was linked with the national diabetes register SCI-diabetes network using the community health index number (CHI) to allow the identification of pregnancies in mothers with diabetes and subsequent perinatal outcomes. GDM diagnosis was coded in both clinical records of SCI-DM and SMR02. Data was extracted from the SCI-diabetes dataset in March 2017, which was then linked to the SMR02 hospital episode dataset by NHS Greater Glasgow and Clyde Safe Haven. This linkage produced a dataset with no patient-identifiable information and was provided to the researcher through secure Safe haven access.

3.4.2 Demographic information and clinical outcomes

The following demographic information was extracted from SMR02 for every pregnant woman: maternal age, parity, height, weight, ethnicity, smoking status during pregnancy and deprivation category, at booking visit (~14 weeks pregnant). Maternal age was 100% complete in our cohort while there was only 0.2% missing information regarding parity. Maternal smoking information during pregnancy was 81% complete in SMR02 and coded as current smoker, current non-smoker and unknown (Information Services Division Scotland, 2010).

Maternal BMI was calculated from the mother's height and weight (weight (kilograms)/ height^2 (meter^2), which were measured at the booking visit by a trained midwife. Within SMR02, height and weight were 72-79% complete (Information Services Division Scotland, 2010). This is due to some hospitals incorrectly mis-assigned data for weight and duration of labour which lead to miss recorded BMI within the dataset. Furthermore, height was an optional data item which contributed to the missing BMI data. In our study height values were deemed valid within the range 100–250 cm, and weight was considered valid within the range 30–250 kg. Women were grouped into six BMI categories based on the WHO classification: underweight (UW<18.5 kg/m^2), normal weight (NW=18.5–24.9 kg/m^2), overweight (OW=25–29.9 kg/m^2), obese class 1 (OB1=30–34.9 kg/m^2), obese class 2 (OB2=35–39.9 kg/m^2) and obese class 3 (OB3≥40 kg/m^2) (World Health Organization, 2000). The reference group for all analysis were women with normal weight. We also used BMI as a continuous variable to investigate associations over the entire range of body weight.

Maternal deprivation was categorized using a tool for measurement of social deprivation, the SIMD 2009 deciles corrected version 2 (SIMD 2009v2), with 1 being the most deprived and 10 being the least deprived. The SIMD dataset identifies small areas where there are concentrations of multiple deprivations. It combines many indicators in several domains as a measure of multiple deprivation such as income, employment, education, housing, health, crime, and geographical access. Each data zone was ranked according to the overall score from a range of indicators which together creates the index. Ranks were grouped in categories such as deciles and quantiles. The SIMD 2009v2 used 2001 Data Zones which were introduced in 2004 to replace post code as the key small area geography in Scotland (Information

Services Division Scotland, 2019). These Data Zones were divided in to 6,505 Data Zones containing 350 households and mean population size 800 people. In the current study, SIMD 2009v2-population weighted deciles that were calculated by ranking all Data Zones in Scotland from most to least deprived and then splitting them into ten deprivation deciles with approximately 10% of the Scottish population in each decile were used. This data item has only 3.6% missing information in our cohort.

Maternal ethnicity was retrieved from SMR02 record and there was 33.8% missing information in our cohort. It was originally coded in 35 detailed categories which were then collapsed into the following seven categories of interest: White, South Asian, East Asian, (Caribbean, African or Black), Arab, mixed or other ethnicity (Figure 3-1). Ethnic group was the fifth most poorly recorded data item in SMR02 with only 11% matching the information on the medical record/SWHMR (Information Services Division Scotland, 2010). This data item was mostly assessed by midwifes but not entered in the hospital Patient Administration System (PAS). Therefore, ISD strongly recommended after the latest data quality assurance in 2010 that ethnic group is recorded in the medical record SWHMR/PAS for SMR02 purposes.

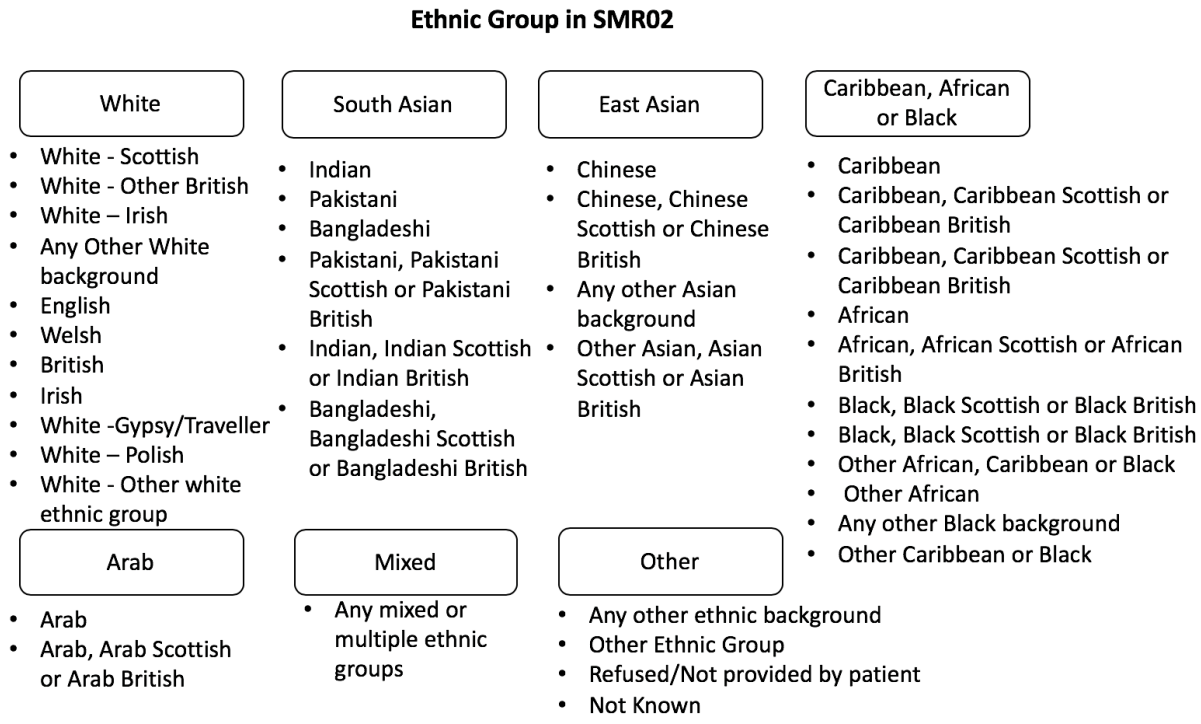


Figure 3-1 Ethnic group categorization in the current study based on SMR02 codes of ethnicity.

Maternal outcomes included mode of delivery (codes of interest were elective caesarean section (ELCS) and emergency caesarean section (EMCS)) and diabetes status (codes of interest were pre-existing DM and GDM). Mode of delivery and diabetes data items were retrieved from SMR02 and was 100% complete in our cohort and 85% complete in the most recent data quality assurance, compared to the SWHMR record (Information Services Division Scotland, 2010). Pregnant women with GDM were identified using SCI-diabetes and/or SMR02 diagnosis of GDM. In our cohort, we examined the accuracy levels of SMR02 in recording pre-existing diabetes and GDM compared to SCI-diabetes record diagnosis for n= 63,476 women after data cleaning and before application of the current study exclusion criteria detailed below in section 3.4.4. The number of women identified as cases in both datasets compared to the total number of cases identified in SCI-diabetes diagnosis, were for type 1 diabetes mellitus (T1DM) 68/102, T2DM 52/101, maturity onset diabetes 1/4 and GDM 158/ 237. The accuracy of SMR02 in recording pre-existing diabetes and GDM compared to SCI-Diabetes are 58.4% and 66.7% respectively Table 3-1. However, there was only 237 GDM cases recorded in SCI-Diabetes compared to 1024 GDM cases recoded in SMR02 and it could be that GDM diagnosis in SCI-diabetes is not as complete for the other types of diabetes in the SCI-diabetes record. Therefore, although SCI-Diabetes is the gold standard source for pre-existing diabetes diagnosis it is a poor source of GDM diagnosis.

Type of diabetes	**SMR02**	**SCI-DM**	**Number of cases identified in both**	**Missing in SMR02 (but identified in SCI-DM)**	**Missing in SCI-DM (but identified in SMR02)**
Type 1 diabetes	Pre-existing DM= 268*	102	68 (66.7%)	34 (33.3%)	Pre-existing DM= 61 (22.7%)*
Type 2 diabetes	Pre-existing DM= 268*	101	52 (51.5%)	49 (43.6%)	Pre-existing DM= 61 (22.7%)*
Maturity onset diabetes	Pre-existing DM= 268*	4	1 (25%)	3 (75%)	Pre-existing DM= 61 (22.7%)*
Gestational diabetes	1024	237	158 (66.7%)	79 (50%)	866 (84.5%)

Table 3-1 Diabetes recording in SMR02 in comparison to SCI-Diabetes.
Total number of subjects was (n= 63,476). Diabetes recording in SMR02 in comparison to SCI-Diabetes as carried out after cleaning the data and before application of the current study exclusion criteria to include subjects with pre-existing diabetes in the comparison. * Type 1 diabetes, type 2

diabetes and maturity onset diabetes was recorded in one category in SMR02 as pre-existing diabetes.

Offspring outcomes were extracted from the SMR02 record and included gestational age, birthweight, birth outcome (codes of interest were live birth, stillbirth and perinatal death), feeding intention at discharge (coded as breast, formula, mixed breast and formula or unknown) and APGAR score at 5 minutes (coded as score from 1 to 10), all were more than 99% complete in the cohort. Gestational age at birth was reported in completed weeks as estimated by the clinicians based on ultrasound dating scan at early pregnancy and last menstrual period date. Gestational age confirmation by ultrasound in early pregnancy is the usual practice in the UK and it has been more than 95% complete for pregnant women since the early 1990s (Campbell and Soothill, 1993). Gestational age at delivery was deemed valid if it was ≤ 43 and ≥ 22 weeks of gestation, 22 weeks is the lowest gestational age for infant's survival after the recent advances in neonatal intensive care. Infant APGAR score was used as an index to evaluate the neonate's overall health status and response to resuscitation. The resulting scores ranged from zero to 10 and were evaluated at 5 minutes after birth. Low infant APGAR score at 5 minutes is associated with an increased risk of neonatal and infant death (Zhu et al., 2015).

Birth weight z score was calculated and adjusted for gestational age, sex and parity using a set of standard LMS-tables derived from Scottish data on 100,133 singleton births from the years 1998–2003 (Bonellie et al., 2008). Birthweight centiles are used to monitor the birthweight of an individual offspring in the context of the mean population birthweight when plotted against gestational age. These centiles were calculated using the LMS method which uses the Box-Cox power transformation to obtain normally distributed data within each group (Cole, 1988). This involves estimating three sets of values for each gestational age group, (L) the power transformation used to achieve normality, (M) the median birthweight and (S) the coefficient of variation of the data. The L, M and S values are estimated for each gestational age and then smoothed curves are fitted using cubic splines to give L(t), M(t) and S(t) where t is the gestational age (Bonellie et al., 2008). There were 227 (0.6%) missing birthweight z scores due to missing information on birthweight, gestational age, baby sex and/or parity.

The selection of covariates was based on the published literature, and bi-variable logistic regression analysis was performed to identify potentially confounding

factors. We considered the following factors to be potential confounders: continuous factors (maternal age at delivery, parity and z score of birth weight) and categorical variables (smoking during pregnancy, ethnicity, SIMD and APGAR score).

3.4.3 Definitions

Stillbirth was defined as a child born after 24 weeks of gestation, who did not breathe or show signs of life.

Perinatal death was considered as a combination of stillbirth and death in the first week of life.

Large for gestational age (LGA) infants were those weighing above the 90th centile at birth, corrected for gestational age, sex and parity.

Delivery was considered preterm if gestation was less than 37 weeks and considered very preterm if gestation was less than 32 weeks.

The APGAR score used as an index to evaluate the neonate's overall health and response to resuscitation and is evaluated at 1, 5 and 10 minutes after birth.

3.4.4 Data linkage

The original dataset for analysis included 144,167 pregnancies. After exclusion of non-delivery admission (identified with missing delivery date), 65,054 pregnancies were identified in SMR02 between 1 January 2009 and 1 January 2016 (Figure 3-2). We excluded 354 pregnancies, as the maternal weight and height were mis-recorded and out of the height (100–250 cm) and weight ranges (30–250 kg) we considered. Data for 64,700 pregnancies were merged with the SCI-diabetes database using patient ID. A further 968 pregnancies were removed due to twin pregnancy. Gestational age was either less than 22 weeks or more than 43 weeks for 192 pregnancies, which were removed. We included only the first pregnancy for each woman; therefore, 13,615 pregnancies, for women who had an additional pregnancy over the study period were not included. Due to the low number of pregnancies recorded in SMR02 in 2009 and 2016, those two calendar years were excluded from our analysis (Table 3-2).

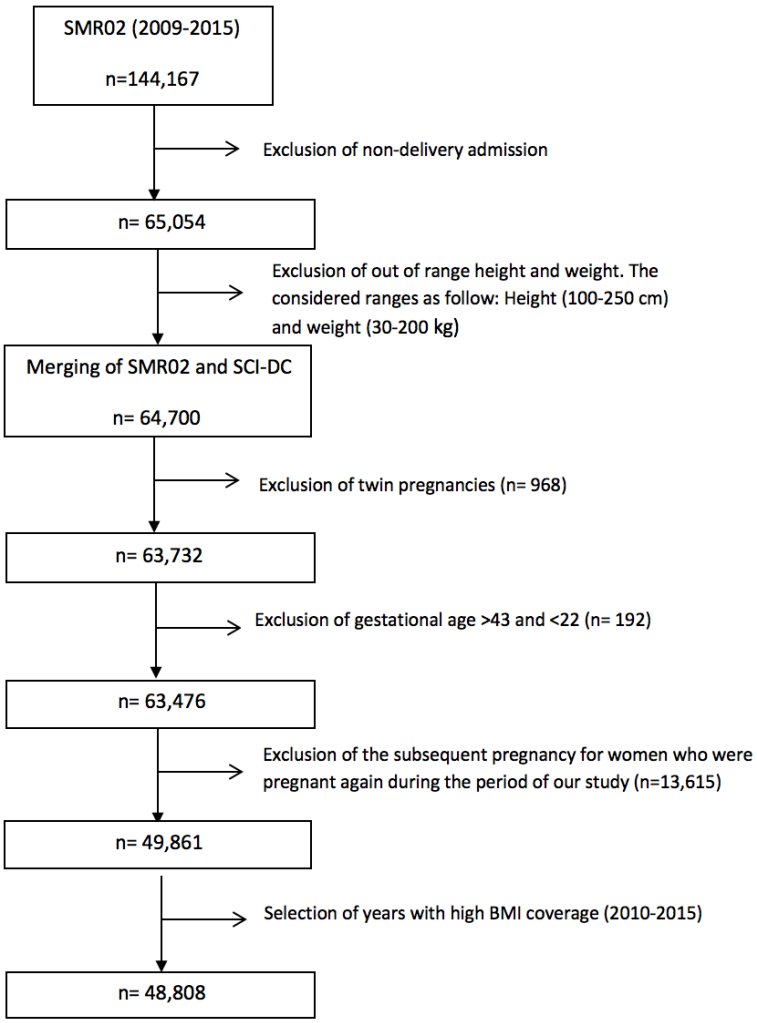


Figure 3-2 Flow diagram for data cleaning and linkage.

Table 3-2 BMI coverage over the study period after data merge (n= 64,700)

BMI category	2009	2010	2011	2012	2013	2014	2015	2016
Missing BMI	831 (40.3)	3122 (44.6)	3757 (33)	4759 (41.8)	1468 (14.3)	149 (1.3)	98 (0.9)	0
Normal weight (BMI 18.5- 24.9)	667 (32.2)	1891 (27)	3726 (32.7)	3148 (27.6)	4301 (41.8)	5421 (47.9)	5290 (46.8)	2
Underweight (BMI< 18.5)	13 (0.6)	33 (0.5)	63 (0.6)	57 (0.5)	109 (1.1)	135 (1.2)	133 (1.2)	0
Overweight (BMI 25.0- 29.9)	314 (15.2)	1043 (14.9)	2209 (19.4)	1933 (16.9)	2492 (24.2)	3059 (27.1)	3166 (28)	2
Obesity class 1 (BMI 30- 34.9)	150 (7.2)	535 (7.6)	1039 (9.1)	941 (8.3)	1198 (11.6)	1579 (14)	1581 (14)	5
Obesity class 2 (BMI 35- 39.9)	64 (3.1)	260 (3.7)	411 (3.6)	387 (3.4)	505 (4.9)	644 (5.7)	675 (6)	0
Obesity class 3 (BMI≥ 40)	25 (1.2)	113 (1.6)	167 (1.5)	147 (1.3)	216 (2.1)	311 (2.8)	356 (3.1)	0
Total number of recorded pregnancies	2064	6997	11372	11372	10289	11298	11299	9

3.4.5 Statistical analysis

The association between maternal BMI and various maternal demographic characteristics and pregnancy outcomes were compared using ANOVA or univariate logistic regression analysis when appropriate. Descriptive data analysis was performed for all maternal and fetal variables of the study final dataset in section 1.5.2. Continuous variables were summarised by mean ±SD and categorical variables by counts and percentages. Multivariate logistic regression models were applied to identify risk factors for GDM diagnosis, EMCS, ELCS, stillbirth, perinatal mortality, LGA infants, less than 7 APGAR score, preterm and very preterm delivery, adjusting for potential confounders. The relationship of maternal BMI category with several maternal, fetal, perinatal complications was expressed as an odds ratio (OR) and 95% confidence interval (95% CI) before and after adjustment for confounders. A two-sided p value < 0.05 was considered statistically significant. No imputations were made for missing data. Data handling and analysis was performed using RStudio version 3.3.0.

3.5 Results

3.5.1 Final dataset for analysis

Over the study period, there were a total of 48,808 singleton deliveries (Figure 3-2). Maternal BMI was our primary dependent variable; therefore, all pregnancies with missing maternal weight or height recording (n=10,319) were excluded from our analysis (Figure 3-3). Of the 38,489 pregnancies that fulfilled our criteria, 311 pregnancies were further excluded because they were identified as cases of pre-existing diabetes using SMR02 (n=232) and SCI-Diabetes (n=79) codes (Figure 3-3). The final cohort consisted of 38,178 singleton pregnancies recorded in SMR02 with complete BMI data and no pre-existing diabetes.

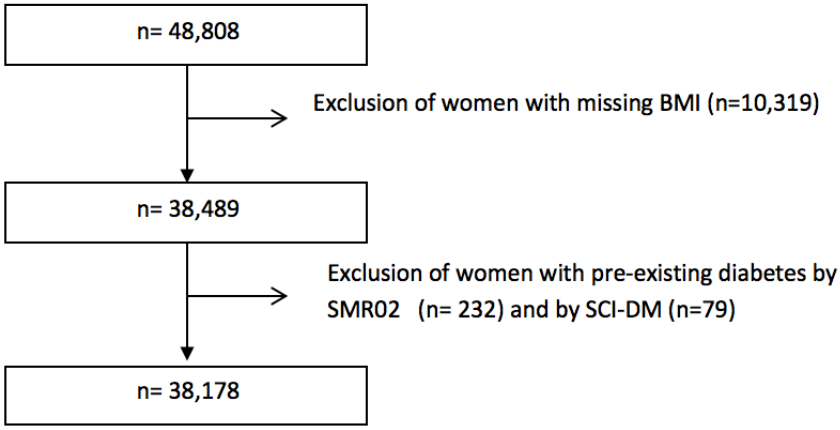


Figure 3-3 Flow diagram for the study exclusions.

3.5.2 Maternal BMI and clinical outcomes

The maternal characteristics and the prevalence of maternal, delivery and neonatal outcomes are summarised in (Table 3-3). Fifty percent of pregnancies were classified as overweight or obese from the total cohort: 28.2% were overweight, 13.8% were obese class 1, 5.9% were obese class 2, and 2.7% were obese class 3. Pregnant women with overweight and obesity were older and more multiparous compared with normal-weight women. Furthermore, underweight, overweight and obese women formed a higher proportion of women in the most deprived SIMD and formed a lower proportion of women in the least deprived SIMD, compared with women of normal weight ($P<$ 0.001). Mothers of African ethnic background was more likely to be overweight or obese, while, mothers of South Asian ethnicity were more likely to be underweight or overweight, but not obese. The proportion of maternal smoking during pregnancy appeared higher among underweight women but did not reach statistical significance.

There were marked differences in pregnancy outcomes between overweight and obese mothers versus normal-weight mothers. Severely obese or underweight women delivered earlier than normal-weight women. Among them, the proportion of preterm delivery significantly increased, but not the proportion of very preterm delivery. Overweight and obese mothers formed a much greater proportion of those who had undergone ELCS and EMCS. Despite earlier delivery, mean birth weight was higher for the offspring of overweight and obese mothers (OW, 109g; OB1, 132g; OB2, 157g; OB3, 148g higher compared to normal-weight mothers' offspring mean birth weight). The same pattern was observed for birthweight adjusted for

baby sex, gestational age and parity, with the offspring of overweight women, obese class 1, obese class 2 and obese class 3 born at average weight 0.16 SD, 0.24 SD, 0.34 SD, 0.41 SD higher than the offspring of normal-weight mothers, respectively. Similarly, the proportion of LGA infants was significantly higher (OW, 12%; OB1, 14%; OB2, 17%; OB3, 20% vs NW 8%) across overweight and obese BMI categories. Furthermore, breast feeding intention rates at discharge were significantly lower, and there was a greater risk of breast-feeding cessation and early formula feeding in overweight and obese women. Unfortunately, there were 84 stillborn infants over the study period, 41 infants were born to overweight and obese mothers, which was not statistically different from the normal-weight population. Similarly, there were 105 perinatal deaths during the study; 54 infants were born to overweight and obese mothers, which was not statistically different from figures for normal-weight mothers.

Table 3-3 Maternal characteristics and obstetric outcomes over the study period (2010–2015)

Variable	Under weight	Normal weight	Over weight	Obese 1	Obese 2	Obese 3	Total	*P* Value
Number of pregnancies, %(n)	1.04 (400)	48.4 (18504)	28.1 (10745)	13.8 (5269)	5.9 (2235)	2.7 (1025)	38,178	
Gestational diabetes, %(n)	1 (4)	0.8 (151)	1.7 (193)***	3.5 (184)***	7.1 (156)***	14.8 (152)***	840	<0.001
Maternal age at delivery, years	26.9± 5.5§§§	29.7± 5.9	30.6± 5.9***	30.4± 5.8***	30.4± 5.7***	30.9± 5.6***	*	<0.001
Parity, %(n)†								
Nulliparous®	87.7 (351)	85.1 (15718)	80.7 (8656)	77 (4056)	76.8 (1713)	73.1 (747)	31,241	ref
Multiparous	12.3 (49)	14.8 (2743)	19.2 (2064)***	22.9 (1207)***	23.2 (517)***	26.9 (275)***	6,855	<0.001
Ethnicity/ Race, %(n)†								
White®	72.6 (199)	77.1 (9266)	78.2 (5540)	80.5 (2895)	80.2 (1273)	83.7 (620)	19,793	ref
Mixed ethnicity	0.4 (1)	0.5 (69)	0.5 (34)	0.6 (21)	0.3 (4)	0 (0)	129	
South Asian	8.4 (23)*	5.04 (606)	5.9 (415)*	4.9 (175)	4.7 (75)	3.5 (26)	1,320	<0.001
East Asian	5.1 (14)§§	2.4 (293)	1.8 (130)§§	0.8 (30)§§§	0.8 (13)§§§	0.8 (6)§§§	486	<0.001
Caribbean, African or Black	1.1 (3)	1.3 (153)	2 (142)***	2.7 (96)***	2.3 (37)***	2.3 (17)*	448	<0.001
Arab	0 (0)	0.11 (12)	0.1 (8)	0.1 (5)	0.3 (4)	0 (0)	29	<0.001
Other ethnicity	12.4 (34)	13.4 (1606)	11.5 (816)	10.3 (371)§§§	11.3 (180)§	9.7 (72)§§	3,079	<0.001
SIMD, % (n)†								
SIMD1 most deprived	**32.6 (126)*** **	23 (4093)	**24.5 (2538)** **	**28.4 (1449)*** **	**31.9 (690)*** **	**37.2 (371)*** **	9,267	<0.001
SIMD2	16 (62)	13.2 (2351)	**14.3 (1488)** **	**16.4 (837)*** **	**17.7 (384)*** **	**17.9 (178)*** **	5,300	<0.001
SIMD3	9.3 (36)	9.3 (1653)	9.9 (1033)	**10.4 (528)***	9.8 (212)	10.9 (109)	3,571	<0.001
SIMD4	9.3 (36)	7.7 (1374)	7.8 (811)	8.3 (422)	7.7 (166)	6.6 (66)	2,875	<0.001
SIMD5	5.9 (23)	7.2 (1270)	**8 (838)** **	**8 (412)***	7.9 (171)	6.7 (67)	2,781	<0.001
SIMD6	6.4 (25)	7 (1245)	7.4 (763)	6.6 (335)	**5.8 (126)§**	**5.1 (51)§**	2,545	<0.001
SIMD7	3.6 (14)	5.1 (914)	**4.6 (476)§**	**4.4 (225)§**	**4.1 (89)§**	3.8 (38)	1,756	<0.001
SIMD8	**4.1 (16)§**	7.3 (1308)	7 (728)	**5.4 (275)§§§**	**5.3 (115)§§§**	**4.2 (42)§§§**	2,484	<0.001
SIMD9	**5.4 (21)§**	8.9 (1574)	**7.9 (814)§§**	**5.7 (291)§§§**	**5.2 (112)§§§**	**4.8 (48)§§§**	2,860	<0.001
SIMD10®	7.2 (28)	11.2 (1992)	8.4 (870)	6.4 (329)	4.6 (100)	2.7 (27)	3,346	ref
Maternal smoking in pregnancy,% (n)	26.3 (105)	18.4 (3413)	16.8 (1806)	18.6 (980)	17 (380)	16.7 (171)	6,855	not sig.
Mode of delivery, %(n)								
ELCS	11.2 (45)	11.2 (2070)	14.9 (1601)***	18.6 (979)***	21 (470)***	26.8 (275)***	5,440	<0.001
EMCS	13.2 (53)	15.6 (2883)	19 (2044)***	20.8 (1095)***	25 (558)***	26.4 (271)***	6,904	<0.001

*significant increase p<0.05, **p<0.01, ***p<0.001 §significant reduction p<0.05, § § p<0.01, § § § p<0.001 ®reference category

Table 3-4 Fetal outcomes over the study period (2010–2015)

Variable	Under weight	Normal weight	Over weight	Obese 1	Obese 2	Obese 3	Total	*P* Value
Stillbirths, n (n per 1000 births)	3 (7.5)*	40 (2.2)	20 (1.9)	12 (2.3)	6 (2.7)	3 (1.9)	84	<0.05
Perinatal mortality, n (n per 1000 births)	3 (7.5)	50 (2.7)	26 (2.4)	15 (2.8)	8 (3.6)	5 (4.9)	107	not sig.
Birthweight, g†	3076.8± 558.5§§§	3324.1± 562.2	3433.1± 561.6***	3456.5± 580.5***	3481.2± 606.5***	3472.8± 615.9***	*	<0.001
Z score for birthweight†	-0.42± 0.97§§§	-0.05± 0.95	0.16± 0.97***	0.24± 0.99***	0.34± 1.04***	0.4± 1.06***	*	<0.001
LGA, % (n)	4.5 (18)§	8 (1483)	12.1 (1294)***	14.2 (746)***	17.1 (380)***	20.2 (206)***	4,127	<0.001
Gestational age at delivery, weeks	38.7± 1.8§§§	39.1± 1.8	39.1± 1.8	39.1± 1.9	38.9± 1.9§§§	38.6± 1.9§§§	*	<0.001
Preterm delivery (<37 weeks), %(n)	9 (36)*	5.9 (1098)	5.6 (607)	6.6 (348)	7.1 (159)*	8.1 (83)*	2,331	<0.001
Very Preterm delivery (<32 weeks), %(n)	0.5 (2)	0.8 (152)	0.6 (72)	1.02 (54)	1.2 (26)	1.2 (12)	318	0.06
Baby feeding on discharge, %(n)								
Breast only®	28.8 (115)	42.3 (7834)	38.9 (4177)	32.1 (1694)	27 (604)	21 (216)	14,640	ref
Formula only	57.7 (230)***	42.7 (7900)	44.2 (4760)**	51.8 (2730)***	55.9 (1251)***	61.6 (631)***	17,502	<0.001
Mixed breast and formula	6 (24)	7.4 (1365)	8.9 (965)***	8.3 (439)*	8.5 (191)*	9.2 (94)*	3,078	<0.001
APGAR score, %(n)†								
< 3 at 5 minutes	0.5 (2)	0.2 (37)	0.2 (20)	0.3 (13)	0.1 (3)	0.1 (1)	76	not sig.
< 7 at 5 minutes	1.3 (5)	1 (185)	0.9 (97)	1.2 (63)	1.1 (25)	1.7 (17)*	392	<0.001

*significant increase p<0.05, **p<0.01, ***p<0.001 §significant reduction p<0.05, § § p<0.01, § § § p<0.001 ®reference category

Unadjusted odds ratios for the association between maternal BMI and several obstetric outcomes are shown in Table 3-5. Maternal overweight and obesity was strongly associated with risk of ELCS, EMCS and LGA. Mothers with morbid obesity are at greater risk of having an infant with APGAR score below 7, but this relationship disappeared after adjustment for confounders. Underweight women had a greater risk for stillbirth, which disappeared after adjustment for confounders.

Table 3-5 Unadjusted odds ratios for the relationship of maternal BMI category and several maternal and fetal outcomes

Population and BMI category	No. of participants	GDM		ELCS		EMCS		stillbirth		Perinatal mortality		LGA		Preterm		very preterm	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
BMI (continous variable)	38178	1.14***	1.12, 1.16	1.05	1.05, 1.06	1.04***	1.03, 1.04	0.99	0.96, 1.03	1.007	0.97, 1.04	1.06***	1.05, 1.06	1.008*	1.001, 1.02	1.02*	1.002, 1.04
Normal weight (BMI 18.5- 24.9	18504	1		1		1		1		1		1		1		1	
Underweight (BMI< 18.5)	400	1.49***	0.08, 6.97	1.01	0.72, 1.3	0.83	0.61, 1.09	3.49*	0.84, 9.64	2.79	0.68, 7.63	0.54*	0.32, 0.89	1.57*	1.09, 2.19	0.61	0.10, 1.91
Overweight (BMI 25.0- 29.9)	10745	2.79***	1.78, 4.41	1.39***	1.29, 1.40	1.27***	1.19, 1.35	0.86	0.49, 1.45	0.89	0.55, 1.43	1.57***	1.45, 1.69	0.95	0.86, 1.05	0.81	0.61, 1.07
Obese (BMI≥ 30)																	
Obesity class 1 (BMI 30- 34.9	5269	4.56***	2.86, 7.34	1.81***	1.67, 1.97	1.42***	1.31, 1.53	1.05	0.53, 1.95	1.05	0.57, 1.83	1.89***	1.72, 2.08	1.12	0.99, 1.27	1.25	0.91, 1.69
Obesity class 2 (BMI 35- 39.9	2235	13.64***	8.74, 21.59	2.11***	1.89, 2.36	1.80***	1.62, 1.99	1.24	0.47, 2.72	1.32	0.58, 2.64	2.35***	2.08, 2.66	1.21*	1.01, 1.43	1.42	0.91, 2.12
Obesity class 3 (BMI≥ 40)	1025	22.94***	14.24, 37.25	2.91***	2.51, 3.36	1.95***	1.68, 2.25	1.35	0.33, 3.74	1.81	0.62, 4.13	2.89***	2.46, 3.40	1.39**	1.09, 1.75	1.43	0.75, 2.47

In multivariate logistic regression analysis, maternal overweight and obesity remained strongly associated with the risk of ELCS, EMCS and LGA, after adjusting for maternal age, parity, smoking, ethnicity, and deprivation category (Figure 3-4, Figure 3-5, Figure 3-6). Only underweight (OR=1.91 [95%CI: 1.27, 2.77]) and obese class 2 (OR=1.26 [95%CI: 1.02, 1.53]) mothers showed a significant association with the risk of preterm, but not very preterm, delivery (Figure 3-7). Maternal BMI was not associated with less than 7 APGAR score, stillbirth or perinatal mortality in multivariate analysis (Figure 3-8, Figure 3-9, Figure 3-10). High parity was strongly associated with increased risk of ELSC (OR=1.35 [95%CI: 1.30, 1.39]) and significant reduction in EMCS risk (OR=0.48 [95%CI: 0.45, 0.49]) and reduced birth weight Z score (OR=0.92 [95%CI: 0.88, 0.96]). Smoking during pregnancy was associated with increased risk of EMCS (OR=1.16 [95%CI: 1.06, 1.27]) and premature delivery (OR=1.42 [95%CI: 1.25, 1.62]) and significant reduction in ELCS (OR=0.83 [95%CI: 0.74, 0.92]) and low birthweight z score (OR=0.54 [95%CI: 0.47, 0.61]).

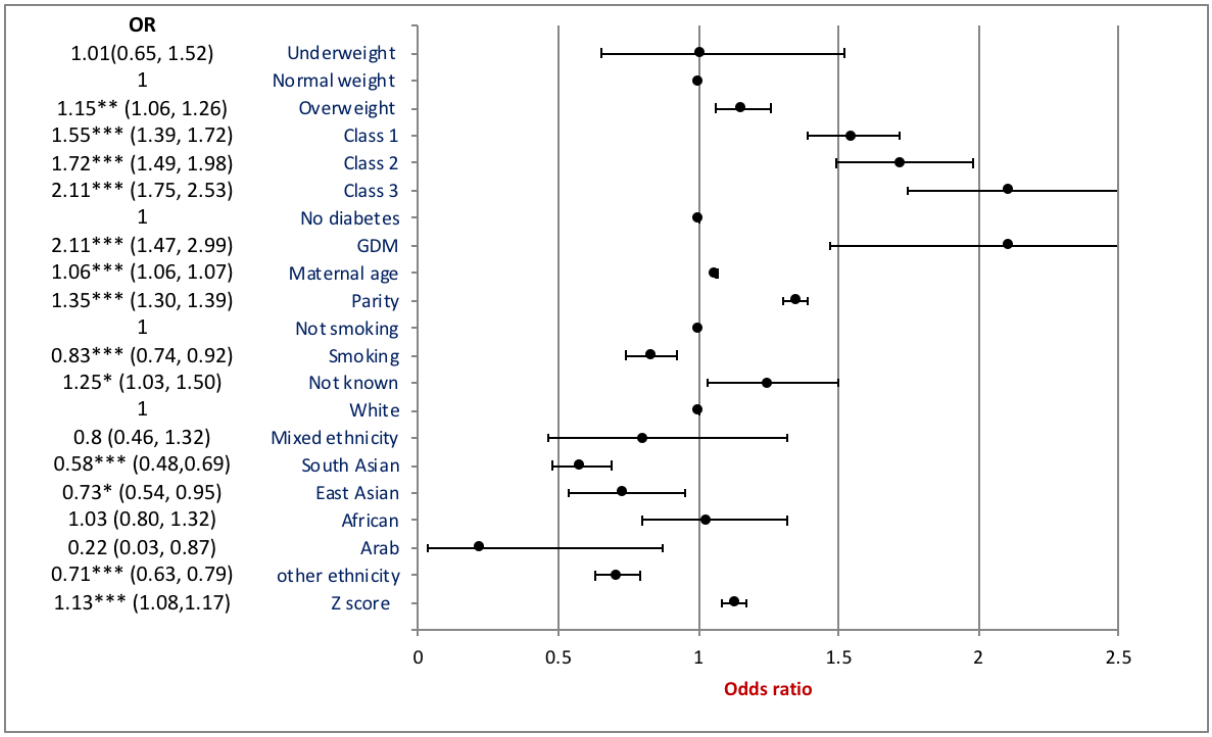


Figure 3-4 Multiple logistic regression analysis evaluating the risk of elective caesarean section.
*p<0.05, **p<0.01, ***p<0.001

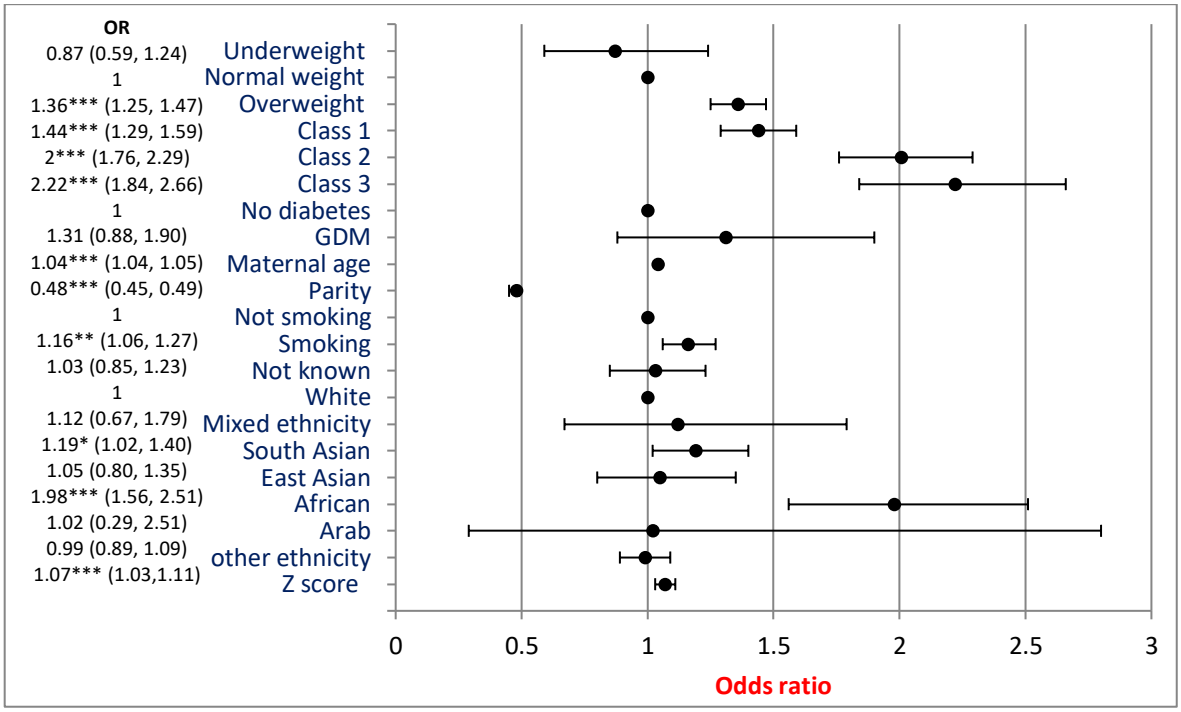


Figure 3-5 Multiple logistic regression analysis evaluating the risk of emergency caesarean section.
*p<0.05, **p<0.01, ***p<0.001

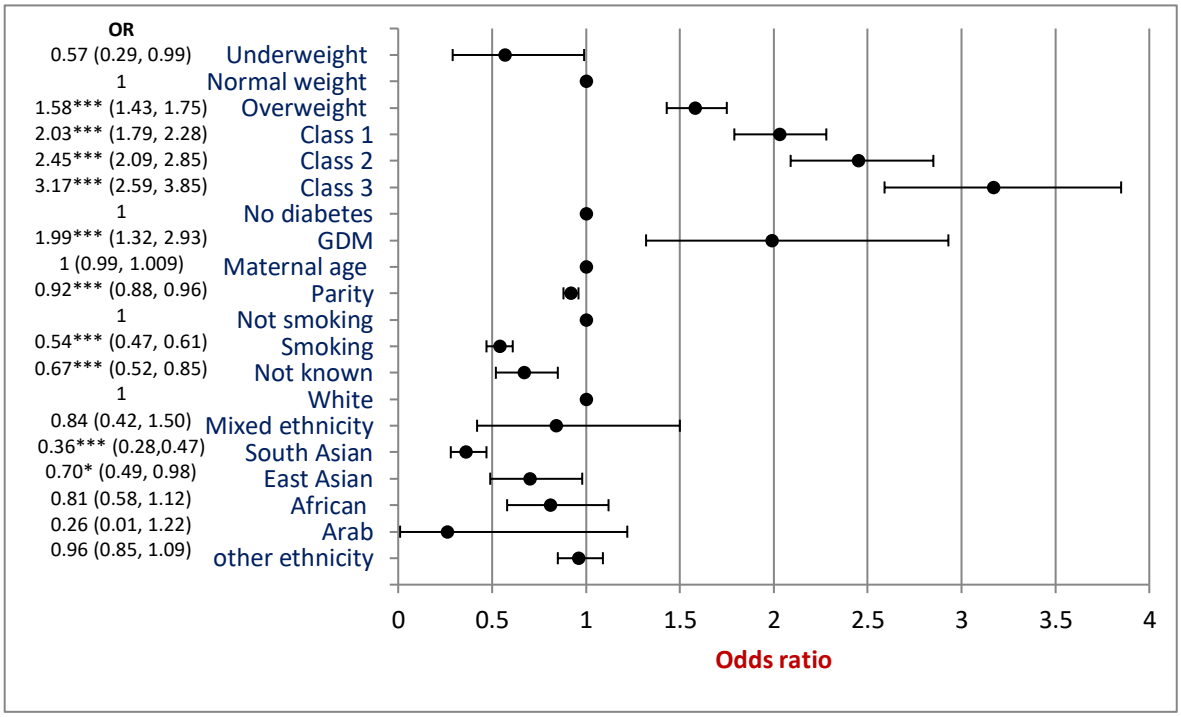


Figure 3-6 Multiple logistic regression analysis evaluating the risk of large for gestational age (LGA).
*p<0.05, **p<0.01, ***p<0.001

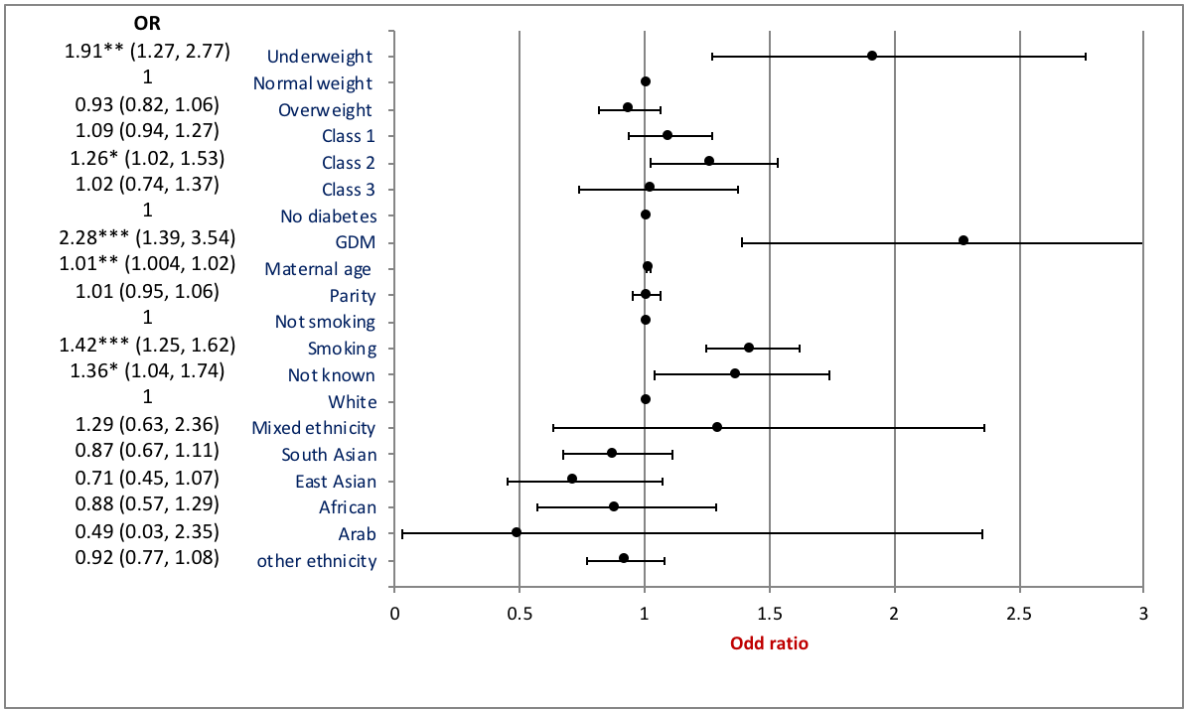


Figure 3-7 Multiple logistic regression analysis evaluating the risk of preterm delivery.
*p<0.05, **p<0.01, ***p<0.001

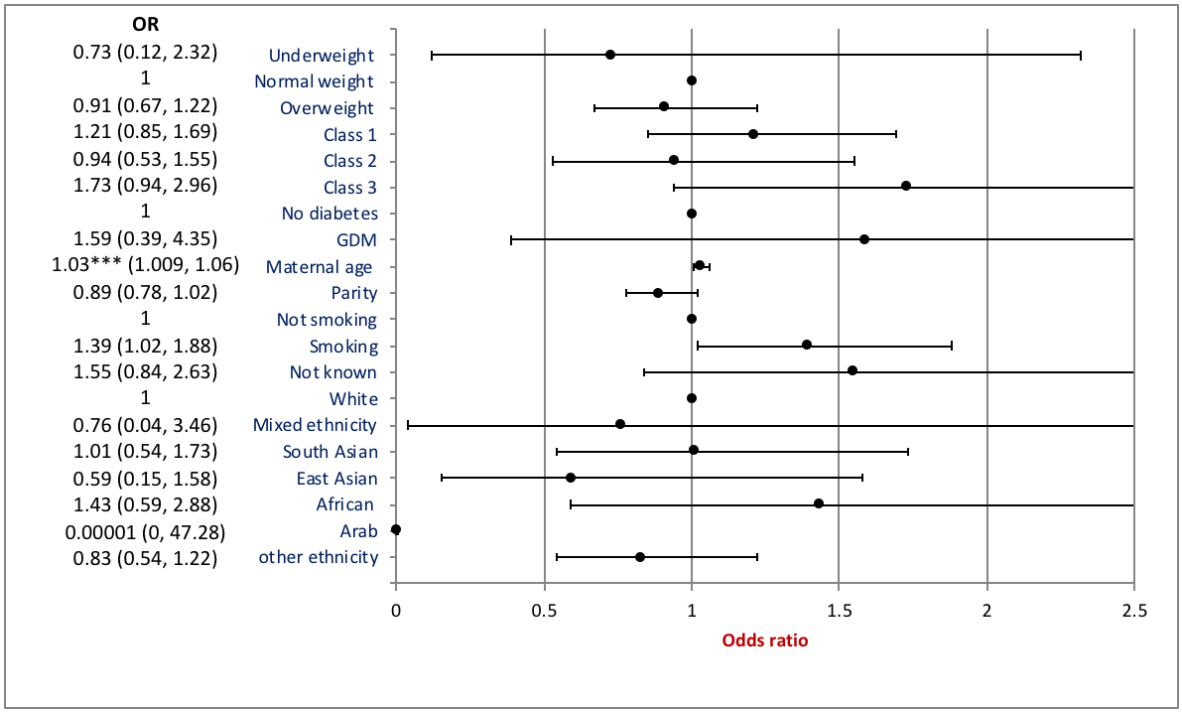


Figure 3-8 Multiple logistic regression analysis evaluating the risk of APGAR <7.
*p<0.05, **p<0.01, ***p<0.001

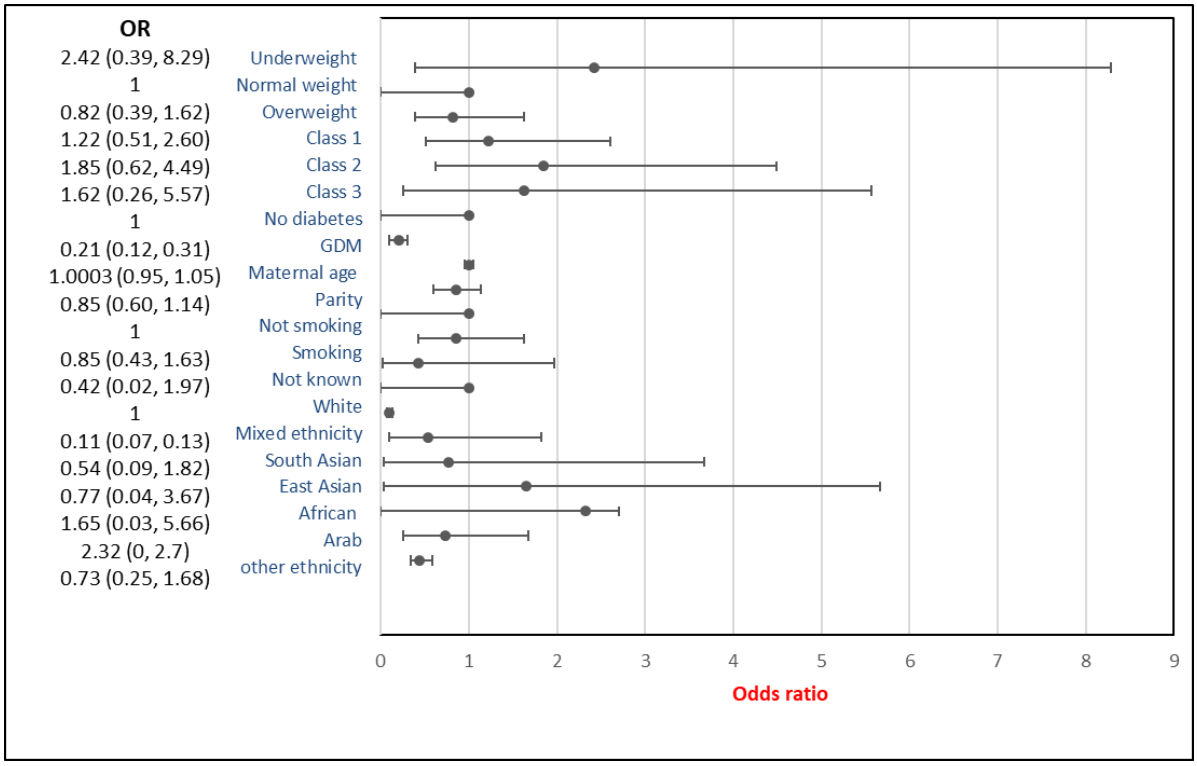


Figure 3-9 Multiple logistic regression analysis evaluating the risk of stillbirth

*p<0.05, **p<0.01, ***p<0.001

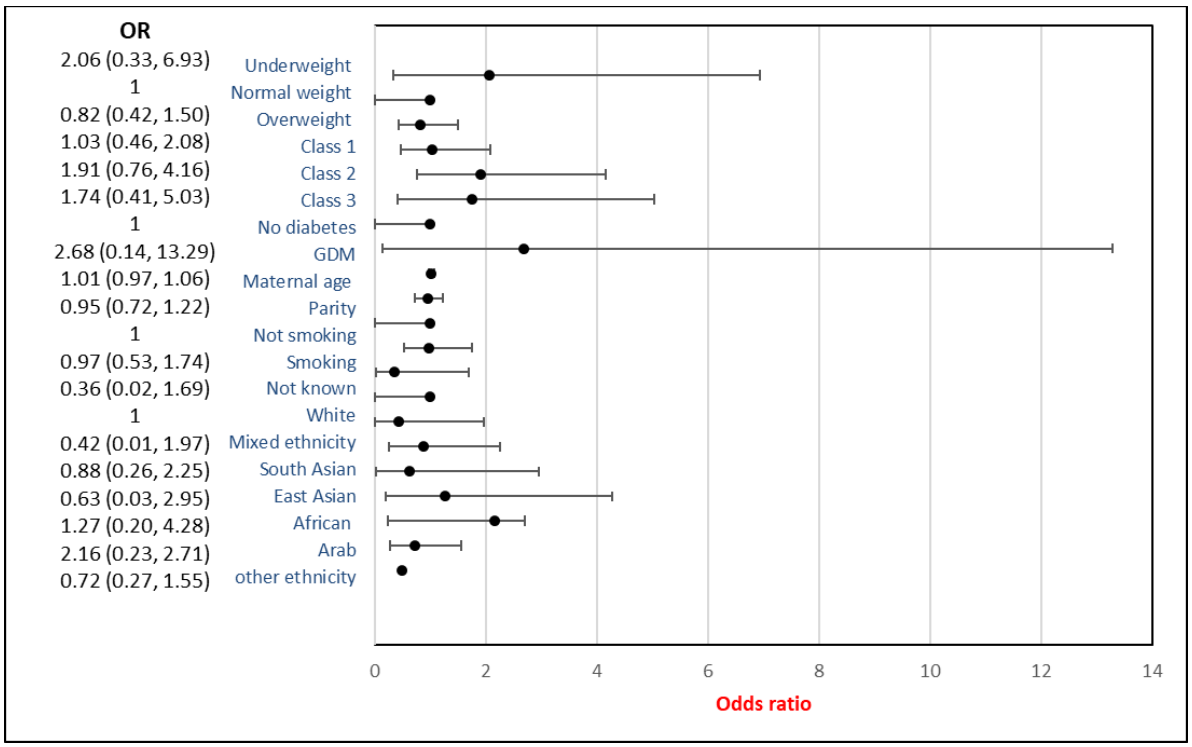


Figure 3-10 Multiple logistic regression analysis evaluating the risk of perinatal mortality

*p<0.05, **p<0.01, ***p<0.001

3.5.3 GDM and clinical outcomes

GDM affected 2.2% (840/38178) of pregnancies from the total cohort, and 3.6% (685/19274) of the overweight and obese pregnancies. Univariate logistic regression analysis for the likelihood of GDM and maternal BMI at booking showed a significant graded increased risk of GDM for overweight and obesity. GDM diagnosis was an independent risk factor for ELCS (OR=3.01 [95%CI: 2.5, 3.6]), EMCS (OR=1.95 [95%CI: 1.6, 2.3]) and preterm delivery (OR=1.9 [95%CI: 1.5, 2.3]).

The greatest increase in adjusted odds ratios for a complication associated with maternal overweight and obesity was observed for GDM after adjustment for maternal age at delivery, parity, smoking, ethnicity, and SIMD (Figure 3-11). Accordingly, a woman with booking BMI≥ 40 kg/m^2 has a risk of GDM almost nineteen times greater than women of normal weight, after adjustment for confounders. Interestingly, South Asian (OR=3.19 [95%CI: 1.89, 5.14]) and Arab ethnicity (OR=14.79 [95%CI: 2.21, 56.54]) women were at significantly increased risk of GDM compared to those of white ethnicity. However, there were too few participants (n= 29) of Arab ethnicity for a meaningful multi-variable analysis. GDM risk increased significantly in mothers with the most deprived SIMD scores, (SIMD1, OR=3.43 [95%CI: 1.14, 14.43]; SIMD2, 7.66[95%CI: 2.72, 32.21]; SIMD3, 6.02[95%CI: 2.04, 25.74]).

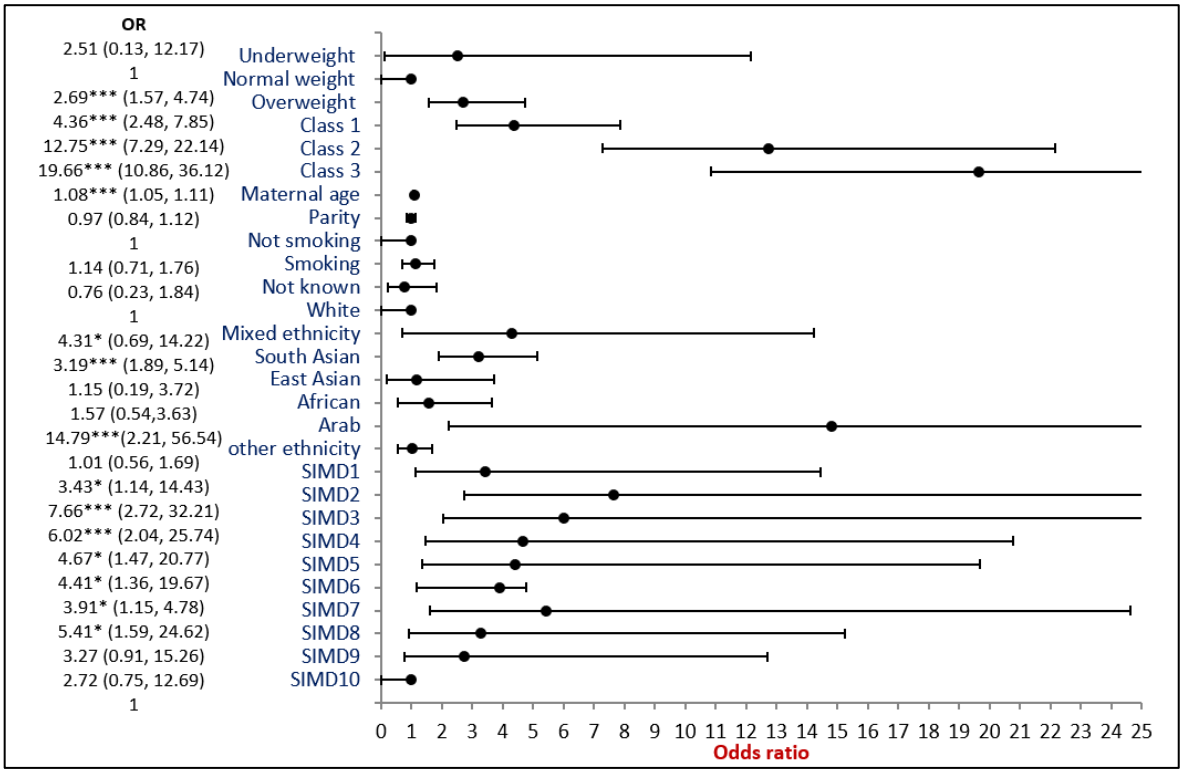


Figure 3-11 Multiple logistic regression analysis evaluating the risk of GDM during pregnancy. *p<0.05, **p<0.01, ***p<0.001

3.6 Discussion

The current study demonstrates that both high and low BMI are associated with increased odds of pregnancy adverse outcomes. The prevalence of maternal obesity was 22.3% which has increased compared to the previous analysis conducted in the Glasgow population in 2005 (Kanagalingam et al., 2005). Higher maternal BMI was strongly associated with several risks such as GDM, EMCS, ELCS and LGA babies. The prevalence of GDM was 2.2% in our cohort, however, although this is a low GDM prevalence but it has slightly increased by 0.3% compared to the Scottish prevalence since 2012 (Collier et al., 2017). However, GDM rate is very dependent on the screening pattern used and the rates of the current study could be an underestimate compared to populations implying the universal screening. The ATLANTIC Diabetes in Pregnancy (DIP) program identified 12.4% prevalence of GDM within a cohort of Irish women in which universal screening was applied using the IADPSG criteria. In the current study pregnant women with GDM had increased risk of ELCS, EMCS, LGA baby and preterm delivery in our cohort. The present study confirmed the association of GDM with maternal BMI, maternal age, social deprivation and ethnicity. The cohort for this study was taken from a national dataset with regular proven data quality assessment which enabled us to obtain a large number of patients' representative of the general

population. In particular, the results of this study were not confounded by other forms of pre-existing diabetes and twin pregnancy. Furthermore, each woman accounts for one pregnancy only and the effect of consecutive pregnancies during the study period was accounted for.

The results of this study showed that 50.4% of the maternity population living in Greater Glasgow and Clyde were overweight or obese at the first trimester of their pregnancy, of which 28.1% were overweight and 22.3% were obese. From 2002 to 2004, a previous observational study reported maternal obesity prevalence rate of 18% in the Princess Royal Maternity Unit (PRMU) at Glasgow Royal Infirmary (Kanagalingam et al., 2005). However, the previous study cohort was randomly selected by medical record staff from one hospital in Glasgow. The current rates of maternal obesity confirm that the maternal obesity prevalence rate in the Glasgow population continues to be on a slow increasing trend over the last 15 years.

Maternal obesity in the first trimester has significant implications for clinical practice. It results in a higher demand for high dependency care, and the complications that can arise for mother or child need to be managed. The NICE guideline recommends that pregnant women whose BMI is greater than 30 kg/m^2 should be placed under the care of a consultant rather than a midwife, which places a huge burden on resources in maternity units (National Collaborating Centre for Women's and Children's Health, 2008). In our cohort, overweight and obese women are at higher risk of operative delivery (both ELCS and EMCS) and LGA infants. This possibly could lead to further complications such as shoulder dystocia and instrumental delivery due to LGA infants and wound infection following operative delivery. Only obese class 2 women showed a slight increase in risk of preterm delivery. However, different types of preterm birth (i.e. after spontaneous preterm labour, preterm premature rupture of membranes, or indicated labour) were not distinguished in our study. Secondary analysis of the Maternal–Fetal Medicine Units Network (MFMUN) study showed that pre-pregnancy obesity was associated with a lower rate of spontaneous preterm birth (Hendler et al., 2005). Therefore, the higher preterm delivery risk observed in the current study among obese women class 2 could be secondary to other obstetric complications such as GDM and PE. In our cohort, first-trimester obesity was not associated with stillbirth, perinatal mortality or APGAR score <7. Contrary to our results, a meta-analysis concluded that even modest increases in maternal BMI were associated with risk of stillbirth and perinatal

mortality suggesting that our study could be underpowered to observe this association (Aune et al., 2014).

Maternal underweight, overweight and increasing levels of obesity show a striking positive relationship with social deprivation. The reverse was also true, higher SIMD levels were associated with a significant reduction in the risk of being underweight, overweight or obese. This highlights the inequalities in health that have been reported widely in previous research. A similar association was observed in a retrospective cohort of women living in Scotland from 2003–2010 (Denison et al., 2014). Therefore, the mothers at higher clinical risk (severely obese) are those facing a higher level of health inequality. In our study, the most deprived women are at increased risk of GDM, EMCS and preterm delivery, and reduced risk of ELCS and LGA infants. Moreover, the association between deprivation and preterm delivery in our cohort was striking. We observed increasing risk of preterm delivery with increasing deprivation status. Although social deprivation, underweight and smoking were individually associated with preterm delivery, the risk between deprivation status and preterm delivery could be mediated via underweight and smoking. However, underweight and smoking, both modifiable risk factors, could be an important determinant of preterm delivery in the most deprived population. Although association does not infer causation, possible interventions to reduce maternal underweight and promote smoking cessation among the deprived population may be highly beneficial in adverse obstetric outcome reduction. The recent research of Mehra et al. (2019) observed a similar association, and suggested that hypertension and infection moderately determine this association. However, other factors related to area-level conditions, such as inadequate housing, transportation and violence, may influence pregnancy through psychological stress (Messer et al., 2013). In line with our results, a meta-analysis of seven studies on neighbourhood deprivation found that the risk for preterm birth was significantly increased in the most deprived neighbourhood quantiles, compared to the least (OR=1.23 [95%CI: 1.18, 1.25]) (Vos et al., 2014). The higher risk for EMCS among the socially deprived population could be related to the observed higher incidence of metabolic complications such as maternal obesity and GDM in this group.

Ethnic minorities in Europe have been shown to have higher susceptibility to T2DM (Meeks et al., 2016) and CVD (Tran et al., 2011). Pregnancy can be considered to be a stress test to unmask the mother’s metabolic dysfunction and predict future

morbidity patterns that will affect both maternal and offspring health. There was a positive relationship between obesity and African ethnicity, which is representative of the black ethnicity relationship with obesity in the general population, where Black African and Black Caribbean women show the highest prevalence of obesity (Sproston and Mindell, 2006). While African mothers are very likely to be overweight or obese, GDM risk in the current study population disappeared after adjustment for confounders. Contrary to our results, a larger study in London, including 76,158 pregnancies, found that women of Afro-Caribbean origin had increased risk of GDM in multivariate analysis (OR=1.44 [95%CI: 1.25, 1.65] (Khalil et al., 2013). The same study observed three-fold increased risk of PE in women of Afro-Caribbean origin. Although we did not have data on PE, but this could possibly explain the observed higher EMCS rate among African population in our study.

Interestingly, our study showed a positive relationship between South Asian ethnicity and being underweight or overweight, but not obese pregnant women. There was an increased relationship between obesity and women of Asian ethnicity in the general population (Sproston and Mindell, 2006). In our study, although South Asian mothers tended to have lower BMI compared to the white population, they had significantly increased risk of GDM and EMCS probably reflecting increased testing for this population, and reduced risk of LGA infants and ELCS. On the other hand. Heslehurst et al. (2010b) observed a significantly reduced risk of being overweight or obese for pregnant Asian women in England. However, Heslehurst et al. (2010b) study involved a larger sample size, and this might have contributed to this inverse relationship which we did not observe. In addition, their study did not observe South Asian ethnicity as a separate group from other Asian backgrounds. Unfortunately, we did not possess data on PE, but it would be interesting to examine whether it is related to the increased risk of EMCS in South Asian and black mothers.

At the other end of the spectrum, underweight women represented the only BMI category at significantly increased risk of stillbirth in our cohort, which remained consistent after adjustment for confounders. Underweight women are at greater risk of preterm delivery and reduced z score for their infant's birthweight. These findings were consistent with other meta-analysis studies for different populations, including low- and middle-income countries (Rahman et al., 2015). However, these results should be interpreted with caution, due to the low number of events (3 stillbirth and 9 preterm birth) among underweight women.

The results of this analysis have shown that the prevalence of GDM was 2.2% in our cohort. The Scottish prevalence of GDM was 1.9% on 2012 (Collier et al., 2017). This confirms that the reporting of GDM is low in the Glasgow population in similar rates to the national data although it is not clear whether this is probably due to lower reporting or testing or not. In our cohort, GDM diagnosis was associated with maternal BMI, maternal age, social deprivation and ethnicity. A study by Collier et al. (2017) showed similar associations although ethnicity was not studied in their analysis. They showed that maternal macrosomia was not associated with GDM. However, in our cohort, women with GDM had increased risk for a LGA baby. This is probably because we have used adjusted birthweight for gestational age, sex and parity rather than birth weight only for offspring of GDM mothers who tend to deliver earlier than healthy mothers. In this situation the birthweight alone may not be reflecting the real impact. Furthermore, we have used both SMR02 and SCI-diabetes dataset diagnosis of GDM and excluded mothers with pre-existing diabetes from our analysis which could improve our GDM diagnosis accuracy.

Overweight and obese women start pregnancy with greater insulin resistance than normal weight women (Catalano, 2010), and become highly insulin-resistant around mid- to late-gestation, which then can lead to metabolic complications such as GDM and PE. In the current cohort, the association between first-trimester BMI and GDM was strongly significant, with increasing levels of risk among higher BMI categories, and remained consistent after adjustment for confounders. South Asian women had a three-fold increased risk of GDM despite being at lower BMI. This observation was in line with the finding of Khalil et al. (2013), which also pointed to women of South Asian origin developing GDM at a younger age compared to those from Caucasian ethnic backgrounds. This suggests that lower BMI cut-off for obesity diagnosis in women of South Asian origin might not be sufficient to identify women at higher risk. The observed difference in body fat distribution between South Asian and Caucasian ethnicity could probably explain the previous results in which South Asians were found to accumulate fat more centrally rather mostly in subcutaneous fat such as Caucasians. Therefore, lower BMI cut off points were recommended to be used in South Asian population due to their high risk to develop T2DM at lower BMI compared to Caucasians. Another relevant aspect for ethnic differences is that a two- to three-fold higher prevalence of GDM is reported when applying IADPSG criteria, rather than WHO criteria, due to a lower fasting glucose cut-off in the former (Jenum et al., 2012). Previous evidence has shown that women of South Asian

origin have more abnormal fasting plasma glucose levels (Agarwal et al., 2010). Therefore, the application of IADPSG criteria to our cohort could be responsible for the observed association. In addition to adiposity and ethnicity, low socio-economic status in our cohort was strongly associated with GDM diagnosis. It could be that there are area level factors that we did not measure, such as the density of retail outlets for alcohol, tobacco, and unhealthy and healthy foods, which might explain this association.

A GDM pregnancy implies a substantial risk of T2DM development later in life. Neonates of women of South Asian origin are of lower birth weight compared to the general population. When exposed to the current obesogenic environment, those neonates could be at higher risk of later obesity, T2DM and CVD. This study draws awareness to the notion that pregnancy can be considered as a natural stress test to reveal the maternal risk for future T2DM and CVD development. The increasing trend for maternal obesity and GDM prevalence among ethnic minority and socially deprived groups is of great concern.

There are several limitations in our study. First, this study used routinely collected data, which relied on reliable coding, and did not include a laboratory measure of GDM diagnosis. Second, our analysis was adjusted for several maternal demographic confounders but not for maternal pre-existing conditions other than diabetes, such as operative delivery, which could probably amplify the effect of obesity in those individuals. Third, we used BMI as a measure of obesity. Emerging evidence has suggested that measures of abdominal obesity may be more important as an indicator of risk for metabolic disease than general adiposity. Several alternative anthropometric measures such as waist circumference, waist/hip ratio and skin fold thickness could have been used but these can be unreliable in pregnant women and difficult to record at a population level. Therefore, although BMI has limitations, it remains the practical option for large epidemiological studies. Finally, this study might not be representative of other settings, or generalizable to other populations but it does confirm the work of multiple previous investigations.

A particular strength of our study is that it provides detailed demographic and obstetrics outcomes analysis among the greatly diverse Greater Glasgow and Clyde population, and highlights subgroups at higher risk of facing health inequalities.

Furthermore, we examined the associations between maternal obesity and a wide range of adverse pregnancy outcomes among ethnic minorities, an area which is still sparsely documented. Our analysis included social economic status and the effect of race on various obstetric outcomes which are not usually accounted for in previous research. We merged SMR02 and SCI-diabetes to obtain accurate data on diabetes status to diagnose GDM and accurately exclude woman with pre-existing diabetes from confounding our findings.

In conclusion, our study showed that in a single health board of Scotland's population, there was a notable risk of maternal obesity and GDM, which predisposes mothers to several obstetric complications and adverse outcomes. Mothers with obesity and GDM receive higher obstetric interventions, including operative and earlier delivery. Average birthweight for the offspring of overweight and obese mothers is significantly increased, despite the increased rate of medical interventions among this group. Among ethnic minority groups and the most deprived areas, there was a significant risk of both adverse maternal and fetal outcomes. To reduce the risk of these adverse outcomes, it is important to identify the modifiable risk factors that can potentially be targeted for interventions. Behavioural interventions to control maternal weight during pregnancy have been shown to be significantly reduce the maternal weight gain during pregnancy but did not reduce the adverse pregnancy outcomes (Poston et al., 2015), therefore, effective intervention strategies are needed for weight control prior to pregnancy. Targeted intervention approaches towards the low-income population and ethnic minorities at higher metabolic risk could be highly effective in improving pregnancy outcomes. Wider analysis of maternal obesity rates and obstetric outcomes at national level could be beneficial in informing our clinical practice in the management of maternal obesity during pregnancy. Future research is required to address the economic costs associated with obesity and GDM during pregnancy including maternal hospitals costs for the mother and her neonate.

Chapter 4 Adipocyte lipolytic function in pregnancies complicated with gestational diabetes mellitus compared to healthy pregnancy

4.1 Introduction

Pregnancy requires substantial maternal metabolic adaptation to accommodate the demands of the growing fetus. Late pregnancy is characterized by physiological insulin resistance to adapt with increasing fetal demand to preserve glucose as the primary fuel for the fetus. Insulin resistance is a condition of reduced responsiveness to insulin in several tissues such liver, muscle and adipose tissue. As a result, insulin production from β-cells increases to maintain normal blood glucose levels. Insulin resistance is present in several conditions including T2DM and obesity. For obese women with subclinical decreased insulin sensitivity, pregnancy represents a metabolic stress test which may lead to development of pregnancy complications such as GDM and PE due to exaggerated insulin resistance. The decrease in insulin sensitivity with advancing gestation is not limited only to disturbed glucose metabolism but is also is observed in relation to lipid metabolism (Catalano et al., 2002). There is a two to threefold increase in basal triglyceride and cholesterol concentrations with advancing gestation. The increases are more pronounced in women with GDM as compared with healthy pregnant women (Catalano et al., 2002). The increase in plasma FFA concentration is related to the decreased ability of insulin to suppress lipolysis in late gestation.

During pregnancy, there is an increased need to expand adipose tissue (lipogenesis) in early pregnancy and increase FA availability (lipolysis) in late pregnancy. These physiological changes are of great interest to study, in particular how adipocytes respond to these changes. The size of the adipocytes can change throughout life, depending on the amount of lipid stored. From *in vitro* experiments in non-pregnant individuals, it is well established that larger adipocytes are less sensitive to insulin action (Salans and Dougherty, 1971). Abdominal SAT adipocytes size was shown to be predictive of T2DM in non-pregnant women (Lonn et al., 2010). In severely obese non-pregnant individuals both SAT and VAT adipocytes size was correlated with fatty liver (O'Connell et al., 2010). Despite the great impact of maternal obesity on gestational insulin resistance and the development of

subsequent metabolic complications among the mother and her fetus, there are few studies of human adipose tissue morphology and function during healthy and complicated pregnancy. Longitudinal studies of gluteal adipose tissue biopsies show that there was larger gluteal adipocytes with increased numbers of adipocytes during late gestation compared to early and pre-pregnancy samples (Resi et al., 2012). Inherent defective adipocyte expansion in the face of pre-pregnancy obesity may be unmasked during pregnancy leading to pregnancy metabolic complications such as GDM. The functional measures of adipocytes were rarely studied in women with GDM such as adipocyte size and lipolytic function. Rojas-Rodriguez et al. (2015) found that women with GDM had omental (visceral) adipocyte hypertrophy and decreased capillary density compared to pregnant women with normal glucose tolerance.

Insulin suppression of lipolysis is a direct measure of adipocyte insulin sensitivity, which was shown to be a major contributor to whole body insulin sensitivity. There was increased adipocytes sensitivity to insulin in adipocytes from non-pregnant females compared to males (Macotela et al., 2009). The adipocytes insulin sensitivity in females may account for their lower level of insulin resistance and diabetes risk despite similar or higher fat content than in males (Macotela et al., 2009). Adipose tissue of obese non-pregnant subjects showed reduced response to insulin (Salans et al., 1968). After weight loss and reduction in adipose cell size, insulin sensitivity of the adipose tissue of obese patients was restored to normal. Adipose tissue insulin sensitivity was impaired in non-pregnant T2DM subjects (Kotronen et al., 2008). In the third trimester of pregnancy, there is enhanced insulin resistance. Pregnant normal weight women in the third trimester were shown to have increased SAT adipocytes lipolysis measured by tracer catheterization (Diderholm et al., 2005) compared to the non-pregnant data (Kalhan et al., 2001). In overweight and obese pregnant women, it was demonstrated that there is resistance to insulin suppression of lipolysis and fat oxidation during euglycemic-hyperinsulinemic clamping in third trimester compared to second trimester and postpartum (Sivan et al., 1999). Therefore, defective insulin suppression of lipolysis is suggested in pregnancies complicated with GDM. Moreover, the maternal dyslipidemia observed in GDM may result from enhanced subcutaneous adipocyte lipolysis in obese mothers. however, there are no previous studies on adipocyte lipolysis in women with GDM. Thus, we hypothesized that there is adipocyte

hypertrophy, adipocyte insulin resistance and increased adipocyte lipolysis in GDM over and above the effects of obesity.

It has been suggested that SAT adipocyte storage capacity is diminished due to SAT adipocyte dysfunction in terms of hypertrophy and increased lipolysis, which lead to ectopic fat deposition in other organs. VAT can be regarded as an ectopic fat depot which acts as a source of excessive FA release and inflammatory cytokines to the liver through the portal circulation leading to hepatic fat accumulation. This in turn affect glucose and lipoprotein metabolism and contributes to the development of metabolic diseases. Fatty liver was independently associated with impaired glucose tolerance and T2DM, an association that was increased by adipose tissue insulin resistance (Jorge-Galarza et al., 2016). Recently hepatic steatosis index (HSI) was studied in women at early pregnancy and the results were consistent with non-alcoholic fatty liver disease (NAFLD) diagnosis by ultrasound. Women with higher HSI score had significantly increased risk of developing GDM later in pregnancy (Lee et al., 2019). More interestingly, this study demonstrated that abnormal hepatic steatosis indices correlated positively with GDM risk when the analysis was confined to those women with normal liver ultrasound (Lee et al., 2019). The mechanism linking the development of GDM and hepatic steatosis during pregnancy is not known yet. However, it is likely that these are two metabolic diseases that share a metabolic dysfunction such as insulin resistance.

Overweight and obesity increases the risk for GDM substantially and also independently affects adipocyte function. To account for this, the GDM and the control groups in this study were BMI-matched. I collected adipose tissue biopsies at Caesarean section in order to obtain larger adipose tissue biopsies than could be obtained by needle biopsy and to allow collection of both SAT and VAT biopsies to address the regional differences in adipocyte size and *in vitro* lipolytic function of isolated adipocytes. Since dysfunctional adipocyte lipolysis is key to my central hypothesis, I aimed to study isolated adipocytes and their basal and beta adrenergic-stimulated lipolysis and the inhibitory effects of insulin. To isolate adipocytes, I selected to use the collagenase digestion method, a standard method carried out by the landmark original studies into lipolytic function (Rodbell, 1964) and the more recent study of adipocyte function in pre-eclampsia carried out in my laboratory (Huda et al., 2014). I was also able to measure adipocyte size. Glycerol and NEFA are the end products of TAG hydrolysis such that their release from

adipocytes can be used as an index of adipocyte lipolysis. There is no consensus in the literature on the presentation of lipolysis data in terms of correcting for cell number/ or tissue weight and most lipolysis data are corrected for different factors such as tissue weight, cell number, lipid weight or DNA content in different studies. In this study, DNA content was used as an index of the tissue cellularity which enabled us to directly compare metabolic effects independently of fat cell size i.e. on a per adipocyte basis. It could be argued that lipolysis rates measured in isolated adipocytes in cellular preparations are not similar to lipolysis in adipose tissue pieces *ex vivo* due to presence of other SVF cells which could contribute to the lipolytic response. However, it was reported previously that there is strong correlation between basal lipolysis rate of isolated adipocyte and *ex vivo* adipose tissue samples (Rydén and Arner, 2017). Furthermore, my hypothesis focusses on the adipocyte in the key initiator of adipose tissue dysfunction. While adipocytes are capable of NEFA re-uptake and re-esterification, this is not the case for glycerol due to the limited availability of the glycerol kinase enzyme in adipocytes. Thus, glycerol is suggested to be a better measure of total lipolysis (Rydén et al., 2019) while NEFA measures net lipolysis. NEFA/ glycerol ratio is a measure of FA reuptake where a lower ratio indicates re-uptake of NEFA and the maximum value of 3.0 indicates full breakdown of the triglyceride molecule. Whether depot differences in insulin action on lipolysis and NEFA re-esterification occur in adipocytes from different depot remains to be established (Zierath et al., 1998).

To explore the relationship between adipocyte cell size, lipolytic function and fat deposition in the liver during pregnancy, a relationship not explored before to our knowledge, hepatic steatosis index (HSI) was used. This is a simple non-invasive screening tool for non-alcoholic fatty liver disease (NAFLD) previously used in pregnancy (Lee et al., 2019). Moreover, it was suggested that HSI could have more predictive value in women with mild or moderate NAFLD (Lee et al., 2019) than ultrasound assessment since ultrasound is only able to detect positive results when the liver fat content exceeds 30% (McCullough, 2004). Plasma lipids, insulin, glucose, and liver function enzymes were measured and their relationship to adipocyte function was explored.

Integrating these structural and functional elements of adipocyte function, along with a biomarker of liver fat accumulation, this study was performed to determine the

relative contribution of adipocyte function to the underlying pathophysiology of GDM compared to a healthy BMI-matched control group.

4.2 Aim

The aim of this chapter was to compare subcutaneous (SAT) and visceral (VAT) adipocyte diameter and lipolytic function and a biomarker of liver fat accumulation (HIS) in pregnant women with and without GDM

4.2.1 Hypotheses

- That women with GDM have larger subcutaneous adipocytes than healthy BMI-matched controls

- That SAT adipocytes from GDM women have higher basal lipolysis than those from healthy BMI matched controls

- That adipocytes from women with GDM have higher NEFA release in response to catecholamine stimulation of lipolysis than those from healthy BMI matched controls

- That adipocytes from women with GDM have lower insulin suppression of lipolysis those from healthy BMI matched controls i.e. are more insulin resistant.

- That women with GDM have higher HSI compared healthy controls, i.e. have higher liver fat.

4.2.2 Specific research questions

- How are adipocyte diameter and volume affected by GDM?

- Which adipose tissue depot is more dysfunctional in women with GDM?

- Do adipocytes from women with GDM have increased insulin resistance resulting in increased lipolysis compared to BMI matched controls?

- What are the depot differences in lipolytic function?

- How are plasma lipids and liver function affected if there is enhanced adipocyte lipolysis in GDM?

4.3 Methods

4.3.1 Adipocyte isolation and sizing

The study participants were recruited as outlined in the Methods Chapter section 2.1.1. Subcutaneous and visceral adipose tissue samples were processed as outlined in section 2.2. After two hours incubation, two 300µl aliquots of the medium below the adipocyte suspensions were collected and stored at -20°C for later analysis of NEFA and glycerol concentration as outlined in section 2.4.3. Glycerol and NEFA concentrations were corrected for cell number by dividing by DNA concentration of a known volume of adipocytes as outlined in method section 2.2.3. The values were then expressed in mmol/hr/ug of DNA. Adipocyte sizing was carried out as detailed in section 2.3. Maternal lipids, markers of insulin sensitivity and liver function enzymes were measured as outlined in section 2.7.

4.3.2 Adipocyte lipolysis assay

Adipocyte lipolysis assay was carried out as outlined in 2.4.The degree of lipolysis stimulation by isoproterenol was calculated as the percentage of release of NEFA or glycerol in the presence of isoprotenerol relative to basal lipolysis i.e. Percentage stimulation= ((lipolysis rate in presence of isoproterenol – basal lipolysis) / basal lipolysis)*100. The degree of lipolysis suppression by insulin was calculated as the percentage of release of NEFA or glycerol in the presence of insulin relative to basal release i.e. ((basal lipolysis – lipolysis rate in the presence of insulin) / basal lipolysis)*100. FCISI is a direct measure of adipocyte insulin sensitivity. It can be calculated as the percentage suppression of isoproterenol stimulated lipolysis by insulin measured either as NEFA or glycerol release i.e. FCISI= ((lipolysis rate in presence of isoproterenol – lipolysis rate in presence of isoproterenol and insulin) / (lipolysis rate in presence of isoproterenol – basal lipolysis))*100.

4.3.3 Hepatic steatosis index (HSI)

HSI was calculated using the following equation (Lee et al., 2010):

HSI= 8*ALT (IU/l) / AST (IU/l) + BMI (kg/m^2) + 2 (if T2DM) + 2 (if female)

Where ALT is alanine aminotransferase, AST is aspartate aminotransferase and T2DM is type 2 diabetes. Taking the conservative approach, GDM women in our study were not considered as T2DM in the above equation. The participants were classified into three groups according to the HSI: low risk of steatosis (HSI <30); intermediate risk (30≤ HSI ≤36) and high risk (HSI >36).

4.3.4 Statistical analysis

The statistical analysis was carried out as outlined in section 2.9. The following variables were log transformed: fasting glucose level at OGTT, plasma insulin, HOMA-IR, GGT, HSI, SAT basal lipolysis (SAT-basal), SAT lipolysis rate in presence of isoprotenerol (SAT-ISO), SAT lipolysis rate in presence of insulin and isoprotenerol (SAT-ISO+INS), VAT basal lipolysis (VAT-basal), VAT lipolysis rate in presence of isoprotenerol (VAT-ISO) and VAT lipolysis rate in presence of insulin and isoprotenerol (VAT-ISO+INS). The following variables were square root transformed: SAT total volume and VAT total volume. The following variables were not normally distributed and analyzed non-parametrically: plasma glucose, plasma glycerol, ALT, AST, SAT lipolysis rate in presence of insulin (SAT-INS), VAT lipolysis rate in presence of insulin (VAT-INS), percentage stimulation of lipolysis by isoprotenerol, percentage suppression of lipolysis by insulin and fat cell insulin sensitivity index (FCISI).

4.4 Results

4.4.1 Study participants

The study participants in GDM (n=22) and control group (n=22) had similar BMI and age reflecting the study design (table 1-1). Ethnicity, parity, smoking history, blood pressure, birthweight and fetal sex did not differ between control and GDM pregnancy. GDM mothers delivered six days earlier than controls (p=0.001). Diagnostic OGTT tests were carried out in the second trimester and as expected, women in the GDM group had higher fasting OGTT blood glucose (p= 0.0001). However, there was only 4 women in the control group who were screened for GDM due to having an obese BMI and a family history of T2DM.

Table 4-1 Characteristics of GDM women and BMI matched controls

Demographic	Control (n=22)	GDM (n=22)	P-value
Age (years)	33.6(4.9)	34.9(4.6)	0.35
BMI (kg/m^2)	31.3(7.5)	32.2(8.0)	0.72
Ethnicity*** n (%)			
Caucasians	19(86.4)	9(40.9)	0.066
South Asians	3(13.6)	6(27.3)	
Other	0(0)	1(4.5)	
Missing	0(0)	7 (31.8)	
Parity*** n (%)			
Primiparous	16(72.7)	10(45.5)	0.066
Multiparous	6(27.2)	12(54.5)	
Smoking history*** n (%)			
Yes	5(22.7)	6(27.3)	0.46
No	9(40.9)	6(27.3)	
Missing	8(36.3)	10(45.5)	
SBP at delivery (mmHg)	116.9(14.8)	123.7(16.6)	0.24
DBP at delivery (mmHg)	75.6(10.1)	71.8(10.6)	0.31
Treatment for GDM n (%)			
Diet only		7(31.8)	
Metformin	-	11(50)	-
Insulin		3(13.6)	
Both insulin and metformin		1(4.5)	
OGTT (second trimester)			
Fasting*	4.7(0.3)	5.5 (0.3)	0.0001
2 hours	6.0(0.79)	7.5 (1.9)	0.035
Gestational age at delivery (weeks)	38.9(0.5)	38.3(0.7)	0.001
Birth weight(g)	3563(446)	3349(362)	0.061
Fetal sex*** n (%)			
Female	9(40.9)	12(54.5)	0.16
Male	13(59.1)	7(31.8)	
Missing	0(0)	3(13.6)	

Data are expressed as mean (SD) for continuous variables. Categorical variables are expressed as number (percent). Comparisons were made by unpaired two sample t-test except ***chi-squared test. only 4 women in the control group had an OGTT test. BMI=body mass index; SBP=systolic blood pressure and DBP=diastolic blood pressure prior to caesarean section.

4.4.2 Maternal plasma glucose, lipids, insulin sensitivity and markers of liver function

Plasma glucose and HOMA-IR were significantly higher in the GDM group compared to the control group. Maternal third trimester plasma triglycerides at term

were significantly higher in women with GDM compared to control (p= 0.045). Plasma cholesterol, NEFA and glycerol were not different between groups. Plasma levels of liver enzymes GGT, ALT and AST were not different between the groups (Table 4-2).

Table 4-2 Maternal plasma lipids, markers of insulin resistance and liver function test for GDM and control groups at third trimester

Plasma marker	Control (n=22)	GDM (n=22)	P-value
Glucose (mmol/L)**	4.37(0.36)	4.92(0.56)	0.012
insulin (uU/ml)*	11.3(7.0)	17.9(19.3)	0.053
HOMA-IR*	2.3(1.4)	3.9(4.8)	0.041
Triglycerides (mmol/L)	2.61(0.55)	3.12(0.81)	0.045
Cholesterol (mmol/L)	6.31(1.15)	5.90(1.01)	0.28
plasma glycerol (umol/L)**	86.0 (51.8)	90.2(28.6)	0.61
plasma NEFA (mmol/L)	0.79(0.31)	0.86(0.26)	0.52
GGT (U/L)*	10.0(8.8)	10.5(9.5)	0.31
ALT (U/L)**	13.0(11.3)	15.3(6.5)	0.54
AST (U/L)**	20.9(8.4)	19.6(7.9)	0.16
HSI	39.7(9.6)	41.7(9.8)	0.46
Estradiol (nmol/L)	0.11(0.07)	0.09(0.08)	0.67
Progesterone (nmol/L)	683(178)	761(182)	0.22

Data are expressed as mean (SD) for continuous variables. Comparisons were made by two sample t-test except **Mann-Whitney. HOMA-IR= homeostasis model assessment for insulin resistance, NEFA= non-esterified fatty acids, GGT= gamma- glutamyl transferase, ALT= alanine aminotransferase, AST= aspartate aminotransferase. *log transformed variable.

4.4.3 Hepatic steatosis index (HSI)

In our cohort, HSI was not different between the control and the GDM group (39.7[9.6] vs 41.7[9.8], p=0.46) although both groups were classified as high risk. HSI correlated positively with maternal BMI in the control group (r=0.86, p=0.0001) and the GDM group (r= 0.96, p=0.0001) (Figure 4-1), insulin level in the control group (r=0.68, p=0.004) and the GDM group (r= 0.65, p=0.004) (Figure 4-2) and HOMA-IR in the control group (r=0.55, p=0.017) and the GDM group (r= 0.70, p=0.002) (Figure 4-3). Only in the GDM group was maternal glucose level positively correlated with HSI (r= 0.52, p=0.039) (Figure 4-4).

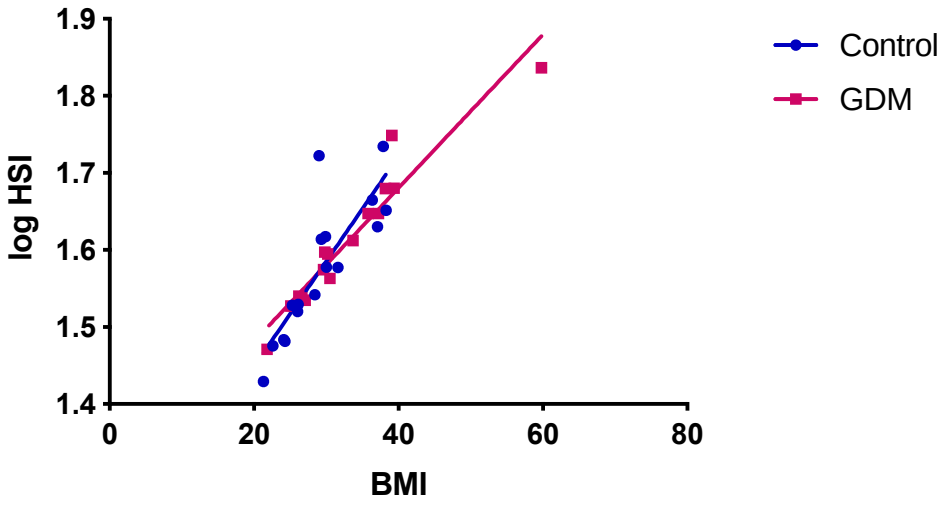


Figure 4-1 The relationship maternal BMI and HSI in GDM and control groups
Maternal BMI correlated positively with HSI in the control and GDM group (control group: r=0.96, p=0.0001) (GDM group: r= 0.86, p=0.0001)

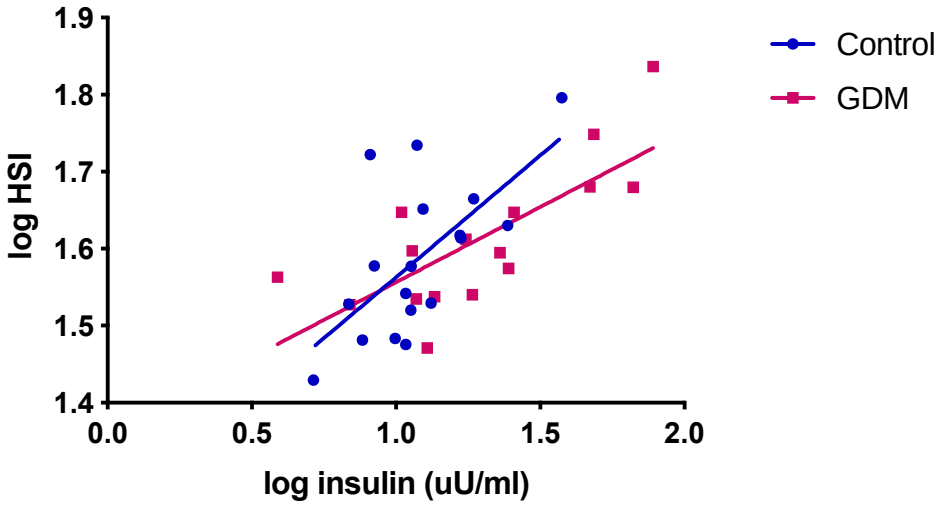


Figure 4-2 The relationship maternal insulin level and HSI in GDM and control group
Maternal BMI correlated positively with HSI in the control and GDM group (control group: r=0.68, p=0.004) (GDM group: r= 0.65, p=0.004)

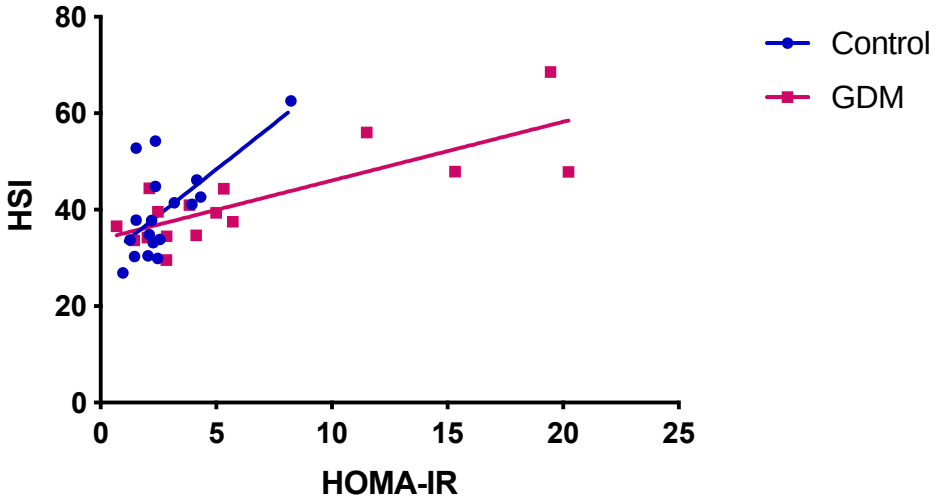


Figure 4-3 The relationship maternal HOMA-IR and HSI in GDM and control group
Maternal HOMA-IR correlated positively with HSI in the control and GDM group (control group: r=0.70, p=0.017) (GDM group: r= 0.55, p=0.002)

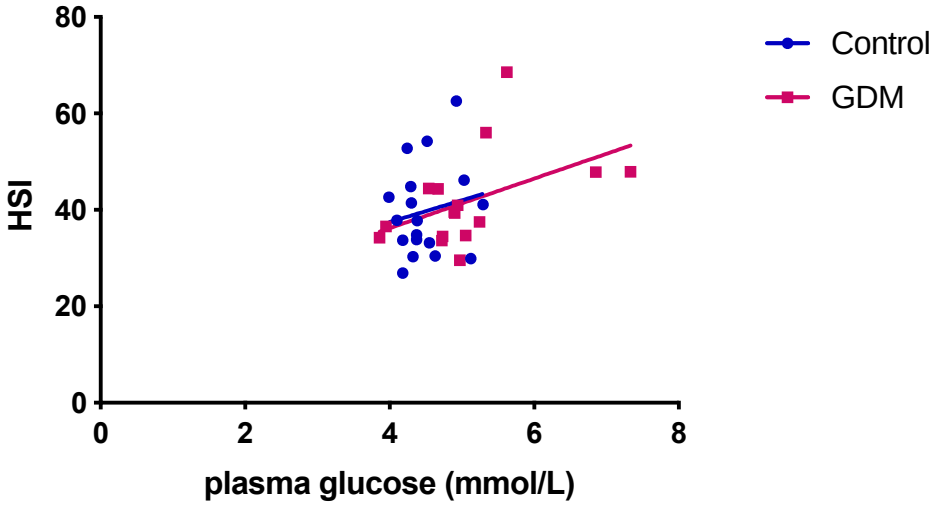


Figure 4-4 The relationship maternal glucose level and HSI in GDM and control group
Maternal BMI correlated positively with HSI in the GDM group only (r= 0.52, p=0.039)

4.4.4 Adipocyte cell size and volume in GDM and healthy pregnancy

4.4.4.1 Subcutaneous adipocytes

SAT adipocyte diameter was not different between control and GDM pregnancy (85.9[12.7] vs 91.9[10.2] µm, p=0.11) (Figure 4-5).

SAT adipocyte total volume was not different between control and GDM pregnancy (0.0002[0.0000005] vs 0.0002[0.0000003] mm^3, p=0.14) (Figure 4-6). Adipocyte

volume distribution across the range of cell diameters was investigated to examine whether how much lipid was stored in cells of different diameter. SAT total volume distribution potentially demonstrated a small shift to the right in GDM group compared to control but the mean volume was not different between groups(Figure 4-6).

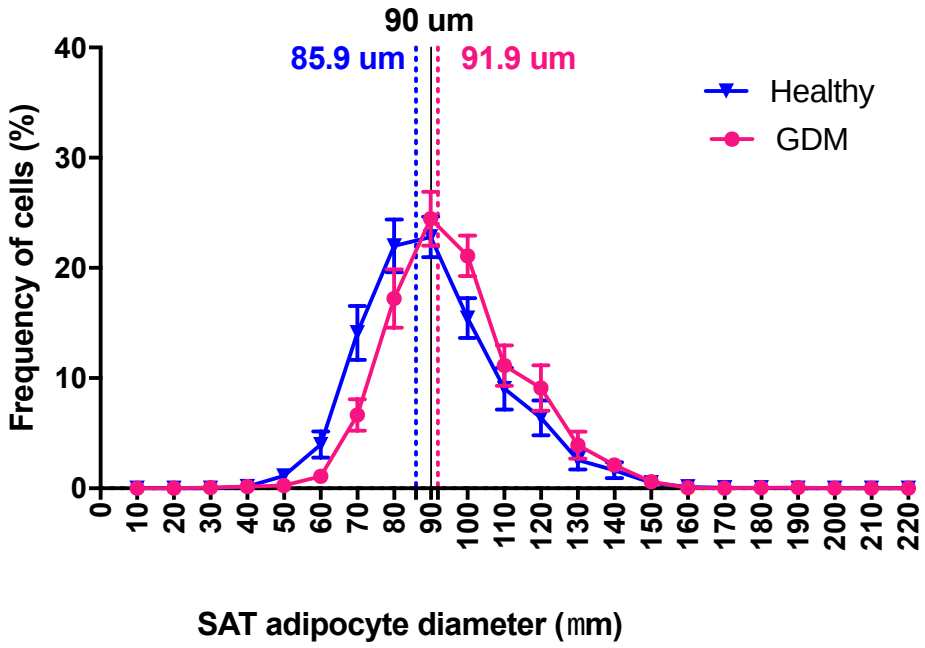


Figure 4-5 Subcutaneous adipocyte size distribution in GDM and controls
There was no difference in mean SAT adipocyte diameter between control and GDM pregnancy (GDM: n=22, control: n=22). Comparisons were made using two sample t test and results were displayed as mean.

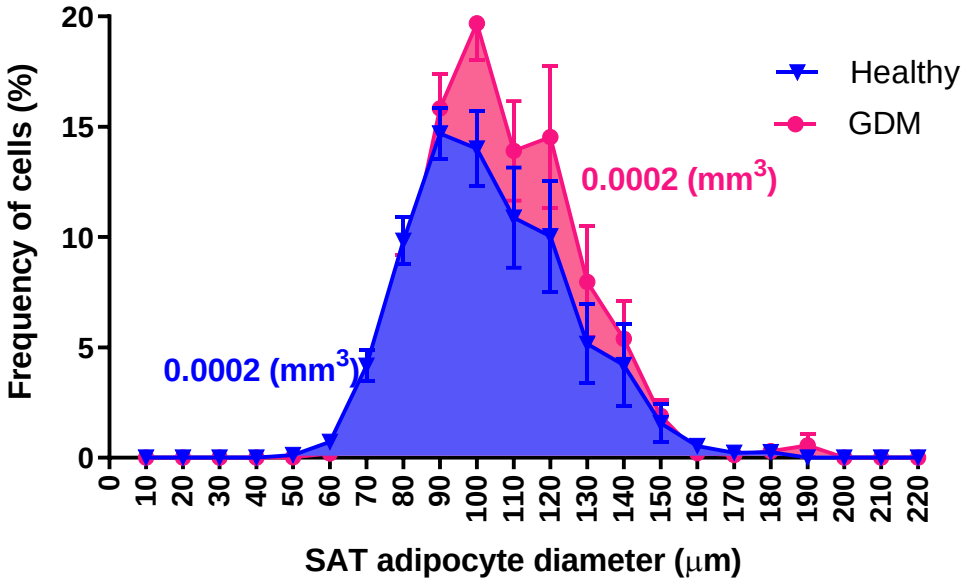


Figure 4-6 Subcutaneous adipocyte volume distribution in GDM and controls
There is no difference in SAT total adipocyte volume in control and GDM pregnancy (GDM: n=22, control: n=22). However, SAT volume distribution was slightly shifted to the right in GDM compared to control group. Comparisons were made using two sample t test and results were displayed as mean.

4.4.4.2 Visceral adipocytes

VAT adipocyte diameter was significantly larger in the GDM group compared to the control group ((control) 62.9[3.8] vs (GDM) 75.5[11.6] µm, p=0.004) (Figure 4-7). Similarly, VAT adipocyte total volume was greater in GDM compared to control group ((control) 0.00007[0.00000002] vs (GDM) 0.00012[0.0000005] mm^3, p=0.003) (Figure 4-8). GDM VAT adipocyte volume distribution showed a clear shift to the right compared to controls hence the mean volume was different between groups (Figure 4-8).

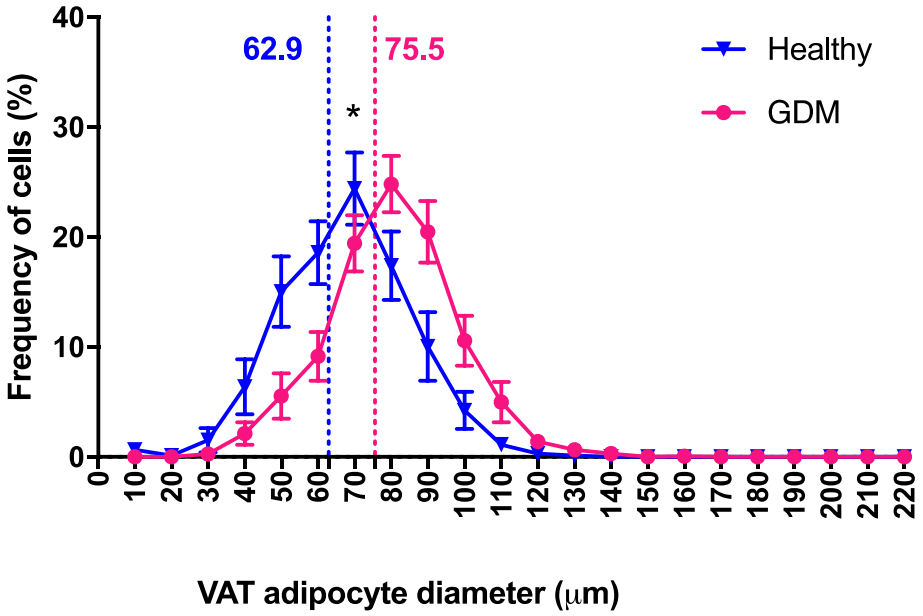


Figure 4-7 Visceral adipocyte size distribution in GDM and controls
VAT adipocyte diameter in GDM group was significantly larger than control group (p=0.004) (GDM: n=22, control: n=22). Comparisons were made using two sample t test and results were displayed as mean.

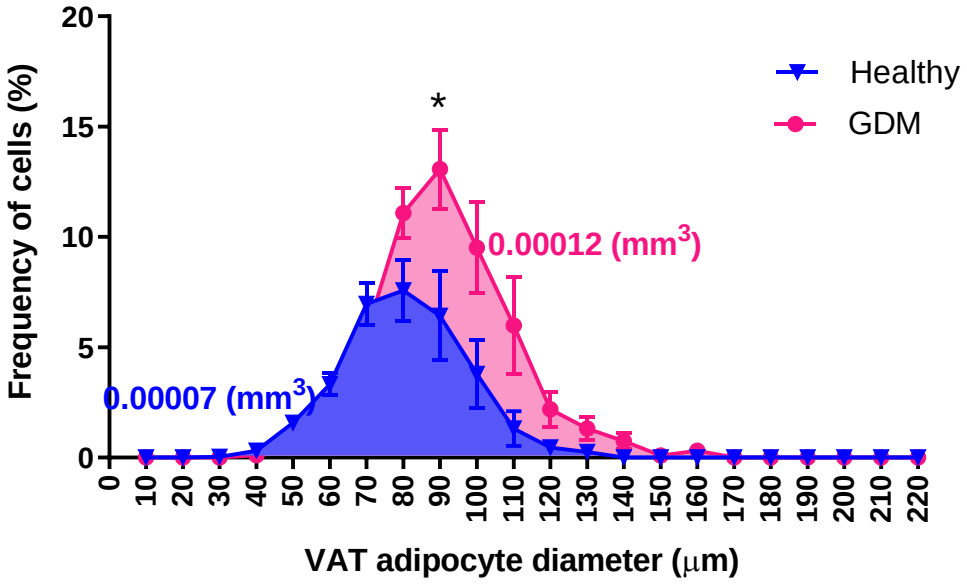


Figure 4-8 Visceral adipocyte volume distribution in GDM and controls
VAT adipocyte volume was significantly greater in GDM compared to control group (p= 0.003) (GDM: n=22, control: n=22). Similarly, VAT volume distribution was shifted to the right in GDM compared to control group. Comparisons were made using two sample t test and results were displayed as mean.

4.4.5 *In vitro* adipocyte lipolysis in GDM and in healthy pregnancy

4.4.5.1 *In vitro* SAT adipocyte lipolysis in GDM compared to healthy pregnancy

Isoprotenerol (200nM) significantly stimulated basal total and net lipolysis rate in SAT adipocytes from GDM and control groups ($p<0.0001$). Insulin (10nM) significantly suppressed basal total and net lipolysis rate in SAT adipocytes from GDM and control groups ($p<0.0001$).

There was no difference in basal SAT lipolysis between isolated adipocytes from GDM and controls when expressed as either total lipolysis (0.12[0.11] vs 0.16[0.13] glycerol mmol/hr/ug of DNA, p=0.10) (Figure 4-9) or net lipolysis (0.24[0.44] vs 0.58[0.44] NEFA mmol/hr/ug of DNA, p=0.33) (Figure 4-10). Similarly, lipolysis rates in the presence of 200nM isoprotenerol were not different between GDM and controls when expressed as either total lipolysis (0.36[0.23] vs 0.42[0.19] glycerol mmol/hr/ug of DNA, p=0.18) (Figure 4-9) or net lipolysis (0.24[0.44] vs 0.58[0.44] NEFA mmol/hr/ug of DNA, p=0.25) (Figure 4-10).

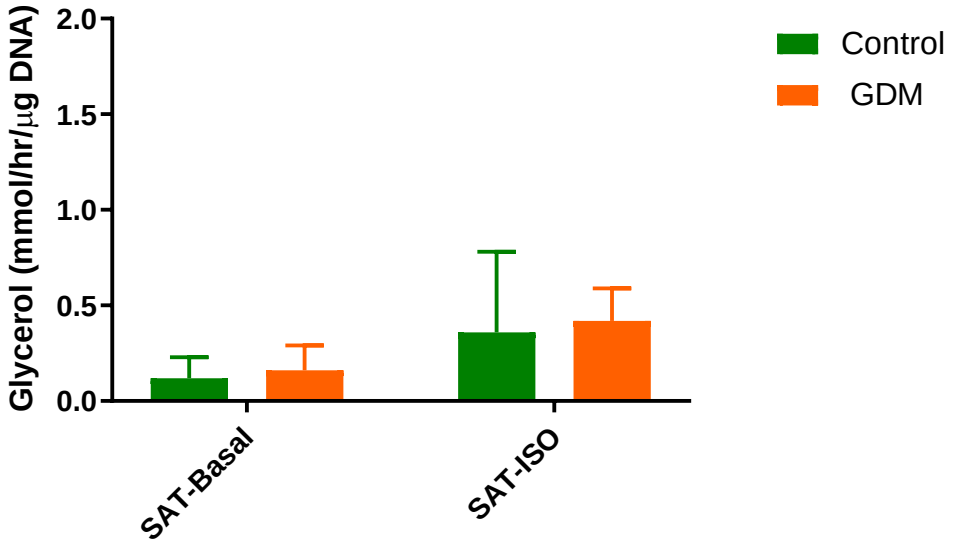


Figure 4-9 Total basal lipolysis and isoprotenerol-stimulated total lipolysis in SAT adipocytes from GDM and control groups.
There was no significant difference in SAT adipocyte total basal or isoprotenerol-stimulated lipolysis in GDM compared to the control group (GDM: n=22, control: n=22). Comparisons were made using two sample t test on log transformed data and results were displayed as mean (SD). SAT-Basal= SAT basal release of glycerol and SAT-ISO= lipolysis rate in presence of 200nM isoproterenol.

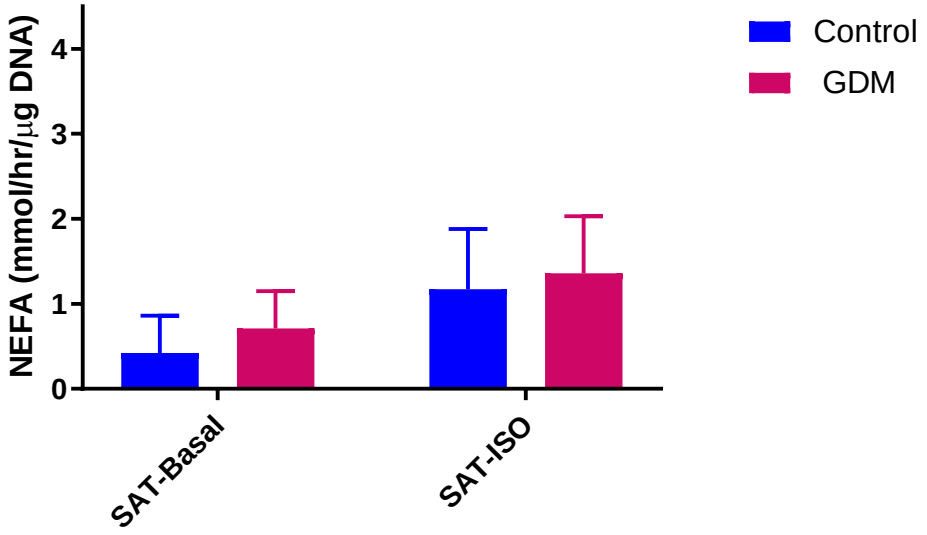


Figure 4-10 Net basal and net isoprotenerol-stimulated lipolysis in SAT adipocytes from GDM and control groups.
There was no significant difference in SAT adipocyte net basal lipolysis or isoprotenerol-stimulated lipolysis in GDM compared to the control group (GDM: n=22, control: n=22). Comparisons were made using two sample t test on log transformed data and results were displayed as mean (SD). SAT-Basal= SAT basal release of NEFA and SAT-ISO= lipolysis rate in presence of 200nM isoprotenerol.

Basal lipolysis rates in the presence of 10nM insulin were not different between GDM and control groups when expressed as either total lipolysis (0.07[0.06] vs 0.11[0.09] glycerol mmol/hr/ug of DNA, p=0.081) (Figure 4-11) or net lipolysis (0.22[0.24] vs 0.39[0.30] NEFA mmol/hr/ug of DNA, p=0.59) (Figure 4-12) release. In the presence of both 200nM isoprotenerol and 10nM insulin, lipolysis rates were not different between the GDM and the control groups when expressed as either total lipolysis (0.28[0.18] vs 0.33[0.18] glycerol mmol/hr/ug of DNA, p=0.27) (Figure 4-11) or net lipolysis(0.93[0.58] vs 1.13[0.61] NEFA mmol/hr/ug of DNA, p=0.31) (Figure 4-12) release.

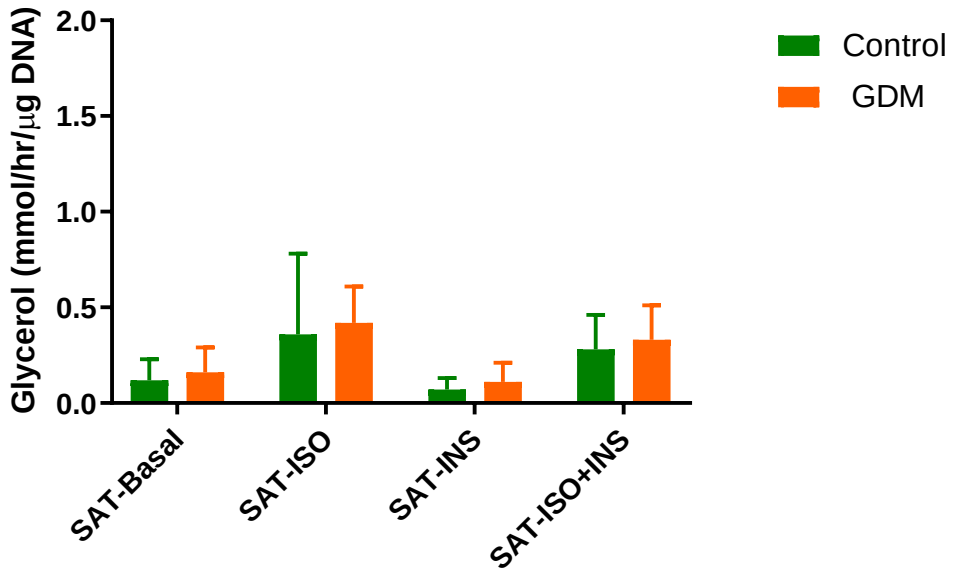


Figure 4-11 Basal total lipolysis, total lipolysis rate in presence of 200nM isoprotenerol, total lipolysis rate in the presence of 10nM insulin and total lipolysis rate in the presence of of 200nM isoprotenerol and 10nM insulin conditions in SAT adipocytes from GDM and control groups.

SAT total lipolysis in all experimental conditions was not different between GDM and control group (GDM: n=22, control: n=22). Comparisons were made using two sample t tests on log transformed data and results were displayed as mean (SD). SAT-Basal= SAT adipocytes basal (unstimulated) release of glycerol, SAT-ISO= SAT adipocytes lipolysis rate in presence of 200nM isoprotenerol, SAT-INS= SAT adipocytes lipolysis rate in presence of 10nM insulin and SAT-ISO+INS= SAT adipocytes lipolysis rate in presence of 200nM isoprotenerol and 10nM insulin.

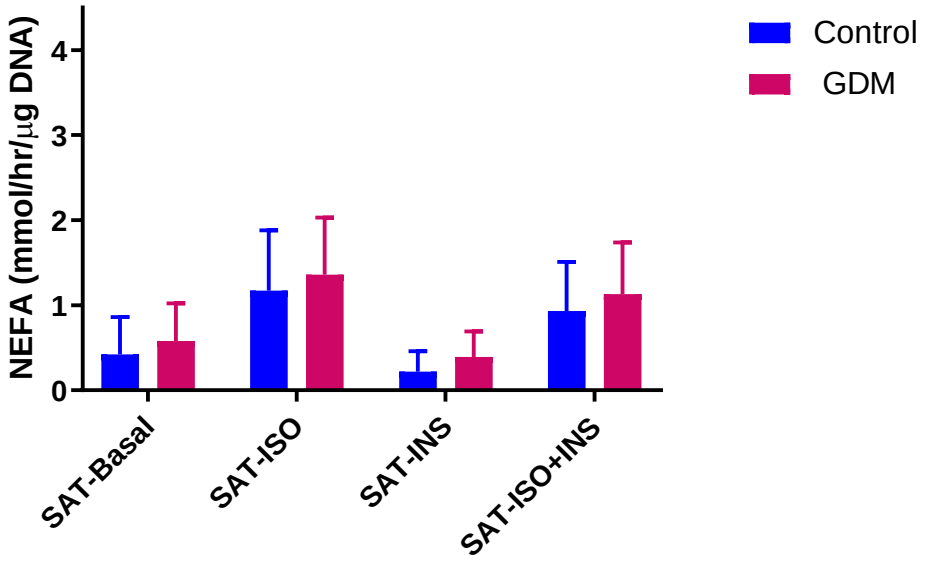


Figure 4-12 Basal net lipolysis, net lipolysis rate in presence of 200nM isoprotenerol, net lipolysis rate in presence of 10nM insulin and net lipolysis rate in presence of 200nM isoprotenerol and 10nM insulin conditions measured by NEFA release in SAT adipocytes from GDM and control group.

SAT adipocytes lipolysis in all experimental conditions was not different between GDM and control group (GDM: n=22, control: n=22). Comparisons were made using two sample t test on log transformed data and results were displayed as mean (SD). SAT-Basal= SAT adipocytes basal (unstimulated) release of NEFA, SAT-ISO= SAT adipocytes lipolysis rate in presence of 200nM isoprotenerol, SAT-INS= SAT adipocytes lipolysis rate in presence of 10nM insulin and SAT-ISO+INS= SAT adipocytes lipolysis rate in presence of 200nM isoprotenerol and 10nM insulin.

The percentage stimulation of SAT adipocytes total and net lipolysis by 200nM isoprotenerol was not different between the GDM and the control groups (350[279] vs 248[206] %glycerol, p=0.36) and (224[250] vs 215[206] %NEFA, p=0.95) (Figure 4-13) respectively.

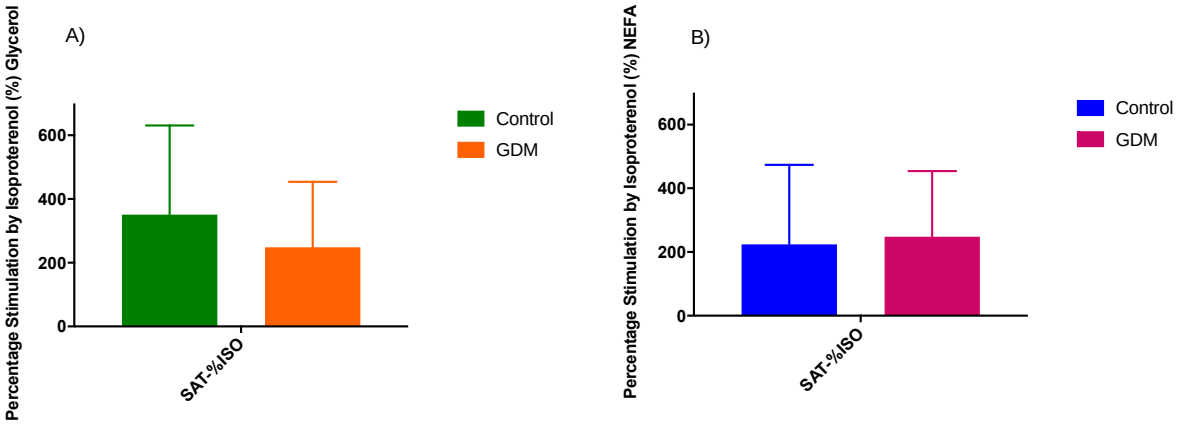


Figure 4-13 Percentage stimulation of lipolysis by 200nM isoprotenerol expressed as A) total lipolysis and B) net lipolysis in SAT adipocytes from GDM and controls
There was no difference in percentage stimulation of total and net lipolysis by 200nM isoprotenerol in SAT adipocytes between GDM and control groups (GDM: n=22, control: n=22). Comparisons were made using two sample t test on log transformed data and results were displayed as mean (SD). SAT-%ISO= SAT adipocyte percentage stimulation of lipolysis by 200nM isoprotenerol measured by glycerol or NEFA release.

Percentage insulin suppression of SAT adipocyte basal total lipolysis was not different between GDM and control groups (33[38] vs 25[33] %glycerol, p=0.27) (Figure 4-14). GDM SAT adipocytes had lower percentage insulin suppression of net lipolysis than controls but this failed to reach statistical significance (46[36] vs 21[38] %NEFA, p=0.050) (Figure 4-14).

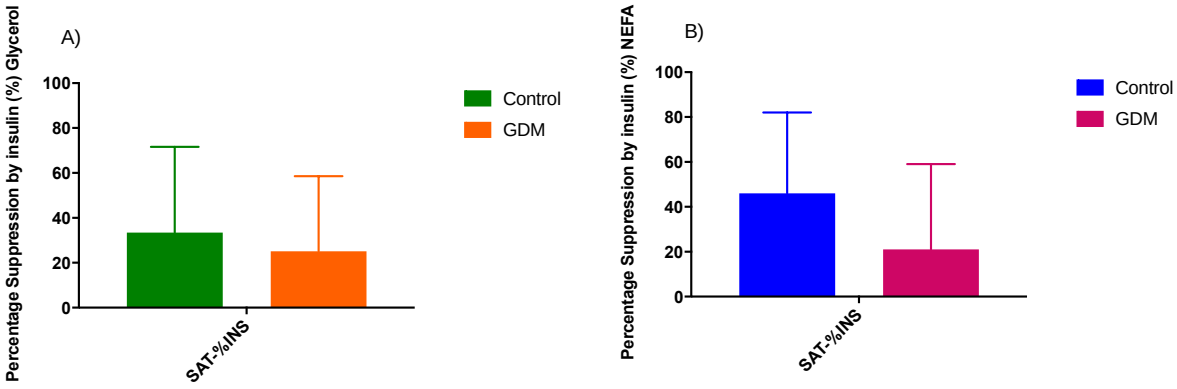


Figure 4-14 Percentage suppression of SAT adipocyte lipolysis by 10nM insulin expressed as A) total lipolysis and B) net lipolysis in SAT adipocytes from GDM and controls
There was no difference in percentage suppression of total lipolysis by 10nM insulin in SAT adipocytes between GDM and control groups as expressed by glycerol release. However, the GDM group had reduced percentage insulin suppression of net lipolysis compared to controls but failed to reach statistical significance (46[36] vs 21[38] %NEFA, p=0.050) (GDM: n=22, control: n=22). For glycerol SAT-%INS, comparisons were made using Mann-Whitney test for non-parametric data and results were displayed as mean (SD). For NEFA SAT-%INS, comparisons were made using two sample t test on log transformed data and results were displayed as mean (SD). SAT-%INS= SAT percentage suppression of lipolysis by 10nM insulin measured by glycerol or NEFA release.

There was no difference in SAT adipocyte FCISI between the GDM and the control groups when expressed as total lipolysis (33[23] vs 39[26] FCISI, p=0.83) (Figure 4-15) or as net lipolysis (33[23] vs 31[28] FCISI, p=0.51) (Figure 4-15).

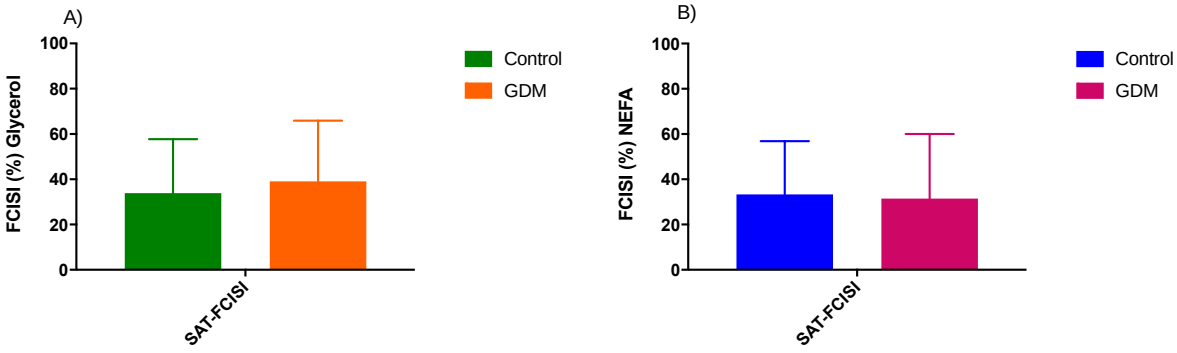


Figure 4-15 SAT adipocyte FCISI expressed as A) total lipolysis and B) net lipolysis in GDM and controls
There was no difference in SAT adipocyte FCISI between GDM and control groups (GDM: n=22, control: n=22). Comparisons were made using Mann-Whitney test and results were displayed as mean (SD). SAT-FCISI= SAT adipocyte fat cell insulin sensitivity index measured by glycerol or NEFA release.

4.4.5.2 *In vitro* VAT adipocyte lipolysis in GDM compared to healthy pregnancy

Isoprotenerol (200nM) significantly stimulated basal total and net lipolysis in VAT adipocytes from the GDM and control groups (p<0.0001). Insulin (10nM)

suppressed basal total and net lipolysis in VAT adipocytes from GDM and control groups ($p<0.0001$).

Basal VAT adipocyte total and net lipolysis was significantly higher in the GDM compared to the control group (0.02[0.02] vs 0.07[0.07] glycerol mmol/hr/ug of DNA, $p=0.001$) (Figure 4-16) and (0.06[0.07] vs 0.26[0.30] NEFA mmol/hr/ug of DNA, $p=0.004$) (Figure 4-17) respectively. Isoprotenerol-stimulated total and net lipolysis was significantly higher in the GDM compared to the control group (0.15[0.13] vs 0.32[0.34] glycerol mmol/hr/ug of DNA, $p=0.014$) (Figure 4-16) and (0.50[0.39] vs 1.09[1.01] NEFA mmol/hr/ug of DNA, $p=0.023$) (Figure 4-17) respectively.

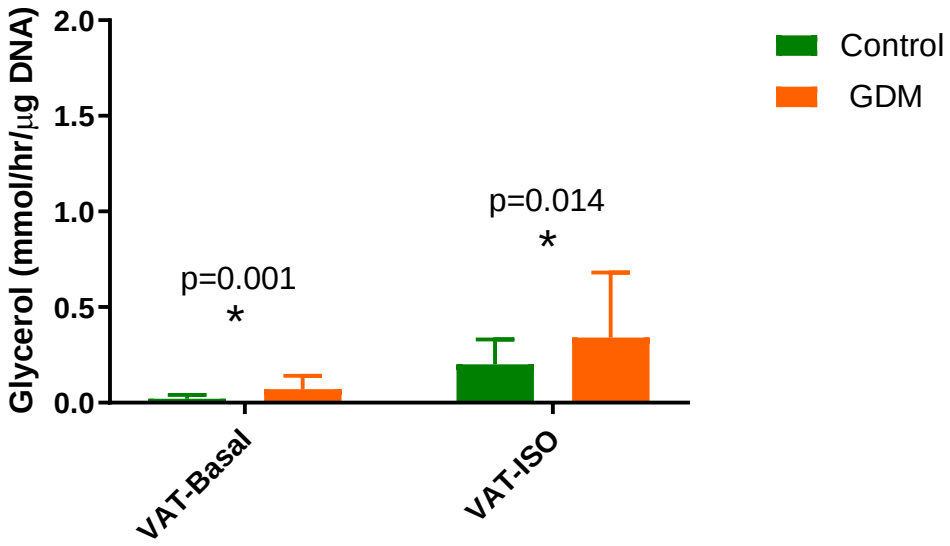


Figure 4-16 Total basal lipolysis and total lipolysis rate in presence of 200nM isoprotenerol in VAT adipocytes from GDM and control groups.
VAT adipocyte total basal and total lipolysis rate in presence of 200nM isoprotenerol were significantly higher in the GDM compared to control group (GDM: n=22, control: n=22). Comparisons were made using two sample t test on log transformed data and results were displayed as mean (SD). VAT-Basal= VAT adipocytes basal release of glycerol and VAT-ISO= VAT adipocytes lipolysis rate in presence of 200nM isoprotenerol.

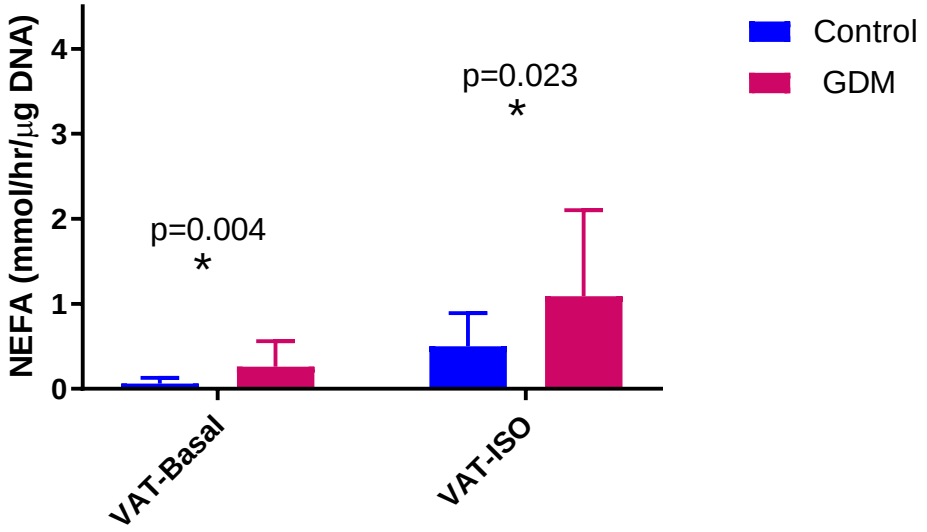


Figure 4-17 Net basal lipolysis and net lipolysis rate in presence of 200nM isoprotenerol in VAT adipocytes from GDM and control group.
VAT adipocytes net basal lipolysis and net lipolysis rates in presence of 200nM isoprotenerol was significantly greater in GDM compared to control group (GDM: n=22, control: n=22). Comparisons were made using two sample t test on log transformed data and results were displayed as mean (SD). VAT-Basal= VAT adipocytes basal release of NEFA and VAT-ISO= VAT adipocyets lipolysis rate in presence of 200nM isoprotenerol.

Total lipolysis in the presence of 10nM insulin was significantly higher in the GDM compared to the control group (0.03[0.03] vs 0.06[0.07] glycerol mmol/hr/ug of DNA, p=0.033) (Figure 4-18) but net lipolysis was not different (0.07[0.10] vs 0.28[0.33] NEFA mmol/hr/ug of DNA, p=0.12) (Figure 4-19).

Isoprotenerol-stimulated total and net lipolysis In presence of 10nM insulin was significantly higher in the GDM group compared to the control group (0.13[0.10] vs 0.27[0.29] glycerol mmol/hr/ug of DNA, p=0.021) (Figure 4-18) and (0.43[0.31] vs 0.96[0.91] NEFA mmol/hr/ug of DNA, p=0.010) (Figure 4-19) respectively.

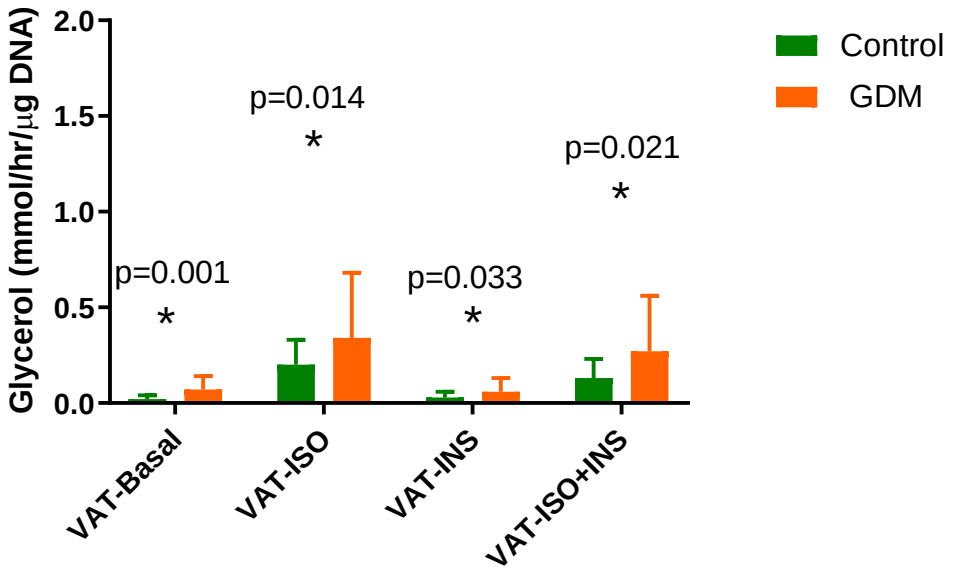


Figure 4-18 Total basal lipolysis, total lipolysis in presence of 200nM isoprotenerol, total lipolysis in presence of 10nM insulin and total lipolysis in presence of 200nM isoprotenerol and 10nM insulin conditions in VAT adipocytes from GDM and control group.
Lipolysis rate in presence of 10nM insulin only and both 200nM isoprotenerol and 10nM insulin was significantly higher in GDM compared to control group (GDM: n=22, control: n=22). Comparisons were made using two sample t test on log transformed data and results were displayed as mean (SD). VAT-Basal= VAT adipocytes basal release of glycerol, VAT-ISO= VAT adipocytes lipolysis in presence of 200nM isoprotenerol, VAT-INS= VAT adipocytes lipolysis in presence of 10nM insulin and VAT-ISO+INS= VAT adipocytes lipolysis in presence of 200nM isoprotenerol and 10nM insulin, all expressed as glycerol release.

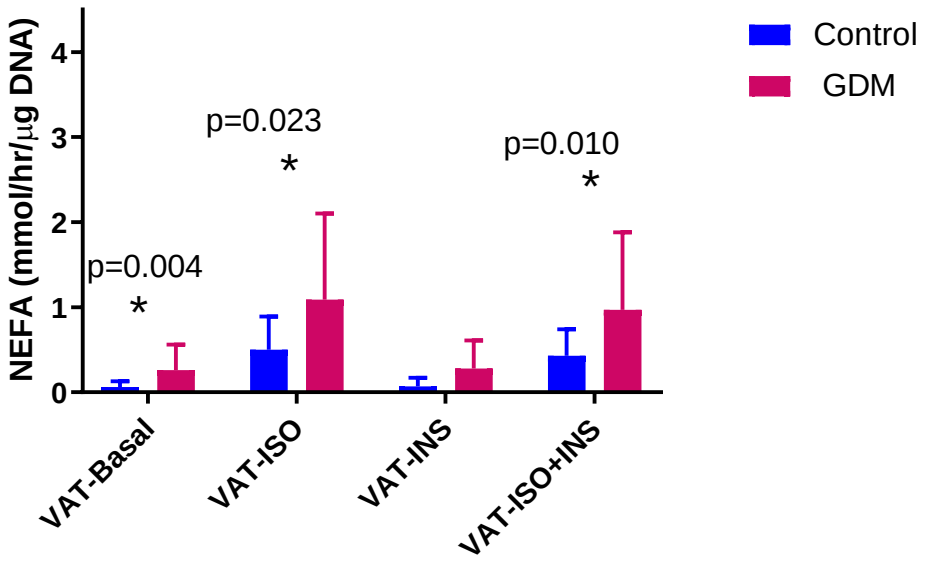


Figure 4-19 Net basal lipolysis, net lipolysis in presence of 200nM isoprotenerol, net lipolysis in presence of 10nM insulin and net lipolysis rate in presence of 200nM isoprotenerol and 10nM insulin conditions in VAT adipocytes from GDM and control group.
Lipolysis rate in presence of both 200nM isoprotenerol and 10nM insulin was significantly higher in GDM compared to control group (GDM: n=22, control: n=22). Comparisons were made using two sample t test on log transformed data and results were displayed as mean (SD). VAT-Basal= VAT adipocytes basal release of NEFA, VAT-ISO= VAT adipocytes lipolysis in presence of 200nM isoprotenerol, VAT-INS= VAT adipocytes lipolysis in presence of 10nM insulin and VAT-ISO+INS= VAT adipocytes lipolysis in presence of 200nM isoprotenerol and 10nM insulin.

The percentage stimulation of VAT adipocyte total and net lipolysis by 200nM isoproterenol is not different between GDM and control groups (729[579] vs 467[397] %glycerol, p=0.14) and (315[406] vs 230[293] %NEFA, p=1.00) (Figure 4-20) respectively.

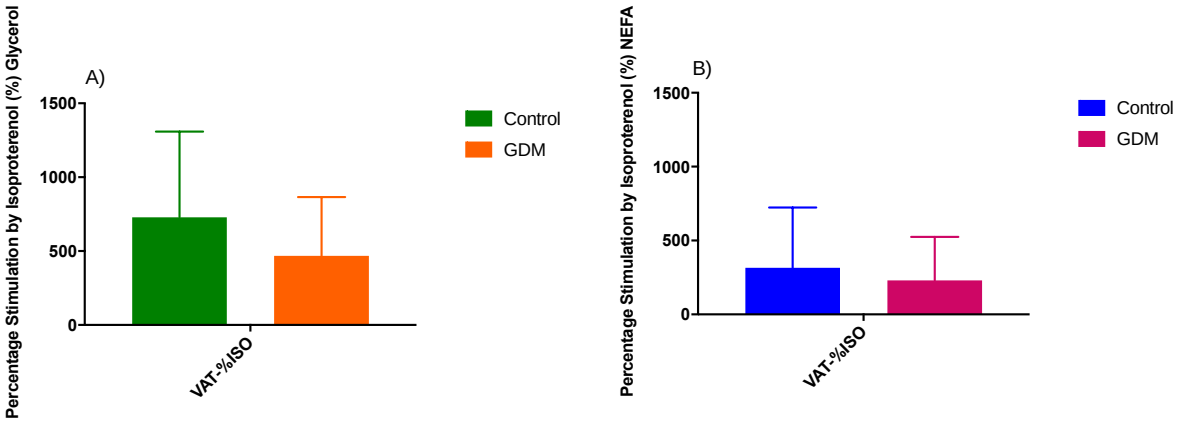


Figure 4-20 Percentage stimulation of lipolysis by 200nM isoproterenol expressed as A) total lipolysis and B) net lipolysis in VAT adipocytes from GDM and controls
There is no difference in percentage stimulation of total and net lipolysis by 200nM isoproterenol in VAT adipocytes between GDM and control groups (GDM: n=22, control: n=22). Comparisons were made using Mann-Whitney test for non-parametric data and results were displayed as mean (SD). VAT-%ISO= VAT adipocytes percentage stimulation of lipolysis by 200nM isoproterenol measured by glycerol or NEFA release.

The percentage suppression by insulin of total and net VAT adipocyte basal lipolysis is not different between the GDM and control groups (1[60] vs -4[90] %glycerol, p=0.72) and net lipolysis (19[75] vs 9[57] %NEFA, p=0.69) (Figure 4-21) respectively.

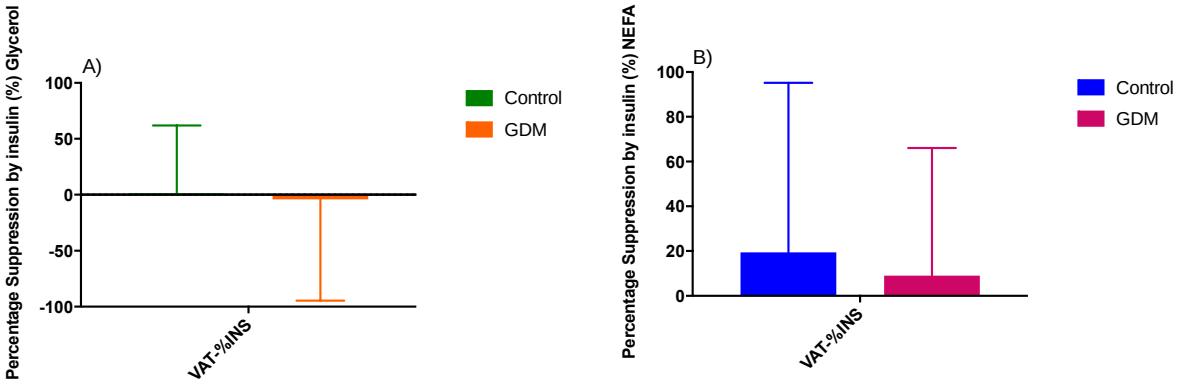


Figure 4-21 The percentage suppression of lipolysis by 10nM insulin expressed as A) total lipolysis and B) net lipolysis in VAT adipocytes from GDM and controls
There is no difference in percentage suppression of total and net lipolysis by 10nM insulin in VAT adipocytes between GDM and control group (GDM: n=22, control: n=22). Comparisons were made using Mann-Whitney test for non-parametric data and results were displayed as mean (SD). VAT-%INS= VAT adipocytes percentage suppression of lipolysis by insulin measured by glycerol or NEFA release.

VAT adipocyte FCISI was not different between GDM and controls when measured as total lipolysis (62[204] vs 35[52] FCISI, p=0.61) (Figure 4-22) or net lipolysis (64[219] vs 11[32] FCISI, p=0.86) (Figure 4-22).

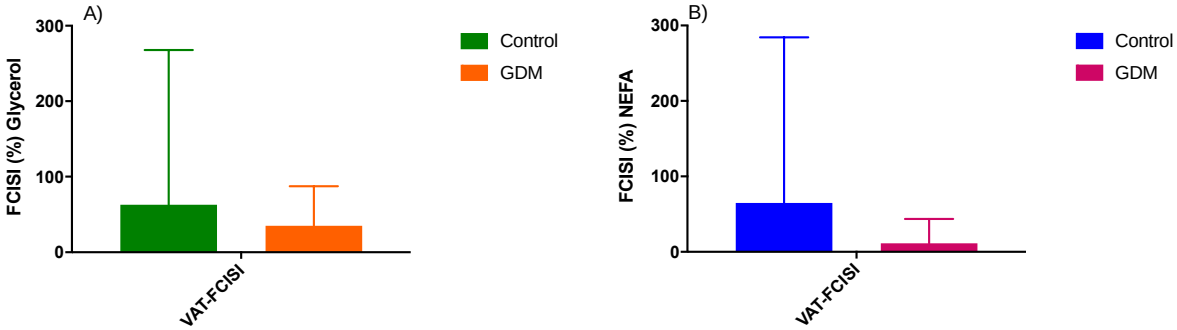


Figure 4-22 VAT adipocytes FCISI expressed as A) total lipolysis and B) net lipolysis in GDM and controls
There is no difference in VAT adipocytes FCISI between GDM and control group (GDM: n=22, control: n=22). Comparisons were made using Mann-Whitney test for non-parametric data and results were displayed as median (IQR). VAT-FCISI= VAT adipocytes fat cell insulin sensitivity index measured by glycerol or NEFA release.

4.4.6 Depot specific differences in adipocyte size and lipolysis in the healthy control group

SAT adipocyte diameter was significantly larger than VAT adipocyte diameter in the control group ((SAT) 85.9[12.7] vs (VAT) 62.9[13.8] µm, p<0.0001). Similarly, SAT adipocyte total volume was greater than VAT adipocytes total volume in the control group ((SAT) 0.08[0.03] vs (VAT) 0.03[0.02] mm^3, p<0.0001). Regarding the depot differences in adipocyte lipolytic function between SAT and VAT in the control group, SAT adipocytes had significantly higher basal lipolysis compared to VAT adipocytes (Table 4-3). Although control VAT FCISI was double that in SAT adipocytes from the control group, it did not reach statistical significance. SAT adipocytes also had a higher percent stimulation of lipolysis in response to isoprotenerol that was not significant.

Table 4-3 Lipolysis rates in SAT and VAT adipocytes from healthy pregnancy

Treatment	Total lipolysis (Glycerol release)		P-value	Net lipolysis (NEFA release)		P-value
	SAT (n=22)	VAT (n=22)		SAT (n=22)	VAT (n=22)	
Lipolysis rate, mmol/hr/ug of DNA						
SAT basal lipolysis *	0.12(0.11)	0.02(0.02)	<0.0001	0.42(0.44)	0.06(0.07)	<0.0001
SAT lipolysis in presence of isoprotenerol*	0.36(0.23)	0.15(0.13)	<0.0001	1.17(0.71)	0.50(0.39)	<0.0001
SAT lipolysis in presence of insulin**	0.07(0.06)	0.03(0.03)	0.002	0.22(0.24)	0.07(0.10)	0.082
SAT lipolysis in presence of isoprotenerol and insulin*	0.28(0.18)	0.13(0.10)	0.001	0.93(0.58)	0.43(0.31)	0.001
Relative lipolysis (%)						
Percent stimulation of lipolysis by isoprotenerol**	350(279)	729(579)	0.019	224(250)	315(406)	0.78
Percent suppression of lipolysis by insulin	33(38)	1(60)	0.11	46(36)	19(75)	0.25
FCISI**	33(23)	62(204)	0.24	33(23)	64(219)	0.057

Data are expressed as mean (SD). Comparisons were made by paired t-test except **Mann-Whitney. *log transformed variable.

4.4.7 Depot specific differences in adipocyte size and lipolysis in the GDM group

SAT adipocyte diameter was significantly larger than VAT adipocyte diameter in the GDM group ((SAT) 91.9[10.2] vs (VAT) 75.5[11.6] µm, p<0.0001). Similarly, SAT adipocyte total volume was greater than VAT adipocyte total volume in the GDM group ((SAT) 0.09[0.03] vs (VAT) 0.05[0.02] mm^3, p<0.0001). SAT adipocytes had significantly higher basal total, but not net, lipolysis compared to VAT adipocytes (Table 4-4). VAT FCISI was 67% lower that SAT FCISI for net lipolysis, suggesting

that there is a trend for lower NEFA re-esterification in VAT adipocytes compared to SAT adipocytes from women with GDM.

Table 4-4 Lipolysis rates in SAT and VAT adipocytes from GDM pregnancy

Treatment	Total lipolysis (Glycerol release)		P-value	Net lipolysis (NEFA release)		P-value
	SAT (n=22)	VAT (n=22)		SAT (n=22)	VAT (n=22)	
Lipolysis rate, mmol/hr/ug of DNA						
SAT basal lipolysis *	0.16(0.13)	0.07(0.07)	0.002	0.58(0.44)	0.26(0.30)	0.072
SAT lipolysis in presence of isoprotenerol*	0.42(0.19)	0.32(0.34)	0.034	1.36(0.67)	1.09(1.02)	0.047
SAT lipolysis in presence of insulin**	0.11(0.10)	0.06(0.07)	0.017	0.39(0.30)	0.28(0.33)	0.068
SAT lipolysis in presence of isoprotenerol and insulin*	0.33(0.18)	0.27(0.29)	0.108	1.13(0.61)	0.97(0.91)	0.15
Relative lipolysis (%)						
Percent stimulation of lipolysis by isoprotenerol**	248(206)	467(397)	0.071	215(206)	230(293)	0.53
Percent suppression of lipolysis by insulin	25(33)	-4(90)	0.39	21(38)	9(57)	0.48
FCISI**	39(26)	35(52)	0.145	31(28)	11(32)	0.062

Data are expressed as mean (SD). Comparisons were made by paired t-test except **Mann-Whitney. *log transformed variable.

4.4.8 Relationships between SAT and VAT adipocyte cell size and adipocyte characteristics in control and GDM pregnancies

4.4.8.1 Adipocyte size and maternal BMI

In controls, higher BMI was associated with larger adipocyte diameter in both SAT (SAT diameter: r=0.57, p=0.006: Figure 4-23) and VAT (VAT diameter: r=0.60, p=0.005: Figure 4-24). There was no suggestion of a different relationship of adipocyte diameter to BMI in women with GDM compared to controls (interaction term GDM*BMI: SAT p=0.80; VAT p=0.35).

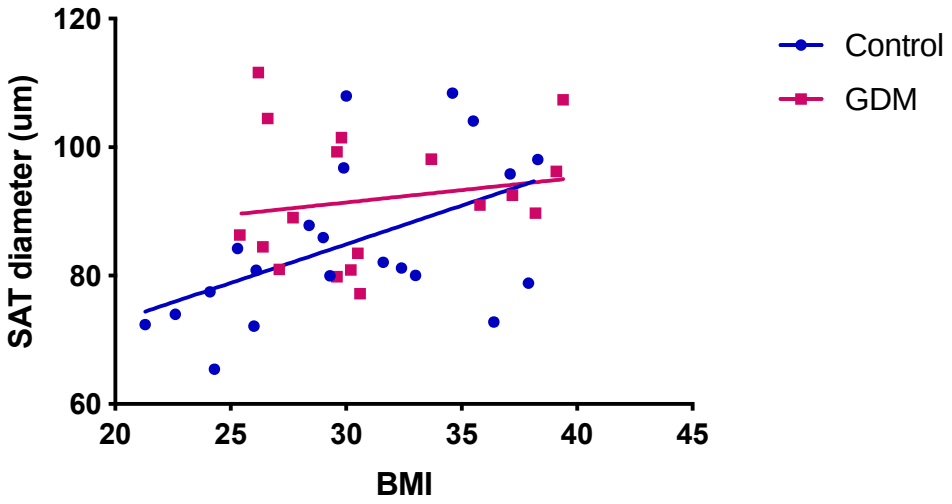


Figure 4-23 The relationship between BMI and SAT adipocyte diameter in GDM and control groups
SAT diameter correlated positively with BMI in the control group (r=0.57, p=0.006). There was no similar correlation in GDM group (r=0.29, p=0.26) with no suggestion of different relationship between BMI and SAT diameter in women with GDM vs controls (interaction term GDM*BMI: p=0.80)

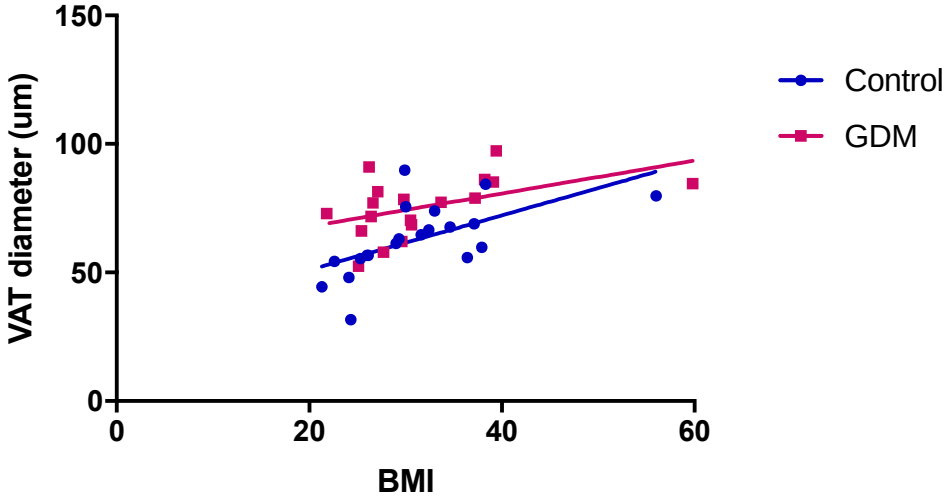


Figure 4-24 The relationship between BMI and VAT adipocyte diameter in GDM and control groups
VAT diameter correlated positively with BMI in the control group (r=0.60, p=0.005). Correlation in GDM group (r=0.48, p=0.042) with no suggestion of different relationship between BMI and VAT diameter in women with GDM vs controls (interaction term GDM*BMI: p=0.35)

4.4.8.2 Adipocyte size and insulin sensitivity measures

SAT adipocyte diameter was not associated with maternal HOMA-IR and insulin level in both groups. In contrast, VAT adipocyte diameter was closely related to measures of maternal whole body insulin sensitivity including insulin levels (Figure 4-25) and HOMA-IR (Figure 4-26) in the control and GDM group. There was a suggestion of different relationship between VAT adipocyte diameter and HOMA-

IR in women with GDM compared to controls, but not with insulin level (interaction term GDM*insulin level: VAT p=0.22, GDM*HOMA-IR: VAT p=0.12).

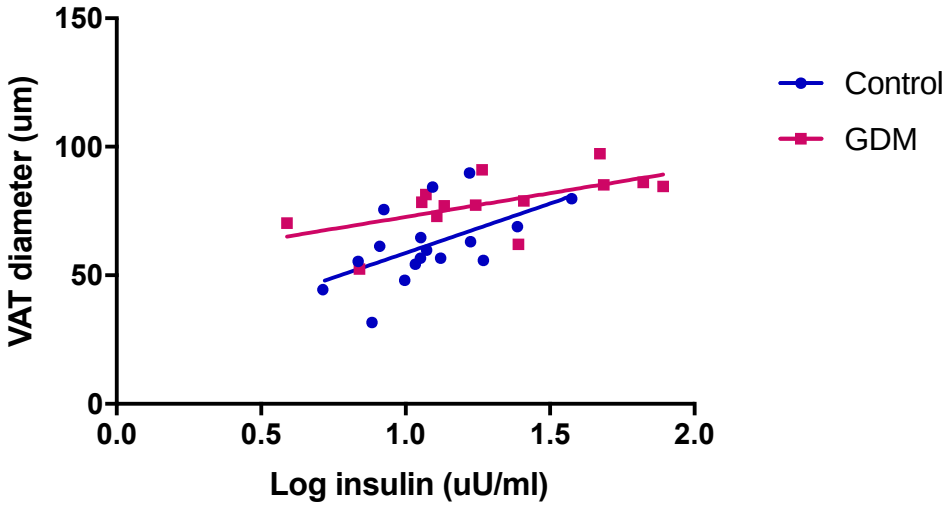


Figure 4-25 The relationship between VAT adipocytes diameter and maternal insulin level in GDM and control group
Maternal insulin level correlated positively with VAT adipocytes diameter in the control and GDM group (control group: r=0.55, p=0.021) (GDM group: r= 0.61, p=0.022) with no suggestion of different relationship between insulin level and VAT diameter in women with GDM vs controls (interaction term GDM*insulin level: p=0.22)

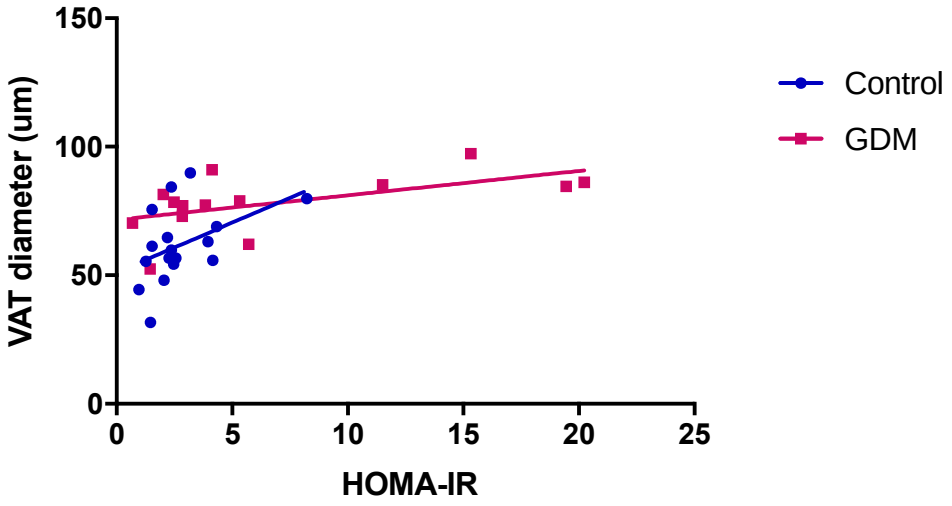


Figure 4-26 The relationship between VAT adipocytes diameter and maternal HOMA-IR in GDM and control group
Maternal HOMA-IR correlated positively with VAT adipocytes diameter in the control and GDM group (control group: r=0.51, p=0.037) (GDM group: r= 0.65, p=0.012) with a suggestion of different relationship between HOMA-IR and VAT diameter in women with GDM vs controls (interaction term GDM*HOMA-IR: p=0.12)

4.4.8.3 Adipocyte size and maternal plasma lipids (glycerol, NEFA and TAG)

There was no correlation between SAT and VAT adipocyte diameter and maternal plasma NEFA, glycerol and TAG levels in either group.

4.4.8.4 Adipocyte size and maternal liver function enzymes (ALT, AST and GGT) and hepatic steatosis index

SAT and VAT adipocyte size was not related to liver enzymes. In controls, higher HSI was associated with larger adipocyte diameter in both the SAT (SAT diameter: r=0.53, p=0.025: Figure 4-27) and VAT (VAT diameter: r=0.61, p=0.010: Figure 4-28). There was no suggestion of a different relationship of adipocyte diameter to HSI in women with GDM compared to controls (interaction term GDM*HSI: SAT p=0.26; VAT p=0.47). There were no correlations between SAT and VAT adipocyte size and HSI in the GDM group.

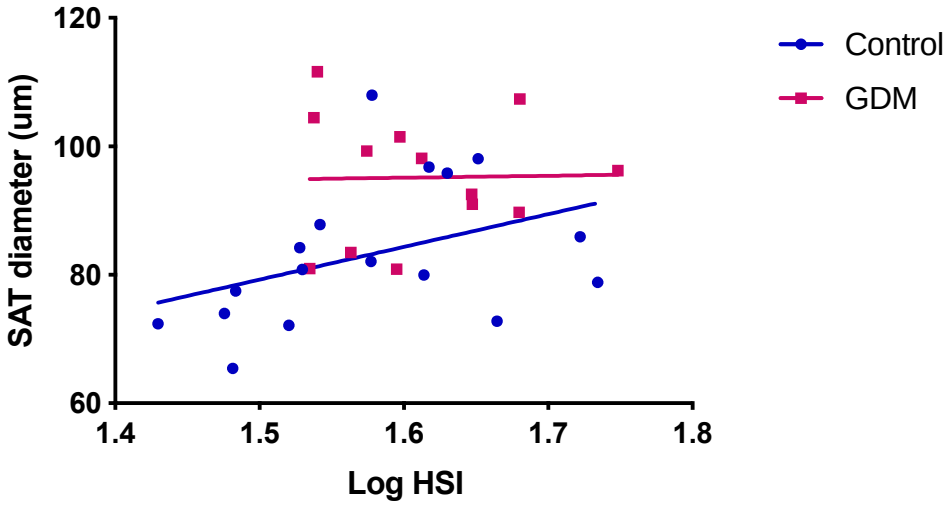


Figure 4-27 The relationship between SAT adipocytes diameter and maternal HSI in GDM and control group
SAT diameter correlated positively with HSI in the control group (r=0.53, p=0.025). There was no similar correlation in GDM group (r=0.02, p=0.95) with no suggestion of different relationship between HSI and SAT diameter in women with GDM vs controls (interaction term GDM*HSI: p=0.47)

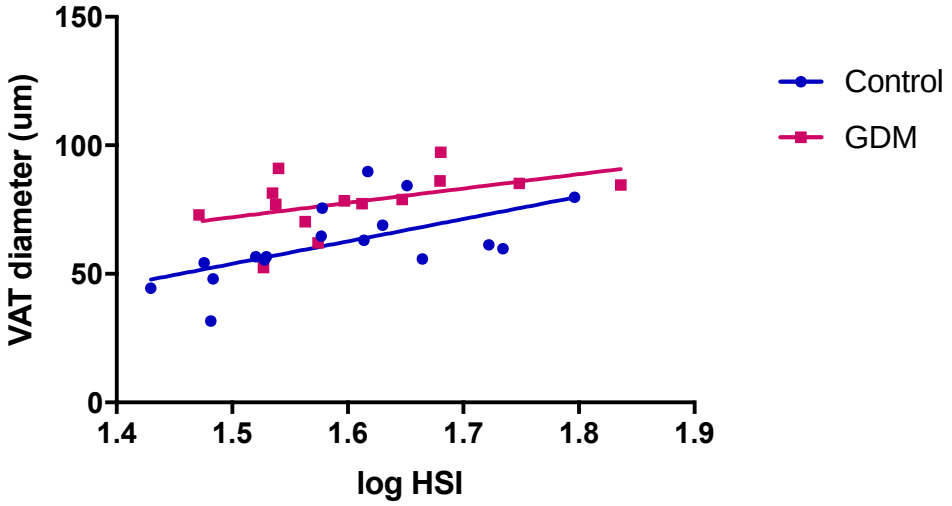


Figure 4-28 The relationship between VAT adipocytes diameter and maternal HSI in GDM and control group
VAT diameter correlated positively with HSI in the control group (r=0.61, p=0.010). There was no similar correlation in GDM group (r=0.48, p=0.08) with no suggestion of different relationship between HSI and VAT diameter in women with GDM vs controls (interaction term GDM*HSI: p=0.26)

4.4.8.5 Adipocyte size and pregnancy hormones (estradiol and progesterone)

There were no correlations between SAT and VAT adipocyte diameter and estradiol or progesterone levels in either control or GDM groups.

4.4.9 Relationships between SAT and VAT adipocyte lipolytic function and maternal characteristics in control and GDM pregnancies

4.4.9.1 Adipocyte lipolytic function and maternal characteristics

The percentage of isoprotenerol stimulation of lipolysis, the percentage suppression of insulin suppression of lipolysis and FCISI was not correlated with maternal BMI in SAT and VAT adipocytes in both groups.

4.4.9.2 Adipocyte lipolytic function and maternal insulin sensitivity measures

In GDM group, higher HOMA-IR, insulin and glucose level was associated with lower percentage stimulation of net lipolysis by isoprotenerol SAT depot (HOMA-IR: r=-0.79, p=0.001: Figure 4-29) (insulin: r=-0.77, p=0.002: Figure 4-30) (glucose: r=-0.76, p=0.002: Figure 4-31). There was no suggestion of a different relationship of HOMA-IR, insulin or glucose level to percentage stimulation of net lipolysis by isoprotenerol in SAT in women with GDM compared to controls (interaction term

GDM*HOMA-IR: p=0.64; GDM*insulin: p=0.95; GDM*glucose: p=0.47). Maternal insulin sensitivity measures were not correlated with VAT net lipolysis measures in all conditions. There were no correlations between SAT and VAT adipocyte total lipolysis rates and measures of insulin sensitivity in both groups.

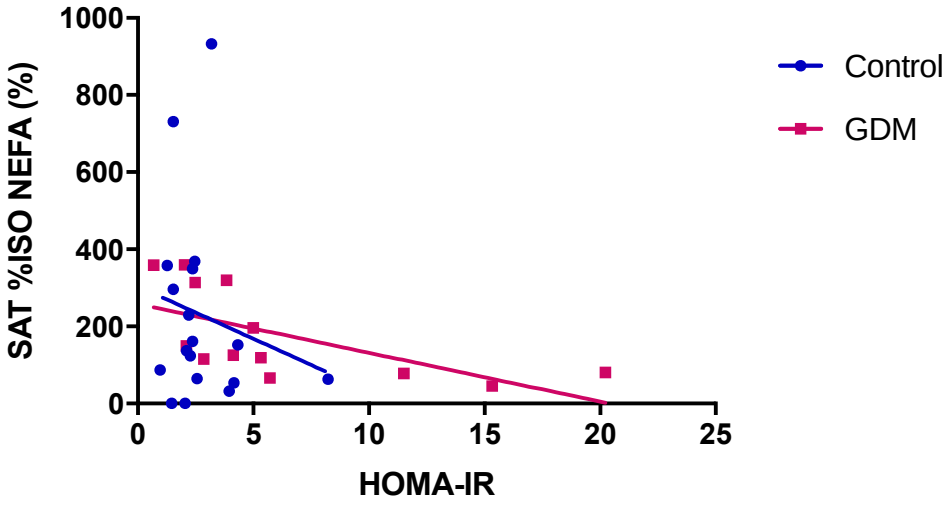


Figure 4-29 The relationship between maternal HOMA-IR and SAT percentage stimulation of lipolysis by isoprotenerol expressed as NEFA release in GDM and control group
Maternal HOMA-IR correlated negatively with SAT percentage stimulation of lipolysis by isoprotenerol in the GDM group only (r= -0.79, p=0.001). Correlation in the control group (r=-0.11, p=0.65) with no suggestion of different relationship of HOMA-IR to percentage stimulation of net lipolysis by isoprotenerol in SAT depot in women with GDM vs controls (interaction term GDM*HOMA-IR: p=0.64)

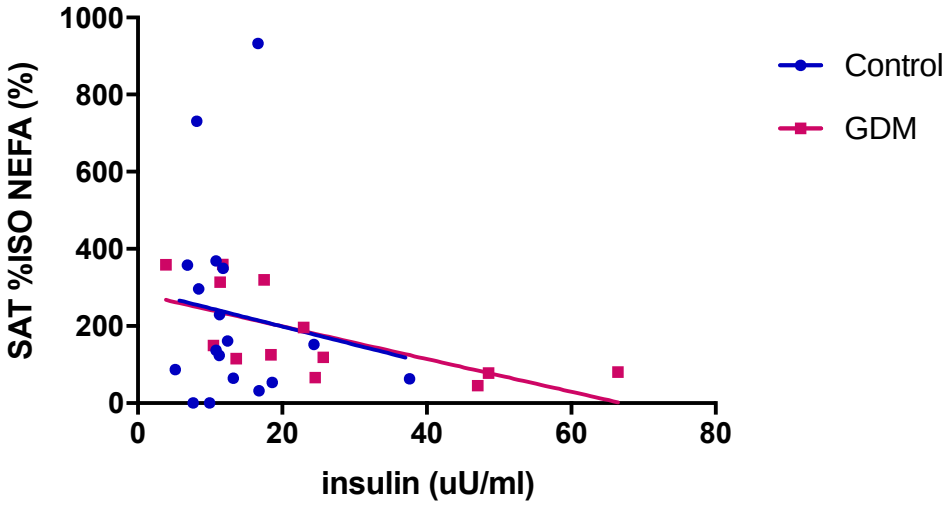


Figure 4-30 The relationship between maternal insulin level and SAT percentage stimulation of lipolysis by isoprotenerol expressed as NEFA release in GDM and control group
Maternal insulin level correlated negatively with SAT percentage stimulation of lipolysis by isoprotenerol in the GDM group only (r= -0.77, p=0.002). Correlation in the control group (r=-0.15, p=0.55) with no suggestion of different relationship of insulin level to percentage stimulation of net lipolysis by isoprotenerol in SAT depot in women with GDM vs controls (interaction term GDM*insulin: p=0.95)

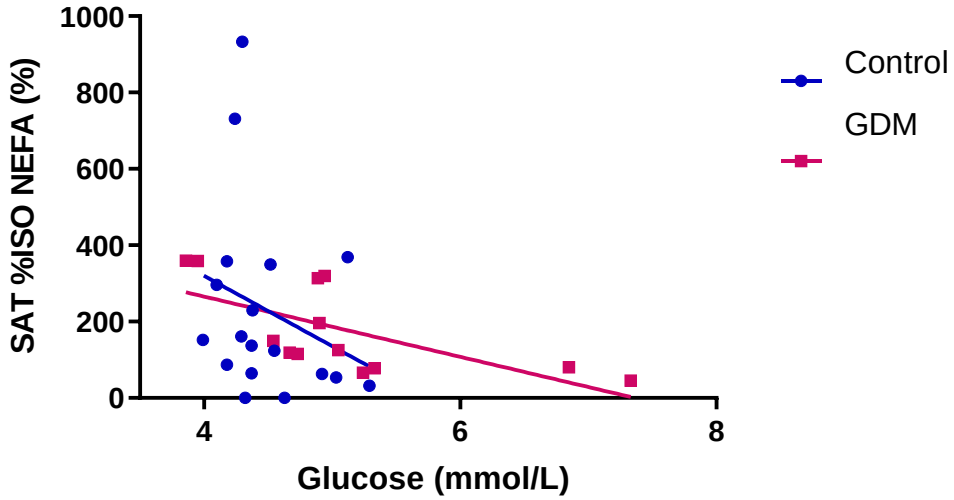


Figure 4-31 The relationship between maternal glucose level and SAT percentage stimulation of lipolysis by isoprotenerol expressed as NEFA release in GDM and control group
Maternal glucose level correlated negatively with SAT percentage stimulation of lipolysis by isoprotenerol in the GDM group only (r= -0.76, p=0.002). Correlation in the control group (r=-0.39, p=0.11) with no suggestion of different relationship of glucose level to percentage stimulation of net lipolysis by isoprotenerol in SAT depot in women with GDM vs controls (interaction term GDM*glucose: p=0.47).

4.4.9.3 Adipocyte lipolytic function and maternal plasma lipids (plasma glycerol, NEFA and TAG)

Interestingly, maternal plasma glycerol level was consistently positively correlated with SAT lipolytic function including total basal lipolysis (Figure 4-32), total lipolysis rate in presence of isoprotenerol (Figure 4-33) and total lipolysis rate in presence of both isoprotenerol and insulin (Figure 4-34) in the control group only. This association was lacking for SAT in GDM group. However, maternal plasma glycerol level was not correlated with SAT net lipolysis in both groups. Maternal plasma glycerol level was not correlated with VAT total lipolysis. Maternal NEFA and TAG levels was not associated with SAT or VAT adipocyte lipolytic function in both groups.

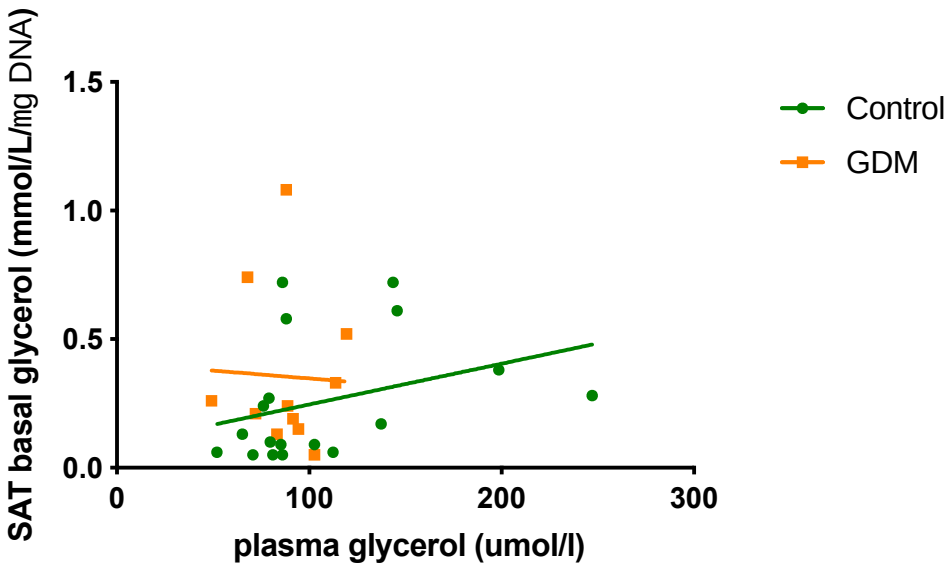


Figure 4-32 The relationship between maternal plasma glycerol level and SAT basal release of glycerol in GDM and control group
Maternal plasma glycerol level correlated positively with SAT basal glycerol release in the control group only (r=0.52, p=0.028). The correlation in the GDM group (r=-0.21, p=0.49)

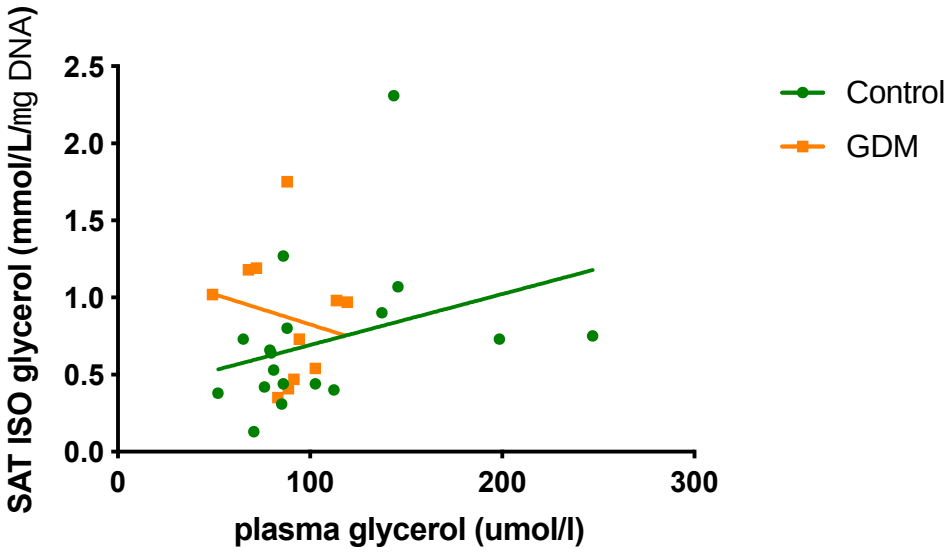


Figure 4-33 The relationship between maternal plasma glycerol level and SAT glycerol release in presence of isoproterenol in GDM and control group
Maternal plasma glycerol level correlated positively with SAT glycerol release in presence of isoproterenol in the control group only (r=0.58, p=0.011). The correlation in the GDM group (r=-0.15, p=0.62)

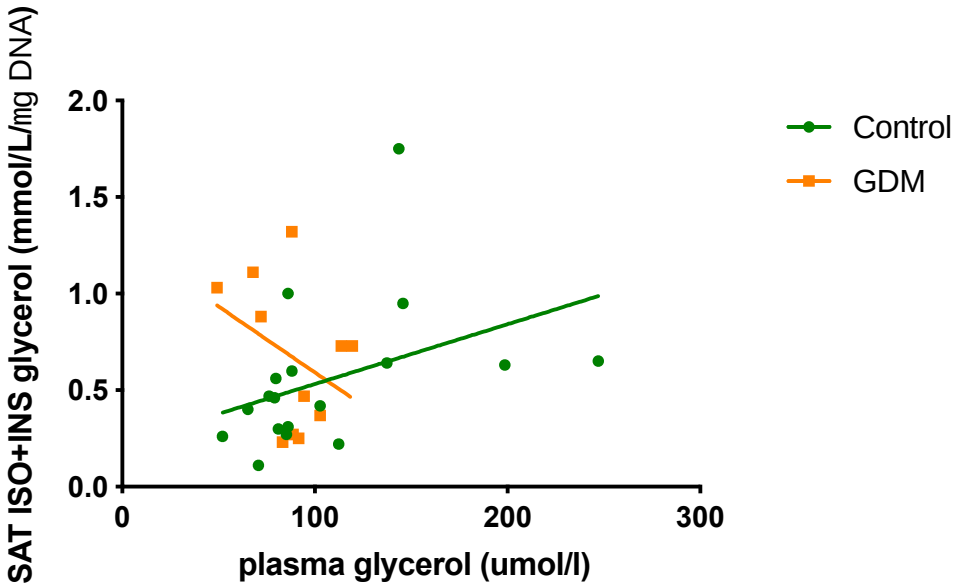


Figure 4-34 The relationship between maternal plasma glycerol level and SAT glycerol release in presence of isoproterenol and insulin in GDM and control group
Maternal plasma glycerol level correlated positively with SAT glycerol release in presence of isoproterenol and insulin in the control group only (r=0.62, p=0.006). The correlation in the GDM group (r=-0.21, p=0.48)

4.4.9.4 Adipocyte lipolytic function and maternal liver function enzymes (ALT, AST and GGT) and hepatic steatosis index

Liver enzymes including AST, ALT and GGT was not associated with SAT and VAT adipocytes lipolytic function in both groups. However, HSI showed a consistent very strong positive correlation with SAT adipocytes lipolytic function in GDM group including basal lipolysis (Figure 4-35) (Figure 4-36), lipolysis rate in presence of isoprotenerol (Figure 4-37) (Figure 4-38), lipolysis rate in presence of insulin (Figure 4-39) (Figure 4-40) and lipolysis rate in presence of isoprotenerol and insulin (Figure 4-41) (Figure 4-42) total and net lipolysis release. The percentage of stimulation of lipolysis by isoprotenerol and the percentage suppression of lipolysis by insulin of SAT and VAT adipocytes was not associated with HSI in both groups. VAT adipocytes lipolytic function was not associated with HSI in both groups.

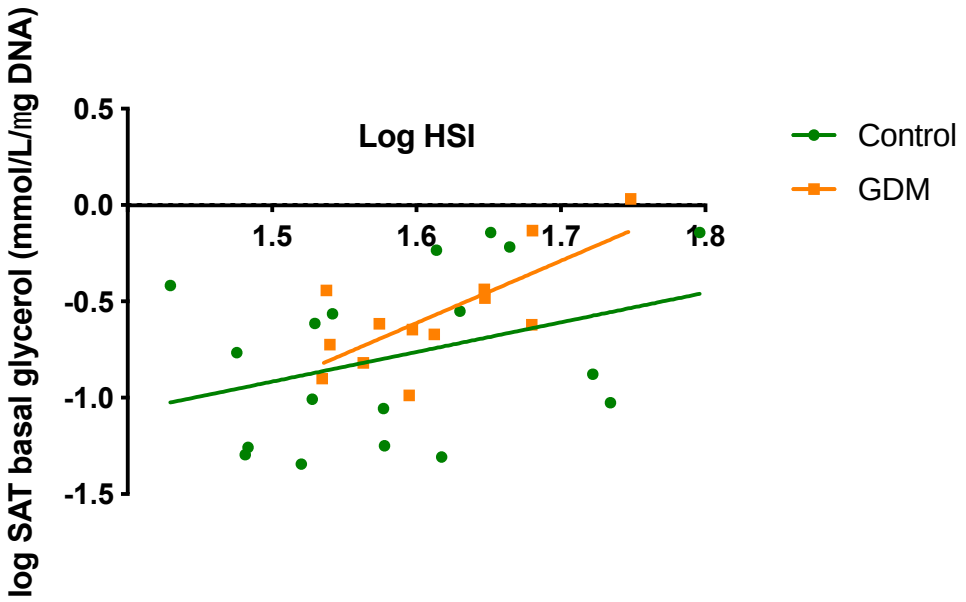


Figure 4-35 The relationship between maternal HSI and SAT adipocytes total basal lipolysis in GDM and control group
Maternal HSI correlated positively with SAT adipocytes total basal lipolysis in GDM group only (r= 0.73, p=0.004). The correlation in the control group (r=0.36, p=0.15)

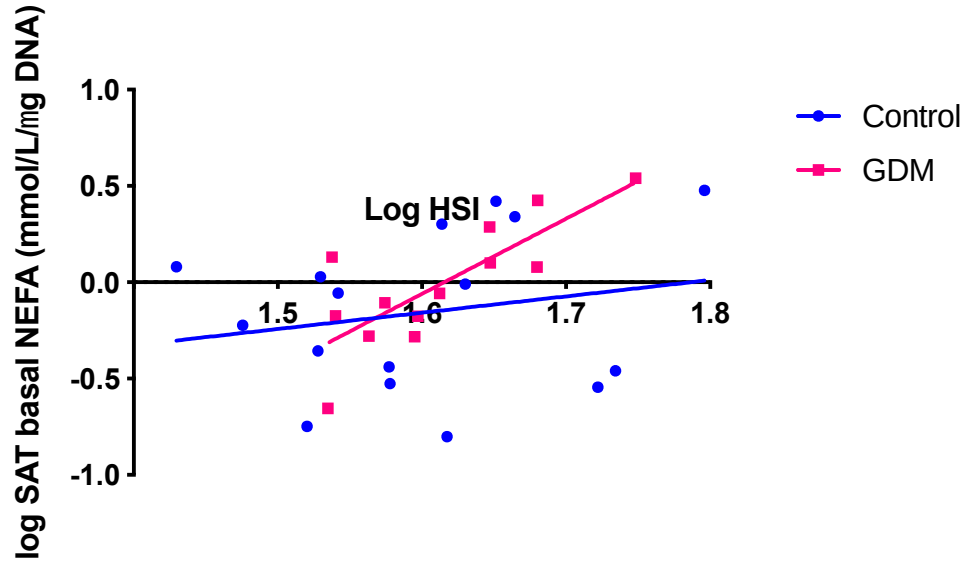


Figure 4-36 The relationship between maternal HSI and SAT adipocytes net basal lipolysis in GDM and control group
Maternal HSI correlated positively with SAT adipocytes net basal lipolysis in GDM group only (r= 0.79, p=0.001). The correlation in the control group (r=0.20, p=0.45)

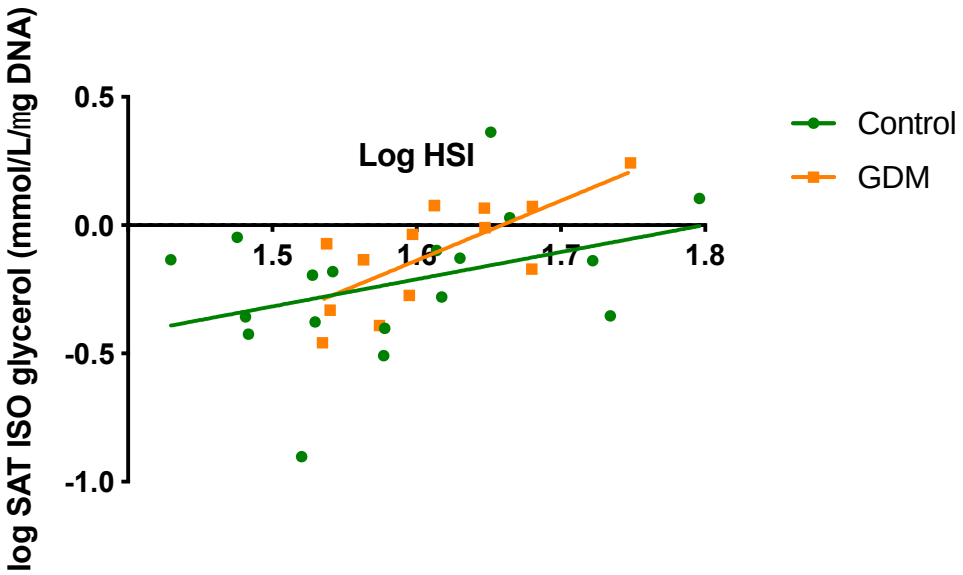


Figure 4-37 The relationship between maternal HSI and SAT adipocytes total lipolysis in presence of 200nM isoproterenol in GDM and control group
Maternal HSI correlated positively with SAT adipocytes total lipolysis rate in presence of 200nM isoprotenerol expressed as glycerol release in GDM group only (r= 0.73, p=0.004). The correlation in the control group (r=0.39, p=0.11)

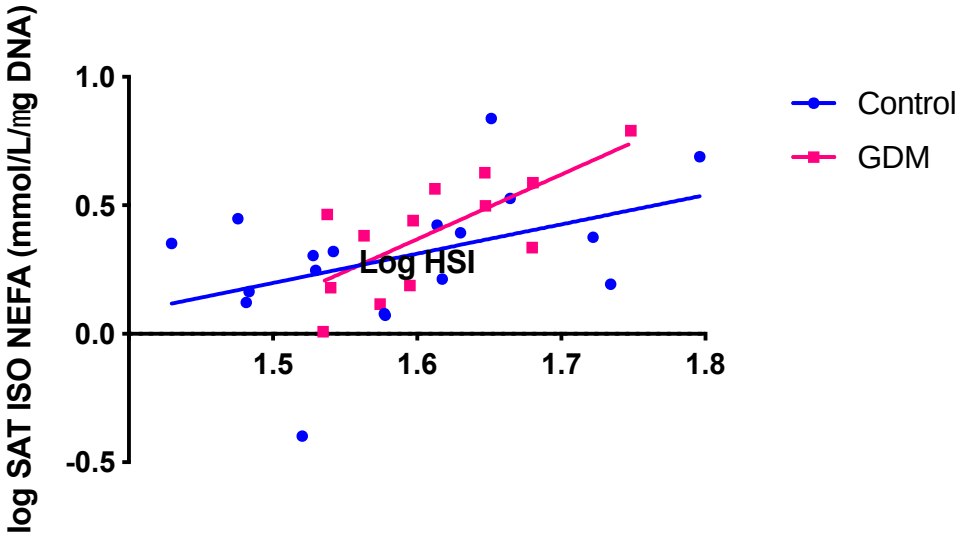


Figure 4-38 The relationship between maternal HSI and SAT adipocytes net lipolysis in presence of 200nM isoproterenol in GDM and control group
Maternal HSI correlated positively with SAT adipocytes net lipolysis rate in presence of 200nM isoprotenerol expressed as NEFA release in GDM group only (r= 0.73, p=0.005). The correlation in the control group (r=0.43, p=0.08).

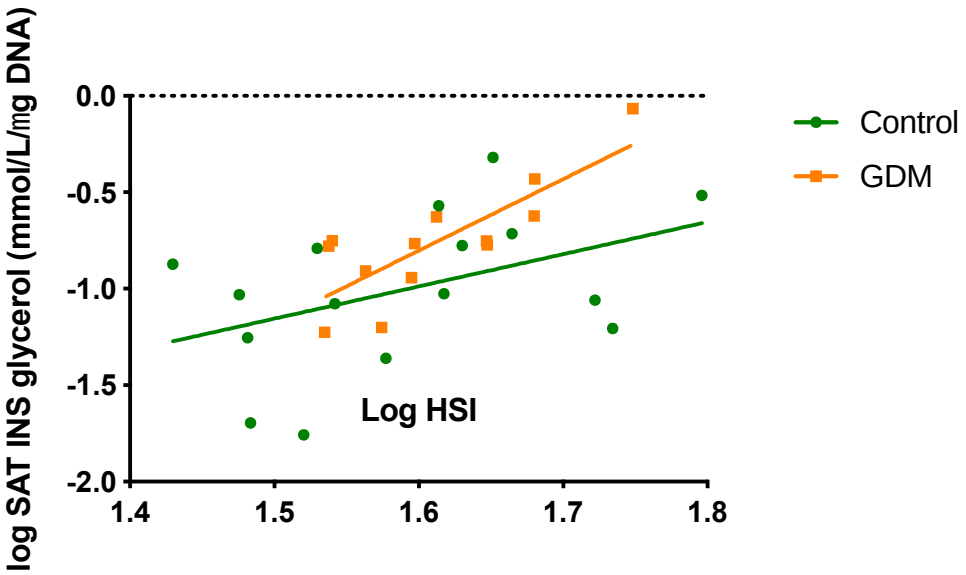


Figure 4-39 The relationship between maternal HSI and SAT adipocytes total lipolysis in presence of 10nM insulin in GDM and control group
Maternal HSI correlated positively with SAT adipocytes total lipolysis rate in presence of 10nM insulin expressed as glycerol release in GDM group only (r= 0.80, p=0.001). The correlation in the control group (r=0.44, p=0.09)

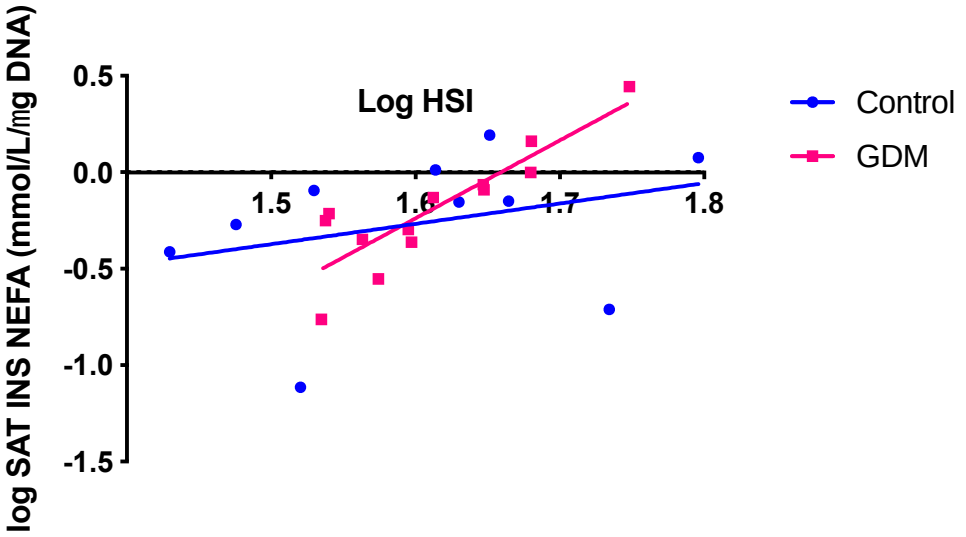


Figure 4-40 The relationship between maternal HSI and SAT adipocytes net lipolysis in presence of insulin in GDM and control group
Maternal HSI correlated positively with SAT adipocytes net lipolysis rate in presence of 10nM insulin expressed as NEFA release in GDM group only (r= 0.87, p=0.0001). The correlation in the control group (r=0.31, p=0.39)

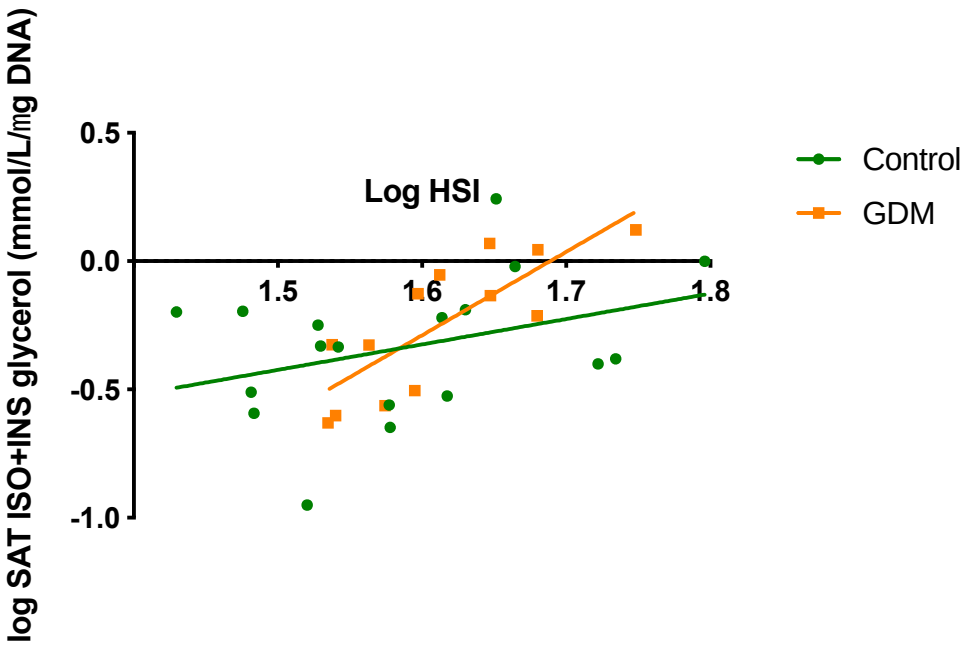


Figure 4-41 The relationship between maternal HSI and SAT adipocytes total lipolysis in presence of 200nM isoproterenol and 10nM insulin in GDM and control group
Maternal HSI correlated positively with SAT adipocytes total lipolysis rate in presence of 200nM isoprotenerol and 10nM insulin expressed as glycerol release in GDM group only (r= 0.81, p=0.001). The correlation in the control group (r=0.36, p=0.14)

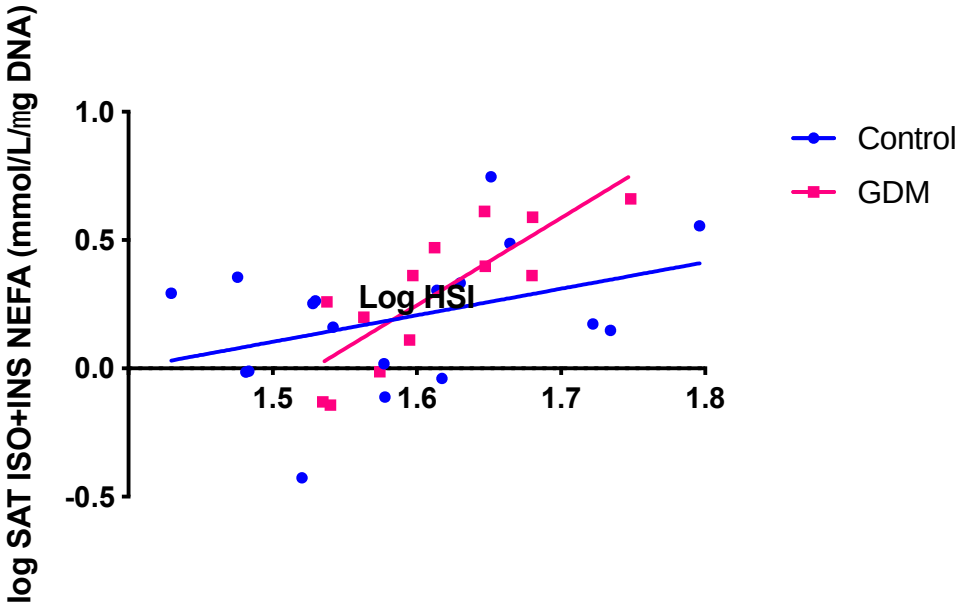


Figure 4-42 The relationship between maternal HSI and SAT adipocytes net lipolysis in presence of 200nM isoproterenol and 10nM insulin in GDM and control group
Maternal HSI correlated positively with SAT adipocytes net lipolysis rate in presence of 200nM isoprotenerol and 10nM insulin expressed as NEFA release in GDM group only (r= 0.82, p=0.001). The correlation in the control group (r=0.38, p=0.12)

4.4.9.5 Adipocyte lipolytic function and pregnancy hormones (estradiol and progesterone)

There was no correlation between progesterone level and SAT lipolytic function in both groups. In GDM group, higher progesterone level was associated with lower net lipolysis FCISI (progesterone: r=-0.68, p=0.005: Figure 4-43) in VAT depot. There was no suggestion of a different relationship of progesterone level to net lipolysis FCISI in VAT in women with GDM compared to controls (GDM*VAT net lipolysis FCISI: p=0.17). Maternal estradiol level was not associated with adipocytes lipolytic function in SAT and VAT in both groups.

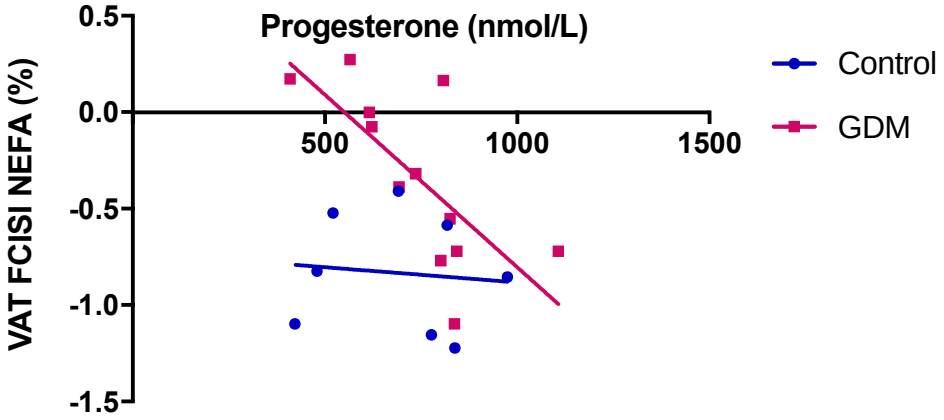


Figure 4-43 The relationship between maternal progesterone level and VAT adipocytes net lipolysis FCISI expressed as NEFA release in GDM and control group
Maternal plasma progesterone level correlated negatively with VAT adipocytes net lipolysis FCISI in the GDM group only (r= -0.68, p=0.005). Correlation in the control group (r=0.03, p=0.92) with no suggestion of different relationship of progesterone level to net lipolysis FCISI in VAT depot in women with GDM vs controls (interaction term GDM*net lipolysis FCISI in VAT: p=0.17)

4.5 Discussion

To the best of our knowledge, this is the first study reporting detailed data on adipocyte lipolytic function in pregnancies complicated with GDM and exploring the relationship between depot-specific adipose tissue phenotype (i.e. adipocyte size and lipolysis) and whole-body insulin resistance (HOMA-IR), plasma lipids (TAG, NEFA and glycerol) and fatty liver (HSI). A key finding of this study was that in GDM, there was a significant increase in VAT adipocyte diameter compared to BMI-matched healthy controls. This was a surprising finding, as it suggests there is significant hypertrophy of VAT adipocytes in response to the metabolic challenge of GDM rather than SAT adipocytes hypertrophy as was hypothesised. Contrary to our

expectation, VAT adipocytes (rather than SAT) had higher total and net basal lipolysis rate in women with GDM compared to the control group. However, FCISI was not different in SAT and VAT adipocytes between the GDM and the control group.

Although a 6 µm increase in SAT adipocyte diameter demonstrated a trend towards a larger SAT adipocyte in the GDM group compared to the healthy controls, this difference was not significant. Under the proposed hypothesis, women with GDM were expected to have larger SAT adipocyte diameter, resulting from hypertrophic expansion of SAT adipocyte in the face of the metabolic challenge of GDM to store excess lipid, consequently leading to adipocyte dysfunction and spill over of fatty acids in VAT adipocytes and maybe storage ectopically in other organs resulting in systemic insulin resistance and lipotoxicity. The observed increase in VAT adipocyte diameter in GDM and the notable shift to the right of VAT adipocyte volume distribution suggest that excess lipid is indeed stored in the VAT depot in GDM. Possibly we didn't observe SAT adipocytes hypertrophy because SAT adipocytes were already in a state of hypertrophy caused by obesity in our BMI matched control group. Therefore, another control group of pregnant women with normal BMI could be more informative in drawing conclusion regarding SAT adipocytes size in GDM. The observed VAT adipocyte hypertrophy could be a consequence of the limited ability of SAT to recruit new adipocytes or to further becoming hypertrophic. Rojas-Rodriguez et al. (2015) observed similar VAT adipocyte hypertrophy in a smaller sample of women (n=5) with GDM compared to healthy pregnant women. The previous results suggest that VAT depots are dysfunctional in women with GDM and ectopic fat storage might be the consequence.

Interestingly, in our cohort, maternal BMI was closely related to SAT and VAT adipocyte diameter in the control group. The loss of this relationship with SAT adipocyte in the GDM group suggests that SAT adipocytes were not able to increase in diameter with increasing BMI in the GDM group probably because they are already large at each given BMI. Additionally, in our cohort, only VAT adipocyte diameter was consistently strongly positively associated with maternal insulin level and HOMA-IR in the control and GDM group with significant interactive term of 0.12 as evidence for a different relationship between control and GDM group. The present findings indicate that probably adipocyte hypertrophy in VAT during pregnancy in overweight and obese women is associated with whole-body insulin

resistance. however, the direction of the association cannot be inferred. A similar association between VAT hypertrophy and adverse metabolic health was observed previously in obese individuals (O'Connell et al., 2010). Moreover, VAT cell size increases the total amount of the VAT depot. Although we haven't measured the total size of VAT depot, it was previously shown that VAT thickness measured by ultrasound is associated with insulin resistance in early pregnancy (De Souza et al., 2014) and impaired glucose homeostasis in mid-pregnancy (De Souza et al., 2016) (D'Ambrosi et al., 2018) independent of BMI and other risk factors for GDM.

This study sheds light on the possible aetiological role of defective VAT adipocyte expansion in the development of GDM. In this study, several lipolysis measures were examined (basal, catecholamine stimulation and insulin suppression of lipolysis). Although all measures of lipolysis and SAT FCISI were not different in SAT adipocytes in the GDM compared to the control group, both groups had one fold lower SAT FCISI net lipolysis when our results were compared with the lean control group of a previous similar study conducted by our group on women with PE (Huda et al., 2014). The possible explanation for not observing a difference in SAT lipolysis between this study groups might be explained by the similar degree of SAT adipocytes insulin resistance in both groups due to BMI matching. This is suggesting there could be no GDM effect above the obesity effect in SAT adipocytes insulin resistance and hypertrophy. Similar to this finding but in non-pregnant T2DM, Verboven et al. (2018) observed a similar degree of SAT adipocytes hypertrophy in obese men with and without diabetes but this was significantly larger than the mean SAT adipocyte diameter of the lean control group in their study. VAT adipocytes in GDM were 6 times more insulin resistant compared to control group but failed to reach statistical significance probably due to low numbers. This finding was different from what our group found previously in PE, in PE there was significant insulin resistance in SAT adipocytes compared to healthy women (Huda et al., 2014). PE is a metabolic pregnancy complication that shares accelerated insulin resistance and poor lipid metabolism handling with GDM. Although PE and GDM are the most common metabolic pregnancy complications, it is likely that are both share an underlying SAT adipocytes dysfunction but may be at different steps in the subsequent pathway. PE is a pregnancy complication with more systemic involvement than GDM and SAT adipocytes insulin suppression of lipolysis was shown to be defective (Huda et al., 2014). However, GDM is a metabolic complication of a milder form than PE with hepatic insulin resistance affecting

glucose tolerance. Therefore, the observed VAT adipocyte dysfunction in women with GDM in our study could be the missing link between SAT adipocytes and the underlying pathophysiology.

The observed higher basal lipolysis rate in VAT adipocytes in the GDM compared to the control group suggests that dysregulation of VAT adipocyte basal lipolysis may be an important secondary event to SAT adipocyte hypertrophy contributing to the emergence of metabolic dysfunction in women with GDM. Regarding the association between lipolytic function and plasma lipid level, only maternal glycerol level was associated with SAT basal lipolysis, lipolysis rate in presence of isoprotenerol and lipolysis rate in presence of both isoprotenerol and insulin in the control group suggesting that SAT adipocytes glycerol release is an important determinant of plasma glycerol level in pregnancy. The maternal plasma NEFA and glycerol level was not elevated in the GDM group and did not correlate with HOMA-IR suggesting that it could be there is a compensatory increase in FA clearance from plasma as protective mechanism to reduce the deleterious effects of circulating NEFA.

The adipocyte size and lipolysis results when interpreted together suggest that in GDM, in order to store excess lipids which were unable to be stored in SAT adipocytes probably due to defective expansion, VAT adipocytes are responding by hypertrophy resulting in higher basal lipolysis rates compared to the control group. VAT is considered as an alternative depot for fat storage to minimize the deleterious effect of circulating NEFA. However, the data does not distinguish whether adipocyte dysfunction precedes the insulin resistance in GDM or the opposite. A major determinant of metabolic health is SAT's ability to expand and store excess fat safely rather than its accumulation in ectopic sites. Ectopic fat accumulation in insulin sensitive organs lead to insulin resistance and T2DM (Ravussin and Smith, 2002). Thus, it is important to recognize that SAT has a "fat buffering" property, which is key to preserving normal level of insulin sensitivity and appropriate metabolic responses.

Data from the current study suggest that expansion of VAT, probably due to underlying SAT defective expansion, might lead to consequent ectopic fat deposition in other organs (Figure 4-44). Hepatic fat measured by HSI and its relationship with adipocyte size during normal or complicated pregnancy was not

assessed before in women with GDM. Although HSI was not different between the groups, both groups demonstrated high HSI score which could be driven by their BMI as we have shown the strong relationship between HSI and BMI. In our cohort, HSI is closely related to maternal adiposity and measures of insulin sensitivity in normal pregnancy and GDM. Furthermore, SAT and VAT adipocyte diameter was closely positively associated with HSI in the control group highlighting the importance of adipocyte size as a determinant of metabolic health possibly by affecting subsequent lipid deposition in the liver during obese pregnancy following adipocytes defective expansion. The strong consistent relationship between HSI and SAT lipolytic function in GDM supports the importance of lipid release from SAT adipocytes in determining the lipid deposition in the liver and this could be the underlying pathophysiology for the previously observed liver insulin resistance in GDM pregnancy in several studies (Retnakaran et al., 2011) (Tiikkainen et al., 2002). A study by Armstrong et al. (2014) represents the first *in vivo* evidence of abdominal SAT dysfunction in the inflammatory form of NAFLD, non-alcoholic steatohepatitis (NASH). It was previously reported that the majority of lipid accumulation in NASH was attributed to adipose tissue derived NEFA (59%) rather than *de novo* lipogenesis in the liver (26%) (Donnelly et al., 2005). Subcutaneous adipose tissue is 10 fold larger than VAT, therefore, it was suggested that there is an important SAT mass effect and its lipolytic action compared to VAT. Serum NEFA was shown to be strongly related to SAT basal lipolysis in a large cohort of 1066 subjects (Rydén and Arner, 2017). The previous findings suggest that SAT adipocytes may not have a direct impact on metabolic dysfunction, but they still may play a key role as the initiating factor in the process of the hypothesized fat overflow to ectopic sites.

VAT adipocyte hypertrophy would be expected to lead to detrimental metabolic effects such as fatty liver, possibly due to the drainage of VAT into the portal circulation. VAT adipocytes hypertrophy and disturbed adipokine secretion which could be transported to the liver through portal circulation may be the link between NAFLD and metabolic diseases such as GDM. It was shown in a large population study (n=1115) that GDM is strongly associated with non-alcoholic fatty liver (NAFLD) disease, and GDM diagnosis could represent an opportunity to prevent the development of NAFLD later in life (Ajmera et al., 2016). It was shown that fatty liver precedes T2DM in non-pregnant population (Lim et al., 2011). However, similar to other parameters of metabolic health, not all women with history of GDM develop

NAFLD or T2DM. Other potential sites of ectopic fat deposition such as pancreas, heart and placenta are beyond the scope of this study and require further investigation. However, this pathway could be anticipated by the observed maternal hypertriglyceridemia in the GDM group in our cohort.

A limitation of the present study was the cross-sectional design as it will not provide information about adipose tissue changes during pregnancy. However, a longitudinal design was difficult to perform in pregnant women, especially for VAT samples because a repeated intra-abdominal biopsy would be needed to get access to adipose tissue, a procedure unlikely to be tolerated by pregnant women. The collagenase digestion method was used to obtain mature adipocytes for this study, however, it was reported previously that collagenase could lead to small cells disruption (Taylor, 1987). Therefore, the same batch of collagenase was used for this experiment. Isolated adipocyte lipolytic function may not exactly represent *in vivo* adipose tissue lipolysis because it excludes the extracellular matrix and SVF effect. However, *in vivo* measurement of adipose tissue lipolysis involves cumbersome methods such as tracer catheterization which require continuous infusion of glucose and glycerol and frequent venous blood sampling which could challenging to consent for large sample of pregnant women. Furthermore, this technique assesses whole body adipose tissue lipolysis and is not depot specific. Another *in vivo* lipolysis assay is microdialysis, which requires probe insertion in the abdomen for minimum of one-centimeter depth and frequent sampling is required which can be time consuming and uncomfortable for pregnant women. Furthermore, microdialysis allows investigation of SAT only and it is difficult to evaluate VAT lipolysis using this technique (Frayn et al., 1997). However, several studies have found similar results when SAT lipolysis was compared in vivo (Kolehmainen et al., 2000) and in vitro (Agustsson et al., 2007). This study involved a small cohort of women and there were not enough numbers to look at the effect of GDM treatment and glycemic control on adipose tissue lipolysis. The original study was powered for 30 participants per group but I only managed to collect 22 participants per group, which could affect achieving significance level in some of the parameters such as FCISI. HSI was used to assess for fatty liver however, liver biopsy is the most sensitive measure to assess for fatty liver, but it can be more challenging to perform. In this study, it is not possible to exclude the GDM treatment effect on adipose tissue function but it was not possible to look at this effect due to relatively small sample

of women in each treatment group whether diet only, metformin, insulin or both metformin and insulin.

The strength of this study is the collection of both SAT and VAT samples at Caesarean section, while many studies assessing the role of adipose tissue in metabolic disease are limited to the study of SAT biopsies obtained by needle or by the practicalities and ethics of access to VAT samples. Moreover, access to fat samples during intra-abdominal procedures provided us with samples consistently biopsied unlike needle biopsy which can provide either superficial or deep SAT samples. By design, no differences in age, BMI and most other characteristics between the GDM and control groups were observed allowing findings to be interpreted independently of BMI and other confounders. The GDM group confirms the described metabolic phenotype for pregnancies complicated with GDM. They had higher fasting OGTT results with exaggerated hyperlipidemia of pregnancy consisting of higher plasma TAG compared to controls. Furthermore, GDM mothers were insulin resistant with higher maternal HOMA-IR. As expected, women with GDM tend to undergo Caesarean section a couple of days earlier than controls while there was no difference in the birthweight between the groups. Adipocyte sizing was carried out by two observers and both were blinded to participant grouping.

In summary, the results of this chapter support the proposed hypothesis of impaired adipose tissue expansion as a component of the underlying pathophysiology of GDM (Figure 4-44). An additional adipocyte function component was assessed by the study of lipolytic function with observed trend for VAT adipocyte insulin resistance in women with GDM. Elevated VAT adipocyte basal lipolysis could be an important feature of pregnancies complicated with GDM. VAT adipocytes are hypertrophic in women with GDM compared to the healthy BMI matched controls as shown in previous study (Rojas-Rodriguez et al., 2015). Although this finding is contrary to the proposed hypothesis of SAT adipocytes dysfunction rather than VAT adipocytes, but FA accumulation in VAT adipocytes could be a consequence of SAT adipocytes dysfunction we did not observe due to BMI matching of the study groups. Adding pregnant women with normal weight control group, could enhance our understanding of obesity effect on adipocytes and further metabolic disturbance in the GDM group. SAT adipocyte lipolytic function was significantly associated with the degree of fat in the liver in women with GDM but had no association with other metabolic parameters. The immediate consequence of SAT defective storage is

outflow of FA to expand the VAT depot or it gets stored in non-adipose tissue organs such as the liver. Therefore, longitudinal studies to look at adipose tissue function throughout pregnancy are needed, combined with liver ultrasound assessment for fatty liver and liver and placenta biopsy at term to assess the evidence for ectopic fat deposition in GDM. Studies of a larger cohort of women with GDM would make it possible to look at adipose tissue response to GDM treatment. Although placentas from GDM pregnancies have been shown to have altered fatty acid composition and lipid transporter expression (Segura et al., 2017), an investigation to quantify placental lipid storage in GDM compared to controls would be valuable to provide evidence for the remaining component of the hypothesised pathway; ectopic fat accumulation and any pathological effects it may have on the placenta of women with GDM.

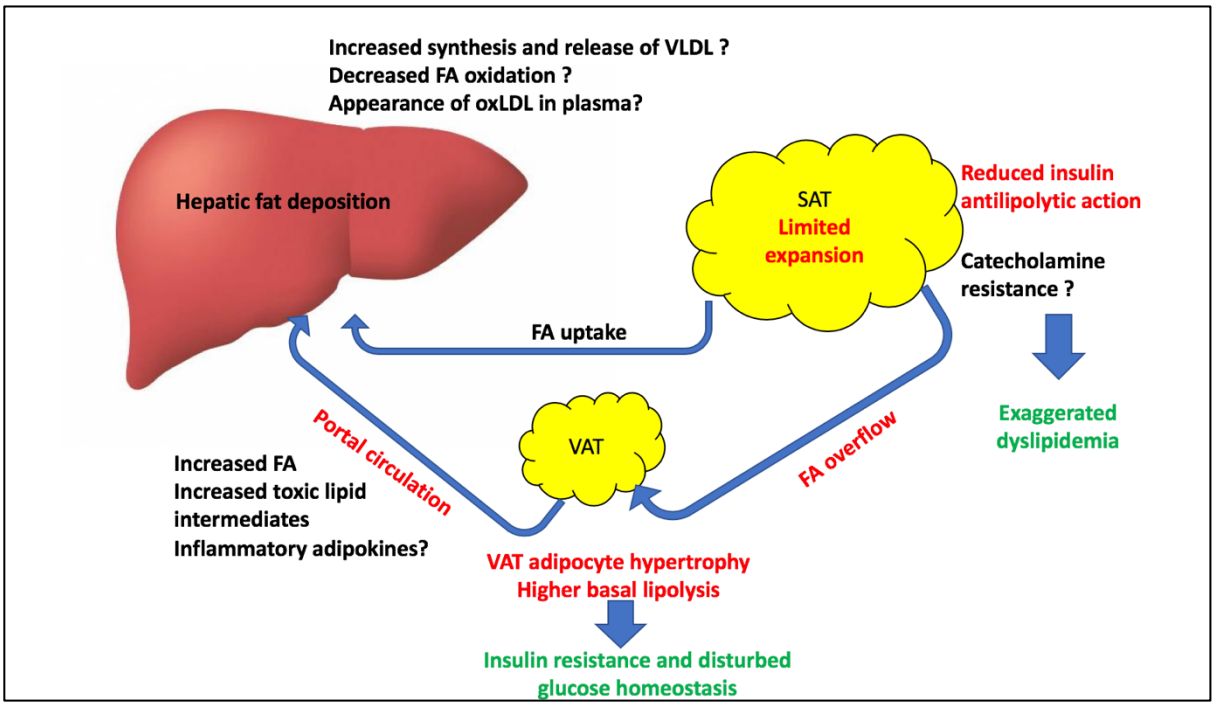


Figure 4-44 Regional differences in adipose tissue expansion in GDM
SAT limited expandability leads to overflow of FA in VAT and other organs such as liver. This possibly could lead to rise in plasma lipid level in women with GDM. Adipocytes in VAT depot responded by adipocyte hypertrophy and higher basal lipolysis. Increased FA flow in portal circulation could lead to fatty liver and increased production of VLDL. Reduced clearance of VLDL particles plasma can lead to production of oxLDL.

Chapter 5 Adipose tissue inflammation in gestational diabetes mellitus

5.1 Introduction

Disturbed adipocyte function in the form of adipocyte hypertrophy and enhanced basal lipolysis in VAT adipocytes in women with GDM compared to healthy controls was observed in the previous chapter. I hypothesised that dysregulated adipokine secretion from hypertrophic VAT adipocytes would occur and aimed to examine it in this chapter. There is emerging evidence suggesting a role for plasma adipokines in the development of insulin resistance and the subclinical inflammation observed in women with GDM as discussed in the Introduction Chapter section 1.7. Plasma levels of several adipokines such as TNF-α and IL-6 were found to be elevated in women with GDM (Christian and Porter, 2014). Adipose tissue has been suggested as a source of higher plasma inflammatory cytokine levels in women with GDM. While most of the published literature has been about the dysregulated inflammatory cytokines markers, direct measurement of adipocytes cytokine secretion was not studied before in GDM. Moreover, the underlying mechanism for the increased inflammatory cytokine release in women with GDM is not completely understood but infiltration of immune cells such as macrophages infiltration in adipose tissue was one of the suggested mechanisms.

It was previously identified that GDM is associated with increased macrophage infiltration in omental fat (VAT) rather than SAT compared to healthy control pregnancy (Harlev et al., 2014). In the same study, omental fat macrophages correlated positively with HOMA-IR suggesting that low grade inflammation contributes to the development of insulin resistance in GDM. A similar observation in non-pregnant centrally obese individuals suggests that, in GDM, there is a similar inflammatory stress in the VAT depot. During pregnancy, macrophage phenotype shifts from resident M2 anti-inflammatory phenotype to M1 pro-inflammatory phenotype which secretes several pro-inflammatory cytokines as discussed in the general introduction through activation of the TLR4/NF-κB pathway (Shi et al., 2006). In adipose tissue, hypertrophic adipocytes release FA, which in turn stimulate the TLR4/NF-κβ pathway in the macrophages, thus, participating in a deleterious paracrine loop between adipocytes and macrophages (Suganami et al., 2009).

Proinflammatory stimuli such as LPS and the proinflammatory cytokines such as IL-6 and TNFα cause phosphorylation of inhibitor NF-κB (IκB) and subsequent translocation of the nuclear transcription factor NF-κB to the nucleus. Intra-nuclear NFKB induces transcription of proinflammatory cytokines such as TNF-α, IL-6, IL-1β, adhesion molecules such as ICAM-1 and VCAM-1, chemokines such as MCP-1. However, the adipocyte's contribution to the previously observed higher plasma levels of adipokines (independent of the paracrine effect of SVF and macrophages) has not been studied previously in GDM. In women with PE, where isolated SAT and VAT adipocyte secretory function was studied in basal and in stimulated conditions (in response to 10μM LPS) there was higher VAT adipocyte release of TNF-α and IL-6 in response to LPS stimulation (Huda et al., 2017). In the same cohort, a negative correlation was noted between TNF-α secretion from VAT adipocytes in response to LPS and VAT adipocyte insulin sensitivity suggesting a role of adipocyte inflammation, lipolysis and insulin resistance in the development of PE (Huda et al., 2017). TNF-α is known to be an important mediator of insulin resistance during pregnancy and in women with GDM (Long et al., 2009). Furthermore, TNF-α has a potent lipolytic activity possibly through down-regulation of perilipin expression (Souza et al., 2003). Therefore, in addition to the previously observed higher VAT basal lipolysis in GDM (chapter 4), it is plausible that there could be upregulation of adipokine secretion in VAT adipocytes associated with the development of GDM, a condition with accelerated maternal insulin resistance and higher plasma level of inflammatory markers.

Adipocyte size is an important determinant of the expression and secretion of adipokines from isolated adipocytes (Skurk et al., 2007). In particular, Skurk et al. (2007) have suggested that the secretion of pro-inflammatory adipokines such as leptin, IL-6, IL-8, TNF-α, MCP-1, IL-1ra, and adiponectin was significantly elevated in larger adipocytes compared to small adipocytes and was positively correlated with cell size. In addition, there was a decrease of anti-inflammatory IL-10 secretion with increasing cell size. Therefore, the observed VAT adipocytes hypertrophy in women with GDM could be hypothesised to regulate the expression level of these adipokines in isolated VAT adipocytes.

In adipocytes, exogenous signals such as fatty acids or LPS induce an inflammatory response through activation of TLR4 and the associated intracellular pathway NF-κB (Song et al., 2006). Therefore, in the current study LPS was used to mimic

inflammation in isolated adipocytes as previously used in Huda et al. (2017). I measured the secretion by adipocytes of several proinflammatory cytokines known to be induced in response to NF-κB activation such as TNFα, IL-6, IL-1β, MCP-1 and IFN-gamma. Other anti-inflammatory cytokines such as IL-8 and IL-10 and growth factors such as VEGF and PIGF were assessed. Adipokine secretion from adipocytes treated with or without LPS was measured using the multiplex array technology which allowed detection of numerous analytes in small volume of sample, while possible analytes to study can be limited by sample volume when using traditional ELISA. Limited by the cost of multiplex technology, adipokine secretion was studied in VAT adipocytes only due to the previously observed adipocyte dysfunction in the previous chapter, otherwise SAT adipokine secretory profile will be of great interest. Furthermore, our group's previous study in GDM showed that the inflammatory dysfunction was found primarily in VAT adipocytes (Huda et al., 2014). However, due to the late instigation of LPS stimulated experiment conditions throughout our fat biopsy collection, a smaller subset from the original cohort used in the lipolysis study (GDM n=22 and control n=22) was available for the analysis of adipokine secretion experiment (GDM n=15 and control n=12) as detailed in the methods section below.

In the non-pregnant population different adipose tissue depots are known to differentially regulate the expression of inflammatory markers, adipokines, and several other genes involved in adipocyte differentiation, lipid storage and insulin signalling. There are some data available in GDM. The mRNA expression of the anti-inflammatory insulin sensitizing agent, adiponectin is reduced in whole adipose tissue from both SAT and VAT in pregnancies complicated with GDM (Lappas et al., 2005). In GDM VAT samples, there was increased expression of leptin and the proinflammatory cytokines IL-6 and IL-8 (Kleiblova et al., 2010). However, the contribution of isolated adipocytes rather than whole adipose tissue to the observed disturbed adipokine release and subsequent insulin resistance in women with GDM has not been studied previously to the best of our knowledge. Whole adipose tissue sample inflammatory mediator production is confounded by the presence of immune cells, particularly macrophages in the SVF.

Adipose tissue expands in the face of a requirement to store additional energy by increasing lipid storage in existing adipocytes leading to adipocyte hypertrophy or by increasing adipocyte differentiation and recruitment of new adipocytes. PPARγ

mRNA expression in abdominal SAT tissue samples has been shown to be reduced by 38% in pregnant women and 48% in women with GDM compared to non-pregnant control women group suggesting a reduced SAT adipocyte differentiation ability in the face of the metabolic challenge and insulin resistance of pregnancy and GDM (Catalano et al., 2002). Furthermore, this was suggested to be part of the molecular mechanisms to accelerate fat catabolism to meet the fetal nutritional demands.

In adipocytes, the insulin receptors are essential to transmit insulin effects on glucose and lipid metabolism. There are two isoforms of insulin receptor; insulin receptor A (IR-A) and insulin receptor B (IR-B) based on alternative splicing of exon 11. IR-A promotes cell growth while IR-B is the more active isoform in insulin signalling and glucose homeostasis (Belfiore et al., 2009). Insulin receptor dysfunction has been implicated in cellular insulin resistance and disturbed metabolic function. Few studies have investigated the role of insulin receptor different isoforms and its downstream effects on insulin sensitive tissues such as muscle and SAT. In women with GDM, there was reduced mRNA expression levels of IRB in both SAT and VAT whole tissue samples compared to healthy pregnant women. However, this was more pronounced in VAT and the reduction of IRB was independent of maternal BMI (Ott et al., 2018). The depot specific regional variation of adipocytes insulin receptors isoform expression in women with GDM was not studied before to the best of our knowledge.

Various metabolic pathways are involved in the development of metabolic disturbances in women with GDM. The adipocytes intracellular mechanisms responsible for insulin suppression of lipolysis are initiated when insulin bind to its receptor resulting in tyrosine phosphorylation of insulin receptor substrate 1 (IRS1) by insulin receptor tyrosine kinase. Tyrosine phosphorylated IRS1 activates phosphoinositide 3-kinase (PI3K) (Czech and Corvera, 1999). Insulin activates PI3K activity and mediates the activation of protein kinase Akt which subsequently phosphorylates and activates cAMP-phosphodiesterase (PDE3B)(Okada et al., 1994). This kinase is activated by insulin through binding to insulin receptors (PDE3B is the substrate) thus, initiating the antilipolytic effect of insulin. The PI3K/AKT pathway further stimulates the translocation of GLUT4 vesicles to the plasma membrane and allows the uptake of glucose into the adipose cells. In GDM, defective post receptor insulin signalling was suggested in several studies. Studies

on adipose and muscle tissue from pregnant GDM women revealed that there was defective post-receptor insulin signalling demonstrated as reduced IRS1 activity (Friedman et al., 1999). The protein expression of IRS1 was 43% lower in abdominal SAT whole tissue from women with GDM compared to healthy pregnant controls (Catalano et al., 2002). In addition, reduced GLUT4 translocation to plasma membrane was demonstrated in women with GDM (Garvey et al., 1993).

The changes in lipid and glucose metabolism during GDM pregnancy is consistent with increased maternal insulin resistance leading to increased FA and TAG availability for the fetus (Colomiere et al., 2010). Offspring of GDM mothers were shown to have greater risk of obesity and insulin resistance later in life (Catalano et al., 2003). Therefore, further understanding adipose tissue lipid metabolism and function has become increasingly important in the light of rising obesity and GDM incidence. Furthermore, the differential expression of genes involved in other adipose tissue functions such as adipose tissue angiogenesis and adipocyte apoptosis was of interest to the current study.

Microarray analysis of genes was a potential method for assessing differences in gene expression between healthy control and GDM adipocytes, but this was not accessible due to the particularly expensive cost for adipocytes due to the need for pre-amplification step and limited fund availability. Alternatively, to avoid selection only of genes already reported previously to be differentially expressed in GDM in the literature and to explore new pathways, a number of genes was selected by Ingenuity pathway analysis (QIAGEN) (discussed in the Methods Chapter section 1.5.1) involved in several pathways. To quantitate the mRNA expression of target genes Taqman qRT PCR was used. Messenger RNA was extracted from isolated adipocytes from SAT and VAT rather than whole adipose tissue samples to avoid the confounding effects of SVF cells including macrophages. Preamplification of cDNA targets for gene expression analysis was required due to the very small quantity of RNA that can be isolated from adipocytes. The uniformity of preamplification of genes of interest was controlled for by A $\Delta\Delta$ C_T method, using *CDKN1B* (the recommended endogenous uniformity reference gene). Calculated C_T values for the target genes and *CDKN1B* were obtained for different dilutions of pre-amplified and non-pre-amplified cDNA. $\Delta\Delta$ C_T value close to zero indicates preamplification uniformity, with values within ±1.5 being acceptable. The cohort used for SAT (GDM n=15 and control n=18) and VAT (GDM n=14 and control n=11)

gene expression analysis utilizes samples available from the original cohort of lipolysis experiments (GDM n=22 and control n=22). There were some samples not available for SAT and VAT gene expression analysis from the original cohort and this was limited by smaller fat biopsies provided to the researcher which limited the number of adipocytes that could be isolated. Priority was given to lipolysis assays. Another frequent reason for missing samples especially in VAT depot, was wound closure by the surgeon before remembering to take the VAT sample.

This study explored the expression of several genes involved in different adipocyte functions as shown in Table 5-1.

Table 5-1 Genes included in gene expression analysis

Gene symbol	Protein name
Adipocyte inflamation genes	
TNF	Tumour necrosis factor alpha
IL-6	Interleukin 6
CCL2	C-C Motif Chemokine Ligand 2
PTGS2	Prostaglandin-Endoperoxide Synthase 2
Adipocytes lipolysis genes	
ADRA2A	adrenreceptor alpha 2 A
ADRB1	adrenoreceptor β1
ADRB2	adrenoreceptor β2
ADRB3	adrenoreceptor β3
LIPE	Lipase E, Hormone Sensitive Type
ATGL	Adipose tissue triglyceride lipase
Adipocytes differentiation genes	
IGF1	Insulin-like growth factor 1
PPARG	Peroxisome proliferator- activated receptor gamma
FOS	Fos protein
IGFBP2	Insulin-like growth factor- binding protein 2
IGFBP3	Insulin-like growth factor- binding protein 3
IGFBP5	Insulin-like growth factor- binding protein 5
CEBPB	CCAAT/Enhancer binding protein beta
MAPK8	Mitogen-activated protein kinase 8
TGFB1	Transforming growth factor beta 1
Lipid storage genes	
SCD	Stearoyl-CoA desaturase
SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1
CIDEC	Cell death activator CIDE-3
FOXO1	Forkhead box protein 01
SREBF1	Sterol regulatory element- binding protein 1
LPL	Lipoprotein lipase
CD36	Platelet glycoprotein 4
Insulin signaling genes	
INSR	Insulin receptor
IR-A	Insulin receptor isoform A
IR-B	Insulin receptor isoform B
IRS1	Insulin receptor substrate 1
IRS2	Insulin receptor substrate 2
AKT1	RAC-alpha serine/threonine- protein kinase
Glucose metabolism genes	
PCK1	Phosphoenolpyruvate carboxykinase 1
Angiogenesis genes	
VEGFA	Vascular endothelial growth factor A
ICAM1	Intercellular adhesion molecule 1
NOS3	Nitric Oxide Synthase 3
PTGS1	Prostaglandin-Endoperoxide Synthase 2
F3	Coagulation factor 3
Apoptosis genes	
DDIT3	DNA Damage Inducible Transcript 3
CASP3	Caspase 3

5.2 Aim

- The aim was to compare visceral adipocyte (VAT) basal and LPS-stimulated adipokine release in pregnant women with and without GDM.

- To compare SAT and VAT adipocyte gene expression of thirty-five genes involved in adipose tissue inflammation, adipocyte differentiation, lipid storage, insulin signaling, glucose metabolism, angiogenesis, and apoptosis in pregnant women with and without GDM.

5.2.1 Hypotheses

- Women with GDM have higher levels of VAT adipocyte pro-inflammatory and lower anti-inflammatory adipokine release under basal and LPS stimulated conditions compared to BMI-matched controls

- There is enhanced expression of genes involved in inflammation, lipid storage and apoptosis in VAT adipocytes in women with GDM compared to a BMI-matched control group and gene expression are related to adipocyte hypertrophy as assessed by adipocyte diameter.

- Adipocytes in GDM have lower expression of genes involved in adipocyte differentiation, angiogenesis and insulin signaling compared to BMI-matched controls.

5.2.2 Specific research questions

- Is the release of IL-6, IL-8, IL-10, IL-1β,TNF-α and adiponectin from VAT adipocytes altered in women with GDM compared to control? In basal or LPS stimulated conditions?

- Is basal or LPS stimulated release of MCP-1, VEGF, PlGF and INF-gamma from VAT adipocytes altered in women with GDM?

- How does basal or stimulated VAT adipocyte adipokine release relate to adipocyte size and lipolytic function?

- How does basal or stimulated VAT adipocyte adipokine release relate to maternal BMI, whole body insulin sensitivity or plasma cytokine levels?

- Is there is reduced SAT and VAT adipocyte insulin receptor expression in women with GDM compared to healthy BMI matched controls? Which depot is affected more?

- Is the SAT and VAT adipocyte expression level of adipokines and inflammatory markers different in women with GDM compared to healthy controls?

5.3 Methods

A subgroup of the original cohort outlined in Chapter 2 were used for the adipokine secretion from adipocytes experiment (GDM n=15 and control n=12). The study participants were recruited as outlined in the Methods Chapter section 2.1.1. Adipose tissue samples were processed as outlined in the Methods Chapter 2.2. After 2 hours incubation, two aliquots of the medium below the adipocyte suspension were collected for later adipokines analysis using multiplex technology as outlined in the Methods Chapter section 2.6.2. The analytes (IFN-γ, IL-1β, IL-8, IL-6, IL-10, MCP-1, VEGF) were measured in one multiplex kit MILLIPEX Map Human Cytokine/Chemokine Magnetic Bead Panel (EMD Millipore, Merck, HCYTOMAG-60K). PlGF was measured in a singleplex kit MILLIPEX Map Human Cardiovascular Disease (CVD) Magnetic Bead Panel 1 to avoid cross reactivity (EMD Millipore, Merck, HCVD1MAG-67K). Adiponectin and TNF-α were measured by ELISA due to raw material issue with Merck company. Medium adipokines concentrations were corrected for the cell number using DNA concentration in a known volume of adipocytes as outlined in the Methods Chapter section 2.2.3 and expressed as pg/ml/µg DNA. Due to the expense of these kits and limited funds available near the end of the project, adipokine analysis was carried out only for VAT samples. For adipokine secretion analysis, paired t test was used to compare basal and LPS-stimulated release of adipokines within the same group. Two sample t test was used to compare the basal release of adipokines between the GDM and control group as well as the LPS stimulated adipokine release between the groups.

For gene expression analysis, both SAT (GDM n=15 and control n=18) and VAT (GDM n=14 and control n=11) adipocytes gene expression was studied in the samples available from the original cohort outlined in chapter 2 (Figure 5-1). Isolation of RNA was carried out as outlined in the Methods Chapter section 2.5.2. The isolated RNA was then reverse transcribed to cDNA. Preamplification of cDNA targets for gene of interest and test of preamplification uniformity was then carried out as outlined in the Methods Chapter section 2.5.5. The pre-amplified cDNA was quantified using TaqMan technology as outlined in the Methods Chapter section 2.5.7. This analysis was carried out for both SAT and VAT depot.

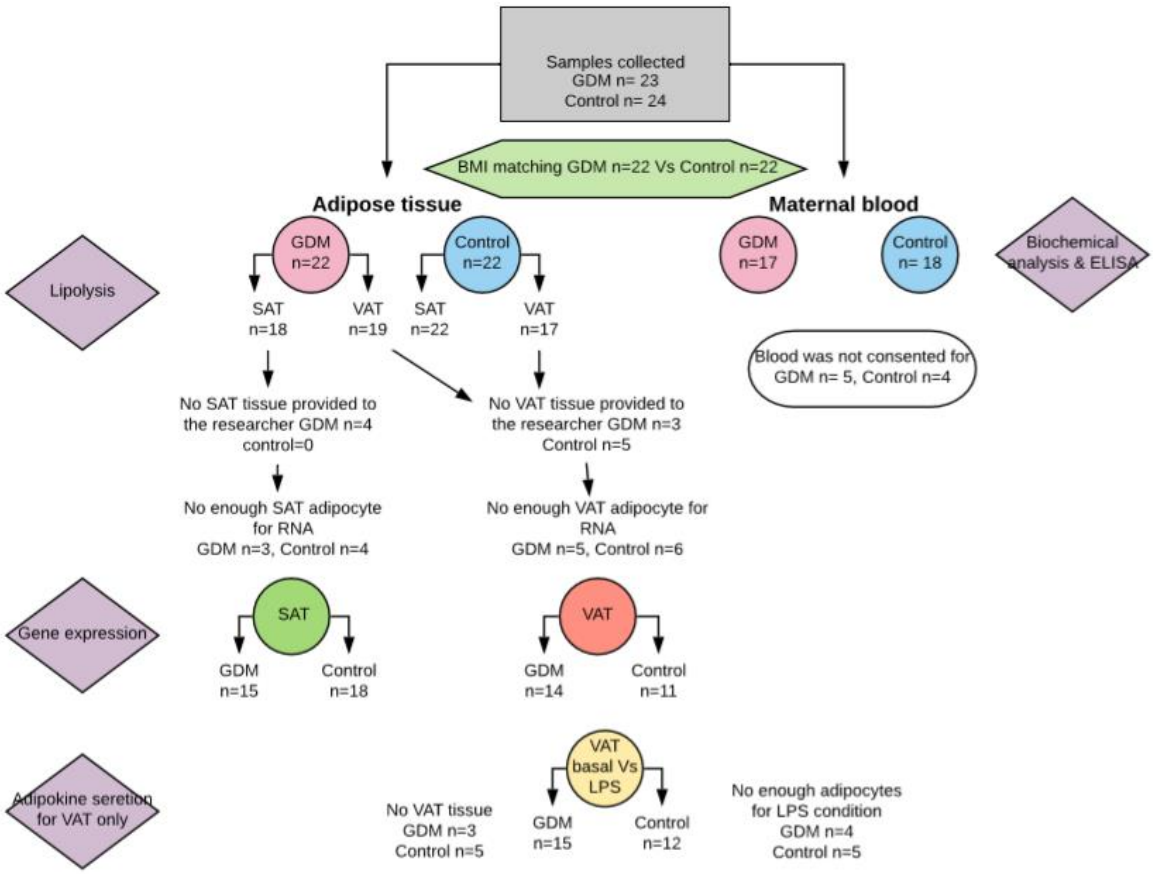


Figure 5-1 Flowchart for the sample number at lipolysis, adipokine secretions and gene expression experiment

The statistical analysis was carried out as outlined in the Methods Chapter section 2.9. Comparisons were made using unpaired 2 sample student t test on log transformed data for the following genes: *TNF, IL-6, MCP-1, PTGS2, IGFBP3, IGFBP5, CEBPB, TGFβ1, SCD, CIDEC, FOXO1, LPL, INSR, IRA, IRS1, LIPE* and

ICAM1. Two 2 sample student t test on square root transformed data was used for IRB and VEGF genes. Unpaired 2 sample student t test on raw normally distributed data for the following genes: *IGF-1, PPARG, MAPK8, CD36* and *IRS1*. Mann-Whitney test for non-parametric data for the following genes: *Akt1, FOS, IGFBP2, SREBF1, SLC2A1, PCK1, NOS3, PTGS1, F3, DDIT3* and *CASP3* at significance level of p value of <0.05. A targeted research question approach was used when examining the correlation analysis in adipokine secretions from VAT adipocytes experiment. Correlation were considered significant if the p value was <0.010 to avoid random associations.

5.4 Results

5.4.1 Study participants included for adipocyte adipokine secretion experiments

The study participants had similar BMI and age (Table 5-2). Blood pressure did not differ between control and GDM pregnancy. GDM mothers delivered six days earlier than controls (p= 0.0009). Women diagnosed with GDM in our cohort undergo diagnostic OGTT test in the second trimester of their pregnancy. However, only four women in the control group was screened for GDM and the results were negative and the test was indicated due to high maternal BMI or family history of diabetes. Diagnostic OGTT test results as expected, women in the GDM group had higher fasting blood glucose (p= 0.0001) and higher 2 hours OGTT blood glucose (p=0.035). Women with GDM had babies of lower birthweight compared to controls (p=0.006). This subgroup demographic characteristics were similar to the original cohort apart from the tendency of women with GDM to have babies of lower birth weight compared to controls.

Table 5-2 Demographic characteristics of GDM pregnancies and BMI matched controls for adipocyte adipokine secretion experiments

Demographic	Control (n=12)	GDM (n=15)	P-value
Age (years)	33.0(5.2)	35.2(5.6)	0.29
BMI (kg/m^2)	31.2(5.1)	32.9(9.2)	0.55
SBP at delivery (mmHg)	114.4(11.6)	122.5(16.8)	0.19
DBP at delivery (mmHg)	73.7(9.1)	70.5(10.9)	0.45
Treatment for GDM n (%)			
Diet only		6(40)	
Metformin	-	6(40)	-
insulin		2(13.3)	
Both insulin and metformin		1(6.7)	
OGTT (second trimester)			
Fasting* (mmol/L)	4.6(0.2)	5.6 (0.6)	0.0001
2 hours (mmol/L)	6.0(0.79)	7.6(1.9)	0.035
Gestation at dilvery (weeks)	39.0(0)	38.3(0.16)	0.0009
Birth weight(g)	3748(481)	3277(347)	0.006

Data are expressed as mean (SD) for continuous variables. Categorical variables are expressed as number (percent). Comparisons were made by two sample t-test except ***chi-squared test. BMI=body mass index; SBP=systolic blood pressure, DBP=diastolic blood pressure prior to caesarean section and OGTT= oral glucose tolerance test. *log transformed variable.

Maternal third trimester plasma triglycerides were higher in women with GDM compared to controls. Plasma cholesterol, NEFA and glycerol level were not different between women with GDM and the control group. Similarly, plasma glucose and HOMA-IR were not different between GDM and control groups, however, this was contrary to what we have observed in the original cohort of lipolysis study. Plasma markers of inflammation such as CRP, TNF-α, IL-6, oxLDL and the anti-inflammatory marker adiponectin were not different between the groups(Table 5-3).

Table 5-3 Maternal lipids, plasma markers of insulin resistance and plasma cytokines for GDM and control group

Plasma marker	Control (n=12)	GDM (n=15)	P-value
Glucose (mmol/L)**	4.38(0.31)	4.96(0.59)	0.07
insulin (uU/ml)*	13.68(4.58)	29.61(24.31)	0.093
HOMA-IR**	2.42(0.95)	3.99(11.80)	0.07
Triglycerides (mmol/L)	2.69(0.65)	2.93(0.77)	0.044
Cholesterol (mmol/L)	6.67(1.31)	5.91(1.15)	0.17
plasma glycerol (umol/L)**	86.72 (64.1)	89.85(37.32)	0.60
plasma NEFA (mmol/L)	0.83(0.31)	0.84(0.26)	0.91
CRP** (mg/L)	2.66(4.43)	2.32(1.24)	0.53
TNF-alpha** (pg/ml)	1.08(0.22)	0.99(0.20)	0.20
IL-6* (pg/ml)	3.15(2.57)	2.34(1.02)	0.41
oxLDL* (U/L)	16.76(3.27)	14.85(3.51)	0.11
Adiponectin** (ng/ml)	179.5(100.5)	260.0(110.1)	0.53

Data are expressed as mean (SD) for continuous variables. Categorical variables are expressed as number (percent). Comparisons were made by two sample t-test except **Mann-Whitney. HOMA-IR= homeostasis model assessment for insulin resistance, NEFA= non-esterified fatty acids, CRP= C reactive protein, TNF-alpha= tumour necrosis factor alpha, IL-6= interleukin 6 and oxLDL= oxidized low density lipoprotein. *log transformed variable.

5.4.2 Adipokine release from VAT adipocytes in women with GDM compared to controls

Although statistical difference in plasma cytokines were not detected between women with GDM and healthy controls in our cohort, it is possible that there could be altered adipokine secretion from adipocytes within the adipose tissue contributing to adipose tissue inflammation, in a paracrine action. Therefore, adipocyte adipokine secretion was studied in VAT adipocytes.

5.4.2.1 LPS stimulation of basal lipolysis of SAT and VAT adipocytes in GDM compared to controls

LPS (10μM) significantly stimulated basal net lipolysis in SAT adipocytes from the control ($p<0.0001$) and GDM ($p<0.0001$) group. Furthermore, SAT adipocytes from women with GDM had higher LPS stimulated lipolysis compared to the controls ($p=0.009$) (Figure 5-2).

In contrast in VAT, adipocyte lipolysis was not stimulated by LPS in both GDM and controls. Both basal ($p=0.020$) and LPS- stimulated (0.040) lipolysis was higher in VAT adipocytes from the GDM group compared to the controls (Figure 5-3).

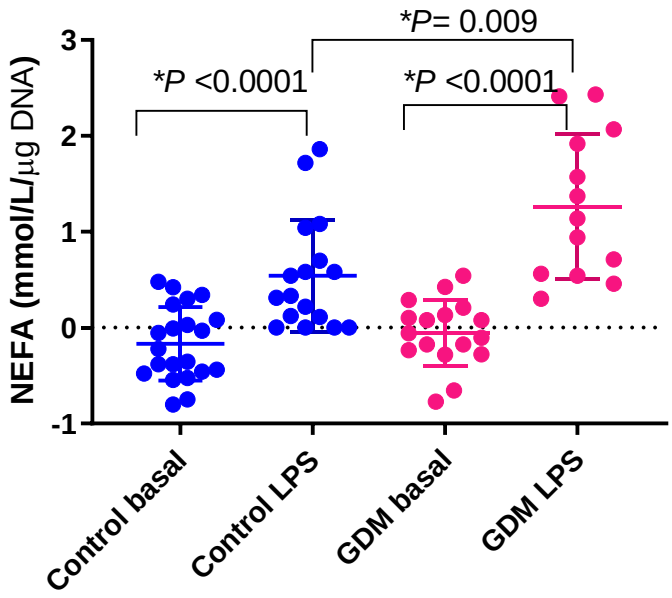


Figure 5-2: Net basal and net lipolysis rate in presence of LPS expressed as NEFA release in SAT adipocytes from GDM and control groups.
SAT adipocytes net basal lipolysis was significantly stimulated by the presence of LPS in both GDM ($p<0.0001$) and control group ($p<0.0001$). Comparisons were made using paired two sample student t test on log transformed basal lipolysis data and normally distributed raw LPS data. Results were displayed as mean (SD).

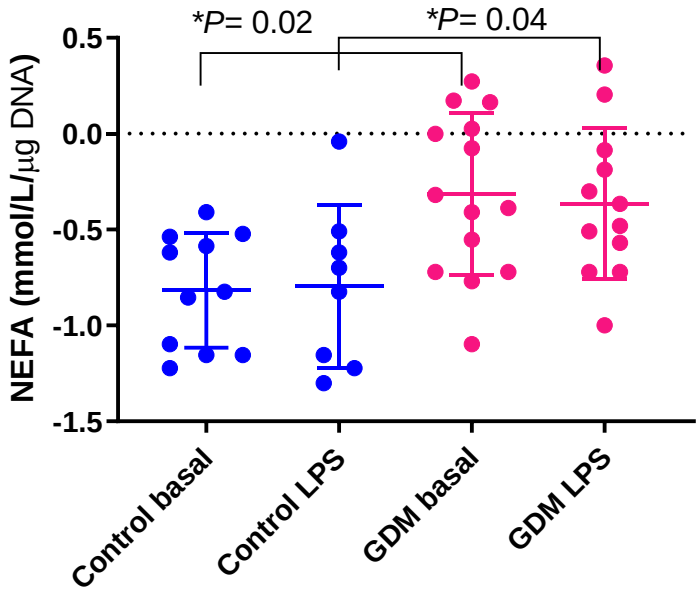


Figure 5-3: Net basal and net lipolysis rate in presence of LPS expressed as NEFA release in VAT adipocytes from GDM and control groups.
VAT adipocytes net basal lipolysis was not stimulated by the presence of LPS in both GDM and control group. Comparisons were made using paired two sample student t test on log transformed basal lipolysis data and normally distributed raw LPS data. Results were displayed as mean (SD).

5.4.2.2 VAT adipocyte TNF-α release in GDM compared to controls

There was no difference between VAT basal and LPS stimulated release of TNF-α in the control group (p=0.15). Similarly, there was no difference between VAT basal and LPS stimulated release of TNF-α in the GDM group (p=0.24). There was no difference in VAT basal (p= 0.18) or LPS stimulated (p= 0.36) release of TNF-α between the GDM and control groups (Figure 5-4).

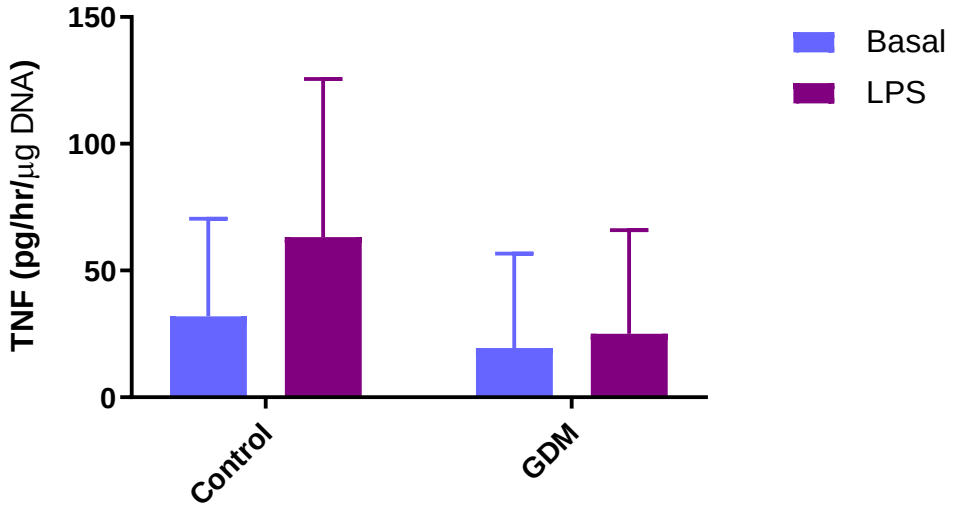


Figure 5-4 Comparison of basal and LPS stimulated release of TNF-α in VAT adipocytes
Secretion of TNF-α was not different between basal and LPS stimulated lipolysis in both groups. Analysis was carried out on log transformed data by paired t-test. There was no difference in TNF-α secretion in basal lipolysis between GDM and control group. Similarly, there was no difference in TNF-α secretion in LPS stimulated lipolysis between GDM and control group, analysis by two sample student t-test on log transformed data. Raw data are presented, and data are expressed as mean and SD.

5.4.2.3 VAT adipocyte IL-6 release in GDM compared to controls

There was no difference between VAT basal and LPS stimulated release of IL-6 in the control group (p=0.59). Similarly, there was no difference between VAT basal and LPS stimulated release of IL-6 in the GDM group (p=0.59). There was no difference in VAT basal (p= 0.33) or LPS stimulated (p= 0.40) release of IL-6 between the GDM and control group (Figure 5-5).

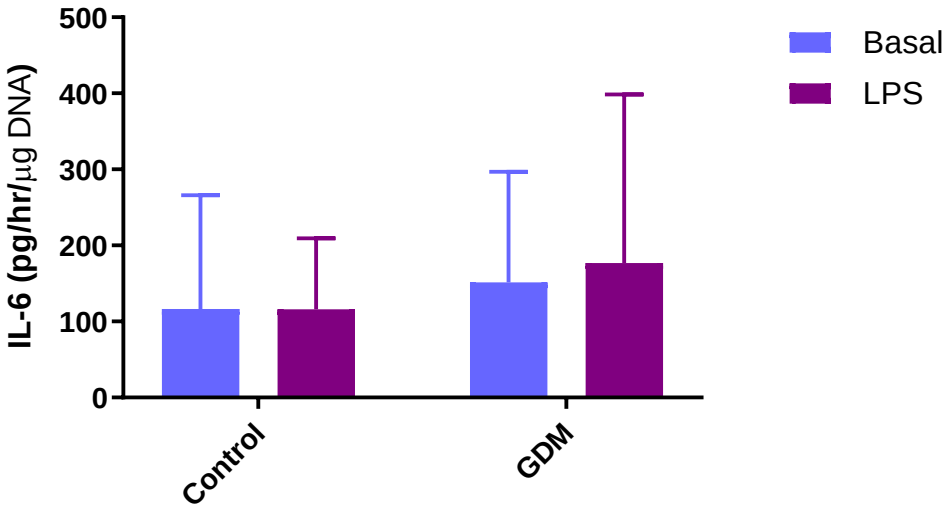


Figure 5-5 Comparison of basal and LPS stimulated release of IL-6 in VAT adipocytes
Secretion of IL-6 was not different between basal and LPS stimulated lipolysis in both groups. Analysis by two samples t test on log transformed data. There was no difference in IL-6 secretion in basal lipolysis between GDM and control group. Similarly, there was no difference in IL-6 secretion in LPS stimulated lipolysis between GDM and control group, analysis by two sample t-test. Raw data are presented, and data are expressed as mean and SD.

5.4.2.4 VAT adipocyte IL-1β release in GDM compared to controls

There was no difference between VAT basal and LPS stimulated release of IL-1β in the control group (p=0.53). Similarly, there was no difference between VAT basal and LPS-stimulated release of IL-1β in the GDM group (p=0.14). There was no difference in VAT basal (p= 0.52) or LPS-stimulated (p= 0.36) release of IL-1β between the GDM and control group (Figure 5-6).

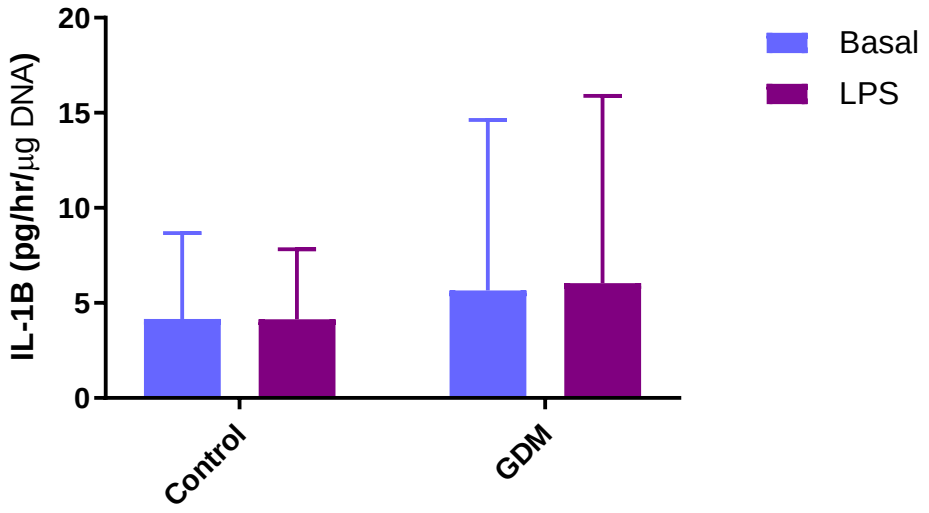


Figure 5-6 Comparison of basal and LPS stimulated release of IL-1β in VAT adipocytes
Secretion of IL-1β was not different between basal and LPS stimulated lipolysis in both groups. Analysis by paired t-test was carried out on log transformed data. There was no difference in IL-1β secretion in basal lipolysis between GDM and control group. Similarly, there was no difference in IL-1β secretion in LPS stimulated lipolysis between GDM and control group, analysis by two sample student t-test on log transformed data. Raw data are presented, and data are expressed as mean and SD.

5.4.2.5 VAT adipocyte IFN-gamma release in GDM compared to controls

There was no difference between VAT basal and LPS-stimulated release of IFN-gamma in the control group (p=0.33). Similarly, there was no difference between VAT basal and LPS-stimulated release of IFN-gamma in the GDM group (p=0.26). There was no difference in VAT basal (p= 0.44) or LPS-stimulated (p= 0.33) release of IFN-gamma between the GDM and control group (Figure 5-7).

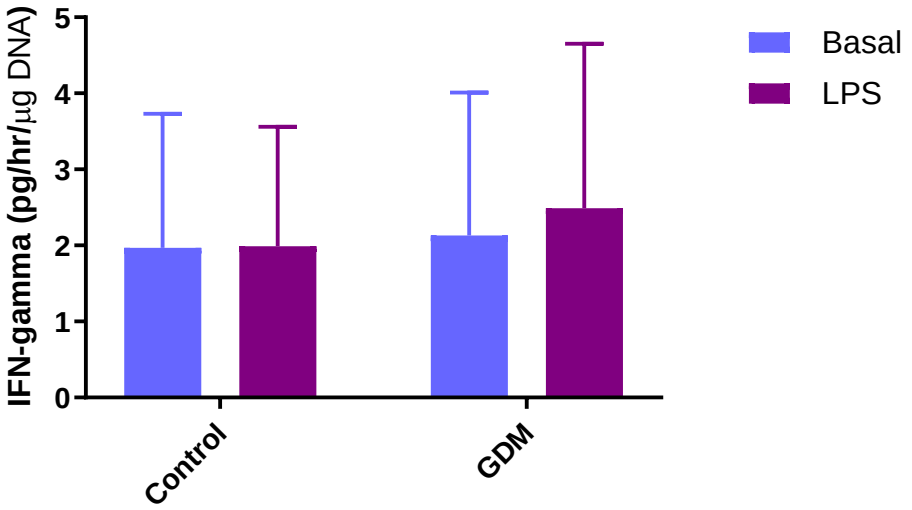


Figure 5-7 Comparison of basal and LPS stimulated release of IFN-gamma in VAT adipocytes Secretion of IFN-gamma was not different between basal and LPS stimulated lipolysis in both groups. Analysis by paired t-test was carried out on log transformed data. There was no difference in IFN-gamma secretion in basal lipolysis between GDM and control group. Similarly, there was no difference in IFN-gamma secretion in LPS stimulated lipolysis between GDM and control group, analysis by two sample t-test was carried out. Raw data are presented, and data are expressed as mean and SD.

5.4.2.6 VAT adipocyte MCP-1 release in GDM compared to controls

There was no difference between VAT basal and LPS-stimulated release of MCP-1 in the control group (p=0.61). Similarly, there was no difference between VAT basal and LPS-stimulated release of MCP-1 in the GDM group (p=0.67). There was no difference in VAT basal (p= 0.21) or LPS-stimulated (p= 0.45) release of MCP-1 between the GDM and control group (Figure 5-8).

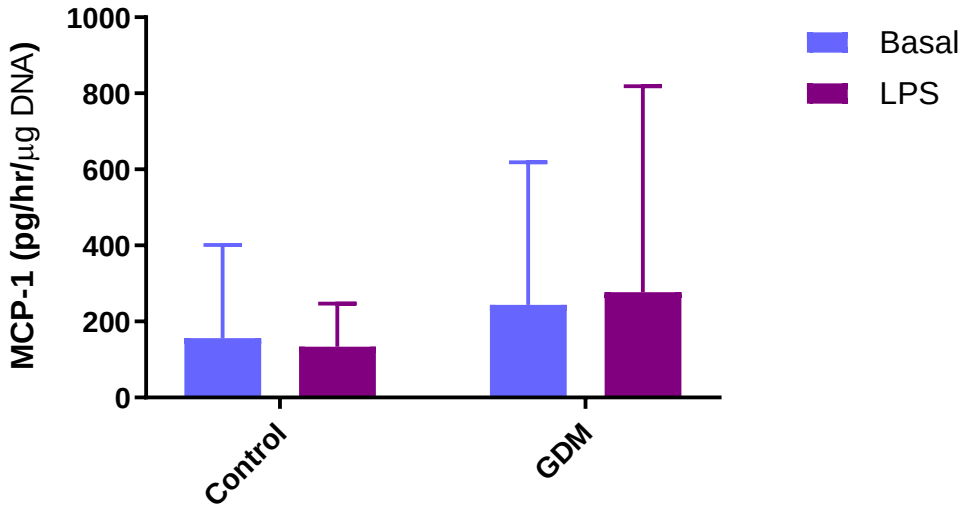


Figure 5-8 Comparison of basal and LPS stimulated release of MCP-1 in VAT adipocytes
Secretion of MCP-1 was not different between basal and LPS stimulated lipolysis in both groups. Analysis by paired t-test was carried out on log transformed data. There was no difference in MCP-1 secretion in basal lipolysis between GDM and control group. Similarly, there was no difference in MCP-1 secretion in LPS stimulated lipolysis between GDM and control group. Analysis by two sample t-test was carried out for (LPS MCP-1) and Mann Whitney test for non-parametric data (basal MCP-1). Raw data are presented, and data are expressed as mean and SD.

5.4.2.7 VAT adipocyte Adiponectin release in GDM compared to controls

There was no difference between VAT basal and LPS-stimulated release of adiponectin in the control group (p=0.35). In the GDM group, VAT LPS-stimulated release of adiponectin was higher compared to basal release (basal: 0.27[0.46] vs LPS: 0.43[0.33] pg/ml/ug of DNA, p=0.017). There was no difference in VAT basal (p= 0.70) or LPS-stimulated (p= 0.93) release of adiponectin between the GDM and control group (Figure 5-9).

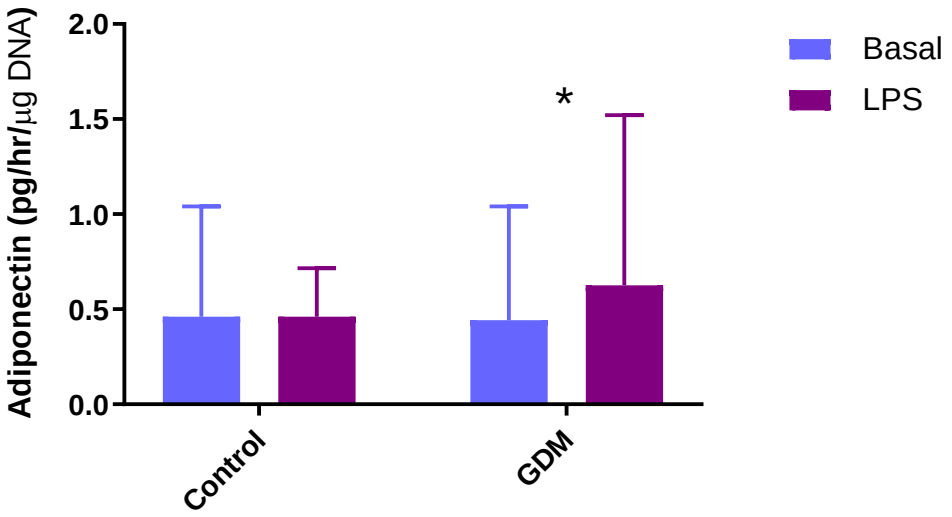


Figure 5-9 Comparison of basal and LPS stimulated release of adiponectin in VAT adipocytes Secretion of adiponectin was significantly increased after LPS stimulation of lipolysis in the GDM group (p=0.017) but not in the control group. Analysis was carried by Wilcoxon signed rank test. There was no difference in adiponectin secretion in basal lipolysis between GDM and control group. Similarly, there was no difference in adiponectin secretion in LPS stimulated lipolysis between GDM and control group. Analysis by two sample t-test was carried out for (LPS adiponectin) and Mann Whitney test for non-parametric data (basal adiponectin). Raw data are presented, and data are expressed as mean and SD *p≤ 0.05.

5.4.2.8 VAT adipocyte IL-8 release in GDM compared to controls

There was no difference between VAT basal and LPS-stimulated release of IL-8 in the control group (p=0.67). Similarly, there was no difference between VAT basal and LPS-stimulated release of IL-8 in the GDM group (p=0.13). There was no difference in VAT basal (p= 0.68) or LPS-stimulated (p= 0.51) release of IL-8 between the GDM and control group (Figure 5-10).

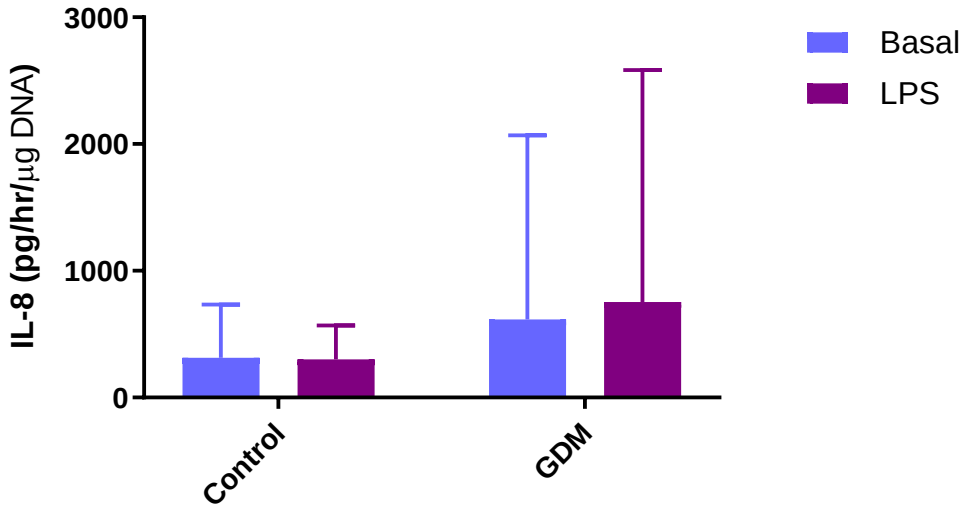


Figure 5-10 Comparison of basal and LPS stimulated release of IL-8 in VAT adipocytes
Secretion of IL-8 was not different between basal and LPS stimulated lipolysis in both groups. Analysis by Wilcoxon signed rank test was carried out. There was no difference in IL-8 secretion in basal lipolysis between GDM and control group. Similarly, there was no difference in IL-8 secretion in LPS stimulated lipolysis between GDM and control group., analysis by Mann Whitney test for non-parametric data was carried out. Raw data are presented, and data are expressed as mean and SD.

5.4.2.9 VAT adipocyte IL-10 release in GDM compared to controls

There was no difference between VAT basal and LPS-stimulated release of IL10 in the control group (p=0.41). Similarly, there was no difference between VAT basal and LPS-stimulated release of IL-10 in the GDM group (p=0.27). There was no difference in VAT basal (p= 0.27) or LPS-stimulated (p= 0.34) release of IL-10 between the GDM and control group (Figure 5-11).

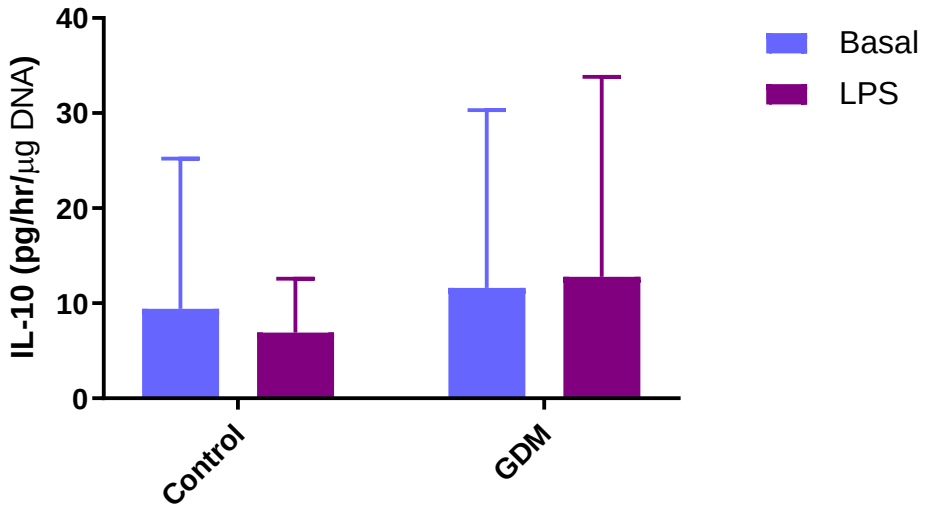


Figure 5-11 Comparison of basal and LPS stimulated release of IL-10 in VAT adipocytes
Secretion of IL-10 was not different between basal and LPS stimulated lipolysis in both groups. Analysis by Wilcoxon signed rank test was carried out. There was no difference in IL-10 secretion in basal lipolysis between GDM and control group. Similarly, there was no difference in IL-10 secretion in LPS stimulated lipolysis between GDM and control group, analysis by Mann Whitney test for non-parametric data was carried out. Raw data are presented, and data are expressed as mean and SD.

5.4.2.10 VAT adipocyte VEGF release in GDM compared to controls

There was no difference between VAT basal and LPS-stimulated release of VEGF in the control group (p=0.70). Similarly, there was no difference between VAT basal and LPS-stimulated release of VEGF in the GDM group (p=0.99). There was no difference in VAT basal (p= 0.68) or LPS-stimulated (p= 0.86) release of VEGF between the GDM and control group (Figure 5-12).

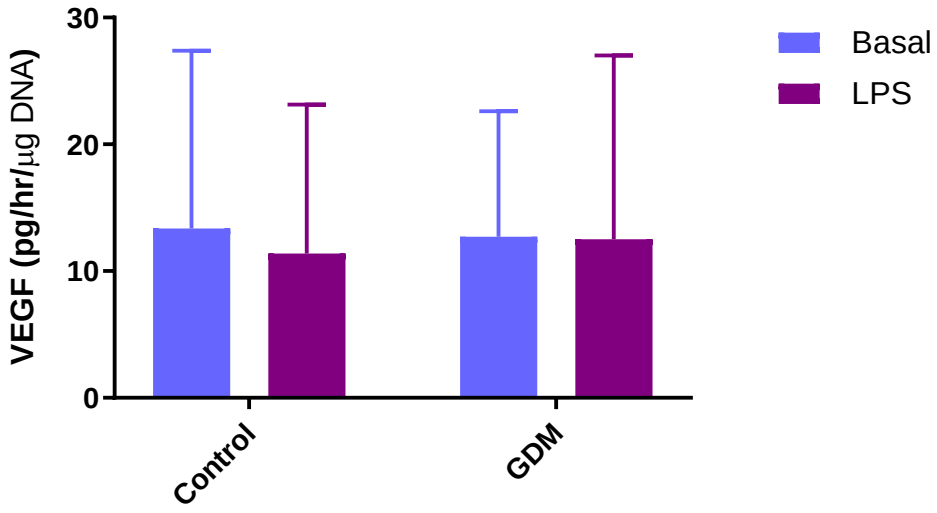


Figure 5-12 Comparison of basal and LPS stimulated release of VEGF in VAT adipocytes
Secretion of VEGF was not different between basal and LPS stimulated lipolysis in both groups. Analysis by paired t-test was carried out on log transformed data. There was no difference in VEGF secretion in basal lipolysis between GDM and control group. Similarly, there was no difference in VEGF secretion in LPS stimulated lipolysis between GDM and control group analysis by two sample t-test was carried out. Raw data are presented, and data are expressed as mean and SD.

5.4.2.11 VAT adipocyte PlGF release in GDM compared to controls

There was no difference between VAT basal and LPS-stimulated release of PlGF in the control group (p=0.98). Similarly, there was no difference between VAT basal and LPS-stimulated release of PlGF in the GDM group (p=0.52). There was no difference in VAT basal (p= 0.89) or LPS-stimulated (p= 0.79) release of PlGF between the GDM and control group (Figure 5-13).

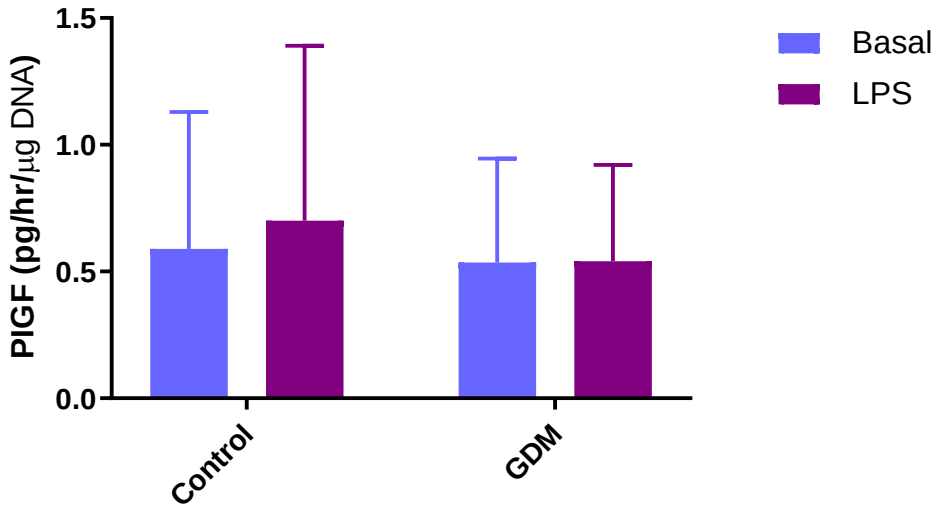


Figure 5-13 Comparison of basal and LPS stimulated release of PIGF in VAT adipocytes
Secretion of PIGF was not different between basal and LPS stimulated lipolysis in both groups. Analysis by paired t-test was carried out on log transformed data. There was no difference in PIGF secretion in basal lipolysis between GDM and control group. Similarly, there was no difference in PIGF secretion in LPS stimulated lipolysis between GDM and control group. Analysis by two sample t-test was carried out. Raw data are presented, and data are expressed as mean and SD.

5.4.3 Relationship between VAT adipocyte adipokine release and VAT adipocyte morphological and lipolytic characteristics

Several correlation analysis were carried out relating VAT adipocyte adipokine release with VAT adipocyte diameter and lipolytic function (basal lipolysis, FCISI, percentage stimulation of lipolysis by isoprotenerol and percentage suppression of lipolysis by insulin). There were no significant correlations at $P<0.010$ for any of the adipokines except TNF-α. In the control group, VAT FCISI was positively correlated with LPS stimulated TNF-α release (Figure 5-14).

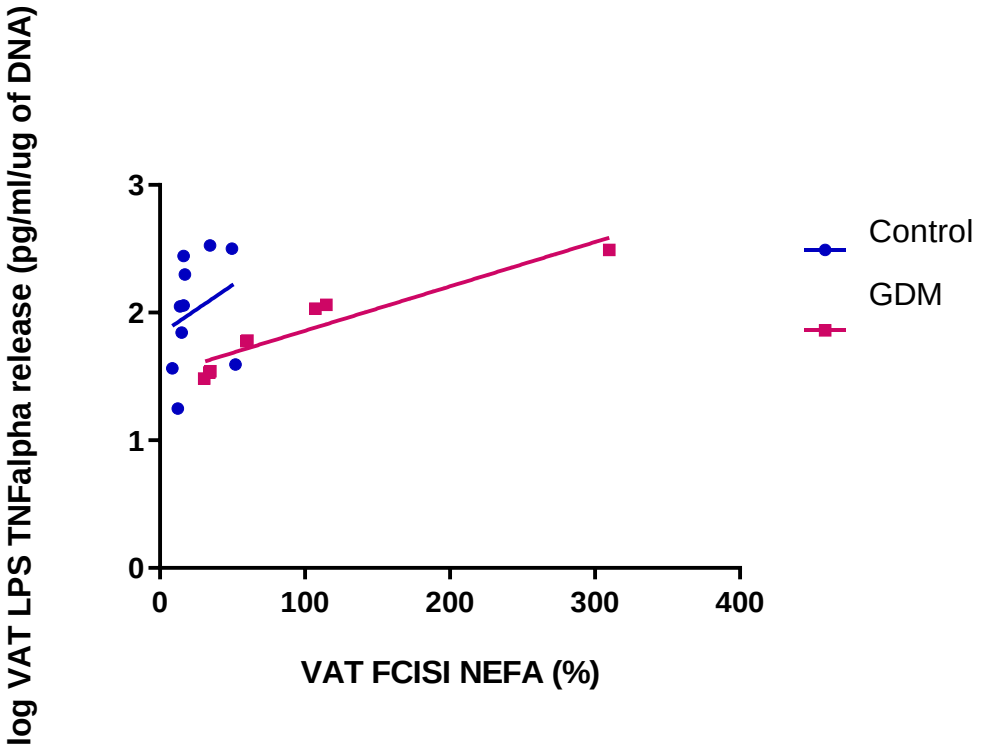


Figure 5-14 The relationship between VAT LPS stimulated TNF-α release and FCISI in GDM and control group
VAT FCISI expressed as NEFA release correlated positively with LPS stimulated release of TNF-α in the control group only (r= 0.77, p=0.004). Lower number of data points was noted in both groups due to zero release of TNF-α from adipocytes of some study subjects. No interaction p=0.41

5.4.4 Relationship between VAT adipocyte adipokine release and maternal BMI and HOMA-IR

Maternal BMI and HOMA-IR level were not related to VAT adipocyte adipokine release in basal (Figure 5-15) or LPS stimulated condition (Figure 5-16) in both groups.

	BMI		HOMA-IR	
Adipokine	Control	GDM	Control	GDM
VAT basal TNF-α	Not sig.	Not sig.	P=0.019, r=-0.72	Not sig.
VAT basal IL-6	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal IL-1β	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal IFN-gamma	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal MCP-1	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal adiponectin	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal IL-8	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal IL-10	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal VEGF	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal PIGF	Not sig.	Not sig.	Not sig.	Not sig.

Figure 5-15 VAT adipocyte basal adipokine release and maternal BMI and HOMA-IR relationship

	BMI		HOMA-IR	
Adipokine	Control	GDM	Control	GDM
VAT LPS TNF-α	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS IL-6	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS IL-1β	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS IFN-gamma	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS MCP-1	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS adiponectin	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS IL-8	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS IL-10	Not sig.	Not sig.	Not sig.	P=0.025, r=-0.69
VAT LPS VEGF	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS PIGF	Not sig.	Not sig.	Not sig.	Not sig.

Figure 5-16 VAT adipocyte LPS adipokine release and maternal BMI and HOMA-IR relationship

5.4.5 Relationship between VAT adipocyte adipokine release and pregnancy hormones

Maternal plasma estradiol and progesterone concentrations were not related to adipokine release in basal (Figure 5-17) or LPS stimulated condition (Figure 5-18) in both groups .

	Esterogen		Progesterone	
Adipokine	Control	GDM	Control	GDM
VAT basal TNF-α	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal IL-6	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal IL-1β	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal IFN-gamma	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal MCP-1	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal adiponectin	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal IL-8	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal IL-10	Not sig.	Not sig.	Not sig.	Not sig.
VAT basal VEGF	Not sig.	Not sig.	Not sig.	P=0.012, r=10.72
VAT basal PIGF	Not sig.	Not sig.	Not sig.	Not sig.

Figure 5-17 VAT adipocyte basal adipokine release and pregnancy hormones relationship

	Estrogen		Progesterone	
Adipokine	Control	GDM	Control	GDM
VAT LPS TNF-α	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS IL-6	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS IL-1β	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS IFN-gamma	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS MCP-1	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS adiponectin	Not sig.	Not sig.	Not sig.	P=0.046, r=-0.58
VAT LPS IL-8	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS IL-10	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS VEGF	Not sig.	Not sig.	Not sig.	Not sig.
VAT LPS PIGF	Not sig.	Not sig.	Not sig.	Not sig.

Figure 5-18 VAT adipocyte LPS adipokine release and pregnancy hormones relationship

5.4.6 Study participants included for adipocyte gene expression analysis

5.4.6.1 SAT adipocyte gene expression experiment

This subgroup was similar to the original cohort for BMI and age matching between the groups. Similarly, the higher plasma TAG and glucose levels in the GDM group compared to controls was previously observed in the original lipolysis study cohort (Table 5-4).

Table 5-4: Demographic characteristics and plasma markers of GDM pregnancies and BMI matched controls for SAT adipocytes gene expression experiment

Demographic	Control (n=18)	GDM (n=15)	P-value
Age (years)	33.8(5.4)	34.9(5.3)	0.58
BMI (kg/m^2)	32.1(7.8)	31.8(4.8)	0.89
Glucose (mmol/L)**	4.56(0.37)	5.2(0.9)	0.045
insulin (uU/ml)*	14.8(7.6)	25.9(18.7)	0.075
HOMA-IR**	3.02(1.7)	6.60(5.96)	0.066
Triglycerides (mmol/L)	2.61(0.59)	3.26(0.87)	0.04
Cholesterol (mmol/L)	6.34(1.23)	5.91(1.17)	0.37
plasma NEFA (mmol/L)	0.78(0.33)	0.88(0.27)	0.37

Data are expressed as mean (SD) for continuous variables; median (interquartile range) for *log transformed and nonparametric data. Categorical variables are expressed as number (percent). Comparisons were made by two sample t-test except **Mann-Whitney. HOMA-IR= homeostasis model assessment for insulin resistance and NEFA= non-esterified fatty acids.

5.4.6.2 VAT adipocyte gene expression experiment

This subgroup was similar to the original cohort for BMI and age. Similarly, the higher TAG in GDM group compared to controls was previously observed in the original lipolysis study cohort. However, this subgroup plasma glucose concentration and HOMA-IR was not different between women with GDM and controls in contrast to what observed in the original lipolysis study (Table 5-5).

Table 5-5: Demographic characteristics and plasma markers of GDM pregnancies and BMI matched controls for VAT adipocytes gene expression experiment

Demographic	Control (n=11)	GDM (n=14)	P-value
Age (years)	33.8(5.4)	34.9(5.3)	0.20
BMI (kg/m^2)	32.1(7.8)	31.8(4.8)	0.87
Glucose (mmol/L)**	4.56(0.37)	5.2(0.9)	0.059
insulin (uU/ml)*	14.8(7.6)	25.9(18.7)	0.14
HOMA-IR**	3.02(1.7)	6.60(5.96)	0.092
Triglycerides (mmol/L)	2.61(0.59)	3.26(0.87)	0.15
Cholesterol (mmol/L)	6.34(1.23)	5.91(1.17)	0.18
plasma NEFA (mmol/L)	0.78(0.33)	0.88(0.27)	0.97

Data are expressed as mean (SD) for continuous variables; median (interquartile range) for *log transformed and nonparametric data. Categorical variables are expressed as number (percent). Comparisons were made by two sample t-test except **Mann-Whitney. HOMA-IR= homeostasis model assessment for insulin resistance and NEFA= non-esterified fatty acids.

5.4.7 Gene expression of inflammatory genes in SAT and VAT adipocytes from women with GDM compared to controls

5.4.7.1 TNF

There was no difference in the percentage expression of *TNF* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (1.1(0.6) vs 1.1(0.9) *TNF* relative to *PPIA* percentage expression, p= 0.45) (Figure 5-19) or VAT (2.7(1.3) vs 2.3(1.9) *TNF* relative to *PPIA* percentage expression, p= 0.19) (Figure 5-20)

5.4.7.2 IL-6

There was no difference in the percentage expression of *IL-6* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (10.4(8.5) vs 12.3(12.9) *IL-6* relative to *PPIA* percentage expression, p= 0.96) (Figure 5-19) or VAT (28.5(17.4) vs 28.0(18.5) *IL-6* relative to *PPIA* percentage expression, p= 0.75) (Figure 5-20).

5.4.7.3 MCP-1 (CCL2)

There was no difference in the percentage expression of *MCP-1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (20.9(12.6) vs 21.7(18.2) *MCP-1* relative to *PPIA* percentage expression, p= 0.81) (Figure 5-19) or VAT (43.4(26.3) vs 42.4(2.1) *MCP-1* relative to *PPIA* percentage expression, p= 0.61) (Figure 5-20).

5.4.7.4 PTGS2

There was no difference in the percentage expression of *PTGS2* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (1.0(0.7) vs 1.9(2.5) *PTGS2* relative to *PPIA* percentage expression, p= 0.21) (Figure 5-19) or VAT (5.8(4.1) vs 5.8(5.6) *PTGS2* relative to *PPIA* percentage expression, p= 0.48) (Figure 5-20).

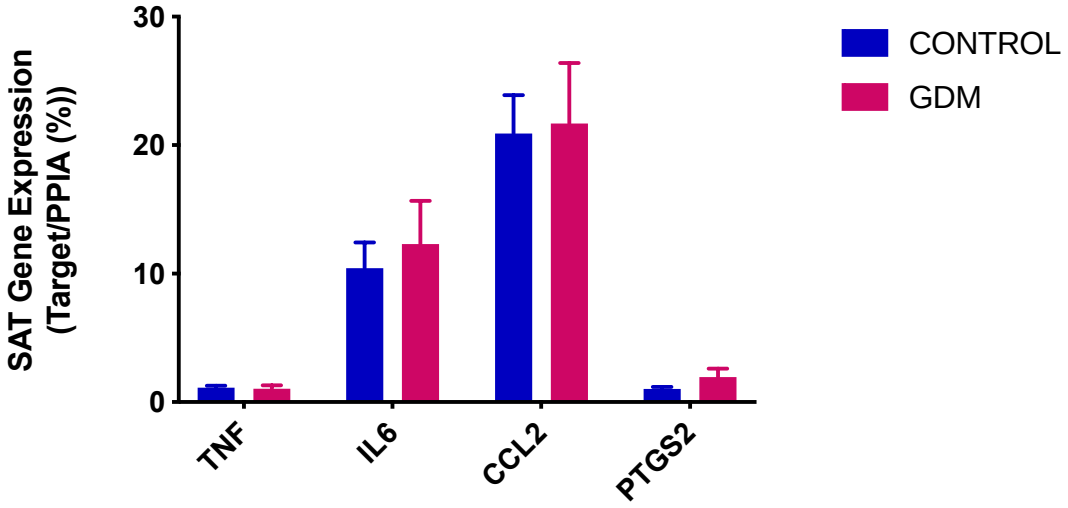


Figure 5-19 Gene expression of inflammatory genes in SAT adipocytes in controls and GDM group
Inflammatory genes expression relative to the endogenous control gene PPIA in SAT adipocytes from women with GDM (n=15) compared to control group (n=18) analyzed by RT-qPCR. The percentage expression of *TNF-α, IL-6, CCL2, PTGS2* genes relative to PPIA are shown and are expressed as mean (SD).

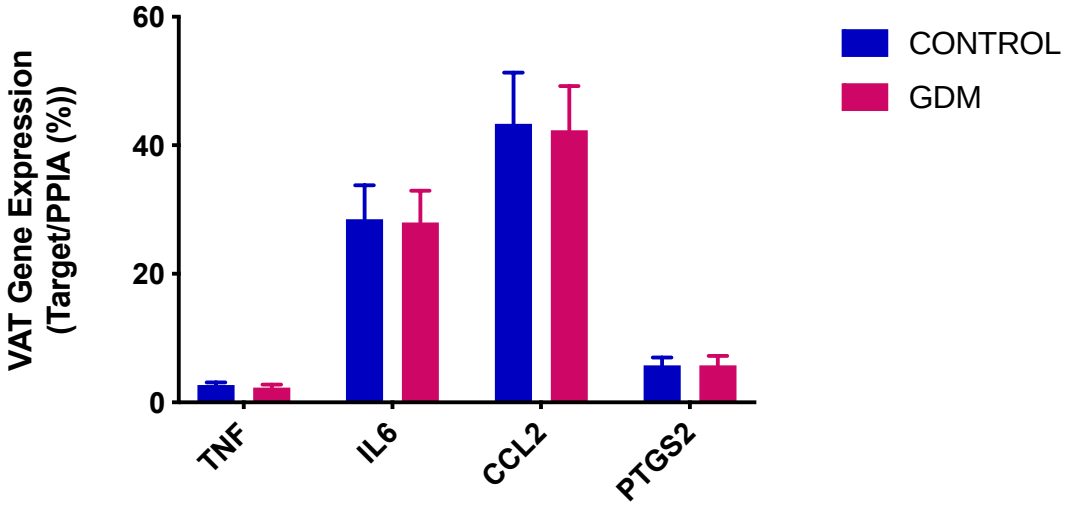


Figure 5-20 Gene expression of inflammatory genes in VAT adipocytes in controls and GDM group
Inflammatory genes expression relative to the endogenous control gene PPIA in VAT adipocytes from women with GDM (n=14) compared to control group (n=11) analyzed by RT-qPCR. The percentage expression of *TNF-α, IL-6, CCL2, PTGS2* genes relative to PPIA are shown and are expressed as mean (SD).

5.4.8 Gene exprssion of genes involved in adipocyte lipolysis genes in SAT and VAT adipocytes from women with GDM compared to controls

5.4.8.1 LIPE

There was no difference in the percentage expression of *LIPE* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (0.6(0.4) vs 0.5(0.4) *LIPE* relative to *PPIA* percentage expression, p= 0.55) (Figure 5-21) or VAT (1.4(1.1) vs 0.9(0.6) *LIPE* relative to *PPIA* percentage expression, p= 0.24) (Figure 5-22).

5.4.8.2 ATGL

There was no difference in the percentage expression of *ATGL* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (166.9(70.5) vs 157.6(52.1) *ATGL* relative to *PPIA* percentage expression, p= 0.65) (Figure 5-21) or VAT (166.5(57.6) vs 209.3(186.5) *ATGL* relative to *PPIA* percentage expression, p= 0.65) (Figure 5-22).

5.4.8.3 ADRA2A

There was no difference in the percentage expression of *ADRA2A* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (0.2(0.2)

vs 0.2(0.08) *ADRA2A* relative to *PPIA* percentage expression, p= 0.55) (Figure 5-23) or VAT (0.1(0.09) vs 0.2(0.07) *ADRA2A* relative to *PPIA* percentage expression, p= 0.25) (Figure 5-24).

5.4.8.4 ADRB1

There was no difference in the percentage expression of *ADRB1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (4.9(2.6) vs 3.4(1.1) *ADRB1* relative to *PPIA* percentage expression, p= 0.065) (Figure 5-23) or VAT (7.6(2.8) vs 6.7(5.9) *ADRB1* relative to *PPIA* percentage expression, p= 0.18) (Figure 5-24).

5.4.8.5 ADRB2

There was no difference in the percentage expression of *ADRB2* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (20.5(9.1) vs 18.4(7.9) *ADRB2* relative to PPIA percentage expression, p= 0.43) (Figure 5-23) or VAT (24.9(7.6) vs 24.9(12.4) *ADRB2* relative to PPIA percentage expression, p= 0.72) (Figure 5-24).

5.4.8.6 ADRB3

There was no difference in the percentage expression of *ADRB3* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (2.2(2.0) vs 1.6(0.9) *ADRB3* relative to *PPIA* percentage expression, p= 0.48) (Figure 5-23) or VAT (2.0(2.1) vs 0.9(0.4) *ADRB3* relative to *PPIA* percentage expression, p= 0.26) (Figure 5-24).

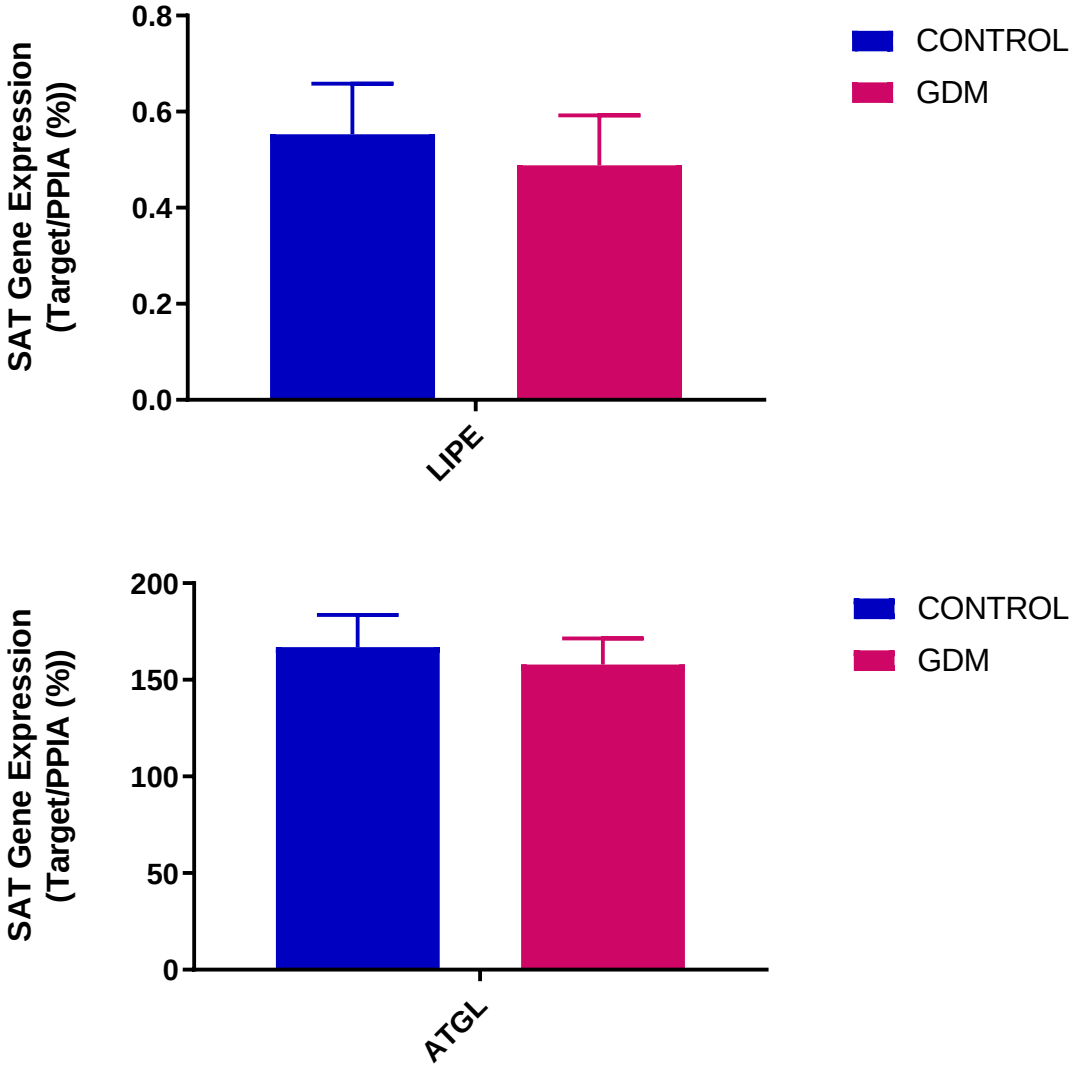


Figure 5-21 Gene expression of lipolytic enzymes genes in SAT adipocytes in controls and GDM group

Lipolytic enzymes genes expression relative to the endogenous control gene PPIA in SAT adipocytes from women with GDM (n=15) compared to control group (n=18) analyzed by RT-qPCR. The percentage expression of *LIPE* and *ATGL* genes relative to *PPIA* are shown and are expressed as mean (SD).

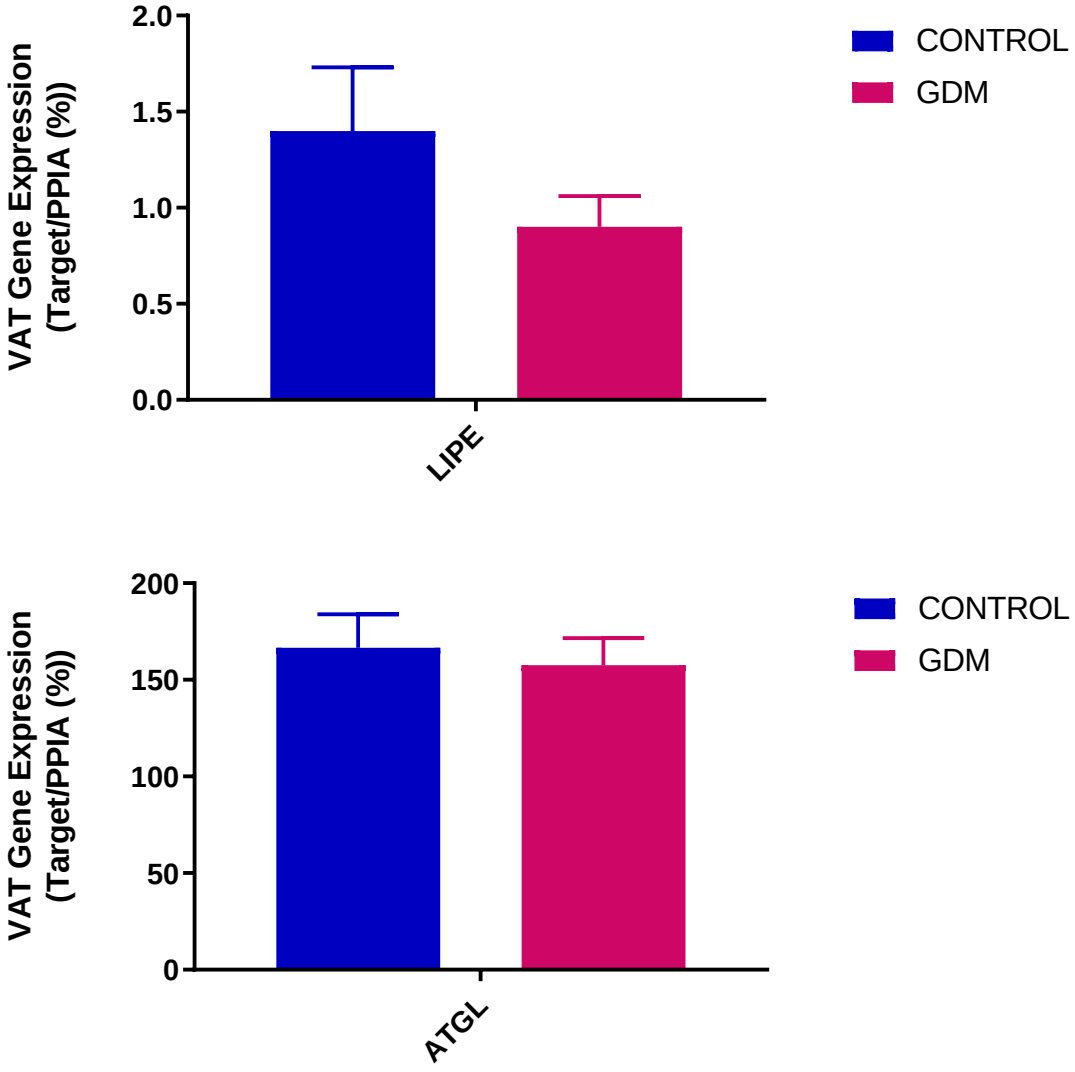


Figure 5-22 Gene expression of lipolytic enzymes genes in VAT adipocytes in controls and GDM group

Lipolytic enzymes genes expression relative to the endogenous control gene PPIA in VAT adipocytes from women with GDM (n=14) compared to control group (n=11) analyzed by RT-qPCR. The percentage expression of *LIPE* and *ATGL* genes relative to *PPIA* are shown and are expressed as mean (SD).

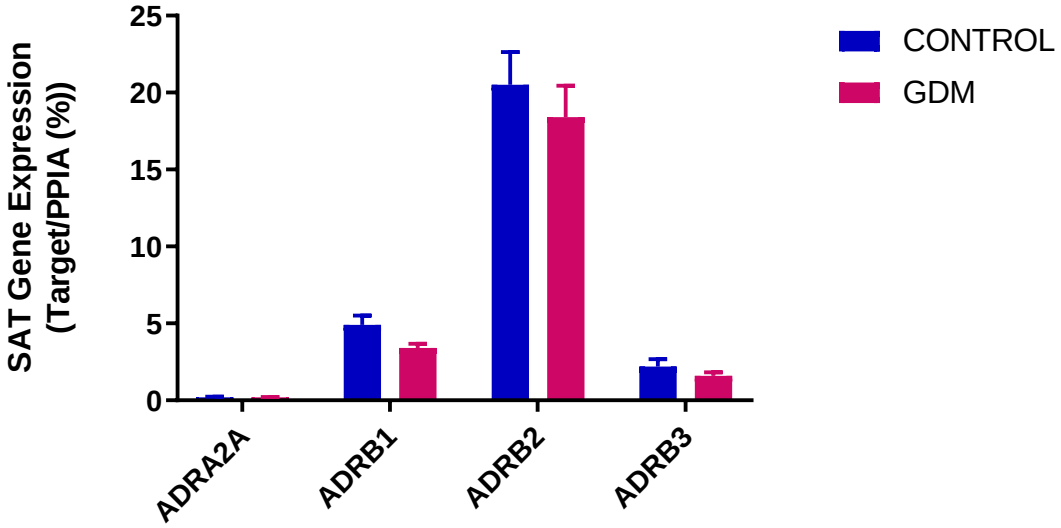


Figure 5-23 Gene expression of adrenoreceptors genes in SAT adipocytes in controls and GDM group
Adrenoreceptors gene expression relative to the endogenous control gene PPIA in SAT adipocytes from women with GDM (n=15) compared to control group (n=18) analyzed by RT-qPCR. The percentage expression of *ADRA2A, ADRB1, ARB2* and *ADRB3* genes relative to *PPIA* are shown and are expressed as mean (SD).

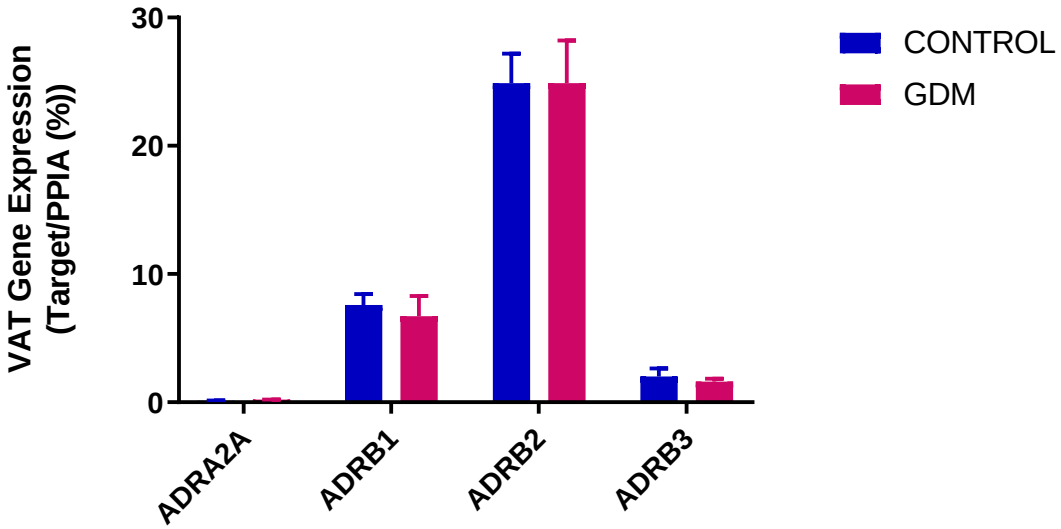


Figure 5-24 Gene expression of adrenoreceptors genes in VAT adipocytes in controls and GDM group
Adrenoreceptors gene expression relative to the endogenous control gene PPIA in VAT adipocytes from women with GDM (n=14) compared to control group (n=11) analyzed by RT-qPCR. The percentage expression of *ADRA2A, ADRB1, ARB2* and *ADRB3* genes relative to *PPIA* are shown and are expressed as mean (SD).

5.4.9 Gene expression of genes involved in insulin signaling in SAT and VAT adipocytes from women with GDM compared to controls

5.4.9.1 INSR

There was higher percentage expression of *INSR* mRNA expression relative to *PPIA* in GDM relative to the control group in SAT (9.0(3.1) vs 11.7(3.7) *INSR* relative to *PPIA* percentage expression, p= 0.031) (Figure 5-25) and VAT (10.8(3.6) vs 15.8(5.6) *INSR* relative to *PPIA* percentage expression, p= 0.022) (Figure 5-26).

5.4.9.2 IRA

There was no difference in the percentage expression of *IRA* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (3.8(1.7) vs 4.1(1.3) *IRA* relative to *PPIA* percentage expression, p= 0.38) (Figure 5-25) or VAT (8.9(2.5) vs 10.0(5.0) *IRA* relative to *PPIA* percentage expression, p= 0.91) (Figure 5-26).

5.4.9.3 IRB

There was no difference in the percentage expression of *IRB* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (21.6(9.8) vs 23.9(8.4) *IRB* relative to *PPIA* percentage expression, p= 0.37) (Figure 5-25) or VAT (25.1(6.9) vs 28.3(12.9) *IRB* relative to *PPIA* percentage expression, p= 0.54) (Figure 5-26).

5.4.9.4 IRS1

There was no difference in the percentage expression of *IRS1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (9.1(3.4) vs 7.3(5.6) *IRS1* relative to *PPIA* percentage expression, p= 0.45) (Figure 5-25) or VAT (7.8(2.9) vs 8.9(10.1) *IRS1* relative to *PPIA* percentage expression, p= 0.71) (Figure 5-26).

5.4.9.5 IRS2

There was no difference in the percentage expression of *IRS2* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (39.8(9.2) vs 47.6(15.5) *IRS2* relative to *PPIA* percentage expression, p= 0.10) (Figure 5-25) or

VAT (49.3(11.4) vs 61.3(47.7) *IRS2* relative to *PPIA* percentage expression, p= 0.97) (Figure 5-26).

5.4.9.6 AKT1

There was no difference in the percentage expression of *AKT1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (18.5(3.5) vs 18.8(3.4) *AKT1* relative to *PPIA* percentage expression, p= 0.59) (Figure 5-25) or VAT (14.7(4.8) vs 19.4(14.6) *AKT1* relative to *PPIA* percentage expression, p= 0.37) (Figure 5-26).

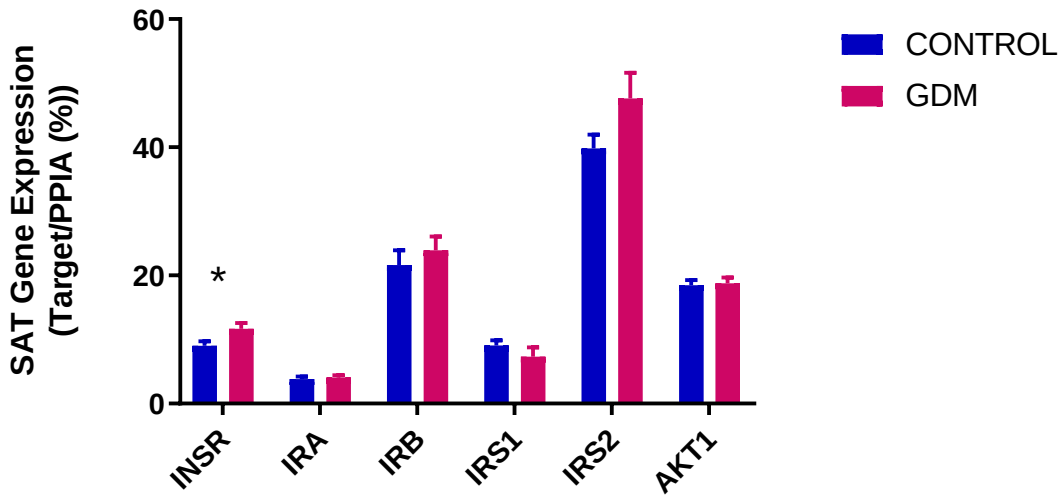


Figure 5-25 Gene expression of insulin signaling genes in SAT adipocytes from controls and GDM group
Insulin receptors genes expression relative to the endogenous control gene PPIA in SAT adipocytes from women with GDM (n=15) compared to control group (n=18) analyzed by RT-qPCR. The percentage expression of *INSR, IRA*, *IRB*, *IRS-1*, *IRS-2* and *AKT1* genes relative to *PPIA* are shown and are expressed as mean (SD).

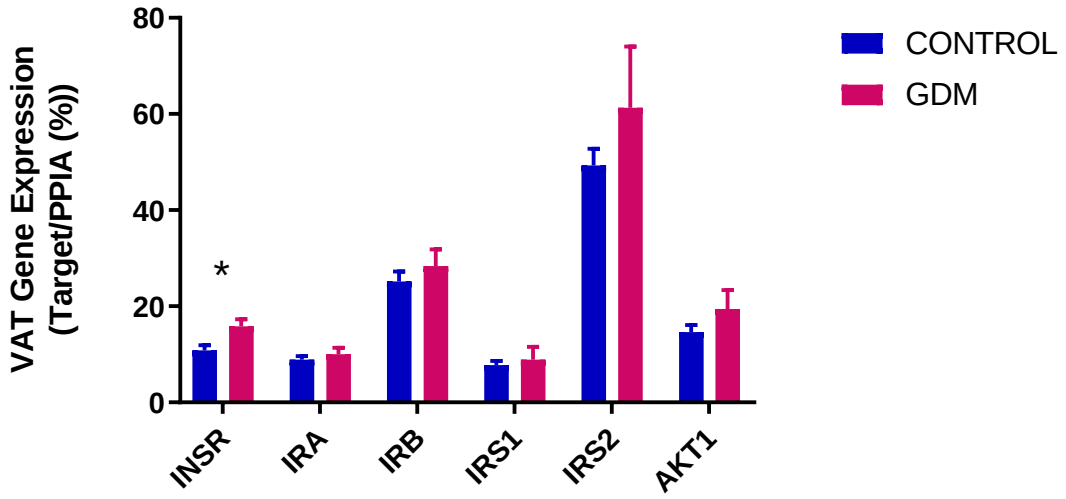


Figure 5-26 Gene expression of insulin signaling genes in VAT adipocytes from controls and GDM group
Insulin receptors genes expression relative to the endogenous control gene PPIA in VAT adipocytes from women with GDM (n=14) compared to control group (n=11) analyzed by RT-qPCR. The percentage expression of *INSR, IRA*, *IRB*, *IRS-1*, *IRS-2* and AKT1 genes relative to *PPIA* are shown and are expressed as mean (SD).

5.4.10 Gene expression of glucose metabolism genes in SAT and VAT adipocytes from women with GDM compared to controls

5.4.10.1 PCK1

There was no difference in the percentage expression of *PCK1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (19.3(33.6) vs 45.9(66.9) *PCK1* relative to *PPIA* percentage expression, p= 0.12) (Figure 5-27) or VAT (2.2(7.0) vs 8.9(27.3) *PCK1* relative to *PPIA* percentage expression, p= 0.11) (Figure 5-28).

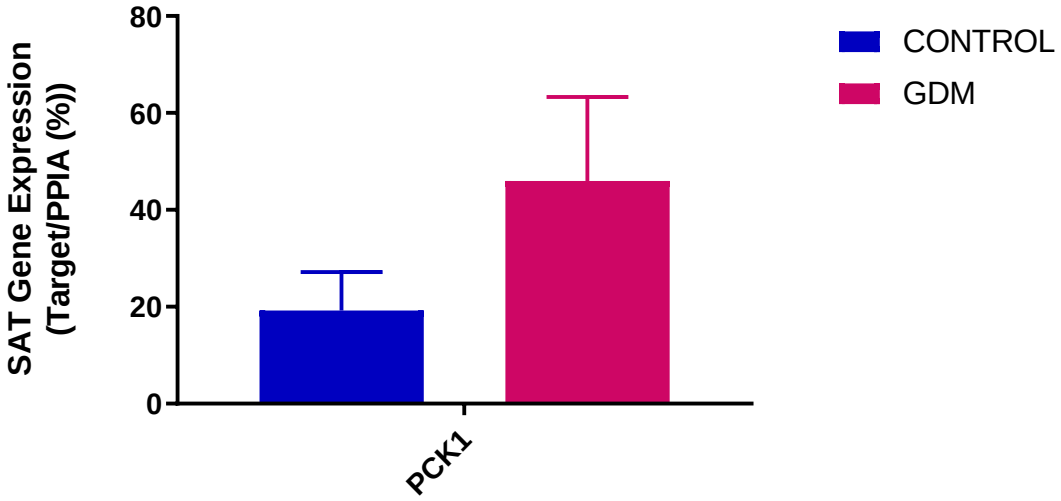


Figure 5-27 Gene expression of glucose metabolism genes in SAT adipocytes from controls and GDM group
Glucose metabolism genes expression relative to the endogenous control gene PPIA in SAT adipocytes from women with GDM (n=15) compared to control group (n=18) analyzed by RT-qPCR. The percentage expression of PCK1 gene relative to *PPIA* are shown and are expressed as mean (SD).

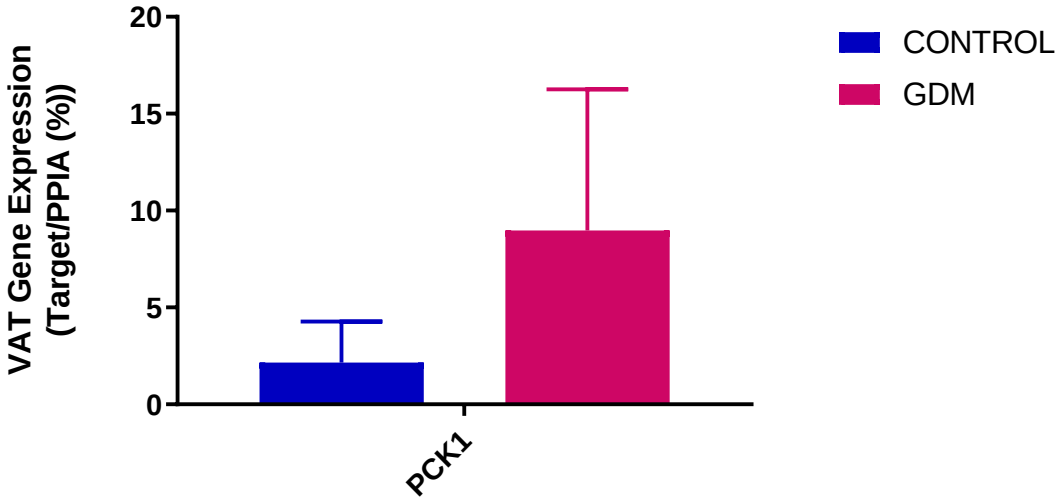


Figure 5-28 Gene expression of glucose metabolism genes in VAT adipocytes from controls and GDM group
Glucose metabolism genes expression relative to the endogenous control gene PPIA in VAT adipocytes from women with GDM (n=14) compared to control group (n=11) analyzed by RT-qPCR. The percentage expression of PCK1 gene relative to *PPIA* are shown and are expressed as mean (SD).

5.4.11 Gene expression of adipocyte differentiation genes in SAT and VAT adipocytes from women with GDM compared to controls

5.4.11.1 IGF-1

There was no difference in the percentage expression of *IGF-1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (74.8(20.1) vs 70.4(20.5) *IGF-1* relative to *PPIA* percentage expression, p= 0.56) (Figure 5-29) or VAT (49.5(24.4) vs 54.0(21.4) *IGF-1* relative to *PPIA* percentage expression, p= 0.65) (Figure 5-30).

5.4.11.2 PPARG

There was no difference in the percentage expression of *PPARG* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (57.9(11.7) vs 57.4(10.5) *PPARG* relative to *PPIA* percentage expression, p= 0.90) (Figure 5-29) or VAT (69.6(14.8) vs 58.8(21.9) *PPARG* relative to *PPIA* percentage expression, p= 0.18) (Figure 5-30).

5.4.11.3 FOS

There was no difference in the percentage expression of *FOS* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (420.1(154.0) vs 387.8(132.2) *FOS* relative to *PPIA* percentage expression, p= 0.65) (Figure 5-29) or VAT (441.9(109.9) vs 429.8(237.4) *FOS* relative to *PPIA* percentage expression, p= 0.20) (Figure 5-30).

5.4.11.4 IGFBP2

There was no difference in the percentage expression of *IGFBP2* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (1.4(1.0) vs 2.4(2.3) *IGFBP2* relative to *PPIA* percentage expression, p= 0.11) (Figure 5-29) or VAT (26.5(12.9) vs 44.1(42.4) *IGFBP2* relative to *PPIA* percentage expression, p= 0.82) (Figure 5-30).

5.4.11.5 IGFBP3

There was no difference in the percentage expression of *IGFBP3* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (20.5(14.1) vs 38.8(38.2) *IGFBP3* relative to *PPIA* percentage expression, p= 0.051) (Figure 5-29) or VAT (56.4(34.8) vs 90.3(84.3) *IGFBP3* relative to *PPIA* percentage expression, p= 0.31) (Figure 5-30).

5.4.11.6 IGFBP5

There was no difference in the percentage expression of *IGFBP5* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (86.4(50.5) vs 87.7(47.4) *IGFBP5* relative to *PPIA* percentage expression, p= 0.91) (Figure 5-29) or VAT (96.5(32.3) vs 123.1(77.1) *IGFBP5* relative to *PPIA* percentage expression, p= 0.55) (Figure 5-30).

5.4.11.7 CEBPB

There was no difference in the percentage expression of *CEBPB* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (60.1(30.1) vs 70.6(27.9) *CEBPB* relative to *PPIA* percentage expression, p= 0.17) (Figure 5-29) or VAT (63.7(35.4) vs 104.9(86.7) *CEBPB* relative to *PPIA* percentage expression, p= 0.24) (Figure 5-30).

5.4.11.8 MAPK8

There was no difference in the percentage expression of *MAPK8* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (1.9(0.4) vs 1.9(0.5) *MAPK8* relative to *PPIA* percentage expression, p= 0.89) (Figure 5-29) or VAT (2.4(0.5) vs 2.3(0.7) *MAPK8* relative to *PPIA* percentage expression, p= 0.61) (Figure 5-30).

5.4.11.9 TGFβ1

There was no difference in the percentage expression of *TGFβ1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (1.4(0.7) vs 1.6(0.7) *TGFβ1* relative to *PPIA* percentage expression, p= 0.45) (Figure 5-29) or VAT

(4.8(2.1) vs 5.3(3.1) *TGFβ1* relative to *PPIA* percentage expression, p= 0.69) (Figure 5-30).

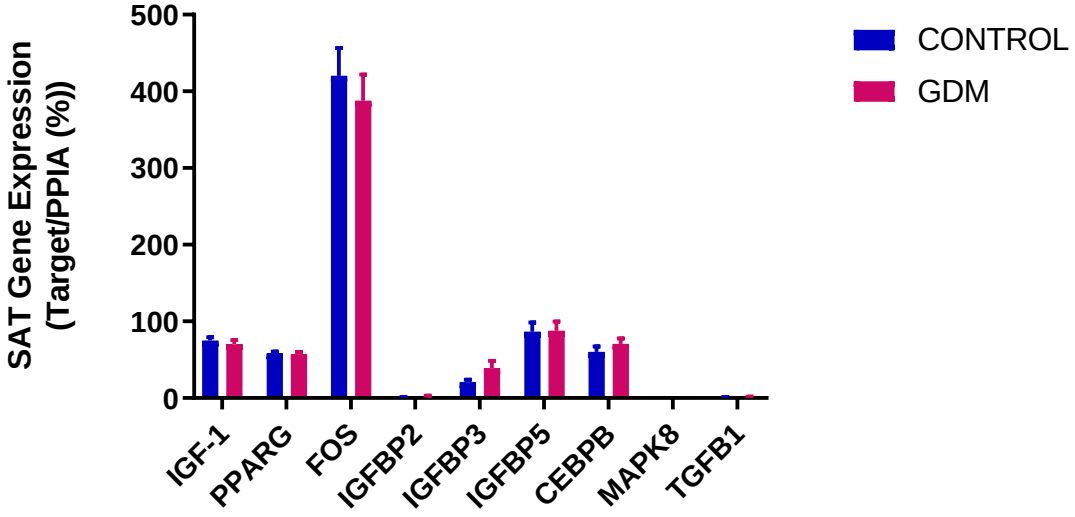


Figure 5-29 Gene expression of adipocytes differentiation genes in SAT adipocytes from controls and GDM group
Adipocyte differentiation genes expression relative to the endogenous control gene PPIA in SAT adipocytes from women with GDM (n=15) compared to control group (n=18) analyzed by RT-qPCR. The percentage expression of *IGF-1, PPARG, FOS, IGFBP2, IGFBP3, IGFBP5, CEBPB, MAPK8, TGFβ1* genes relative to *PPIA* are shown and are expressed as mean (SD).

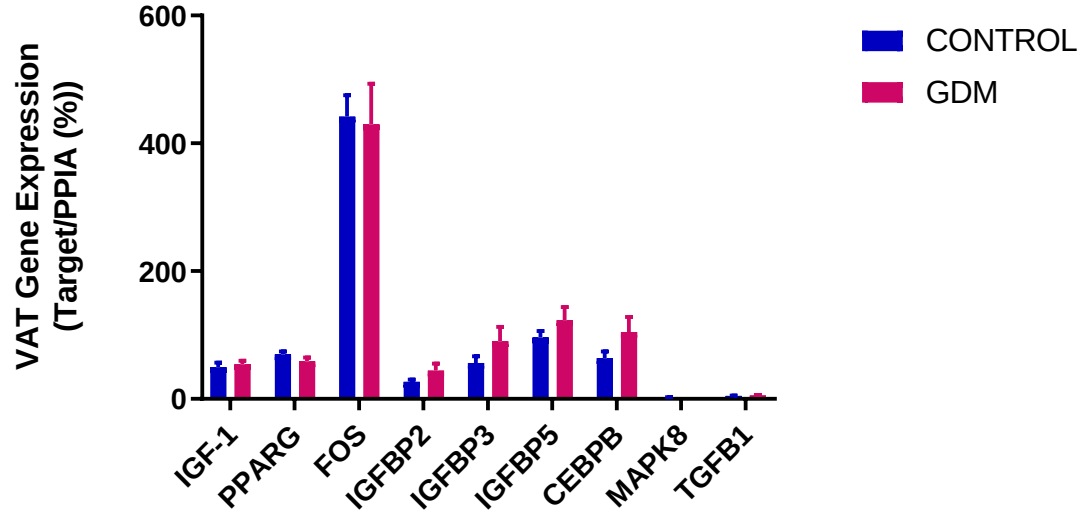


Figure 5-30 Gene expression of adipocytes differentiation genes in VAT adipocytes from controls and GDM group
Adipocyte differentiation genes expression relative to the endogenous control gene PPIA in VAT adipocytes from women with GDM (n=14) compared to control group (n=11) analyzed by RT-qPCR. The percentage expression of *IGF-1, PPARG, FOS, IGFBP2, IGFBP3, IGFBP5, CEBPB, MAPK8, TGFβ1* genes relative to *PPIA* are shown and are expressed as mean (SD).

5.4.12 Gene expression of lipid storage genes in SAT and VAT adipocytes from women with GDM compared to controls

5.4.12.1 SCD

There was no difference in the percentage expression of *SCD* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (577.7(219.7) vs 690.3(297) *SCD* relative to *PPIA* percentage expression, p= 0.38) (Figure 5-31) or VAT (751.1(327.7) vs 579.3(524.1) *SCD* relative to *PPIA* percentage expression, p= 0.17) (Figure 5-32).

5.4.12.2 CIDEC

There was no difference in the percentage expression of *CIDEC* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (420.5(132.0) vs 445.4(141.7) *CIDEC* relative to *PPIA* percentage expression, p= 0.69) (Figure 5-31) or VAT (272.9(94.1) vs 341.7(160.1) *CIDEC* relative to *PPIA* percentage expression, p= 0.31) (Figure 5-32).

5.4.12.3 FOXO1

There was no difference in the percentage expression of *FOXO1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (22.3(7.1) vs 30.5(19.3) *FOXO1* relative to *PPIA* percentage expression, p= 0.25) (Figure 5-31) or VAT (30.7(9.7) vs 33.9(16.8) *FOXO1* relative to *PPIA* percentage expression, p= 0.95) (Figure 5-32).

5.4.12.4 SREBF1

There was no difference in the percentage expression of *SREBF1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (24.7(9.4) vs 20.2(9.5) *SREBF1* relative to *PPIA* percentage expression, p= 0.26) (Figure 5-31) or VAT (22.5(10.9) vs 33.7(49.9) *SREBF1* relative to *PPIA* percentage expression, p= 0.77) (Figure 5-32).

5.4.12.5 LPL

There was no difference in the percentage expression of *LPL* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (394.7(103.1) vs

423.7(141.5) *LPL* relative to *PPIA* percentage expression, p= 0.64) (Figure 5-31) or VAT (382.8(89.2) vs 437.1(279.2) *LPL* relative to *PPIA* percentage expression, p= 0.97) (Figure 5-32).

5.4.12.6 CD36

There was no difference in the percentage expression of *CD36* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (317.5(71.1) vs 320.3(83.8) *CD36* relative to *PPIA* percentage expression, p= 0.92) (Figure 5-31) or VAT (263.4(71.5) vs 247.9(103.0) *CD36* relative to *PPIA* percentage expression, p= 0.69) (Figure 5-32).

5.4.12.7 SLC2A1

There was no difference in the percentage expression of *SLC2A1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (2.5(0.9) vs 3.9(3.4) *SLC2A1* relative to *PPIA* percentage expression, p= 0.14) (Figure 5-31) or VAT (7.9(4.6) vs 10.4(7.6) *SLC2A1* relative to *PPIA* percentage expression, p= 0.62) (Figure 5-32).

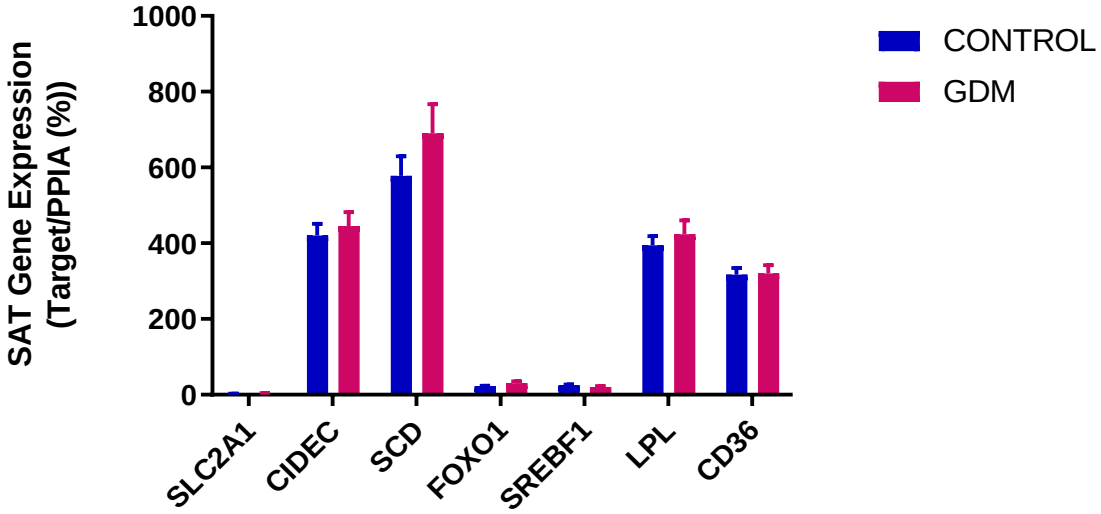


Figure 5-31 Gene expression of lipid storage genes in SAT adipocytes from controls and GDM group
Lipid storage genes expression relative to the endogenous control gene PPIA in SAT adipocytes from women with GDM (n=15) compared to control group (n=18) analyzed by RT-qPCR. The percentage expression of *SCD, SLC2A1, CIDEC, FOXO1, SREBF1, LPL* and *CD36* genes relative to *PPIA* are shown and are expressed as mean (SD).

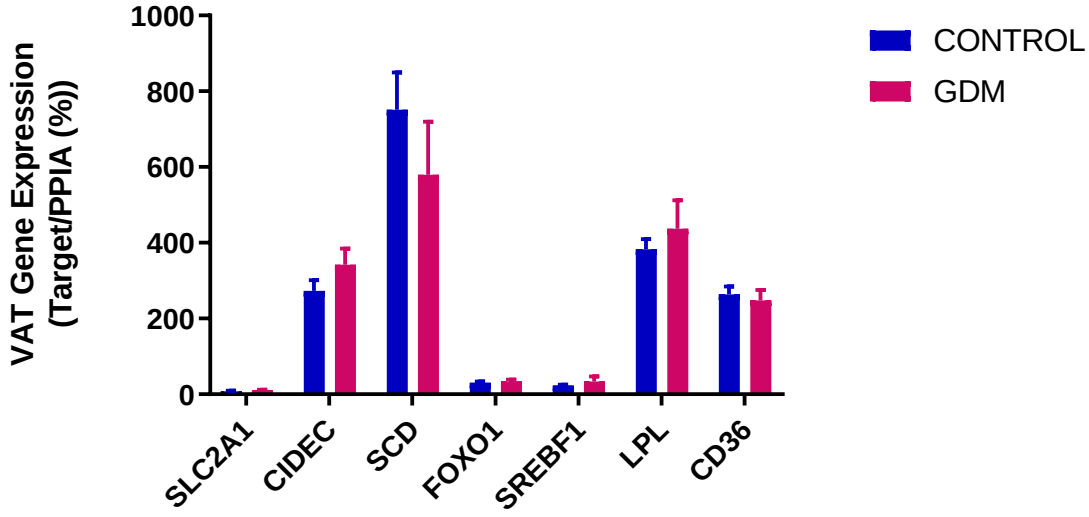


Figure 5-32 Gene expression of lipid storage genes in VAT adipocytes from controls and GDM group
Lipid storage genes expression relative to the endogenous control gene *PPIA* in VAT adipocytes from women with GDM (n=14) compared to control group (n=11) analyzed by RT-qPCR. The percentage expression of *SCD, SLC2A1, CIDEC, FOXO1, SREBF1, LPL and CD36* genes relative to *PPIA* are shown and are expressed as mean (SD).

5.4.13 Gene expression of angiogenesis genes in SAT and VAT adipocytes from women with GDM compared to controls

5.4.13.1 VEGFA

There was no difference in the percentage expression of *VEGFA* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (71.7(51.7) vs 90.4(87.9) *VEGFA* relative to *PPIA* percentage expression, p= 0.34) (Figure 5-33) or VAT (169.4(85.6) vs 166.4(90.5) *VEGFA* relative to *PPIA* percentage expression, p= 0.88) (Figure 5-34).

5.4.13.2 ICAM1

There was no difference in the percentage expression of *ICAM1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (8.8(5.8) vs 13.2(12.3) *ICAM1* relative to *PPIA* percentage expression, p= 0.46) (Figure 5-33) or VAT (36.8(23.8) vs 48.4(38.5) *ICAM1* relative to *PPIA* percentage expression, p= 0.99) (Figure 5-34).

5.4.13.3 NOS3

There was no difference in the percentage expression of *NOS3* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (0.5(1.5) vs 0.2(0.2)

NOS3 relative to *PPIA* percentage expression, p= 0.30) (Figure 5-33) or VAT (0.8(0.8) vs 1.12(1.04) *NOS3* relative to *PPIA* percentage expression, p= 0.62) (Figure 5-34).

5.4.13.4 PTGS1

There was no difference in the percentage expression of *PTGS1* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (1.0(0.7) vs 1.9(2.5) *PTGS1* relative to *PPIA* percentage expression, p= 0.62) (Figure 5-33) or VAT (0.9(0.6) vs 0.8(0.7) *PTGS1* relative to *PPIA* percentage expression, p= 0.58) (Figure 5-34).

5.4.13.5 F3

There was no difference in the percentage expression of *F3* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (25.5(6.9) vs 31.2(17.5) *F3* relative to *PPIA* percentage expression, p= 0.80) (Figure 5-33) or VAT (58.9(19.2) vs 58.5(18.9) *F3* relative to *PPIA* percentage expression, p= 0.62) (Figure 5-34).

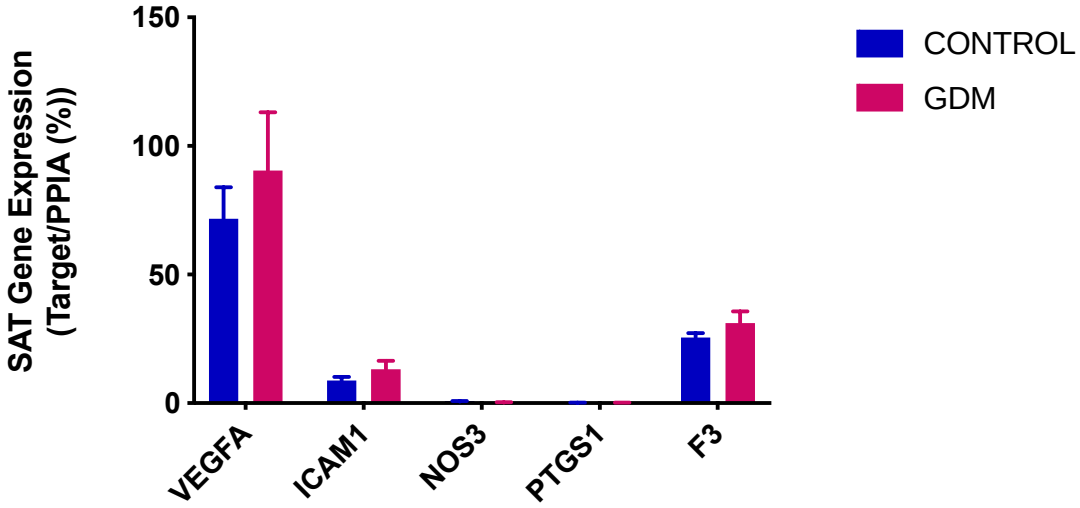


Figure 5-33 Gene expression of angiogenesis genes in SAT adipocytes from controls and GDM group
Angiogenesis genes expression relative to the endogenous control gene PPIA in SAT adipocytes from women with GDM (n=15) compared to control group (n=18) analyzed by RT-qPCR. The percentage expression of *VEGFA, ICAM1, NOS3, PTGS1* and *F3* genes relative to *PPIA* are shown and are expressed as mean (SD).

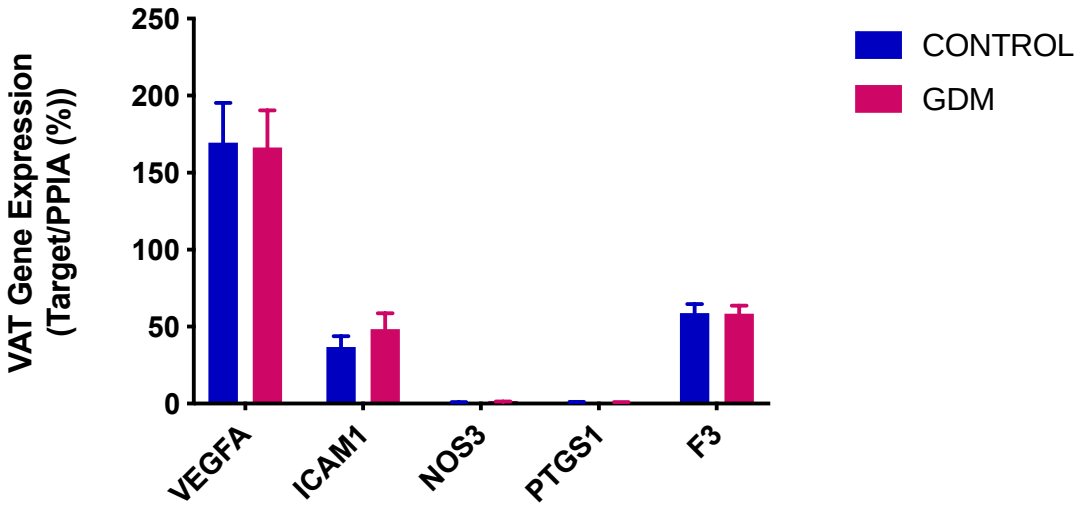


Figure 5-34 Gene expression of angiogenesis genes in VAT adipocytes from controls and GDM group
Angiogenesis genes expression relative to the endogenous control gene PPIA in VAT adipocytes from women with GDM (n=14) compared to control group (n=11) analyzed by RT-qPCR. The percentage expression of *VEGFA, ICAM1, NOS3, PTGS1* and *F3* genes relative to *PPIA* are shown and are expressed as mean (SD).

5.4.14 Gene expression of apoptosis genes in SAT and VAT adipocytes from women with GDM compared to controls

5.4.14.1 DDIT3

SAT mRNA expression of *DDIT3* was down regulated in the GDM compared to control group (6.8(3.2) vs 5.3(3.3) *DDIT3* relative to *PPIA* percentage expression, p= 0.041) (Figure 5-35). There was no difference in the percentage expression of *DDIT3* mRNA expression relative to *PPIA* between GDM and control groups in VAT (7.5(3.4) vs 7.8(8.1) *DDIT3* relative to *PPIA* percentage expression, p= 0.20) (Figure 5-36).

5.4.14.2 CASP3

There was no difference in the percentage expression of *CASP3* mRNA expression relative to *PPIA* between GDM and control groups in either SAT (3.7(0.5) vs 3.9(1.3) *CASP3* relative to *PPIA* percentage expression, p= 0.48) (Figure 5-35) or VAT (4.0(0.9) vs 4.2(2.1) *CASP3* relative to *PPIA* percentage expression, p= 0.72) (Figure 5-36).

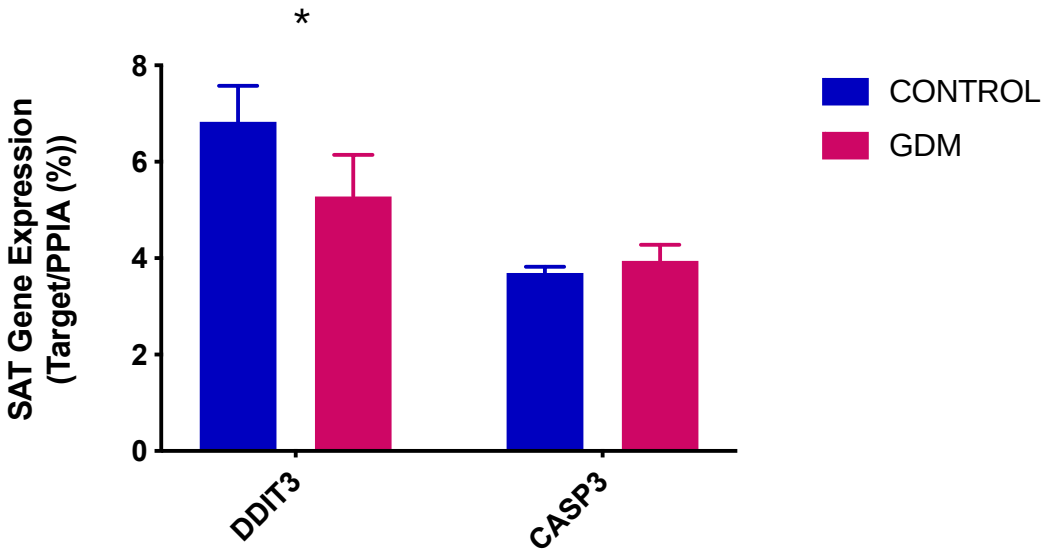


Figure 5-35 Gene expression of angiogenesis genes in SAT adipocytes from controls and GDM group
Apoptosis genes expression relative to the endogenous control gene PPIA in SAT adipocytes from women with GDM (n=15) compared to control group (n=18) analyzed by RT-qPCR. The percentage expression of *DDIT3* and *CASP3* genes relative to *PPIA* are shown and are expressed as mean (SD).

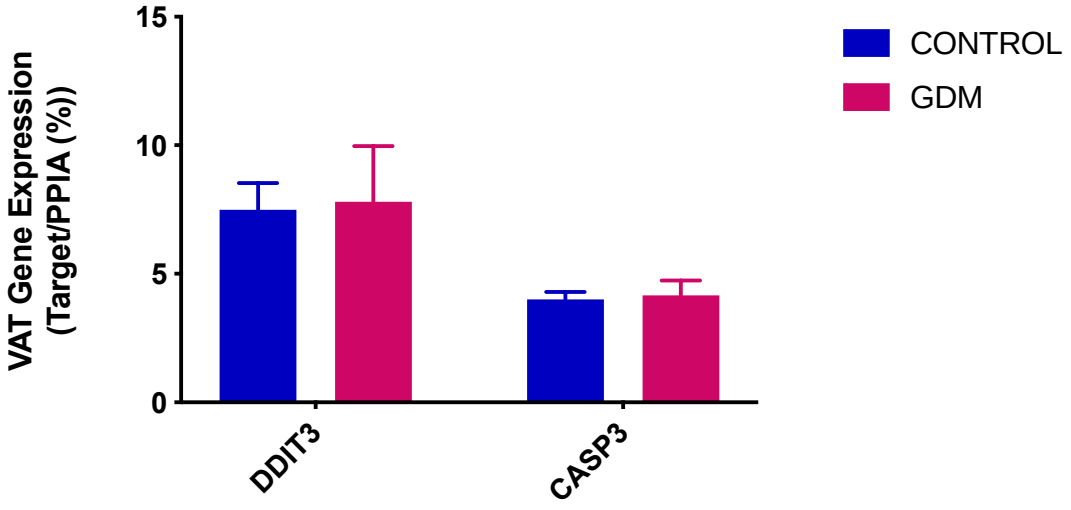


Figure 5-36 Gene expression of apoptosis genes in VAT adipocytes from controls and GDM group
Apoptosis genes expression relative to the endogenous control gene PPIA in VAT adipocytes from women with GDM (n=14) compared to control group (n=11) analyzed by RT-qPCR. The percentage expression of *DDIT3* and *CASP3* genes relative to *PPIA* are shown and are expressed as mean (SD).

5.5 Discussion

The key observation of this study is that VAT adipocytes from women with GDM are less responsive to LPS stimulation in terms of NEFA release compared to SAT adipocytes in both GDM and control groups. However, against our hypothesis, there was no evidence for higher VAT adipocytes inflammatory adipokine release in either basal conditions or in response to LPS in GDM compared to controls. On the

contrary, there was higher release of adiponectin, an anti-inflammatory agent, from VAT adipocytes in response to LPS in women with GDM compared to controls. Consistent with the adipokine secretion from VAT adipocytes findings, there was no evidence for differential inflammatory gene expression in either SAT or VAT adipocytes from GDM compared to controls. Similarly, other genes involved in adipocyte differentiation, lipid storage, lipid and glucose metabolism and angiogenesis gene expression were not different in GDM compared to control. However, there was a higher expression of the insulin receptor gene INSR in both SAT and VAT adipocytes from women with GDM compared to controls. In addition, the apoptosis gene DDIT3 was downregulated in SAT adipocytes from women with GDM compared to controls.

In this study, VAT adipocyte inflammatory cytokine release was not responsive to LPS stimulation in either GDM or controls. It has previously been observed in lean non-pregnant women that SAT explants demonstrated a greater sensitivity to LPS induced NF-κB activation whereas VAT explants were only mildly responsive to LPS (Vatier et al., 2012). Higher SAT sensitivity to LPS led to reduced NEFA re-esterification and increased FA release, potentially resulting in the expansion of VAT and ectopic fat deposition in other organs (Vatier et al., 2012). Furthermore, the expression of TLR4 in adipocytes from different depots was not studied previously. The lack of response to LPS in VAT adipocytes may indicate a lack of TLR4 receptors. Our previous study in PE showed that VAT adipocytes were responsive to LPS stimulation in terms of higher release of TNF-α and IL-6 in PE compared to controls, a phenomena that were not seen in SAT adipocytes in PE (Huda et al., 2017). A potential explanation could be that in PE SAT adipocytes are insulin resistant (Huda et al., 2014) leading to VAT expansion and progression of inflammatory VAT adipocytes demonstrating higher sensitivity to LPS and possibly contributing to ectopic fat. However, in VAT adipocytes of GDM women, we have not observed higher release of inflammatory adipokines in response to LPS. It could be that GDM is a metabolic complication of milder form than PE affecting glucose tolerance and increased VAT adipocyte release of FA, rather than inflammatory adipokines. However, this conclusion should be drawn with caution due low sample number in our study and there was no difference in the metabolic parameters between the healthy and GDM group including plasma glucose level and HOMA-IR. Therefore, more sample size is needed. Although TNFα secretion in response to LPS was one-fold higher in GDM this was not statically significant and was 88%

powered. However, there was higher release of adiponectin in response to LPS in GDM compared to controls. This release was not correlated with adipocyte diameter and does not suggest its related to hypertrophied VAT adipocytes. Probably, the higher adiponectin release from VAT adipocytes in our cohort can be related to the treatment of GDM with metformin or insulin. Metformin has anti-inflammatory properties which are found to be exerted irrespective of the diabetes status (Cameron et al., 2016). Furthermore, insulin, another GDM treatment, was found to antagonize IL-6 signaling and it has anti-inflammatory effect on 3T3-L1 adipocytes (Andersson et al., 2007). Adipokine release from VAT adipocytes was not correlated with maternal BMI, HOMA-IR, adipocyte size and lipolytic function.

The lack of any differences in gene expression of inflammatory genes is consistent with our observation of lack of differences in VAT adipocytes adipokine release of women with GDM compared to controls. There was no difference in SAT and VAT adipocytes inflammatory gene expression including TNF between GDM and controls . Dong et al. (2018) have observed increased macrophage infiltration and gene expression of TNFα from VAT explants in women with GDM compared to pregnant healthy controls. Similarly, Rancourt et al. (2020) recently have shown increased gene expression of the inflammatory markers TNFα and suppressor of cytokine signaling (SOCS3) in VAT tissue from women with GDM compared to healthy pregnant controls. However, the previous studies used whole adipose tissue samples rather than isolated adipocytes which are confounded by the demonstrated increased VAT macrophage infiltration in GDM. Contrary to our results, increased gene expression of IL-6 was demonstrated previously in SAT tissue samples in women with GDM compared to controls (Kleiblova et al., 2010).

Lipolytic activity *in vitro* and *in vivo* depends on the functional efficiency of lipolytic and antilipolytic regulators of lipolysis such as lipases and adrenoreceptors. Expression of the lipolysis enzymes *ATGL* and *LIPE* were not different between GDM and controls in both SAT and VAT adipocytes. Similarly, gene expression of lipolytic adrenoreceptors (*ADRB1* and *ADRB2*) and the antilipolytic adrenoreceptor (*ADRA2A*) were not different between GDM and controls in both SAT and VAT adipocytes. However, although the gene expression of *ADRB3* was one-fold lower in VAT adipocytes in GDM compared to controls, this was not statically significant with 78% power. These results did not explain the observed higher VAT adipocytes basal lipolysis in GDM compared to controls (Chapter 4), however, other changes

at protein or receptor activity level cannot be ruled out. Furthermore, other underlying mechanisms such as the role perilipin1 and other lipid droplet associated proteins have not explored in this project. Gene expression of perilipin1 was not measured in this study and would be of interest to find out if its expression is related to higher VAT adipocyte lipolysis in GDM compared to controls.

In the insulin signalling pathway, there was higher expression of the insulin receptor gene *INSR* in SAT and VAT adipocytes in GDM compared to controls. Then, using the method described by Huang et al. (2011) I designed probes to measure the two insulin receptor isoforms *IRA* and *IRB* gene expression in order to have a better understanding of whether there is a role for a specific isoform in the observed increase in *INSR* expression in SAT and VAT adipocytes in GDM. There was no difference in IRA and IRB gene expression in GDM compared to controls in both SAT and VAT adipocytes. This is suggesting there is total increase in insulin receptors in SAT and VAT adipocytes from women with GDM compared to controls without specific increase in one of the insulin receptors isoforms. The higher expression of insulin receptors in both SAT and VAT adipocytes in women with GDM could be an adaptive mechanism to the accelerated insulin resistance especially for IR-B. Furthermore, this possibly could explain why we did not find evidence for VAT adipocytes insulin resistance (FCISI) in our cohort in the previous chapter. We also cannot rule out the effect of GDM treatment among our treated cohort. The insulin sensitizing effects of both metformin (Cameron et al., 2016) and insulin (Das, 2001) could contribute to improvement of adipocyte insulin sensitivity and availability of insulin receptors. Alterations in INSR splicing are associated with insulin resistance and T2DM (Sesti et al., 1991). However, other studies did not show any significant alterations in the *IRA* and *IRB* expression in various conditions of insulin resistance (Moller et al., 1989). The role of the distinct insulin receptors isoforms in the function of human adipose tissue is not yet fully understood. Gene expression of insulin receptors substrates *IRS1* and *IRS2* was not different in either SAT or VAT adipocytes in GDM compared to control group. However, we did not measure the protein level of IRS1 which has been reported previously to be 43% lower in abdominal SAT tissue from women with GDM compared to healthy pregnant controls (Catalano et al., 2002).This reduction in IRS1 expression was suggested to be responsible for the failure of insulin to suppress lipolysis as well as reduced insulin-stimulated glucose transport in GDM in adipose tissue. Similarly, in isolated SAT adipocytes from T2DM patients, IRS-1 protein expression is markedly reduced

in SAT adipocytes, resulting in decreased IRS-1-associated PI3-kinase activity (Rondinone et al., 1997).

Previous studies have shown that the roles of PCK1 in white adipose tissue in glyceroneogenesis and re-esterification of FFA to generate TAG are vital for the prevention of T2DM (Millward et al., 2010). Glyceroneogenesis is the de novo synthesis of glycerol-3-phosphate from gluconeogenic precursors such as pyruvate, alanine and lactate. Glyceroneogenesis is considered to be a shorter version of gluconeogenesis that is induced by fasting in both liver and adipose tissue. Each glycerol-3-phosphate product is used to esterify three fatty acyl-CoAs to form a molecule of TAG as explained in the introduction Chapter. The function of adipose tissue glyceroneogenesis is to regulate the release of FAs via a TAG/FA cycle within the adipocyte. In the current study, PCK1 gene expression demonstrated a trend for higher expression in SAT and VAT adipocytes from GDM compared to controls but this was not a statically significant difference in both SAT and VAT. *PCK1* encodes an essential enzyme involved in the NEFA re-esterification (glyceroneogensis). Increased expression of *PCK1* decrease serum FA while increasing stored TAG and promote obesity. In T2DM, regions near the PCK1 locus on chromosome 20 have also been implicated in obesity. Furthermore, mice that overexpress *PCK1* in adipocytes have reduced circulating FFA levels and normal insulin sensitivity, which has been associated with increased fat depots due to enhanced FA re-esterification (Franckhauser et al., 2002). The gene expression of the PCK1 was found to be reduced in SAT and VAT explants from lean women in response to LPS induced inflammation consequently, the FA release during lipolysis was higher, SAT was far more sensitive than VAT (Vatier et al., 2012).

Although the results from the expression analysis of genes involved in adipocyte differentiation present no statistically significant difference to support our hypothesis of failure of adipocytes differentiation in GDM, it should be considered that by investigating mature adipocytes which are terminally differentiated, as we did in our experiment, we may be investigating the wrong stage of the cell. Deficiencies in potential for differentiation could be detected in preadipocytes which are present in the stromal vascular fraction of the adipose tissue explants. Decreased preadipocyte differentiation capacity has been demonstrated in in T2DM (van Tienen et al., 2011). To evaluate whether the adipocyte differentiation process is impaired in GDM and limiting the hyperplastic expansion of the adipose tissue, it will

be more valuable to assess preadipocyte gene expression analysis and *in vitro* differentiation capacity in future studies.

Gene expression of adipogenic and angiogenic genes was not different in both SAT and VAT adipocytes in GDM compared to controls. Apoptosis gene expression DDIT3 was lower in SAT adipocytes from women with GDM compared to controls. Several autophagic genes were found to be down regulated during obesity in non-pregnant population including Atg7, Atg5 and Ulk1. AMPK positively regulate autophagy to decrease the accumulation of mitochondrial ROS by increased clearance of dysfunctional mitochondria (Egan et al., 2011). Saturated FA decrease AMPK activity leading to defective autophagy response and increased ROS in adipocytes (Wen et al., 2011). Thus, the measurement of ROS in the current study isolated adipocytes will be an interesting follow on study.

This study main limitation was the small sample size especially for subgroup analysis when we had less than 50% of the proposed n=30 per group. There was an inherent difficulties in VAT tissue collection such as the closure of wound by the surgeon before remembering to take the VAT samples in the way out. Furthermore, this study was limited by the measurement of adipokine release from one depot (VAT adipocytes) rather than both depots (SAT and VAT adipocytes). This was due to limited resources. SAT adipocytes adipokine release would be of a great interest to reflect if there is underlying SAT adipocyte inflammation. Furthermore, we used a GDM treated population therefore, our adipokine release and gene expression results could be confounded by this. However, the number of GDM women at each treatment category (diet only, metformin only, insulin only and both metformin and insulin) were too small to look at statically meaningful analysis for treatment effect. Alternatively, we could have used women treated with diet only, but this would have lengthen the time period for tissue collection. With regards to gene expression analysis, microarray analysis would be of great interest to this project, but we were again limited by resource because of the requirement form pre-amplification of cDNA. therefore, Ingenuity Pathway Analysis to look for novel genes was chosen as an alternative approach. Collection of placenta and cord blood samples would have enhanced our results to look at other potential source of inflammation in GDM other than adipose tissue but this was not accessible during this project due to limited personnel available to handle fresh samples.

A particular strength of the present study that to the best of our knowledge this is the first study that examined isolated VAT adipocytes adipokine release in both basal and LPS stimulated conditions. Furthermore, I used isolated VAT adipocytes rather than whole adipose tissue samples to examine adipocyte *per se* contribution to the hypothesized adipose tissue inflammation in women with GDM. The multiplex technique was used to look at several adipokines using small volume rather than using ELISA to look at different adipokine release which requires larger volume which might limit the adipokines we are able to investigate. The differential expression of insulin receptors isoforms IRA and IRB has not previously been studied in adipocytes from women with GDM.

In conclusion, although our data present no evidence for VAT adipocytes inflammation in women with GDM, however, we had a small sample size in this subgroup analysis which might limit our ability to detect significant differences. SAT adipocytes adipokines secretion was not studied yet, but gene expression analysis did not show that there could be SAT adipocytes contribution to inflammation in GDM. Other potential mechanism than inflammation could be underlying adipose tissue dysfunction in women with GDM. However, other potential sites such as placenta could be contributing to the observed higher inflammatory markers in GDM (Feng et al., 2016).

Chapter 6 Maternal VLDL lipid and fatty acid composition in early pregnancy

6.1 Introduction

The long chain polyunsaturated fatty acids (LC-PUFA), particularly docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) are of particular importance in fetal neurological development (Sattar et al., 1998). AA is particularly important for the synthesis of eicosanoids, which are involved in the development of the fetal nervous, visual, and immune systems (Duttaroy, 2009). DHA is a key membrane component of nervous tissue (Parletta et al., 2013). DHA is highly concentrated in the fetal brain and retina and is essential for the development of the fetal nervous and visual system (Duttaroy, 2009). The importance of maternal DHA mobilisation at latter stages of pregnancy are well established as demonstrated by the link between premature birth and neurodevelopmental disorders (Johnson et al., 2015).

During pregnancy, maternal transport of DHA to the rapidly growing fetus via the placenta is not very well understood (Freeman and Meyer, 2017). Knowing that the first primitive fetal brain cells are formed around 15 days of gestation and that neural tube closure occurs at 28 days of gestation, early DHA availability for the growing fetus appears to be very critical. The maternal-fetal circulation is established around 9-13 weeks of gestation to allow transport of nutrients to the growing fetus. The placenta has been shown to preferentially transport DHA from the maternal plasma to the fetus, suggesting an important role for DHA in fetal development (Al et al., 1995). However, the precursors of LC-PUFA, linoleic acid (LA, 18:2n6) and alpha-linolenic acid (ALA, 18:3n-3) are at best minimally transferred to the fetus from maternal plasma (De Groot et al., 2004).

Essential fatty acids derived from maternal diet are transported as TAG in TAG-rich lipoproteins in maternal plasma. There is no direct placental transfer of maternal lipoproteins and essential FA need to be made available to the fetus (Herrera et al., 2006). The presence of the scavenger-receptors for VLDL, LDL and HDL allows these lipoproteins to be taken up by the placenta. In addition, placental tissue expresses LPL activity which may release FA from maternal plasma lipoproteins (Haggarty, 2002). Subsequently, the constituent FA of maternal TAG in plasma

lipoproteins are re-esterified to synthesize glycerolipids in the fetal circulation (Coleman and Haynes, 1987). Subsequent hydrolysis of glycerolipids releases FA into fetal plasma, where they bind to the α-fetoprotein and are transported to the fetal liver. Although LC-PUFAs may be taken up by the syncytiotrophoblast via passive diffusion, the placental plasma membrane fatty acid binding protein (pFABPpm), which has a preferential affinity for AA and DHA is also implicated in this transport (Araújo et al., 2013). Moreover, intracellular fatty acid binding proteins (FABPs) are responsible for guiding FA to their specific intracellular locations (Duttaroy, 2009). The exact mechanisms by which these protein carriers facilitate the transcellular transport of LC-PUFAs remain not well understood.

Luteinizing hormone (LH) is hormone secreted by the gonadotropic cells in the anterior pituitary gland. LH acute rise (LH surge) in females trigger ovulation. The LH surge biologically indicates day zero of pregnancy whereas the clinical day zero of pregnancy is taken as the day of the last menstrual period, approximately 14 days prior to the LH surge. It was previously shown that the plasma concertation of DHA was uniquely increased, compared to other LC-PUFA, prior to 28 day of gestation (between 18 and 29 days post-LH surge, in women who had a successful pregnancy after frozen embryo transfer (FET) (Meyer et al., 2016). This higher DHA concentration coincides with the time of neural tube closure at early pregnancy. Maternal erythrocyte DHA content, an indicator of maternal DHA plasma concentration over the previous three months, was found to be increased by 17% at the end of the first trimester (Stewart et al., 2007a). Thus, maternal DHA concentrations in plasma and erythrocytes are increased by the end of the first trimester and are available for placental transport to the fetus. This could potentially be very important for early fetal neurological development.

The early increase in maternal plasma DHA concentration could be achieved either through increased DHA release from maternal stores, increased DHA *de novo* synthesis in the liver or from increased dietary consumption. There are several observations from the Meyer et al. (2016) study that suggest there is an increased synthesis of DHA from its precursors (ALA) at the critical time of neural tube closure. Firstly, the increase in DHA concentration between 18 and 29 days post-LH surge was correlated positively with an index of delta-6 desaturase activity (20:3n6/ 18:2n6 ratio) during this time point only. Secondly, there was a significant 10% reduction in plasma LA, the precursor for the n-6 series of LC-PUFA, in those women who

became pregnant after FET but not in those who did not, from pre-pregnancy to 18 days post-LH surge. Together these findings suggest an increased ability to convert ALA to DHA in very early pregnancy.

The main plasma carrier that delivers LC-PUFA in the maternal circulation to the placenta is not yet known. DHA is primarily transported on HDL particles in the non-pregnant population (Augustine et al., 2014). However, during pregnancy, there is a three-fold increase in VLDL synthesis by the liver (Huda et al., 2009). Moreover, the liver is the primary site for *de novo* LC-PUFA synthesis which is suggested to be increased in early pregnancy (Meyer et al., 2016). Thus, VLDL may be an important transporter of plasma LC-PUFA during pregnancy. I hypothesised that in pregnancy, as compared to the non-pregnant state, DHA is carried in higher concentration than other FA in the VLDL lipoprotein fraction. The assessment of maternal FA changes from pre-pregnancy to the first weeks of gestation is difficult in a free-living population. Therefore, these changes were assessed in a population of women undergoing natural cycle-frozen embryo transfer as a means of achieving accurately timed periconceptual sampling.

6.2 Aim

The aim of this study was to determine the distribution of fatty acids, particularly DHA, in the VLDL plasma fraction prior to pregnancy and throughout early gestation.

6.2.1 Hypothesis

1. That pregnant women have higher VLDL particle number compared to non-pregnant controls.

2. That pregnant women have VLDL enriched with TAG compared to non-pregnant controls.

3. That maternal VLDL is enriched in DHA relative to other FA.

6.2.2 Specific research questions

- Is there is an early pregnancy increase in VLDL particle number and/or TAG enrichment?

- How is VLDL composition related to plasma pregnancy hormone concentrations?

- How does VLDL FA composition change in early pregnancy?

- Is there DHA enrichment of VLDL in early pregnancy?

6.3 Methods

This study utilized plasma samples from an archival collection of women undergoing frozen embryo transfer (FET) (Meyer et al., 2016) as discussed in the Methods Chapter sections 2.8.1 and 2.8.2. Fasting blood samples were collected approximately day 10 after last menstrual period (LMP) (pre-LH surge) and on days 18, 29, and 45 post-LH surge. Plasma was collected by low-speed centrifugation and frozen at -80°C within 2 hours. At day 18 post-LH surge the women who were not successful in getting pregnant tended to not come for more study visits and withdrew from the study. Therefore, there were no blood samples for non-pregnant women at days 29 and 45 post-LH surge. VLDL isolation was carried out by sequential density ultracentrifugation as outlined in the Methods Chapter section 2.8.2.2. VLDL lipoprotein fraction triglyceride (TAG), phospholipid (PL), total cholesterol (TC), free cholesterol (FC), apo-B and cholesteryl ester (CE), were analysed by autoanalyzer as outlined in Methods Chapter section 2.8.2.3. Apo-B is an indicator of VLDL particle number because each VLDL particle contains a single molecule of apo-B. VLDL TAG/Apo-B ratio and VLDL CE/Apo-B ratio (VLDL TAG and CE concentrations divided by VLDL Apo-B concentration respectively) were calculated to express TAG and CE content by VLDL particle number. FA extraction was carried out as outlined in the Methods Chapter section 2.8.3. VLDL FA composition was analysed by flame-ionization gas chromatography as outlined in the Methods Chapter section 2.8.4.

Data were tested for normal distribution using a Ryan Joiner test and log or square root transformed when necessary. Values for continuous variables are given as mean (SD) or number (%) for categorical variables. Differences in VLDL apo-B concentration, VLDL TG/Apo-B ratio and VLDL CE/Apo-B ratio over time were assessed by one-way ANOVA for repeated measures, with between group comparison using *post hoc* Tukey-Kramer honest significant difference (HSD) test.

The rate of change of fatty acids between time points was calculated as the difference between the fatty acid concentration at the second time point and at the first time point divided by the difference in time (days) between the two time points. The results are expressed as nmol/mL plasma per day. Incremental area under the concentration (pre-LH surge to 45 d post-LH surge) x time curve (iAUC) were calculated using the trapezium method for FA change in concentration (Matthews et al., 1990). The results are expressed as nmol/mL x day. Differences in concentrations of fatty acids over time were assessed by one-way ANOVA for repeated measures, with between-group comparison using post-hoc Tukey-Kramer HSD. Statistical analysis was performed using Minitab version 18.

6.4 Results

6.4.1 Study participants

A total of 196 FET cycles were started in the original study cohort (Meyer et al., 2016), of which 161 were completed, from which there were 38 pregnancies: two were excluded as being a repeat attempt at FET treatment within the study and nine were excluded as they did not have complete data for all visits; therefore, 27 pregnant women were included. Of the 161 FET cycles completed, there were 123 failed pregnancies, of which 35 had full fatty-acid data (Figure 6-1). Frozen plasma aliquots were available from these n=27 pregnant women and n=35 non-pregnant women and were used for the current analysis.

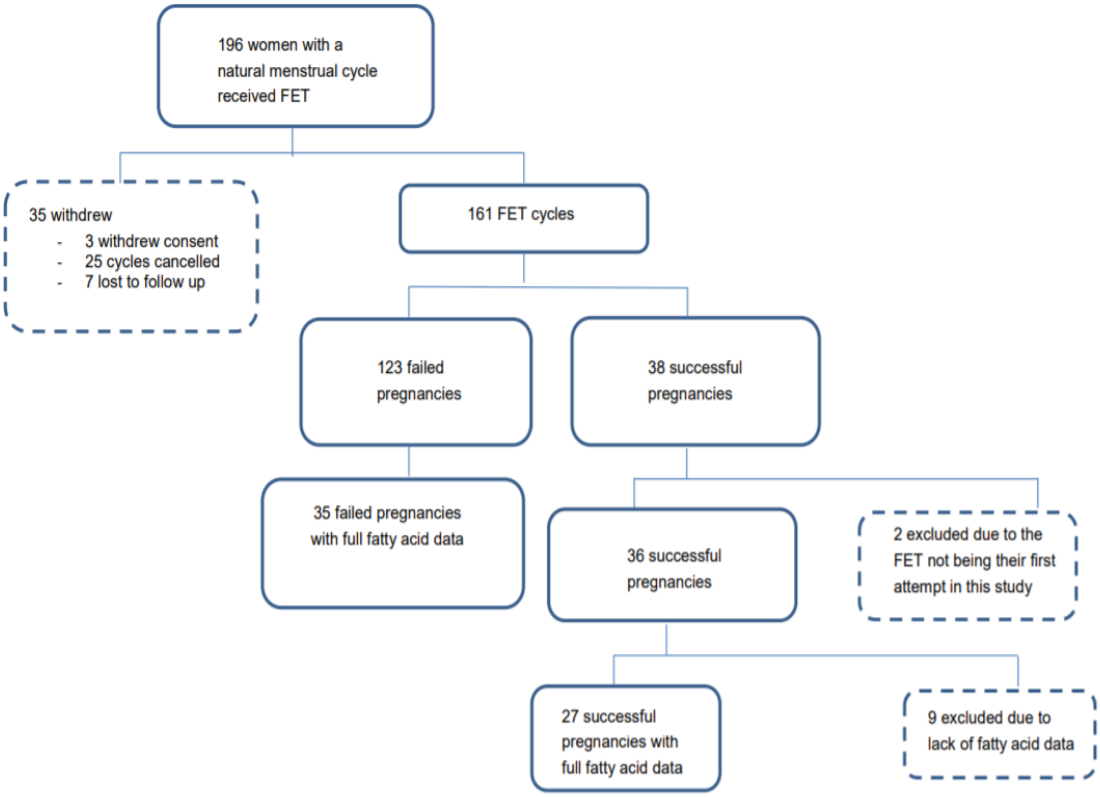


Figure 6-1 Consort diagram of the study
Consort diagram illustrating women enrolled in the study, showing unsuccessful and successful pregnancies as well as reasons for withdrawal and exclusions. Taken with the publisher permission (pending) from (Meyer et al., 2016)

The demographic characteristics of the 27 pregnant women and 35 women not successful in getting pregnant are shown in Table 6-1. There were no significant differences between the two groups of pregnant and non-pregnant women entering the study.

Table 6-1 Demographic characteristics of the study participants

Demographic	Pregnant (n=27)	non-pregnant (n=35)	P-value
Age at FET (years)	34.5(4.1)	33.6(5.2)	0.81
BMI (kg/m^2)	26.0(3.7)	26.7(5.2)	0.91
BMI category, n (%)			
Normal weight	12(44.4)	15(42.9)	0.41
Overweight	9(33.3)	14(40.0)	
Obese	5(18.5)	6(17.1)	
SIMD quantiles, n (%)			
Q1: least deprived	2(7.4)	8(22.9)	
Q2	8(29.6)	8(22.9)	0.32
Q3	7(25.9)	5(14.3)	
Q4	3(11.1)	5(14.3)	
Q5: Most deprived	7(25.9)	9(25.7)	
SBP (mmHg)	115.2(14.0)	116.1(11.5)	0.77
DBP (mmHg)	64.4(6.7)	66.4(9.7)	0.21
Smoking, n (%)			
Current smoker	3(11.1)	6(17.1)	0.25
Non-smoker	24(88.8)	27(77.1)	
History of smoking	0(0)	2(5.7)	

Values are mean and standard deviation (SD) for continuous variables or number (%) for categorical variables.

6.4.2 Maternal pregnancy hormones, plasma lipids, insulin and HOMA-IR

As expected, the pregnancy hormones estradiol, progesterone and human chorionic gonadotropin (HCG) levels rise progressively throughout gestation (Table 6-2). Plasma TAG show significant rise at 45 days post-LH surge compared to pre-LH levels. Plasma total cholesterol (TC) levels were lower in 18, 29, 45 days post-LH surge compared to pre-LH levels. Plasma insulin and HOMA-IR were not changed across the study period in pregnant women.

Table 6-2 Maternal pregnancy hormones, plasma lipids, insulin and HOMA, mean (SD) across the study period (from pre-LH surge to 45 days post-LH surge).

Plasma variable	Pre-LHS P	18 Days Post-LHS P	29 Days Post-LHS P	45 Days Post-LHS P	*P* value P
Estradiol (pmol/L)*	594(482)[a]	820(344)[b]	1473(584)[b,c]	3701(2110)[c]	<0.0001
Progesterone (nmol/L)*	0.53(1.28)[a]	73.65 (39.16)[a]	66.84 (25.62)[a]	64.69(17.50)[b]	<0.0001
HCG (IU/L)	0 (0)[a]	686(602)[b]	21706(13541)[b]	102046(60756)[b]	<0.0001
Plasma TAG (mmol/L)[#]	1.10(0.40)[a]	0.86(0.38)[a,b]	0.96(0.39)[a,b]	1.18(0.41)[b]	0.025
Plasma TC (mmol/L)	4.80(0.65)[a]	4.28(0.58)[b]	4.32(0.62)[b]	4.30(0.59)[b]	0.008
Plasma insulin (mU/L)	8.99(8.76)	11.87(10.61)	10.38(7.86)	11.41(10.62)	0.69
HOMA-IR	1.96(1.96)	2.75(2.64)	2.22(1.72)	2.72(2.82)	0.53

Values are mean and standard deviation (SD) for continuous variables or number (%) for categorical variables. a,b indicate differences between individual groups using post-hoc Tukey-Kramer at significance level P < .05, there is a significant difference where superscript letters are different and where superscript letters are shared there is no difference. # log transformed * square root transformed variable.

6.4.3 Plasma VLDL composition in pregnant and non-pregnant women

6.4.3.1 VLDL particle number

In a pure VLDL fraction, apo-B concentration is indicative of VLDL particle number. In pregnant women, apo-B concentration at 18 days post LH surge were significantly lower than baseline pre-pregnant levels ($p<0.001$) then levels started to rise at 29 days post LH surge but did not reach statistical significance until 45 days post LH surge compared to 18 days post-LH surge level ($p<0.003$) (Figure 6-2). There was no significant difference in apo-B concentration between visits in non-pregnant women.

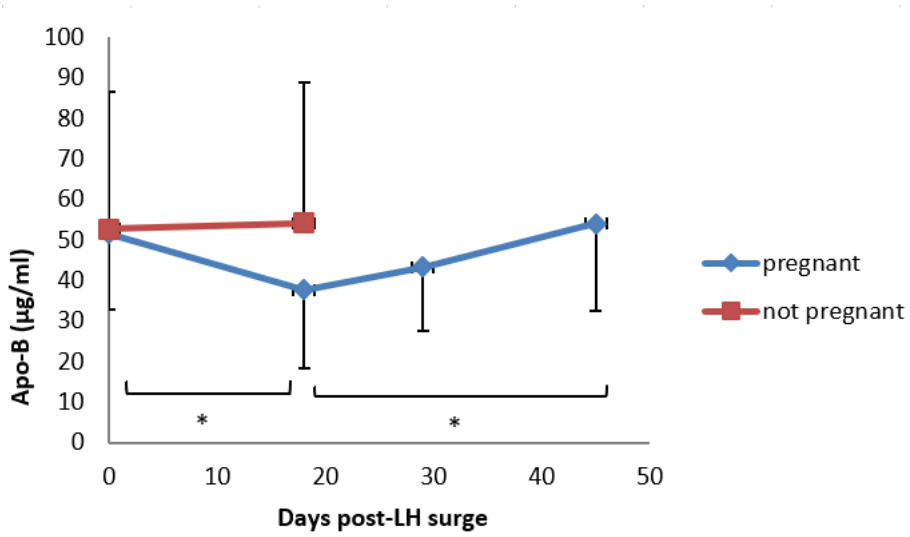


Figure 6-2 VLDL apo-B concentration
Plotted data are Mean (SD). One-way ANOVA was used to study the variance across timeline.

6.4.3.2 VLDL lipid content

In pregnant women, VLDL TC concentration at 18 days was significantly lower than baseline levels ($p<0.001$) (Figure 6-3). Then, the levels in 29 and 45 days remain not significantly different from the baseline levels. There was no significant difference in VLDL TC concentration between visits in non-pregnant women.

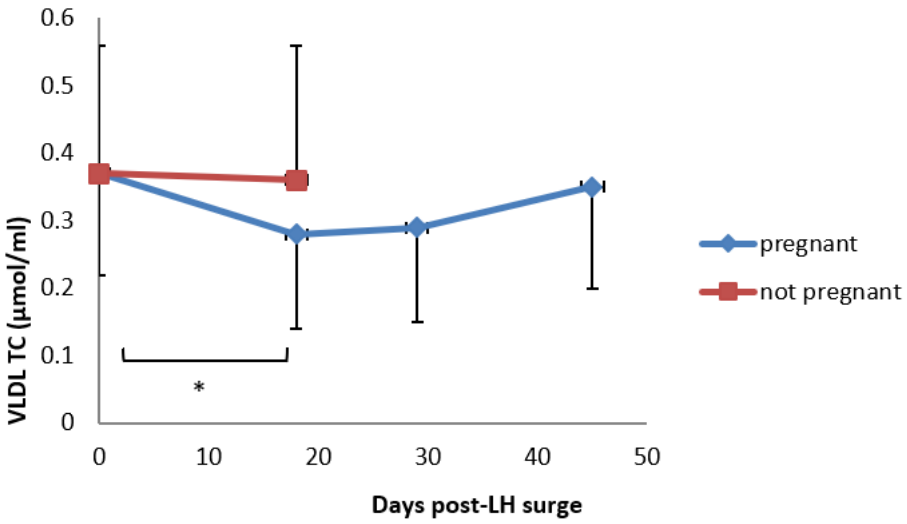


Figure 6-3 VLDL TC concentration
Plotted data are Mean (SD). One-way ANOVA was used to study the variance across timeline.

In pregnant women, VLDL TAG concentration at 18 days were significantly lower than baseline levels ($p<0.001$) (Figure 6-4). At 29 and 45 days post-LH surge VLDL TG remained similar to baseline levels. There was no significant difference in VLDL TG concentration between visits in non-pregnant women.

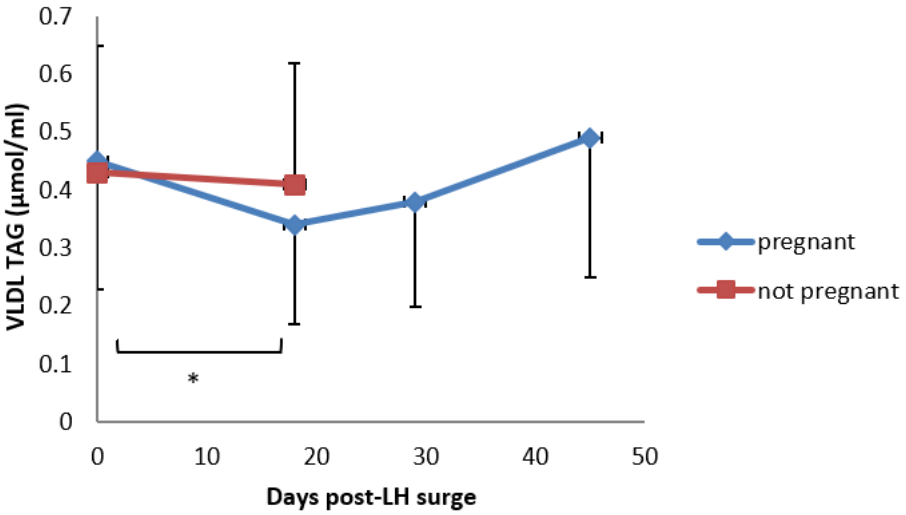


Figure 6-4 VLDL TAG concentration
Plotted data are Mean (SD). One-way ANOVA was used to study the variance across timeline.

6.4.3.3 VLDL particle composition

VLDL TAG/Apo-B ratio and VLDL CE/Apo-B ratio are shown in (Figure 6-5 and Figure 6-6). There was no significant difference in TAG/Apo-B or CE/Apo-B between visits in pregnant women. There was no significant difference in TAG/Apo-B and CE/Apo-B ratios between visits in non-pregnant women.

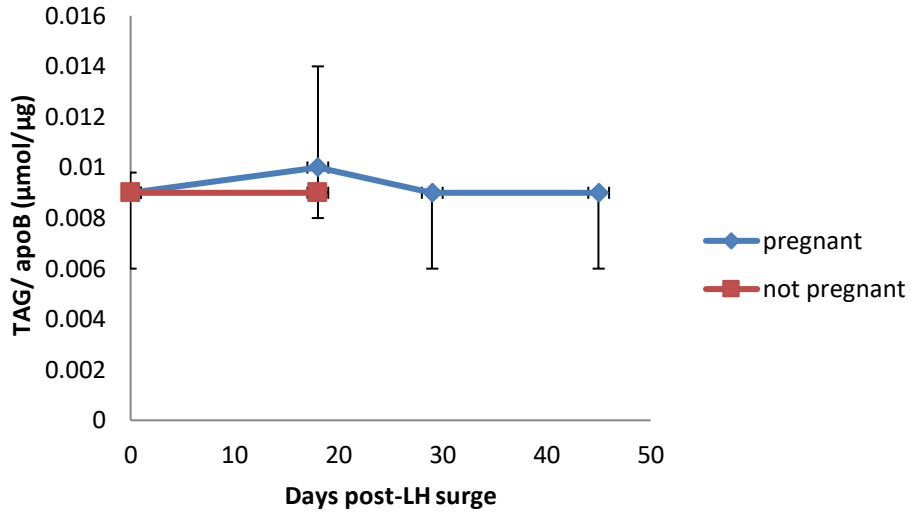


Figure 6-5 TAG/ apo-B ratio
Plotted data are Mean (SD). One-way ANOVA was used to study the variance across timeline.

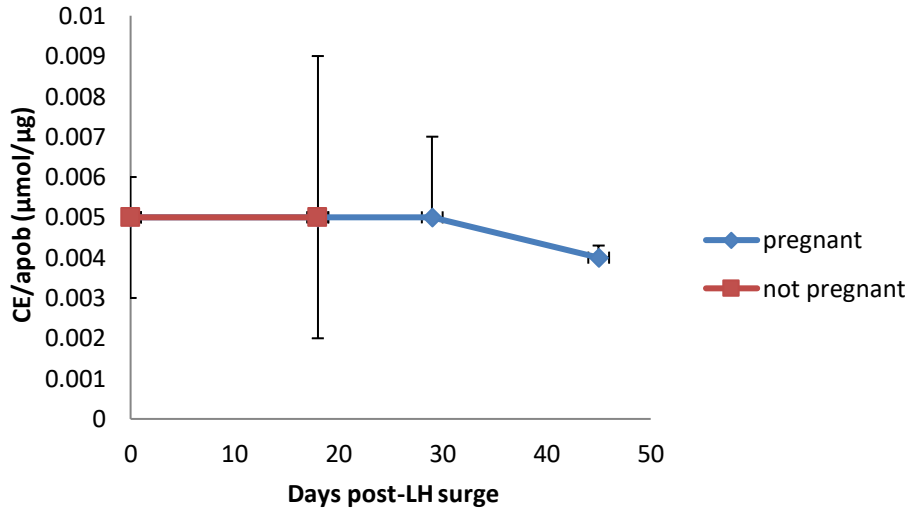


Figure 6-6 CE/apo-B ratio
Plotted data are Mean (SD). One-way ANOVA was used to study the variance across timeline.

6.4.3.4 Relationship between pregnancy hormones, VLDL composition and plasma lipids

The relationship between the pregnancy hormones estradiol, progesterone and human chorionic gonadotropin (HCG) and VLDL composition at all time points is shown in Table 6-3. The significance level considered is p <0.010. The pregnancy hormones did not correlate with VLDL apo-B, VLDL TAG and VLDL TC. However, plasma TAG levels correlated positively with VLDL apo-B, VLDL TAG and VLDL TC levels. Moreover, the plasma insulin and HOMA-IR levels correlated positively with plasma TAG levels at early pregnancy. Similarly, VLDL TAG correlated with plasma insulin and HOMA-IR levels.

Table 6-3 Correlations between pregnancy hormones (estrogen, progesterone and HCG), VLDL composition (VLDL apo-B, VLDL TAG and VLDL TC) and plasma lipids (TAG and TC) at all time points.

	VLDL apo-B		VLDL TAG		VLDL TC		Plasma TAG		Plasma TC	
Plasma variable	R^2 Adj	P value	R^2 Adj	P value	R^2 Adj	P value	R^2 Adj	P value	R^2 Adj	P value
Estradiol (pmol/L)*	0.0%	0.49	0.0%	0.89	0.8%	0.18	0.0%	0.41	1.2%	0.14
Progesterone (nmol/L)*	0.4%	0.23	0.5%	0.22	3.3%	0.040	0.6%	0.19	6.6%	0.006
HCG (IU/L)	0.6%	0.2	1.9%	0.091	0.0%	0.85	1.2%	0.14	0.7%	0.19
Plasma TAG (mmol/L)#	53.1%	<0.0001	78.2%	<0.0001	55.3%	<0.0001	-	-	-	-
Plasma TC (mmol/L)	5.4%	0.010	1.7%	0.97	2.9%	0.046	-	-	-	-
Plasma insulin (mU/L)	0.8%	0.17	9.9%	0.001	4.2%	0.022	17.6%	<0.0001	0.0%	0.38
HOMA-IR	1.0%	0.16	10.9%	<0.0001	4.7%	0.016	17.8%	<0.0001	0.0%	0.42

log transformed * square root transformed variable. Significance level p<0.010.

6.4.3.5 VLDL FA concentrations over the first 45 days post-LH surge

VLDL FA concentrations over time for women who were successful in getting pregnant and non-pregnant women are shown in Table 6-4..

Table 6-4 VLDL FA concentrations from pre-LH surge to 45 days post-LH surge in pregnant women (P, n=27) and women who were unsuccessful in getting pregnant (NP, n=35)

Fatty Acid (nmol/mL)	Pre-LHS P	18 Days Post-LHS P	29 Days Post-LHS P	45 Days Post-LHS P	*P* value P	Pre-LHS NP	18 Days Post-LHS NP	P value NP
SAFA								
16:0 palmitic	505 (223)[a]	375 (108)[a]	395 (207)[a]	525 (284)[a]	0.037	457 (276)	454 (243)	0.91
18:0 stearic	127 (40)[a]	102 (40)[a]	102 (38)[a]	124 (50)[a]	0.047	115 (45)	124 (70)	0.70
24:0 lignoceric	3.95 (2.54)[a]	2.54 (1.41)[a,b]	2.86 (1.47)[a,b]	3.15 (1.96)[b]	0.039	3.52 (1.82)	3.43 (2.23)	0.64
MUFA								
18:1n-9 oleic	556 (274)	404 (214)	453 (220)	591 (317)	0.064	545 (316)	612 (473)	0.76
PUFA n-6								
18:2n-6 linoleic	395 (191)	291 (141)	331 (152)	384 (201)	0.13	382 (203)	418 (335)	0.75
18:3n-6 Y- linolenic	9 (6)	6 (5)	7 (4)	6 (4)	0.12	10 (8)	10 (9)	0.91
20:3n-6 dihomo-Y-linolenic	16 (11)	13 (9)	14 (7)	19 (12)	0.17	15 (9)	17 (16)	0.67
20:4n-6 arachidonic	59 (27)	47 (25)	51 (24)	62 (32)	0.17	53 (31)	60 (50)	0.89
22:4n-6 adrenic	3 (1)	3 (1)	2 (1)	3 (1)	0.46	3 (1)	3 (2)	0.083
22:5n-6 docosapentaenoic	1 (1)	1 (1)	2 (1)	3 (1)	0.11	1 (1)	1 (1)	0.93
PUFA n-3								
18:3n-3 α-linolenic	21 (13)	16 (10)	16 (11)	16 (11)	0.074	23 (16)	26 (25)	0.56
20:5n-3 eicosapentaenoic	21 (57)	11 (11)	29 (69)	11 (13)	0.44	59 (119)	36 (96)	0.37
22:5n-3 docosapentaenoic	8 (4)	6 (3)	6 (3)	7 (4)	0.24	8 (4)	8 (6)	0.93
22:6n-3 docohexaenoic	21 (10)	17 (8)	22 (12)	26 (14)	0.17	17 (13)	25 (34)	0.15

Abbreviations: LHS, luteinizing hormone surge; MUFA, monounsaturated fatty acids; NP, not successful in becoming pregnant; P, successful pregnancy; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids. Means (SD) are shown. Differences between concentrations were tested using one-way ANOVA for repeated measures, across sampling from pre-LH surge (pre-pregnancy) to 18, 29, and 45 d post-LH surge and P values are given. NP women withdrew from the study after blood pregnancy testing at day 18 post-LH surge and therefore 29 and 45 d post-LH surge samples are not available for these women. a,b indicate differences between individual groups using post-hoc Tukey-Kramer at significance level $P < .05$, there is a significant difference where superscript letters are different and where superscript letters are shared there is no difference.

In pregnant women, lignoceric acid (24:0) concentration significantly changed over time in VLDL particles of pregnant women, with 45 days post-LH surge being significantly lower than the pre-LH surge level. Although palmitic (16:0) and stearic (18:0) acid concentration changed significantly over time in VLDL particles, there were no subgroup differences in the post-hoc analysis. For the monounsaturated fatty acid (MUFA), n−6 PUFAs and n−3 PUFAs, there was no change over time in FAs concentrations in VLDL in pregnant women. Interestingly, DHA (22:6n−3) concentration in VLDL particles was not changed from pre-LH surge to 45 days post-LH surge. There was no change in DHA (22:6n−3) concentration per apo-B (p= 0.29) (Figure 6-7). In the women who were not successful in getting pregnant, there were no significant changes in plasma VLDL FA concentration from pre-LH surge to day 18 post-LH surge.

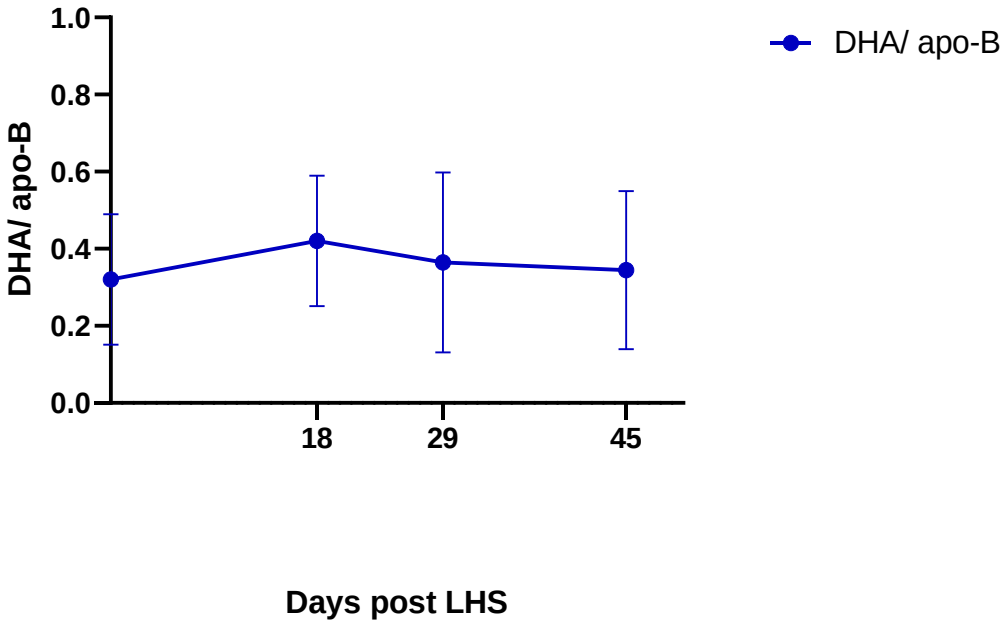


Figure 6-7 DHA per apo-B
Plotted data is Mean. One-way ANOVA was used to study the variance across timeline.

6.4.3.6 Rate of change of maternal VLDL FA concentrations and iAUC over the first 45 days post-LH surge

The rate of change in concentration in VLDL lignoceric acid (24:0) significantly decreased from 18 days to 45 days post-LH surge (p=0.031) (Figure 6-8). The iAUC was negative indicating that VLDL lignoceric acid concentration decreased over this time period (Figure 6-9). The rate of change in VLDL palmitic and stearic acid concentration was not significantly changed.

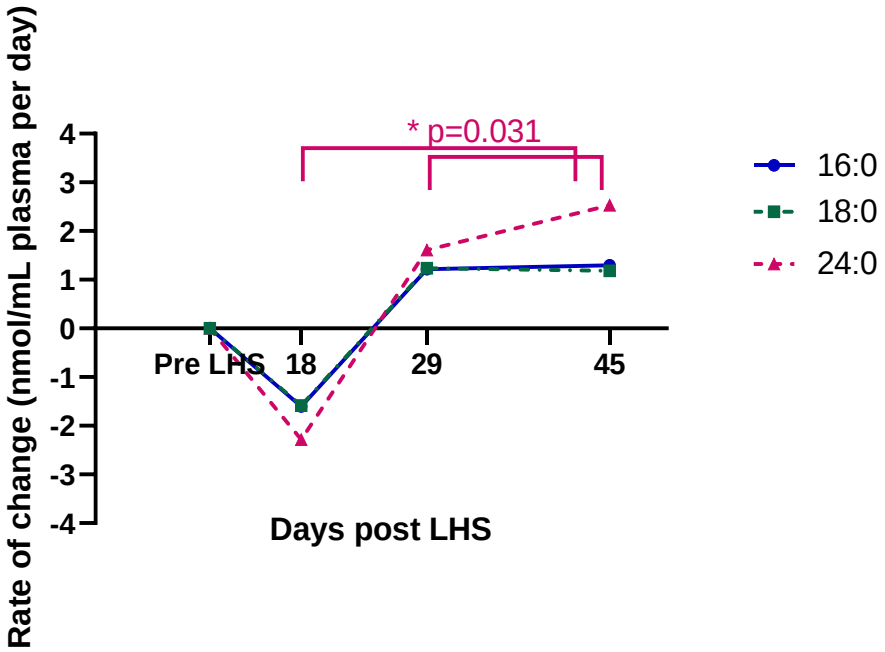


Figure 6-8 Rates of change of maternal FA concentration in VLDL:16:0, 18:0 and 24:0
The rate of change of fatty acids were calculated as the difference between the fatty acid on day 18 post-LH surge and pre-LH surge and then divided by the difference in time (day) between day 18 post-LH surge and pre-LH surge. This was then repeated for all other time points, ie, day 29 post-LH surge and day 18 post-LH surge and then day 45 post-LH surge and day 29 post-LH surge. The results are expressed as nmol/mL plasma per day. One-way ANOVA was used to study the variance across timeline.

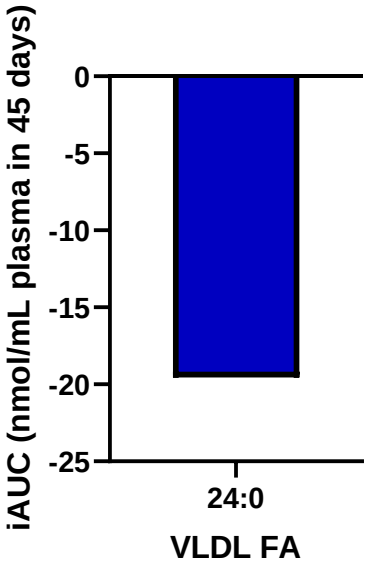


Figure 6-9 iAUC of maternal FA concentration in VLDL lignoceric acid (24:0)
Incremental areas under the time (pre-LH surge to 45 d post-LH surge) x concentration curve was calculated using the trapezium method

6.5 Discussion

Our main finding is that there was no change in VLDL DHA concentration or VLDL DHA enrichment from pre-LH surge to 45 days post-LH surge. However, there was reduced VLDL particle number by 18 days post-LH surge in pregnant women only. These findings suggest that VLDL is not the main lipoprotein carrier for DHA which was contrary to our hypothesis. Other lipoproteins such as HDL could be an important carrier of DHA similar to the non-pregnant population. Therefore, isolation of other lipoprotein fractions throughout gestation and the subsequent study of their FA composition would be of interest to identify the primary carrier of DHA in maternal plasma.

The reduction in VLDL particle number by 18 days post-LH surge was an unexpected finding. VLDL assembly and secretion is a process highly regulated by TAG availability in the liver (Fisher and Ginsberg, 2002). The TAG sources for the liver include circulating FA bound to albumin, VLDL and chylomicron remnants and *de novo* hepatic synthesis of TAG. TAG entry into the pool for VLDL secretion is regulated by insulin and microsomal triglyceride transfer protein (MTTP) (Pan and Hussain, 2007). In the presence of lipids, nascent apo-B is lipidated by MTTP to increase VLDL secretion by the liver (Fisher et al., 2001). In contrast, the absence of lipids leads to a reduction in MTTP activity, subsequent apo-B degradation and reduced VLDL secretion (Fisher et al., 2001). In early pregnancy, there is a physiological increase in insulin sensitivity which leads to increased LPL activity and increased FA storage. Furthermore, the enhanced insulin sensitivity in early pregnancy inhibits adipocyte lipolysis by inhibition of HSL. Interestingly, plasma TAG levels were reduced by 18- and 29-days post-LH surge. However, the underlying mechanism for this early reduction in plasma TAG is not clear but it could be used as an energy source for the rapidly growing fetus. The reduction in plasma TAG was partially mediated by HOMA-IR and plasma insulin level in early pregnancy. Moreover, the reduction in TAG availability to the liver in early pregnancy might contributed to the observed reduction in VLDL particle number by subsequent apo-B degradation and reduced VLDL secretion. This relationship was suggested by the observed correlation between VLDL particle number and plasma TAG. Thus, we hypothesise that the early pregnancy changes in insulin sensitivity and the subsequent reduction in TAG availability to the liver could probably explain the current study observation of reduced VLDL particle number at 18 days post-LH

surge, an effect probably mediated through reduced MTTP activity and subsequent apo-B degradation.

Other lipoprotein fractions were not studied by the researcher. However, this has since been carried out in collaboration with Wollongong University. The current study cohort was analysed along with other study cohort plasma samples collected longitudinally throughout gestation at 16, 25, 35 weeks of gestation and 13 weeks postnatal to examine the lipoprotein fractions VLDL, IDL, LDL, HDL and LPDP FA composition prior to pregnancy, throughout gestation and in the post-partum period. The results revealed that DHA is carried primarily in HDL throughout gestation and the HDL particles are enriched in DHA similar to the non-pregnant population (Augustine et al., 2014). This was observed as a consistently increasing concentration of DHA carried by HDL peaking at 25 weeks of gestation, a higher proportion of DHA carried in the HDL fraction than any other lipoprotein fraction and the progressive enrichment of HDL in DHA peaking at 25 weeks of gestation (Samani et al unpublished data). In contrast, DHA concentrations in the other lipoprotein fractions do not change throughout pregnancy. HDL is an ideal vehicle to carry the additional DHA required for transfer to the fetus because of its very well-known anti-oxidant and anti-inflammatory properties which are increased during pregnancy as a protective mechanism against the pregnancy associated oxidative stress (Sulaiman et al., 2016). The observed peak concentration of DHA carried by HDL at 25 weeks of gestation coincides with the previously observed higher CETP activity in the second trimester which then declines in third trimester (Iglesias et al., 1994). Therefore, it is possible that the maternal DHA packaged into VLDL is incorporated into HDL by the transfer from other lipoproteins via CETP. Furthermore, the observed increased concentration of DHA carried by HDL peaking at 25 weeks of gestation (Samani et al unpublished data), coincides with a second peak of increased head circumference growth velocity which peaks twice during gestation, (14 weeks and 19-21 weeks of gestation) (Grantz et al., 2018). This evidence supports the importance of HDL in delivering the DHA required for fetal neurological development.

The concentration of DHA in neonates of mothers with GDM have been found to be low compared to healthy women (Thomas et al., 2005). Several studies have related reduced DHA concentration to reduced placental transfer to the fetus (Wijendran et al., 2000). GDM pregnancy is characterized by greater FA flux from adipose tissue

stores as we have shown in Chapter 4. The maternal FA flux affects the placental lipid uptake. In GDM, there was a higher accumulation of lipid droplets in placental tissue (Tewari et al., 2011) and reduced mitochondrial FA oxidation by 20% in placenta, contributing to enhanced TAG content within the placenta. This might affect the placental function and may affect the fetal levels of important FA such as DHA. These changes could adversely affect the fetal metabolism and predispose the fetus to future metabolic diseases and other adverse implications on the fetal neurodevelopment. However, another study has attributed the reduced DHA concentration in neonates of mothers with GDM to increased fetal utilization (Ortega-Senovilla et al., 2009). Additionally, the previous study proposed that augmented utilization of LC-PUFA could contribute to increase in the fetus body fat mass and subsequent tendency to develop macrosomia (Ortega-Senovilla et al., 2009). The study of HDL FA composition, the main carrier of DHA in pregnancy, to determine the GDM effect on DHA concentration compared to healthy pregnancy was not carried out before.

A particular strength of the current study was the prospective design and repeated within individual sampling up to 45 days post-LH surge. This early time period is rarely studied in early pregnancy because it is difficult to assess in a free-living population and is very critical for embryo neuronal development. Additionally, the comprehensive analysis of VLDL FA measuring absolute concentrations allowed me to draw conclusions regarding different subtypes of FAs. The main limitation of the current study was that the women participants were undergoing *in vitro* fertilization, and this might not be considered a “normal” pregnancy population. However, the women had a natural menstrual cycle, meaning there was no interference of exogenous hormones used in cycle reconstruction on their metabolic adaptation to pregnancy. Indeed, the study of this population provided us with accurately timed periconceptual and early pregnancy blood samples that would be extremely difficult to achieve from a free-living population. Unfortunately, there was no dietary intake data, no direct measure of δ6-desaturase activity and no assessment of embryonal accrual of FA. Inferences on changes in VLDL FA metabolism were made using serial steady state plasma VLDL FA concentrations. Although I could observe increases and decreases in plasma VLDL FA concentration, it does not fully establish whether these changes in concentration were due to changes in rates of entry into or removal from plasma compartments as

would be described by kinetic tracer studies, which are clearly extremely difficult to perform in pregnant women.

In conclusion, my results have shown that contrary to our hypothesis, VLDL is not the carrier for DHA in the first 45 days post-LH surge as evidenced by unchanged DHA concentration in VLDL, unchanged DHA enrichment per VLDL particle and reduced VLDL particle number. Subsequent analysis of the study cohort by our collaborator has shown that HDL, and not VLDL, is the preferred lipoprotein in terms of DHA transport across the pregnancy timeline possibly because of HDL important antioxidant and anti-inflammatory properties that may protect DHA from oxidation.

Chapter 7 Discussion

Given the increased obesity rates among the general population, it is unsurprising that maternal obesity rates are on an increasing trend. In Scotland, the proportion of women who were obese at their booking visit rose from 9.4% to 18.9% between 1990 and 2002–2004 (Kanagalingam et al., 2005). Similar trends were also observed in England (Heslehurst et al., 2010b). Given the association between maternal obesity and maternal and fetal adverse outcomes, this rising trend is a major concern. The increase in adiposity has been linked to several cardiometabolic complications, including insulin resistance and impaired glucose and lipid metabolism. The main and unique function of adipocytes is the storage of FFA under conditions of positive energy supply, and their release during conditions of negative energy balance. However, the concept of a primary mechanistic role for adipose tissue in the development of metabolic abnormalities has emerged over the last decade, which involves adipose tissue expandability, fat distribution (specifically the role of VAT), ectopic fat deposition, lipotoxicity and low-grade inflammation. The work presented in this thesis firstly aimed to examine the current trends in maternal obesity and GDM rates at the population level among pregnant women in Greater Glasgow and Clyde. The recent advances in robust recording of maternal BMI and its associated adverse events make this a timely analysis. Secondly, the thesis aimed to test the hypothesis that in women with GDM, SAT adipocytes show adipocyte hypertrophy and increased lipolysis, which would provide evidence for the failure of adipocyte expansion in GDM, consistent with that observed in T2DM. Thirdly, the work presented in this thesis aimed to assess the role of VLDL in early pregnancy as a potential maternal plasma carrier of the extremely important long chain polyunsaturated fatty acid docosahexaenoic acid (DHA), required by the fetus for neuronal development. Infants born to mothers with GDM had lower DHA levels, and were shown to have lower cognitive performance, partly attributed to lower placental transfer of DHA in GDM. However, the underlying mechanism for defective DHA metabolism and transport in GDM pregnancy is not fully understood.

The first objective of this thesis was to explore the epidemiological evidence that among the population of the overall study, women with maternal obesity are at greater risk of GDM and adverse maternal and fetal outcomes. This analysis provided robust background information on the clinical characteristics of the population in which the adipocyte function and DHA transportation studies were

undertaken. The prevalence of maternal obesity in the current study was 22.3%, suggesting that maternal obesity is on an increasing trend in the Glasgow population when compared to earlier studies (Kanagalingam et al., 2005). Although the prevalence of GDM (2.2%) remains low in the Glasgow population compared to the worldwide prevalence of GDM (from 0.5 to 15%), the associated risks observed represent a great burden on NHS resources. The current study showed that mothers with obesity and GDM receive a higher number of obstetric interventions, including operative and earlier delivery. Moreover, despite the higher rate of medical interventions among obese and GDM mothers, the average birthweight for offspring is significantly higher for GDM mothers. Further analysis of this data to address the economic costs associated with obesity and GDM diagnosis during pregnancy, including maternal hospital costs for the mother and her neonate, will provide insights to inform clinical practice decision-making and national pregnancy care planning. Wider analysis of maternal obesity rates at a national level could be beneficial in informing clinical practice for the management of maternal obesity during pregnancy. Moreover, a national analysis of maternal obesity rates and subsequent outcomes will provide an evaluation of the current therapeutic strategies, and might provide firm evidence regarding the optimal treatment/management for maternal obesity during pregnancy and better direction of NHS resources. Prospective follow-up analysis of the current study cohort might provide more insight about the long-term complications in the mother and her fetus in this population. Future experimental studies are required to provide better understanding of the underlying pathophysiology linking high maternal BMI and the associated adverse pregnancy outcomes.

The second objective of this thesis was to ascertain whether adipocyte size and adipocyte lipolytic function (basal and β-adrenergic-stimulated lipolysis, and insulin suppression of lipolysis) in SAT and VAT adipocytes were different between women with GDM and healthy BMI-matched controls. In this study, contrary to our hypothesis that adipocyte hypertrophy would be observed in SAT adipocytes, significantly higher adipocyte diameter and basal lipolysis were observed in VAT adipocytes in GDM women than in BMI-matched healthy controls. This suggests that failure of adipocyte expansion occurs in the VAT depot in women with GDM. Due to the study design (cross-sectional in the third trimester), pre-gestation or early gestation hypertrophic changes in SAT adipocytes caused by overweight and obesity, which might have proceeded VAT adipocyte hypertrophy, would not be

observed. Thus, our revised hypothesis is that VAT adipocyte hypertrophy in the third trimester of pregnancy could be a consequence of failed SAT expandability, which occurs earlier and represents SAT adipocyte overspill stored “ectopically” in VAT adipocytes. Studying another control group of healthy pregnant women with normal BMI would help in drawing conclusions regarding hypertrophy in SAT adipocytes. One could hypothesise that in healthy-weight women in pregnancy, SAT hypertrophy may occur in response to gestational weight gain, at least in a subset of women, but may never reach the stage of fatty acid overspill. In such healthy women with low pre-pregnancy BMI or limited gestational weight gain, either they have full potential for adipocyte differentiation to occur, or, even in a situation of limited adipocyte differentiation capacity, hypertrophy of SAT is sufficient to buffer excessive gestational fatty acid flux without VAT hypertrophy. In healthy pregnant women with normal BMI, SAT hypertrophy could be reversed after delivery (Figure 7-1). The failure of adipocyte expansion results from a reduced ability to recruit new adipocytes. Adipocyte differentiation was not measured in the current study, and assessment of this in pre-adipocytes collected pre-pregnancy, in early gestation and in late gestation, would be very informative. This would be a valuable, but difficult to execute, next study.

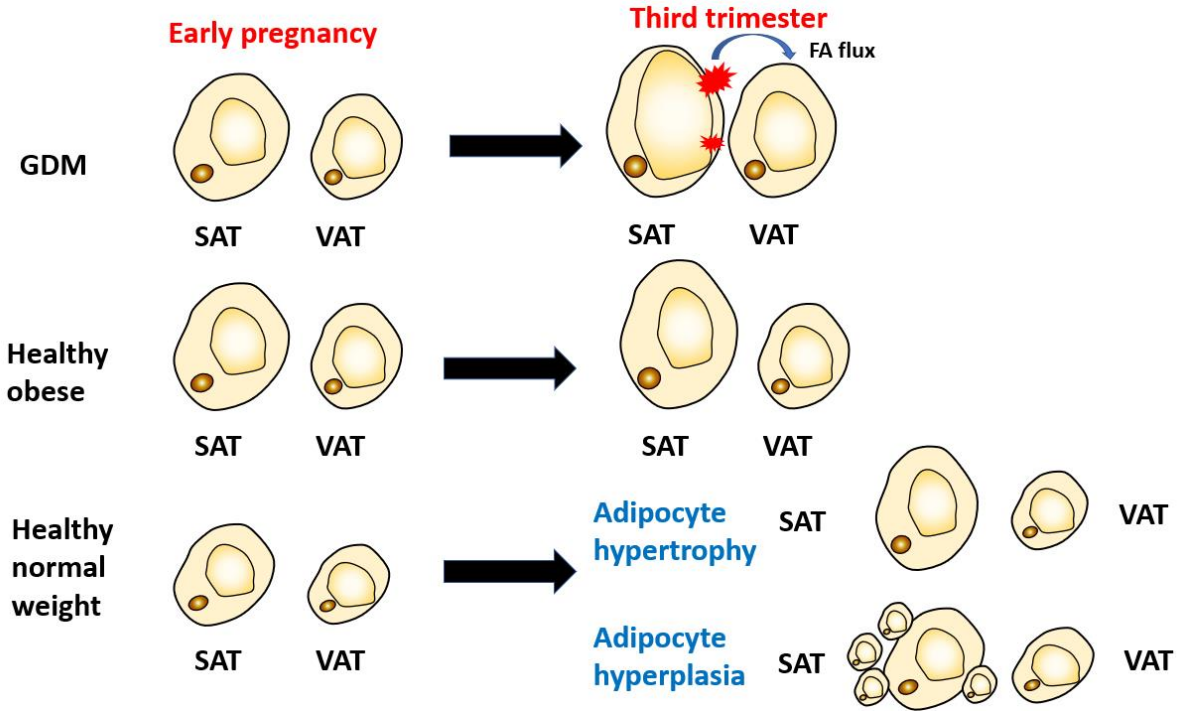


Figure 7-1 Revised hypothesis of adipocyte expansion during pregnancy in GDM, healthy obese and normal-weight pregnancy.
In GDM, there is defective SAT adipocyte expansion in response to increased FA storage in early pregnancy. Subsequently, SAT defective expansion results in FA overspill in VAT adipocytes with subsequent VAT adipocyte hypertrophy and higher basal lipolysis. In healthy obese pregnancy there is SAT adipocyte hypertrophy in response to increased FA storage in early pregnancy, but it does not reach the stage of FA overspill into VAT. In healthy normal-weight pregnancy, there is efficient FA storage, either by SAT hypertrophy or hyperplasia in response to increased FA storage in early pregnancy, but there is no VAT storage of NEFA.

The finding that there was higher basal lipolysis in VAT adipocytes from women with GDM than controls suggests that there is reduced ability for efficient storage and retention of FFA in VAT adipocytes in GDM. Thus, dysfunctional VAT adipocyte fat storage may be an important factor contributing to ectopic fat accumulation in other tissues, leading to the emergence of metabolic dysfunction in women with GDM. Increased VAT mass in obese insulin-resistant individuals has been proposed to represent a marker of reduced SAT ability to store fat (Després and Lemieux, 2006). Increased VAT mass could also be a surrogate marker, rather than a necessary causal factor for downstream ectopic fat accumulation and the development of metabolic risk. When the storage capacity of adipocytes is exceeded, increased FFA flux to ectopic tissues may not be balanced by an increase in FA oxidation in those tissues, leading to accumulation of unoxidised FFA in non-adipose tissue, as suggested by the “spill over” hypothesis. Lipids can be deposited in other organs, such as the liver, pancreas, and skeletal and cardiac muscle, which can lead to the

development of metabolic syndrome features. According to the portal hypothesis, the anatomical location of the VAT depot exposes the liver to increased flux of FFA and adipokines via the portal vein. Higher VAT adipocyte FFA release potentially contributes to ectopic fat deposition in the liver, and to hepatic insulin resistance (Boden, 2008). This is consistent with the current study, finding that both women with GDM and overweight/obese control groups had high HSI scores. In our cohort, HSI was closely related to maternal adiposity and measures of insulin sensitivity in normal pregnancy and GDM. There was a strong, consistent relationship between SAT adipocyte lipolysis and HSI score in women with GDM, probably suggesting the importance of SAT adipocyte expansion ability as an initiating factor for the subsequent increase in VAT mass and ectopic fat deposition in the liver. Therefore, the measurement of liver fat during routine antenatal ultrasound for pregnant women at risk of pregnancy metabolic complications may be of clinical utility. Further studies that examine maternal liver fat deposition longitudinally throughout gestation in women with GDM, in comparison to a healthy pregnant control group using magnetic resonance spectroscopy, are needed to assess the evidence for ectopic fat deposition in the liver in women with GDM.

This thesis provided support for the theory that adipose tissue distribution during pregnancy is associated with maternal metabolic health. The importance of visceral obesity as either a marker of or facilitator in developing subsequent maternal and fetal complications should be recognised among clinicians. Visceral distribution of fat pre-pregnancy, or accumulation of visceral fat during pregnancy, may identify women most at risk of an adverse outcome. If so, there is a need to revise the clinical approach towards obesity categorisation and treatment during pregnancy. Measuring VAT could be a novel strategy that would assist in more effectively directing medical resources to those women at greater risk. Screening for visceral adiposity in pregnant populations is not an easy task. BMI does not account for differences in body fat distribution among different populations. BMI is not a precise measure of body composition, and cannot distinguish lean or fat tissue or the location of fat. Abdominal waist circumference has been used as a measure of central obesity and a marker of visceral fat in large-scale epidemiological studies. Sattar et al. (2001) assessed abdominal waist circumference at 16 weeks' gestation, and found that larger waist circumferences (>80cm) were associated with an increased risk of pregnancy-induced hypertension and pre-eclampsia. At 20–24 weeks of gestation, waist circumference values in the range of 86–88 cm were

shown to represent a predictor of GDM in the population of Brazilian women (Bolognani et al., 2014). Sattar et al. (2001) suggested that waist circumference is a simple measure which could form the basis for health promotion among women who are planning to get pregnant, indicating a need for weight reduction. Further epidemiological studies are warranted to determine whether abdominal waist circumference is as sensitive as BMI for predictions of pregnancy complications associated with maternal obesity and GDM. Newer techniques to measure visceral fat, which are convenient and quick to perform in busy antenatal clinics, include the use of bioimpedance assessment (Khalil et al., 2014). Bioimpedance analysis can be used to identify the visceral fat compartment, which highly correlates with VAT measurements obtained by computed tomography (Yamakage et al., 2014). A new screening tool – used to define the obesity-associated risk of adverse pregnancy outcomes using a composite phenotype measure, taking into account maternal BMI, plasma TAG as a surrogate marker for effective fat storage in adipocytes, adipose tissue distribution, adipose tissue inflammation status, and ectopic fat – would represent a novel strategy and could be highly beneficial.

Adipose tissue is an active endocrine organ, secreting many adipokines shown to be linked to obesity, insulin resistance and T2DM in the non-pregnant population. This thesis explored isolated VAT adipocyte adipokine secretion in basal or activated (LPS-stimulated) conditions in healthy and BMI-matched GDM women. In this study, there was no evidence for higher VAT adipocyte inflammatory adipokine release in either basal or stimulated conditions in GDM compared to controls. However, this conclusion can only be drawn with caution, as there was insufficient recruitment for the study to be sufficiently representative to identify significant differences. If similar results were confirmed with larger sample size, this data might suggest that adipokine release is related to obesity, and due to BMI-matching of our groups we did not observe differences between GDM and control groups. Alternatively, adipokine release could have been altered in SAT, which was not assessed in this study. Furthermore, pregnancy is considered to be a state of low-grade inflammation, especially in the third trimester, and it is not clear whether there is a difference in adipokine levels from adipocytes in response to obesity in pregnancy. In the current study, consistent with the adipokine secretion from VAT adipocyte findings, there were no significant differences in inflammatory gene expression in either SAT or VAT adipocytes from GDM compared to controls. Similarly, other genes involved in adipocyte differentiation, lipid storage, lipid and

glucose metabolism, and angiogenesis gene expression were not different in GDM compared to controls.

The data collected on adipocyte inflammation is limited by the incomplete analysis of all tissues, and low sample size. At present, the data provides no evidence for the role of VAT inflammation in GDM. This could suggest that adipocytes are not the main contributing factor *per se* to the low-grade inflammation observed in obese and GDM pregnancy. This is probably because infiltrating inflammatory cells may be of more importance than adipocyte cytokine production. Other studies have focused more on the role of inflammatory cells that infiltrate adipose tissue. Gene profile analysis of isolated SAT adipocytes and SVF from obese non-pregnant individuals, after following a very low-calorie diet for 28 days, revealed that only 13% of differentially expressed genes related to inflammation were expressed in adipocytes in obese individuals compared to lean controls (Clément et al., 2004). The inflammation-related genes are mainly expressed in cells of the SVF of adipose tissue, which include the macrophages (Clément et al., 2004). However, it would be useful to discount the adipocyte's role in inflammatory cytokine production if the results presented here could be confirmed by a larger sample size. Further studies of inflammatory gene expression on the isolated SVF fraction or whole adipose tissue explants could provide insight into adipose tissue inflammation in GDM. Adipocytes could be viewed as the primary initiating factor for macrophage activation and recruitment when adipose tissue becomes dysfunctional, which subsequently leads to adipose tissue inflammation through enhanced macrophage and immune cell recruitment. Therefore, the determination of adipose tissue cell densities of activated (cfms+) and total (CD68+) macrophages could be an interesting analysis to perform on the current study samples. Furthermore, a wide range of protein factors, including various chemokines, which are known to be secreted from adipose tissue to attract immune cells, could be studied further, such as MCP-1 and Stromal cell-derived factor 1 (SDF1). SDF1 is a novel chemotactic factor secreted from adipose tissue, mainly by cells of SVF, and might be involved in the recruitment of lymphocytes.

The placenta is another potential site of inflammation during pregnancy. The placenta is able to produce inflammatory cytokines, and has the capability to respond to a variety of inflammatory stimuli. It was found that the secretory function of the placenta contributes to local and systemic cytokine levels. The placenta

secretes cytokines similar to those derived from adipose tissue. Under the hypothesis suggesting that the activation of inflammatory pathways is necessary to induce gestational insulin resistance towards the third trimester, placental cytokines could contribute to low-grade inflammation at the third trimester of pregnancy. Alternatively, placental hormones could enhance the pre-pregnancy inflammatory status of adipose tissue in pregnancies complicated by maternal obesity and GDM. However, the relative contribution of the placenta to low-grade inflammation in obese and GDM pregnancy is yet to be determined. Under the proposed overspill hypothesis, ectopic fat in the placenta could enhance the inflammatory status. FFA deposition in the placenta could potentially contribute to macrophage accumulation in the placenta. Thus, the study of the placental contribution to low-grade inflammation in women with GDM might provide insight into the potential mechanisms for communicating the metabolic disease to the fetus. Unfortunately, in the current study, we could not collect placenta samples due to having limited lab personnel able to assist with its collection.

The final objective of this thesis was to test whether VLDL could be the main carrier for DHA at the critical time of neural tube closure. Given that the liver is the primary site for *de novo* LC-PUFA synthesis, and there was a three-fold increase in VLDL synthesis by the liver during pregnancy, we hypothesised that DHA is carried out by VLDL in early pregnancy. However, our analysis showed that, contrary to our hypothesis, DHA was not carried out by VLDL in early pregnancy. Subsequent analysis of these samples by our collaborator showed that HDL is the primary carrier of DHA throughout gestation, similar to the non-pregnant population. HDL is an ideal carrier for DHA at the very important time of neuronal development, as its antioxidant and anti-inflammatory properties could protect DHA from oxidation. Considering the important role played by LC-PUFAs in fetal visual, behavioural and cognitive development, the study of DHA metabolism in pregnancies complicated by GDM is of interest. Thomas et al. (2005) showed that offspring of mothers with GDM have lower plasma DHA level than neonates of healthy mothers. Infants born to mothers with GDM demonstrate lower cognitive performance (Hod et al., 1999), attention span, and face more than twice the risk of language impairment than infants born to mothers without diabetes (Dionne et al., 2008). This was attributed to reduced placental transfer of DHA in GDM (Wijendran et al., 2000). However, the underlying mechanisms for reduced placental DHA transfer to the fetus of GDM mothers are not known yet. It is not known whether placental inflammation interferes

with lipid metabolism pathways, affecting the transfer of DHA in women with GDM. Thus, further investigation of the influence of placental inflammation on the regulation of maternal–fetal DHA transport is warranted. Furthermore, the study of HDL composition in GDM pregnancy to determine the DHA concentration compared to healthy pregnancy has not yet been researched. DHA supplementation of 600 mg DHA was found to enhance maternal but not fetal DHA status in pregnancy complicated by GDM (Min et al., 2016). This suggests that the transfer of DHA across the placenta is impaired in GDM. Further investigation of the clinical implications of reduced DHA level in offspring of mothers with GDM, and the optimal clinical management to reduce the neurodevelopmental impact on GDM neonates, is warranted.

There were significant strengths to this study. The epidemiology study provided detailed demographic and obstetric outcomes analysis among the greatly diverse Greater Glasgow and Clyde population, using data linkage analysis of two robust datasets. The associations between maternal obesity and a wide range of adverse pregnancy outcomes were examined, and the effect of race and social economic status on various obstetric outcomes was accounted for – factors which were not usually studied in previous research. The study of SAT and VAT adipocytes in women with GDM compared to BMI-matched controls provided, for the first time, extensive adipocyte functional measures in women with GDM, including adipocyte size, and lipolytic and secretory function. We have studied isolated adipocytes rather than whole adipose tissue samples to clearly identify the role of the adipocyte in the development of insulin resistance during GDM pregnancy. Thus, our results were not confounded by immune and other cells in the adipose tissue. This is an area where the current available literature is very limited, especially in the context of pregnancy and GDM. The VLDL FA composition study provided samples from the early pregnancy period, which is rarely studied in pregnancy, because it is difficult to assess in a free-living population, a very critical for embryo neuronal development. The strengths of the methodologies employed in this study were discussed in detail in the relevant chapters.

The limitations of the current study should also be considered. In the epidemiology study, BMI was used as a measure of obesity. However, alternative measures of abdominal obesity such as waist circumference, waist/hip ratio and skin-fold thickness could have been used, but were not available in the dataset I used, are

difficult to record at a population level, and can be unreliable in pregnant women. Moreover, this study might not be representative of other settings, or generalisable to other populations, but it does confirm the work of multiple previous investigations. The study of adipocyte function in GDM was limited by the cross-sectional design, which does not provide any information about the adipose tissue changes during pregnancy. However, a longitudinal study design would be difficult to conduct with pregnant women, because the required repeated intra-abdominal procedure during pregnancy would be difficult for pregnant women to tolerate. Another limitation involved adipose tissue inflammation only being assessed in adipocytes, which may not be the main cell contributing to adipokine secretion in adipose tissue. The main limitation of the VLDL FA composition in the early pregnancy study was that the women participants were undergoing *in vitro* fertilisation, and this might not be considered a "normal" pregnancy population. However, the women had a natural menstrual cycle, meaning there was no interference of exogenous hormones used in cycle reconstruction on their metabolic adaptation to pregnancy. The study of this population provided us with accurately timed periconceptual and early pregnancy blood samples that would be extremely difficult to obtain from a free-living population.

In this thesis, much useful data was collected, which has a bearing on current ongoing research in maternal obesity, GDM and clinical practice. Although the epidemiology study provided evidence that GDM prevalence remains low in the Greater Glasgow and Clyde population, maternal obesity and its associated risks still represent a great burden on clinical resources. The study of adipocyte function in GDM adds evidence to the existing literature, showing that adipocyte dysfunction in GDM could be one of the underlying pathophysiological mechanisms for GDM and adverse pregnancy complications among obese mothers. The early pregnancy study provides evidence that VLDL is not the carrier of DHA in early pregnancy.

In conclusion, in a single health board of Scotland's population, there was a notable risk of maternal obesity and GDM, which predisposes mothers to several obstetric complications and adverse outcomes. Furthermore, this study provides evidence that VAT adipocytes are dysfunctional in GDM pregnancy compared to healthy BMI-matched controls. Our data did not provide evidence of adipocyte inflammation in GDM pregnancy. However, the effects of SVF and macrophages were not studied; therefore, their possible contribution to adipose tissue inflammation cannot be

disregarded. Further collection of samples is recommended to improve the power of the current study in order to reach conclusions regarding VAT FCISI and adipocyte inflammation, as the current study was underpowered with regard to detecting significant differences. Finally, the data presented in this thesis has shown that HDL, and not VLDL, is the preferred lipoprotein in terms of DHA transport across the pregnancy timeline, possibly because of HDL's important antioxidant and anti-inflammatory properties that may protect DHA from oxidation. Therefore, the study of HDL metabolism in GDM pregnancy could be of particular importance to provide insight into the neurodevelopmental impacts of reduced DHA levels in the neonates of mothers with GDM.

Appendices

PATIENT INFORMATION LEAFLET

Adipose Tissue, Blood Vessel and Placental Function in Pregnancy (Pregnant Participants)

Researchers: **Dr Amaal Alrehaili** **Postgraduate Researcher** **Dr Sharon Mackin** **Postgraduate Researcher** **Dr Patamat Patapirunhakit** **Postgraduate Researcher** **Dr Kirsten Mitchell** **Postgraduate Researcher** **Mrs Fiona Jordan** **Senior Research Technician** **Institute of Cardiovascular and Medical Sciences** **Level 5, Gardiner Lab (Lab 535),** **Wolfson Link Building** **University of Glasgow,** **G12 8QQ, Glasgow** **Tel: 0141 330 2821**	**Dr Dilys Freeman BSc (Hons), PhD** **Senior Lecturer, Principal Investigator** **Institute of Cardiovascular and Medical Sciences** **Room 534, Wolfson Link Building** **University of Glasgow,** **G12 8QQ, Glasgow** **Tel: 0141 330 2299**

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part.
Part 2 gives you more detailed information about the conduct of the study.
Part 3 provides information on the General Data Protection Regulations (GDPR)

Ask us if there is anything that is not clear, or, if you would like more information. Take time to decide whether or not you wish to take part.

PART 1

1. What is the purpose of the study?
We are interested in how blood, fat tissue and the afterbirth (placenta) function in pregnancy and how these tissues can influence blood vessel function in the mother and the baby. This is important as blood, fat tissue and blood vessel function may play a prominent role in achieving a successful outcome i.e. a healthy mother and baby. In some instances, we are interested in obtaining fat tissue from different body

locations. In other words, our question is does fatty tissue from the lower part of the body influence blood vessels in a different way to fat from the abdomen?" The effects of blood, fat tissue on the blood vessels may produce conditions such as high blood pressure in pregnancy (preeclampsia), diabetes in pregnancy and problems affecting the growth of the baby. We are keen to understand how these and other conditions develop through vessels not working properly or failing to form new blood vessels. Sometimes we use the tissue to provide training and to set up the techniques used in this research.

2. Why have I been chosen?
We wish to study pregnant women at third trimester, like you, with no current medical conditions unrelated to pregnancy, who are undergoing either elective or emergency caesarean-section. We are recruiting women of different ages and weights. We are recruiting mothers with a healthy pregnancy and mothers with pregnancies complicated by preeclampsia, gestational diabetes mellitus and intrauterine growth restriction.

3. Do I have to take part?
No.
It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

4. What will happen to me if I take part?
If you choose to take part in the study we will collect a sample of blood, about 2 tablespoons, at the time that you have a drip inserted as part of the preparation to give you an anaesthetic. This is part of the routine procedure prior to caesarean section.

We will then collect some fat tissue samples during your C-section and we will collect placenta, umbilical cord tissue and cord blood after your baby is delivered.

5. How will the tissue samples be collected?
(A) Fat tissue samples
We are interested in how fat cells and the blood vessels that supply them work in different sites of the body. We would like to obtain small samples (a biopsy) of fat at your C-section delivery while you are still under anaesthesia. At most we would take four small samples although this may not be required in every patient.

The first and second samples are taken from just under the skin (subcutaneous fat) after the C-section cut has been made. The third is from fat tissue within the abdominal cavity (visceral fat) after the womb has been stitched closed, but before the skin is stitched closed. This is an additional procedure that takes approximately 4-5 minutes. It will not significantly lengthen the time of your operation.

In some instances, the fourth will be a biopsy of fat from your upper, outer thigh using a biopsy needle and syringe. This procedure will take approximately 5 minutes.

If you require a caesarean section because of concern about you or your baby's wellbeing or your surgeon does not consider it appropriate, we may not collect any/some of these samples.

(B)Samples from the placenta (afterbirth)
We would like to gain information about how the placenta (afterbirth) functions during pregnancy and we would like to use umbilical cord cells to understand how blood vessels function. Normally the placenta and its attached umbilical cord are delivered after the baby and it is discarded because it has completed its function. In our study, instead of the afterbirth being discarded, the blood retained in the cord will be collected and the placental and umbilical cord tissue will be passed onto the laboratory where it will be studied.

6. What do I have to do?
Your participation in the study will end once the aforementioned samples have been collected.

7. What are the possible disadvantages and risks of taking part?
There are no disadvantages to your baby by taking part in this study. There is a minimal extra risk to you of abdominal bleeding from small vessels in the fat layers over and above that associated with a Caesarean section when the fat samples are collected. As noted above it may also extend your operation by a few minutes. There is a very small risk of bruising associated with the needle biopsy from your upper thigh should this sample be collected; however this is no greater than what would be expected following a blood sample taken from the arm.

8. What are the possible benefits of taking part?
There is no direct benefit for you in taking part in this study. We hope that the information we get from this study will help improve the care of women who develop problems in pregnancy.

10. What if there is a problem?
The samples will be collected at the discretion of the surgeon responsible for your care. If at any point the surgeon feels it is not appropriate to take samples then they will not be taken. If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. The contact numbers are provided on this form. If you remain unhappy and wish to complain formally, you can do so through the NHS complaints procedure. Details can be obtained from the hospital.

11. Will my taking part in the study be kept confidential?
Yes. All the information which we collect will remain completely confidential.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 and Part 3 before making any decision.

PART 2

12. What if relevant new information becomes available?
Since your participation in the study involves a one-off collection of tissue future new information will not affect the tissue collection. New information may suggest modifications and improvement to our laboratory analysis which will not directly affect you.

13. What will happen if I don't want to carry on with the study?
If you withdraw from the study, we will retain the data that has been collected up to your withdrawal. However, should you wish for samples and data to be destroyed we will comply with this request.

14. What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. The contact numbers are provided on this form. If you remain unhappy and wish to complain formally, you can do so through the NHS complaints procedure. Details can be obtained from the hospital.

In the event that something does go wrong and you are harmed during the research study, there are no special compensation arrangements. If you are harmed and this is due to someone's negligence, then you may have grounds for a legal action for compensation against NHS Greater Glasgow and Clyde, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

15. Will my taking part in this study be kept confidential?
Yes. All the information which we collect will remain completely confidential. Any information about you which leaves the hospital will have your name and address removed so you cannot be identified from it.

If you join the study, some of the data collected for the study may be looked at by authorised persons from the University of Glasgow. They may also be looked at by people or representatives of regulatory authorities and by authorised people to check that the study is being carried out correctly.

16. What will happen to any tissue samples or data that I give?
Data and tissue samples collected from the study are retained by the University of Glasgow. Sometimes new research indicates further tests that would expand the knowledge coming from the study and we can use the archived material to carry out additional tests. This allows us to maximise the amount of information on pregnancy complications that we can get from the study. Anonymised data and samples may be shared with collaborators in other institutions who may be able to offer specialised techniques that we do not have in Glasgow.

17. What will happen to the results of the research study?
New information that we gain from the study will be published in scientific journals. No specific individual from whom we have collected tissue will be identified in these publications. These publications are available for all to read.

18. Who is organising and funding the research?

This research will be funded by grants from the Saudi Arabian Cultural Bureau, the Siriraj hospital, Mahidol University, Thailand and the Glasgow Children's Hospital Charity to the University of Glasgow. This work is supervised by Dr Dilys Freeman from Institute of Cardiovascular and Medical Sciences, University of Glasgow.

19. Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the West of Scotland Research Ethics Committee 4.

20. Further Information

Further information about this study may be obtained from Dr Amaal Alrehaili, Dr Sharon Mackin, Dr Patamat Panatapirunhakit, Dr Kirsten Mitchell or Dr Dilys Freeman

Should you require independent advice regarding this study you can contact

Professor Mary Ann Lumsden **Level 2, Reproductive & Maternal Medicine, Glasgow Royal Infirmary, New Lister Building** **Tel: 0141 201 8616**	

PART 3 General Data Protection Regulation (GDPR) Information

NHS Greater Glasgow & Clyde is the sponsor for this study based in Scotland. We will be using information from your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. NHS Greater Glasgow and Clyde will keep identifiable information about you for 10 years after the study has finished.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information by contacting Dr Dilys Freeman

NHS Greater Glasgow and Clyde will collect information about you for the Adipose Tissue, Blood Vessel and Placental Function in Pregnancy from your maternity medical notes. This information will include your name, NHS number, postcode and date of birth and health information, which is regarded as a special category of information. We will use this information to relate our study findings to maternal characteristics (e.g. how does maternal age affect the research findings?) and to delivery characteristics (e.g. how are the research finding's related to baby birth weight?).

When you agree to take part in a research study, the information about your health and care may be provided to researchers running other research studies in this organisation and in other organisations. These organisations may be universities, NHS organisations or companies involved in health and care research in this country or abroad. Your information will only be used by organisations and researchers to conduct research in accordance with the UK Policy Framework for Health and Social Care Research**.**

This information will not identify you and will not be combined with other information in a way that could identify you. The information will only be used for the purpose of health and care research, and cannot be used to contact you or to affect your care. It will not be used to make decisions about future services available to you, such as insurance.

University of Glasgow

STUDY IDENTIFICATION NUMBER_____

Adipose Tissue, Blood Vessel and Placental Function in Pregnancy (Pregnant Participants)

Your Consent	Please initial box
I confirm that I have read and understand the patient information sheet dated 18th September 2018 (Pregnant Participants, Version 7) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw without giving any reason, without my medical care or legal rights being affected.	
I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the University of Glasgow, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
I understand that data and samples used in this study may be used in relevant future research. I give my consent for this.	
I consent to the collection of a blood sample before delivery and tissue samples at delivery. These samples will be retained by the Institute of Cardiovascular and Medical Sciences (ICAMS) at the University of Glasgow.	
I consent to the collection, processing, reporting and transfer within and outside Europe of my anonymised data for healthcare and/or medical research purposes.	
I agree to take part in the above study	

_____ _____ _____ Name of Patient

Date Signature

_____ _____ _____

Name of Person obtaining consent | Date | Signature

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

List of References

ABRAMS, B. & PARKER, J. 1988. Overweight and pregnancy complications. *International journal of obesity,* 12**,** 293-303.

ADAMS, K. M., LI, H., NELSON, R. L., OGBURN, P. L. & DANILENKO-DIXON, D. R. 1998. Sequelae of unrecognized gestational diabetes. *American journal of obstetrics and gynecology,* 178**,** 1321-1332.

AGARWAL, M. M., DHATT, G. S. & SHAH, S. M. 2010. Gestational diabetes mellitus: simplifying the international association of diabetes and pregnancy diagnostic algorithm using fasting plasma glucose. *Diabetes care,* 33**,** 2018-2020.

AGUSTSSON, T., RYDÉN, M., HOFFSTEDT, J., VAN HARMELEN, V., DICKER, A., LAURENCIKIENE, J., ISAKSSON, B., PERMERT, J. & ARNER, P. 2007. Mechanism of increased lipolysis in cancer cachexia. *Cancer research,* 67**,** 5531-5537.

AJMERA, V. H., GUNDERSON, E. P., VANWAGNER, L. B., LEWIS, C. E., CARR, J. J. & TERRAULT, N. A. 2016. Gestational Diabetes Mellitus Is Strongly Associated With Non-Alcoholic Fatty Liver Disease. *American Journal of Gastroenterology,* 111**,** 658-664.

AKASH, M. S. H., REHMAN, K. & LIAQAT, A. 2018. Tumor Necrosis Factor-Alpha: Role in Development of Insulin Resistance and Pathogenesis of Type 2 Diabetes Mellitus. *Journal of Cellular Biochemistry,* 119**,** 105-110.

AL, M. D., VAN HOUWELINGEN, A. C., KESTER, A. D., HASAART, T. H., DE JONG, A. E. & HORNSTRA, G. 1995. Maternal essential fatty acid patterns during normal pregnancy and their

relationship to the neonatal essential fatty acid status. *British Journal of Nutrition,* 74**,** 55-68.

ALVAREZ, J., MONTELONGO, A., IGLESIAS, A., LASUNCION, M. & HERRERA, E. 1996. Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. *Journal of lipid research,* 37**,** 299-308.

AMERICAN DIABETES ASSOCIATION 2015. Classification and diagnosis of diabetes. *Diabetes Care,* 38**,** S8-S16.

ANDERSSON, C. X., SOPASAKIS, V. R., WALLERSTEDT, E. & SMITH, U. 2007. Insulin antagonizes interleukin-6 signaling and is anti-inflammatory in 3T3-L1 adipocytes. *Journal of Biological Chemistry,* 282**,** 9430-9435.

ANGULO, P. 2002. Nonalcoholic fatty liver disease. *New England Journal of Medicine,* 346**,** 1221-1231.

ANWAR, H., FISCHBACHER, C., LEESE, G., LINDSAY, R., MCKNIGHT, J. & WILD, S. 2011. Assessment of the under-reporting of diabetes in hospital admission data: a study from the Scottish Diabetes Research Network Epidemiology Group. *Diabetic Medicine,* 28**,** 1514-1519.

ARAÚJO, J. R., CORREIA-BRANCO, A., RAMALHO, C., KEATING, E. & MARTEL, F. 2013. Gestational diabetes mellitus decreases placental uptake of long-chain polyunsaturated fatty acids: involvement of long-chain acyl-CoA synthetase. *The Journal of Nutritional Biochemistry,* 24**,** 1741-1750.

ARMSTRONG, M., HAZLEHURST, J., HULL, D., GUO, K., BORROWS, S., YU, J., GOUGH, S., NEWSOME, P. & TOMLINSON, J. 2014.

Abdominal subcutaneous adipose tissue insulin resistance and lipolysis in patients with non-alcoholic steatohepatitis. *Diabetes, Obesity and Metabolism,* 16**,** 651-660.

ARNER, P., ANDERSSON, D. P., THÖRNE, A., WIRÉN, M., HOFFSTEDT, J., NÄSLUND, E., THORELL, A. & RYDÉN, M. 2013. Variations in the size of the major omentum are primarily determined by fat cell number. *The Journal of Clinical Endocrinology & Metabolism,* 98**,** E897-E901.

AUGUSTINE, A. H., LOWENSTEIN, L. M., HARRIS, W. S., SHEARER, G. C. & BLOCK, R. C. 2014. Treatment with omega-3 fatty acid ethyl-ester alters fatty acid composition of lipoproteins in overweight or obese adults with insulin resistance. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 90**,** 69-75.

AUNE, D., SAUGSTAD, O. D., HENRIKSEN, T. & TONSTAD, S. 2014. Maternal body mass index and the risk of fetal death, stillbirth, and infant death: a systematic review and meta-analysis. *Jama,* 311**,** 1536-1546.

BARKER, D. J. 1998. In utero programming of chronic disease. *Clinical science,* 95**,** 115-128.

BASU, S., HAGHIAC, M., SURACE, P., CHALLIER, J. C., GUERRE-MILLO, M., SINGH, K., WATERS, T., MINIUM, J., PRESLEY, L. & CATALANO, P. M. 2011. Pregravid obesity associates with increased maternal endotoxemia and metabolic inflammation. *Obesity,* 19**,** 476-482.

BELFIORE, A., FRASCA, F., PANDINI, G., SCIACCA, L. & VIGNERI, R. 2009. Insulin receptor isoforms and insulin receptor/insulin-like growth factor

receptor hybrids in physiology and disease. *Endocrine reviews,* 30**,** 586-623.

BELLAMY, L., CASAS, J.-P., HINGORANI, A. D. & WILLIAMS, D. 2009. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *The Lancet,* 373**,** 1773-1779.

BERKOWITZ, G. S., LAPINSKI, R. H., WEIN, R. & LEE, D. 1992. Race/Ethnicity and Other Risk Factors for Gestational Diabetes. *American Journal of Epidemiology,* 135**,** 965-973.

BILHARTZ, T. D., BILHARTZ, P. A., BILHARTZ, T. N. & BILHARTZ, R. D. 2011. Making use of a natural stress test: pregnancy and cardiovascular risk. *Journal of Women's Health,* 20**,** 695-701.

BILOUS, R. 2015. Diagnosis of gestational diabetes, defining the net, refining the catch. *Diabetologia,* 58**,** 1965-1968.

BLÜHER, M. 2013. Adipose tissue dysfunction contributes to obesity related metabolic diseases. *Best practice & research Clinical endocrinology & metabolism,* 27**,** 163-177.

BODEN, G. 2008. Obesity and Free Fatty Acids. *Endocrinology and Metabolism Clinics of North America,* 37**,** 635-646.

BODNAR, L. M., NESS, R. B., MARKOVIC, N. & ROBERTS, J. M. 2005. The risk of preeclampsia rises with increasing prepregnancy body mass index. *Annals of epidemiology,* 15**,** 475-482.

BOLOGNANI, C. V., DE SOUSA MOREIRA REIS, L. B., DE SOUZA, S. S., DIAS, A., RUDGE, M. V. C. & DE MATTOS PARANHOS CALDERON, I. 2014. Waist circumference in predicting gestational diabetes mellitus. *The Journal of Maternal-Fetal & Neonatal Medicine,* 27**,** 943-948.

BONELLIE, S., CHALMERS, J., GRAY, R., GREER, I., JARVIS, S. & WILLIAMS, C. 2008. Centile charts for birthweight for gestational age for Scottish singleton births. *BMC Pregnancy and Childbirth,* 8**,** 5.

BOULOUMIÉ, A., LOLMEDE, K., SENGENES, C., GALITZKY, J. & LAFONTAN, M. Angiogenesis in adipose tissue. Annales d'endocrinologie, 2002. 91.

BOZKURT, L., GÖBL, C. S., TURA, A., CHMELIK, M., PRIKOSZOVICH, T., KOSI, L., WAGNER, O., RODEN, M., PACINI, G. & GASTALDELLI, A. 2012. Fatty liver index predicts further metabolic deteriorations in women with previous gestational diabetes. *PloS one,* 7.

BRASAEMLE, D. L. 2007. Thematic review series: adipocyte biology. The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis. *Journal of lipid research,* 48**,** 2547-2559.

BRASAEMLE, D. L., RUBIN, B., HARTEN, I. A., GRUIA-GRAY, J., KIMMEL, A. R. & LONDOS, C. 2000. Perilipin A increases triacylglycerol storage by decreasing the rate of triacylglycerol hydrolysis. *Journal of Biological Chemistry,* 275**,** 38486-38493.

BRAUN, J. E. & SEVERSON, D. L. 1992. Regulation of the synthesis, processing and translocation of lipoprotein lipase. *Biochemical Journal,* 287**,** 337-347.

BRAVO-FLORES, E., MANCILLA-HERRERA, I., ESPINO Y SOSA, S., ORTIZ-RAMIREZ, M., FLORES-RUEDA, V., IBARGÜENGOITIA-OCHOA, F., IBAÑEZ, C., ZAMBRANO, E., SOLIS-PAREDES, M. & PERICHART-PERERA, O. 2018.

Macrophage Populations in Visceral Adipose Tissue from Pregnant Women: Potential Role of Obesity in Maternal Inflammation. *International journal of molecular sciences,* 19**,** 1074.

BUCHANAN, T. A. 2001. Pancreatic B-cell defects in gestational diabetes: Implications for the pathogenesis and prevention of type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism,* 86**,** 989-993.

BURDGE, G. C. & WOOTTON, S. A. 2002. Conversion of α-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *British Journal of Nutrition,* 88**,** 411-420.

CAMERON, A. R., MORRISON, V. L., LEVIN, D., MOHAN, M., FORTEATH, C., BEALL, C., MCNEILLY, A. D., BALFOUR, D. J., SAVINKO, T. & WONG, A. K. 2016. Anti-inflammatory effects of metformin irrespective of diabetes status. *Circulation research,* 119**,** 652-665.

CAMPBELL, F. M., GORDON, M. J. & DUTTA-ROY, A. K. 1996. Preferential uptake of long chain polyunsaturated fatty acids by isolated human placental membranes. *Molecular and Cellular Biochemistry,* 155**,** 77-83.

CAMPBELL, S. & SOOTHILL, P. 1993. Detection and management of intrauterine growth retardation: a British approach. *Ultrasound in obstetrics and gynaecology,* 2**,** 1432-5.

CATALANO, P. M. 2010. Obesity, insulin resistance, and pregnancy outcome. *Reproduction,* 140**,** 365-371.

CATALANO, P. M., KIRWAN, J. P., HAUGEL-DE MOUZON, S. & KING, J. 2003. Gestational Diabetes and Insulin Resistance: Role in Short-

and Long-Term Implications for Mother and Fetus. *The Journal of Nutrition,* 133**,** 1674S-1683S.

CATALANO, P. M., NIZIELSKI, S. E., SHAO, J., PRESTON, L., QIAO, L. & FRIEDMAN, J. E. 2002. Downregulated IRS-1 and PPARγ in obese women with gestational diabetes: relationship to FFA during pregnancy. *American Journal of Physiology-Endocrinology and Metabolism,* 282**,** E522-E533.

CATALANO, P. M., ROMAN-DRAGO, N. M., AMINI, S. B. & SIMS, E. A. 1998. Longitudinal changes in body composition and energy balance in lean women with normal and abnormal glucose tolerance during pregnancy. *American journal of obstetrics and gynecology,* 179**,** 156-165.

CATALANO, P. M., TYZBIR, E. D., WOLFE, R. R., ROMAN, N. M., AMINI, S. B. & SIMS, E. A. 1992. Longitudinal changes in basal hepatic glucose production and suppression during insulin infusion in normal pregnant women. *American journal of obstetrics and gynecology,* 167**,** 913-919.

CEDERGREN, M. I. 2004. Maternal morbid obesity and the risk of adverse pregnancy outcome. *Obstetrics & Gynecology,* 103**,** 219-224.

CHALLIER, J., BASU, S., BINTEIN, T., MINIUM, J., HOTMIRE, K., CATALANO, P. & HAUGUEL-DE MOUZON, S. 2008. Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. *Placenta,* 29**,** 274-281.

CHALLIS, J. R., LOCKWOOD, C. J., MYATT, L., NORMAN, J. E., STRAUSS, J. F. & PETRAGLIA, F. 2009. Inflammation and pregnancy. *Reproductive Sciences,* 16**,** 206-215.

CHAPPELL, L. C., ENYE, S., SEED, P., BRILEY, A. L., POSTON, L. & SHENNAN, A. H. 2008. Adverse perinatal outcomes and risk factors for preeclampsia in women with chronic hypertension: a prospective study. *Hypertension,* 51**,** 1002-1009.

CHAVARRO, J. E., RICH-EDWARDS, J. W., ROSNER, B. A. & WILLETT, W. C. 2007. Diet and lifestyle in the prevention of ovulatory disorder infertility. *Obstetrics and Gynecology,* 110**,** 1050-1058.

CHEN, Y., QUICK, W. W., YANG, W., ZHANG, Y., BALDWIN, A., MORAN, J., MOORE, V., SAHAI, N. & DALL, T. M. 2009. Cost of gestational diabetes mellitus in the United States in 2007. *Population health management,* 12**,** 165-174.

CHRISTIAN, L. M. & PORTER, K. 2014. Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: effects of maternal body mass index. *Cytokine,* 70**,** 134-140.

CINTI, S., MITCHELL, G., BARBATELLI, G., MURANO, I., CERESI, E., FALOIA, E., WANG, S., FORTIER, M., GREENBERG, A. S. & OBIN, M. S. 2005. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *Journal of lipid research,* 46**,** 2347-2355.

CLAUSEN, T., DJUROVIC, S. & HENRIKSEN, T. 2001. Dyslipidemia in early second trimester is mainly a feature of women with early onset pre-eclampsia. *British Journal of Obstetrics and gynaecology,* 108**,** 1081-1087.

CLÉMENT, K., VIGUERIE, N., POITOU, C., CARETTE, C., PELLOUX, V., CURAT, C. A., SICARD, A., ROME, S., BENIS, A., ZUCKER, J. D., VIDAL, H., LAVILLE, M., BARSH, G. S.,

BASDEVANT, A., STICH, V., CANCELLO, R. & LANGIN, D. 2004. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. *FASEB Journal,* 18**,** 1657-1669.

COCKELL, A. P. & POSTON, L. 1997. Flow-mediated vasodilatation is enhanced in normal pregnancy but reduced in preeclampsia. *Hypertension,* 30**,** 247-251.

COLE, T. 1988. Fitting smoothed centile curves to reference data. *Journal of the Royal Statistical Society: Series A (Statistics in Society),* 151**,** 385-406.

COLEMAN, R. A. & HAYNES, E. B. 1987. Synthesis and release of fatty acids by human trophoblast cells in culture. *Journal of lipid research,* 28**,** 1335-1341.

COLLIER, A., ABRAHAM, E. C., ARMSTRONG, J., GODWIN, J., MONTEATH, K. & LINDSAY, R. 2017. Reported prevalence of gestational diabetes in Scotland: The relationship with obesity, age, socioeconomic status, smoking and macrosomia, and how many are we missing? *Journal of diabetes investigation,* 8**,** 161-167.

COLOMIERE, M., PERMEZEL, M. & LAPPAS, M. 2010. Diabetes and obesity during pregnancy alter insulin signalling and glucose transporter expression in maternal skeletal muscle and subcutaneous adipose tissue. *J Mol Endocrinol,* 44**,** 213-223.

COLOMIERE, M., PERMEZEL, M., RILEY, C., DESOYE, G. & LAPPAS, M. 2009. Defective insulin signaling in placenta from pregnancies complicated by gestational diabetes mellitus. *European Journal of Endocrinology,* 160**,** 567-578.

COMBS, T. P., PAJVANI, U. B., BERG, A. H., LIN, Y., JELICKS, L. A., LAPLANTE, M., NAWROCKI, A. R., RAJALA, M. W., PARLOW, A. F. & CHEESEBORO, L. 2004. A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. *Endocrinology,* 145**,** 367-383.

CROWTHER, C. A., HILLER, J. E., MOSS, J. R., MCPHEE, A. J., JEFFRIES, W. S. & ROBINSON, J. S. 2005. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *New England Journal of Medicine,* 352**,** 2477-2486.

CURRY, A., VOGEL, I., SKOGSTRAND, K., DREWS, C., SCHENDEL, D., FLANDERS, W., HOUGAARD, D. M. & THORSEN, P. 2008. Maternal plasma cytokines in early-and mid-gestation of normal human pregnancy and their association with maternal factors. *Journal of reproductive immunology,* 77**,** 152-160.

CZECH, M. P. & CORVERA, S. 1999. Signaling mechanisms that regulate glucose transport. *Journal of Biological Chemistry,* 274**,** 1865-1868.

D'AMBROSI, F., CROVETTO, F., COLOSI, E., FABIETTI, I., CARBONE, F., TASSIS, B., MOTTA, S., BULFONI, A., FEDELE, L., ROSSI, G. & PERSICO, N. 2018. Maternal Subcutaneous and Visceral Adipose Ultrasound Thickness in Women with Gestational Diabetes Mellitus at 24-28 Weeks' Gestation. *Fetal Diagnosis and Therapy,* 43**,** 143-147.

DAS, U. N. 2001. Is insulin an antiinflammatory molecule? *Nutrition,* 17**,** 409-413.

DE GROOT, R. H., HORNSTRA, G., VAN HOUWELINGEN, A. C. & ROUMEN, F. 2004.

Effect of α-linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome. *The American journal of clinical nutrition,* 79**,** 251-260.

DE SOUZA, L. R., BERGER, H., RETNAKARAN, R., MAGUIRE, J. L., NATHENS, A. B., CONNELLY, P. W. & RAY, J. G. 2016. First-trimester maternal abdominal adiposity predicts dysglycemia and gestational diabetes mellitus in midpregnancy. *Diabetes Care,* 39**,** 61-64.

DE SOUZA, L. R., KOGAN, E., BERGER, H., ALVES, J. G., LEBOVIC, G., RETNAKARAN, R., MAGUIRE, J. L. & RAY, J. G. 2014. Abdominal adiposity and insulin resistance in early pregnancy. *Journal of Obstetrics and Gynaecology Canada,* 36**,** 969-975.

DENISON, F., NORWOOD, P., BHATTACHARYA, S., DUFFY, A., MAHMOOD, T., MORRIS, C., RAJA, E., NORMAN, J., LEE, A. & SCOTLAND, G. 2014. Association between maternal body mass index during pregnancy, short-term morbidity, and increased health service costs: a population-based study. *BJOG: An International Journal of Obstetrics & Gynaecology,* 121**,** 72-82.

DESPRÉS, J.-P. & LEMIEUX, I. 2006. Abdominal obesity and metabolic syndrome. *Nature,* 444**,** 881-887.

DIAZ-RUIZ, A., GUZMAN-RUIZ, R., MORENO, N. R., GARCIA-RIOS, A., DELGADO-CASADO, N., MEMBRIVES, A., TÚNEZ, I., EL BEKAY, R., FERNÁNDEZ-REAL, J. M. & TOVAR, S. 2015. Proteasome dysfunction associated to oxidative stress and proteotoxicity in adipocytes

compromises insulin sensitivity in human obesity. *Antioxidants & redox signaling,* 23**,** 597-612.

DIDERHOLM, B., STRIDSBERG, M., EWALD, U., LINDEBERG-NORDÉN, S. & GUSTAFSSON, J. 2005. Increased lipolysis in non-obese pregnant women studied in the third trimester. *BJOG: An International Journal of Obstetrics & Gynaecology,* 112**,** 713-718.

DIONNE, G., BOIVIN, M., SÉGUIN, J. R., PÉRUSSE, D. & TREMBLAY, R. E. 2008. Gestational diabetes hinders language development in offspring. *Pediatrics,* 122**,** e1073-e1079.

DIVELLA, R., DE LUCA, R., ABBATE, I., NAGLIERI, E. & DANIELE, A. 2016. Obesity and cancer: the role of adipose tissue and adipo-cytokines-induced chronic inflammation. *Journal of Cancer,* 7**,** 2346.

DJELMIS, J., PAVIĆ, M., MULLIQI KOTORI, V., PAVLIĆ RENAR, I., IVANISEVIC, M. & ORESKOVIC, S. 2016. Prevalence of gestational diabetes mellitus according to IADPSG and NICE criteria. *International Journal of Gynecology & Obstetrics,* 135**,** 250-254.

DONG, Y., CHAUHAN, M., BETANCOURT, A., BELFORT, M. & YALLAMPALLI, C. 2018. Adipose tissue inflammation and adrenomedullin overexpression contribute to lipid dysregulation in diabetic pregnancies. *The Journal of Clinical Endocrinology & Metabolism,* 103**,** 3810-3818.

DONNELLY, K. L., SMITH, C. I., SCHWARZENBERG, S. J., JESSURUN, J., BOLDT, M. D. & PARKS, E. J. 2005. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *The Journal of clinical investigation,* 115**,** 1343-1351.

DROLET, R., RICHARD, C., SNIDERMAN, A., MAILLOUX, J., FORTIER, M., HUOT, C., RHEAUME, C. & TCHERNOF, A. 2008. Hypertrophy and hyperplasia of abdominal adipose tissues in women. *International journal of obesity,* 32**,** 283-291.

DUBÉ, E., ETHIER-CHIASSON, M. & LAFOND, J. 2013. Modulation of cholesterol transport by insulin-treated gestational diabetes mellitus in human full-term placenta. *Biology of reproduction,* 88**,** 16, 1-10.

DUTTAROY, A. K. 2009. Transport of fatty acids across the human placenta: A review. *Progress in Lipid Research,* 48**,** 52-61.

EGAN, D. F., SHACKELFORD, D. B., MIHAYLOVA, M. M., GELINO, S., KOHNZ, R. A., MAIR, W., VASQUEZ, D. S., JOSHI, A., GWINN, D. M. & TAYLOR, R. 2011. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science,* 331**,** 456-461.

EHRENBERG, H. M., HUSTON-PRESLEY, L. & CATALANO, P. M. 2003. The influence of obesity and gestational diabetes mellitus on accretion and the distribution of adipose tissue in pregnancy. *American journal of obstetrics and gynecology,* 189**,** 944-948.

EL-CHAAR, D., FINKELSTEIN, S. A., TU, X., FELL, D. B., GAUDET, L., SYLVAIN, J., TAWAGI, G., WEN, S. W. & WALKER, M. 2013. The impact of increasing obesity class on obstetrical outcomes. *Journal of Obstetrics and Gynaecology Canada,* 35**,** 224-233.

ELLIS, J. M., FRAHM, J. L., LI, L. O. & COLEMAN, R. A. 2010. Acyl-coenzyme A synthetases in

metabolic control. *Current opinion in lipidology,* 21**,** 212.

ENDEMANN, D. H. & SCHIFFRIN, E. L. 2004. Endothelial dysfunction. *Journal of the American Society of Nephrology,* 15**,** 1983-1992.

ENGELI, S., FELDPAUSCH, M., GORZELNIAK, K., HARTWIG, F., HEINTZE, U., JANKE, J., MÖHLIG, M., PFEIFFER, A. F., LUFT, F. C. & SHARMA, A. M. 2003. Association between adiponectin and mediators of inflammation in obese women. *Diabetes,* 52**,** 942-947.

FAHRAEUS, L., LARSSON-COHN, U. & WALLENTIN, L. 1985. Plasma lipoproteins including high density lipoprotein subfractions during normal pregnancy. *Obstetrics and gynecology,* 66**,** 468-472.

FANTUZZI, G. 2005. Adipose tissue, adipokines, and inflammation. *Journal of Allergy and Clinical Immunology,* 115**,** 911-919.

FARLEY, D., TEJERO, M. E., COMUZZIE, A. G., HIGGINS, P. B., COX, L., WERNER, S. L., JENKINS, S. L., LI, C., CHOI, J. & DICK, E. 2009. Feto-placental adaptations to maternal obesity in the baboon. *Placenta,* 30**,** 752-760.

FENG, H., SU, R., SONG, Y., WANG, C., LIN, L., MA, J. & YANG, H. 2016. Positive correlation between enhanced expression of TLR4/MyD88/NF-κB with insulin resistance in placentae of gestational diabetes mellitus. *PloS one,* 11.

FISHER, E. A. & GINSBERG, H. N. 2002. Complexity in the secretory pathway: the assembly and secretion of apolipoprotein B-containing lipoproteins. *Journal of Biological Chemistry,* 277**,** 17377-17380.

FISHER, E. A., PAN, M., CHEN, X., WU, X., WANG, H., JAMIL, H., SPARKS, J. D. & WILLIAMS, K. J. 2001. The triple threat to nascent apolipoprotein B Evidence for multiple, distinct degradative pathways. *Journal of Biological Chemistry,* 276**,** 27855-27863.

FRANCKHAUSER, S., MUÑOZ, S., PUJOL, A., CASELLAS, A., RIU, E., OTAEGUI, P., SU, B. & BOSCH, F. 2002. Increased fatty acid re-esterification by PEPCK overexpression in adipose tissue leads to obesity without insulin resistance. *Diabetes,* 51**,** 624-630.

FRAYN, K., FIELDING, B. & SUMMERS, L. 1997. Obesity and the adipocyte. Investigation of human adipose tissue metabolism in vivo. *Journal of Endocrinology,* 155**,** 187-189.

FREEMAN, D. J. 2010. Effects of maternal obesity on fetal growth and body composition: implications for programming and future health. *Seminars in Fetal and Neonatal Medicine,* 15**,** 113-118.

FREEMAN, D. J. & MEYER, B. J. 2017. Brain food for babies. *The Biochemist,* 39**,** 26-29.

FRIEDMAN, J. E., ISHIZUKA, T., SHAO, J., HUSTON, L., HIGHMAN, T. & CATALANO, P. 1999. Impaired glucose transport and insulin receptor tyrosine phosphorylation in skeletal muscle from obese women with gestational diabetes. *Diabetes,* 48**,** 1807-1814.

GARTON, A. J., CAMPBELL, D. G., COHEN, P. & YEAMAN, S. J. 1988. Primary structure of the site on bovine hormone-sensitive lipase phosphorylated by cyclic AMP-dependent protein kinase. *FEBS letters,* 229**,** 68-72.

GARVEY, W. T., MAIANU, L., ZHU, J.-H., HANCOCK, J. A. & GOLICHOWSKI, A. M. 1993. Multiple

defects in the adipocyte glucose transport system cause cellular insulin resistance in gestational diabetes: heterogeneity in the number and a novel abnormality in subcellular localization of GLUT4 glucose transporters. *Diabetes,* 42**,** 1773-1785.

GEALEKMAN, O., GUSEVA, N., HARTIGAN, C., APOTHEKER, S., GORGOGLIONE, M., GURAV, K., TRAN, K.-V., STRAUBHAAR, J., NICOLORO, S. & CZECH, M. P. 2011. Depot-specific differences and insufficient subcutaneous adipose tissue angiogenesis in human obesity. *Circulation,* 123**,** 186-194.

GESINK LAW, D., MACLEHOSE, R. F. & LONGNECKER, M. P. 2006. Obesity and time to pregnancy. *Human Reproduction,* 22**,** 414-420.

GIACCA, A., XIAO, C., OPRESCU, A. I., CARPENTIER, A. C. & LEWIS, G. F. 2011. Lipid-induced pancreatic β-cell dysfunction: focus on in vivo studies. *American Journal of Physiology-Endocrinology And Metabolism,* 300**,** E255-E262.

GOLDRICK, R. & MCLOUGHLIN, G. 1970. Lipolysis and lipogenesis from glucose in human fat cells of different sizes: Effects of insulin, epinephrine, and theophylline. *The Journal of clinical investigation,* 49**,** 1213-1223.

GOOSSENS, G. H. 2008. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiology & behavior,* 94**,** 206-218.

GOURGARI, E., PLAYFORD, M. P., CAMPIA, U., DEY, A. K., COGEN, F., GUBB-WEISER, S., METE, M., DESALE, S., SAMPSON, M. & TAYLOR, A. 2018. Low cholesterol efflux capacity and abnormal lipoprotein particles in youth with

type 1 diabetes: a case control study. *Cardiovascular diabetology,* 17**,** 158.

GRANNEMAN, J. G., MOORE, H.-P. H., KRISHNAMOORTHY, R. & RATHOD, M. 2009. Perilipin controls lipolysis by regulating the interactions of AB-hydrolase containing 5 (Abhd5) and adipose triglyceride lipase (Atgl). *Journal of Biological Chemistry,* 284**,** 34538-34544.

GRANTZ, K. L., KIM, S., GROBMAN, W. A., NEWMAN, R., OWEN, J., SKUPSKI, D., GREWAL, J., CHIEN, E. K., WING, D. A. & WAPNER, R. J. 2018. Fetal growth velocity: the NICHD fetal growth studies. *American journal of obstetrics and gynecology,* 219**,** 285. e1-285. e36.

GREENBERG, A. S., EGAN, J. J., WEK, S. A., GARTY, N. B., BLANCHETTE-MACKIE, E. J. & LONDOS, C. 1991. Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets. *Journal of Biological Chemistry,* 266**,** 11341-11346.

GRIFFIN, B. A., FREEMAN, D. J., TAIT, G. W., THOMSON, J., CASLAKE, M. J., PACKARD, C. J. & SHEPHERD, J. 1994. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk. *Atherosclerosis,* 106**,** 241-253.

GUENTHER, M., JAMES, R., MARKS, J., ZHAO, S., SZABO, A. & KIDAMBI, S. 2014. Adiposity distribution influences circulating adiponectin levels. *Translational Research,* 164**,** 270-277.

GUESNET, P. & ALESSANDRI, J.-M. 2011. Docosahexaenoic acid (DHA) and the developing

central nervous system (CNS)–implications for dietary recommendations. *Biochimie,* 93**,** 7-12.

GUILHERME, A., VIRBASIUS, J. V., PURI, V. & CZECH, M. P. 2008. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nature reviews Molecular cell biology,* 9**,** 367-377.

GUSTAFSON, B., GOGG, S., HEDJAZIFAR, S., JENNDAHL, L., HAMMARSTEDT, A. & SMITH, U. 2009. Inflammation and impaired adipogenesis in hypertrophic obesity in man. *American Journal of Physiology-Endocrinology and Metabolism,* 297**,** E999-E1003.

HAGGARTY, P. 2002. Placental regulation of fatty acid delivery and its effect on fetal growth—a review. *Placenta,* 23**,** S28-S38.

HAJRI, T. & ABUMRAD, N. A. 2002. Fatty acid transport across membranes: relevance to nutrition and metabolic pathology. *Annual review of nutrition,* 22**,** 383-415.

HANEBUTT, F. L., DEMMELMAIR, H., SCHIESSL, B., LARQUÉ, E. & KOLETZKO, B. 2008. Long-chain polyunsaturated fatty acid (LC-PUFA) transfer across the placenta. *Clinical Nutrition,* 27**,** 685-693.

HARLEV, A., ARICHA-TAMIR, B., SHACO-LEVY, R., TARNOVSCKI, T., BASHAN, N., RUDICH, A., SHEINER, E., PRESS, F. & WIZNITZER, A. 2014. Macrophage infiltration and stress-signaling in omental and subcutaneous adipose tissue in diabetic pregnancies. *The Journal of Maternal-Fetal & Neonatal Medicine,* 27**,** 1189-1194.

HENDLER, I., GOLDENBERG, R. L., MERCER, B. M., IAMS, J. D., MEIS, P. J., MOAWAD, A. H., MACPHERSON, C. A., CARITIS, S. N.,

MIODOVNIK, M. & MENARD, K. M. 2005. The Preterm Prediction Study: association between maternal body mass index and spontaneous and indicated preterm birth. *American journal of obstetrics and gynecology,* 192**,** 882-886.

HERNÁEZ CAMBA, Á., SORIA FLORIDO, M. T., SCHRÖDER, H., ROS RAHOLA, E., PINTÓ SALA, X., ESTRUCH RIBA, R., SALAS SALVADÓ, J., CORELLA PIQUER, D., ARÓS, F. & SERRA MAJEM, L. 2019. Role of HDL function and LDL atherogenicity on cardiovascular risk: a comprehensive examination. *PLoS One, 2019, vol. 14, núm. 6: e0218533*.

HERRERA, E. 2002a. Implications of Dietary Fatty Acids During Pregnancy on Placental, Fetal and Postnatal Development—A Review. *Placenta,* 23**,** S9-S19.

HERRERA, E. 2002b. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. *Endocrine,* 19**,** 43-55.

HERRERA, E., AMUSQUIVAR, E., LÓPEZ-SOLDADO, I. & ORTEGA, H. 2006. Maternal Lipid Metabolism and Placental Lipid Transfer. *Hormone Research in Paediatrics,* 65(suppl 3)**,** 59-64.

HERRERA, E. & ORTEGA-SENOVILLA, H. 2018. Implications of lipids in neonatal body weight and fat mass in gestational diabetic mothers and non-diabetic controls. *Current diabetes reports,* 18**,** 7.

HESLEHURST, N., RANKIN, J., WILKINSON, J. & SUMMERBELL, C. 2010a. A nationally representative study of maternal obesity in England, UK: trends in incidence and demographic inequalities in 619 323 births, 1989–

2007. *International Journal of Obesity,* 34**,** 420-428.

HESLEHURST, N., RANKIN, J., WILKINSON, J. & SUMMERBELL, C. 2010b. A nationally representative study of maternal obesity in England, UK: trends in incidence and demographic inequalities in 619 323 births, 1989–2007. *International journal of obesity,* 34**,** 420.

HESLEHURST, N., SIMPSON, H., ELLS, L. J., RANKIN, J., WILKINSON, J., LANG, R., BROWN, T. J. & SUMMERBELL, C. D. 2008. The impact of maternal BMI status on pregnancy outcomes with immediate short-term obstetric resource implications: a meta-analysis. *Obesity reviews,* 9**,** 635-683.

HOCKING, S. L., WU, L. E., GUILHAUS, M., CHISHOLM, D. J. & JAMES, D. E. 2010. Intrinsic depot-specific differences in the secretome of adipose tissue, preadipocytes, and adipose tissue–derived microvascular endothelial cells. *Diabetes,* 59**,** 3008-3016.

HOD, M., LEVY-SHIFF, R., LERMAN, M., SCHINDEL, B., BEN-RAFAEL, Z. & BAR, J. 1999. Developmental outcome of offspring of pregestational diabetic mothers. *Journal of Pediatric Endocrinology and Metabolism,* 12**,** 867-872.

HOFFSTEDT, J., ARNER, E., WAHRENBERG, H., ANDERSSON, D., QVISTH, V., LÖFGREN, P., RYDEN, M., THÖRNE, A., WIREN, M. & PALMER, M. 2010. Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity. *Diabetologia,* 53**,** 2496-2503.

HORVATH, K., KOCH, K., JEITLER, K., MATYAS, E., BENDER, R., BASTIAN, H., LANGE, S. &

SIEBENHOFER, A. 2010. Effects of treatment in women with gestational diabetes mellitus: systematic review and meta-analysis. *Bmj,* 340**,** c1395.

HOTAMISLIGIL, G. S., BUDAVARI, A., MURRAY, D. & SPIEGELMAN, B. M. 1994. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. *The Journal of Clinical Investigation,* 94**,** 1543-1549.

HUANG, J., MOREHOUSE, C., STREICHER, K., HIGGS, B. W., GAO, J., CZAPIGA, M., BOUTRIN, A., ZHU, W., BROHAWN, P. & CHANG, Y. 2011. Altered expression of insulin receptor isoforms in breast cancer. *PloS one,* 6**,** e26177.

HUDA, S. S., FORREST, R., PATERSON, N., JORDAN, F., SATTAR, N. & FREEMAN, D. J. 2014. In preeclampsia, maternal third trimester subcutaneous adipocyte lipolysis is more resistant to suppression by insulin than in healthy pregnancy. *Hypertension,* 63**,** 1094-1101.

HUDA, SHAHZYA S., JORDAN, F., BRAY, J., LOVE, G., PAYNE, R., SATTAR, N. & FREEMAN, DILYS J. 2017. Visceral adipose tissue activated macrophage content and inflammatory adipokine secretion is higher in pre-eclampsia than in healthy pregnancys. *Clinical Science,* 131**,** 1529-1540.

HUDA, S. S., SATTAR, N. & FREEMAN, D. J. 2009. Lipoprotein metabolism and vascular complications in pregnancy. *Clinical Lipidology,* 4**,** 91-102.

IGLESIAS, A., MONTELONGO, A., HERRERA, E. & LASUNCIÓN, M. A. 1994. Changes in cholesteryl ester transfer protein activity during normal

gestation and postpartum. *Clinical biochemistry,* 27**,** 63-68.

INFORMATION SERVICES DIVISION SCOTLAND. 2010. *Data Quality Assurance Assessment of Maternity Data (SMR02) 2008-2009* [Online]. Available: https://www.isdscotland.org/data_quality_assurance/DQA-Assessment-of-Maternity-Data-SMR02-2008-to-2009.pdf [Accessed 21-05-2019].

INFORMATION SERVICES DIVISION SCOTLAND. 2019. *DEPRIVATION GUIDANCE FOR ANALYSTS* [Online]. Available: https://www.isdscotland.org/Products-and-Services/GPD-Support/Deprivation/_docs/PHI-Deprivation-Guidance-version-3-2.pdf [Accessed 21-05-2019].

INKSTER, M. E., FAHEY, T. P., DONNAN, P. T., LEESE, G. P., MIRES, G. J. & MURPHY, D. J. 2006. Poor glycated haemoglobin control and adverse pregnancy outcomes in type 1 and type 2 diabetes mellitus: systematic review of observational studies. *BMC Pregnancy and Childbirth,* 6**,** 1.

INNIS, S. M. 1991. Essential fatty acids in growth and development. *Progress in lipid research,* 30**,** 39-103.

INTERNATIONAL ASSOCIATION OF DIABETES AND PREGNANCY STUDY GROUPS 2010. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes care,* 33**,** 676-682.

JEFFERY, E., CHURCH, C. D., HOLTRUP, B., COLMAN, L. & RODEHEFFER, M. S. 2015. Rapid depot-specific activation of adipocyte precursor

cells at the onset of obesity. *Nature cell biology,* 17**,** 376-385.

JENSEN, M. D. 2008. Role of body fat distribution and the metabolic complications of obesity. *The Journal of Clinical Endocrinology & Metabolism,* 93**,** s57-s63.

JENUM, A. K., MØRKRID, K., SLETNER, L., VANGE, S., TORPER, J. L., NAKSTAD, B., VOLDNER, N., ROGNERUD-JENSEN, O. H., BERNTSEN, S. & MOSDØL, A. 2012. Impact of ethnicity on gestational diabetes identified with the WHO and the modified International Association of Diabetes and Pregnancy Study Groups criteria: a population-based cohort study. *European journal of endocrinology,* 166**,** 317-324.

JOCKEN, J. W., LANGIN, D., SMIT, E., SARIS, W. H., VALLE, C., HUL, G. B., HOLM, C., ARNER, P. & BLAAK, E. E. 2007. Adipose triglyceride lipase and hormone-sensitive lipase protein expression is decreased in the obese insulin-resistant state. *The journal of clinical endocrinology & metabolism,* 92**,** 2292-2299.

JOE, A. W., YI, L., EVEN, Y., VOGL, A. W. & ROSSI, F. M. 2009. Depot-specific differences in adipogenic progenitor abundance and proliferative response to high-fat diet. *Stem cells,* 27**,** 2563-2570.

JOHNSON, S., EVANS, T. A., DRAPER, E. S., FIELD, D. J., MANKTELOW, B. N., MARLOW, N., MATTHEWS, R., PETROU, S., SEATON, S. E. & SMITH, L. K. 2015. Neurodevelopmental outcomes following late and moderate prematurity: a population-based cohort study. *Archives of Disease in Childhood-Fetal and Neonatal Edition,* 100**,** F301-F308.

JORGE-GALARZA, E., MEDINA-URRUTIA, A., POSADAS-SÁNCHEZ, R., POSADAS-ROMERO, C., CARDOSO-SALDAÑA, G., VARGAS-ALARCÓN, G., CARACAS-PORTILLA, N., GONZÁLEZ-SALAZAR, C., TORRES-TAMAYO, M. & JUÁREZ-ROJAS, J. G. 2016. Adipose tissue dysfunction increases fatty liver association with pre diabetes and newly diagnosed type 2 diabetes mellitus. *Diabetology & metabolic syndrome,* 8**,** 73.

KAC, G., DOS SANTOS VAZ, J., SCHLÜSSEL, M. M. & MOURA, A. S. 2011. C-reactive protein and hormones but not IL-6 are associated to body mass index in first trimester of pregnancy. *Archives of gynecology and obstetrics,* 284**,** 567-573.

KALHAN, S. C., MAHAJAN, S., BURKETT, E., RESHEF, L. & HANSON, R. W. 2001. Glyceroneogenesis and the source of glycerol for hepatic triacylglycerol synthesis in humans. *Journal of Biological Chemistry,* 276**,** 12928-12931.

KANAGALINGAM, M. G., FOROUHI, N. G., GREER, I. A. & SATTAR, N. 2005. Changes in booking body mass index over a decade: retrospective analysis from a Glasgow Maternity Hospital. *BJOG: An International Journal of Obstetrics & Gynaecology,* 112**,** 1431-1433.

KARIHTALA, P. & SOINI, Y. 2007. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. *Apmis,* 115**,** 81-103.

KAUTZKY-WILLER, A., KRSSAK, M., WINZER, C., PACINI, G., TURA, A., FARHAN, S., WAGNER, O., BRABANT, G., HORN, R. & STINGL, H. 2003.

Increased intramyocellular lipid concentration identifies impaired glucose metabolism in women with previous gestational diabetes. *Diabetes,* 52**,** 244-251.

KERSHAW, E. E. & FLIER, J. S. 2004. Adipose tissue as an endocrine organ. *The Journal of Clinical Endocrinology & Metabolism,* 89**,** 2548-2556.

KHALIL, A., REZENDE, J., AKOLEKAR, R., SYNGELAKI, A. & NICOLAIDES, K. 2013. Maternal racial origin and adverse pregnancy outcome: a cohort study. *Ultrasound in Obstetrics & Gynecology,* 41**,** 278-285.

KHALIL, S. F., MOHKTAR, M. S. & IBRAHIM, F. 2014. The theory and fundamentals of bioimpedance analysis in clinical status monitoring and diagnosis of diseases. *Sensors,* 14**,** 10895-10928.

KIM, C., NEWTON, K. M. & KNOPP, R. H. 2002. Gestational Diabetes and the Incidence of Type 2 Diabetes A systematic review. *Diabetes care,* 25**,** 1862-1868.

KIM, J. I., HUH, J. Y., SOHN, J. H., CHOE, S. S., LEE, Y. S., LIM, C. Y., JO, A., PARK, S. B., HAN, W. & KIM, J. B. 2015. Lipid-overloaded enlarged adipocytes provoke insulin resistance independent of inflammation. *Molecular and cellular biology,* 35**,** 1686-1699.

KIM, S. M., LUN, M., WANG, M., SENYO, S. E., GUILLERMIER, C., PATWARI, P. & STEINHAUSER, M. L. 2014. Loss of white adipose hyperplastic potential is associated with enhanced susceptibility to insulin resistance. *Cell metabolism,* 20**,** 1049-1058.

KIM, S. S., ZHU, Y., GRANTZ, K. L., HINKLE, S. N., CHEN, Z., WALLACE, M. E., SMARR, M. M., EPPS, N. M. & MENDOLA, P. 2016. Obstetric and

Neonatal Risks Among Obese Women Without Chronic Disease. *Obstetrics and gynecology*.

KINOSHITA, T. & ITOH, M. 2006. Longitudinal variance of fat mass deposition during pregnancy evaluated by ultrasonography: the ratio of visceral fat to subcutaneous fat in the abdomen. *Gynecologic and obstetric investigation,* 61**,** 115-118.

KLEIBLOVA, P., DOSTALOVA, I., BARTLOVA, M., LACINOVA, Z., TICHA, I., KREJCI, V., SPRINGER, D., KLEIBL, Z. & HALUZIK, M. 2010. Expression of adipokines and estrogen receptors in adipose tissue and placenta of patients with gestational diabetes mellitus. *Molecular and cellular endocrinology,* 314**,** 150-156.

KNOCK, G. A., MCCARTHY, A. L., LOWY, C. & POSTON, L. 1997. Association of gestational diabetes with abnormal maternal vascular endothelial function. *BJOG: An International Journal of Obstetrics & Gynaecology,* 104**,** 229-234.

KNOPP, R. H., BONET, B. & ZHU, X. 1998. Lipid metabolism in pregnancy. *Principles of Perinatal—Neonatal Metabolism.* Springer.

KOLEHMAINEN, M., OHISALO, J., KAARTINEN, J., TUONONEN, V., PÄÄKKÖNEN, M., POIKOLAINEN, E., ALHAVA, E. & UUSITUPA, M. 2000. Concordance of in vivo microdialysis and in vitro techniques in the studies of adipose tissue metabolism. *International journal of obesity,* 24**,** 1426.

KOLETZKO, B. & BRAUN, M. 1991. Arachidonic acid and early human growth: is there a relation? *Annals of nutrition and metabolism,* 35**,** 128-131.

KOTRONEN, A., JUURINEN, L., TIIKKAINEN, M., VEHKAVAARA, S. & YKI–JÄRVINEN, H. 2008. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. *Gastroenterology,* 135**,** 122-130.

KUBOTA, N., TERAUCHI, Y., YAMAUCHI, T., KUBOTA, T., MOROI, M., MATSUI, J., ETO, K., YAMASHITA, T., KAMON, J. & SATOH, H. 2002. Disruption of adiponectin causes insulin resistance and neointimal formation. *Journal of Biological Chemistry,* 277**,** 25863-25866.

KUSMINSKI, C. M., HOLLAND, W. L., SUN, K., PARK, J., SPURGIN, S. B., LIN, Y., ASKEW, G. R., SIMCOX, J. A., MCCLAIN, D. A. & LI, C. 2012. MitoNEET-driven alterations in adipocyte mitochondrial activity reveal a crucial adaptive process that preserves insulin sensitivity in obesity. *Nature medicine,* 18**,** 1539.

KUSMINSKI, C. M., SHETTY, S., ORCI, L., UNGER, R. H. & SCHERER, P. E. 2009. Diabetes and apoptosis: lipotoxicity. *Apoptosis,* 14**,** 1484-1495.

KWAK, S. H., CHOI, S. H., JUNG, H. S., CHO, Y. M., LIM, S., CHO, N. H., KIM, S. Y., PARK, K. S. & JANG, H. C. 2013. Clinical and genetic risk factors for type 2 diabetes at early or late post partum after gestational diabetes mellitus. *The Journal of Clinical Endocrinology & Metabolism,* 98**,** E744-E752.

LAFONTAN, M. & BERLAN, M. 1993. Fat cell adrenergic receptors and the control of white and brown fat cell function. *Journal of lipid research,* 34**,** 1057-1091.

LANDON, M. B., SPONG, C. Y., THOM, E., CARPENTER, M. W., RAMIN, S. M., CASEY, B., WAPNER, R. J., VARNER, M. W., ROUSE, D. J.

& THORP JR, J. M. 2009. A multicenter, randomized trial of treatment for mild gestational diabetes. *New England Journal of Medicine,* 361**,** 1339-1348.

LANGER, O., RODRIGUEZ, D. A., XENAKIS, E. M., MCFARLAND, M. B., BERKUS, M. D. & ARREDONDO, F. 1994. Intensified versus conventional management of gestational diabetes. *American journal of obstetrics and gynecology,* 170**,** 1036-1047.

LAPPAS, M. 2014a. Activation of inflammasomes in adipose tissue of women with gestational diabetes. *Molecular and cellular endocrinology,* 382**,** 74-83.

LAPPAS, M. 2014b. NOD1 expression is increased in the adipose tissue of women with gestational diabetes. *Journal of endocrinology,* 222**,** 99-112.

LAPPAS, M., YEE, K., PERMEZEL, M. & RICE, G. E. 2005. Release and regulation of leptin, resistin and adiponectin from human placenta, fetal membranes, and maternal adipose tissue and skeletal muscle from normal and gestational diabetes mellitus-complicated pregnancies. *Journal of endocrinology,* 186**,** 457-465.

LAURENCIKIENE, J., SKURK, T., KULYTÉ, A., HEDÉN, P., ÅSTRÖM, G., SJÖLIN, E., RYDÉN, M., HAUNER, H. & ARNER, P. 2011. Regulation of Lipolysis in Small and Large Fat Cells of the Same Subject. *The Journal of Clinical Endocrinology & Metabolism,* 96**,** E2045-E2049.

LEE, J.-H., KIM, D., KIM, H. J., LEE, C.-H., YANG, J. I., KIM, W., KIM, Y. J., YOON, J.-H., CHO, S.-H., SUNG, M.-W. & LEE, H.-S. 2010. Hepatic steatosis index: A simple screening tool reflecting

nonalcoholic fatty liver disease. *Digestive and Liver Disease,* 42**,** 503-508.

LEE, S. M., KWAK, S. H., KOO, J. N., OH, I. H., KWON, J. E., KIM, B. J., KIM, S. M., KIM, S. Y., KIM, G. M. & JOO, S. K. 2019. Non-alcoholic fatty liver disease in the first trimester and subsequent development of gestational diabetes mellitus. *Diabetologia,* 62**,** 238-248.

LEKVA, T., BOLLERSLEV, J., GODANG, K., CECILIE, M., ROLAND, P. & FRIIS, C. 2015. B-cell dysfunction in women with previous gestational diabetes is associated with visceral adipose tissue distribution. *Eur J Endocrinol,* 173**,** 63-70.

LEPAGE, G. & ROY, C. C. 1986. Direct transesterification of all classes of lipids in a one-step reaction. *Journal of lipid research,* 27**,** 114-120.

LIM, E. L., HOLLINGSWORTH, K. G., ARIBISALA, B. S., CHEN, M. J., MATHERS, J. C. & TAYLOR, R. 2011. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia,* 54**,** 2506-2514.

LOGUE, J. & SATTAR, N. 2011. Childhood obesity: a ticking time bomb for cardiovascular disease? *Clinical Pharmacology & Therapeutics,* 90**,** 174-178.

LONG, Y., SU, K., ZHOU, Y., LU, Y., LIN, F., YU, J. & PENG, Y. 2009. Relationship between the adiponectin and TNF-α with insulin resistance in patients with gestational diabetes mellitus. *Shandong Medical Journal,* 49**,** 26-28.

LONN, M., MEHLIG, K., BENGTSSON, C. & LISSNER, L. 2010. Adipocyte size predicts

incidence of type 2 diabetes in women. *The FASEB journal,* 24**,** 326-331.

LÖNNQVIST, F., NORDFORS, L., JANSSON, M., THÖRNE, A., SCHALLING, M. & ARNER, P. 1997. Leptin secretion from adipose tissue in women. Relationship to plasma levels and gene expression. *The Journal of clinical investigation,* 99**,** 2398-2404.

MACOTELA, Y., BOUCHER, J., TRAN, T. T. & KAHN, C. R. 2009. Sex and depot differences in adipocyte insulin sensitivity and glucose metabolism. *Diabetes,* 58**,** 803-812.

MARCHI, J., BERG, M., DENCKER, A., OLANDER, E. & BEGLEY, C. 2015. Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews. *Obesity Reviews,* 16**,** 621-638.

MARTINEZ, M. 1992. Tissue levels of polyunsaturated fatty acids during early human development. *The Journal of pediatrics,* 120**,** S129-S138.

MASON, T. M. 1998. The Role of Factors that Regulate the Synthesis and Secretion of Very-Low-Density Lipoprotein by Hepatocytes. *Critical Reviews in Clinical Laboratory Sciences,* 35**,** 461-487.

MATARESE, G. & LA CAVA, A. 2004. The intricate interface between immune system and metabolism. *Trends in Immunology,* 25**,** 193-200.

MATARESE, G., MOSCHOS, S. & MANTZOROS, C. S. 2005. Leptin in immunology. *The Journal of Immunology,* 174**,** 3137-3142.

MATTHEWS, D., HOSKER, J., RUDENSKI, A., NAYLOR, B., TREACHER, D. & TURNER, R. 1985. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma

glucose and insulin concentrations in man. *Diabetologia,* 28**,** 412-419.

MATTHEWS, J., ALTMAN, D. G., CAMPBELL, M. & ROYSTON, P. 1990. Analysis of serial measurements in medical research. *Bmj,* 300**,** 230-235.

MAURIEGE, P., GALITZKY, J., BERLAN, M. & LAFONTAN, M. 1987. Heterogeneous distribution of beta and alpha-2 adrenoceptor binding sites in human fat cells from various fat deposits: functional consequences. *European journal of clinical investigation,* 17**,** 156-165.

MCCULLOUGH, A. J. 2004. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clinics in liver disease,* 8**,** 521-533.

MCKNIGHT, J., MORRIS, A., CLINE, D., PEDEN, N., FISCHBACHER, C. & WILD, S. 2008. Implementing a national quality assurance system for diabetes care: the Scottish Diabetes Survey 2001–2006. *Diabetic Medicine,* 25**,** 743-746.

MEEK, C. L., LEWIS, H. B., PATIENT, C., MURPHY, H. R. & SIMMONS, D. 2015. Diagnosis of gestational diabetes mellitus: falling through the net. *Diabetologia,* 58**,** 2003-2012.

MEEKS, K. A., FREITAS-DA-SILVA, D., ADEYEMO, A., BEUNE, E. J., MODESTI, P. A., STRONKS, K., ZAFARMAND, M. H. & AGYEMANG, C. 2016. Disparities in type 2 diabetes prevalence among ethnic minority groups resident in Europe: a systematic review and meta-analysis. *Internal and emergency medicine,* 11**,** 327-340.

MEHMOOD, S., MARGOLIS, M., YE, C., MAPLE-BROWN, L., HANLEY, A. J., CONNELLY, P. W., SERMER, M., ZINMAN, B. & RETNAKARAN, R. 2018. Hepatic fat and glucose tolerance in women

with recent gestational diabetes. *BMJ Open Diabetes Research and Care,* 6.

MEHRA, R., SHEBL, F. M., CUNNINGHAM, S. D., MAGRIPLES, U., BARRETTE, E., HERRERA, C., KOZHIMANNIL, K. B. & ICKOVICS, J. R. 2019. Area-level deprivation and preterm birth: results from a national, commercially-insured population. *BMC Public Health,* 19**,** 236.

MESSER, L. C., MAXSON, P. & MIRANDA, M. L. 2013. The Urban Built Environment and Associations with Women's Psychosocial Health. *Journal of Urban Health,* 90**,** 857-871.

METZGER, B., GABBE, S., PERSSON, B., BUCHANAN, T., CATALANO, P., DAMM, P., DYER, A., LEIVA, A., HOD, M. & KITZMILER, J. 2010. International Association of Diabetes and Pregnancy Study Groups Consensus Panel. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care,* 33**,** 676-682.

METZGER, B. E., LOWE, L. P., DYER, A. R., TRIMBLE, E. R., SHERIDAN, B., HOD, M., CHEN, R., YOGEV, Y., COUSTAN, D. R. & CATALANO, P. M. 2008. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations with neonatal anthropometrics. *Diabetes,* 14 Nov.

MEYER, B. J. 2016. Australians are not meeting the recommended intakes for omega-3 long chain polyunsaturated fatty acids: results of an analysis from the 2011–2012 national nutrition and physical activity survey. *Nutrients,* 8**,** 111.

MEYER, B. J. & FREEMAN, D. J. 2017. Maternal plasma docosahexaenoic acid (DHA)

concentrations increase at the critical time of neural tube closure. *Fats of Life*.

MEYER, B. J., ONYIAODIKE, C. C., BROWN, E. A., JORDAN, F., MURRAY, H., NIBBS, R. J., SATTAR, N., LYALL, H., NELSON, S. M. & FREEMAN, D. J. 2016. Maternal plasma DHA levels increase prior to 29 days post-LH surge in women undergoing frozen embryo transfer: a prospective, observational study of human pregnancy. *The Journal of Clinical Endocrinology & Metabolism,* 101**,** 1745-1753.

MILLWARD, C. A., DESANTIS, D., HSIEH, C.-W., HEANEY, J. D., PISANO, S., OLSWANG, Y., RESHEF, L., BEIDELSCHIES, M., PUCHOWICZ, M. & CRONIGER, C. M. 2010. Phosphoenolpyruvate carboxykinase (Pck1) helps regulate the triglyceride/fatty acid cycle and development of insulin resistance in mice. *Journal of lipid research,* 51**,** 1452-1463.

MIN, Y., DJAHANBAKHCH, O., HUTCHINSON, J., ERAM, S., BHULLAR, A. S., NAMUGERE, I. & GHEBREMESKEL, K. 2016. Efficacy of docosahexaenoic acid-enriched formula to enhance maternal and fetal blood docosahexaenoic acid levels: Randomized double-blinded placebo-controlled trial of pregnant women with gestational diabetes mellitus. *Clinical Nutrition,* 35**,** 608-614.

MOKKALA, K., VAHLBERG, T., PELLONPERÄ, O., HOUTTU, N., KOIVUNIEMI, E. & LAITINEN, K. 2019. Distinct Metabolic Profile in Early Pregnancy of Overweight and Obese Women Developing Gestational Diabetes. *The Journal of Nutrition,* 150**,** 31-37.

MOLLER, D. E., YOKOTA, A., CARO, J. F. & FLIER, J. S. 1989. Tissue-Specific Expression of Two Alternatively Spliced Insulin Receptor mRNAs in Man. *Molecular Endocrinology,* 3**,** 1263-1269.

MORRISON, J. L., HODGSON, L. A., LIM, L. L. & AL-QURESHI, S. 2016a. Diabetic retinopathy in pregnancy: a review. *Clinical & experimental ophthalmology,* 44**,** 321-334.

MORRISON, J. L., HODGSON, L. A., LIM, L. L. & AL-QURESHI, S. 2016b. Diabetic retinopathy in pregnancy: a review. *Clinical & experimental ophthalmology*.

MYERS, J., HALL, C., WAREING, M., GILLHAM, J. & BAKER, P. 2006. The effect of maternal characteristics on endothelial-dependent relaxation of myometrial arteries. *European Journal of Obstetrics & Gynecology and Reproductive Biology,* 124**,** 158-163.

NANKERVIS, A., MCINTYRE, H., MOSES, R., ROSS, G., CALLAWAY, L., PORTER, C. & JEFFRIES, W. 2014. ADIPS consensus guidelines for the testing and diagnosis of gestational diabetes mellitus in Australia. *Australas Diabetes Pregnancy Soc, ,* 1-8 . Modified June.

NATIONAL COLLABORATING CENTRE FOR WOMEN'S AND CHILDREN'S HEALTH 2008. Antenatal Care: Routine Care for the Healthy Pregnant Woman. RCOG Press, London.

NEVILLE, M. J., COLLINS, J. M., GLOYN, A. L., MCCARTHY, M. I. & KARPE, F. 2011. Comprehensive human adipose tissue mRNA and microRNA endogenous control selection for quantitative real-time-PCR normalization. *Obesity,* 19**,** 888-892.

NOHR, E. A., BECH, B. H., DAVIES, M. J., FRYDENBERG, M., HENRIKSEN, T. B. & OLSEN, J. 2005. Prepregnancy obesity and fetal death: a study within the Danish National Birth Cohort. *Obstetrics & Gynecology,* 106**,** 250-259.

NUTTALL, F. Q. 2015. Body mass index: obesity, BMI, and health: a critical review. *Nutrition today,* 50**,** 117.

O'CONNELL, J., LYNCH, L., CAWOOD, T. J., KWASNIK, A., NOLAN, N., GEOGHEGAN, J., MCCORMICK, A., O'FARRELLY, C. & O'SHEA, D. 2010. The relationship of omental and subcutaneous adipocyte size to metabolic disease in severe obesity. *PloS one,* 5**,** e9997.

O'REILLY, J. R. & REYNOLDS, R. M. 2013. The risk of maternal obesity to the long-term health of the offspring. *Clinical endocrinology,* 78**,** 9-16.

O'BRIEN, T. E., RAY, J. G. & CHAN, W.-S. 2003. Maternal body mass index and the risk of preeclampsia: a systematic overview. *Epidemiology,* 14**,** 368-374.

OBSTETRICIANS, A. C. O. & GYNECOLOGISTS 2013. Practice bulletin no. 137: gestational diabetes mellitus. *Obstet Gynecol,* 122**,** 406-416.

OGURTSOVA, K., DA ROCHA FERNANDES, J., HUANG, Y., LINNENKAMP, U., GUARIGUATA, L., CHO, N. H., CAVAN, D., SHAW, J. & MAKAROFF, L. 2017. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes research and clinical practice,* 128**,** 40-50.

OKADA, T., KAWANO, Y., SAKAKIBARA, T., HAZEKI, O. & UI, M. 1994. Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat

adipocytes. Studies with a selective inhibitor wortmannin. *Journal of Biological Chemistry,* 269**,** 3568-3573.

OKEREKE, N. C., HUSTON-PRESLEY, L., AMINI, S. B., KALHAN, S. & CATALANO, P. M. 2004. Longitudinal changes in energy expenditure and body composition in obese women with normal and impaired glucose tolerance. *American Journal of Physiology-Endocrinology and Metabolism,* 287**,** E472-E479.

ORTEGA-SENOVILLA, H., ALVINO, G., TARICCO, E., CETIN, I. & HERRERA, E. 2009. Gestational diabetes mellitus upsets the proportion of fatty acids in umbilical arterial but not venous plasma. *Diabetes Care,* 32**,** 120-122.

OTODA, T., TAKAMURA, T., MISU, H., OTA, T., MURATA, S., HAYASHI, H., TAKAYAMA, H., KIKUCHI, A., KANAMORI, T. & SHIMA, K. R. 2013. Proteasome dysfunction mediates obesity-induced endoplasmic reticulum stress and insulin resistance in the liver. *Diabetes,* 62**,** 811-824.

OTT, R., MELCHIOR, K., STUPIN, J. H., ZISKA, T., SCHELLONG, K., HENRICH, W., RANCOURT, R. C. & PLAGEMANN, A. 2018. Reduced insulin receptor expression and altered DNA methylation in fat tissues and blood of women with GDM and offspring. *The Journal of Clinical Endocrinology & Metabolism,* 104**,** 137-149.

OUCHI, N. & WALSH, K. 2007. Adiponectin as an anti-inflammatory factor. *Clinica chimica acta,* 380**,** 24-30.

PAN, X. & HUSSAIN, M. M. 2007. Diurnal regulation of microsomal triglyceride transfer protein and plasma lipid levels. *Journal of Biological Chemistry,* 282**,** 24707-24719.

PARLETTA, N., MILTE, C. M. & MEYER, B. J. 2013. Nutritional modulation of cognitive function and mental health. *The Journal of nutritional biochemistry,* 24**,** 725-743.

PASARICA, M., SEREDA, O. R., REDMAN, L. M., ALBARADO, D. C., HYMEL, D. T., ROAN, L. E., ROOD, J. C., BURK, D. H. & SMITH, S. R. 2009. Reduced Adipose Tissue Oxygenation in Human Obesity. *Evidence for Rarefaction, Macrophage Chemotaxis, and Inflammation Without an Angiogenic Response,* 58**,** 718-725.

PERSSON, M., JOHANSSON, S., VILLAMOR, E. & CNATTINGIUS, S. 2014. Maternal overweight and obesity and risks of severe birth-asphyxia-related complications in term infants: a population-based cohort study in Sweden. *PLoS medicine,* 11**,** e1001648.

PESSIN, J. E. & KWON, H. 2013. Adipokines mediate inflammation and insulin resistance. *Frontiers in endocrinology,* 4**,** 71.

PHILLIPS, C. M., SHIVAPPA, N., HÉBERT, J. R. & PERRY, I. J. 2018. Dietary inflammatory index and biomarkers of lipoprotein metabolism, inflammation and glucose homeostasis in adults. *Nutrients,* 10**,** 1033.

PONTES, I. E., AFRA, K. F., SILVA JR, J. R., BORGES, P. S., CLOUGH, G. F. & ALVES, J. G. 2015. Microvascular reactivity in women with gestational diabetes mellitus studied during pregnancy. *Diabetology & metabolic syndrome,* 7**,** 27.

POSTON, L., BELL, R., CROKER, H., FLYNN, A. C., GODFREY, K. M., GOFF, L., HAYES, L., KHAZAEZADEH, N., NELSON, S. M. & OTENG-NTIM, E. 2015. Effect of a behavioural intervention

in obese pregnant women (the UPBEAT study): a multicentre, randomised controlled trial. *The lancet Diabetes & endocrinology,* 3**,** 767-777.

POSTON, L., CALEYACHETTY, R., CNATTINGIUS, S., CORVALÁN, C., UAUY, R., HERRING, S. & GILLMAN, M. W. 2016. Preconceptional and maternal obesity: epidemiology and health consequences. *The Lancet Diabetes & Endocrinology,* 4**,** 1025-1036.

PRENTKI, M. & NOLAN, C. J. 2006. Islet β cell failure in type 2 diabetes. *Journal of Clinical Investigation,* 116**,** 1802-1812.

RAHMAN, M., ABE, S., KANDA, M., NARITA, S., RAHMAN, M., BILANO, V., OTA, E., GILMOUR, S. & SHIBUYA, K. 2015. Maternal body mass index and risk of birth and maternal health outcomes in low-and middle-income countries: a systematic review and meta-analysis. *Obesity reviews,* 16**,** 758-770.

RANCOURT, R. C., OTT, R., ZISKA, T., SCHELLONG, K., MELCHIOR, K., HENRICH, W. & PLAGEMANN, A. 2020. Visceral Adipose Tissue Inflammatory Factors (TNF-Alpha, SOCS3) in Gestational Diabetes (GDM): Epigenetics as a Clue in GDM Pathophysiology. *International Journal of Molecular Sciences,* 21**,** 479.

RAO, R., SEN, S., HAN, B., RAMADOSS, S. & CHAUDHURI, G. 2014a. Gestational diabetes, preeclampsia and cytokine release: similarities and differences in endothelial cell function. *Advances in Fetal and Neonatal Physiology.* Springer.

RAO, R., SEN, S., HAN, B., RAMADOSS, S. & CHAUDHURI, G. 2014b. Gestational diabetes, preeclampsia and cytokine release: similarities

and differences in endothelial cell function. *Advances in Fetal and Neonatal Physiology.* Springer, New York, NY.

RAVUSSIN, E. & SMITH, S. R. 2002. Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. *Annals of the New York Academy of Sciences,* 967**,** 363-378.

RAYANAGOUDAR, G., HASHI, A. A., ZAMORA, J., KHAN, K. S., HITMAN, G. A. & THANGARATINAM, S. 2016a. Quantification of the type 2 diabetes risk in women with gestational diabetes: a systematic review and meta-analysis of 95,750 women. *Diabetologia* 59, 1403–1411(2016).

RAYANAGOUDAR, G., HASHI, A. A., ZAMORA, J., KHAN, K. S., HITMAN, G. A. & THANGARATINAM, S. 2016b. Quantification of the type 2 diabetes risk in women with gestational diabetes: a systematic review and meta-analysis of 95,750 women. Springer.

REBUFFE-SCRIVE, M., ENK, L., CRONA, N., LÖNNROTH, P., ABRAHAMSSON, L., SMITH, U. & BJÖRNTORP, P. 1985. Fat cell metabolism in different regions in women. Effect of menstrual cycle, pregnancy, and lactation. *The Journal of clinical investigation,* 75**,** 1973-1976.

RESI, V., BASU, S., HAGHIAC, M., PRESLEY, L., MINIUM, J., KAUFMAN, B., BERNARD, S., CATALANO, P. & HAUGUEL-DE MOUZON, S. 2012. Molecular inflammation and adipose tissue matrix remodeling precede physiological adaptations to pregnancy. *American Journal of*

Physiology-Endocrinology and Metabolism, 303**,** E832-E840.

RETNAKARAN, R., QI, Y., YE, C., HANLEY, A. J., CONNELLY, P. W., SERMER, M. & ZINMAN, B. 2011. Hepatic insulin resistance is an early determinant of declining β-cell function in the first year postpartum after glucose intolerance in pregnancy. *Diabetes Care,* 34**,** 2431-2434.

RIBET, C., MONTASTIER, E., VALLE, C., BEZAIRE, V., MAZZUCOTELLI, A., MAIRAL, A., VIGUERIE, N. & LANGIN, D. 2010. Peroxisome proliferator-activated receptor-α control of lipid and glucose metabolism in human white adipocytes. *Endocrinology,* 151**,** 123-133.

RODBELL, M. 1964. METABOLISM OF ISOLATED FAT CELLS. I. EFFECTS OF HORMONES ON GLUCOSE METABOLISM AND LIPOLYSIS. *The Journal of biological chemistry,* 239**,** 375.

ROJAS-RODRIGUEZ, R., LIFSHITZ, L. M., BELLVE, K. D., MIN, S. Y., PIRES, J., LEUNG, K., BOERAS, C., SERT, A., DRAPER, J. T. & CORVERA, S. 2015. Human adipose tissue expansion in pregnancy is impaired in gestational diabetes mellitus. *Diabetologia,* 58**,** 2106-2114.

RONDINONE, C. M., WANG, L.-M., LONNROTH, P., WESSLAU, C., PIERCE, J. H. & SMITH, U. 1997. Insulin receptor substrate (IRS) 1 is reduced and IRS-2 is the main docking protein for phosphatidylinositol 3-kinase in adipocytes from subjects with non-insulin-dependent diabetes mellitus. *Proceedings of the National Academy of Sciences,* 94**,** 4171-4175.

ROWAN, J. A., HAGUE, W. M., GAO, W., BATTIN, M. R. & MOORE, M. P. 2008. Metformin versus insulin for the treatment of gestational diabetes.

New England Journal of Medicine, 358**,** 2003-2015.

RUPNICK, M. A., PANIGRAHY, D., ZHANG, C.-Y., DALLABRIDA, S. M., LOWELL, B. B., LANGER, R. & FOLKMAN, M. J. 2002. Adipose tissue mass can be regulated through the vasculature. *Proceedings of the National Academy of Sciences,* 99**,** 10730-10735.

RUSTAN, A. C. & DREVON, C. A. 2001. Fatty acids: structures and properties. *Nature Publishing, London,* 30

RYDÉN, M. & ARNER, P. 2017. Subcutaneous adipocyte lipolysis contributes to circulating lipid levels. *Arteriosclerosis, thrombosis, and vascular biology,* 37**,** 1782-1787.

RYDÉN, M., PETRUS, P., ANDERSSON, D. P., MEDINA-GÓMEZ, G., ESCASANY, E., CORRALES CORDÓN, P., DAHLMAN, I., KULYTÉ, A. & ARNER, P. 2019. Insulin action is severely impaired in adipocytes of apparently healthy overweight and obese subjects. *Journal of internal medicine,* 285**,** 578-588.

SAARELAINEN, H., LAITINEN, T., RAITAKARI, O. T., JUONALA, M., HEISKANEN, N., LYYRA-LAITINEN, T., VIIKARI, J. S., VANNINEN, E. & HEINONEN, S. 2006. Pregnancy-related hyperlipidemia and endothelial function in healthy women. *Circulation Journal,* 70**,** 768-772.

SALANS, L. B. & DOUGHERTY, J. W. 1971. The effect of insulin upon glucose metabolism by adipose cells of different size: influence of cell lipid and protein content, age, and nutritional state. *The Journal of clinical investigation,* 50**,** 1399-1410.

SALANS, L. B., KNITTLE, J. L. & HIRSCH, J. 1968. The role of adipose cell size and adipose tissue

insulin sensitivity in the carbohydrate intolerance of human obesity. *The Journal of Clinical Investigation,* 47**,** 153-165.

SANCHALIKA, A. & TERESA, J. 2015. Risk of gestational diabetes among South Asian immigrants living in New Jersey—a retrospective data review. *Journal of racial and ethnic health disparities,* 2**,** 510-516.

SANTOS, M., FERNANDES, V., MARQUES, O. & PEREIRA, M. 2016a. Effect of maternal body mass index and weight gain in women with gestational diabetes on the incidence of large-for-gestational-age infants. *Diabetes & Metabolism*.

SANTOS, M., FERNANDES, V., MARQUES, O. & PEREIRA, M. 2016b. Effect of maternal body mass index and weight gain in women with gestational diabetes on the incidence of large-for-gestational-age infants.

SATTAR, N., BERRY, C. & GREER, I. A. 1998. Essential fatty acids in relation to pregnancy complications and fetal development. *BJOG: An International Journal of Obstetrics & Gynaecology,* 105**,** 1248-1255.

SATTAR, N., CLARK, P., HOLMES, A., LEAN, M. E., WALKER, I. & GREER, I. A. 2001. Antenatal waist circumference and hypertension risk. *Obstetrics & Gynecology,* 97**,** 268-271.

SAVVIDOU, M., KAMETAS, N., DONALD, A. & NICOLAIDES, K. 2000. Non-invasive assessment of endothelial function in normal pregnancy. *Ultrasound in obstetrics & gynecology,* 15**,** 502-507.

SCHAEFER-GRAF, U. M., GRAF, K., KULBACKA, I., KJOS, S. L., DUDENHAUSEN, J., VETTER, K. & HERRERA, E. 2008. Maternal lipids as strong

determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus. *Diabetes care,* 31**,** 1858-1863.

SCHAEFER-GRAF, U., MEITZNER, K., ORTEGA-SENOVILLA, H., GRAF, K., VETTER, K., ABOU-DAKN, M. & HERRERA, E. 2011. Differences in the implications of maternal lipids on fetal metabolism and growth between gestational diabetes mellitus and control pregnancies. *Diabetic medicine,* 28**,** 1053-1059.

SCHLING, P. & LÖFFLER, G. 2018. Cross talk between adipose tissue cells: impact on pathophysiology. *Physiology*.

SEBIRE, N., JOLLY, M., HARRIS, J., REGAN, L. & ROBINSON, S. 2001a. Is maternal underweight really a risk factor for adverse pregnancy outcome? A population-based study in London. *BJOG: an international journal of obstetrics & gynaecology,* 108**,** 61-66.

SEBIRE, N. J., JOLLY, M., HARRIS, J., WADSWORTH, J., JOFFE, M., BEARD, R., REGAN, L. & ROBINSON, S. 2001b. Maternal obesity and pregnancy outcome: a study of 287 213 pregnancies in London. *International journal of obesity,* 25**,** 1175.

SEGURA, M. T., DEMMELMAIR, H., KRAUSS-ETSCHMANN, S., NATHAN, P., DEHMEL, S., PADILLA, M. C., RUEDA, R., KOLETZKO, B. & CAMPOY, C. 2017. Maternal BMI and gestational diabetes alter placental lipid transporters and fatty acid composition. *Placenta,* 57**,** 144-151.

SESTI, G., MARINI, M. A., TULLIO, A. N., MONTEMURRO, A., BORBONI, P., FUSCO, A., ACCILI, D. & LAURO, R. 1991. Altered expression of the two naturally occurring human insulin

receptor variants in isolated adipocytes of non-insulin-dependent diabetes mellitus patients. *Biochemical and Biophysical Research Communications,* 181**,** 1419-1424.

SHAW, J. E., SICREE, R. A. & ZIMMET, P. Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes research and clinical practice,* 87**,** 4-14.

SHI, H., KOKOEVA, M. V., INOUYE, K., TZAMELI, I., YIN, H. & FLIER, J. S. 2006. TLR4 links innate immunity and fatty acid–induced insulin resistance. *The Journal of clinical investigation,* 116**,** 3015-3025.

SHIMABUKURO, M., ZHOU, Y.-T., LEVI, M. & UNGER, R. H. 1998. Fatty acid-induced β cell apoptosis: A link between obesity and diabetes. *Proceedings of the National Academy of Sciences,* 95**,** 2498-2502.

SHULMAN, G. I. 2014. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *New England Journal of Medicine,* 371**,** 1131-1141.

SIVAN, E., HOMKO, C. J., CHEN, X., REECE, E. A. & BODEN, G. 1999. Effect of insulin on fat metabolism during and after normal pregnancy. *Diabetes,* 48**,** 834-838.

SJÖSTRÖM, L., SMITH, U., KROTKIEWSKI, M. & BJÖRNTORP, P. 1972. Cellularity in different regions of adipose tissue in young men and women. *Metabolism,* 21**,** 1143-1153.

SKURK, T., ALBERTI-HUBER, C., HERDER, C. & HAUNER, H. 2007. Relationship between adipocyte size and adipokine expression and secretion. *The Journal of Clinical Endocrinology & Metabolism,* 92**,** 1023-1033.

SOHN, J. H., LEE, Y. K., HAN, J. S., JEON, Y. G., KIM, J. I., CHOE, S. S., KIM, S. J., YOO, H. J. & KIM, J. B. 2018. Perilipin 1 (Plin1) deficiency promotes inflammatory responses in lean adipose tissue through lipid dysregulation. *Journal of Biological Chemistry,* 293**,** 13974-13988.

SONG, M. J., KIM, K. H., YOON, J. M. & KIM, J. B. 2006. Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. *Biochemical and biophysical research communications,* 346**,** 739-745.

SOUZA, S. C., PALMER, H. J., KANG, Y. H., YAMAMOTO, M. T., MULIRO, K. V., ERIC PAULSON, K. & GREENBERG, A. S. 2003. TNF-α induction of lipolysis is mediated through activation of the extracellular signal related kinase pathway in 3T3-L1 adipocytes. *Journal of cellular biochemistry,* 89**,** 1077-1086.

SPROSTON, K. & MINDELL, J. 2006. Health Survey for England 2004. The health of minority ethnic groups.

STEFAN, N., KANTARTZIS, K., MACHANN, J., SCHICK, F., THAMER, C., RITTIG, K., BALLETSHOFER, B., MACHICAO, F., FRITSCHE, A. & HÄRING, H.-U. 2008. Identification and characterization of metabolically benign obesity in humans. *Archives of internal medicine,* 168**,** 1609-1616.

STEVENS-SIMON, C., THUREEN, P., BARRETT, J. & STAMM, E. 2001. Skinfold caliper and ultrasound assessments of change in the distribution of subcutaneous fat during adolescent pregnancy. *International journal of obesity,* 25**,** 1340-1345.

STEWART, F., RODIE, V. A., RAMSAY, J. E., GREER, I. A., FREEMAN, D. J. & MEYER, B. J.

2007a. Longitudinal assessment of erythrocyte fatty acid composition throughout pregnancy and post partum. *Lipids,* 42**,** 335-344.

STEWART, F. M., FREEMAN, D. J., RAMSAY, J. E., GREER, I. A., CASLAKE, M. & FERRELL, W. R. 2007b. Longitudinal assessment of maternal endothelial function and markers of inflammation and placental function throughout pregnancy in lean and obese mothers. *The Journal of Clinical Endocrinology & Metabolism,* 92**,** 969-975.

STONE, J. 1994. Risk factors for severe preeclampsia. *Obstet. Gynecol,* 83**,** 357-61.

STRAUGHEN, J., TRUDEAU, S. & MISRA, V. 2013. Changes in adipose tissue distribution during pregnancy in overweight and obese compared with normal weight women. *Nutrition & diabetes,* 3**,** e84-e84.

SUGANAMI, T., YUAN, X., SHIMODA, Y., UCHIO-YAMADA, K., NAKAGAWA, N., SHIRAKAWA, I., USAMI, T., TSUKAHARA, T., NAKAYAMA, K. & MIYAMOTO, Y. 2009. Activating transcription factor 3 constitutes a negative feedback mechanism that attenuates saturated Fatty acid/toll-like receptor 4 signaling and macrophage activation in obese adipose tissue. *Circulation research,* 105**,** 25-32.

SULAIMAN, W. N. W., CASLAKE, M. J., DELLES, C., KARLSSON, H., MULDER, M. T., GRAHAM, D. & FREEMAN, D. J. 2016. Does high-density lipoprotein protect vascular function in healthy pregnancy? *Clinical Science,* 130**,** 491-497.

SUMMERS, S. A. 2006. Ceramides in insulin resistance and lipotoxicity. *Progress in lipid research,* 45**,** 42-72.

SVENSSON, H., WETTERLING, L., BOSAEUS, M., ODÉN, B., ODÉN, A., JENNISCHE, E., EDÉN, S., HOLMÄNG, A. & LÖNN, M. 2016. Body fat mass and the proportion of very large adipocytes in pregnant women are associated with gestational insulin resistance. *International journal of obesity,* 40**,** 646.

SZCZEPANIAK, L. S., VICTOR, R. G., ORCI, L. & UNGER, R. H. 2007. Forgotten but Not Gone. *Circulation Research,* 101**,** 759-767.

TAKEUCHI, K. & REUE, K. 2009. Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis. *American Journal of Physiology-Endocrinology And Metabolism,* 296**,** E1195-E1209.

TANSEY, J., SZTALRYD, C., GRUIA-GRAY, J., ROUSH, D., ZEE, J., GAVRILOVA, O., REITMAN, M., DENG, C.-X., LI, C. & KIMMEL, A. 2001. Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proceedings of the National Academy of Sciences,* 98**,** 6494-6499.

TANSEY, J., SZTALRYD, C., HLAVIN, E., KIMMEL, A. & LONDOS, C. 2004. The central role of perilipin a in lipid metabolism and adipocyte lipolysis. *IUBMB life,* 56**,** 379-385.

TAYLOR, R. 1987. 11 Use of adipose tissue for metabolic studies. *Bailliere's clinical endocrinology and metabolism,* 1**,** 1023-1035.

TCHOUKALOVA, Y. D., KOUTSARI, C., VOTRUBA, S. B., TCHKONIA, T., GIORGADZE, N., THOMOU, T., KIRKLAND, J. L. & JENSEN, M. D. 2010a. Sex-and depot-dependent differences in

adipogenesis in normal-weight humans. *Obesity,* 18**,** 1875-1880.

TCHOUKALOVA, Y. D., VOTRUBA, S. B., TCHKONIA, T., GIORGADZE, N., KIRKLAND, J. L. & JENSEN, M. D. 2010b. Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. *Proceedings of the National Academy of Sciences,* 107**,** 18226-18231.

TEWARI, V., TEWARI, A. & BHARDWAJ, N. 2011. Histological and histochemical changes in placenta of diabetic pregnant females and its comparision with normal placenta. *Asian Pacific Journal of Tropical Disease,* 1**,** 1-4.

THE SCOTTISH INTERCOLLEGIATE GUIDELINES NETWORK 2010. Management of diabetes, a national clinical guideline.

THOMAS, B. A., GHEBREMESKEL, K., LOWY, C., OFFLEY-SHORE, B. & CRAWFORD, M. A. 2005. Plasma fatty acids of neonates born to mothers with and without gestational diabetes. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 72**,** 335-341.

THOMPSON, D., BERGER, H., FEIG, D., GAGNON, R., KADER, T., KEELY, E., KOZAK, S., RYAN, E., SERMER, M. & VINOKUROFF, C. 2013. Diabetes and Pregnancy. *Canadian Journal of Diabetes,* 37, Supplement 1**,** S168-S183.

TIIKKAINEN, M., TAMMINEN, M., HÄKKINEN, A. M., BERGHOLM, R., VEHKAVAARA, S., HALAVAARA, J., TERAMO, K., RISSANEN, A. & YKI-JÄRVINEN, H. 2002. Liver-fat accumulation and insulin resistance in obese women with previous gestational diabetes. *Obesity research,* 10**,** 859-867.

TOESCU, V., NUTTALL, S., MARTIN, U., KENDALL, M. & DUNNE, F. 2002. Oxidative stress and normal pregnancy. *Clinical endocrinology,* 57**,** 609-613.

TONTONOZ, P., HU, E. & SPIEGELMAN, B. M. 1994. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. *Cell,* 79**,** 1147-1156.

TRAN, A. T., STRAAND, J., DIEP, L. M., MEYER, H. E., BIRKELAND, K. I. & JENUM, A. K. 2011. Cardiovascular disease by diabetes status in five ethnic minority groups compared to ethnic Norwegians. *BMC public health,* 11**,** 554.

TRAYHURN, P. 2013. Hypoxia and adipose tissue function and dysfunction in obesity. *Physiological reviews,* 93**,** 1-21.

VAN DER ZIJL, N. J., GOOSSENS, G. H., MOORS, C. C., VAN RAALTE, D. H., MUSKIET, M. H., POUWELS, P. J., BLAAK, E. E. & DIAMANT, M. 2011. Ectopic fat storage in the pancreas, liver, and abdominal fat depots: impact on β-cell function in individuals with impaired glucose metabolism. *The Journal of Clinical Endocrinology & Metabolism,* 96**,** 459-467.

VAN TIENEN, F. H., VAN DER KALLEN, C. J., LINDSEY, P. J., WANDERS, R. J., VAN GREEVENBROEK, M. M. & SMEETS, H. J. 2011. Preadipocytes of type 2 diabetes subjects display an intrinsic gene expression profile of decreased differentiation capacity. *International Journal of Obesity,* 35**,** 1154-64.

VATIER, C., KADIRI, S., MUSCAT, A., CHAPRON, C., CAPEAU, J. & ANTOINE, B. 2012. Visceral and subcutaneous adipose tissue from lean women respond differently to lipopolysaccharide-induced

alteration of inflammation and glyceroneogenesis. *Nutrition & diabetes,* 2**,** e51-e51.

VEILLEUX, A., CARON-JOBIN, M., NOËL, S., LABERGE, P. Y. & TCHERNOF, A. 2011. Visceral adipocyte hypertrophy is associated with dyslipidemia independent of body composition and fat distribution in women. *Diabetes,* 60**,** 1504-1511.

VERBOVEN, K., WOUTERS, K., GAENS, K., HANSEN, D., BIJNEN, M., WETZELS, S., STEHOUWER, C., GOOSSENS, G., SCHALKWIJK, C. & BLAAK, E. 2018. Abdominal subcutaneous and visceral adipocyte size, lipolysis and inflammation relate to insulin resistance in male obese humans. *Scientific reports,* 8**,** 4677.

VESCO, K., LEO, M., FRANCISCO, M., MARCHALL, N. & RONNEY, W. 2019. 1386-P: A Comparison of Ectopic Fat Stores and Insulin Sensitivity among Women With and Without Gestational Diabetes. (2019): 1386-P.

VESCO, K., LEO, M. C., FRANCISCO, M., BAETSCHER, E., ROONEY, W., MARSHALL, N. E. & PURNELL, J. Q. 2018a. Is Gestational Weight Gain Associated with Change in Ectopic Lipid Stores? *American Diabetes Association* (2018): 1407-P.

VESCO, K., MARSHALL, N. E., ROONEY, W., LEO, M. C., BAETSCHER, E., FRANCISCO, M. & PURNELL, J. Q. 2018b. Are Changes in Whole-Body Insulin Sensitivity during Pregnancy Related to Changes in Ectopic Fat Stores? *American Diabetes Association* (2018): 1408-P.

VOS, A. A., POSTHUMUS, A. G., BONSEL, G. J., STEEGERS, E. A. & DENKTAŞ, S. 2014.

Deprived neighborhoods and adverse perinatal outcome: a systematic review and meta-analysis. *Acta obstetricia et gynecologica Scandinavica,* 93**,** 727-740.

WAHRENBERG, H., LÖNNQVIST, F. & ARNER, P. 1989. Mechanisms underlying regional differences in lipolysis in human adipose tissue. *The Journal of clinical investigation,* 84**,** 458-467.

WALLACE, T. M., LEVY, J. C. & MATTHEWS, D. R. 2004. Use and abuse of HOMA modeling. *Diabetes care,* 27**,** 1487-1495.

WANG, B., WOOD, I. S. & TRAYHURN, P. 2007. Dysregulation of the expression and secretion of inflammation-related adipokines by hypoxia in human adipocytes. *Pflügers Archiv-European Journal of Physiology,* 455**,** 479-492.

WANG, H., HU, L., DALEN, K., DORWARD, H., MARCINKIEWICZ, A., RUSSELL, D., GONG, D., LONDOS, C., YAMAGUCHI, T. & HOLM, C. 2009. Activation of hormone-sensitive lipase requires two steps, protein phosphorylation and binding to the PAT-1 domain of lipid droplet coat proteins. *Journal of Biological Chemistry,* 284**,** 32116-32125.

WANG, Y., SULLIVAN, S., TRUJILLO, M., LEE, M. J., SCHNEIDER, S. H., BROLIN, R. E., KANG, Y. H., WERBER, Y., GREENBERG, A. S. & FRIED, S. K. 2003. Perilipin expression in human adipose tissues: effects of severe obesity, gender, and depot. *Obesity research,* 11**,** 930-936.

WEINBERG, J. 2006. Lipotoxicity. *Kidney international,* 70**,** 1560-1566.

WEISBERG, S. P., MCCANN, D., DESAI, M., ROSENBAUM, M., LEIBEL, R. L. & FERRANTE, A. W. 2003. Obesity is associated with

macrophage accumulation in adipose tissue. *The Journal of clinical investigation,* 112**,** 1796-1808.
WELCH, H. G. 2011. *Overdiagnosed: making people sick in the pursuit of health,* Boston, Mass. , Beacon Press.
WEN, H., GRIS, D., LEI, Y., JHA, S., ZHANG, L., HUANG, M. T.-H., BRICKEY, W. J. & TING, J. P. 2011. Fatty acid–induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nature immunology,* 12**,** 408.
WERNER, E. F., PETTKER, C. M., ZUCKERWISE, L., REEL, M., FUNAI, E. F., HENDERSON, J. & THUNG, S. F. 2012. Screening for gestational diabetes mellitus: are the criteria proposed by the International Association of the Diabetes and Pregnancy Study Groups cost-effective? *Diabetes care,* 35**,** 529-535.
WHITE, U. & RAVUSSIN, E. 2019. Dynamics of adipose tissue turnover in human metabolic health and disease. *Diabetologia,* 62**,** 17-23.
WIJENDRAN, V., BENDEL, R. B., COUCH, S. C., PHILIPSON, E. H., CHERUKU, S. & LAMMI-KEEFE, C. J. 2000. Fetal erythrocyte phospholipid polyunsaturated fatty acids are altered in pregnancy complicated with gestational diabetes mellitus. *Lipids,* 35**,** 927-931.
WORLD HEALTH ORGANIZATION 2000. OBESITY: PREVENTING AND MANAGING THE GLOBAL EPIDEMIC. *Report of a WHO Consultation,* WHO Technical Report Series 894, Geneva.
WORLD HEALTH ORGANIZATION. 2013. *Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy* [Online]. Available: https://www.who.int/diabetes/publications/Hypergl

ycaemia_In_Pregnancy/en/ [Accessed October 11 2019].

YAMAKAGE, H., ITO, R., TOCHIYA, M., MURANAKA, K., TANAKA, M., MATSUO, Y., ODORI, S., KONO, S., SHIMATSU, A. & SATOH-ASAHARA, N. 2014. The utility of dual bioelectrical impedance analysis in detecting intra-abdominal fat area in obese patients during weight reduction therapy in comparison with waist circumference and abdominal CT. *Endocrine journal*, EJ14-0092.

YANG, L., CALAY, E. S., FAN, J., ARDUINI, A., KUNZ, R. C., GYGI, S. P., YALCIN, A., FU, S. & HOTAMISLIGIL, G. S. 2015. S-Nitrosylation links obesity-associated inflammation to endoplasmic reticulum dysfunction. *Science,* 349, 500-506.

YE, J., GAO, Z., YIN, J. & HE, Q. 2007. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. *American Journal of Physiology-Endocrinology and Metabolism,* 293, E1118-E1128.

YOGEV, Y. & LANGER, O. 2008. Pregnancy outcome in obese and morbidly obese gestational diabetic women. *European Journal of Obstetrics & Gynecology and Reproductive Biology,* 137, 21-26.

ZEYDA, M. & STULNIG, T. M. 2007. Adipose tissue macrophages. *Immunology letters,* 112, 61-67.

ZHOU, Z., CONNELL, M. C. & MACEWAN, D. J. 2007. TNFR1-induced NF-κB, but not ERK, p38MAPK or JNK activation, mediates TNF-induced ICAM-1 and VCAM-1 expression on endothelial cells. *Cellular signalling,* 19, 1238-1248.

ZHU, T., TANG, J., ZHAO, F., QU, Y. & MU, D. 2015. Association between maternal obesity and

offspring Apgar score or cord pH: a systematic review and meta-analysis. *Scientific reports,* 5**,** 18386.

ZHU, Y. & ZHANG, C. 2016. Prevalence of Gestational Diabetes and Risk of Progression to Type 2 Diabetes: a Global Perspective. *Current diabetes reports,* 16**,** 1-11.

ZIERATH, J., LIVINGSTON, J., THÖRNE, A., BOLINDER, J., REYNISDOTTIR, S., LÖNNQVIST, F. & ARNER, P. 1998. Regional difference in insulin inhibition of non-esterified fatty acid release from human adipocytes: relation to insulin receptor phosphorylation and intracellular signalling through the insulin receptor substrate-1 pathway. *Diabetologia,* 41**,** 1343-1354.

Accompanying Material

Increased risk of gestational diabetes, caesarean delivery and large for gestational age infants among overweight and obese women in Greater Glasgow and Clyde

Amaal alrehaili[1], Dilys Freeman[1] and Robert Lindsay[1]

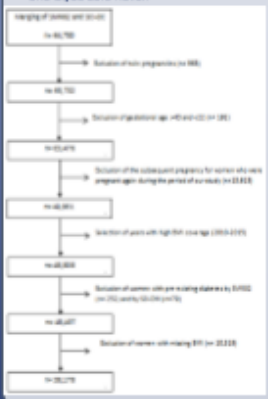
[1]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow UK

Introduction

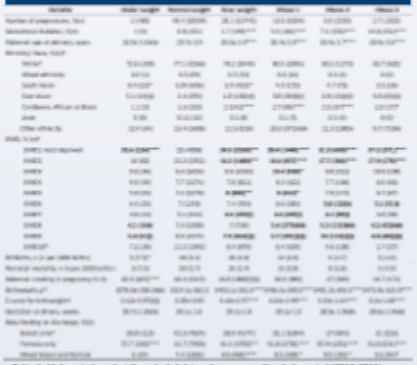
- The rapidly increasing ratio of obesity worldwide are a major health concern
- Maternal obesity can result in maternal and fetal complications including preeclampsia (PE) and gestational diabetes (GDM)
- In Scotland, the Maternity Inpatient and Day Case dataset (SMR02) collects episodic level data every time a mother goes in for an obstetric event and includes information on mother and baby characteristics, birth weight, gestational age, mode of delivery, induction and outcome of pregnancy and where a baby is delivered
- Data on diabetes diagnoses were obtained from the Scottish Care Information – Diabetes Collaboration (SCI-DM)
- We aimed to examine outcomes of pregnancy in women who are overweight or obese allowing for the presence of gestational diabetes

Materials and method

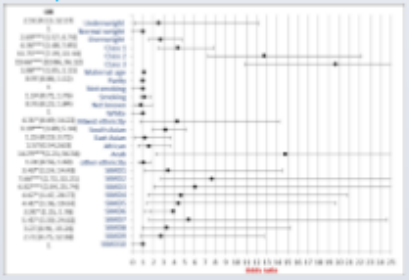
- Maternal BMI was classified according to the WHO classification in to the following classes: underweight (<18.5 kg/m²), normal weight (18.5-24.9 kg/m²), over weight (25- 29.9 kg/m²), obese class 1 (30- 34.9 kg/m²), obese class 2 (35- 39.9 kg/m²) and obese class 3 (≥40 kg/m²).
- Data was collected retrospectively from the SMR02 submitted by maternity hospitals to ISD Scotland
- Information from the SMR02 was linked with the national diabetes register, (SCI-DM) network using the community health index number (CHI) to allow identification of pregnancies in mothers with diabetes and subsequent perinatal outcomes
- Approval for data linkage and analysis was obtained from NHS Greater Glasgow and Clyde Safe Haven

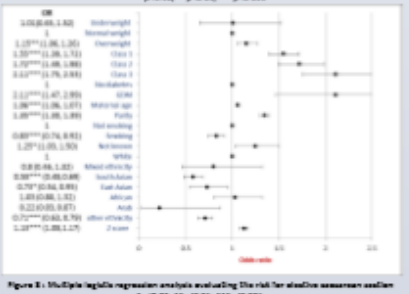


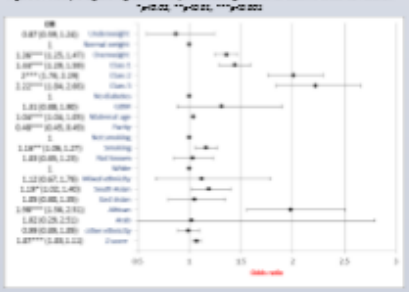
Results



Maternal obesity is a risk factor for GDM and operative delivery:

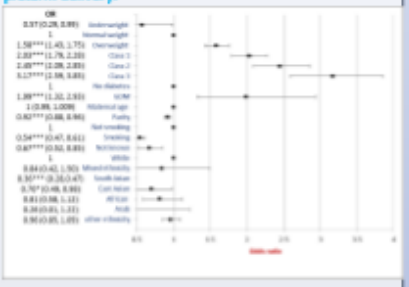


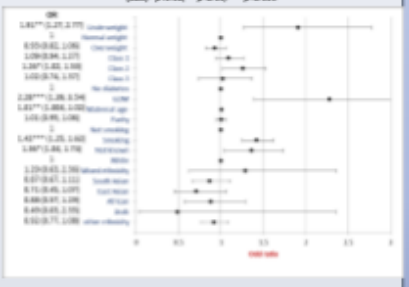




Results

Maternal obesity increase the risk of LGA infants and preterm delivery:





Conclusion

- Our population has a high percentage of women with obesity and severe obesity who are at high risk of several pregnancy complications
- Effective intervention strategies are needed for weight control prior to pregnancy.
- Targeted intervention approaches towards low income population and ethnicities at higher metabolic risk could be highly effective in improving pregnancy outcomes
- Mothers with obesity and GDM are receiving higher obstetric interventions including operative and earlier delivery
- Average birthweight for the offspring of overweight and obese mothers is significantly increased despite the increased rate of medical interventions among this group
- Wider analysis at national level can be beneficial in informing our clinical practice in management of maternal obesity during pregnancy

Acknowledgements

- NHS Greater Glasgow and Clyde Safe Haven
- Ministry of Education and Taibah University, Saudi Arabia

Maternal VLDL composition in early pregnancy and its relationship to estradiol and progesterone

Amaal F. Alrehaili[1], Josephine Cooney[1], Fiona Jordan[1], Barbara J. Meyer[2] and Dilys J. Freeman[1]

[1]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow UK.
[2]Metabolic Research Centre, University of Wollongong, Wollongong, Australia.

Introduction

- In maternal obesity and gestational diabetes (GDM) there is insulin resistance and hyperglycaemia
- The main triglyceride (TG) carrier in plasma is very low density lipoprotein (VLDL) which in later gestation is very important for delivering TG to the placenta for transport to the fetus
- We have shown that the maternal plasma concentration of a fatty acid , critical for neuronal development (docosahexaenoic acid), is increased as early as 18 days of gestation (figure-1)
- We hypothesised that plasma VLDL is also increased in early pregnancy in order to transport this key fatty acid. We aimed to determine maternal plasma VLDL concentration and composition in early pregnancy.

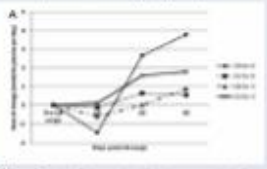


Figure 1: Rates of change (percent plasma per day) of maternal plasma polyunsaturated fatty acids at early pregnancy (Meyer et al. JCEM 2010)

Design

Derived from a prospective study of prediction of pregnancy success, plasma from 27 women who underwent a successful natural cycle frozen embryo transfer was sampled prior to pregnancy and at 18, 29 and 45 days post luteinising hormone surge.

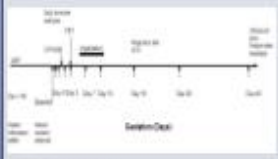


Figure 2: Study design

Methods

VLDL was isolated at 1.006 g/mL using sequential ultracentrifugation. VLDL total cholesterol (TC), triglyceride and apo-lipoprotein B (apoB) were assessed using auto-analyzer. Plasma estradiol and progesterone levels were assessed by Clinical Biochemistry Department at Royal Infirmary Hospital in Glasgow.

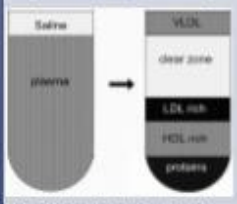


Figure 3: VLDL isolation by ultracentrifugation

Results – VLDL composition

Using repeated measures ANOVA, VLDL TC and apoB (a measure of VLDL particle number) were lower than baseline at 18 days. By 45 days, both had recovered to baseline levels. VLDL TG and TG per VLDL particle (TG/apoB) were unchanged over this time period.

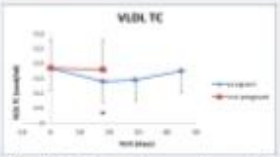


Figure 4: VLDL TC changes between pre LH surge and 45 days post LH surge (* p< 0.05 compared to baseline level)

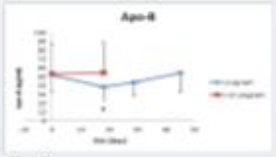


Figure 5: VLDL apoB changes between pre LH surge and 45 days post LH surge (* p< 0.05 compared to baseline level)

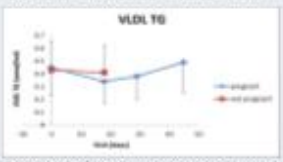


Figure 6: VLDL TG level changes between pre LH surge and 45 days post LH surge

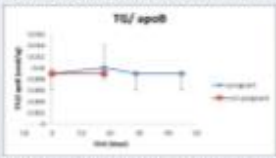


Figure 7: VLDL TG/apoB changes between pre LH surge and 45days post LH surge

This brief reduction in VLDL particle number at 18 days of gestation may be due to decreased liver production or increased maternal utilisation. Embryo energy requirements may cause an initial reduction in plasma VLDL prior to a compensatory increase in liver production by 45 days of gestation. The decrease in VLDL concentration may result from the stimulatory effect of low estradiol concentration on lipoprotein lipase activity leading to increased VLDL lipolysis.

VLDL and its relationship to pregnancy hormone

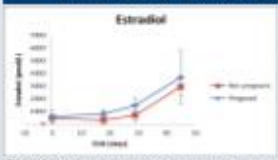


Figure 8: Estradiol level changes between pre LH surge and 45 days post LH surge

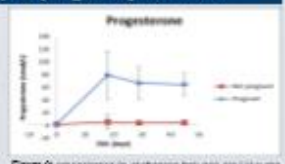


Figure 9: progesterone level changes between pre LH surge and 45 days post LH surge

To assess the relationship between changes in VLDL and plasma pregnancy hormone concentration correlation analysis was carried out

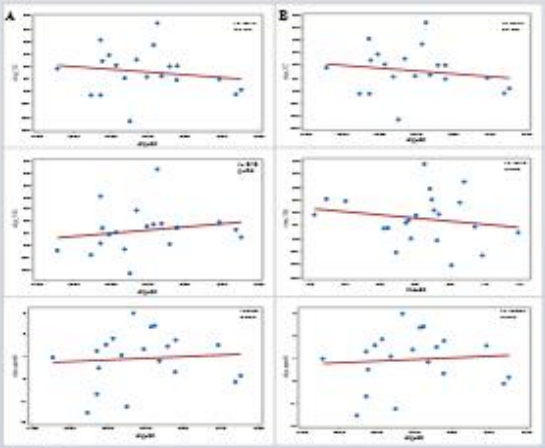


Figure 10: A- Correlation analysis for the dip in VLDL lipids with the change in estradiol levels in that time period (baseline-day18). B- Correlation analysis for the rise in VLDL lipids with the change in estradiol levels in that time period (day 45 - day 29).

VLDL lipids relationship to the rise in progesterone level

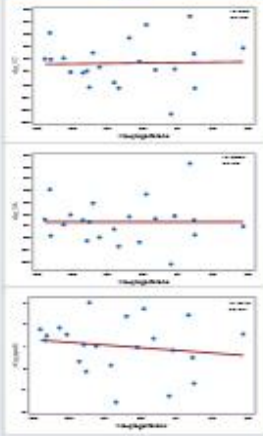


Figure 11: Correlation analysis for the rise in VLDL lipids with the change in progesterone levels in that time period (day 45 - day 29).

Conclusion & future work

- The composition of VLDL particles were not different compared to non-pregnant women at early pregnancy
- Contrary to our expectation, there was a brief reduction in VLDL particle number at 18 days post LH surge. This reduction was not correlated with changes in pregnancy hormones.
- Our data suggest that VLDL production may not be responsible for transporting plasma docosahexaenoic acid (DHA) in early pregnancy.
- Further LDL (low density lipoprotein), HDL (high density lipoprotein) and LPDS (lipoprotein deficient serum) isolation and fatty acid composition will be carried out

Acknowledgements

- Ministry of Higher Education and Taibah University, Saudi Arabia.
- Wellbeing of Women RG939/07

WELLBEING OF WOMEN