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Anatomical fat distribution and accumulation and lipotoxicity in lean and obese pregnancy

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Abstract

Maternal obesity has been at the forefront of pregnancy-related research in recent times. The impact of this chronic health condition has been highlighted in reports on maternal mortality (CEMACH, 2007, CEMACH, 2011), where 30% of mothers who died from pregnancy related causes were obese (CEMACH, 2011). The importance of maternal obesity and how it affects maternal adaptation to pregnancy is well documented with obese women exhibiting low grade inflammation, greater coagulability and poorer improvement in vascular function during pregnancy compared to lean women (Stewart et al., 2007a). These findings suggest that obese women display similar characteristics to the non-pregnant adult metabolic syndrome and these attributes may be important in explaining why obese pregnancies have higher rates of obstetric complications including gestational diabetes (GDM) and pre-eclampsia (PET). In non-pregnant adult obesity it has been found that central or truncal adiposity is associated with increased NEFA (non-esterified fatty acids) turnover and ectopic fat (especially liver) deposition. It has been suggested that obese pregnant women may also preferentially gain fat in central depots and this may be the mechanism by which poor vascular improvement and inflammation are initiated.

The aims of this thesis were to assess subcutaneous fat accumulation and distribution throughout pregnancy in both lean and OW/OB women. Furthermore this thesis aimed to acquire a better understanding of the impact of anatomical fat deposition on metabolic and vascular function during pregnancy. A final aim was to assess vascular function and evidence of lipotoxicity during pregnancy and test whether the site of fat accumulation and distribution was associated with gestational improvement of vascular function.

A longitudinal study was performed and anthropometric data was collected from 26 lean and 16 OW/OB women at three antenatal time points (15, 25 and 35 weeks' gestation) during pregnancy. Direct measurements of energy metabolism (basal metabolic rate, substrate utilisation, physical activity and diet) were also collected to assess the impact of energy metabolism on fat accumulation and distribution. A comprehensive panel of plasma markers of carbohydrate and lipid metabolism (fasting glucose, fasting insulin, total cholesterol [TC], total triglyceride [TG], high density lipoprotein [HDL] and NEFA) and inflammatory

(C-reactive protein [CRP], interleukin-6 [IL6] and tumour necrosis factor alpha [TNFα]) were quantitated at each study appointment. Endothelial function was measured using laser Doppler imaging (LDI). Measurement of plasma and urinary biomarkers of endothelial function and lipotoxicity including soluble intracellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule-1 (sVCAM-1), oxidised low density lipoprotein (oxLDL), plasma superoxide and urinary isoprostanes were undertaken.

Lean and OW/OB women gained similar amounts of total body weight and fat mass during pregnancy. Only in lean women was there an anatomical preference for site of fat storage and this was in the upper peripheral subcutaneous depots. In healthy OW/OB pregnancy no such anatomical preference of fat deposition was found.

The study of energy metabolism found that OW/OB women had higher basal metabolic rate and higher fat oxidation than lean women, whilst lean women had higher rates of carbohydrate oxidation and physical activity than OW/OB women. In the lean and OW/OB groups dietary macronutrient intakes were similar. Overall the parameters of energy metabolism were not associated with overall fat mass accumulation or distribution.

During pregnancy, OW/OB women were more insulin resistant and pro-inflammatory (CRP and TNFα) than lean women and lean women had higher concentrations of plasma HDL. Interestingly the lean group had higher plasma concentrations of IL6 which may be a result of higher rates of vascular remodelling and may reflect a physiological rather than pathological process. In both lean and OW/OB pregnancies the gestational increase in subcutaneous adipose depots was not associated with the gestational changes in markers of carbohydrate, lipid or inflammatory profiles.

Both lean and OW/OB women exhibited similar gestational improvement in endothelial microvascular function. During pregnancy both groups showed an increase in markers of lipotoxicity but levels were not associated with vascular function. Changes in anatomical subcutaneous fat distribution were also not associated with the changes in vascular function during pregnancy.

In conclusion, in pregnancy, only lean women exhibit an anatomical site-specific fat accumulation. Although the OW/OB group displayed some aspects of the metabolic syndrome in general the OW/OB women studied here adapted to pregnancy in a similar way to lean women in terms of vascular function and levels of lipotoxicity. However, visceral adiposity was not assessed and OW/OB women with larger visceral adipose stores may exhibit a more lipotoxic phenotype and more pathological adaptation to pregnancy that may make them susceptible to metabolic complications of pregnancy. This study highlights the heterogeneity of maternal obesity and suggests that further studies into 'metabolically healthy' and 'metabolically unhealthy' lean and OW/OB women is warranted.

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Anatomical Adiposity & Metabolic Response in Lean and Non-Lean Pregnancies, American Diabetic Association Annual Conference Diabetes June 2012 supplement.

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Longitudinal assessment of energy metabolism: Comparison between lean and obese pregnancies in a Scottish Population. Jarvie E, Gill J, Lovegrove J, Meyer B, Freeman D. International Association for the Study of Obesity, Boston, USA, May 2013.

The effect of distribution and accumulation of body fat in pregnancy on peripheral microvascular function. Jarvie E, Ferrell W, Gill J, Freeman D. Annual Academic Obstetric & Gynaecology Meeting/Blair Bell Conference, RCOG In December 2011.

Distribution and accumulation of body fat in pregnancy has an effect on peripheral microvascular function. Jarvie E, Ferrell W, Gill J, Freeman D. International Keystone Symposium on Lipid Biology and Lipotoxicity, Killarney, Ireland May 2011.

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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 patient recruitment and consent, anthropometric, energy metabolism and microvascular function studies and ELISA techniques.

I would therefore like to acknowledge the contributors below for their assistance in the laboratory techniques used. Lipid (total cholesterol, total triglyceride, non-esterified fatty acids and HDL cholesterol) and C-reactive protein (CRP) assays were performed by Josephine Cooney from the University of Glasgow Vascular Biochemistry department.

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Superoxide levels were detected in whole blood samples by Jim McCulloch and Elaine Friel from the Institute of Cardiovascular & Medical Sciences University of Glasgow

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List of Abbreviations

ACH	acetyl choline
ABS	abdominal skinfolds
ADP	air displacement plethysmography
Apo-A1	apolipoprotein A1
Apo-B	apolipoprotein-B
ATP	adenosine triphosphate
AUC	area under the curve
BMR	basal metabolic rate
BMI	body mass index
BHT	butylated hydroxytoluene
CETP	cholesteryl transfer protein
CHO	carbohydrate
CoA	coenzyme A
CRP	C-reactive protein
CV	coefficient of variation
DRV	dietary reference values
EDMVF	endothelium dependent microvascular function
EDTA	Ethylenediaminetetraacetic acid
EIMVF	endothelium independent microvascular function
eNOS	endothelial isoform NO synthase
EPR	electron paramagnetic resonance
$FECO_2$	fraction of expired carbon dioxide
FEO_2	fraction of expired oxygen
FMD	flow mediated dilation
GDM	gestational diabetes mellitus
GGT	gamma glutamyl transferase
GTT	glucose tolerance test
GWG	gestational weight gain
HEAT	Health improvement, efficiency and access to services and treatment
HDL	high density lipoprotein
HOMA	homeostatic model assessment of insulin resistance

IL6	interleukin 6
IOM	Institute of Medicine
IUGR	intrauterine growth restriction
kcal	kilocalories
kJ	kilojoules
LBS	lower body skinfolds
LC-PUFA	long chain polyunsaturated fatty acids
LDI	laser doppler imaging
LDL	low density lipoprotein
LXR	liver X receptor
MJ	megajoules
MVPA	moderate and vigorous physical activity
NADPH	nicotinamide adenine dinucleotide phosphate
NEFA	non-esterified fatty acids
NO	nitric oxide
NPRER	non-protein respiratory exchange ratio
$NPVCO_2$	non-protein carbon dioxide production ml/min
$NPVO_2$	non-protein oxygen consumption ml/min
oxLDL	oxidised low density lipoprotein
PAI-1	plasminogen activator inhibitor 1
PAL	physical activity level
PAT	peripheral artery tonometry
PCOS	polycystic ovarian syndrome
PET	pre-eclampsia
PPAR-γ	peroxisome proliferator activated receptor γ
PU	perfusion units
RER	respiratory exchange ratio
ROI	region of interest
ROS	reactive oxygen species
SEM	standard error of the mean
SD	standard deviation
sICAM-1	soluble intercellular adhesion molecule-1
SMID	Scottish multiple index of deprivation
SNP	sodium nitroprusside
SREBP-1c	sterol regulatory element binding protein 1c

sVCAM-1	soluble vascular cell adhesion molecule-1
TBS	total body skinfolds
TC	total cholesterol
TEM	technical error of the mean
TG	total triglycerides
TNFα	tumour necrosis factor alpha
UBPS	upper body peripheral skinfolds
VCO$_2$	volume of carbon dioxide produced in ml/min
VEGF	vascular endothelial growth factor
VLDL	very low density lipoprotein
VO$_2$	volume of oxygen consumed in ml/min
WHR	waist hip ratio

Chapter 1 - Introduction

1.1 The obesity problem

In 2008 the World Health Organisation estimated that over 200 million men and approximately 300 million women were obese, with the worldwide prevalence of obesity having doubled from 1980 to 2008 (WHO, 2013).

In 2006 the Department of Health predicted that if the trend for the increase in obesity was to continue, there would be a further 1 230 573 women of reproductive age who were obese by 2010, estimating that in England alone there would be approximately six million obese women (Zaninotto et al., 2006). In 2009, 23% of all adults in England were obese, and although the trend appeared to have slowed, the obesity prevalence was higher than other developed counties (DOH, 2010). Recent epidemiological modelling has suggested that by 2030 approximately 45% of men and 40% of women may be obese (Wang et al., 2011).

1.2 Body composition in the general adult population and metabolic risk

Human body fat compartments can be pragmatically based on anatomical site and can be divided into subcutaneous and visceral compartments. These sites can be further demarcated as upper body subcutaneous fat, intra-abdominal and visceral fat and lower body subcutaneous fat (Jensen, 2008). Often the term 'central obesity' does not differentiate between visceral and abdominal subcutaneous adipose tissue. However, whilst central obesity can be measured using waist circumference (Anuradha et al., 2012) visceral obesity relies on advanced visualisation techniques such as magnetic resonance imaging (Abate et al., 1997). In this thesis, central obesity will refer to both visceral and abdominal subcutaneous fat depots.

In addition, adipose tissue can be classified based on its function. Importantly in the context of adult obesity, classification can be based on fat depot sensitivity to insulin-induced lipolysis. Central adiposity is recognised as a core feature of insulin resistance in obese men (Ross et al., 2002a) and premenopausal women (Ross et al., 2002b) and is a stronger predictor of coronary heart disease than

BMI (body mass index) alone (Canoy et al., 2007). In the general adult population, both central obesity, and more specifically visceral obesity, are documented risk factors for the development of both diabetes mellitus and coronary heart disease in both men and women (Sattar et al., 2008, Canoy, 2008).

Adult central obesity is associated with insulin resistance, high levels of plasma triglycerides, low HDL, increased small dense low density lipoprotein (LDL) concentration, low grade inflammation and development of a prothrombotic environment. This has been collectively termed the metabolic syndrome (Huang, 2009).

An excess of upper body subcutaneous (abdominal and truncal) fat is associated with an abnormal metabolic and adipokine profile but the relative contributions of subcutaneous and visceral fat are unclear. Numerous groups (Hutley and Prins, 2005, Despres et al., 2008, Despres and Lemieux, 2006) have discussed the role of visceral adipose tissue in the production of inflammatory cytokines such as tumour necrosis factor alpha (TNFα) and interleukin 6 (IL6). TNFα is known to further stimulate the production of leptin and IL6. In addition, TNFα stimulates atherosclerosis formation through the activation of soluble vascular cell adhesion molecule 1 (sVCAM-1) and soluble intercellular adhesion molecule 1 (sICAM-1), as well as reducing the bioavailability of nitric oxide (NO) for vasodilatation, thus further perpetuating endothelial dysfunction (Lau et al., 2005). TNFα is not only important in inflammatory and vascular pathways but interferes with insulin-induced glucose uptake in adipocytes and appears to inhibit phosphorylation of the insulin receptor at least *in vitro* (Hotamisligil et al., 1994).

IL6 has been proposed to play a role in stimulating the production of acute-phase inflammatory proteins such as C-reactive protein (CRP) which are found at higher levels in obese patients (Fantuzzi, 2005). *In vitro* studies comparing the production of IL6 found that visceral fat produces higher levels of this inflammatory cytokine compared with subcutaneous abdominal adipocytes (Fain et al., 2004, Fried et al., 1998). However, abdominal subcutaneous adipose tissue, along with visceral fat, has been shown to be positively associated with biomarkers inflammation and oxidative stress such as CRP, IL6, sICAM-1, and TNFα (Pou et al., 2007). Subcutaneous adipose tissue has also been shown to be

strongly associated with insulin resistance, even when analysis adjusted for the impact of visceral adiposity (Goodpaster et al., 1997). Interestingly, recent data has found the positive association between abdominal subcutaneous fat thickness and insulin resistance and plasma triglycerides in much younger cohorts (ages 6-18 years) (Kelly et al., 2014), as well as abdominal subcutaneous fat depots being inversely correlated with HDL cholesterol (Kelly et al., 2014).

In adult central obesity there are elevated levels of circulating NEFA which results in insulin resistance and dysfunction of adipocytes (Frayn, 2000). Both upper body subcutaneous and visceral fat is relatively resistant to insulin suppression of lipolysis and consequently is estimated to be the source of around 60% of circulating NEFA (Jensen, 2008). Subcutaneous adipose tissue could be considered the main source of circulating NEFA because NEFA from visceral fat is transported directly to the liver via the portal circulation (Bevilacqua et al., 1987). In animal studies, visceral fat adipose tissue is strongly correlated with the NEFA concentrations seen in hepatic vein samples (Jensen et al., 2003). Therefore, because NEFA from visceral fat drains into the portal circulation there is no uptake by peripheral tissues before they reach the liver unlike NEFA from subcutaneous fat sources (Jensen et al., 2003). In obese women with a propensity for upper body obesity it has been confirmed that in the postprandial state, elevated levels of plasma NEFA originated from upper body fat but not visceral fat (Guo et al., 1999). Therefore, both visceral and subcutaneous fat depots are involved in abnormal lipid, inflammatory profiles and the promotion of insulin resistance.

Conversely lower body fat is considered more sensitive to insulin suppression of lipolysis, and contributes only 15-20% of circulating NEFA (Jensen, 2008). It has been proposed that lower body adipose tissue depots are associated with more efficient storage of dietary fat as a result of lower body subcutaneous fat being more sensitive to lipoprotein lipase activity in the post prandial state than either upper body subcutaneous or visceral adipocytes (McCarty, 2003, Votruba et al., 2007). There are differences in the storage capacity of lower body fat based on gender and BMI. In published literature it has been shown that lean women can store fatty acids more efficiently in the lower body fat compartments in the post prandial state than either obese women or men of any level of obesity (Santosa

et al., 2008). In the general adult population, smaller lower body depots, mainly thigh fat, have been highlighted as a risk factor for increased levels of triglycerides, lower HDL and hyperglycaemia (Snijder et al., 2005). Further studies by the same group have confirmed a reduced risk of carbohydrate and lipid dysregulation with greater lower body fat adiposity (Bos et al., 2005, Snijder et al., 2004a, Snijder et al., 2004b). Published data has suggested that larger hip circumference measurements had an inverse association with cardiovascular disease and diabetes which was stronger than the positive correlations between these diseases and waist circumference (Lissner et al., 2001). This proposed preference for lean women to store fat in lower body depots could be a reason for their relative protection against metabolic risk and endothelial dysfunction compared to men and obese women.

1.3 Metabolic syndrome and the development of lipotoxicity

Central obesity in adults is associated with hypertrophy of adipocytes. This hypertrophic obesity results in the reduced uptake and storage of fatty acids along with increased lipolysis, inflammatory cell infiltration and adipokine secretion (Jensen, 2008). In addition, in adult obesity, there is a reduced capacity of the pre-adipocytes population to undergo differentiation to mature adipocytes in abdominal subcutaneous adipose tissue (Gustafson et al., 2009). In insulin resistant obese adults, there are a disproportionately high number of both small preadipocytes and large hypertrophic adipocytes which have a reduced capacity to store fatty acids. These two populations of cells which are at the extremes of adipocyte size are found to positively correlate with whole body insulin resistance (McLaughlin et al., 2007). Thus obesity and insulin resistance are associated with defects of adipose tissue function where there is a failure to expand the capacity of the tissue to store additional triglyceride via developing more normal size adipocytes, in combination with a propensity to over fill the adipocytes which are available.

In the metabolic syndrome, which arises secondary to central obesity, the abnormal insulin resistance leads to an increase in plasma NEFA concentrations. This excessively high level of NEFA has further detrimental effects on adipocyte

function. In the adipocyte, the storage capacity for fatty acids is limited by triglyceride synthesising capacity and the ability of the adipocyte to expand as discussed above. If excessive fatty acid levels are present this overwhelms the triglyceride biosynthesis ability and storage capacity, leading to fatty acid accumulation and a reduced cellular homeostatic capacity of both the endoplasmic reticulum and mitochondria which will lead to adipocyte dysfunction (Garbarino and Sturley, 2009).

In addition to elevated NEFA having a detrimental effect of adipocyte function excessive circulating NEFA have been shown to effect end organ function. In the context of lipotoxicity, excessive NEFA accumulates at ectopic or non-adipose end organ sites such as skeletal muscle, liver and heart. When excess fatty acids from adipose tissue are transported to the liver, the storage and utilisation capacity of the hepatocytes is overwhelmed resulting in impaired signalling, cellular dysfunction and possible cell death (Trauner et al., 2010). In skeletal muscle the initial rise in fatty acid metabolites (palmitoyl carnitine, palmitoyl-coenzyme (CoA) and oleoyl-CoA) stimulate adenosine triphosphate (ATP) synthesis within the mitochondria via fatty acid oxidation. However, eventually saturation of the electron transport chain leads to a dose-dependent inhibition of ATP synthesis (Abdul-Ghani et al., 2008). Within myocytes, this affects the mitochondrial production of ATP, leading to a rise in intramyocellular fatty acid CoA and subsequent abnormal glucose oxidation and insulin resistance.

As is well documented in the literature, liver triglyceride content has been found to be elevated in people with type 2 diabetes (Ryysy et al., 2000). Clinical experiments have shown that reducing the hepatic triglyceride content in type 2 diabetic patients using pioglitazone (a thiazolididinedione known to reduce hepatic triglyceride levels) for 16 weeks significantly improved fasting and postprandial glucose levels (Ravikumar et al., 2008). In addition it has been shown in type 2 diabetic patients, adherence to a very low energy dense diet (2.5MJ or 600 kcal/day) for eight weeks led to normalisation of pancreatic beta cell function, improved hepatic insulin sensitivity and reduced liver and pancreatic triacylglycerol stores (Lim et al., 2011, Taylor, 2013). Therefore, although the excessive deposition of NEFA in the liver leads to hepatocyte dysfunction and insulin resistance, correction of insulin resistance improves fatty liver lipid accumulation. This would suggest that both ectopic fat accumulation

at the liver as well as insulin resistance perpetuate each other and further promote further dysregulation in insulin and triglyceride metabolism (Taylor, 2008).

In adult type 2 diabetic populations, elevated gamma-glutamyl transferase (GGT) has been linked to ectopic deposition of fat in the liver. It is thought to contribute to the development of insulin resistance (Gohel and Chacko, 2013) and has been proposed to be a marker of oxidative stress (Lim et al., 2004) and atherosclerosis (Bradley et al., 2014).

High plasma triglyceride concentration can lead to formation of small dense LDL, a highly atherogenic lipoprotein particle readily susceptible to oxidation forming oxidised LDL (oxLDL) because in atherosclerotic plaque formation these low density lipoproteins in the intimal layer of blood vessels are a focus for oxidation. This process leads to the formation of oxLDL which promotes sterol accumulation in macrophages and subsequent foam cell formation but also leads to increased expression of sVCAM-1 and macrophage proliferation both of which are important in the early inflammatory process seen in the vascular wall (Jessup et al., 2004). Pro-inflammatory lipoproteins such as oxLDL have been shown to promote endothelial damage through oxidative stress mediated DNA damage (Ding et al., 2013). In addition, oxLDL promotes the production of reactive oxidative and superoxide species which again are linked to endothelial dysfunction and damage. Other *in vivo* markers such as urinary isoprostanes have also been suggested to be markers of vascular damage and oxidative stress although there role in clinical disease is still being investigated (Minuz et al., 2006).

Oxidised metabolites of cholesterol, referred to as oxysterols have been shown to have beneficial effects but also to play a role in lipotoxicity (Bjorkhem and Diczfalusy, 2002). They can decrease cholesterol biosynthesis via interaction with sterol regulatory element binding protein 1c (SREBP-1c) and are also potent transactivators of the nuclear receptors liver X α and ß (LXRα and ß) (Peet et al., 1998). LXRα is involved in the regulation of steroid hormone biosynthesis, bile acid synthesis, conversion of lanosterol to cholesterol, cellular cholesterol efflux and lipid mobilising proteins such as cholesterylester transfer protein

(CETP) (Bjorkhem and Diczfalusy, 2002). However, oxysterols can be toxic to cells inducing inflammation, oxidative stress and apoptosis (Bjorkhem and Diczfalusy, 2002, Brown and Jessup, 2009, van Reyk et al., 2006). Whilst oxysterols pay a key role in steroid metabolism through the LXR receptor they will also induce expression of inflammatory markers in endothelial cells via LXR-independent mechanisms (Morello et al., 2009). Therefore they can lead to damage of the vascular endothelium via both oxidative and inflammatory means (Vejux and Lizard, 2009, Zhou et al., 2000).

The abnormal oxidation of fatty acids in the liver will also lead to the production of reactive oxygen species (ROS), disturbances in cellular membrane fatty acids and phospholipid composition, alterations in cholesterol content and ceramide signalling (Trauner et al., 2010). Elevated plasma NEFA levels can also induce nitroxide radical formation in smooth muscle and endothelial cells, induce respiratory burst in white cells and serve as substrates for oxidation themselves leading to propagation of lipid peroxides (Trauner et al., 2010). High levels of intracellular triglycerides can lead to mitochondrial accumulation of electrons within the electron transport chain which when it reacts with oxygen forms superoxide radicals. Interestingly, high levels of intracellular triglycerides will also directly stimulate the production of reactive oxygen species. Therefore, central obesity is linked to oxidative stress as a result of increased production of ROS (Pou et al., 2007, Vincent and Taylor, 2005).

Therefore the excessive plasma levels of NEFA observed in central obesity and the subsequent spillover of NEFA into the circulation with the deposition of lipids in non-adipose tissue sites can be considered the starting point of lipotoxicity.

1.4 **Lipotoxicity and endothelial dysfunction**

Sustained elevation of NEFA can lead to a reduction in insulin-mediated vasodilation (Jensen, 2008). Pathophysiological levels of fatty acids, such as those seen in central obesity, have been shown to directly affect vascular reactivity and through impaired activity of the endothelial isoform of nitric oxide synthase (eNOS)(Williams et al., 2002). In addition, vascular function can be blunted by the activation of nicotinamide adenine dinucleotide (NADPH) oxidase leading to the generation of superoxides in the vascular wall and the mitochondrial electron transport chain leading to endothelial oxidative stress (Williams et al., 2002).

In human obesity, oxidative stress is thought to lead to endothelial dysfunction through mechanisms which promote inflammation and lipid accumulation in the vascular wall (Matsuura et al., 2006). Using non-invasive techniques it has been shown that endothelial dysfunction is associated with raised plasma triglycerides concentrations (Rasmussen et al., 2009a) probably due to the formation of oxLDL. OxLDL can induce activation of the pro-inflammatory pro-thrombotic plasminogen activator inhibitor 1 (PAI-1) in endothelial cells (Zhao et al., 2009). Elevated levels of oxLDL can also lead to endothelial cell dysfunction via NADPH oxidase ROS production (Zhao et al., 2009, Silver et al., 2007). Therefore, in the context of lipotoxicity, hyperlipidaemia can lead to vascular damage either directly or via ROS generation.

The peroxidation of lipids is associated with oxidative stress and subsequent endothelial dysfunction. Lipid peroxidation occurs when free radicals come into contact with lipoproteins (Davies and Guo, 2014). These free radicals are produced as a results of normal respiration and the resulting effects of both the free radical and the lipid peroxides are normally counterbalanced by antioxidant defence systems (Little and Gladen, 1999). However when this system is out of equilibrium the toxic effect of these lipid peroxides cannot be blunted and oxidative stress results.

It has been suggested that 7ß-hydroxycholesterol may be a marker of oxLDL, lipid peroxidation and oxidative stress (Bjorkhem and Diczfalusy, 2002, Yoshida and Niki, 2006, Iuliano et al., 2003, Vejux and Lizard, 2009). Direct attack on

cholesterol by ROS can lead to formation of the relatively stable 7α- and 7ß -hydroxyl cholesterol, 7-ketocholesterol and 5,6-epoxycholesterol. Both 7-ketocholesterol and 7-hydroxycholesterol have been shown to have a toxic effect on porcine aortic smooth muscle cells which may contribute to atherosclerosis (Hughes et al., 1994).

Plasma oxysterol concentrations have been shown to be raised in metabolic syndrome, diabetes and hypercholesterolaemia (Endo et al., 2008). In adolescent females plasma oxysterols correlate with BMI, waist circumference and fasting plasma insulin and are suggested to be early markers of oxidative stress-mediated metabolic dysregulation (Alkazemi et al., 2008). High intracellular levels of cholesterol can also lead to tissue oxysterol production (e.g. 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, 25-epoxycholesterol) via specific enzymes in liver, brain and macrophages and these oxysterols are detectable in plasma. Within the diabetic population, 7-ketocholesterol levels were reported to be significantly higher, and subjects exhibited increased oxidation potential (Murakami et al., 2000). Oxysterols may also interfere with the production of long chain polyunsaturated fatty acids (LC-PUFA) which are protective for the circulation (Risé et al., 2004).*Williams* et al explored the impact of adipose tissue distribution on endothelial function and found an increased waist-hip ratio (as a marker of central obesity), was an independent positive predictor of endothelial dysfunction in healthy study participants (Williams et al., 2006). These findings together with others described above may suggest that lower body fat may be protective against vascular dysfunction (Lissner et al., 2001, Bos et al., 2005, Snijder et al., 2004b).

Therefore, although oxysterols have important physiological roles in steroid metabolism, in the context of obesity, oxysterols along with oxLDL and the promotion of ROS or superoxides will lead to an increase oxidative stress and continued endothelial dysfunction.

1.5 **Weight gain and body composition in pregnancy**

During pregnancy women will gain weight in response to the increased vascular and metabolic demands on the maternal system as well as supporting the development and growth of the fetus. The amount of weight a woman gains during pregnancy varies greatly. Gestational weight gain (GWG) is markedly increased in multiple pregnancies (15-22kg) and adolescents (14.0-18.0kg) compared to singleton adult pregnancy (range 10.0-16.7kg) and is inversely correlated to prepregnancy BMI (IOM, 2009).

Recent guidelines produced by the Institute of Medicine (IOM) have recommended total gestational weight gain based on prepregnancy BMI (IOM, 2009). The IOM guidelines on gestational weight gain (IOM, 2009) have prompted suggested lifestyle interventions to reduce the incidence of adverse pregnancy outcome in the obese pregnant population (Nelson et al., 2010).

Table 1.1: Gestational weight gain recommendations. This table illustrates the published recommendations for gestational weight gain by the Institute of Medicine, 2009

BMI group	Total gestational weight gain recommendations (kg)
$<18.5kg/m^2$	12.5-18.0
$18.5-24.9kg/m^2$	11.5-16.0
$25.0-29.9kg/m^2$	7.0-11.5
$>30kg/m^2$	$\leq$5-9

Current research suggest that the impact of excessive GWG is variable (Rasmussen et al., 2009b). Some authors suggests that the evidence linking excessive GWG (defined as being above the IOM GWG recommendations) to gestational diabetes and antenatal hypertensive disorders at best 'weak' due to methodological variations. However, women who gain excessive weight in pregnancy have an increased likelihood to retain this weight in the postpartum. For these women the retention of this weight is significant as they then enter their next pregnancy with a higher BMI leading to an increased risk of hypertensive disorders, gestational diabetes, caesarean section, stillbirth and

macrosomia (Villamor and Cnattingius, 2006). Published data has suggested that in an obese obstetric population a gestational weight gain of less than 7kg reduced the risk of pre-eclampsia, caesarean section and large for gestational age birth weights but a higher risk of small for gestational age infants (Kiel et al., 2007). A more recent study also found the increased risk of small for gestational age birth weight in obese women who gained less than 5kg during pregnancy and urges caution in recommending very low gestational weight gains in the obese and overweight population (Catalano et al., 2014).

Previous studies have shown that in healthy pregnancy there is a mean total gestational weight gain of 10.4$\pm$0.53kg, with 6.08kg gained between 12 to 26 weeks (Pipe et al., 1979). Total maternal fat mass increased rapidly in the first trimester and peaked at 28 weeks before falling slightly towards the end of pregnancy (Pipe et al., 1979). Interestingly the increase seen in the visceral fat compartment was concentrated in the third trimester when assessed longitudinally during pregnancy using ultrasonography (Kinoshita and Itoh, 2006). Assessment of the subcutaneous fat compartment has suggested that, consistent with the increase in total body weight, the main increase in this fat depot was seen between 10-30 weeks' gestation (Taggart et al., 1967).

There is evidence that during pregnancy obese women have similar or smaller increases in total weight, fat mass, % fat and total body fat compared to lean women (Catalano et al., 1998, Catalano et al., 1999, Ehrenberg et al., 2003, Soltani and Fraser, 2000). Anthropometric studies of skinfold thickness have shown that obese women put on more fat in the upper body subcutaneous compartment (suprailiac and subscapular skinfold), and that lean women put on more fat in the lower body compartment (mid-thigh skinfold) (Taggart et al., 1967, Soltani and Fraser, 2000). What is not known is the precise anatomical distribution of gestational fat accumulation and how this may be linked to metabolic risk.

In late pregnancy and the postpartum period, peripheral fat stores in the thighs and triceps are utilized to a greater extent (Sidebottom et al., 2001). In women who chose to breastfeed there was greater mobilisation of fat from the thighs compared to those who did not (Kramer et al., 1993). In addition, for the women who did breastfeed, there was a reduction in waist hip ratio (WHR). This

was not found in women who did not breastfeed suggesting that breastfeeding improves utilisation of peripheral adipose tissue in the postpartum period (Kramer et al., 1993).

1.6 **The impact of energy metabolism during pregnancy**

There are important confounding variables which may play a role in fat accumulation and distribution. Basal metabolic rate as well as diet and exercise are important factors in fat accumulation in the general population. It would be anticipated that these aspects of energy metabolism would be important in body fat accumulation and distribution during pregnancy.

For a well-nourished woman the energy costs of pregnancy can be considered as comprising of three components. First there is the energy cost of accumulating new tissue in the conceptus (including the fetus, placenta, amniotic fluid and the expansion of blood volume) approximated at 20 megajoules (MJ). Secondly, the energy required to deposit fat in mother and fetus (150 MJ), and finally the energy spent on maintaining this new tissue (150 MJ) (Prentice and Goldberg, 2000). In order to support the energy cost of these three components, the maternal basal metabolic rate (BMR) must increase during pregnancy (Forsum and Lof, 2007). The timing of the reported increase in BMR varies, some authors suggest that this is concentrated in the third trimester – presumably as an anticipation to labour and breastfeeding (Lof et al., 2005). Other authors have found that the BMR increases throughout gestation (Butte et al., 2004), and that women with higher BMI have higher BMR. Further published data supports the theory that during pregnancy obese women show a significantly greater rise in their basal metabolic rate than non-obese women (Bronstein et al., 1996). This would seem logical as in the general adult population an increased BMI is associated with a rise in BMR.

Previous assessment of maternal diet in pregnancy in humans has concentrated on the general obstetric population (Rogers and Emmett, 1998), the effect of social deprivation (Mouratidou et al., 2006) and maternal diet in the developing world (Cheng et al., 2009). These data, which largely predate the obesity epidemic, have been extremely informative in the context of healthy eating, but

little comparison of patterns of dietary intake in lean and obese pregnant populations has been performed.

In the context of obese pregnancy, certain aspects of maternal diet are problematic, such as folic acid intake, iron status and essential fatty acid profile. Obese women are more at risk of having a pregnancy affected by neural tube defects, and high dose folic acid (5mg daily) has been shown to significantly reduce this risk (Super et al., 1991) and is now recommended (Fitzsimons and Modder, 2010). In the non-pregnant, obese women are more folate-deficient than leaner women (Laraia et al., 2007). Antenatal anaemia has been shown to be extremely common in a British teenage obstetric population and 29.2% of these women were overweight or obese (Baker et al., 2009). Fatty acids, especially LC-PUFA, are essential for the cell membrane synthesis and play important key roles in neural development and maturation in the offspring (Uauy et al., 2000). The fetus is dependent on maternal circulatory delivery of LC-PUFA and the placenta expresses a number of fatty acid specific binding proteins which facilitate transport into the fetal system (Haggarty, 2002). In non-obese pregnancies complicated by pre-eclampsia and intrauterine growth restriction, maternal erythrocyte measurements of LC-PUFA (specifically n-6 and n-3) were over 50% lower than compared to healthy controls (Mackay et al., 2012).

Recent guidance on the management of obese pregnancies (Fitzsimons and Modder, 2010), highlighted the need for advising women about the importance of a healthy diet during pregnancy. The only specific nutritional guidance for obese woman regarded increased supplementation of folic acid and vitamin D (5mg and 10ug daily respectively), and did not discuss other aspects including fat and complex carbohydrate consumption.

British nutritional guidance (Vulliemoz et al., 2010) discusses dietary reference values (DRV) for energy intake and some micronutrients for pregnant women. Interestingly, the recommended increase in energy intake is only required in the third trimester 8117kilojoules [kJ] (1940 kilocalories [kcal]) to 8954kJ (2140 kcal, which translates to an increase of 837kJ (200kcal) (DOH, 1991). There is no distinction for maternal age in the UK recommendations. Recommendations from Australian sources (nhmrc.gov.au, 2006) suggest that although there is no additional energy intake requirement in the first trimester, energy intake should

increase by 1400kJ (335 kcal) in the second trimester, and 1900kJ (454kcal) in the third trimester. Australian recommendations for energy requirements are calculated on predicted basal metabolic rate (BMR) multiplied by physical activity level (PAL) and are also related to body mass index (BMI) of the individual. Thus energy requirement in pregnancy would be 8100kJ, 9500kJ and 10000kJ, and 7900kJ, 9300kJ and 9800kJ respectively for a 19-30 year old and a 31-50 year old expectant mother across gestation.

UK recommendations for daily protein intake suggest an increase from 45g/day to 51g/day. There are no specific values for the daily intake of carbohydrate and total fat and it is recommended that the proportion of energy derived from carbohydrate sources remains at 50% and from fat at 35%.

The Food and Nutritional Board of the Institute of Medicine, published a series of guidance reports on DRV for nutrients in North America and Canada across all ages including pregnancy and lactation (IOM, 2002). During pregnancy, there should be an increase in daily protein (46g to 71g) and carbohydrate (130g to 175g). Fat intake was not determined, on the basis that all individuals should try to keep their dietary intake of cholesterol, trans fatty acids and saturated fats to a minimum.

Another aspect of energy metabolism which can have an impact on gestational weight gain and adipose tissue accumulation is physical activity during pregnancy. Guidance on physical activity during pregnancy encourages exercise on most days but not at maximum aerobic capacity (RCOG, 2006). Currently there is substantial interest in the benefits of exercise maintenance and commencement in pregnancy. Not only is aerobic exercise recommended but resistance training has been found to be safe and beneficial for pregnant women (White et al., 2013). Maintaining exercise programmes and even starting a new regimen during pregnancy has been suggested to decrease depression, anxiety and fatigue (Gaston and Prapavessis, 2013). In terms of physical health, authors have suggested that regular exercise in the second trimester of pregnancy may attenuate GDM-related complications such as fetal macrosomia and the need for caesarean section (Barakat et al., 2013). Investigation in mouse models has also suggested that exercise prevents placental vascular endothelial growth factor (VEGF) gene expression which may have an impact on angiogenesis and pre-

eclampsia risk (Joles and Poston, 2010). The available data which focus on the impact of exercise and diet in obese pregnancy are limited (Gardner et al., 2011). Interventional studies are ongoing (Poston et al., 2013) suggesting a change in dietary (lower glycaemic index and dietary saturated fat), rather than exercise alone may have beneficial effects. For obese women it has been suggested that there are many barriers (available time, concerns regarding fetal health) to taking part in an exercise programme. Obese women who were more likely to partake in exercise were psychologically more positive about the impact of exercise on their health and had better family and health professional support (Sui and Dodd, 2013). Novel factors which appear to improve involvement in exercise in maternal obesity such as dog walking activities have been suggested (Westgarth et al., 2012).

As highlighted above, obese women have similar or smaller increases in percentage fat and total body fat mass than lean women (Kinoshita and Itoh, 2006, Ehrenberg et al., 2003, Catalano et al., 1999, Soltani and Fraser, 2000, Okereke et al., 2004). It is not clear whether minimum gestational weight gain or perhaps loss of weight during pregnancy will necessarily improve metabolic profile and downstream consequences for obese women. In addition, healthy pregnant women store fat earlier in pregnancy despite little change in energy intake (O'Sullivan, 2009). Thus the location and timing of fat accumulation in pregnancy, its association with energy intake and expenditure and its impact of obesity and the subsequent metabolic syndrome of pregnancy has not been explored.

Based on the above evidence diet, exercise and metabolic rate are significant confounding variables on total weight and fat gain during pregnancy and may work with the above interventions or against them within the obese population.

1.7 **Changes in lipid and carbohydrate metabolism during healthy pregnancy**

During pregnancy a multitude of complex alterations in lipid, carbohydrate and inflammatory metabolism take place in order to establish a healthy maternal adaptation to pregnancy. Early pregnancy is characterised by an anabolic state and late pregnancy by a more catabolic profile to optimise energy utilisation by the fetus for growth and development. As mentioned above, maternal fat mass increases significantly in the earlier stages of pregnancy when the fetal energy demands are less. This increase in maternal fat mass may be brought about partially by behavioural changes, including changes in activity and diet, but also as a result of a shift towards lipogenesis promoting the storage of adipose tissue. Plasma triglycerides increase gradually leading to a two- to three-fold increase in plasma concentration which peaks at term before falling to pre-pregnancy levels at six weeks post partum (Salameh and Mastrogiannis, 1994). Total cholesterol levels, including very low density lipoprotein (VLDL), LDL and HDL are increased by 50-60% by term and all lipoprotein particles have increased their cholesterol content by term (Salameh and Mastrogiannis, 1994). In addition, in early pregnancy insulin sensitivity remains normal or slightly improved (Catalano et al., 1991). Together, along with the effects of oestrogen, progesterone and cortisol, adipose tissue accumulation is favoured (Huda et al., 2009).

Healthy pregnancy shows a progressive increase in insulin secretion with a decrease in insulin sensitivity which is approximately half of that of non-pregnant women (Huda et al., 2009). A modest rise in fasting glucose levels is seen in line with the above adaptations but in healthy pregnancy this does not cross the threshold of GDM. Interestingly central obesity in early pregnancy has been shown to be linked to glucose intolerance in late gestation (Martin et al., 2009).

In late pregnancy, when fetal growth demands are at their maximum, the metabolic environment favours lipolysis and an insulin resistant profile that allows the mother to utilise fat as an energy source for labour and lactation and so the fetus can preferentially utilise circulating glucose for growth. In rat

models, there is an increase in mRNA expression hormone-sensitive lipase and a reduction in expression of lipoprotein lipase which promotes the rise in free fatty acids and glycerol seen in pregnancy (Martin-Hidalgo et al., 1994).

1.8 **Vascular adaptation in healthy pregnancy**

The maternal cardiovascular system undergoes many changes from early in pregnancy in order to meet to oxygen demands of both fetus and mother. By eight weeks' gestation, cardiac output has increased by 20% and will increase by a further twenty percent by the third trimester. The change in cardiac output is facilitated by an increase in stroke volume but also by an increase in basal heart rate (Nelson-Piercy, 2006). However the primary vascular adaptation seen in pregnancy is peripheral vasodilatation.

Longitudinal assessment of healthy pregnancies have shown a fall in blood pressure during the second trimester before a rise in the third trimester to pre-pregnancy levels (Grindheim et al., 2012). The peripheral vasodilation which leads to the fall in blood pressure is mediated predominantly by endothelium-dependent factors which are upregulated by oestradiol and prostaglandins (Nelson-Piercy, 2006).

Because endothelial dysfunction in the context of cardiovascular disease is systemic, assessing the peripheral circulation can be used as a surrogate marker for cardiovascular disease (Anderson et al., 1995). Peripheral microvascular function can be assessed by non-invasive or invasive techniques (Kasprzak et al., 2006). Flow mediated dilation (FMD) by brachial artery ultrasonography and peripheral arterial tonometry (PAT) have been used to assess peripheral macro- and microvascular function respectively. Although these techniques have been found to correlate significantly with each other, FMD was found to be more reproducible (Onkelinx et al., 2012). Within pregnancy however, FMD did not reflect the vasodilatation seen as gestation progressed (Miyague et al., 2013). Peripheral arterial tonometry has also been shown to be less reliable in pregnancy as women were more vasodilated and therefore the change in the diameter of peripheral vessels during reactive hyperaemia was not observed (Carty et al., 2012). Other groups have focused on the use of laser doppler imaging (LDI) for microvascular assessment function in pregnancy. This

technique has been shown to be reproducible and reliable (Jadhav et al., 2007, Ramsay et al., 2002b, Ramsay et al., 2002a) This approach uses a vasodilator agent which is delivered transdermally by iontophoresis and the hyperaemia is then captured as an image by the laser. Published data have shown that in the third trimester obese women have a poorer response to acetylcholine (ACH) iontophoresis than lean women, indicating poorer endothelial dependent vasodilatation (Ramsay et al., 2002a). Further studies by the same group assessed microvascular function throughout gestation and found that although both lean and obese women exhibited improvement in endothelial microvascular function, obese women started pregnancy with pre-existing endothelial dysfunction and did not show as much improvement across gestation as lean women (Stewart et al., 2007a).

It is well documented that during pregnancy there is a significant improvement in endothelium dependent vascular function (Ramsay et al., 2002a, Stewart et al., 2007a, Dørup et al., 1999). In healthy pregnancy, the improvement in endothelial function is thought to be related in part to increased nitric oxide activity which contributes to the fall in peripheral resistance in the systemic vascular system (Anumba et al., 1999). In healthy premenopausal women oestrogen has a protective role on vascular health (Barrett-Connor, 2013). It has been reported that in human endothelial cells the G protein-coupled estrogen receptor (GPER) is found in intact intracellular membranes and enhances eNOS activation and NO formation which may provide a pathway for oestrogen mediated vascular protection (Meyer et al., 2014). In addition, oestrogen has been shown to attenuate the effects of vascular injury by inhibiting the expression of TNFα and the cascade of inflammation via the NFκB signalling pathway (Xing et al., 2009). In pregnancy plasma oestrogen concentration rises (Branch, 1992) and therefore the above mechanism may also promote an improvement in vascular function.

In adult populations vascular remodelling and improvement has been stimulated by shear stress at the level of the blood vessel (Rodríguez and González, 2014), and it has been suggested that this pathway can be promoted through regular exercise (Kim et al., 2014). *In vitro* studies have compared subcutaneous arteries from pregnant and non-pregnant women subjected to shear stress

indicated that in pregnancy there is greater relaxation of these vessels (Cockell and Poston, 1997), which is suggested as an important mechanism for the fall in peripheral resistance during pregnancy.

Valdes et al comprehensively described the additional factors which are thought to play a part in the improvement in pregnancy related endothelial function, such as prostacyclin, kallikren, angiotensin and VEGF which appear to inhibit vasoconstriction pathways (Valdes et al., 2009).

1.9 **Clinical impact of maternal obesity**

The increase in maternal obesity is now well established in the pregnant population and obstetric practice, with an estimated one in five pregnant women being obese in the UK (Heslehurst et al., 2010). Epidemiological data from Scotland has indicated that the incidence of maternal obesity in the obstetric population has doubled, rising from 10% to 19% over a ten year period (Kanagalingam et al., 2005). Similar trends have been seen in American populations with a rise of almost 70% in the rate of maternal obesity from 1993 to 2003 (Kim et al., 2007). The impact of maternal obesity on maternal mortality rates was highlighted in 2007, when it was estimated that 15% of women who died from causes directly related to pregnancy were obese (CEMACH, 2007). More recent reports indicate that 30% of mothers who died from pregnancy related causes were obese (CEMACH, 2011). Despite the fact that maternal mortality remains very low in the UK, this is a startling figure to see in black and white.

The increased rate of maternal obesity has had ramifications for all aspects of female reproduction, with maternal adiposity being strongly associated with an increase of essentially all maternal and fetal complications (table 1.2). Observational studies have demonstrated that obesity in pregnancy increases the risk of antenatal complications such as GDM, pregnancy induced hypertension and PET (Gate and Ramsay, 2007). Furthermore, data examining the impact of obesity in women under the age of 19 showed a stepwise increase in the risk of pre-eclampsia and gestational diabetes (Sukalich et al., 2006) with increasing BMI. It is a concern that such a young population, with a long reproductive

potential, are exhibiting such serious obstetric complications at this age. In addition, an increase in BMI between pregnancies has been highlighted as a risk factor for PET, gestational hypertension, GDM, caesarean section and large for gestational age birth weight in a subsequent pregnancy (Villamor and Cnattingius, 2006).

The risk of other pregnancy-related complications such as venous thromboembolism, caesarean section, postpartum haemorrhage, surgical wound infection, post-operative endometritis and prolonger hospital stay are all increased in maternal obesity (Jarvie and Ramsay, 2010). For the fetus, there are increased risks of fetal anomalies, birth trauma, neonatal tachypnoea and admission to the neonatal high dependency units (Gate and Ramsay, 2007, Jarvie and Ramsay, 2010).

Consequently the increased risk of maternal and neonatal complications secondary to maternal obesity results in a significant rise in socioeconomic costs (Chu et al., 2008).

Table 1.2 - Potential effects of maternal obesity. The table below is adapted from *Jarvie E & Ramsay J, Obstetric management of obesity on pregnancy Semin Fetal Neonatal Med.(2010)* and illustrates the impact of maternal obesity at all stages of a woman's reproductive life.

	Medical Complications	**Technical Complications**
Pre-pregnancy	Menstrual disorders	
	Infertility	
Early-pregnancy	Miscarriage	Difficult Ultrasound Examination
	Fetal Anomalies	
Antenatal	Pregnancy induced Hypertension	Auscultation of the fetal heart
	PET	
	GDM	
	Venous Thromboembolic Disease	
Intrapartum	Increased induction of labour	Operative issues surgical access restrictions, increased time of surgery and increased blood loss

	Caesarean Section (elective & emergency)	Anaesthetic issues – difficulties siting regional anaesthesia, increased failed intubation risk Intrapartum fetal monitoring
Postpartum	Haemorrhage	
	Infection	
	Venous Thromboembolic Disease	
Fetal	Macrosomia	Birth injury
	Fetal Distress	
	Perinatal Morbidity/Mortality Reduced breast feeding rate	

1.10   Obesity and the metabolic syndrome of pregnancy

Maternal obesity is linked to increased perinatal complications and there is a body of literature as mentioned which suggests that obese women gain weight in central body compartments. This suggests that obese women are at risk of developing the metabolic syndrome as pregnancy progresses. As metabolic syndrome increases the risk of cardiovascular disease and diabetes in the general adult population due to dysregulated fatty acid metabolism and lipotoxicity, then in pregnancy, lipotoxicity potentially may be a factor in the development of hypertensive disorders and GDM.

Cross sectional studies have indicated that in the third trimester of obese pregnancies there is exaggerated hypertriglyceridaemia associated with an abnormal lipid profile (high VLDL, low HDL concentration), hyperinsulinaemia and increased inflammation (Ramsay et al., 2002a). From early pregnancy, it has been observed that obese mothers are less insulin sensitive than overweight and normal weight mothers (Catalano et al., 1999, Catalano and Ehrenberg, 2006). Further studies have confirmed that, in early pregnancy, obese women had higher triglycerides, increased insulin resistance and higher markers of inflammation compared to lean women (Stewart et al., 2007a). In addition gestational CRP levels in pregnancy were significantly correlated to pre-pregnancy BMI (Retnakaran et al., 2003). High levels of inflammatory markers, such as CRP, correlate with impaired endothelial function which may partly explain the altered vascular function seen in obese pregnancy (Retnakaran et al., 2003). It is unclear whether this is established before pregnancy, with obese women entering pregnancy with a more pro-inflammatory and insulin resistance profile or if there is an exaggerated adaptive response early in gestation.

The abnormal lipid profile in obese pregnancy also includes higher plasma levels of LDL (Ramsay et al., 2002a) particularly of smaller size (Meyer et al., 2013). In addition, the ease at which LDL is oxidised has been shown to directly correlate with maternal BMI (Sánchez-Vera et al., 2007) this may be related to an increased proportional of readily oxidisable small, dense LDL. Interestingly in obese women with GDM the relationship between LDL oxidisability and BMI was strengthened indicating the high risk nature of GDM and also suggesting that

obesity lowers the threshold of the lipotoxic effect of LDL (Sánchez-Vera et al., 2007). When metabolic risk factors (such as insulin resistance, low HDL and raised triglycerides) were measured in early pregnancy, they were found to correlate more strongly with visceral fat thickness than BMI (Bartha et al., 2007) suggesting that body fat distribution in pregnancy plays a key role in the production of lipotoxic biomarkers.
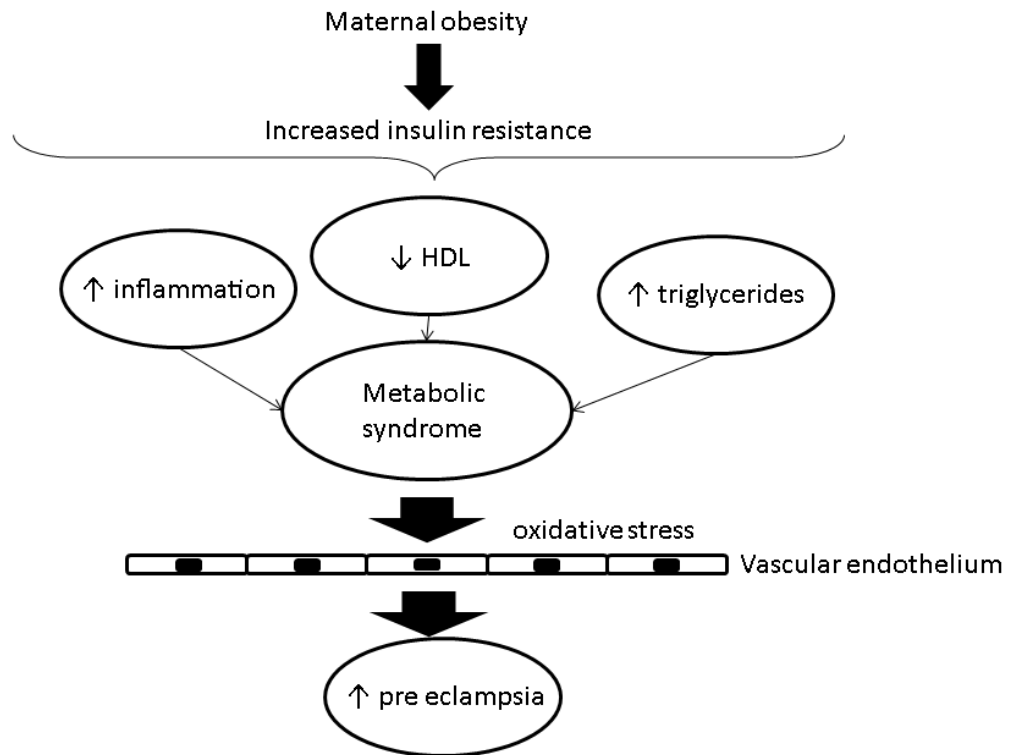
Figure 1.1 Maternal metabolic syndrome. Schematic representation of the effect that maternal obesity has on metabolic and inflammatory pathways which may explain the increased risk of hypertensive disorders observed in obese pregnancies

1.11 Oxidative stress in pregnancy

Oxidative stress has been linked to microvascular damage. A number of molecules have been suggested as markers of oxidative stress (Matsuura et al., 2006) such as ROS and lipid peroxides. It has been proposed that in maternal obesity the poorer endothelial improvement is related to a pathway of increased plasma lipids with the promotion of oxidative stress (Vincent and Taylor, 2005). Endothelial dysfunction can be measured by the physiological and *ex vivo* methods suggested above but also by looking at in vivo biomarkers of endothelial activation such as sVCAM-1 and sICAM-1 which have been found to be elevated in pre-eclamptic pregnancies (Farzadnia et al., 2013), where endothelial damage is a hallmark of the disease.

The impact of pregnancy on the levels of lipid peroxides has been reviewed previously (Little and Gladen, 1999) and the data are rather equivocal. When compared to non-pregnant controls, lipid peroxides in the first trimester were sometimes higher and sometimes lower in the pregnant group. By the second trimester there was an observed increase of 10-50%, and in the third trimester these levels sometimes fell. When compared with healthy pregnancies, all diabetic women (including type 1, type 2 and GDM) had higher levels of lipid peroxides, which may be related to the increased risk of vascular complications in pregnancy associated with diabetes (Toescu et al., 2004). Lipid peroxidation products have been noted to be markers of oxidative stress (Niki, 2008). In circumstances where there are elevated fatty acids and triglyceride concentrations – as in obese pregnancy – lipid peroxidation may be enhanced leading to increased oxidative stress and subsequent endothelial damage. High lipid peroxide levels were particularly apparent in pregnant women with type 2 diabetes– a population characterised by a predisposition to central obesity (Toescu et al., 2004).

The LDL-III subfraction of cholesterol is a readily oxidised form (Griffin, 1999), which can be a potential source of lipotoxic oxysterols for both the fetus and the mother although data is limited. In maternal obesity, approximately a third of women had at least 50% of LDL in the smallest fraction LDL-III, indicative of an atherogenic phenotype, compared to lean women who had no LDL in the LDL-III fraction (Meyer et al., 2013). It is possible that in the context of maternal

obesity, this lipoprotein is an important factor in the development of lipotoxicity.

The literature on oxysterols during pregnancy is limited. Gestational increases in (7-ketocholesterol, 7 alpha and 7 alpha-hydroxycholesterol and the total sum of 5 alpha, 6 alpha and 5 beta, 6 beta-epoxycholesterols) have been reported in healthy pregnancy, most notably in the second and third trimester, and oxysterol levels are higher in pregnancies in women with type 1 diabetes (Bodzek et al., 2002). In non-pregnant type 2 diabetics, high plasma oxysterol levels are particularly observed when plasma LDL levels are above 3.62mmol/L (Endo et al., 2008), and LDL concentrations frequently exceed this level in overweight and obese women in late gestation (Stewart et al., 2007a, Ramsay et al., 2002a).

Oxysterols can bind and activate both the LXRα and LXRβ receptors which are important regulators of lipid and cholesterol metabolism (Jakobsson et al., 2012). Furthermore (Peet et al., 1998) oxLDL can inhibit trophoblastic invasion via interaction with LXRβ receptors expressed by human placenta. Oxysterol activation of the LXRβ receptor could contribute to the observed increase in placental complications such as intrauterine growth restriction (IUGR) and PET in obese pregnancy (Pavan et al., 2004).

In summary, there are significant data which suggest that the biomarkers of lipotoxicity may be linked to endothelial dysfunction in the mother and placenta. Interestingly, little research has looked at the gestational changes in these markers of lipotoxicity and their impact on microvascular function in pregnancy. In addition, what is not explored in the current literature is whether differences between lean and obese women can be explained by either the presence of central adiposity at the beginning of pregnancy or the accretion of adipose tissue in the central distribution during gestation.

1.12   Consequences of lipotoxicity in pregnancy

Obese women enter pregnancy with poorer overall vascular function compared to lean women (Stewart et al., 2007a). In addition, during pregnancy although both lean and obese women showed an improvement in vascular function, the endothelium- dependent microvasculature improvement was blunted in obese pregnancies (Stewart et al., 2007a). A high risk group for early onset coronary artery disease is women with familial hypercholesterolaemia, an autosomal dominant condition characterised by elevated LDL, tendon xanthomas and premature death (Austin et al., 2004). Women with familial hypercholesterolaemia exhibit increased lipid levels, pro-coagulant activity and enhanced endothelial activation during pregnancy (Amundsen et al., 2007). This high-risk group also exhibits a reduction in uterine blood flow during pregnancy which indicates increased uteroplacental vascular resistance with subsequent risk of PET and IUGR (Khoury et al., 2009). As well as an intrinsic elevated cholesterol concentration, in a mouse model of PET it has been shown that a high fat diet can lead to higher levels of fat infiltration in the maternal liver (Sun et al., 2012). However, early onset pre-eclampsia tends to have a more severe clinical impact and thus these findings may not be applicable to other gestational hypertensive disorders. However, these findings do support dysfunctional lipid metabolism consistent with the development of lipotoxicity in early onset PET.

In pregnancies which are complicated by GDM, women are insulin resistant and hyperlipidaemic (Catalano et al., 1999). This group show evidence of increased oxidative stress where the degree of hyperglycaemia correlates with lipid peroxide concentrations (Chen and Scholl, 2005). Pregnancies affected by GDM are also found to have poorer endothelial function than control women (Paradisi et al., 2002).

Although these are two examples of extreme metabolic conditions (GDM and familial hypercholesterolaemia), in conjunction with the data in the non-pregnant population relating high lipid levels to oxidative stress and endothelial dysfunction, the evidence suggests that lipid abnormalities arising from NEFA excess could contribute to the observed endothelial dysfunction of obese pregnancy.

1.13   The potential impact of lipotoxicity on offspring health

Babies born to overweight and obese mothers have been noted to have significantly more total fat mass than babies born to mothers of normal weight (Hull et al., 2008). In addition to having more body fat, babies of obese mothers have been shown to have significantly higher levels of insulin resistance (expressed as HOMA) and inflammation (IL6 levels) in umbilical cord blood samples obtained at delivery compared to the offspring of lean women (Catalano et al., 2009). If these babies are already born with a degree of insulin resistance and chronic inflammation there may to a propensity to childhood obesity and the development of vascular dysfunction in later life (Freeman, 2010). In terms of fetal complications, excessive GWG was strongly correlated with the incidence of babies born small or large for gestational age and the risk of an unplanned caesarean section. Interestingly, excessive GWG-associated risk of excessive fetal growth and large for gestational age babies was linear even within cohorts of women who were of a normal weight (Dietz et al., 2009).

It is now recognised that the intrauterine environment has the potential to impact on early fetal growth and development and subsequent adult ill-health, including potential programming of future obesity (Freeman, 2010, Catalano, 2003). Placentae from obese pregnancies have shown accumulation of macrophages and increased expression of the inflammatory cytokines IL6, TNF-α, IL1 and increased infiltration of CD14+ cells (Challier et al., 2008). In addition, maternal plasma levels of CRP have been shown to be positively associated with the presence of atherosclerosis in childhood aortic samples (Liguori et al., 2008).

It is not only within the maternal and fetal circulation that lipotoxicity may exert an effect. The LXR receptors have been proposed to be important in placental cholesterol transport from the mother to the fetus (Plosch et al., 2007). The LXRα and LXRß receptors have been detected in early gestation placental tissue (Marceau et al., 2005) and could act as a binding site for oxysterols within the placenta. Oxysterols have shown a high affinity to the LXRα receptor and through this mechanism may prevent the esterification of cholesterol, leading to a further build up of oxysterols (Bjorkhem and Diczfalusy, 2002). Oxidised LDL contains a high proportion of oxysterols, and these have an

affinity to the LXRß receptors. Through activation of these receptors, oxysterols and this oxidised LDL have been shown to inhibit trophoblastic cell invasion in human *in vitro* models (Pavan et al., 2004). Inhibition of trophoblast cell invasion would have serious consequences not only for successful early embryonic development but for maintenance of a healthy pregnancy across gestation. However, oxysterols are involved in important pathways such as sonic hedgehog signalling proteins, and deficiencies with in these pathways can lead to fetal structural anomalies such as Smith–Lemli–Opitz syndrome (Javitt, 2007).

In women with type 1 diabetes, a hyperlipidaemic group with a significant increased risk for fetal anomalies, there were 49 alterations in gene expression at important stages of placental energy metabolism. (Radaelli et al., 2009). Two thirds of these alterations took place within lipid pathways and 9% in regulatory glucose pathways. In addition, this data also studied placentae from women with GDM which suggested that the genes responsible for fetoplacental lipid metabolism were also unregulated when compared to non-diabetic pregnancies. In term primary trophoblast cells, raised insulin and NEFA levels enhance the formation of fatty acid droplets within the placentae (Elchalal et al., 2005). In the fetuses of non-diabetic women with established hypercholesterolemia there were significantly more aortic atherosclerotic plaques associated with oxidised LDL in monocytes (Napoli et al., 1997). The presence of LDL in the intima of these aortic samples suggests that risk of cardiovascular disease is established in utero.

In both human and animal models, the placentae could be a site of fatty acid accumulation. In an early onset pre-eclamptic mice models, placental histology has shown an increased in lipid deposition when the dams were exposed to a high fat diet (Sun et al., 2012). In human studies, placentae from obese women have shown a greater accumulation of lipid in placental macrophages in an analogous way to adipose tissue (Challier et al., 2008). Thus the placenta may act as a site for ectopic fat accumulation in obese pregnancy similar to skeletal muscle, liver and brain seen in adult obesity. In non-human animal models a diet which is high in cholesterol can lead to lipotoxicity of the fetal liver (McCurdy et al., 2009). Interestingly, changing the maternal diet to a low fat version improved fetal triglyceride levels, indicating how vulnerable fetal end organs are to lipotoxicity.

1.14   The utility of maternal BMI as an indicator of high risk pregnancy

One in five women of reproductive age is obese, and obstetric clinics often have an antenatal obstetric population where over 20% of patients are obese (Heslehurst et al., 2010) with significant socioeconomic costs directly attributable to the increased risk of maternal and neonatal complications. The current lack of understanding as to how, where and when mothers put fat on during pregnancy makes it difficult to advise obese mothers how to manage their weight during pregnancy. However, not all obese women are at risk of developing antenatal and fetal complications linked to their obesity (Jarvie and Ramsay, 2010) (table 1.2), and in addition, a smaller but still significant number of lean women may develop metabolic complications of pregnancy. Direct evidence for lipotoxicity, which may only occur in a proportion of obese pregnancies, which may link maternal obesity and placentally-related adverse pregnancy outcome, is lacking. The placenta may transmit metabolic abnormalities resulting from lipotoxicity to the offspring via *in utero* programming and hence there could be far-reaching consequences for offspring health.
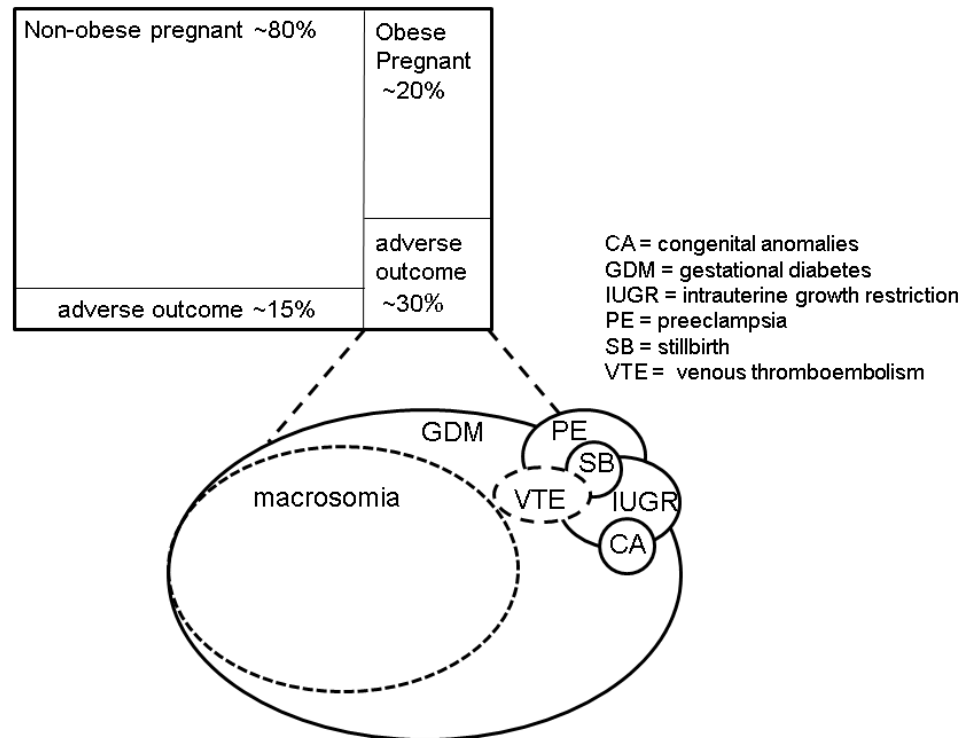
Figure 1.2 Summary of the potential obstetric complications associated with maternal obesity. The diagram illustrates the increased incidence of metabolic obstetric complications which are more common in obese pregnancy, but still occur in a lean obstetric population. These complications include both maternal and fetal conditions which are explained in the figure key for reference.

Identification of women who are at risk of lipotoxicity-mediated pregnancy complications is required in order to ensure that additional antenatal surveillance is focused on the group of women who are at increased risk of these obstetric complications. Research in the field of cardiovascular and diabetic medicine suggests ways of manipulating lipid metabolism using both lifestyle and pharmacological interventions that may have potential utility in obese pregnancy. Because of the potential for harm to both mother and baby, a study of the degree, causes and impact of lipotoxicity in obese pregnancy is warranted

1.15   **Hypothesis**

The 70-100% increase in maternal obesity over the last decade has had
ramifications for all aspects of female reproduction, with maternal adiposity
strongly associated with an increased risk of maternal and fetal complications.
Our hypothesis is that in lean pregnant women, fatty acids required for fetal
growth are efficiently stored and mobilised from lower body fat depots. In obese
pregnant women, lower body fat depots are replete and/or there is a preference
to store fat centrally with the potential of fatty acid spillover and lipotoxicity.
Lipotoxicity can be defined as the vascular, metabolic and ultimately clinical
manifestation of excessive plasma fatty acids and ectopic fat deposition. In this
thesis we suggest that it is lipotoxicity which leads to maternal endothelial
dysfunction, decreases trophoblast invasion and influences placental metabolism
and function (figure 1.3)

This is the pathological link between maternal obesity and adverse "metabolic" pregnancy outcomes such as pregnancy-induced hypertension and PET and may have consequences for the programming of obesity in the offspring.
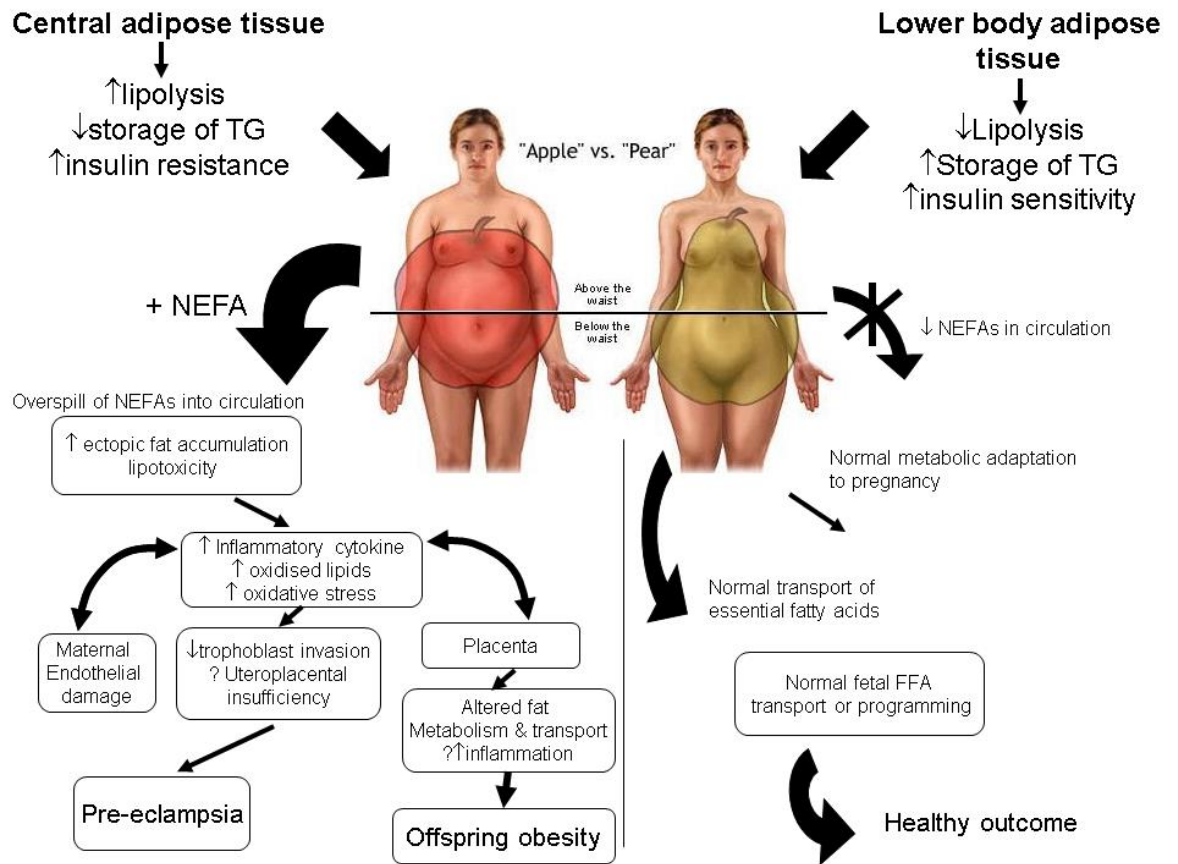
Figure 1.3 Fatty acid metabolism in pregnancy. The figure above is adapted from *Jarvie E et al 'Lipotoxicity in obese pregnancy and its potential role in adverse pregnancy outcome and obesity in the offspring', Clin Sci (Lond). 2010.* Central fat accumulation during pregnancy leads to fatty acid overspill from adipose depots and lipotoxicity. Lipotoxic effects include maternal endothelial dysfunction, decreased trophoblast invasion and altered placental metabolism. These may result in adverse pregnancy outcome (such as pre-eclampsia or miscarriage) and programming of obesity in the offspring. A lower-body fat accumulation allows 'safe' storage of fatty acids and a normal metabolic and physiological adaptation to pregnancy with appropriate nutrient transfer to the offspring.

1.16   **Study aims and objectives**

The aim of the study reported in this thesis was to assess the impact of anatomical fat distribution and accumulation throughout pregnancy in both lean and obese women and examine its importance on metabolic and vascular function. Biomarkers of lipotoxicity and vascular function will be considered the consequence of this process.

Objective 1 (Chapter 3)

To carry out detailed anthropometric assessment of lean and obese women during pregnancy in order to test the hypothesis that the anatomical variation in accumulation of subcutaneous adipose tissue in lean and obese women is different and that this has an impact on the metabolic response seen in pregnancy.

Objective 2 (Chapter 4)

To assess different aspects of energy metabolism in lean and obese women throughout pregnancy to assess whether differences exist between lean and obese pregnancies and if this relates to adipose tissue accretion and distribution during gestation. To record measurements of BMR, substrate utilisation, physical activity and diet throughout pregnancy.

Objective 3 (Chapter 5)

To assess whether gestational changes in markers of lipid and carbohydrate metabolism and inflammatory status are related to site of body fat accumulation in pregnancy. Plasma concentrations of biomarkers of carbohydrate (fasting glucose, fasting insulin), lipid (total cholesterol, total triglyceride, HDL and NEFA) metabolism and inflammatory profiles (CRP, IL6, TNFα) will be quantitated.

Objective 4 (Chapter 6)

To examine the impact of anatomical fat accumulation during pregnancy on the gestational improvement of vascular function and markers of lipotoxicity. To explore whether a difference in body fat distribution between lean and obese women in combination with lipotoxicity may be a mechanism for any differences in vascular function between these groups.

Chapter 2 - General Methods

2.1 Introduction

This chapter discusses the inclusion and exclusion criteria, the formulation of the patient information leaflets and the recruitment process itself. The next section in this chapter describes each of the investigation techniques employed and the protocols which were used in the study. This study was reviewed and approved by the West of Scotland Research Ethics Committee 3 (REC reference 09/S0701/105), and Greater Glasgow & Clyde NHS Research and Development (R&D reference GN09KH553).There were no competing interests.

2.1.1 Inclusion & exclusion criteria

Healthy Caucasian women between the ages of 16-40 years with no significant past medical history were invited to participate in this study. The cohort recruited was further selected based on BMI – either less than $25kg/m^2$ or greater or equal to $27kg/m^2$. The BMI groups were widened (initially $<25kg/m^2$ and ≥ 30 kg/m^2) after the rate of recruitment to the obese group remained poor despite adequate study publicity and additional assistance with recruitment.

Parous women with previous healthy pregnancies with no obstetric or fetal complications were not excluded from participation. This was decided after discussion with senior Obstetricians, as it was felt that a previous healthy pregnancy would not have an impact on the index pregnancy. However, any women was excluded if they had suffered a previous fetal loss beyond 12 weeks' gestation or a miscarriage of any gestation secondary to fetal anomaly (e.g. Edward's syndrome) or complex maternal condition (e.g. confirmed thrombophilia).

Women with known metabolic disease such as diabetes mellitus, thyroid disease or polycystic ovarian syndrome (PCOS) and cardiovascular disease were excluded from recruitment as these women are known to have higher risks of metabolic disease in pregnancy.

Pregnancies achieved through assisted conception, including ovulation induction were excluded because of the potential link with anovulatory infertility and

PCOS. Women with multiple pregnancies were also excluded from the study as these pregnancies are generally more high risk for PET (Duckitt and Harrington, 2005) and gestational diabetes (Santolaya and Faro, 2012).

Any women who developed an obstetric antenatal complication in the current study were excluded from analysis retrospectively.

Table 2.1 study inclusion criteria and rationale

Criteria	Rationale
Parous or primigravid women	uncomplicated previous obstetric history or primiparity will have no effect on index pregnancy
maternal age	poorer outcomes out with 16-40 years of age
Two groups BMI<25 and BMI$\geq$27	Established poorer outcome and greater obstetric risk in overweight obese pregnant population (CEMACH, 2007) WHO criteria for obesity(WHO, 2004)
Caucasian	Predominant ethnic group in Glasgow

2.1.2 Recruitment

Women who booked their pregnancies at either the Princess Royal Infirmary or the Southern General Hospital in Glasgow were recruited at their first antenatal appointments (12-14 weeks' gestation).

Suitable women were identified at the initial antenatal appointment by either the midwifery team or the researcher (Eleanor Jarvie). Following the confirmation of a continuing intrauterine pregnancy and gestational age by ultrasound the potential participants had their BMI calculated (routine clinical practice). If this was within the criteria for the study, the participants had a short consultation with the researcher regarding the study and were issued with an information sheet (Appendix I). A contact number was taken for the potential subject to allow them time to read the sheet and decide whether they wished to participate. Each potential study participant was contacted by the researcher and a date for the first study visit was arranged. This was followed up with a letter and a phone call prior to the visit 1 date to confirm the appointment. Figure 2.1 illustrates the recruitment and retention of participants to the study.
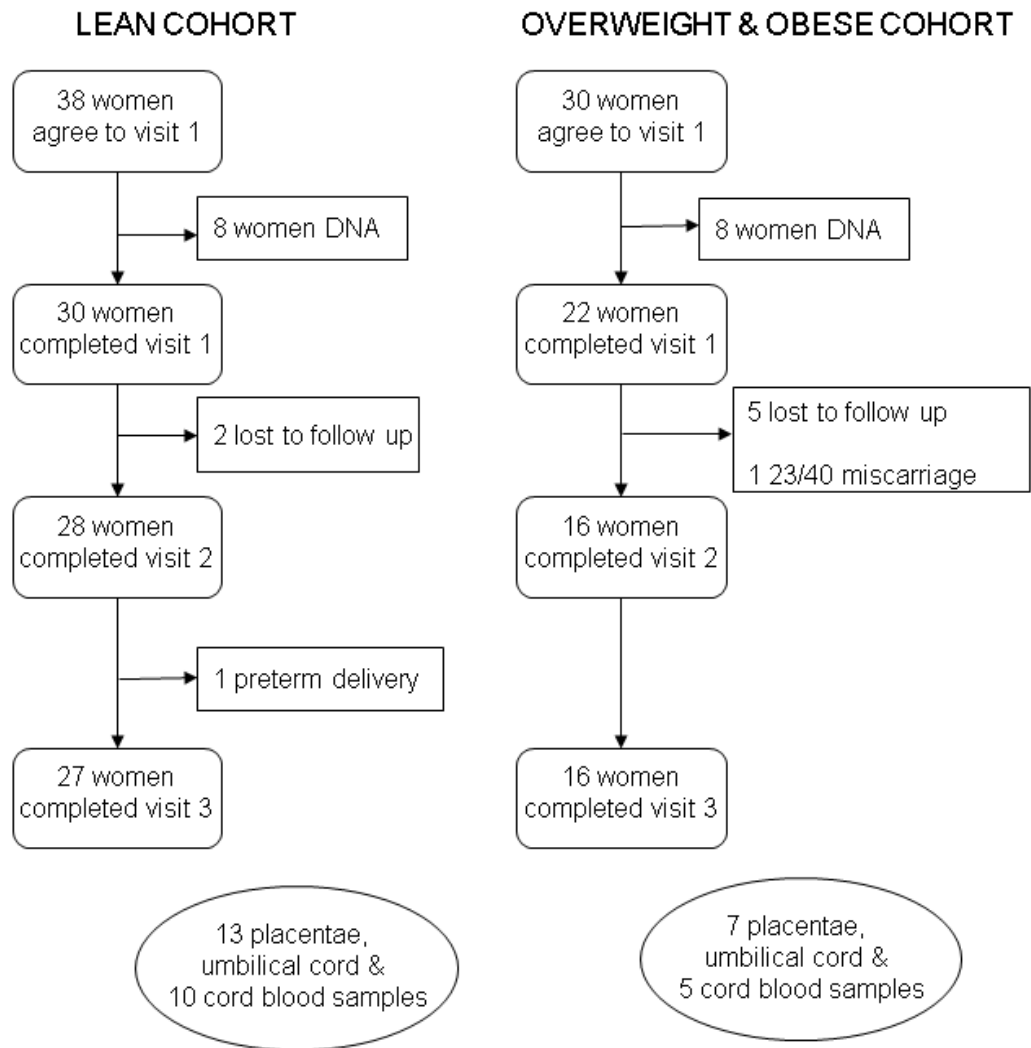
Figure 2.1 Flow diagram of recruitment to study. Figure illustrates the number of participants recruited to the study and the number of participants who attended each of the gestational and postnatal appointments. For each gestational time point the participants excluded or those lost to follow up are included. The abbreviation,.'DNA' refers to 'did not attend'. These women arranged a first visit but did not take part in the study. Indicated in the diagram is also the delivery data and delivery specimens which were obtained.

2.1.3 Study Design

All women underwent the same study appointment protocol throughout the study (figure 2.2, appendix II). Each study participant attended the Metabolic Suite (West Medical Building, University of Glasgow) at 0800 after an overnight fast (participants were advised not to eat after 22:30 the previous night and to only drink water) to undergo assessment. Each participant was asked to attend three antenatal appointments at approximately 15, 25 and 35 weeks' gestation and one postnatal visit at approximately 12 weeks postpartum.

At the initial appointment a consent form (appendix II) was completed, the participant retained a copy and one was kept by the researcher separately from all other visit documentation. At the start of each study appointment the participants' general medical, obstetric, family, social and medication histories were taken (See appendix II for visit documentation). At visits 2, 3 and 4 documentation was updated on any medical or pregnancy-related health problems which had occurred since the last appointment to ensure that the subject was still suitable to be in the study

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┌──────────┐      ┌──────────┐      ┌──────────┐      ┌──────────┐
│ Antenatal│      │ Visit 1  │      │ Visit 2  │      │ Visit 3  │        ⬭
│ Clinic   │      │ Approx 15│      │ Approx 25│      │ Approx 35│      Delivery
│ 12-14 wks│      │ weeks    │      │ weeks    │      │ weeks    │
└──────────┘      └──────────┘      └──────────┘      └──────────┘
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BOX 1: VISIT MEASUREMENTS
Dietary assessment
Indirect calorimetry
Laser Doppler Imaging
Body Composition
Skinfold measurements
Blood and urine sampling
Accelerometer distribution and instructions

Prospective participants identified by midwives. If interested initial discussion with Dr Jarvie. Patient information sheet given

Informed consent obtained Refer to Box 1

Refer to Box 1

Refer to Box 1

Biopsies from umbilical cord, and placenta. Umbilical cord blood sample. Maternal adipose tissue biopsies (visceral and subcuticular) if delivered by elective c-section

Figure 2.2 Study appointment protocol. Detailed above is the protocol time line for study participants, including assessments performed at each visit and tissue samples to be obtained at delivery

2.2 Anthropometric Measurements

2.2.1 Maternal height

Maternal height was measured by the research nurse or the researcher using a stadiometer (Invicta Plastics Ltd, Leicester, UK). The same stadiometer was used for each study participant. The participant was measured without shoes standing with their back and head touching the vertical board and ensuring that the legs, back and head were straight and the feet were flat. The measurements were recorded with participant positioned and relaxed and a moveable horizontal headboard was lowered onto the head with light pressure maintaining the head in the neutral position. The measurements were recorded to the nearest 0.01 metre.

2.2.2 Maternal body mass

Maternal body mass was measured using a calibrated integral system which was part of the BODPOD chamber (COSMED USA Inc, Concord, USA) (see 2.2.5 for details). The same scale was used for each study visit and was periodically calibrated using a 20kg weight as per the manufacturer's guidelines. Study participants were asked to change into shorts and a vest top or similar minimal clothing and were supplied with a swimming cap. Additional clothing and jewellery was removed prior to measurement recording. Their weight was then measured with them standing on the scales for 20 seconds prior to entering the BODPOD chamber. Measurements were recorded to the 0.01kg.

2.2.3 Maternal circumference measurements

All maternal circumference measurements were performed by the researcher in a private setting with the research nurse present documenting the measurements. Measurements were recorded to the nearest 0.1cm using a plastic tape measure. Circumference measurements were taken with the stub of the tape controlled in the left hand using the cross-hand technique and with the right hand stabilising the tape at the correct anatomical point for measurement. All circumference measurements were performed with the subject standing. The two waist measurements were taken at the end of expiration with the abdominal

muscles relaxed. Waist and hip circumferences were measured with the participant's feet together and gluteal and midthigh circumference measurements were measured with the study participant's feet shoulder width apart and weight evenly distributed across both feet.

Minimum waist circumference was measured at the narrowest point of the abdomen between the lower costal edge and the anterior superior iliac spine. Umbilical waist circumference was measured the level of the umbilicus. Hip circumference was measured as the circumference of the buttocks at the level of their greatest protuberance. Two thigh measurements were performed; gluteal thigh circumference was measured 1cm inferior to the gluteal fold and midthigh circumference was measured at the midpoint between the inguinal crease and the proximal edge of the patella (the same point as the midthigh skinfold).

Each circumference was recorded twice and the mean was calculated to be used for analysis. If a difference of >0.5cm was found a third measurement was performed and the mean of the two closest measurements was used for analysis.

The researcher underwent anthropometric training with a Level 3 ISAK (International Society for the Advancement of Kinanthropometry www.isakonline.com) Instructor. Prior to commencement of the study the researcher performed a validation series on healthy volunteers to assess technique. To achieve basic competency (ISAK level 1) required a technical error of the mean (TEM) of circumference measurements to be less than 1.5%. TEM variations for each circumference were waist umbilicus 0.7%, hips 0.5%, gluteal thigh 0.3% and midthigh 0.4%.

2.2.4 Maternal skinfold thickness measurements

All measurements of skinfold thickness were performed by the researcher in a private setting with the research nurse present documenting the measurements. Measurements were recorded to the 0.2mm using Holtain skinfold callipers (Holtain Ltd, Crymych UK) or Harpenden skinfold callipers (Cranlea & Company, Birmingham, UK) when the skinfold was greater than 40mm.

Seven skinfold thickness sites were measured during this study. These sites were based on published work validating the use of skinfold measurement in calculating subcutaneous adipose tissue and total fat in pregnancy (Presley et al., 2000). The skinfold was held between the thumb and the index finger of the left hand and the callipers applied with the right hand. The callipers were held in place for 20 seconds at every measurement throughout the study. The rationale for this technique was to minimise the impact of interstitial oedema observed in late gestation. This phenomenon occurs to a different extent in most women so for consistency this technique was applied to all measurements performed.

All skinfolds were measured on the left hand side of the body. The study participant was standing for the assessment of triceps, biceps, subscapular, costal and suprailiac skinfolds and was sitting with the left leg horizontal and supported at the knee and ankle for midthigh and suprapatellar recordings. The triceps skinfold was measured at the midpoint between the acromion process of the shoulder and the olecranon of the elbow in the longitudinal plane. The biceps skinfold was measured at the midpoint between the acromion process and the antecubital fossa in the longitudinal plane. The subscapular skinfold was measured medial to distal border of the scapular at 45° to the vertical plane. The costal skinfold was measured at the inferior border of the costal margin in the midaxillary line in the horizontal plane. The suprailiac skinfold was measured at the midpoint between the anterior superior iliac spine and the lower edge of the costal margin in the vertical plane. The midthigh skinfold was measured on the front of the thigh at the midpoint between the inguinal crease and the proximal edge of the patella in the vertical plane. The suprapatellar skinfold was measured at the proximal border of the patella in the vertical plane. The researcher stood behind the participant for the triceps and subscapular measurements, on the left hand side of the study participant for the costal, midthigh and suprapatellar measurements and in front of the study participant for the biceps and suprailiac measurements.

Each skinfold measurement was recorded twice and the mean measurement used for analysis. If a difference of 0.8mm or above existed between the recordings

then a third measurement was performed and the mean of the two closest measurements recorded used for analysis.

A similar validation set based on ISAK level 1 competencies was performed for skinfold measurements and the TEM was required to be <7.5% for each skinfold. TEM variations for each of the skinfolds were triceps 1.8%, biceps 3.8% subscapular 2.4%, costal 3.5%, suprailiac 4.8%, midthigh 2.2% and suprapatellar 2.5%.

2.2.5 Air Displacement Plethysmography

Each subject underwent ADP, using the commercially available BODPOD system (COSMED USA Inc, Concord, USA), which calculates fat mass, fat free-mass and percentage body fat. The detailed principles of ADP have been reported by Dempster and Aitkens (Dempster and Aitkens, 1995). ADP works on the principle of Boyle's law, stating that within a fixed volume a change of mass will cause a change of pressure within that fixed volume. This allows for measurement of body volume which is corrected for predicted lung volume based on age and height. From this measurement body density (D_B) is calculated and fat mass and fat-free mass (two compartment model) can be derived using Siri's equation:

$$W_{FM} \text{ (kg)} = W_B/100 \times (495/D_B - 450) \qquad \textbf{(equation 2.1)}$$

Where W_{FM} is fat mass in kilograms, W_B is body weight in kilograms and D_B is body density in kg/m^3.

Subjects were measured in the fasting state wearing either a bathing suit or tight fitting shorts and vest top and an acrylic bathing cap. The subjects' body mass was measured first using the integrated scales to the nearest 0.01kg for 20 seconds. The BODPOD is calibrated with the chamber empty (baseline) and then with a 50 litre aluminium calibration cylinder present in the chamber. Each subject then sat in the chamber with the door closed for two measurements each lasting 45 seconds. These volume measurements were averaged, but if they were not within the reproducibility criteria (150ml or 0.2%), a third

measurement was taken and the three measurements were averaged. If the variation persisted then the system was recalibrated and the measurements repeated. The corrected body volume was calculated using an assumed predicted lung volume and surface area artefact.

The principle of ADP works on the basis that the body can be split into two compartments: fat-free (muscle, bone and water) and fat mass and that the density of these two compartments remain constant. In pregnancy however, the density of each of these compartments changes over the course of gestation as a result of accumulation of interstitial fluid and oedema. Raaij et al (van Raaij et al., 1988) formulated a series of equations to account for this change in body density and therefore data from this cohort was first corrected using these equations prior to analysis.

10 week gestation equation

$$W_{FM} \text{ (kg)} = W_B/100 \times (496.4/D_B - 451.6) \quad \textbf{(equation 2.2)}$$

20 week gestation equation

$$W_{FM} \text{ (kg)} = W_B/100 \times (502.2/D_B - 458.0) \quad \textbf{(equation 2.3)}$$

30 week gestation equation

$$W_{FM} \text{ (kg)} = W_B/100 \times (510.8/D_B - 467.5) \quad \textbf{(equation 2.4)}$$

The above equations were applied to the raw data which was collected from the BODPOD at each antenatal appointment. During the postnatal appointment (visit 4) uncorrected data was used as participants were no longer pregnant and had no observed oedema. Reproducibility of the ADP data has previously show that the typical error is 0.48kg, 0.43kg and 0.56% for fat free mass, fat mass and percentage body fat respectively.

2.3 Energy balance and substrate metabolism in pregnancy

2.3.1 Indirect Calorimetry

Study participants had their BMR and substrate utilisation measured at each study appointment (4 visits). All measurements were performed in the morning following a 10 hour fast using the Oxycon ventilated hood system (Jaegar Oxycon Pro, Hoechberg, Germany). The Oxycon ventilated hood system required to be switched on and calibrated 45 minutes prior to measurement. The equipment was calibrated for ambient atmospheric pressure using a traditional barometer. To ensure that the fraction of expired O_2 (FEO_2) and CO_2 ($FECO_2$) was accurate the gas analyser of the Oxycon was calibrated against a standard gas, and the turbine was manually calibrated for volume. The examination room was kept at a temperature of 23.9+/- 0.8 °C with the researcher and the research nurse present.

Study participants lay in a semi-supine position on an examination couch with adequate support of the lower back. The ventilation hood was placed over their head and shoulders with the plastic skirt covering upper torso and tucked under the pillows (see figure 2.3). The participants in this study did not lie in a supine position due to the potential of the gravid uterus to cause inferior vena cava compression and hypotension. The ventilation hood has two apertures; a cranial aperture which draws air into the hood and a caudal aperture which is attached to the Oxycon ventilated hood system via plastic tubing.

Figure 2.3 Oxycon ventilated hood system. Resting metabolic rate and substrate utilisation being recorded by indirect calorimetry with a volunteer subject.

At each study visit the participants were under the hood for 25 minutes. During the first ten minutes no readings were recorded to allow the participant time to acclimatise to the hood and the environment.

The indirect calorimetry system draws air through the hood and then measures FEO_2 and $FECO_2$. Using these parameters the rate of consumption of oxygen (VO_2, ml/min) and the rate of carbon dioxide production (VCO_2, ml/min) can then be calculated. These parameters were recorded every sixty seconds. Using these values respiratory exchange ratio (RER), substrate utilisation and basal metabolic rate (BMR) are derived from Frayn's (Frayn, 1983) equations for indirect calorimetry.

In order to extrapolate substrate utilisation and basal metabolic rate one must first account for protein metabolism. One method of calculating this is to measure urinary nitrogen, but this was not measured in this study as 24 hour urine collection was impractical. For healthy adults a constant rate of nitrogen excretion may be assumed. Investigation of protein balance and nitrogen metabolism in pregnancy shows that nitrogen excretion changes with gestation and thus a constant cannot be applied to this cohort. Gestational age-specific nitrogen excretion has been described (Mojtahedi et al., 2002). Using these calculated values nitrogen excretion is 0.00014g/kg/min at 12 weeks' gestation, 0.00012g/kg/min at 23 weeks' and 0.00011g/kg/min at 34 weeks'. When

nitrogen excretion (N) is considered a constant at each gestation, then it can be subtracted from Frayn's equations in order to calculate non-protein oxygen consumption ($NPVO_2$) and non-protein carbon dioxide production ($NPVCO_2$), and non-protein respiratory exchange ratio (NPRER), as shown in the following equations:

$NPVO_2$ (ml/min) = VO_2 – 6.04N   **(equation 2.5)**

$NPVCO_2$ (ml/min) = VCO_2 – 4.89N   **(equation 2.6)**

NPRER = $NPVO_2/NPVCO_2$ **(equation 2.7)**

Using the corrected values for $NPVO_2$ and $NPVCO_2$, the individual's substrate utilisation and subsequently basal metabolic rate (expressed as energy expenditure (EE, kilojoules kJ) can be obtained:

Fat oxidation (fat ox) (g/min) = (NVO_2 – $NPVCO_2$)/0.6 **(equation 2.8)**

Carbohydrate oxidation (CHO ox) (g/min) = ($NPVO_2$ – 2.03 x fat ox)/0.746

(equation 2.9)

Estimated protein oxidation (prot ox) (g/min) = N x 6.25

(equation 2.10)

EE (kJ) = (fat x 39.0) + (carbohydrate x 15.5) + (protein x 17.0)

(equation 2.11)

Validation assessment of the Oxycon Pro ventilated hood system was performed by comparing values for VO_2 and VCO_2 obtained using the Oxycon Pro ventilated hood system against values obtained using Douglas bag expired air collections in 11 volunteers in the fasted state (unpublished data). The Pearson correlation coefficients between measurement techniques were 0.964 ($p < 0.0005$) for VO_2 and 0.972 ($p < 0.0005$) for VCO_2.

The reliability of the Oxycon Pro ventilation hood system was determined by comparing resting VO_2 and VCO_2 measurements made in the fasted state in 10 volunteers made on two occasions with an interval of 1-2 weeks (these were different volunteers from those used to determine the system's validity). The Pearson correlation coefficients between measurements were 0.913 ($p < 0.0005$) for VO_2 and 0.943 ($p < 0.0005$) for VCO_2.

2.3.2 Physical Activity

To objectively access physical activity the participants were asked to wear a physical activity monitor during waking hours for seven days following each visit. The monitors used were the Actigraph GT3X or the Actitrainer accelerometer (ActiGraph Pensacola, FL, USA) which were worn on a waist belt or clipped to the waistband. The participants were given a simple diary sheet to indicate when the monitor was worn, and advised to remove it when sleeping, showering or swimming. Once the week of data had been collected the device was sent back in a stamped addressed envelope.

The accelerometer is a small device which detects vertical accelerations at specified time points (referred to as epochs). For this study each 'epoch' is defined as sixty seconds. Each device was initiated using Actilife software Version 5.3.0 (ActiGraph Pensacola, FL, USA) prior to the study visit to record activity every sixty seconds over the course of the seven days. When the data from each device was downloaded this created a CVS Microsoft file which displays a number (displayed in counts/min) for each epoch. Each count/min refers to an intensity level which is defined by the Freedson cut points (Freedson et al., 1998). Therefore sedentary activity <100 counts/min, light activity is 100-1951 counts/min, moderate activity 1952-5724 counts/min and vigorous activity is >5724 counts/min. For the purposes of the current analysis three activity intensities were used; sedentary, light and moderate & vigorous physical activity (MVPA), because as a pregnancy progressed the amount of time spent in vigorous activity alone was expected to be very small and therefore not useful for comparison. Each CVS file was then saved as an excel spreadsheet file.

In order to analyse the data, the seven days of information was first split into each calendar day. Sleep time was removed, guided by the diaries. In addition, if more than one hour of '0' counts (i.e. more that sixty consecutive '0' counts/min) were recorded this was considered non-wear time and was removed from the excel spreadsheet. The Freedson cut points were then applied to the remaining data and the time spent in each of the intensity levels was calculated in minutes as well as an average wear time in minutes for the day. Using each calendar day data an average was calculated for each visit. Previous studies in adults have determined what is considered to be a valid wear time and have suggested that three to five days (Trost et al., 2005) of accelerometer data will provide a valid estimation of physical activity. Other published data on activity levels in pregnancy using accelerometers have suggested using four days for valid activity assessment (Kinnunen et al., 2011). A valid calendar day was considered for the purposes of this study to have 9 hours of wear time data and it was required that at least four days of data were collected. Across gestation and between the two groups there was no difference in valid wear time (appendix III)

2.3.3 Dietary Assessment

At each study visit, participants completed a multiple pass 24 hour dietary recall questionnaire (Conway et al., 2004). The questionnaire (appendix II) recorded everything that the participant had had to eat and drink in the twenty four hours preceding the study visit. The questionnaire was filled out by either the researcher or the research nurse and was administered according to protocols used for the Food Standard Authority's Low Income Diet and Nutrition Survey (Bush, 2008). The multiple pass method is not time consuming (approximately 15-20 minutes to complete) and has been widely implemented in different demographic study groups.

Participants also completed a food frequency intake questionnaire, which has been validated in a Scottish population (Lean et al., 2003). The analysis of the food patterns estimates food intake in relation to national dietary targets of fish, fruit and vegetables, breakfast cereals and intake of fat and sugary foods (appendix II).

Data from the questionnaires was then entered into the Dietplan 6 analysis package (Forestfield Software Ltd, West Sussex, UK). Total energy, macronutrient and micronutrient intake were then exported from this database into Microsoft excel spreadsheets for analysis.

2.4 Peripheral vascular function assessment

Laser Doppler Imaging (LDI) is a non-invasive method for assessing peripheral vascular function. This technique has been used in pregnancy to highlight the differences in vascular function between obese and lean women (Ramsay et al., 2002a, Stewart et al., 2007a).

Before the examination all women were acclimatised in a temperature-controlled room (23.9+/- 0.8 °C) for a ten minute period. Women were asked to recline in a semi-recumbent position with the volar aspect of the forearm exposed resting on an armrest. All participants were fasted.
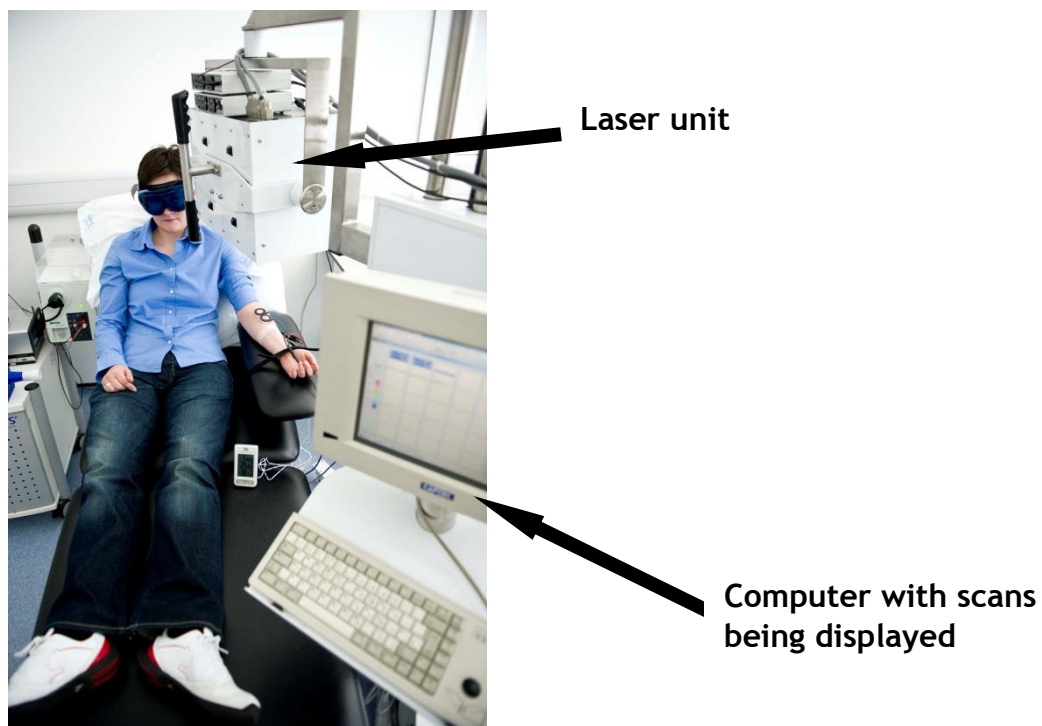
Figure 2.4 Laser Doppler imaging assessment. Volunteer positioned for LDI assessment.

In order to assess peripheral vascular reactivity, iontophoresis of vasoactive solutions was used. Vasodilatation is considered the expression of vascular reactivity, and this response can be considered either endothelium-dependent (mediated through direct stimulation of the endothelial cells) or endothelium-independent (mediated through the effect of NO on vascular smooth muscle cells). Iontophoresis is based on the principle that a charged molecule migrates across the skin under the influence of an applied electrical field. In order to measure endothelium-dependent vasodilatation, acetylcholine (ACH) was used to stimulate the endothelial cells through its binding to muscarinic receptors and the subsequent generation of NO. To assess endothelium-independent vasodilatation, Sodium Nitroprusside (SNP) which acts as a NO donor to vascular smooth muscle cell was used.

Drug delivery was achieved using a battery-powered constant current iontophoresis MIC-1e controller (Moors Instruments Ltd, Axminster, UK). The chambers used for iontophoresis were ION-6 Perspex chambers (Moor Instruments Ltd, Axminster, UK) which had an internal diameter of 22mm and area of $3.8cm^2$ and an internal platinum wire electrode. These chambers were attached using double-sided adhesive disks to the volar aspect of the forearm avoiding any broken skin, hair and superficial veins. These chambers were connected to the iontophoresis controller. In addition, a digital multimeter was connected in parallel to record the voltage across the chambers. This is important as skin resistance has an effect on the delivery of the iontophoresis drugs. As the constant current source is being used, skin resistance can be calculated using Ohm's law where resistance is equal to voltage divided by current. A 2.5ml dose of 1% ACH which is a positively charged ion (Sigma, Poole, UK) was introduced into the anode chamber while a 2.5ml 1% dose of SNP (Sigma, Poole, UK) which is a negatively charged ion was introduced into the cathode chamber. Drug delivery was delivered simultaneously during administration of the current. The vehicle for these drugs was 0.5% sodium chloride (NaCl) in deionised water. Cover slips were placed over each chamber to minimise loss of fluids during the procedure (figure 2.5).
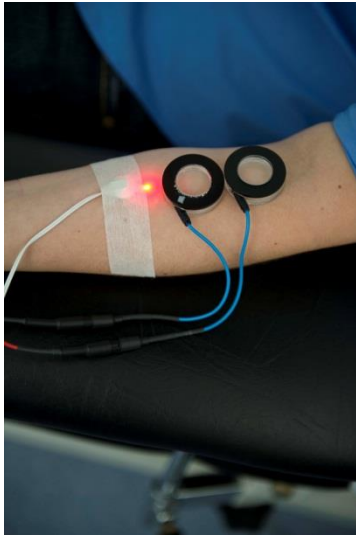
Figure 2.5 LDI laser scanning over the area of interest. The two chambers are attached to the volar aspect of the arm. Each chamber is attached to the iontophoresis unit. The chamber with the white dot indicates the chamber with the SNP solution in it and the other chamber contains the ACH solution. A temperature probe is taped adjacent to the chambers to record skin temperature throughout the experiment.

Measurement of the perfusion of the skin and thus the vasodilatation of the peripheral vascular bed was performed using a laser Doppler imager (Moor Instruments Ltd, Axminster, UK) equipped with a red laser (wavelength, 633 nm; power, 1mW; beam diameter, 1 mm) which was scanned over both chambers through the coverslips. The technique is based on the Doppler shift effect imparted by the moving blood cells in the underlying skin to the backscattered light collected by the photodetectors on the laser unit. The backscattered light is converted into an arbitrary perfusion (or flux) unit (PU) that is displayed as a colour coded image on the computer monitor (figure 2.6). Twenty scans are performed with an incremental iontophoresis current protocol with the first scan indicating baseline perfusion (pre-current administration) followed by an incremental current protocol; four scans at 5 microamps (5 μA), four at 10μA, four at 15μA, two at 20μA and four recovery scans. The total current delivered was 8 milliCoulombs (8 mC).
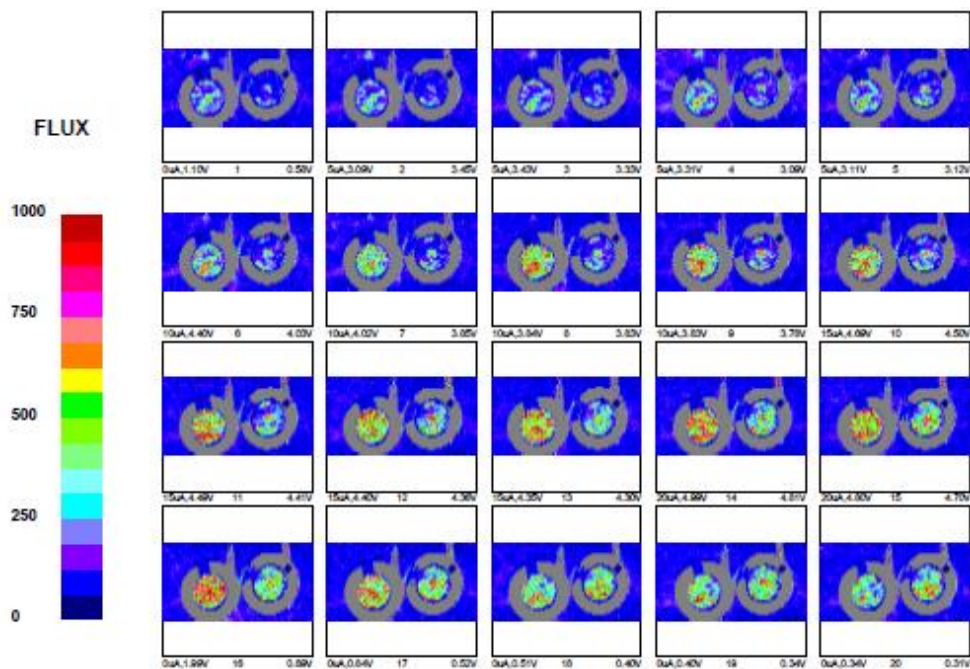
Figure 2.6 LDI scans indicating both endothelium-dependent (ACH left chamber) and -independent (SNP right chamber) vasodilatation. The laser beam and subsequent back scattered light is distorted by the increase in movement of red blood cells through the peripheral circulation, this is then interpreted by the computer software and produces a series of scan images.

Perfusion measurements were performed using the image manufacturer's image analysis software of outlining the region of interest (ROI) around the internal circumference of the chamber. A median value for each scan flux value was calculated across approximately 700 measurement points. The median value was first corrected for skin resistance as described above. The sum of the corrected median values from each scan is referred to as the total perfusion.time area under the curve (AUC). The incremental perfusion.time area under the curve is considered the total perfusion.time curve minus the baseline scan (pre-current administration), illustrated in Figure 2.7.
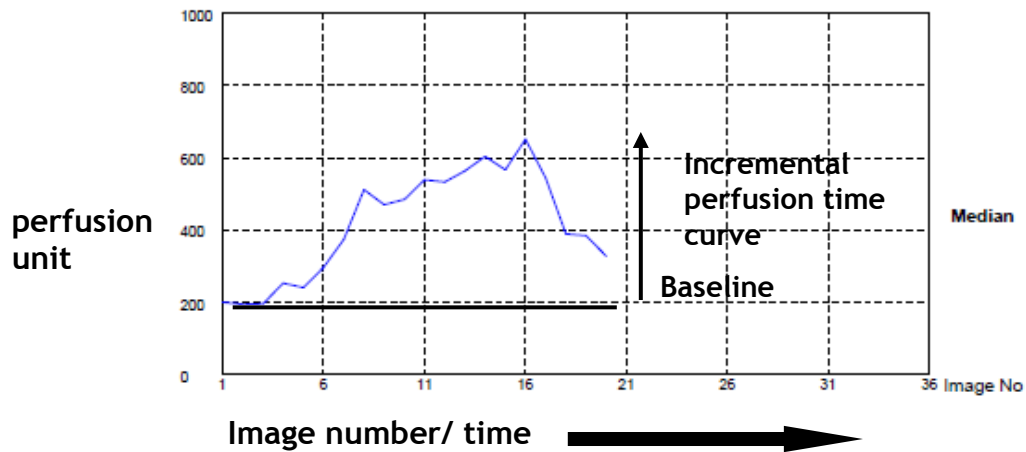
Figure 2.7 Illustration of perfusion.time curve. The above figure relates to the scan displayed in figure 2.6. Total perfusion.time curve is area under the blue line. The incremental perfusion.time curve is the perfusion observed above the baseline only.

In order to calculate the value for the total perfusion.time curves, the sum of these values is multiplied by 5/6 as current delivery for each scan was 50 seconds which can be expressed as 50/60 or 5/6:

Total perfusion.time curve = sum of median values x 5/6   **(equation 2.12)**

In order to calculate the value of the incremental perfusion.time curve, first the baseline median value is subtracted from each of the twenty scans and then multiplied by 5/6:

Incremental perfusion.time curve

$$= \text{median scan}_1 – \text{baseline etc for scans 1-20}$$
$$= \text{sum incremental scan values x5/6} \quad \textbf{(equation 2.13)}$$

Responses were observed with the vehicle alone as a control experiment. The within-day, between site coefficient of variation (CV), measured on the same morning in four subjects, was $2.6 \pm 1.3\%$ for ACH and $1.3 \pm 1.1\%$ for SNP. Previous studies have shown that the mean ($\pm$ SD) between-day CV for the ACH response, measured in four subjects on 2 separate days, was $6.4 \pm 3.3\%$. The within-day, between-site CV, measured in both forearms on the same morning in four subjects, was $8.9 \pm 5.3\%$ (Ramsay et al., 2002a).

2.5 Biological sample analysis

At each study visit blood samples were collected for glucose, insulin, lipid (total cholesterol, total triglycerides, NEFA and HDL cholesterol, oxLDL), CRP, IL6 and TNFα, GGT, sICAM-1, and sVCAM-1 assays. Urine samples were obtained for isoprostanes assessment. Superoxide levels were detected in whole blood samples.

2.5.1 Plasma preparation and storage

Fasting venous blood was collected from each study participant at each visit as follows; 1 x 9 millilitre (ml) lithium heparin tubes, 1 X 5ml serum tubes, 2x9ml EDTA tubes and 1 x 4ml EDTA tube. The lithium heparin and EDTA samples were separated into plasma by centrifugation (1800g) and divided into aliquots. A 950ul lithium heparin sample was removed and transported on ice to complete the superoxide assessment. A proportion of the aliquots were stored under antioxidant conditions (0.01% BHT and the tube filled with nitrogen gas). Buffy coat samples were collected from a proportion of the standard EDTA aliquots. Serum samples were left to stand for 30 minutes before centrifugation and then dispensed into aliquots. All of the above samples were stored in a -80°C freezer within the department until analysis.

2.5.2 Urine sample collection & storage

At each study visit, the participant was requested to provide a sample of urine. Urine aliquots were stored under standard and antioxidant conditions at -80°C.

2.5.3 Routine biochemical assays

Routine assays were carried out on an autoanalyser (ILAB 600 Chemistry Analyser). Total cholesterol, HDL cholesterol, and triglycerides were measured by enzymatic colorimetric methods using commercially available kits (Roche Diagnostics GmbH, Mannheim, Germany). Non-esterified fatty acids (NEFA) concentrations were analysed by enzymatic colorimetric methods using a commercially available kit (Wako Chemicals GmbH, Neuss, Germany). Fasting glucose was measured with the hexokinase/glucose-6-phosphate dehydrogenase

enzymatic method using a commercially available kit (Randox Laboratories Ltd, Co Antrium, UK). Gamma glutamyl transferase (GGT) was assayed by rate reaction absorptive techniques using a commercially available kit (Instrumentation Laboratory, USA). Highly sensitive CRP was analysed by an immunoturbidimetric technique using a commercially available kit (Randox Laboratories, Laboratories Ltd, Co Antrium, UK).

Accuracy and precision of the assays were monitored by internal quality control (Roche Diagnostics GmbH, Mannheim, Germany. Wako Chemicals GmbH, Neuss, Germany. Randox Laboratories Ltd, Co Antrium, UK. Instrumentation Laboratory, USA). Coefficients of the variation were 2.9% for cholesterol, 2.8% for HDL, 3.8% for triglycerides, 5.2% for NEFA, 2.0% for glucose and 2.7% for CRP.

2.5.4 Enzyme linked immunoassays

All kits employed the quantitative sandwich enzyme immunoassay technique. Assays were performed using commercially available kits: insulin by ultrasensitive kit (Mercodia, Uppsala, Sweden), haemoglobin (Universal Biologicals, UK), oxidised LDL (OxLDL), urinary isoprostanes (Oxford Biomedical Research, Oxford, UK) and sICAM-1, sVCAM-1, IL6 and TNFα (R&D Systems, Oxford, UK). With regards to data on urinary isoprostanes, due to the change in glomerular filtration rate during pregnancy these data were corrected for creatinine concentration prior to analysis.

The precision of the kits were monitored by internal quality control (Mercodia, Uppsala, Sweden and R&D Systems, Oxford, UK). Coefficients of the variation for intra-assay were 4.9% for insulin, 4.6% for sICAM-1, 3.1% for sVCAM-1, 7.4% for IL6 and 5.3% for TNFα. Coefficients of the variation for inter-assay were 2.8% for insulin, OxLDL 8%, 5.5% for sICAM, 7.0% for sVCAM, 7.6% for IL6 and 8.4% for TNFα. No coefficients of the variation were quoted in the manufacturer's guidance for haemoglobin or urinary isoprostanes.

2.5.5 Superoxide analysis

Electron paramagnetic resonance (EPR) is a spectroscopic technique which detects species of unpaired electrons, such as those produced by reactive oxygen species (ROS). The method detects the transition of unpaired electrons in an applied magnetic field. Unpaired electrons have a spin which gives them a magnetic property known as a magnetic moment, making the electron behave like a small magnet. When an external magnetic field is applied, the paramagnetic electrons will orientate in either direction parallel or antiparallel to the direction of the magnetic field. This creates two distinct unpaired electron energy levels and the unpaired electrons in the superoxides can then be measured as they are driven between these two levels by the magnetic field. Superoxide levels were detected in whole blood samples using an electron paramagnetic resonance e-scan R Biospin analyser (Bruker GmBH, Osterreich, Austria) with a CPH (1-hyroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine) 500 µM spin probe (Noxygen, Elzach, Germany). Superoxides are measured in whole blood. Throughout pregnancy total blood volume changes. Before analysis was performed on this data, a haematocrit and haemoglobin (Quantichrom, BioAssay Systems, Universal Biologicals Ltd Cambridge, UK) were measured for each sample and the blood volume was corrected as per the method used by Dill and Costill (Dill and Costill, 1974).

2.5.6 Homeostatic Model Assessment

Homeostatic model assessment was performed as a measure of insulin resistance on all plasma samples. The method employed in this thesis was HOMA-1 which has been shown to correlate with the euglycaemic clamp data. Methodology employed in this data analysis was as per Matthew et al (Matthews et al., 1985)

2.6 Statistical Analysis

The numbers recruited were based on power calculations for differences in the anthropometric measures ((Soltani and Fraser, 2000), energy expenditure (Catalano et al., 1998)), endothelial function (Stewart et al., 2007a) and lipotoxic measures (Stewart, 2007)) but not for biological assays.

The number of participants required was worked out was based on the mean obese measurement of the descried parameter minus the mean lean measurement divided by the mean lean. This is described at the standardised delta. The second calculation required for the power was the sigma of the lean group divided by the mean lean. These results were then exported to Minitab vs16 where numbers needed to recruit were based on a 2 sample t power calculation, described in table 2.2. As the largest number needed to recruit was based on endothelial function (n=24), it was decided to aim to recruit 30 women to each BMI group in order to detect a difference and cover any study drop outs.

Table 2.2 Power Calulations

Parameter	Delta (obese minus lean)/ mean lean [standardised]	Sigma of lean group/ mean lean [standardised]	Power 80% (n)	Power 90% (n)	Source
Fat distribution					Soltani 2000
Ratio change in central vs lower body skinfold	+2.31 /2.16 [1.07]	18.61/30.26 [0.62] Used total skinfold SD	7	9	
Lower body fat					Soltani 2000
Skinfold change (mm)	-4/6 [0.67]	2/6 [0.33]	5	7	
Energy expenditure					Catalano 1998, Okereke 2004
Total (kcal/day)	76/373 [0.20]	372/7886 [0.20]	17	23	
CHO (mg/min)	43.7/56.4 [0.77]	59.4/164.1 [0.36]	5	6	
Fat (mg/min)	12.8/6.3 [2.03]	29.2/49.6 [0.59]	3	4	
Endothelial Function					Stewart 2007
Microvascular function – LDI [PUMΩmin]	Trimester 1 5930/15112 [0.39]	Trimester 1 5982/15112 [0.40]	18	24	
Lipotoxic mediators					Frances Stewart
LDLIII mass (mg/dL)	Trimester 3 25.7/37.7 [0.68]	Trimester 3 18.32/37.73 [0.49]	10	12	MD Thesis 2007

In order to normalise data for analysis, raw values were log or square root transformed when necessary. Statistical analysis was performed using a linear mixed model in IBM SPSS Statistics version 19. The statistical model assessed the effect of gestation (quoted as 'p time'), the effect of the group (quoted as 'p type') and their interaction (pattern of change; quoted as 'p time x type') of each variable. For the mixed model effect of time and type were considered significant with a $p < 0.01$, and the interaction model was considered significant with a $p < 0.05$.

Post hoc analysis was performed if a statistically significant difference was found for the interaction. *Post hoc* analysis assessed the change in each variable in early (V1-V2), late (V2-V3) and total (V1-V3) gestation using 2 sample t-tests

(Minitab vs16). This analysis was performed in Minitab vs16 and p<0.05 was considered significant.

Univariate analysis was performed to assess the impact of anatomical fat depots on the total fat mass gained during pregnancy. If significant relationships were found using Pearson's correlations then general linear modelling was performed to assess each sites contribution to the fat mass gained. This analysis was performed in Minitab vs16 and p<0.01 was considered significant.

Any statistical analysis which was specific to a subset of data has been described in the relevant methods section in each results chapter.

Chapter 3 - Relationship between mass and pattern of subcutaneous adipose tissue accumulation in healthy lean and OW/OB pregnancies

3.1 Introduction

The amount of gestational weight which women gain during pregnancy varies greatly. Guidance from the IOM in 2009, recommends that underweight (BMI<18.5kg/m^2) women aim for a total GWG of 12.5-18.0kg, normal weight (BMI18.5-24.9kg/m^2), aim for 11.5-16.0kg, overweight (BMI 25.0-29.9kg/m^2) to aim for 7.0-11.5kg and obese (BMI>30.0 kg/m^2) women aim to gain no more than 5-9kg. Excessive gestational weight gain has been extensively researched and the impact of GWG on maternal obstetric complications is variable. What is known conclusively is that pre-pregnancy obesity is a recognised risk factor for a range of congenital, antenatal, intrapartum and postnatal complications (Jarvie and Ramsay, 2010).

It has been observed that subcutaneous fat stores in the mother increase markedly from 10-30 weeks' gestation and thereafter remain fairly constant until parturition (Taggart et al., 1967). Further examination of the anatomical variation in subcutaneous adiposity has suggested that obese women preferentially store fat in the subcutaneous central compartments when compared to their lean counterparts (Soltani and Fraser, 2000). As highlighted, upper body centrally located fat is associated with an abnormal metabolic profile; although the contribution of the subcutaneous and visceral compartment is unclear. If obese women do store fat preferentially in central compartments then this may be a contributory factor to any abnormal inflammatory and vascular response exhibited (Ramsay et al., 2002a). It has been suggested that, in the non-pregnant population increased thigh circumference may provide protection against cardiovascular disease independent of abdominal and general obesity (Heitmann and Frederiksen, 2009). Furthermore, it has been noted that lower body fat is more sensitive to insulin suppression of lipolysis thus inhibiting the release of NEFA and consequent production of lipotoxic products such as ROS and oxysterols as well as ectopic fat deposition.

There are now established data on the differences seen in gestational weight and fat gain in lean and obese pregnancies. A detailed observation of the impact of maternal booking obesity on the distribution of this gestationally-accumulated fat is now required. Centrally accumulated fat could potentially explain the link between maternal obesity, lipotoxicity and adverse pregnancy outcome.

Within the context of this longitudinal study detailed anthropometric assessment of lean and OW/OB women during pregnancy was performed in order to test the hypothesis that the anatomical variation in accumulation of subcutaneous adipose tissue in lean and OW/OB women was different and that this had an impact on the metabolic response seen in pregnancy. The visceral fat compartment was not measured in this study. Ultrasound assessment was not available and there were ethical consideration in using other imaging modalities in pregnancy – such as the theoretical risk of deafness in the fetus if using magnetic resonance imaging.

3.2 Research Questions

1. Do lean and OW/OB women gain weight and fat mass during pregnancy, and is there a difference in the amount they gain?
2. Does this gain in weight and fat mass happen throughout pregnancy or is it concentrated at a particular gestational time period and is there any difference in pattern between the BMI groups?
3. What is the anatomical pattern of subcutaneous fat accumulation during pregnancy and is it different between lean and OW/OB pregnancies?
4. What contribution does each of the subcutaneous fat depots (upper body peripheral, abdominal and lower body) have to the total gestational fat mass?

3.3 Methods

Anthropometric methodology has been detailed in section 2.2 of the General Methods Chapter. All measurements were performed by the researcher.

3.4 Baseline Demographics

Study participants were recruited as described in section 2.1.2. As described in Chapter 2, recruitment to the obese group was more challenging than anticipated, and the BMI group was extended to include recruitment of primigravid and parous women with a BMI of greater than 27 kg/m^2. The mean BMI of the obese group was 31.5kg/m^2, and the range was 27.1-37.4kg/m^2. For the purposes of this thesis the BMI groups have been referred to as 'lean' and 'overweight/obese' (OW/OB).

For analysis of data, the total number of lean participants was 26 and the OW/OB cohort was 16 unless otherwise stated.

There were no smokers in either of the groups. The baseline demographics of the groups are illustrated in table 3.1. The OW/OB group had a higher baseline systolic and diastolic blood pressure than the lean women. In addition, the OW/OB group had a greater baseline weight, umbilical waist circumference, hip circumference, WHR, fat and fat free mass compared to the lean group.

Table 3.1 Baseline anthropometric characteristics for the lean and OW/OB groups. Statistical analysis was performed for each demographic variable listed below. Analysis was performed using the 2 sample t-test, for parity the assessment used *fishers exact test.

Variable	lean n=26 mean(SD) [range]	OW/OB n=16 mean(SD) [range]	p value
age (years)	30.1 (2.9) [22.0-36.0]	30.8 (4.7) [21.0-37.0]	0.61
parity n prim(%)	26 (100)	14 (88)	0.17*
gestation at visit 1 (weeks)	15.7 (1.1) [14.0-19.0]	16.0 (1.4) [14.0-19.0]	0.42
booking BMI (kg/m^2)	22.0 (1.7) [18.0-24.9]	31.5 (2.7) [27.1-37.4]	<0.0001
booking systolic BP (mmHg)	110 (10) [90-125]	119 (10) [103. -140]	0.011
booking diastolic BP (mmHg)	68 (6) [60-78]	74 (8) [64-89]	0.008
height (m)	1.66 (0.07) [1.56-1.81]	1.67 (0.06) [1.58-1.78]	0.56
weight (kg)	60.7 (7.2) [48.5-75.7]	87.0 (10.2) [72.0-103.3]	<0.0001
umbilical waist circumference (cm)	83.3 (5.6) [75.4-96.0]	103.5 (6.6) [91.7-115.1]	<0.0001
hip circumference (cm)	98.2 (5.3) [89.7-111.6]	113.8 (5.4) [107.0−122.0]	<0.0001
WHR	0.85 (0.04) [0.77-0.91]	0.91 (0.05) [0.83-1.03]	<0.0001
fat mass (kg)	16.4 (4.4) [11.1-26.3]	35.9 (7.3) [25.3-49.1]	<0.0001
% fat mass of total weight (%)	26.9 (5.4) [17.9-37.4]	41.0 (5.0) [34.5-50.5]	<0.0001

3.5 Gestational changes in lean and OW/OB anthropometry

3.5.1 Longitudinal changes in total body weight

In lean and OW/OB pregnancy there was a linear relationship between gestational age and total body weight. Both groups showed a significant increase in total body weight during pregnancy; average visit 1 total body weight 70.7kg (standard deviation [SD] 15.4) versus average visit 3 total weight 80.8kg (SD15.5), p time<0.0001. The OW/OB group was significantly heavier throughout gestation; lean average total body weight 65.8kg (SD8.2) versus OW/OB average total body weight 91.7kg (SD10.9), p type<0.0001. Total gestational weight gain was 10.4kg (SD 2.4) in lean pregnancy and 9.4kg in OW/OB pregnancy; this was not significantly different.

The pattern of increase in total body weight between the two groups was similar (p time x type=0.26).
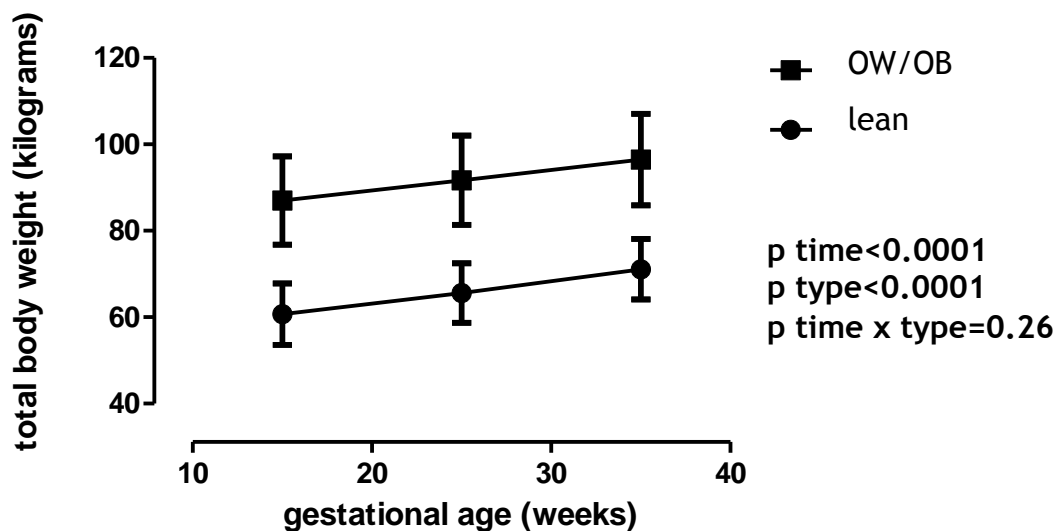
Figure 3.1 Total body weight at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean total body weight (raw data) and standard deviations at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

3.5.2 Longitudinal changes in total fat mass

Both groups showed a significant increase in fat mass during pregnancy; average visit 1 fat mass 23.9kg (SD 11.0) versus average visit 3 27.9kg (SD 11.3), p time<0.0001. The OW/OB group had significantly more body fat throughout gestation; lean average total fat mass 18.8kg (SD 5.2) versus OW/OB average total fat mass 37.9kg (SD 7.4) p type<0.0001. Total gestational fat mass gain was 4.3kg (SD 3.6) in lean pregnancy and 4.0kg (SD 4.0) in OW/OB pregnancy and this was not significantly different between the groups.

As with total body mass, there was a linear relationship between both lean and OW/OB fat mass and gestational age, and the pattern of change was similar (p time x type=0.80).
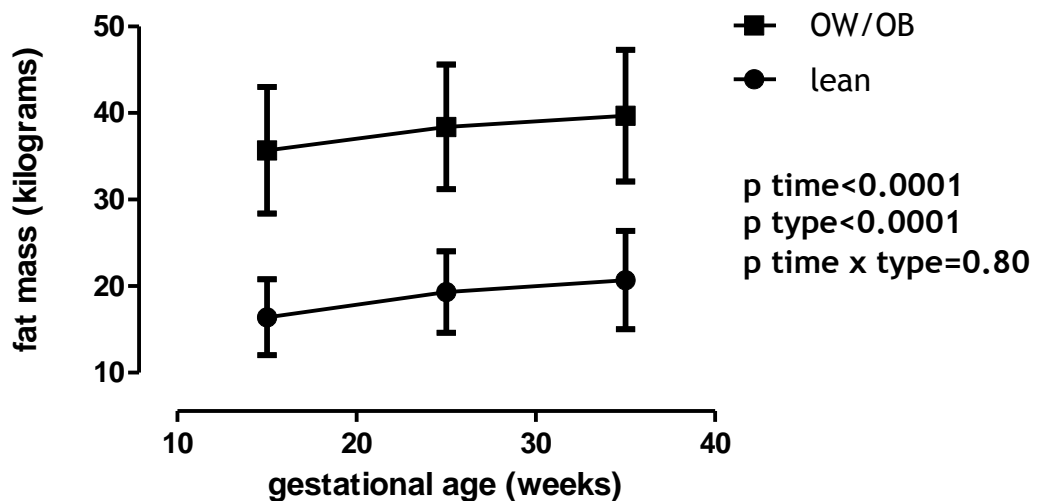
Figure 3.2 Fat mass at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean fat mass (raw data) and standard deviations at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

3.5.3 Longitudinal changes in circumference measurements

Circumference measurements were taken for minimum waist, umbilical waist, hip, upper thigh and midthigh. Methodology of the circumference measurements are detailed in section 2.2.3. During pregnancy lean and OW/OB women had a significant increase in all circumference measurements (data not shown). On reflection, the increases in circumference would not only have represented an increase in the subcutaneous adipose tissue component but also the increase in muscle mass, which has been demonstrated by other authors (Butte et al., 2003). The abdominal measurements would have represented not only maternal circumference increase but growth of the uterus and fetus. For this reason it was decided not to use the circumference measurements in the comparison of the lean and OW/OB cohort.

3.5.4 Longitudinal changes in individual skinfold measurements

Measurements were taken for biceps, triceps, subscapular, costal, suprailiac, midthigh and suprapatella skinfolds. All seven skinfolds measurements were taken at each study appointment. The methodology of the skinfolds assessment is detailed in section 2.2.4. Each individual skinfold measurement increased across pregnancy but there was no difference in the pattern of change observed between the groups (data not shown). The OW/OB groups had significantly larger individual skinfold measurements than lean women.

To assess changes in anatomical distribution of subcutaneous fat during pregnancy, skinfolds were assessed in anatomical groups. The skinfold measurements were assessed in three different ways: total skinfold represented the sum of all skinfolds (biceps, triceps, subscapular, costal, suprailiac, midthigh and suprapatella); upper body peripheral skinfold was the sum of biceps and triceps; upper body truncal skinfold comprised subscapular, costal and suprailiac skinfolds, abdominal skinfold included costal and suprailiac skinfolds; and lower body skinfold comprised midthigh and suprapatella skinfolds.

3.5.5 Longitudinal changes in total skinfold measurements

When total skinfold measurements were assessed there was a significant increase across gestation; mean visit 1 total skinfold measurement 137.6mm (SD 45.3), versus visit 3 mean value 163.3mm (SD 51.2), p time<0.0001. The OW/OB group had thicker total skinfolds than the lean group; total body skinfold 119.3mm (SD 22.8) versus OW/OB 202.9mm (SD 33.5), p type<0.0001.

The interaction model indicated that there was a difference in the pattern of increase in total skinfold measurements between the two groups (p time x type=0.013). In lean pregnancy total skinfold thickness was 107.7mm (SD 21.1), 121.0mm (SD 19.6) and 129.0mm (SD 23.0) at visit 1, 2 and 3 respectively. In OW/OB pregnancy total skinfolds were 186.1mm (SD 28.7), 203.6mm (SD 34.0) and 218.9mm (SD 31.0) at each gestational time point.
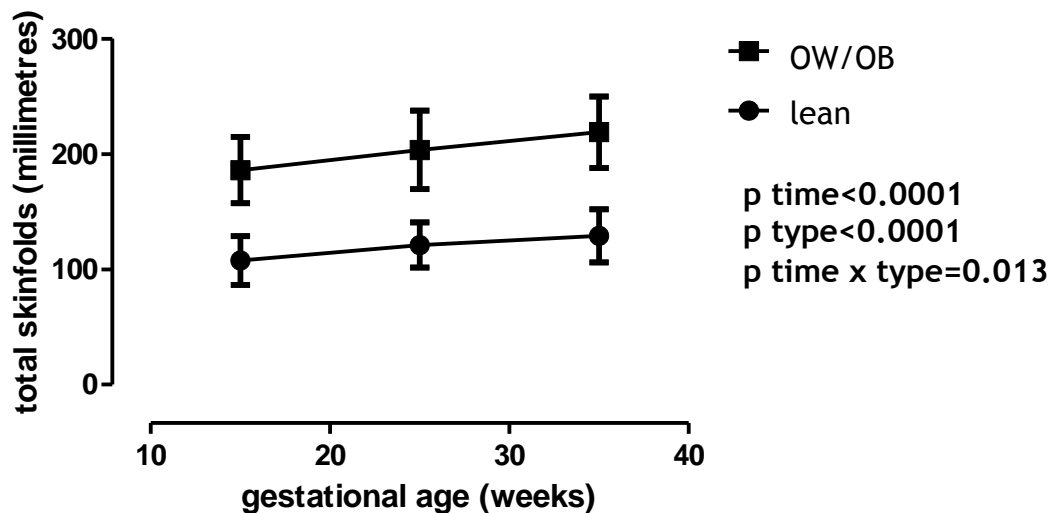
Figure 3.3 Total skinfold measurements at each gestational time point in lean and OW/OB pregnancy. Illustrated are the mean total skinfold measurements (raw data) and standard deviations at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

3.5.6 Longitudinal changes in grouped skinfold measurements

3.5.6.1 Upper body peripheral skinfold measurements

In both groups there was a significant increase in the upper body peripheral skinfold measurements across gestation; average visit 1 upper body peripheral skinfold 37.4mm (SD11.1) versus visit 3 41.0mm (SD 13.2) p time<0.0001. The OW/OB group had significantly thicker upper body peripheral skinfolds throughout pregnancy; lean average 32.1mm (SD 6.4) versus OW/OB 50.9mm (SD 9.1), p type<0.0001.

The interaction model suggested that there was a difference in the pattern of the increase upper body peripheral skinfold thickness (p time x type=0.047). In lean pregnancy, upper body peripheral skinfolds were 30.6mm (SD 6.3), 32.8mm (SD 6.1) and 33.0mm (SD 6.8) at each gestational time point. In OW/OB pregnancy the upper body peripheral skinfolds were 48.4mm (SD 7.8), 50.5mm (SD 8.6) and 54.0mm (SD 10.3) respectively.
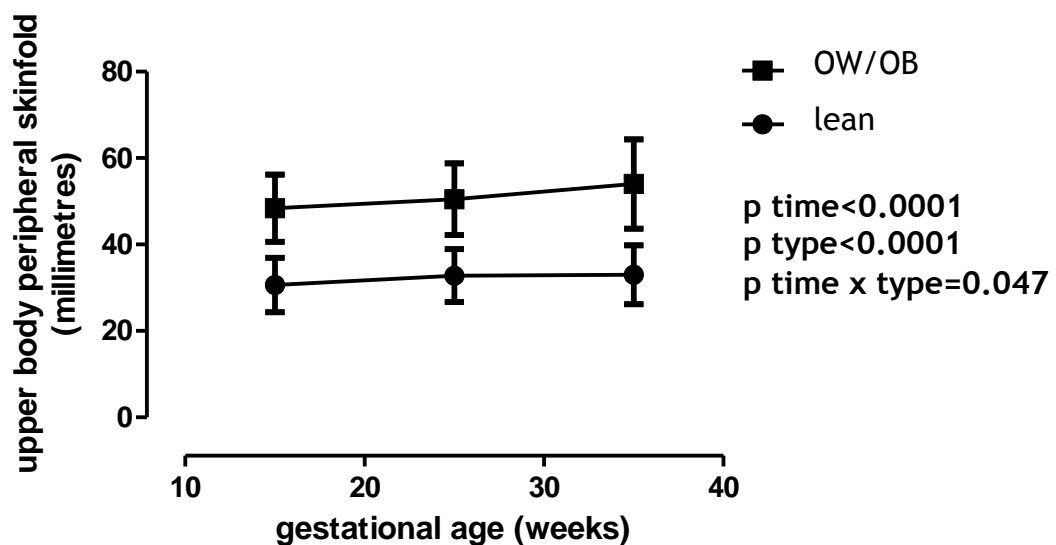
Figure 3.4 Upper body peripheral skinfold measurements at each gestational time point in lean and OW/OB pregnancy. Illustrated are the mean upper body peripheral skinfold measurements (raw data) and standard deviations at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

3.5.6.2 Upper body truncal skinfold measurements

Both groups showed a significant increase in the thickness of upper body truncal skinfold during pregnancy; average visit 1 upper body skinfold 54.9mm (SD 20.5) versus average visit 3 upper body skinfold thickness 66.2mm (SD 22.3), p time<0.0001. The OW/OB group had significantly thicker upper body truncal skinfolds throughout gestation; lean average upper body truncal skinfolds 47.5mm (SD 11.8) versus OW/OB average upper body truncal skinfold 83.0mm (SD 15.2), p type<0.0001.

The pattern of the increase in these skinfolds were similar in both groups, p time x type=0.27.
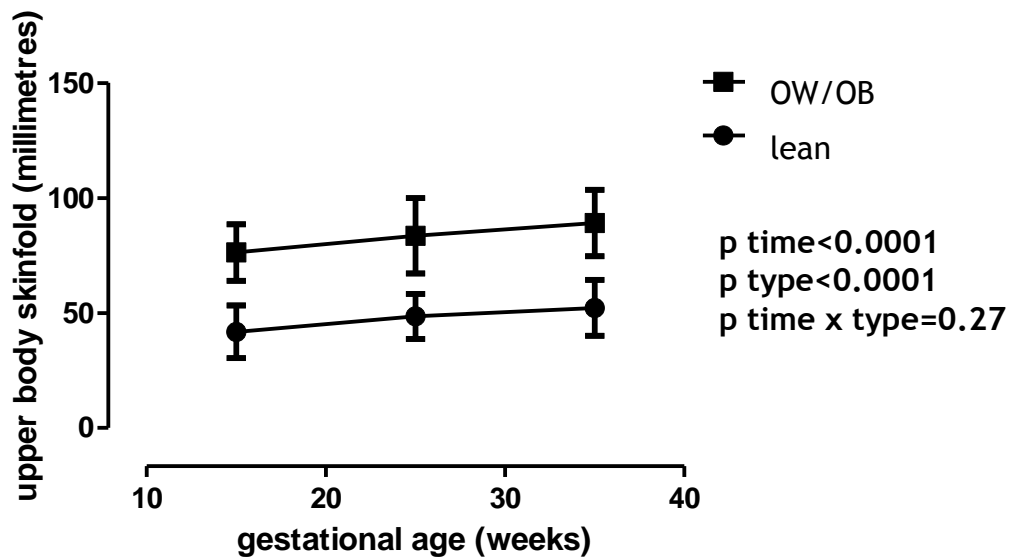
Figure 3.5 Upper body truncal skinfold measurements at each gestational time point in lean and OW/OB pregnancy. Illustrated are the mean upper body truncal skinfold measurements (raw data) and standard deviations at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

3.5.6.3 Abdominal skinfold measurements

Both groups showed a significant increase in the thickness of abdominal skinfold fat during pregnancy; average visit 1 abdominal skinfold 37.5mm (SD 14.1) versus average visit 3 abdominal skinfold thickness 43.6mm (SD 14.5), p time<0.0001. The OW/OB group had significantly thicker abdominal skinfolds throughout gestation; lean average abdominal skinfolds 31.5mm (SD 7.6) versus OW/OB average abdominal skinfold 54.0 (SD 9.6), p type<0.0001.

The pattern of increase in abdominal skinfolds were similar in both groups, p time x type=0.62.
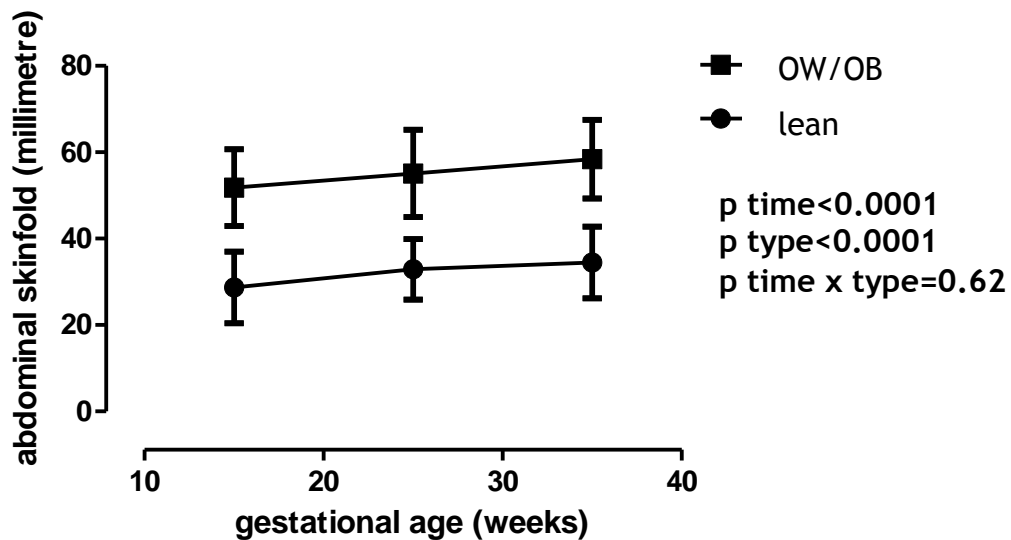
Figure 3.6 Abdominal skinfold measurements at each gestational time point in lean and OW/OB pregnancy. Illustrated are the mean abdominal skinfold measurements (raw data) and standard deviations at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

3.5.6.4 Lower body skinfold measurements

In both groups there was a significant increase in the lower body skinfold measurements across gestation; mean visit 1 lower body skinfold 45.3mm (SD 16.3) versus visit 3 mean value 56.1mm (SD 18.7), p<0.0001. OW/OB women had significantly thicker lower body skinfold than lean women; lean average lower skinfold measurement 36.9mm (SD 8.5) versus OW/OB 69.0mm (SD 14.4), p type<0.0001.

The pattern of increase in lower body skinfolds was different between the two groups (p time x type=0.005). At each gestational time point, in lean pregnancy lower body skinfolds were 35.3mm (SD 7.4), 39.7mm (SD 6.8) and 43.9mm (SD 9.2). In OW/OB pregnancy this was 61.5mm (SD 13.4), 69.6mm (SD 13.9) and 75.8mm (SD 12.8) respectively.
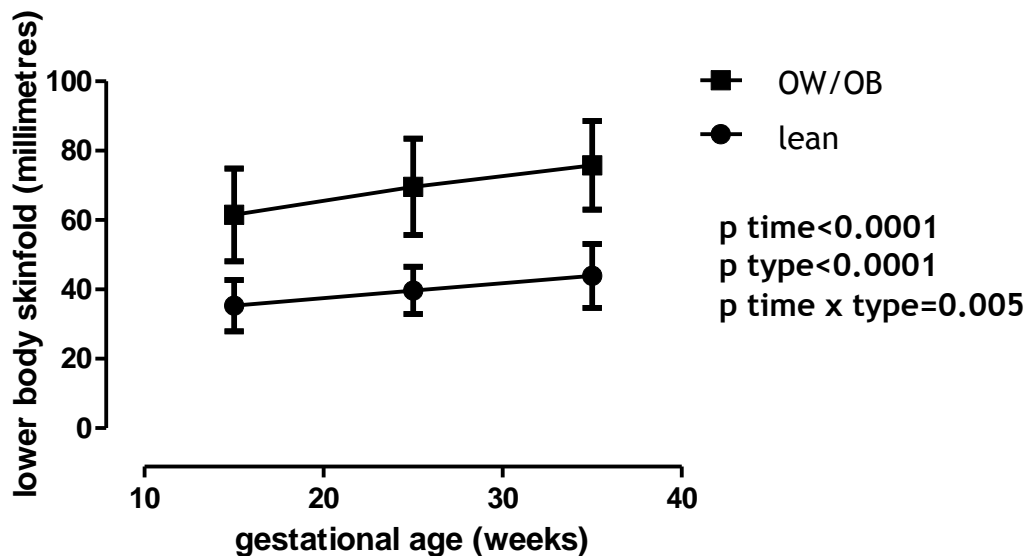
Figure 3.7 Lower body skinfold measurements at each gestational time point in lean and OW/OB pregnancy. Illustrated are the mean lower body skinfold measurements (raw data) and standard deviations at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

3.5.7 Summary of gestational anthropometry in lean and OW/OB pregnancy
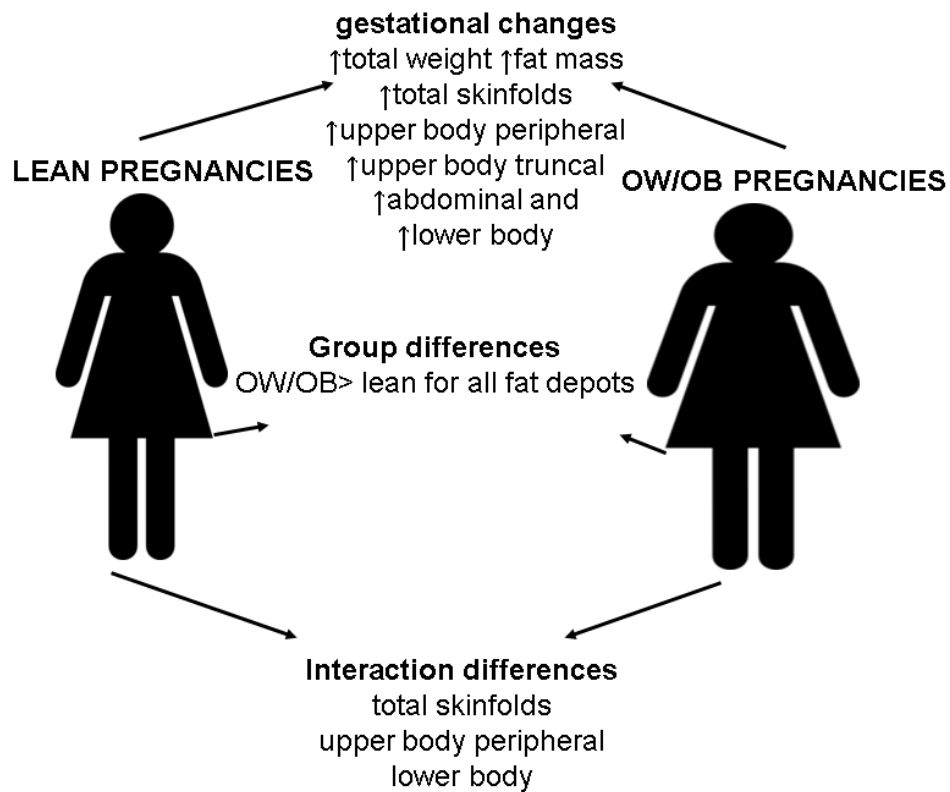
Figure 3.8 Summary of gestational anthropometry in lean and OW/OB pregnancy. This figure summarises the gestational differences (previously expressed as 'p time'), the differences between the groups ('p type') and the differences in the pattern of change ('p time x type') seen in lean and OW/OB pregnancy.

During pregnancy total body weight and fat mass increased. Each of the measured subcutaneous fat depots increases during pregnancy. OW/OB and lean women gain similar amounts of total body weight and fat mass during pregnancy. OW/OB women have thicker subcutaneous fat depots compared to lean women. From the above analysis there appears to be a difference in the pattern of fat accumulation in the total skinfolds, upper body peripheral and lower body skinfolds in lean and OW/OB women.

3.6 Gestational pattern of change in subcutaneous fat accumulation in lean and OW/OB pregnancies

From the above analysis, it would appear that there are difference in the pattern of change seen in total body skinfolds, upper body peripheral skinfolds and lower body skinfolds between the lean and OW/OB groups. Further analysis was performed to assess the pattern of change in early (V1-V2), late (V2-V3) and total gestation (V1-V3) for these grouped skinfolds between lean and OW/OB women.

3.6.1 Total body skinfolds

When total body skinfolds were assessed, as Figure 3.3 suggested, it was the OW/OB women gain more fat in total body skinfolds during pregnancy than lean women (p=0.046).OW/OB.

Table 3.2 Changes in total body skinfold measurements in lean and OW/OB pregnancy during different gestational time periods. Illustrated are the mean measurements (raw data) and standard deviations for the changes seen in total skinfolds during each gestational period. Statistical analysis performed using 2 sample t-test assessing each gestational time period: early (V1-V2), late (V2-V3) and total gestation (V1-V3), where V1=visit 1, V2=visit 2 and V3=visit 3.

anthropometric parameter	visit (gestation wk)	lean mean (SD) (n=26)	OW/OB mean (SD) (n=16)	lean versus OW/OB 2 sample t-test p value
change in total body skinfold (millimetres)	V1-V2	13.2 (12.5)	17.5 (17.8)	0.41
	V2-V3	8.1 (12.0)	15.3 (11.8)	0.065
	V1-V3	21.3 (15.2)	32.8 (18.5)	**0.046**

3.6.2 Upper body peripheral skinfolds

When upper body peripheral skinfolds were assessed, results indicated that OW/OB women gained more fat in upper body peripheral skinfolds in late pregnancy compared to the lean group but this was of borderline significance (p=0.053).

Table 3.3 Changes in upper body peripheral skinfold measurements in lean and OW/OB pregnancy during different gestational time periods. Illustrated are the mean measurements (raw data) and standard deviations for the changes seen in upper body peripheral skinfolds during each gestational period. Statistical analysis performed using 2 sample t-test assessing each gestational time period: early (V1-V2), late (V2-V3) and total gestation (V1-V3), where V1=visit 1, V2=visit 2 and V3=visit 3.

anthropometric parameter	visit (gestation wk)	lean mean (SD) (n=26)	OW/OB mean (SD) (n=16)	lean versus OW/OB 2 sample t-test p value
change in upper body peripheral skinfold (millimetres)	V1-V2	2.2 (4.3)	2.2 (4.6)	0.96
	V2-V3	0.1 (4.9)	3.5 (5.4)	**0.053**
	V1-V3	2.4 (5.2)	5.6 (6.9)	0.12

3.6.3 Lower body skinfolds

When lower body skinfolds were assessed as suggested in Figure 3.5, the OW/OB
women gain more fat in the lower body compartments during pregnancy than
lean women but this is not specific to any gestational time period, (p=0.034).

**Table 3.4 Changes in lower body skinfold measurements in lean and OW/OB pregnancy
during different gestational time periods.** Illustrated are the mean measurements (raw data) and
standard deviations for the changes seen in lower body skinfolds during each gestational period.
Statistical analysis performed using 2 sample t-test assessing each gestational time period: early
(V1-V2), late (V2-V3) and total gestation (V1-V3), where V1=visit 1, V2=visit 2 and V3=visit 3.

anthropometric parameter	visit (gestation wk)	lean mean (SD) (n=26)	OW/OB mean (SD) (n=16)	lean versus OW/OB 2 sample t-test p value
change in lower body skinfold (millimetres)	V1-V2	4.4 ((4.4)	8.1 (8.1)	0.10
	V2-V3	4.3 (4.9)	6.2 (7.1)	0.36
	V1-V3	8.6 (5.2)	14.3 (9.2)	**0.034**

3.7 Gestational pattern of subcutaneous adipose tissue accumulation in lean and OW/OB women

From the previous analysis, there was some evidence that lean and OW/OB women gained subcutaneous fat in different patterns during pregnancy. We wanted to further explore this further and assess the gestational timing of subcutaneous fat accumulation separately within the groups. In order to achieve this we performed repeated measures ANOVA with Tukey *post hoc* analysis.

3.7.1 Lean pregnancy

3.7.1.1 Total skinfolds

In lean pregnancy, repeated measures ANOVA and *post hoc* analysis indicated that there was a significant increase in total body skinfolds in early and late pregnancy and across total gestation (p<0.0001). This would suggest that lean women gain fat in the measured subcutaneous fat depots across gestation, but that there is no specific gestational time period when this gain is concentrated. This suggests that the trend observed in section 3.6.1 is not a true trend.
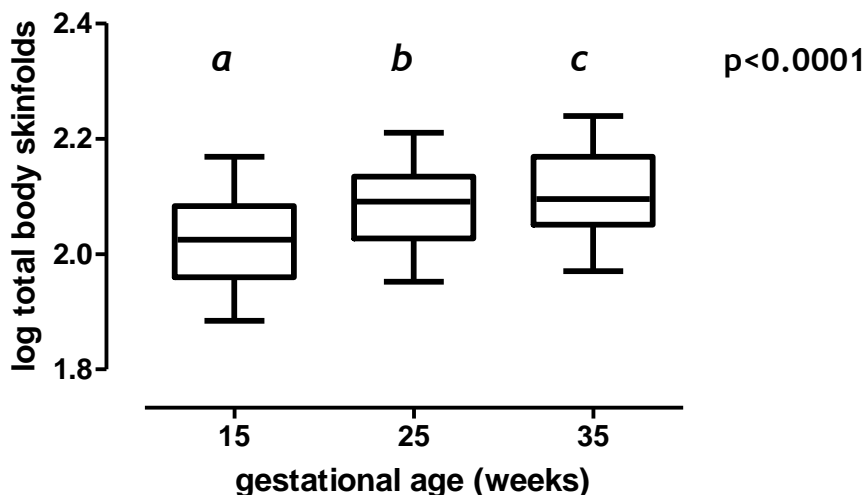
Figure 3.9 Temporal accumulation of adipose tissue in the total skinfolds in lean pregnancy. Gestational time points are illustrated on the x axis and logged values are quotes on the y axis. Analysis performed on logged values following Ryan-Joiner normality testing. Analysis performed using repeated measures ANOVA, significant result if p≤0.01. Data with different superscript levels [a,b,c] are significantly different from each other. There was a significant increase in total skinfolds measurements between 15 weeks and 25 weeks, and between 25 weeks and 35 weeks, and between 15 weeks and 35 weeks gestation in lean pregnancy.

3.7.1.2 Upper body peripheral skinfolds

In lean pregnancy, repeated measures ANOVA and *post hoc* analysis showed that lean women do gain fat in the upper body peripheral skinfolds during pregnancy. Post hoc analysis indicated that this increase was significant in early pregnancy but not late pregnancy. This suggests that lean women gain fat in this depot in early pregnancy but not late pregnancy, confirming the trend seen in section 3.6.2.
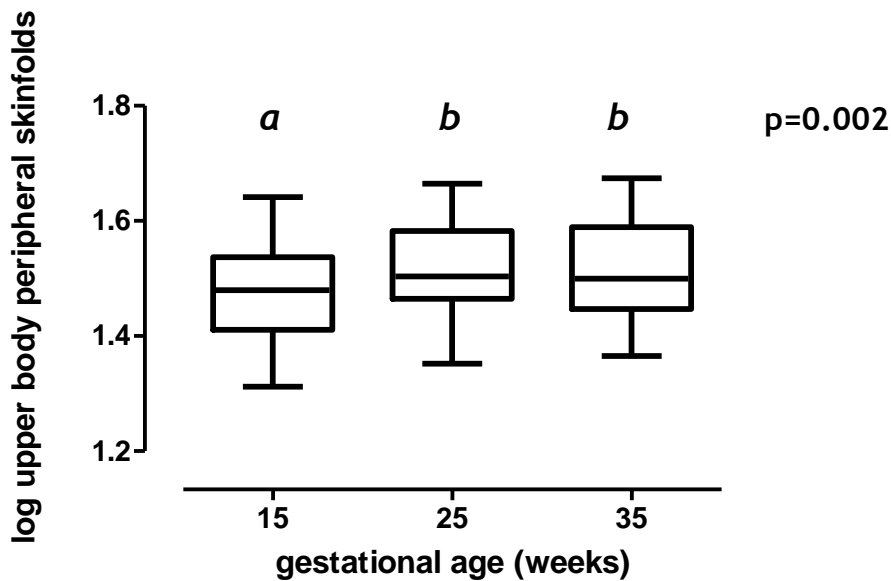
Figure 3.10 Temporal accumulation of adipose tissue in the upper body peripheral skinfolds in lean pregnancy. Gestational time points are illustrated on the x axis and logged values are quotes on the y axis. Analysis performed on logged values following Ryan-Joiner normality testing. Analysis performed using repeated measures ANOVA, significant result if $p \leq 0.01$. Data with different superscript levels [a,b] are significantly different from each other. There was a significant increase in upper body peripheral skinfolds measurements between 15 weeks and 25 weeks and between 15 weeks and 35 weeks gestation, but not between 25 weeks and 35 weeks in lean pregnancy.

3.7.1.3 Lower body skinfolds

In lean pregnancy, repeated measured ANOVA and *post hoc* analysis indicated
that there was a significant increase in lower body skinfolds in early and late
pregnancy and across total gestation (p<0.0001). This would indicate that lean
women accumulate a significant amount of adipose tissue in the lower body
compartment across gestation rather than at a specific gestational time point,
confirming findings in section 3.6.3.
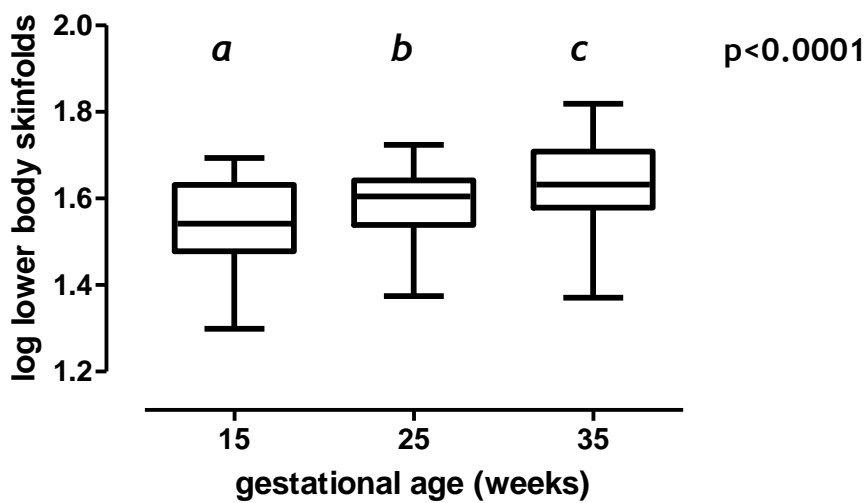
**Figure 3.11 Temporal accumulation of adipose tissue in the lower body skinfolds in lean
pregnancy.** Gestational time points are illustrated on the x axis and logged values are quotes on
the y axis. Analysis performed on logged values following Ryan-Joiner normality testing. Analysis
performed using repeated measures ANOVA, significant result if p≤0.01. Data with different
superscript levels [a,b,c] are significantly different from each other. There was a significant increase in
lower body skinfolds measurements between 15 weeks and 25 weeks, and between 25 weeks and
35 weeks, and between 15 weeks and 35 weeks gestation in lean pregnancy.

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3.7.2 OW/OB pregnancy

3.7.2.1 Total skinfolds

In OW/OB pregnancy, repeated measures ANOVA and *post hoc* analysis indicated that there was a significant increase in total body skinfolds in early and late pregnancy and across total gestation (p<0.0001). This would suggest that OW/OB women gain fat in the measured subcutaneous fat depots across gestation, but that there is no specific gestational time period when this gain is concentrated, confirming findings in section 3.6.1.
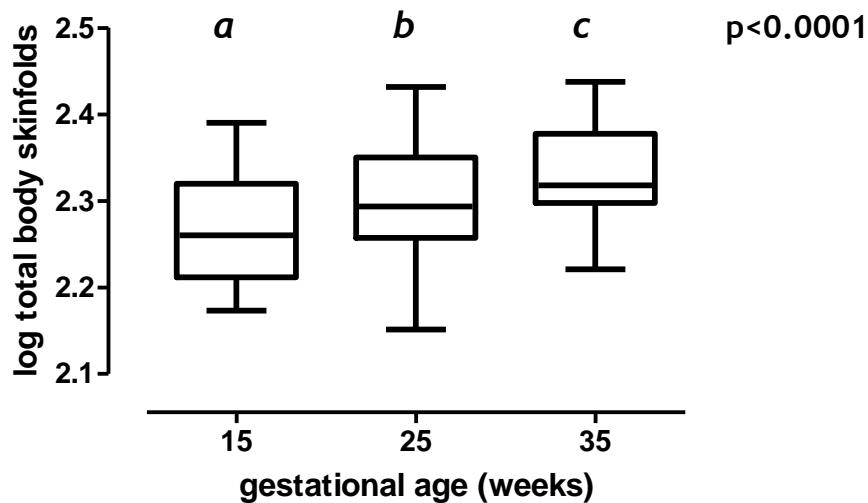
Figure 3.12 Temporal accumulation of adipose tissue in the total skinfolds in OW/OB pregnancy. Gestational time points are illustrated on the x axis and logged values are quotes on the y axis. Analysis performed on logged values following Ryan-Joiner normality testing. Analysis performed using repeated measures ANOVA, significant result if p≤0.01. Data with different superscript levels [a,b,c] are significantly different from each other. There was a significant increase in total skinfolds measurements between 15 weeks and 25 weeks, and between 25 weeks and 35 weeks, and between 15 weeks and 35 weeks gestation in OW/OB pregnancy.

3.7.2.2 Upper body peripheral skinfolds

In OW/OB pregnancy, repeated measures ANOVA and *post hoc* analysis indicated that there was no significant increase in upper body peripheral skinfold thickness during early or late gestation but that the total gestational increase in this fat depot was significant (p=0.002). Therefore, OW/OB women do gain fat in the depot but it is continuous across pregnancy not concentrated in early or late pregnancy, which confirms findings in section 3.6.2.
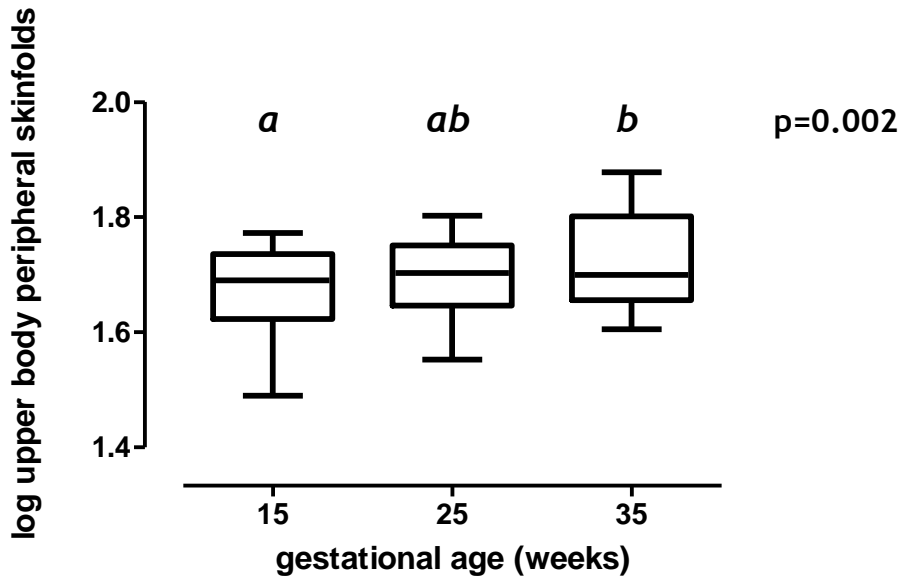
Figure 3.13 Temporal accumulation of adipose tissue in the upper body peripheral skinfolds in OW/OB pregnancy. Gestational time points are illustrated on the x axis and logged values are quotes on the y axis. Analysis performed on logged values following Ryan-Joiner normality testing. Analysis performed using repeated measures ANOVA, significant result if p≤0.01. Data with different superscript levels [a,b,] are significantly different from each other. There was a significant increase in upper body peripheral skinfolds measurements between 15 weeks and 35 weeks, but not between 15 weeks and 25 weeks, and between 25 weeks and 35 weeks gestation in OW/OB pregnancy.

3.7.2.3 Lower body skinfolds

In OW/OB pregnancy, repeated measures ANOVA and *post hoc* analysis indicated that there was a significant increase in lower body skinfolds in early and late pregnancy and across total gestation (p<0.0001). This would indicate that OW/OB women accumulate a significant amount of adipose tissue in the lower body compartment across gestation rather than at a specific gestational time point, which confirms findings in section 3.6.3.
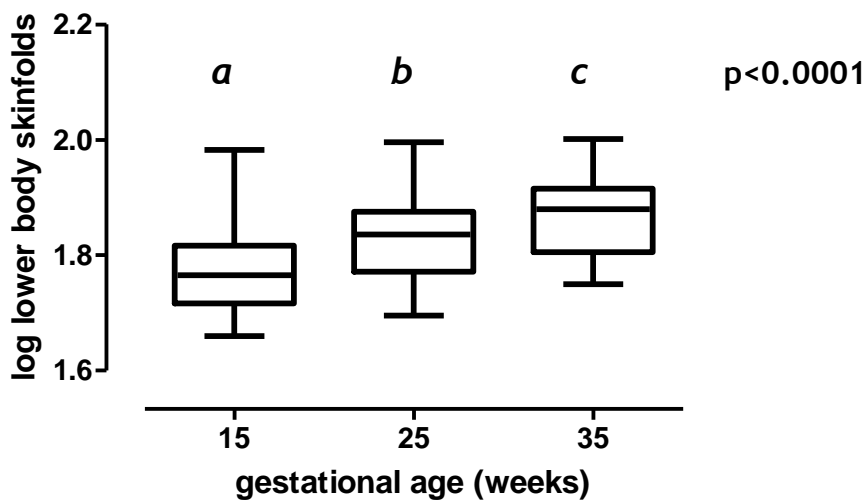
Figure 3.14 Temporal accumulation of adipose tissue in the lower body skinfolds in OW/OB pregnancy. Gestational time points are illustrated on the x axis and logged values are quotes on the y axis. Analysis performed on logged values following Ryan-Joiner normality testing. Analysis performed using repeated measures ANOVA, significant result if p≤0.01. Data with different superscript levels [a,b,c] are significantly different from each other. There was a significant increase in lower body skinfolds measurements between 15 weeks and 25 weeks, and between 25 weeks and 35 weeks, and between 15 weeks and 35 weeks gestation in OW/OB pregnancy.

3.8 The contribution of subcutaneous fat depots to fat mass in pregnancy in lean and OW/OB women

Having assessed the different patterns of fat accumulation between groups, further analysis was performed to assess what contribution all anatomical sites subcutaneous adipose tissue (upper body peripheral, abdominal and lower body) had on the total gestational fat mass gain. This was performed using the Pearson's correlation for univariate associations and General Linear Model for multivariate analysis.

3.8.1 Lean pregnancy

Univariate analysis was performed using Pearson correlation to assess the relationship between the absolute change in grouped skinfolds, total skinfolds and fat mass. The change in upper body peripheral and abdominal but not lower body skinfold thickness significantly correlated with the change seen in fat mass (table 3.5).

Table 3.5 Univariate analysis of the association between gestational change in anthropometric fat depots and the gestational change seen in fat mass in lean pregnancy. Analysis was performed using Pearson's correlation, significant result if p<0.01. Total body skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as LBS.

	TBS (mm)	UPBS (mm)	ABS (mm)	LBS (mm)
V1-V3 fat mass (kg)				
Pearson correlation	0.725	0.508	0.581	0.437
P value	<0.0001	0.009	0.002	0.029

Multivariate analysis using the General Linear Model is illustrated in Table 3.6.

This indicated that there was no anatomical fat depot which was independently

associated with the gain seen in fat mass during gestation in lean pregnancy.

Table 3.6 Multivariate analysis of the contribution of gestational change in anthropometric fat depots to the gestational change seen in fat mass in lean pregnancy. Assessment performed using the General Linear Model, significant result if p<0.01. Upper body peripheral skinfolds as UPBS, abdominal skinfolds as ABS and lower body skinfolds as LBS. The contribution of each component expressed as a percentage (%).

	anthropometric parameter	p value	% contribution
V1-V3 fat mass	UBPS	0.45	0.02%
	ABS	0.11	7.6%
	LBS	0.13	6.6%

3.8.2 OW/OB pregnancy

Univariate analysis was performed using Pearson correlation to assess the relationship between the absolute change in grouped skinfolds, total skinfolds and fat mass (table 3.7). The changes in the grouped anatomical skinfolds did not correlate with the changes seen in fat mass in OW/OB pregnancy. The change in total skinfolds and the change seen in fat mass were of borderline significance.

Table 3.7 Univariate analysis of the association between gestational change in anthropometric fat depots and the gestational change seen in fat mass in OW/OB pregnancy. Analysis was performed using Pearson's correlation, significant result if p<0.01. Total body skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as LBS.

	TBS (mm)	UPBS (mm)	ABS (mm)	LBS (mm)
V1-V3 fat mass (kg)				
Pearson correlation	0.585	0.444	0.310	0.441
P value	0.017	0.085	0.24	0.088

Multivariate analysis using the General Linear Model is illustrated in Table 3.8. This indicated that in OW/OB pregnancy the changes in grouped subcutaneous fat depots contributed little to the overall change seen in fat mass. This may indicate that subcutaneous fat is not the main site of fat accumulation during OW/OB pregnancy.

Table 3.8 Multivariate analysis of the contribution of gestational change in anthropometric fat depots to the gestational change seen in fat mass in OW/OB pregnancy. Assessment performed using the General Linear Model, significant result if p<0.01 Upper body peripheral skinfolds as UPBS, abdominal skinfolds as ABS and lower body skinfolds as LBS. The contribution of each component expressed as a percentage (%).

	anthropometric parameter	p value	% contribution
V1-V3 fat mass	UBPS	0.17	12.2%
	ABS	0.55	2.1%
	LBS	0.47	3.2%

3.9 Discussion

Gestation is associated with a significant increase in total body weight and fat mass in both lean and OW/OB pregnancies. Our results (average increase in total body weight 10.1kg (SD 3.5) and fat mass 4.2kg (3.7) for entire cohort) were consistent with published data. Soltani (Soltani and Fraser, 2000) demonstrated significant increases in total body weight and fat mass accumulation from 13-36 weeks gestation of 10.9kg [SD 4.7]kg (p<0.001) and 4.6 [SD 3.3]kg (p<0.001) respectively. The OW/OB group remained significantly heavier in terms of both total body weight and fat mass throughout pregnancy. Ehrenberg et al (Ehrenberg et al., 2003) showed that lean and OW/OB women gained similar amounts in total body weight (lean 12.3kg versus OW/OB 13kg, p=0.61) and fat mass (lean 4.7kg versus 4.2kg (p=0.58) respectively. When compared, our findings showed that lean and OW/OB women also gained similar amounts of total body weight (lean gain 9.5 [4.8]kg versus OW/OB gain 10.4 SD [2.4]kg p=0.48) and fat mass during pregnancy (lean gain 4.3 [3.6]kg versus OW/OB gain 4.0 [4.0]kg, p=0.80). Thus the data shown here are consistent with the literature.

Compared to the IOM guidelines for GWG, on average the lean group gained less (10.4kg [SD 2.4]) than the minimum recommended GWG of 11.5kg (BMI 18.5-24.9kg/m^2 total recommended GWG 11.5-16kg). When examined further, 19 lean women gained less than the minimum recommended 11.5kg and only 7 lean women gained between the recommended GWG (11.5-16kg). No lean woman gained more than the maximum recommended 16kg. On average the OW/OB group gained more (9.5kg [4.8]) than the maximum of 9kg (BMI$\geq$30kg/m^2 total recommended GWG 5-9kg). Further analysis indicated that 50% of the OW/OB group (n=8) gained more than the maximum 9kg recommended (this included three women with overweight booking BMI of less than 30kg/m^2), 6 women gained between the recommended 5-9kg and 2 women (both booking BMI>35kg/m^2) gained less than the minimum recommended GWG of 5kg. This may illustrate that in a healthy lean Scottish cohort the IOM GWG recommendations are too high and not reflective of this population's behaviour and lifestyle. In the OW/OB population this finding may reflect that the public

health message regarding excessive gestational weight gain is not being acknowledged or in fact being disseminated to this pregnant population. All measured skinfolds increased significantly during gestation indicating that both lean and OW/OB women gain subcutaneous fat in the measured depots during pregnancy. OW/OB women had significantly larger subcutaneous fat depots at all measured gestational time points. Therefore it is likely OW/OB women enter pregnancy with larger subcutaneous fat depots than lean women. The increase in all depots during pregnancy suggests that the measured subcutaneous fat depots were important sites of fat accumulation during pregnancy for both lean and OW/OB women. In lean women, a smaller increase in subcutaneous fat skinfold reflects a larger proportional increase in fat mass compared to the OW/OB counterparts. Taggart found that obese women had smaller relative gains in total skinfolds than lean women during pregnancy (Taggart et al., 1967). Our data does not suggest that OW/OB women gain less in the subcutaneous depot, and *post hoc* analysis of the grouped skinfolds suggests they gain more subcutaneous fat in the lower depots compared to the lean women (14.3 [SD 9.2]mm versus 8.6 [SD5.2]mm respectively (p=0.034).

The contribution of the subcutaneous fat depots to gestational fat mass gained was assessed. In univariate analysis, lean pregnancy fat mass was significantly associated with upper body peripheral skinfolds, abdominal skinfolds and total body skinfolds. However, in multivariate analysis, each anatomical fat depot group did not explain the variation seen in the change in gestational fat seen in lean pregnancy. This may reflect that in lean women subcutaneous fat in general is an important site of fat storage in pregnancy. In OW/OB pregnancy, no specific subcutaneous skinfolds depots or the total skinfolds contributed significantly to gestational fat mass. This finding may suggest that OW/OB women are gaining fat preferentially in other depots such as visceral compartments during pregnancy.

However, as the measured skinfolds are increasing during pregnancy then they must contribute to the increase seen in gestational fat mass to a greater or lesser extent. Therefore this raised the question as it how accurately the methods employed were in measuring fat mass in this study. In terms of skinfold thickness, this seven point method was validated against underwater weighing

by Presley et al (Presley et al., 2000). Total body weight and fat mass was measured by air displacement plethysmography which, because it is a two compartment method, cannot account for the changes in either visceral and subcutaneous depot sites nor the changes which are occurring in the fetal compartment. In addition, assessing the changes in fat accumulation at three gestational points may not be adequate enough, for instance measurements prepregnancy and before 12 weeks of pregnancy may be valuable. This may be a reason why not significant associations were reported in this analysis.

The current analysis, indicated that although the timing of the accumulation of total fat was similar there was a difference between lean and OW/OB women in the anatomical location of fat at different gestations indicated by significant interaction terms in the mixed model.

The pattern of change in the accumulation of fat in the upper body peripheral depots was different between lean and OW/OB women (p=0.047). Post hoc analysis suggested that OW/OB women continued to gain fat in this depot in late pregnancy but the lean did not. This finding was supported by the repeated measures ANOVA analysis which indicated that lean women gained adipose tissue in this depot in early pregnancy but not in late pregnancy. Interestingly, the finding in the lean group of no increase in the upper body peripheral skinfold thickness during late pregnancy, is supportive of one of the outcomes of Taggart's early work which showed no increase in the triceps skinfold in late pregnancy.

For the lower body depots the pattern of accumulation of adipose tissue was the same in the lean and OW/OB groups as both groups continued to accumulate fat in this depot throughout gestation. Therefore, this finding does not support our hypothesis that lean women gain fat preferentially in lower body fat stores.

The lack of correlations between accumulated fat mass and the change in total skinfold in OW/OB women suggests that OW/OB women may accumulate fat in other anatomical sites. In lean women, although there was a correlation between abdominal depots, upper body peripheral depots and fat mass, neither variable explained the increase in gestational fat mass in multivariate analysis.

Therefore the significant correlation could be the result of colinearity between the depots. One subcutaneous fat depot which was not measured directly was the buttock. An indirect assessment of this depot, the hip and upper thigh circumference, was performed. This indicated that there were gestational increases in these depots, but that these were not significantly different from lean women.

Another variable which has not been accounted for in this cohort is the impact of visceral fat accumulation on total body weight and fat mass during pregnancy. Visceral fat is comprised of a variety of potentially functional different depots including omental fat, prepertitoneal fat and adipose tissue attached directly to the organs. Kinoshita (Kinoshita and Itoh, 2006) performed ultrasonographic assessment of preperitoneal fat and showed that there was an increase in this fat depot during pregnancy suggesting that a proportion of the gestational fat mass is gained in the intra-abdominal compartment. Sohlstrom and Forsum (Sohlstrom and Forsum, 1995) used MRI to assess fat accumulation and distribution in a non-obese cohort (prepregnancy BMI 23.3 (SD 3.2) range 17.3-29.1, n=25). They showed that the majority of adipose tissue was accrued in the subcutaneous compartment, and that 68% of this was in the trunk. We did not directly measure lower back adipose tissue skinfolds and thus our assessment of truncal adiposity may be incomplete. Our lack of ultrasound or MRI assessment of visceral fat highlights the difficulty in performing a full anthropometric assessment and suggests that further investigation needs to involve assessment of the visceral fat compartment in a manner which is acceptable to participants and researchers alike.

Another explanation for the lack of association between fat mass and subcutaneous fat depots in the OW/OB group is that during pregnancy although OW/OB women may gain fat in both subcutaneous and visceral sites they store proportionately more fat in the visceral depots as their subcutaneous sites are already replete. If we assume no colinearity then in lean pregnancy, women are able to keep accumulating fat in the subcutaneous depots throughout pregnancy therefore less accretion is seen in the visceral compartment.

The differences in the pattern of subcutaneous fat accumulation seen in the groups may reflect the higher rates of insulin resistance seen in OW/OB pregnancy compared to lean pregnancy in the later stages of pregnancy.

The observation that lean women stop accumulating fat in the upper body peripheral skinfolds between 25 and 35 weeks, whereas OW/OB women do not may suggest that lean women are able to switch easily from a lipogenic to a lipolytic profile in later gestation whereas the OW/OB women remain lipogenic as well as lipolytic i.e. have higher NEFA turnover. If this is the case then this may indicate a lack of metabolic flexibility within the adipose tissue in OW/OB pregnancy, increased NEFA flux and consequently an increase in metabolic risk factors such as hyperinsulinaemia, hypertriglyerideamia and diastolic blood pressure (Bartha et al., 2007).

The strength of the present study was the systematic methodology of the anthropometric measurements. All were performed by the same researcher who was trained in ISAK anthropometric techniques and recorded by the assisting research nurse in order to reduce bias. All study appointments were performed at the same time of day and all study participants attended following an overnight fast. The limitations of this analysis is that we did not measure the visceral compartment and therefore cannot confirm our above discussion points regarding the ratio of visceral to subcutaneous storage of adipose tissue in lean and OW/OB pregnancies. As discussed above, three anatomical groups were used for subcutaneous fat accumulation (upper body peripheral, abdominal and lower body). These groups did not include direct buttock measurements and therefore we may not have collected a data set which is truly representative of the changes in subcutaneous fat accumulation during pregnancy. In addition, due to difficulties recruiting women with very high BMI's our OW/OB group included overweight women as well which will not represent the fat accumulation and distribution of very obese women. Such results may differ from our observations.

In conclusion, as illustrated in figure 3.15, there are certain similarities in anthropometric gestational changes seen in lean and OW/OB pregnancies. Both gain total body weight and fat mass during pregnancy. Lean and OW/OB women gain similar amounts of both total body weight and fat mass during pregnancy.

However, lean women appear to accumulate fat in upper body peripheral storage sites in early pregnancy whereas OW/OB women gain fat globally throughout gestation. Therefore there is a difference in the lipolytic activity of this depot which may relate to metabolic flexibility in pregnancy. There was no significant contribution of the increase in the measured subcutaneous depots to the variation seen in gestational increase in fat mass in either lean or OW/OB pregnancies. This suggests that in both lean and OW/OB pregnancy other sites of fat storage which were not measured were important. Further analysis involving measurement of the visceral compartment would be helpful to explore the importance of this depot in gestational changes in body fat in lean and OW/OB pregnancies.

LEAN PREGNANCIES

- Total body weight, fat mass and increase in pregnancy
- Upper body peripheral depots increase in early but not late pregnancy
- Lower and total skinfolds increase throughout pregnancy

OVERWEIGHT/OBESE PREGNANCIES

- Total body weight, fat mass and increase in pregnancy
- Global increase in all skinfolds thoughout pregnancy
- No gestational time period difference

Figure 3.15 Summary of anthropometric changes seen in healthy lean and OW/OB pregnancy. Summary of the similarities and differences seen in the anthropometric variables measured in healthy lean and OW/OB pregnancies and the impact of these findings on fat mass.

Chapter 4 - Energy metabolism during pregnancy and its relationship with mass and distribution of subcutaneous adipose tissue in lean and OW/OB pregnancy

4.1 Introduction

The energy demands on the mother during pregnancy can be categorised into three components. These are the conceptus (including the fetus, placenta, amniotic fluid and the expansion of blood volume) fat deposition in the mother and finally the energy spent on maintaining this new tissue (Prentice and Goldberg, 2000). It has been recognised that during pregnancy the basal metabolic rate increases and it has been observed to be higher in obese women compared to non-obese controls (Bronstein et al., 1996).

In the past, nutrition and diet in pregnancy were often overlooked as it was assumed that the expectant mother would optimise her health and wellbeing for the good of her unborn child. In the context of the increasing rates of maternal obesity, women are entering pregnancy less healthy and more at risk from a range of serious maternal and fetal complications. Dietary intake during pregnancy should aim to provide enough energy, macro and micronutrients for both the metabolic needs of the mother and fetus without excessive maternal weight gain or fetal growth.

General dietary advice, in the "ready steady baby!" publication (NHS, 2012b), is provided during the course of antenatal care but this does not specifically address the obese population. Recent guidance on the management of obese pregnancies (RCOG, 2010) highlighted the need for advising women about the importance of a healthy diet and exercise during pregnancy in order to avoid excessive weight gain and gestational diabetes. However, this recommendation was within the context of general dietary advice. The only specific nutritional guidance for obese woman was increased supplementation of folic acid and vitamin D (5mg and 10ug daily respectively), and the guidance did not discuss other aspects including fat and complex carbohydrate consumption.
General levels of physical activity among adults have declined dramatically as a result of changes in work practices and technological advances. Previous

recommendations for physical activity during pregnancy were not based on scientific evidence but more on cultural and traditional norms. Current recommendations from the Royal College of Obstetricians & Gynaecologists suggest that exercise activities must balance the benefits and risks to both the mother and fetus (RCOG, 2006). Guidance for exercise intensity suggests a maximum heart rate of 60-70% for women who were sedentary prior to pregnancy. Recommendations from the American College of Obstetricians & Gynecologists suggests that pregnant women may safely engage in $\geq$ 30 minutes of moderate physical activity on most, if not all days of the week (Artal and O'Toole, 2003).

In the current literature, little is known about the impact of diet and exercise on gestational weight gain and their relationship with fat mass and distribution of adipose tissue during pregnancy. Given the current obesity epidemic seen in the obstetric population it is now even more important that these relationships are explored in order to optimise the management of obese pregnancies.

This chapter examines the relationships between energy metabolism components, adiposity and fat distribution during pregnancy.

4.2 Research Questions

1. Do measures of energy metabolism (basal metabolic rate, substrate utilisation and non-protein respiratory exchange ratio) change during pregnancy and do they differ between lean and OW/OB pregnancy?
2. Do physical activity levels change during pregnancy and do they differ between lean and OW/OB women?
3. Does dietary intake change during pregnancy and do they differ between lean and OW/OB pregnancy?
4. If there are significant differences in the above aspects of energy metabolism between lean and OW/OB pregnancy, are these factors related to gestational fat mass accumulation and/or anatomical fat deposition during pregnancy?

4.3 Methods

Basal metabolic rate and substrate utilisation, physical activity and dietary intake assessment methodologies have been detailed in section 2.3 of the General Methods Chapter. All measurements were recorded by the researcher.

Data for macronutrient intake is quoted in kilojoules per day, which is the same unit used for basal metabolic rate and substrate utilisation. The conversion factor for kilojoules to kilocalories is 1kilojoule=0.24 kilocalories. (www.unit-conversion.info).

4.4 Gestational changes in basal metabolic rate and substrate utilisation

4.4.1 Basal metabolic rate

Basal metabolic rate (BMR) was assessed across time and between groups (figure 4.1). Both groups show a significant increase in BMR during pregnancy (p time<0.0001); V1 average 5530 kJ/day (standard error of the mean [SEM] 142) versus V3 average 6080 (SEM 154) kJ/day. The OW/OB group had a significantly higher BMR at all gestational time points (p type<0.0001); the mean lean BMR was 5341 kJ/day (SEM 85) versus OW/OB 6492kJ/day (SEM 127) across gestation.

The pattern of increase in BMR between the two groups was similar (p time x type=0.15).
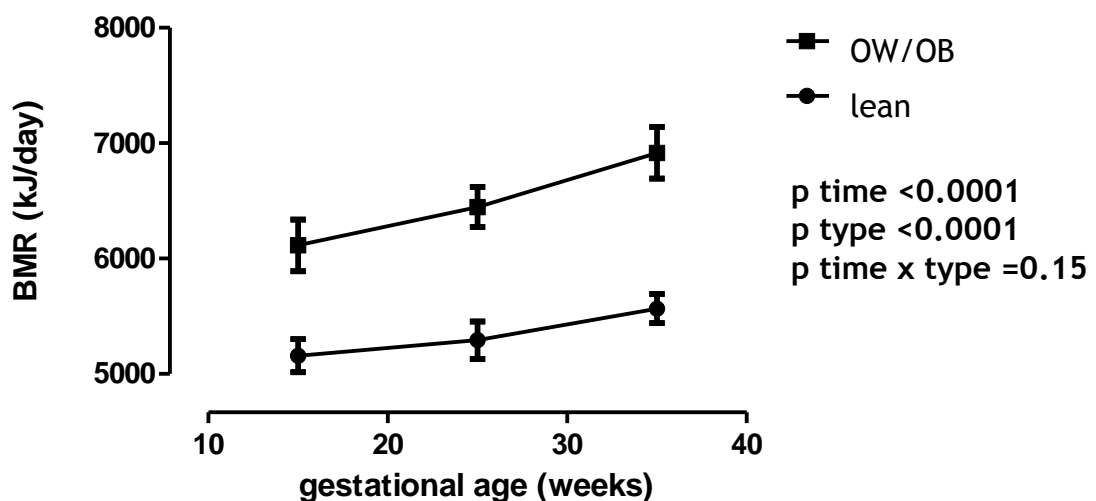
Figure 4.1 Basal metabolic rate at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean basal metabolic rate (BMR) (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.4.2 Basal metabolic rate per kilogram body weight (BMR/kg)

In order to ascertain whether the higher BMR observed in the OW/OB group was reflective of a true difference between the groups or whether this was simply a result of differences in maternal body mass, the BMR/kg body weight was compared between the groups using the linear mixed model.

Across gestation there was no significant change in BMR/kg, p time=0.12. Lean women had a significantly higher BMR per kilogram of weight than OW/OB women; average lean 81.7kJ/Day/kg (SEM 1.3) versus OW/OB 70.9kJ/day/kg (SEM 0.8).

The pattern of change in the BMR per kilogram was not significantly different between the lean and OW/OB pregnancies.
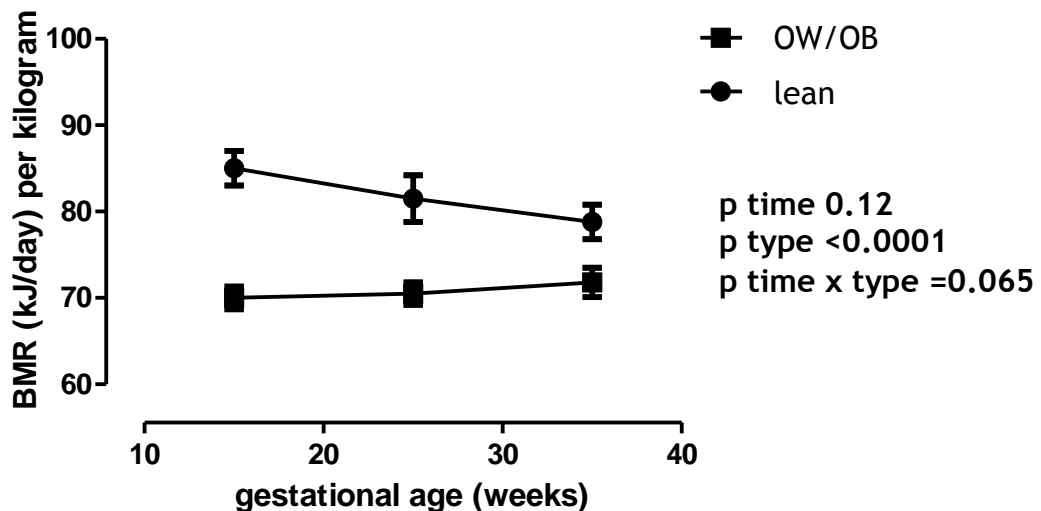
Figure 4.2 Basal metabolic per kilogram at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean basal metabolic rate per kilogram (BMR/kg)) (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.4.3 Non-protein respiratory exchange ratio

Non-protein respiratory energy ratio (NPRER) expresses the ratio of carbohydrate oxidation to fat oxidation by calculating the rate of VO_2 consumption to CO_2 production (see Chapter 2, section 2.3.1). The greater the ratio (i.e. the closer to 1.00 the value is), the higher the proportion of carbohydrates as opposed to fats being utilised as any energy source.

NPRER was assessed across time and between groups (figure 4.3). There was no effect of gestation on NPRER (p time=0.72), There was a significant difference between the two groups (p type<0.0001); average lean NPRER 0.87 (SEM 0.02) versus OW/OB average NPRER 0.81 (SEM 0.08). The pattern of change in NPRER was not significantly different between the groups (p time x type=0.36).
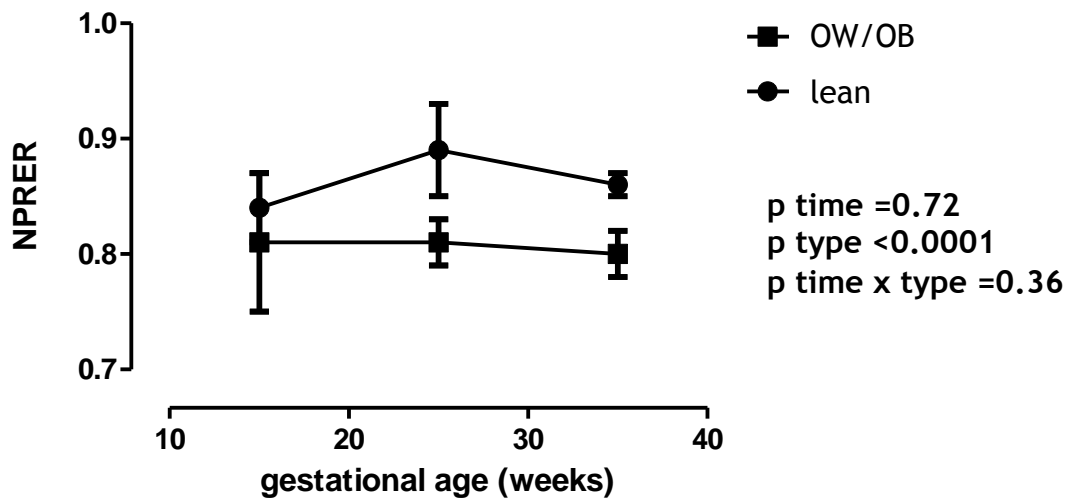
Figure 4.3 NPRER at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean NPRER (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.4.4 Fat oxidation

Using the linear mixed model, fat oxidation was assessed across time and between groups (figure 4.4). There was no effect of gestation on fat oxidation (p time=0.59). Fat oxidation was different between the groups ('p type'). OW/OB women utilised fat as an energy source to a greater extent than lean women; average lean fat oxidation 1947kJ/day (SEM 199) versus OW/OB average fat oxidation 2997 kJ/day (SEM 200).

There was no difference in the fat oxidation across gestation ('p time') or pattern of change ('p time x group').
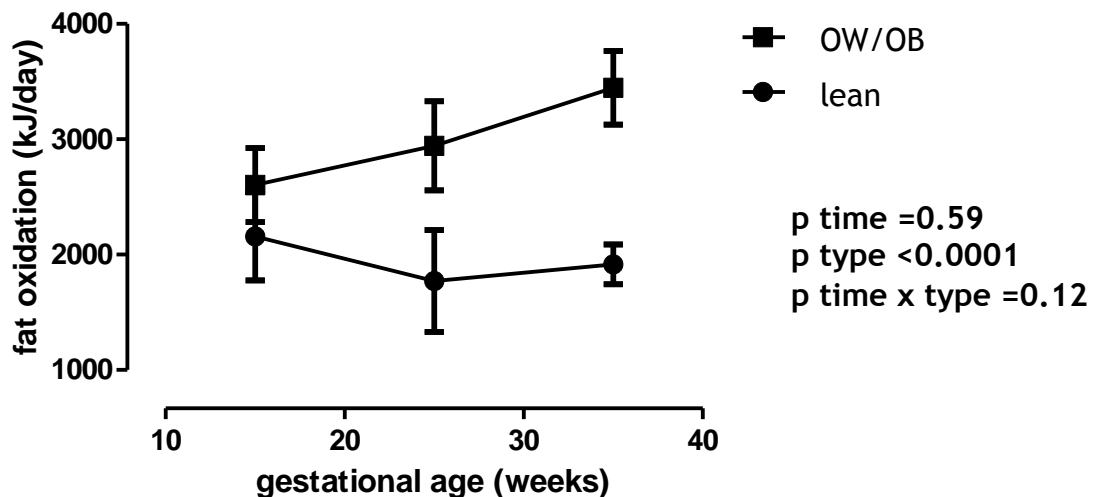
Figure 4.4 Rate of fat oxidation at each gestational time point in lean and OW/OB pregnancy.
Illustrated is the mean rate of fat oxidation (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.4.5 Carbohydrate oxidation

The change in carbohydrate (CHO) oxidation was assessed across time and between the groups (figure 4.5). There was a trend for an increase in CHO utilisation during pregnancy (p time=0.039). There was a significant difference between the groups ('p type' <0.0001); average lean CHO oxidation 2319 kJ/day (SEM 192) versus OW/OB 1864 kJ/day (SEM 211).

The interaction term ('p time x group') was not significant indicating that the lean and OW/OB have similar changes in CHO oxidation during pregnancy.
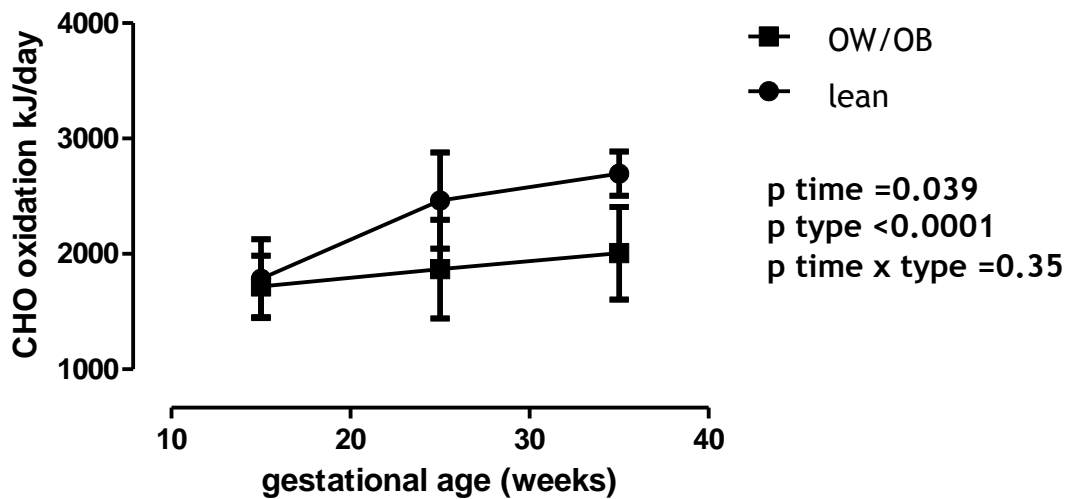
Figure 4.5 Rate of CHO oxidation at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean rate of CHO oxidation (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.5 Longitudinal changes in physical activity

4.5.1 Sedentary activity time

Sedentary activity time was assessed across time and between groups (figure 4.6). There was no effect of gestation on sedentary activity time (p time=0.23). There was no difference in sedentary activity between the groups (p type=0.068).

There was no difference in the pattern of change in sedentary activity time between the two groups, shown as the interaction term ('p time x group').
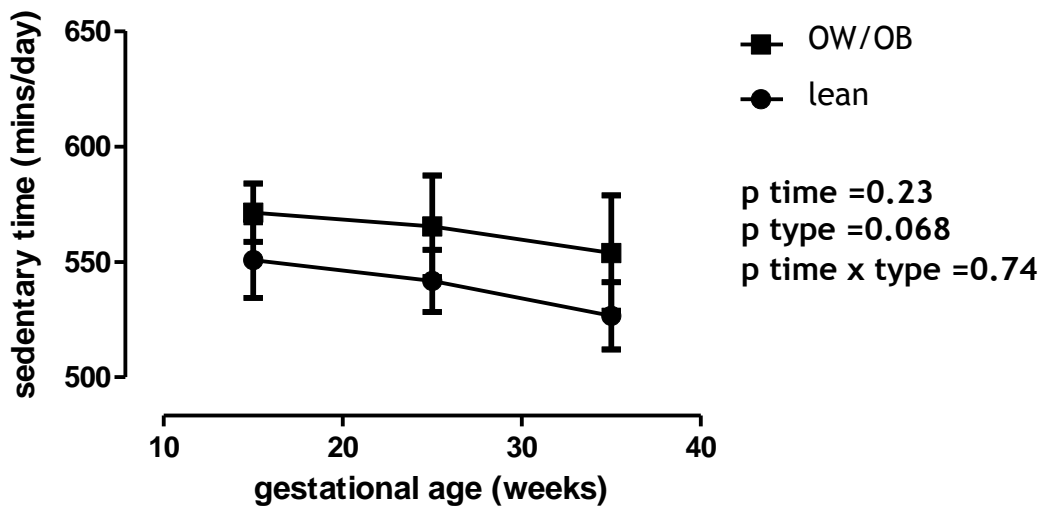
Figure 4.6 Daily sedentary activity time at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean daily sedentary activity time (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.5.2 Light activity time

There was no difference in daily light activity time across gestation and between the groups as shown in figure 4.7. There was no difference in the pattern of change in sedentary activity time between the two groups, show as the interaction term ('p time x group').
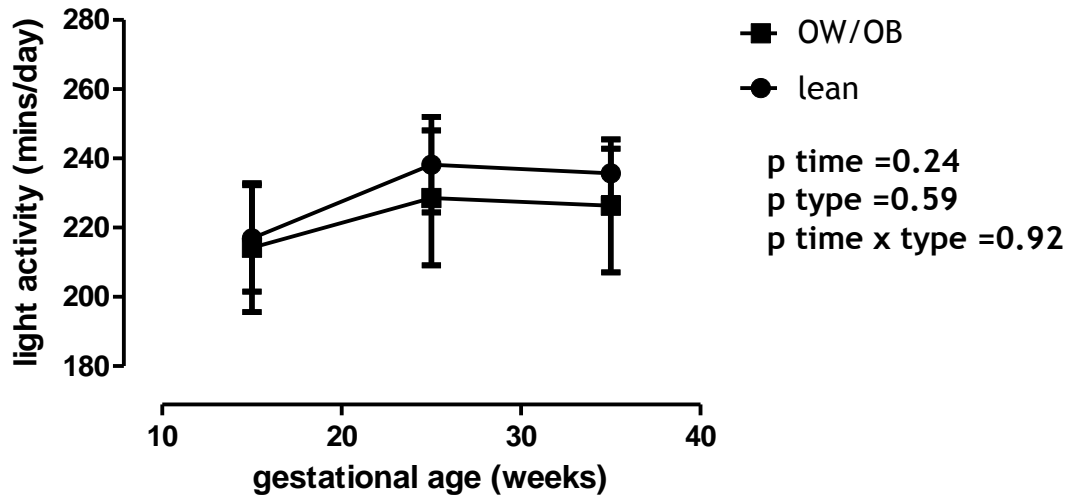
Figure 4.7 Daily light activity time at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean daily light activity time (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.5.3 Moderate & vigorous activity time

Moderate and vigorous physical activity (MVPA) time was assessed across time and between the groups (figure 4.8). Across gestation, there was a significant fall in MVPA, p time=0.008; average visit 1 MVPA 29.4mins/day (SEM 3.0) versus visit 3 average MVPA 18.2mins/day (SEM 2.7). During pregnancy, lean women spent significantly more time in MVPA than OW/OB women; average lean 28.3 mins/day (SEM 2.6) versus 18.6mins/day (SEM 1.8) respectively.

There was no difference in the pattern of change in MVPA between the two groups, show as the interaction term ('p time x group').
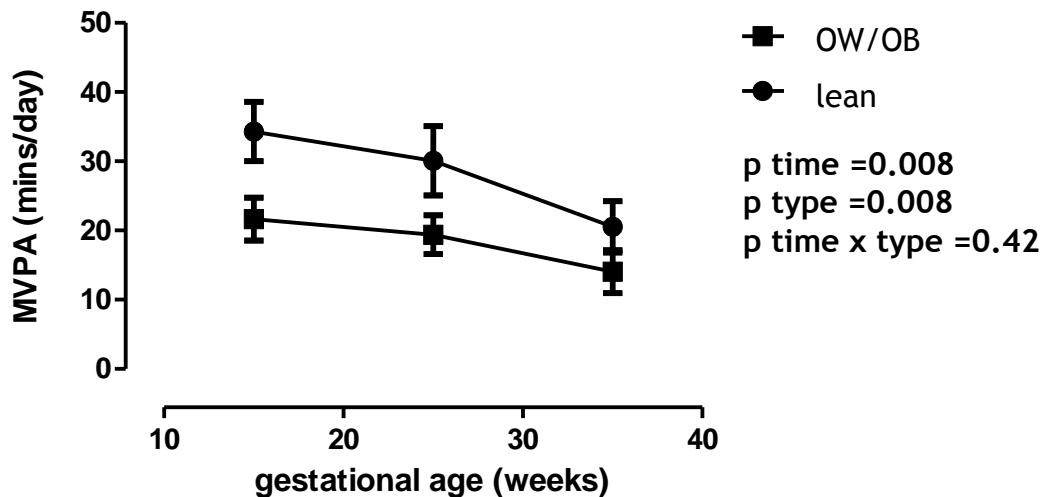
Figure 4.8 Daily MVPA time at each gestational time point in lean and OW/OB pregnancy.
Illustrated is the mean daily MVPA time (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.6 Gestational changes in macronutrient dietary intake

4.6.1 Daily energy intake

Daily energy intake was assessed across time and between groups (figure 4.9). There was no impact of gestation or group on daily energy intake as shown in the figure below.

The pattern of change in daily energy intake seen between the groups was also not significantly different.
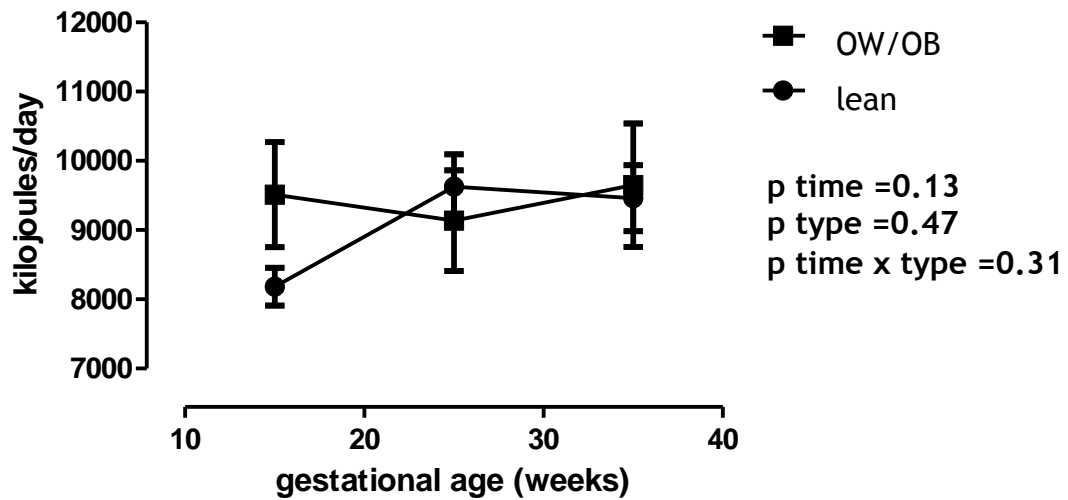
Figure 4.9 Daily energy intake at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean daily energy intake (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.6.2 Daily total fat intake

The change in daily fat intake was assessed across gestation and between the groups (figure 4.10). There was no difference across gestation or between the groups in terms of daily fat intake.

The pattern of change in daily fat intake between the two groups, ('p time x group') was similar.
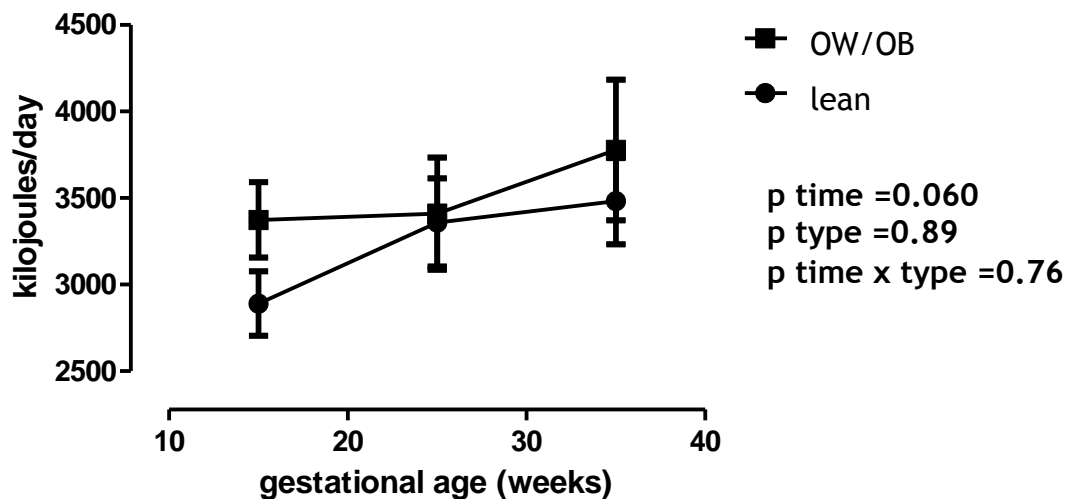
Figure 4.10 Daily fat intake at each gestational time point in lean and OW/OB pregnancy.
Illustrated is the mean daily fat intake (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.6.3 Total carbohydrate intake

The change in daily carbohydrate intake was assessed across gestation and between the groups (figure 4.11). There was no impact of gestation or group on daily carbohydrate intake.

There was no difference across gestation in the pattern of change in daily carbohydrate intake seen between the groups.
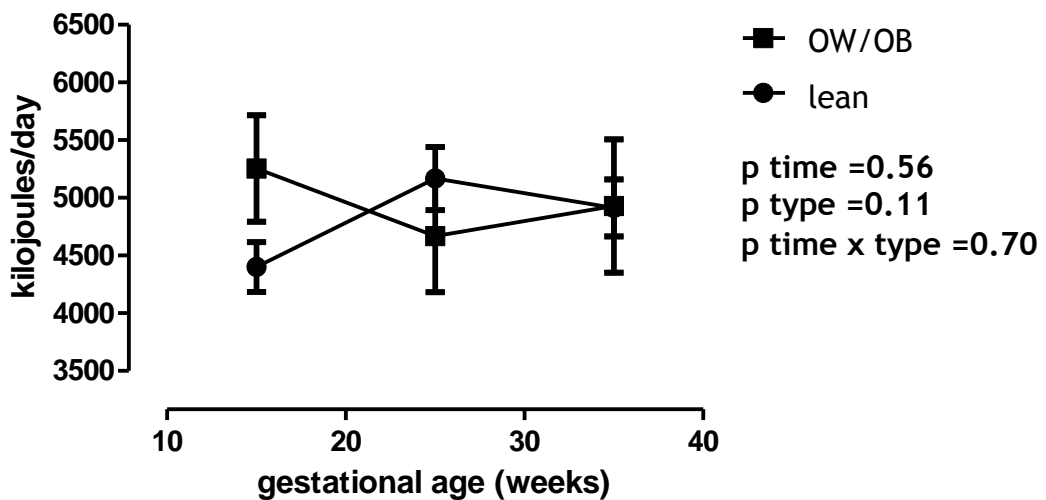
Figure 4.11 Daily carbohydrate intake at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean daily carbohydrate intake (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.6.4 Total protein intake

The change in daily protein intake was assessed across gestation and between the groups (figure 4.12). There was no impact of gestation or group on daily protein intake.

When the statistical model was applied to the data there was no difference in the pattern of change in daily protein intake seen between the groups as shown in figure 4.11.
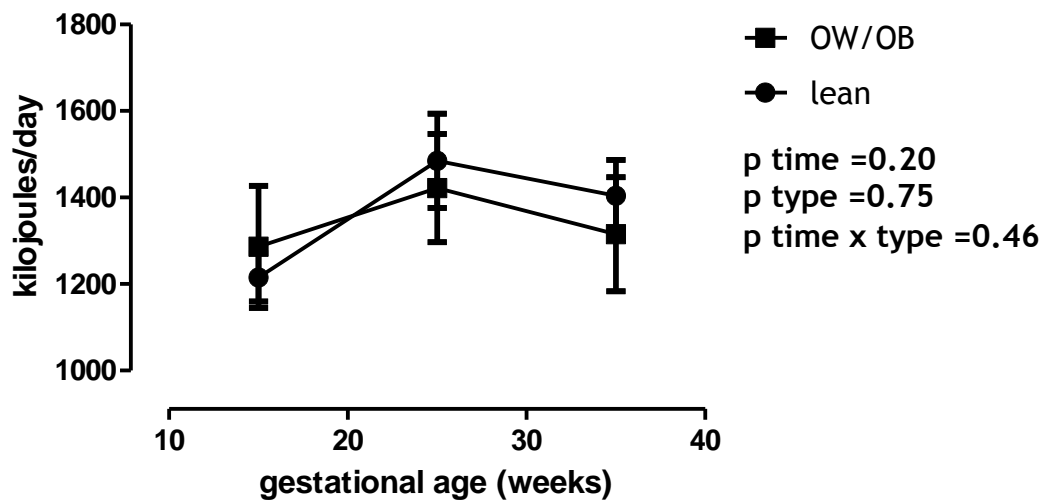
Figure 4.12 Daily protein intake at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean daily protein intake (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

4.7 Summary of gestational changes seen in energy metabolism
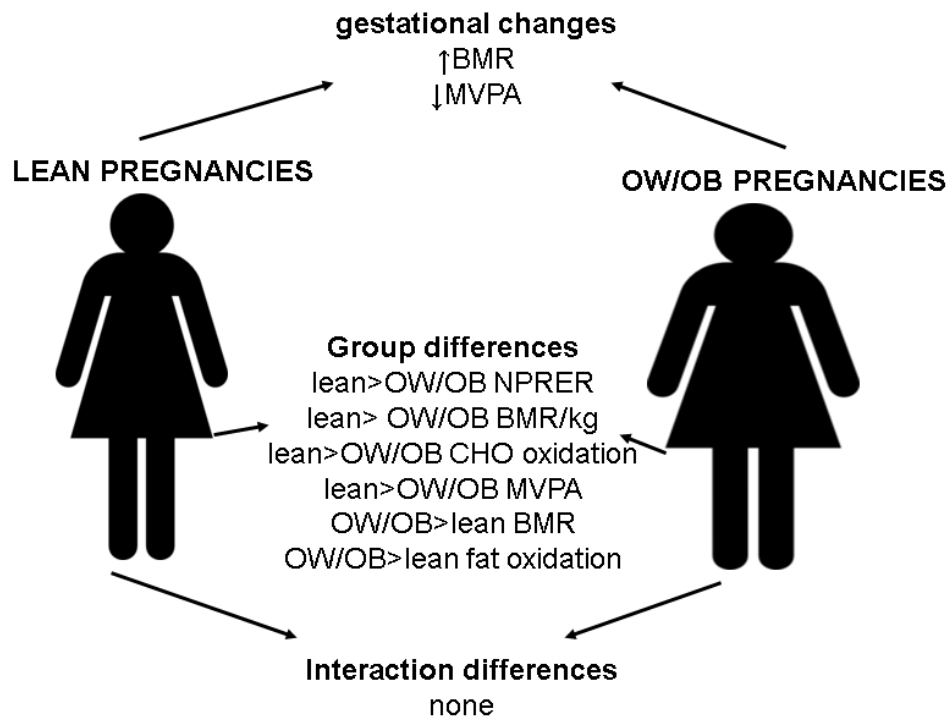
Figure 4.13 Summary of energy metabolism changes seen in healthy lean and OW/OB pregnancy. This figure summarises the gestational differences (previously expressed as 'p time'), the differences between the groups ('p type') and the differences in the pattern of change ('p time x type') seen in lean and OW/OB pregnancy.

Figure 4.13 summarises the analysis of the components of energy metabolism assessed in lean and OW/OB pregnancy. Across gestation BMR rose and MVPA time fell. Lean women had higher BMR/kg, NPRER, and carbohydrate oxidation and lower BMR and fat oxidation compared to OW/OB women. The higher BMR in OW/OB women is due to their greater body mass. After correction for body mass, it was seen that BMR/kg was in fact significantly higher in lean women. In addition lean women were more active than OW/OB women during pregnancy. There were no differences in the pattern of change in the above components of energy metabolism between lean and OW/OB women.

4.8 The contribution of energy metabolism to gestational fat mass and anatomical fat distribution

Further analysis was performed on the components of energy metabolism which changed over gestation or differed between lean and OW/OB mothers and their relationship with observed changes in fat mass and specific subcutaneous fat depots. This analysis was performed using the entire cohort as the lack of interactions for any of the measurements of energy metabolism indicated that lean and OW/OB women responded to pregnancy in the same way. Using the entire cohort increased the sample size and added more power to the analysis. BMI was included in the analysis where appropriate as a covariate.

Univariate analysis was performed using Pearson's correlation to assess the relationships between absolute change in the anthropometric variables and BMR/kg, NPRER, and MVPA. A significant result was taken as $p \leq 0.01$, but trends ($p < 0.05$) were also explored further in multivariate analysis. These variables were chosen as NPRER incorporates both carbohydrate and fat oxidation. BMR/kg was included as this corrected for the increase in BMR associated with increased body mass in the OW/OB group.

If a significant univariate correlation was found, multivariate analysis was performed. This was performed using the General Linear Model (significance level $p < 0.05$) including the above variables and covariates. BMR and BMR/kg are related to therefore they were not analysed in the same model. BMI as a confounding variable was added to a further multivariate analysis when appropriate (i.e. when it was not represented elsewhere in the model).

4.8.1 Gestational changes in energy metabolism and total fat mass

Univariate correlation analysis (table 4.1) was performed to assess the relationship between the absolute change in fat mass and absolute change in components of energy metabolism.

Table 4.1 Univariate analysis of the association between gestational change in fat mass and the gestational change in energy metabolism in the entire cohort. Analysis performed using Pearson's correlation, significant result if $p < 0.01$. The basal metabolic rate/kilogram is shown as BMR/kg, non-protein respiratory exchange ratio as NPRER and moderate & vigorous activity as MVPA.

	BMR/kg V1-V3	NPRER V1-V3	MVPA V1-V3
V1-V3 fat mass			
Pearson correlation	0.111	-0.023	0.186
P value	0.50	0.89	0.33

There were no significant associations between the increase in fat mass during pregnancy and the change in BMR/kg, NPRER or MVPA.

The relationship between BMR/kg and MVPA was explored. The absolute values for BMR/kg and MVPA at each time point during gestation were plotted (figure 4.14). Analysis of the data for all time points showed that BMR/kg was significantly correlated to MVPA. Thus women with a higher BMR/kg are more active. This may explain why the BMR/kg is higher in lean women.
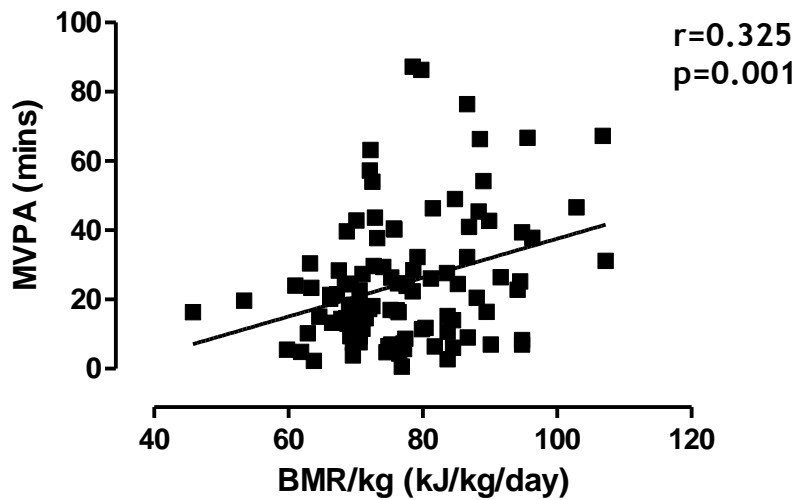
Figure 4.14 Relationship between basal metabolic rate per kilogram versus moderate and vigorous activity for the entire cohort. Assessment performed using Pearson's correlation, significant result if $p \leq 0.01$. The time spent in MVPA in minutes is plotted against the BMR/kg in kilojoule per kilogram per day.. Basal metabolic rate/kilogram is shown as BMR/kg and moderate & vigorous activity is shown as MVPA.

4.8.2 Gestational change in energy metabolism and total body skinfolds

Univariate analysis was performed to assess the relationship between the absolute change in total body skinfolds and gestational changes in energy metabolism (table 4.2). There were no significant association with the increase in total body skinfolds seen during pregnancy and BMR/kg, NPRER or MVPA.

Table 4.2 Univariate analysis of the association between gestational change in total body skinfolds to the gestational change in energy metabolism in entire cohort. Assessment performed using Pearson's correlation, significant result if $p \leq 0.01$. The basal metabolic rate/kilogram is shown as BMR/kg, non-protein respiratory exchange ratio shown as NPRER and moderate & vigorous activity shown as MVPA.

	BMR/kg V1-V3	NPRER V1-V3	MVPA V1-V3
V1-V3 total body skinfolds			
Pearson correlation	0.195	-0.143	-0.113
P value	0.22	0.37	0.55

4.8.3 Gestational change in energy metabolism and upper body peripheral skinfolds

Univariate analysis was performed using Pearson correlation to assess the relationship between the absolute change in upper body peripheral skinfolds and components of energy metabolism (table 4.3). There was a trend for the increase in upper body peripheral skinfolds change to be associated with the change in NPRER during pregnancy.

Table 4.3 Univariate analysis of the association between gestational change in upper body peripheral skinfolds and the gestational change in energy metabolism in the entire cohort. Analysis performed using Pearson's correlation, significant result if p<0.01. The basal metabolic rate/kilogram is shown as BMR/kg, non-protein respiratory exchange ratio as NPRER and moderate & vigorous activity as MVPA.

	BMR/kg V1-V3	NPRER V1-V3	MVPA V1-V3
V1-V3 upper body peripheral skinfolds			
Pearson correlation	0.228	-0.314	-0.004
P value	0.15	0.046	0.98

The relationship between upper body peripheral skinfolds and NPRER was explored. The change in UBPS and the change in NPRER were plotted (figure 4.15). If there is an increase in NPRER across gestation this indicates a move to more carbohydrate oxidation, a decrease in the NPRER suggests more fat oxidation. Therefore, a large increase in UBPS is related to a higher proportion of fat metabolism, whereas a small increase in UBPS is related to a higher degree of carbohydrate metabolism.
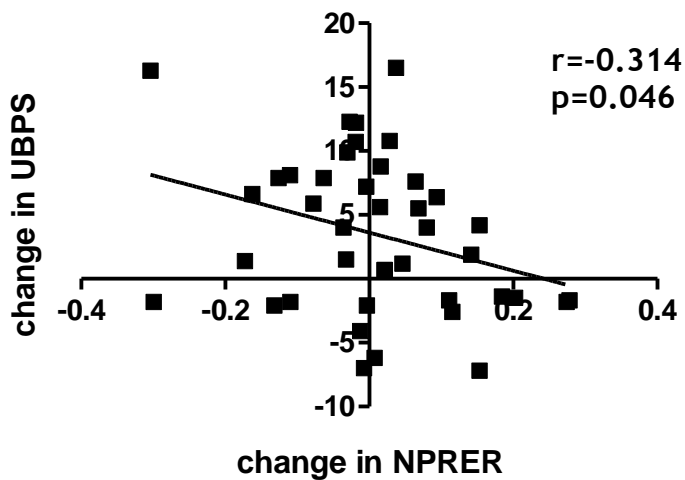
Figure 4.15 Relationship between the change in upper body peripheral skinfolds and the change in NPRER for the entire cohort. Assessment performed using Pearson's correlation, significant result if p≤0.01. The change in UBPS is plotted against the change observed in NPRER during pregnancy for the entire cohort. Upper body peripheral skinfolds is shown as UBPS.

4.8.4 Gestational change in energy metabolism and abdominal skinfolds

Univariate analysis was performed using Pearson correlation to assess the relationship between the absolute change in abdominal skinfolds and components of energy metabolism. No significant associations were found.

Table 4.4 Univariate analysis of the association between gestational change in abdominal skinfolds and the gestational change in energy metabolism in the entire cohort. Analysis performed using Pearson's correlation, significant result if p<0.01. The basal metabolic rate/kilogram is shown as BMR/kg, non-protein respiratory exchange ratio as NPRER and moderate & vigorous activity as MVPA.

	BMR/kg V1-V3	NPRER V1-V3	MVPA V1-V3
V1-V3 abdominal skinfolds			
Pearson correlation	0.038	0.190	-0.127
P value	0.81	0.24	0.50

4.8.5 Gestational change in energy metabolism and lower body skinfolds

Univariate analysis was performed using Pearson correlation to assess the relationship between the absolute change in lower body skinfolds and components of energy metabolism. No significant associations were found.

Table 4.5 Univariate analysis of the association between gestational change in lower skinfolds and the gestational change in energy metabolism in the entire cohort. Analysis performed using Pearson's correlation, significant result if p<0.01. The basal metabolic rate/kilogram is shown as BMR/kg, non-protein respiratory exchange ratio as NPRER and moderate & vigorous activity as MVPA.

	BMR/kg V1-V3	NPRER V1-V3	MVPA V1-V3
V1-V3 lower body skinfolds			
Pearson correlation	0.165	-0.161	-0.217
P value	0.30	0.31	0.25

4.9 Discussion

In our study, there was a significant rise in basal metabolic rate during pregnancy and OW/OB women had higher rates of BMR than lean women. This is consistent with the current literature (Forsum and Lof, 2007, Lof et al., 2005). Although we found the increase in BMR continuous from 15 weeks' gestation, other reports regarding the timing of increases in BMR differ. Some authors have reported that BMR only rises significantly after 32 weeks' gestation (Forsum and Lof, 2007, Lof et al., 2005). Others have detected a rise in BMR from conception and a further increase in the third trimester (Chihara et al., 2002). Our study cannot comment on pre-conceptual or early pregnancy changes, and our results suggest that BMR increases throughout pregnancy rather than just in the third trimester.

In this study, the higher rates of BMR in the OW/OB group simply reflect the fact that these women are significantly heavier throughout pregnancy than the lean women. In order to correct for the impact of higher body mass, basal metabolic rate per kilogram (BMR/kg) was calculated. Lean women had significantly higher BMR/kg throughout pregnancy compared to OW/OB women (p type<0.0001). There was also a trend towards a different pattern of change in BMR/kg between the groups (p=0.065). Across gestation, BMR/kg fell continuously in the lean group but stayed relatively stable in the OW/OB group. Other authors have not discussed BMR/kg (Forsum and Lof, 2007, Chihara et al., 2002) and differences in maternal body mass between different studies may underlie the disparity in reported changes of BMR during pregnancy. As lean and OW/OB women gained the same fat mass and total body weight during pregnancy the observed gestational decline in BMR/kg observed must be explained by another component of energy metabolism.

During pregnancy, both groups show a significant fall in MVPA levels and by the third trimester lean women have similarly low MVPA levels to that observed in OW/OB women. Throughout pregnancy, lean women are on average 37.4% more active during pregnancy than OW/OB women, based on time spent in MVPA. Conversely there was a trend for OW/OB women to be more sedentary than lean women throughout pregnancy but this did not reach significance. In under-

nourished maternal populations, a fall in activity as pregnancy progresses has been thought to relate to conservation of energy in the face of an increasing basal metabolic rate (Lawrence et al., 1987). In affluent mothers, the fall in MVPA has also been suggested to be as a result of physical restrictions in moving because of maternal habitus and avoidance of activity which is considered a risk to fetus (Melzer et al., 2009).

Interestingly in the univariate analysis there were no strong associations between the change in fat mass or total body skinfolds and BMR/kg, NPRER and MVPA. However, because BMR/kg and MVPA were different between the groups it was tested whether higher rates of physical activity accounted for the higher BMR per kilogram in the lean group. Plotting all time points we found that there was a significant association between BMR/kg and MVPA (p=0.001), suggesting that more active women have a higher BMR/kg. In our cohort physical activity appears to be a major determinant of BMR in pregnancy (r=0.325).

In this cohort, we found that there was no difference across gestation or between the groups in terms of total energy and macronutrient intake. Our data suggested that our participants had similarly diets. Previous studies on diets during pregnancy have found very little change in terms of the increase in high energy (cakes, biscuits, processed meats, white bread) diet components (Crozier et al., 2009). Other authors however, have found significant increases in the dietary energy density during pregnancy and that high energy diets were significantly associated with total gestational weight gain (Deierlein et al., 2008). Published data also show that those with a high pre-pregnancy BMI tended to consume a diet with high glycaemic loads (Deierlein et al., 2008). These contradictory findings highlight the difficulties in collecting dietary information. In our study, both groups gain a similar amount of GWG and fat mass during pregnancy which suggests a lack of difference in dietary energy intake. This suggests that managing gestational weight gain in a Scottish population by diet alone may not be successful and guidance should include encouraging OW/OB women to increase their physical activity prior and during pregnancy. It would be interesting to explore associations between insulin resistance and visceral fat accretion and gestational changes in BMR/kg and MVPA to see if this advice would lead to an improved metabolic profile.

As discussed above, there were no associations between the change in fat mass or total body skinfolds and measurements of energy metabolism. It cannot be ruled out that there is an element of fat mass not being assessed which is affected by MVPA; this may be the visceral compartment. Unfortunately the visceral compartment was not measured thus there is no information on this association in the current study. There is an abundance of data which indicates the association between physical activity and visceral fat in the adult non-pregnant population. In inactive adults, prolonged bed rest is associated with an increase in visceral fat depots (Belavy et al., 2014). The converse of this is also true, a recent a meta-analysis showed that moderate and vigorous exercise programmes reduce visceral adiposity more than low-impact aerobic regimens in obese men and women (Vissers et al., 2013). Published data in an overweight and obese pregnant population found that women with the largest fall in MVPA across gestation had higher levels of plasma insulin and triglycerides and worse insulin sensitivity in late pregnancy (van Poppel et al., 2013). Although not examined in this cohort further analysis of this data could assess whether this association exists and if there are differences in any associations in lean pregnancies. Therefore, in an obstetric population whether lean or OW/OB, sedentary lifestyle may be a risk factor for increased deposition of visceral fat.

Carbohydrate oxidation was on average 20% higher in lean women when compared to OW/OB group. The rate of fat oxidation was on average 36% higher in OW/OB pregnancies. These differences in substrate utilisation are reflected in the OW/OB women having lower NPRER. An increased fat oxidation may lead to a 'switching off' of glucose oxidation (Randle et al., 1963). However, in obesity the capacity of tissue, primarily muscle, to utilise fat is also diminished and this further enhances the development of insulin resistance (McGarry, 2002). OW/OB women have been found to be more insulin resistant during pregnancy and their increase in fat oxidation may represent an imbalance in fat and glucose utilisation resulting in increasing insulin resistance. Our results would also suggest that lean women continue to utilise glucose as an energy source even in the third trimester. This may represent a more flexible metabolic response in which they can utilise both fat and carbohydrate utilisation because of relative insulin sensitivity compared to the OW/OB group.

In terms of fat distribution however there was a trend for the change in upper body peripheral skinfolds to be negatively associated with the change in NPRER. Therefore, an increase in UBPS was related to a larger contribution from fat metabolism as pregnancy progresses, whereas a decrease in UBPS was related to a larger contribution from carbohydrate oxidation. These results suggest that there may be a mechanism by which decreases carbohydrate metabolism is associated with a preferential storage in fat in the upper body peripheral depots. OW/OB women had lower contribution from carbohydrate metabolism and lower NPRER during pregnancy and accumulated more fat in this depot that lean women, especially in the latter part of pregnancy. This may be because of other depots such as lower body, truncal and visceral compartments being more replete or that this is the only remaining compartment which can undergo healthy expansion during pregnancy. In non-pregnant OW/OB adults, reduced capacity of a subcutaneous fat depot to expand (using capillary density and quantified capillary branch formation to represent a depots expansion potential), was associated with increased insulin resistance (Gealekman et al., 2011). Therefore, this finding may also reflect changes in insulin sensitivity in different fat depots depending on their expansion during pregnancy. In general adult populations it is recognised that abdominal subcutaneous adipocytes behave differently than visceral adipocytes (Pou et al., 2007, Fried et al., 1998), and that lower body adipose tissue function is different yet again (Jensen, 2008) with gynoid adiposity may be protective to health (McCarty, 2003). In healthy pregnancy, visceral adipocytes are smaller, less lipolytic and more insulin resistant than abdominal subcutaneous fat in the third trimester (Huda et al., 2014). Once again this suggests that any differences in fat distribution seen between the groups could have an impact on metabolic adaptation to pregnancy.

The strength of this study is the comprehensive methodology. The indirect calorimetry measurement technique (Oxycon) employed for basal metabolic rate and substrate utilisation has been compared to the gold standard technique of the Douglas bag (Rosdahl et al., 2010), and is technically reliable and reproducible. Air displacement plethysmography was employed to assess total fat mass, this gives a more objective measurement of fat mass and which was also corrected for gestational changes seen in interstitial fluid and oedema

accumulation. The measurement of physical activity was objective and there were no differences found in the time each accelerometer was worn across gestation or between the groups. Accelerometry has been used by other authors during pregnancy (van Poppel et al., 2013), and our methodology was based on pregnancy-related validation studies for wear time and number of valid wear days (Kinnunen et al., 2011). Dietary analysis is notoriously difficult to optimise due to the potential of recall bias. However, our 24 hour recall dietary questionnaire method has been validated in British populations (Holmes et al., 2008). In order to reduce bias in the recall data protocols from the Food Standard Authority's Low Income Diet and Nutrition Survey were used and the questionnaire conveniently took only 15-20 minutes to complete.

There were limitations to the study. Although a rigorously tested method, air displacement plethysmography is a two compartment model measuring only fat mass and fat free mass. It did not distinguish the fetal and placental compartment from the maternal compartment nor was it able to differentiate between visceral and subcutaneous fat or fat distribution. Skinfold thickness measurements also only assessed selected subcutaneous fat depots and not visceral depots. One element which was included but not quantified is the fetal contribution to metabolism and energy requirements which may, especially in late gestation, impact on both maternal energy metabolism and fat accretion. In addition, power calculations did not include the number needed to recruit in order to find a difference in physical activity. Therefore although a difference was found in MVPA, the lack of differences found in sedentary and light activity may be a result of this data being underpowered.

In conclusion, during pregnancy both lean and OW/OB women exhibited a rise in BMR and a reduction in MVPA. There were no differences in dietary intake either during pregnancy or observed between the lean and OW/OB group. Therefore, both lean and OW/OB women have a similar response in their energy metabolism to pregnancy. During pregnancy, OW/OB women had higher BMR which is due to their heavier total body weight. Lean women have a decline in BMR/kg over gestation that can be explained at least in part by their reduced physical activity. Their higher MVPA and BMR/kg could potentially mean a lesser degree of visceral fat accumulation but we cannot conclude this from our data. Lean

women also start pregnancy with relatively high carbohydrate oxidation (possibly due to greater insulin sensitivity) and are more active than OW/OB women. OW/OB women gain fat in the upper body peripheral fat depots throughout pregnancy. The association between NPRER and upper body peripheral skinfolds may reflect preferential fatty acid storage at this site and a switch to increased fat metabolism associated which may be associated with insulin resistance in OW/OB women. Therefore in healthy pregnancy, despite differences in substrate utilisation, the impact of energy metabolism is similar between lean and OW/OB women and appears to have no impact on overall fat mass accumulation.

Chapter 5 - Gestational carbohydrate, lipid and inflammatory profiles in lean and OW/OB pregnancies

5.1 Introduction

During healthy pregnancy, there are dramatic changes in lipid and carbohydrate metabolism in order to meet the changing metabolic demands for both the mother and fetus. These changes have been well documented in the current literature.

There is a gradual increase in total triglycerides (estimated 2-3 fold increase) which peaks at term and falls to pre-pregnancy levels by about 6 weeks postpartum. Total cholesterol concentrations increase more modestly reaching approximately 50-60% above pre-pregnancy levels. The rise in LDL levels is proportional to the increase in cholesterol and remains elevated until 8 weeks postpartum. HDL exhibits a unique pattern of fluctuation during pregnancy which show a peak concentration at around 20 weeks' gestation before a fall in levels in the third trimester (Sattar et al., 1997). These changes in lipid profiles are thought to be, in part, an oestrogenic response (Salameh and Mastrogiannis, 1994). Gestational changes in lipid metabolism initially allow the accumulation of maternal fat stores before a more lipolytic phase in which there is an increased utilisation of maternal NEFA during late pregnancy in order that the fetus can preferentially use amino acids and glucose as energy sources. Studies have suggested that fatty acids may be important in the endothelial dysfunction seen in pregnancies affected by pre-eclampsia (Robinson, 2009).

Cross-sectional assessment in the third trimester showed that in maternal obesity there is an exaggerated dyslipidaemia (raised TG and lower HDL), hyperinsulinaemia and a low grade inflammatory response (CRP and IL6) compared to lean pregnant women. (Ramsay et al., 2002a). Further longitudinal assessment of lean and OW/OB pregnancies indicated that this pro-inflammatory response exists from the first trimester onwards (Stewart et al., 2007a).

The concept of adipose tissue as an endocrine organ is now firmly established and the dysregulation of adipokine production from fat can lead to the pathogenesis of the metabolic syndrome in the non-pregnant population (Hutley and Prins, 2005). Both visceral and subcutaneous fat have been independently associated with markers of inflammation including CRP and IL6 (Pou et al., 2007).

In current published literature, little has been reported on differences in the pattern of change of markers of lipid, carbohydrate and inflammatory pathways specifically with reference to changes in body fat distribution associated with pregnancy. This chapter assesses these profiles longitudinally and relates them to the anatomical changes seen in fat accumulation during pregnancy.

5.2 Research Questions

1. What are the gestational changes in plasma markers of carbohydrate, lipid and inflammatory profiles in lean and OW/OB pregnancies?
2. What are the differences between lean women and OW/OB women in terms of gestational change in plasma markers of carbohydrate, lipid and inflammation?
3. Does anatomical distribution of subcutaneous fat during pregnancy have an impact on any changes in these plasma markers in lean and OW/OB pregnancies?

5.3 Methods

Plasma collection and methodology have been detailed in section 2.5 of the General Methods Chapter 2. Further information regarding specific analytic techniques has been detailed in each section of this results chapter.

5.4 Gestational changes in plasma markers of carbohydrate metabolism

5.4.1 Fasting glucose

Fasting glucose was assessed across time and between groups (figure 5.1). There was a borderline increase in fasting glucose during pregnancy; mean visit 1 4.6mmol/L (SEM 0.05) versus mean visit 3 4.7mmol/L (SEM 0.07), p time=0.019.

There was no difference between the groups (p type=0.25) and in the pattern of change in fasting glucose between the two groups, shown as the interaction term (p time x group=0.24).
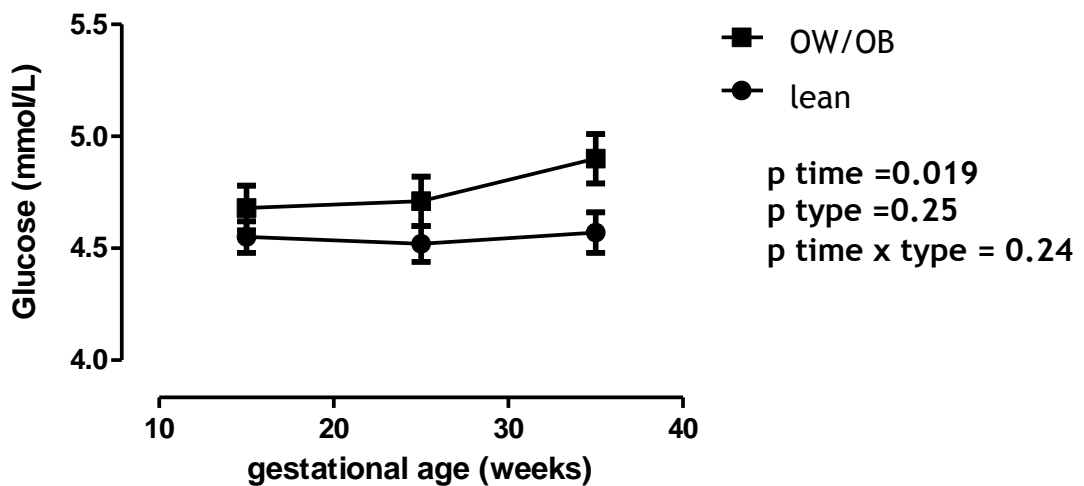
Figure 5.1 Fasting glucose at each gestational time point in lean and OW/OB pregnancy.
Illustrated is the mean fasting glucose (raw data) and standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

5.4.2 Fasting insulin

Fasting insulin was assessed across time and between groups (figure 5.2). There was a significant rise in fasting insulin during pregnancy in both groups; mean visit 1 5.0mU/L (SEM 0.5) versus mean visit 3 11.2mU/L (SEM 1.6), p time<0.0001. OW/OB women had significantly higher levels of fasting insulin during pregnancy compared to lean women; mean OW/OB 11.3mU/L (SEM 0.9) versus mean lean 5.7mU/L (SEM 0.9), p type<0.0001. The pattern of change in this plasma marker was not different between the groups (p time x type=0.76).
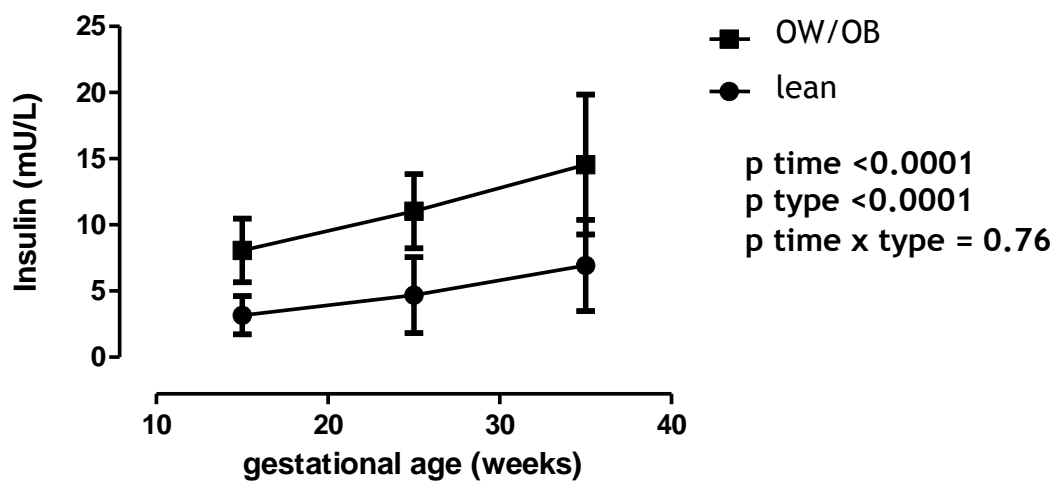
Figure 5.2 Fasting insulin at each gestational time point in lean and OW/OB pregnancy.
Illustrated is the mean fasting insulin (raw data) and standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

5.4.3 Insulin resistance

Insulin resistance was assessed using the Homeostasis Model Assessment (HOMA). Using the linear mixed model HOMA was assessed across time and between groups (figure 5.3). There was a significant rise in insulin resistance during pregnancy in both groups; mean visit 1 HOMA 1.0 (SEM 0.1) versus mean visit 3 HOMA 2.5 (SEM 0.4), p time<0.0001. OW/OB women were significantly more insulin resistant than lean women during pregnancy; mean OW/OB HOMA 2.4 (SEM 0.2) versus mean lean 1.2 (SEM 0.2), p type<0.0001.

Both groups showed a linear relationship between gestation and HOMA thus there was no difference pattern of change (p time x type=0.77) in insulin resistance.
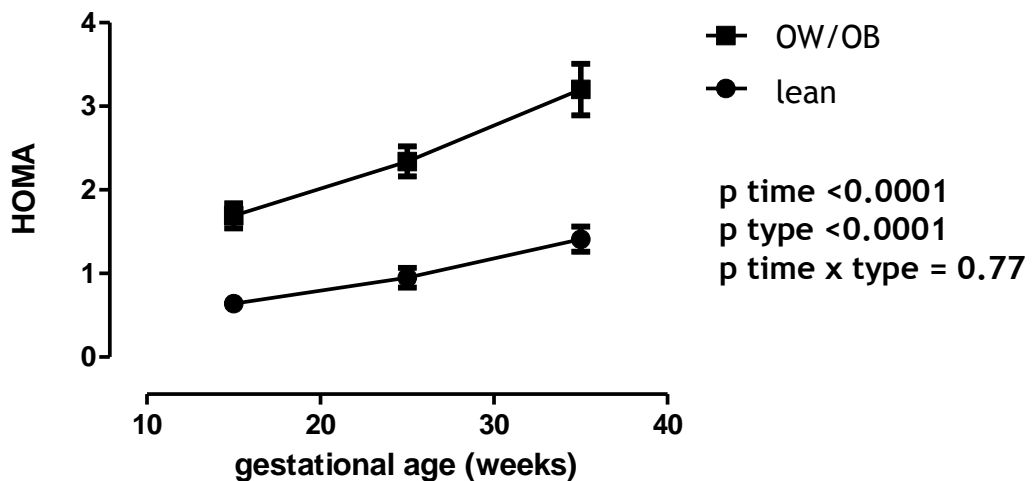
Figure 5.3 Insulin resistance (HOMA) at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean HOMA and standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

5.5 Gestational changes in plasma markers of lipid metabolism

5.5.1 Total cholesterol

The concentration of cholesterol was assessed across time and between the groups (figure 5.4). This indicated that there was a significant increase in total cholesterol during pregnancy; mean visit 1 cholesterol 5.4mmol/L (SEM 0.1) versus visit 3 total cholesterol 6.8mmol/L (SEM 0.2), p time<0.0001. There was no difference observed between the groups (p type=0.46) or in the pattern of change in total cholesterol (p time x type=0.37).
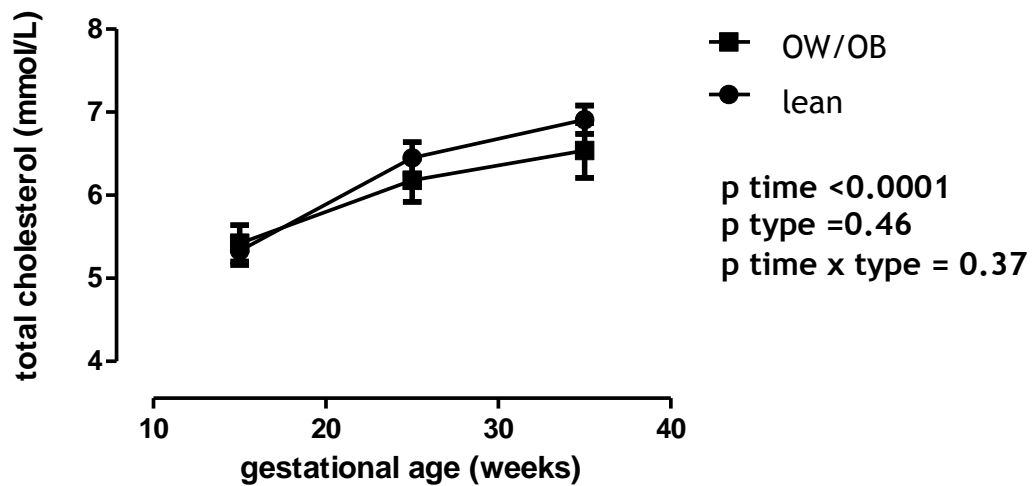
Figure 5.4 Total cholesterol at each gestational time point in lean and OW/OB pregnancy.
Illustrated is the mean total cholesterol concentration (raw data) and standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

5.5.2 High density lipoprotein

The plasma concentration of HDL was assessed across time and between the groups (figure 5.5). Gestation did not have an impact on HDL concentration (p time=0.57). Lean women had significantly higher levels of HDL than OW/OB women during pregnancy; mean lean HDL 2.0mmol/L (SEM 0.04) versus mean OW/OB HDL 1.7mmol/L (SEM 0.04), p type=0.007. There was no difference in the pattern of change seen between the groups (p time x type=0.40).
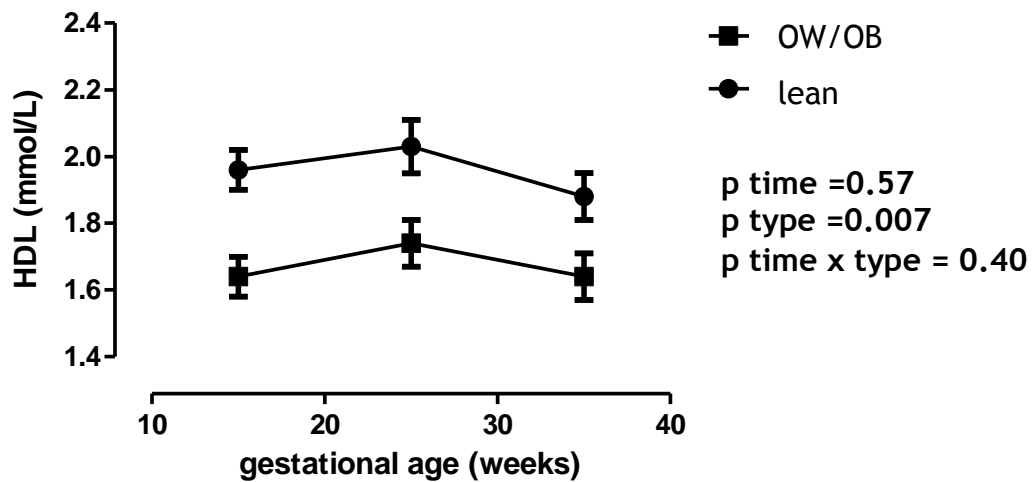
Figure 5.5 HDL at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean HDL concentration (raw data) and standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

5.5.3 Total triglycerides

The total triglyceride concentration was assessed across time and between the groups (figure 5.6). There was a significant increase in total triglycerides during pregnancy; mean visit 1 1.4mmol/L (SEM 0.1) versus mean visit 3 2.6mmol/L (SEM 0.1), p time<0.0001. OW/OB women had borderline higher levels of total triglycerides compared to lean women; mean OW/OB 2.2mmol/L (SEM 0.1) versus lean 1.8mmol/L (SEM 0.1), p type=0.017. There was no difference between the groups in pattern of change (p time x type=0.12).
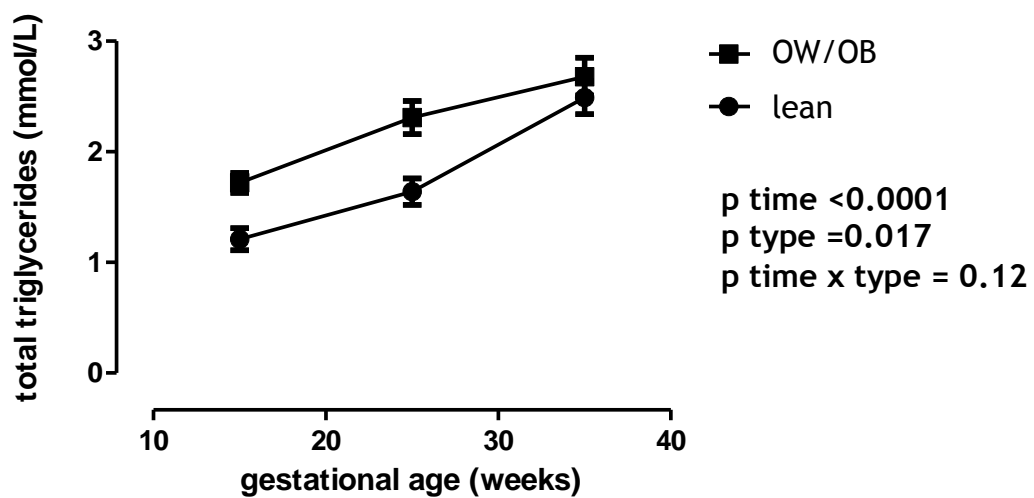
Figure 5.6 Total triglycerides at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean total triglyceride concentration (raw data) and standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

5.5.4 Non-esterified fatty acids

NEFA concentration was assessed across time and between groups (figure 5.7). The levels of NEFA did not change significantly over the course of pregnancy (p time=0.75). Lean women had borderline higher levels of NEFA than OW/OB women during pregnancy; mean lean NEFA 1.4mmol/L (SEM 0.06) versus OW/OB 1.1 (SEM 0.07), p type=0.012.

The pattern of change seen in OW/OB pregnancy was significantly different between the groups ('p time x type'=0.008). In lean pregnancy the mean NEFA level remained essentially static (1.3mmol/L [SEM 0.1], 1.4mmol/L [SEM 0.1], 1.4mmol/L [SEM 0.1] at visit 1, 2 and 3 respectively). In OW/OB women there was a fall in NEFA levels (1.3mmol/L [SEM 0.1], 1.1mmol/L [SEM 0.1] and 0.9mmol/L [SEM 0.1] at visit 1, 2 and 3 respectively).
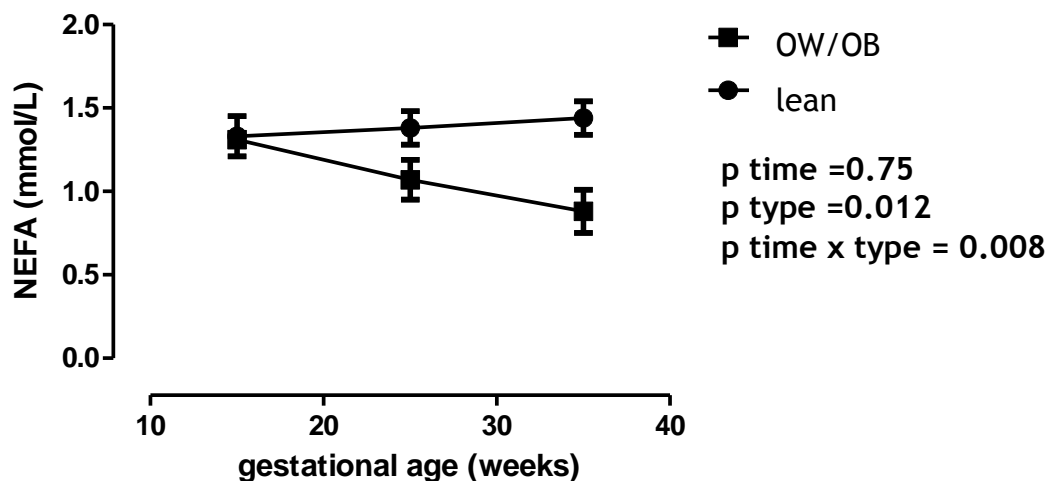
Figure 5.7 NEFA at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean NEFA concentration (raw data) and standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

5.6 Gestational changes in plasma markers of inflammation

5.6.1 C-reactive protein

CRP concentration was assessed across time and between the groups (figure 5.9). Over the course of pregnancy CRP levels fell significantly; mean visit 1 CRP 5.5mg/L (SEM 0.8) versus mean visit 3 CRP 4.2mg/L (SEM 0.5), p time=0.004. During pregnancy CRP was significantly higher in OW/OB women compared to lean women; mean OW/OB CRP 7.7mg/l (SEM 0.7) versus lean 3.5mg/L (SEM 0.4) p type<0.0001.

In both groups although the levels of CRP fell during pregnancy, the pattern of change was significantly different (p time x type=0.003). In lean pregnancy CRP measurements were 3.5mg/L (SEM 0.7), 3.8mg/L (SEM 0.7) and 3.1mg/L (SEM 0.5) at visit 1, 2 and 3 respectively. In OW/OB pregnancy CRP levels were 8.9mg/L (SEM 1.4), 8.5mg/L (SEM 1.2) and 5.7mg/L (SEM 0.8) at each gestational time point.
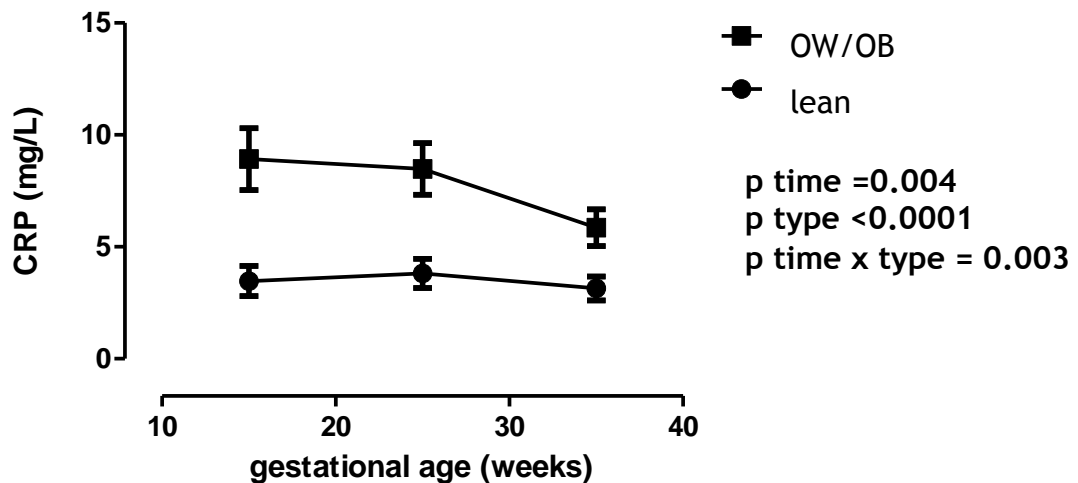
Figure 5.8 CRP at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean CRP concentration (raw data) and standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

5.6.2 Interleukin 6

The change in IL6 concentration was assessed across time and between the groups (figure 5.10). Over the course of pregnancy IL6 levels rose significantly; mean visit 1 IL6 0.8pg/mL (SEM 0.05) versus visit 3 IL6 1.5pg/mL (SEM 0.08), p time<0.0001.

During pregnancy IL6 concentration was different between OW/OB women and lean women, although the overall mean for lean and OW/OB women was similar; lean 1.1pg/mL (SEM 0.06) versus OW/OB 1.1pg/mL (SEM 0.07), p type<0.0001.

In both groups although the levels of IL6 rose during pregnancy, the pattern of change was significantly different (p time x type<0.0001). In lean pregnancy IL6 measurements were 0.8pg/mL (SEM 0.06), 1.0pg/mL (SEM 0.08) and 1.6pg/mL (SEM 0.1) at visit 1, 2 and 3 respectively. In OW/OB pregnancy IL6 levels were 1.0pg/mL (SEM 0.1), 1.1pg/mL (SEM 0.1) and 1.3pg/mL (SEM 0.1) at each gestational time point.
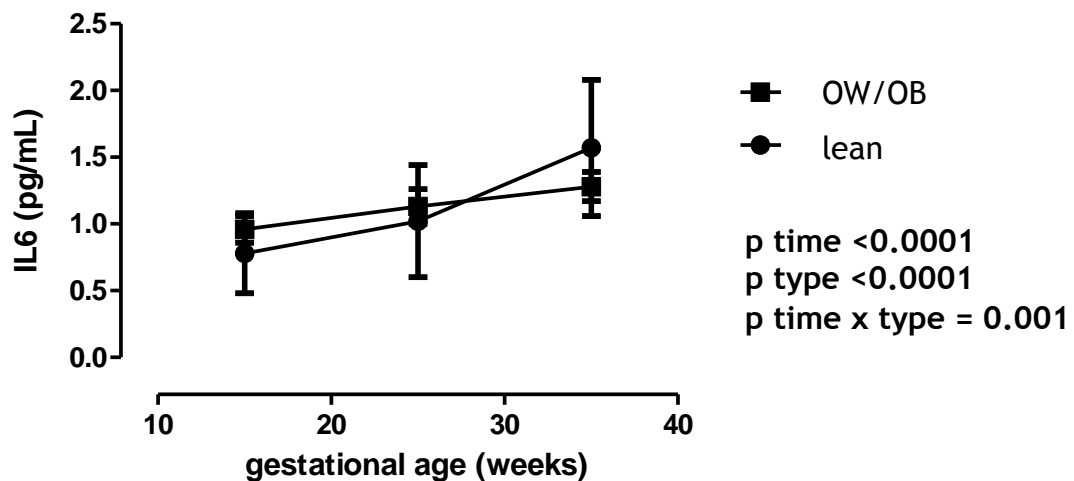
Figure 5.9 IL6 at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean IL6 concentration (raw data) and standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

5.6.3 Tumour necrosis factor alpha

TNFα concentration was assessed across time and between the groups (figure 5.11). Over the course of pregnancy TNFα levels did not change significantly (p time=0.044).

During pregnancy TNFα was significantly higher in OW/OB women compared to lean women; mean OW/OB 1.6pg/mL (SEM 0.1) versus lean 0.8pg/mL (SEM 0.1), p type<0.0001.

The pattern of change in TNFα levels was not different between the groups (p time x type=0.24).
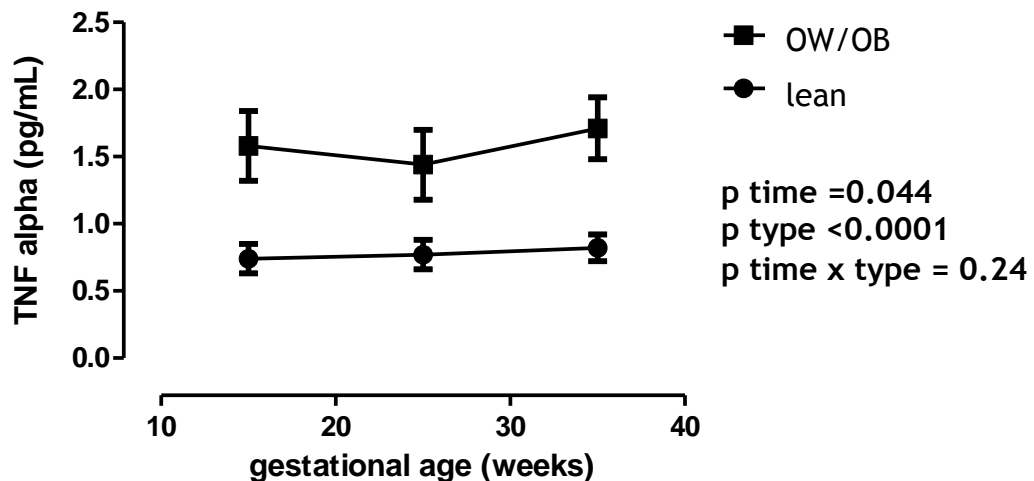
Figure 5.10 TNFα at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean TNFα concentration (raw data) and standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

5.7 Summary of gestational changes seen in carbohydrate & lipid metabolism and inflammatory markers

gestational changes
↑insulin, ↑ HOMA, ↑ TC,
↑ TG, & ↑ IL6
↓CRP

LEAN PREGNANCIES

OW/OB PREGNANCIES

Group differences
Insulin & HOMA OW/OB>lean
CRP, TNFα OW/OB>lean
HDL lean>OW/OB
IL6 lean>OW/OB

Interaction differences
NEFA
CRP
IL6
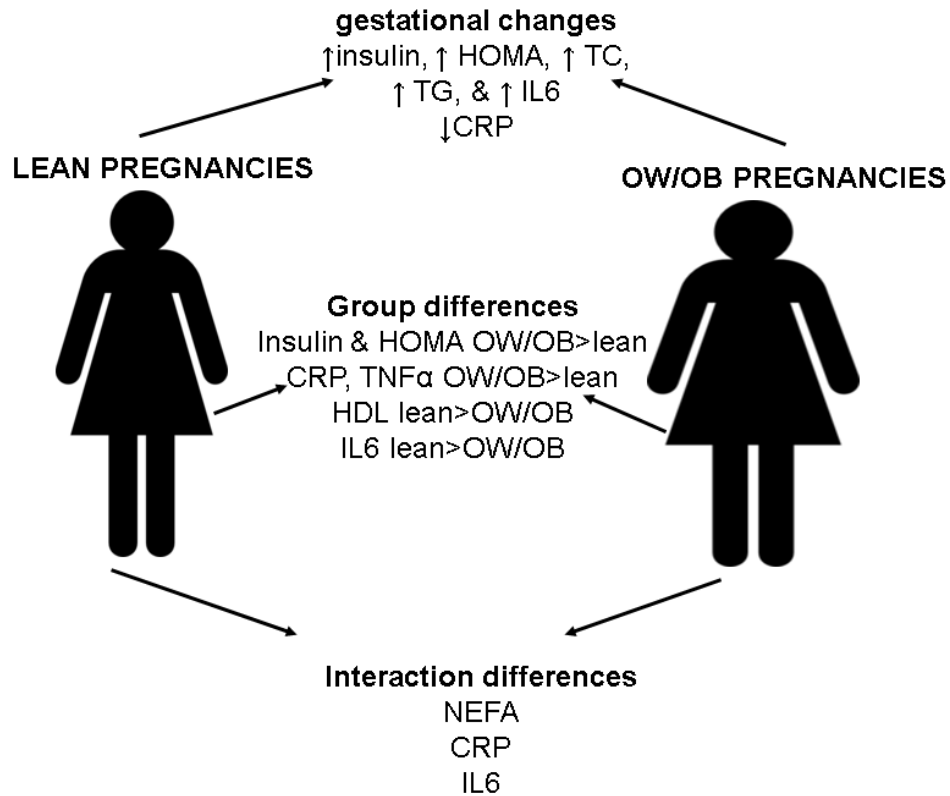
Figure 5.11 Summary of the gestational changes in carbohydrate, lipid and inflammation profiles observed in lean and OW/OB pregnancy. This figure summarises the gestational differences (previously expressed as 'p time'), the differences between the groups ('p type') and the differences in the pattern of change ('p time x type') seen in lean and OW/OB pregnancy.

During pregnancy there are gestational changes which are the same for both lean and OW/OB pregnancy: an increase in insulin, HOMA, total cholesterol, total triglycerides and IL6. Interestingly, CRP falls during pregnancy in both groups. OW/OB women have higher levels of insulin, HOMA, CRP and TNFα and lean women have higher levels of HDL and IL6. From the above analysis the gestational pattern of NEFA, CRP and IL6 change in pregnancy is different between lean and OW/OB pregnancy.

5.8 Gestational pattern of change in plasma markers

From the above analysis, it would appear that there are differences in the pattern of change seen in NEFA, CRP and IL6 in the lean and OW/OB groups. *Post hoc* analysis was carried out to assess the change in each variable across gestation using repeated measures ANOVA and *post hoc* Tukey test. This analysis was performed separately in the lean and OW/OB groups. All analysis was performed in Minitab vs16 and significance was considered as $p \leq 0.01$.

5.8.1 Non-esterified fatty acids

There was no significant change in NEFA concentration across gestation in lean
pregnancy (figure 5.11).

Figure 5.12 Temporal changes in NEFA levels in lean pregnancy. Gestational time points are
illustrated on the x axis and raw values are quotes on the y axis. Analysis performed on raw values
following Ryan-Joiner normality testing. Analysis performed using repeated measures ANOVA,
significant result if p≤0.01. Data with different superscript levels are significantly different from each
other. As each of the means is denoted by [a] this indicates there is no difference between the
mean values at each gestational time point.

OW/OB women showed a significant fall in NEFA levels, (p=0.006), although there was wider variation in the concentration of NEFA in this group as pregnancy progressed Figure 5.12. *Post hoc* analysis suggested that there was a fall across the entire gestational period rather than at a specific time during pregnancy in OW/OB women.
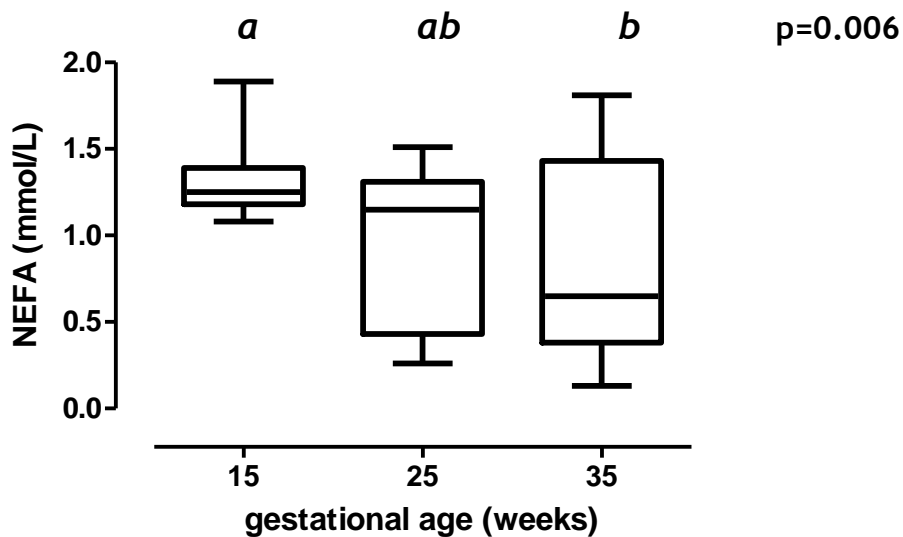
Figure 5.13 Temporal changes in NEFA levels in OW/OB pregnancy. Gestational time points are illustrated on the x axis and raw values are quotes on the y axis. Analysis performed on raw values following Ryan-Joiner normality testing. Analysis performed using repeated measures ANOVA, significant result if p≤0.01. Data with different superscript levels are significantly different from each other. As each of the means is denoted by [a, ab, b] this indicates there is no difference between the mean values at 15 weeks and 25 weeks and between 25 weeks and 35 weeks. However there was a significant difference between 15 weeks and 35 weeks.

Therefore the interaction between the group and time observed ('p time x type') in Figure 5.7 is explained by the fact that NEFA concentration remains stable in lean women but fall in OW/OB women during pregnancy.

5.8.2 C-reactive protein

Lean women did not show a significant change in CRP levels during pregnancy (p=0.31), Figure 5.13.
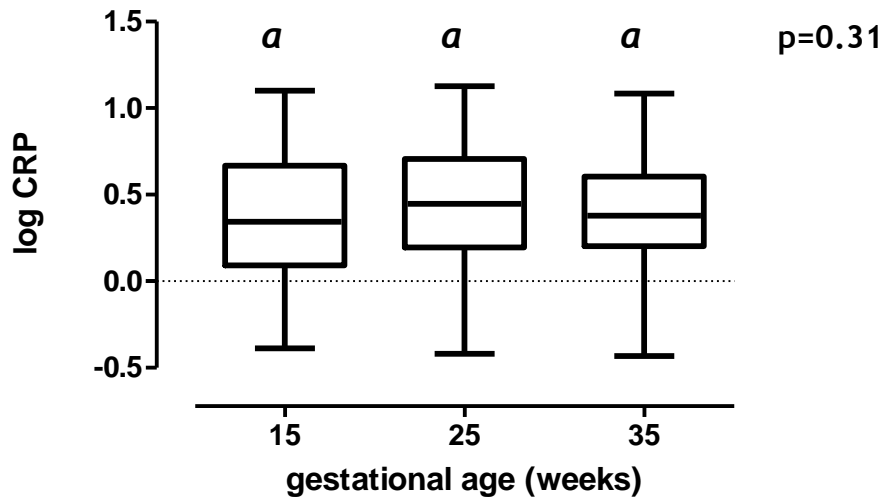
Figure 5.14 Temporal changes in CRP levels in lean pregnancy. Gestational time points are illustrated on the x axis and logged values are quotes on the y axis. Analysis performed on logged values following Ryan-Joiner normality testing. Analysis performed using repeated measures ANOVA, significant result if p≤0.01. Data with different superscript levels are significantly different from each other. As each of the means is denoted by [a] this indicates there is no difference between the mean values at each gestational time point.

OW/OB women had a significant fall in CRP levels as pregnancy progressed (p<0.0001), Figure 5.14, and *post hoc* analysis indicated that the fall in CRP was significant between 25 weeks and 35 weeks.
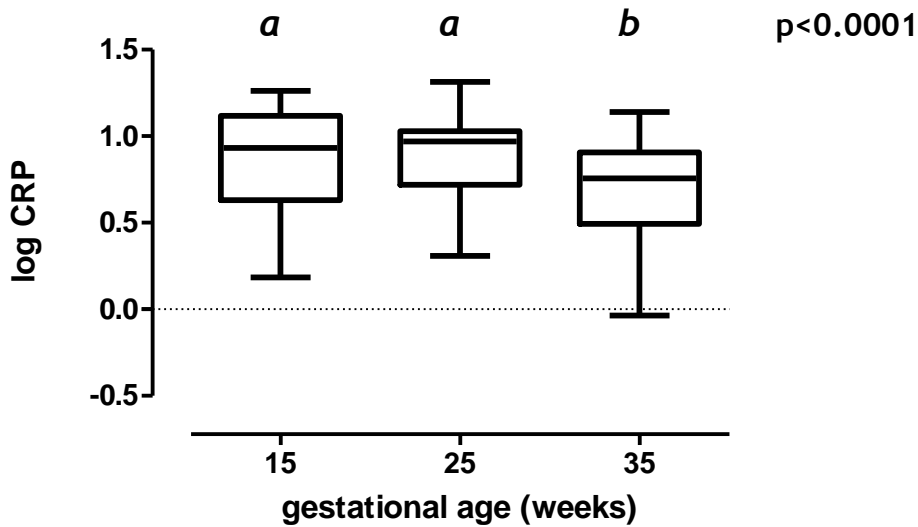
Figure 5.15 Temporal changes in CRP levels in OW/OB pregnancy. Gestational time points are illustrated on the x axis and logged values are quoted on the y axis. Analysis was performed on logged data after a Ryan-Joiner test showed a non-normal distribution. Analysis was performed using repeated measures ANOVA, significance level was p≤0.01. Data with different superscript levels [a,b] are significantly different from each other. There was no difference between the mean CRP at 15 weeks and 25 weeks. Between 15 weeks and 35 weeks and between 25 weeks and 35 weeks there was a significant fall in CRP level.

Therefore the interaction between the group and time observed ('p time x type') in Figure 5.8 is explained by the fact that CRP levels remains stable in lean women but fall in OW/OB women during late pregnancy.

5.8.3 Interleukin-6

Lean women showed a continual significant rise in IL6 levels during pregnancy (p<0.0001), Figure 5.15 which was not specific to either early or late pregnancy.
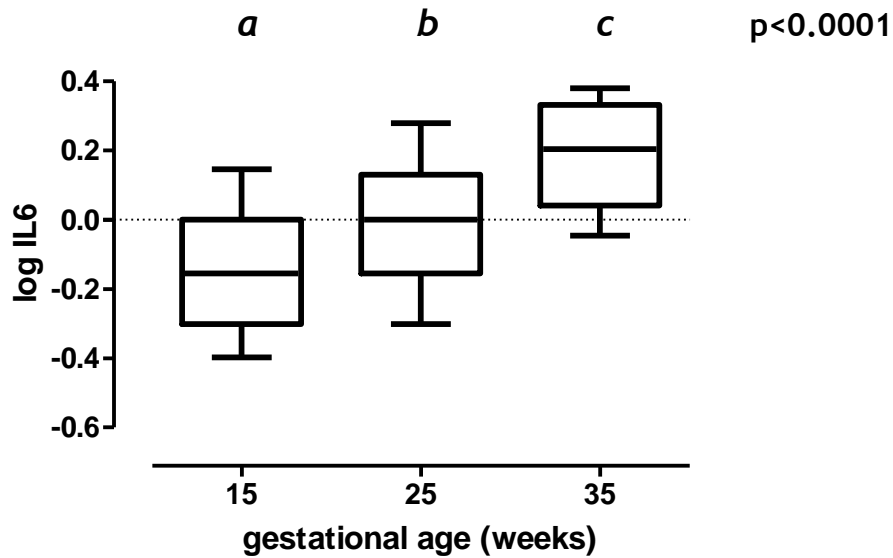
Figure 5.16 Temporal changes in IL6 levels in lean pregnancy. Gestational time points are illustrated on the x axis and logged values are quoted on the y axis. Analysis was performed on logged data after a Ryan-Joiner test showed a non-normal distribution. Analysis was performed using repeated measures ANOVA, significance level was p≤0.01. Data with different superscript levels [a,b,c] are significantly different from each other. There was a significant increase in IL6 between 15 weeks and 25 weeks, and between 25 weeks and 35 weeks, and between 15 weeks and 35 weeks.

OW/OB women also showed a significant rise in IL6 levels as pregnancy progressed (p=0.005), Figure 5.16. *Post hoc* analysis indicates that this rise in IL6 occurs throughout pregnancy rather than a specific gestational time period.
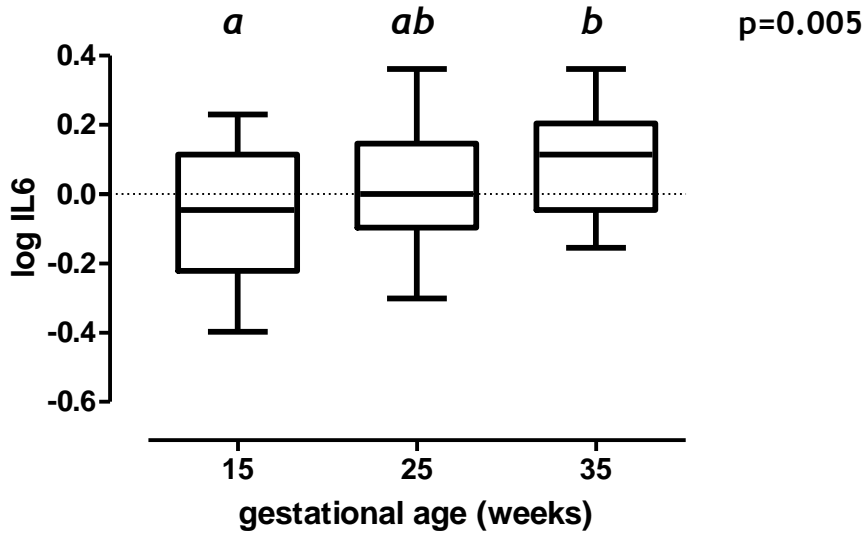
Figure 5.17 Temporal changes in IL6 levels in OW/OB pregnancy. Gestational time points are illustrated on the x axis and logged values are quoted on the y axis. Analysis was performed on logged data after a Ryan-Joiner test showed a non-normal distribution. Analysis was performed using repeated measures ANOVA, significance level was $p \leq 0.01$. Data with different superscript levels [a,b] are significantly different from each other. There was no difference in IL6 concentration between 15 weeks and 25 weeks or between 25 weeks and 35 weeks. However, between 15 weeks and 35 weeks IL6 did increase significantly.

Therefore, both lean and OW/OB women show a rise in IL6 during pregnancy. The significant interaction term in the linear mixed model analysis is explained by there being a greater increase in IL6 in the lean group compared to the OW/OB group.

5.9 Associations between the increase in total fat mass, anatomical fat distribution and plasma markers of carbohydrate & lipid metabolism and inflammatory profiles

Having assessed how plasma markers change in lean and OW/OB pregnancy, further analysis was performed to look for associations between changes in total fat mass and changes in anatomical location of subcutaneous adipose tissue (total body, upper body peripheral and lower body skinfolds) and changes in carbohydrate, lipid or inflammatory plasma markers. Where there were no interactions between group and gestation in the initial analysis described above (fasting glucose, total cholesterol, total triglycerides, fasting insulin, TNFα, and HDL), the lean and OW/OB groups were combined as a single cohort to analyse relationships between changes in body fat distribution and changes in plasma biomarkers. Where an interaction between lean and OW/OB group and gestation existed i.e. for NEFA, CRP and IL6, associations between changes in total fat mass and grouped skinfolds with changes in plasma biomarkers was assessed separately for each group.

Univariate analysis was performed using Pearson's correlations, and significance level was set at $p \leq 0.01$ to mitigate for multiple testing. If a significant univariate association was found, multivariate analysis was performed using the General Linear Model (significance $p \leq 0.05$). The anthropometric measures included in this analysis were fat mass, total body skinfolds, upper body peripheral skinfolds and lower body peripheral skinfolds.

As shown in Table 5.1, there were no significant correlations between the absolute change in plasma markers and absolute change in fat mass and anatomical skinfold groups using the whole cohort. There were trends for negative associations between change in LBS and change in total cholesterol, total triglyceride and a positive association with plasma glucose but these did not reach significance. As there were no significant univariate associations, no further analysis was performed.

Table 5.1 Univariate analysis of the association between the gestational change in fat mass and skinfolds and the change in carbohydrate, lipid and inflammatory profiles. The table shows Pearson correlation and p values for plasma markers which changed significantly across gestation or between the groups. Significant result if $p \leq 0.01$. Total body skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as LBS.

Plasma marker V1-V3	Fat mass V1-V3	TBS V1-V3	UPBS V1-V3	LBS V1- V3
Total cholesterol				
Pearson's correlation	0.082	-0.027	0.254	-0.322
p value	0.62	0.87	0.11	0.043
Total triglycerides				
Pearson's correlation	0.167	-0.125	0.032	-0.297
p value	0.31	0.44	0.84	0.063
HDL				
Pearson's correlation	-0.044	0.046	0.074	0.076
p value	0.79	0.78	0.65	0.64
Fasting glucose				
Pearson's correlation	0.133	0.269	0.029	0.297
p value	0.42	0.093	0.86	0.062
Fasting insulin				
Pearson's correlation	0.143	0.013	-0.150	0.061
p value	0.39	0.94	0.36	0.71
TNFα				
Pearson's correlation	-0.078	0.158	0.234	-0.010
p value	0.65	0.34	0.16	0.95

5.9.1 Association between gestational change in obesity measures and gestational change in NEFA

5.9.1.1 Lean pregnancy

Univariate analysis was performed using Pearson correlation to assess the relationship between the change in NEFA and change in fat mass, total body, upper body peripheral and lower body skinfolds (Table 5.2). There were no significant correlations found in this analysis.

Table 5.2 Univariate analysis of the association between the gestational change in NEFA and the gestational change in anatomical and total fat in lean pregnancy. Analysis was performed using Pearson's correlation, significant result if p<0.01. Total body skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as LBS.

	Fat mass V1-V3	TBS V1-V3	UPBS V1-V3	LBS V1-V3
V1-V3 NEFA				
Pearson correlation	-0.103	-0.277	-0.050	-0.187
P value	0.63	0.18	0.81	0.14

5.9.1.2 OW/OB pregnancy

Univariate analysis was performed using Pearson correlation to assess the relationship between the absolute change in NEFA and change in fat mass, total body, upper body peripheral and lower body skinfolds (Table 5.3). There were no significant relationships between the change in NEFA and the change in fat mass total or grouped skinfolds.

Table 5.3 Univariate analysis of the association between the gestational change in NEFA and the gestational change in anatomical and total fat in OW/OB pregnancy. Analysis was performed using Pearson's correlation, significant result if p<0.01. Total body skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as LBS.

	Fat mass V1-V3	TBS V1-V3	UPBS V1-V3	LBS V1-V3
V1-V3 NEFA				
Pearson correlation	-0.084	-0.069	0.290	-0.330
P value	0.77	0.81	0.30	0.23

5.9.2 Association between gestational change in obesity measures and gestational change in CRP

5.9.2.1 Lean pregnancy

Univariate analysis was performed using Pearson correlation to assess the relationship between the absolute change in CRP and fat mass, total body, upper body peripheral, abdominal and lower body skinfolds (Table 5.4). There were no significant associations between the grouped skinfolds and the change seen in CRP.

Table 5.4 Univariate analysis of the association between the gestational change in CRP and the gestational change in anatomical and total fat in lean pregnancy. Analysis was performed using Pearson's correlation, significant result if p<0.01. Total body skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as LBS.

	Fat mass V1-V3	TBS V1-V3	UPBS V1-V3	LBS V1-V3
V1-V3 CRP				
Pearson correlation	0.145	0.303	0.345	0.229
P value	0.50	0.14	0.091	0.27

5.9.2.2 OW/OB pregnancy

Univariate analysis was performed using Pearson correlation to assess the relationship between the absolute change in CRP and the change in fat mass, total body, upper body peripheral and lower body skinfolds (Table 5.5). There were no significant relationships between the change in CRP and the change in fat mass total or grouped skinfolds.

Table 5.5 Univariate analysis of the association between the gestational change in CRP and the gestational change in anatomical and total fat in OW/OB pregnancy. Analysis was performed using Pearson's correlation, significant result if p<0.01. Total body skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as LBS.

	Fat mass V1-V3	TBS V1-V3	UPBS V1-V3	LBS V1-V3
V1-V3 CRP				
Pearson correlation	0.359	0.021	-0.148	0.045
P value	0.19	0.94	0.60	0.88

5.9.3 Association between gestational change in obesity measures and gestational change in IL6

5.9.3.1 Lean pregnancy

Univariate analysis was performed using Pearson correlation to assess the relationship between the absolute change in IL6 and fat mass, total body, upper body peripheral and lower body skinfolds (Table 5.6). There were no significant associations between gestational change in IL6 and grouped skinfolds in lean pregnancy.

Table 5.6 Univariate analysis of the association between the gestational change in IL6 and the gestational change in anatomical and total fat in lean pregnancy. Analysis was performed using Pearson's correlation, significant result if p<0.01. Total body skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as LBS.

	Fat mass V1-V3	TBS V1-V3	UPBS V1-V3	LBS V1-V3
V1-V3 IL6				
Pearson correlation	0.356	0.289	0.087	0.113
P value	0.088	0.16	0.68	0.59

5.9.3.2 OW/OB pregnancy

Univariate analysis was performed using Pearson correlation to assess the relationship between the absolute change in IL6 and change in fat mass, total body, upper body peripheral and lower body skinfolds (Table 5.7). There were no significant relationships between the change in IL6 and the change in fat mass total or grouped skinfolds in OW/OB pregnancy.

Table 5.7 Univariate analysis of the association between the gestational change in IL6 and the gestational change in anatomical and total fat in OW/OB pregnancy. Analysis was performed using Pearson's correlation, significant result if p<0.01. Total body skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as LBS.

	Fat mass V1-V3	TBS V1-V3	UPBS V1-V3	LBS V1-V3
V1-V3 IL6				
Pearson correlation	-0.068	0.133	0.040	0.011
P value	0.81	0.64	0.89	0.97

5.10 Discussion

There were no associations between the increase in total fat mass, total skinfolds and upper body peripheral skinfolds and changes in plasma biomarkers. There was a trend for the increase in lower body subcutaneous adipose depots to be inversely associated with the change in total cholesterol and total triglycerides, although this did not reach significance. These results may suggest that those women who gain more fat in lower body depots during pregnancy have an adverse lipid response to pregnancy, but this would require to be verified in a larger sample size study. Published data from the general adult population show that lower body fat is more insulin sensitive (Jensen, 2008) and smaller thigh fat depots are considered a risk factor for high levels of plasma triglyceride, low plasma HDL concentration and hyperglycaemia (Snijder et al., 2005).

The gestational increases in both total cholesterol and total triglycerides were as expected and have been observed before (Salameh and Mastrogiannis, 1994). The rise in total triglycerides and fall in plasma HDL is a likely response to the developing gestational insulin resistance secondary to rising levels of pregnancy related hormones (Emet et al., 2013, Salameh and Mastrogiannis, 1994) and patterns of change in HOMA, TG and HDL suggest that obese women have a shift towards a metabolic syndrome profile at all gestational time points. There was a significant rise in total triglycerides across gestation with a trend for the OW/OB group to have higher levels (p=0.017). However, the maximum concentrations reached by 35 weeks were very similar (lean 2.5mmol/L versus OW/OB 2.68mmol/L) in the groups confirming previous observations (Meyer et al., 2013). This suggests that a similar limiting factor, possibly lipoprotein lipase concentration or VLDL secretion rate, in the third trimester limits the maximum plasma triglyceride concentration reached. High plasma triglycerides concentrations are linked with greater oxidative stress and the production of small, dense LDL which are easily oxidised. High concentrations of oxidised lipids are associated with vascular damage (Norata et al., 2003). Current literature has found that in approximately a third of obese mothers there are increased levels of LDL-III in the third trimester (Meyer et al., 2013). It will be interesting to assess small, dense LDL-III levels in the present study and aliquots of plasma

have been retained for future ascertainment. The low HDL and high triglycerides exhibited by the OW/OB women in the current study suggest they have metabolic syndrome but that they are not in the subset of women most at risk of vascular damage through higher levels of LDL-III.

Total cholesterol increased by approximately 25% and interestingly there were no lean/OW/OB group differences as observed for triglycerides. This might suggest that gestational cholesterol mobilisation is not related to gestational insulin resistance. The magnitude of the increase in plasma cholesterol is smaller than that of plasma triglyceride, suggesting a lower extent of cholesterol mobilisation or greater cholesterol utilisation than that for triglycerides by the mother and/or fetus.

Lean women in this study had higher levels of HDL than OW/OB women, which is consistent with the current literature (Scifres et al., 2014, Stewart et al., 2007a, Ramsay et al., 2002a). A large recent study (n=225) (Scifres et al., 2014), has shown, similarly to the current data, that there was a peak in HDL concentration at 25 weeks' gestation and then a fall in the third trimester in all pregnancies. This adds significant support to this finding of a peak HDL concentration mid-gestation. HDL has been shown to offer vascular protection via a number of mechanisms including increased production of NO via upregulation of eNOS expression (Mineo et al., 2006, Besler et al., 2012), preventing the formation of toxic oxidised lipid species (Mackness et al., 1993) and acting as an antioxidant (Besler et al., 2011). These properties suggest that HDL might confer significant protection to the maternal vascular endothelium in the face of the developing gestational hyperlipidaemia. The synthesis of HDL is determined by the production and secretion of apolipoprotein-A1 (Apo-A1), the main protein in HDL. However, the alterations in HDL concentration and function in pregnancy have not been fully investigated (Besler et al., 2012). An impaired improvement in endothelial function is a feature of obese pregnant women (Stewart et al., 2007a). In the current study, although HDL concentrations were significantly higher in lean women, the pattern of change across pregnancy was the same. This suggests that the signal that determines HDL production in pregnancy are the same in both groups, i.e. the biosynthesis of apo-A1 regulated by oestrogens rather than an obesity-driven effect such as the negative impact of

hypertriglyceridaemia on plasma HDL concentration. The higher levels of HDL found in the lean cohort may result in a more pronounced protective effect of HDL on vascular function than that in obesity where lower levels of HDL may lead to an increased susceptibility to vascular damage. This would be an interesting hypothesis to test in this data set at a later date.

There was no significant association of plasma biomarkers with subcutaneous adipose depots. A possible explanation for the lack of association may be that there is an unmeasured fat depot unaccounted for in the present analysis having a confounding impact on these metabolic parameters. Changes in the visceral fat compartment or gluteal skinfolds were not assessed. Recent published data has suggested that gluteal but not abdominal subcutaneous adipose tissue have higher rates of gene expression involved in adipocyte differentiation (Divoux et al., 2014). Data presented in Chapter 3 suggest that there may be a contribution from the visceral compartment which cannot be measured using skinfold analysis or air displacement plethysmography. Alternatively the observed trends may not reach significance because of the small sample size.

Where there was a significant interaction between the lean and OW/OB group then analysis was performed separately within each group, this was performed for CRP, IL6 and NEFA There were no significant associations between total fat mass, total skinfolds and fat distribution with the changes seen in these markers in either lean or OW/OB women suggesting that there is no innate differences in subcutaneous fat mass accumulation between lean and OW/OB women that account for the differences in these plasma markers. However, by separating the lean and OW/OB women for this analysis the sample size did become smaller and differences are more difficult to evaluate.

The majority of the gestational changes (increases in total cholesterol, total triglycerides, fasting insulin, HOMA, and IL6 and fall in CRP) and group differences (higher HOMA, fasting insulin, CRP and TNFα, and lower HDL) in OW/OB pregnancies in plasma biomarkers observed in this study are similar to those previously observed by others (Catalano et al., 1991, Catalano et al., 1999, Salameh and Mastrogiannis, 1994, Meyer et al., 2013, Ramsay et al.,

2002a, Scifres et al., 2014, Stewart et al., 2007a).There are however some interesting findings worth exploring in more detail.

Throughout pregnancy, TNFα remained significantly higher in the OW/OB group but its concentration did not change significantly across gestation. Current literature suggests that during gestation, TNFα rises and peaks in the second trimester (Beckmann et al., 1997) and that this plasma marker correlates significantly with insulin sensitivity in non-diabetic healthy pregnancies (Kirwan et al., 2002). In our study, we did not find a significant association between the change in TNFα and the change in insulin resistance (r=0.016, p=0.92), as TNFα remains stable during pregnancy whereas HOMA increases significantly. This could suggest that in the current study TNFα may not be contributing as much to insulin resistance as in other cohorts (Hotamisligil et al., 1996).

As expected CRP levels were significantly higher in the OW/OB group than the lean group throughout pregnancy (p<0.0001). However, the pattern of change in CRP concentration was different between the groups (p time x type=0.003). When the groups were assessed separately CRP fell significantly in the OW/OB group (p<0.0001) but not the lean group (p=0.57). Consistent with our findings, other authors have seen a fall in CRP during gestation, (Stewart et al., 2007a). It is possible that, in the relatively healthy OW/OB women studied here, in late pregnancy the increase in lipolysis from adipose tissue may relieve the inflammation at the fat depot, and potentially the liver, leading to a fall in plasma CRP. CRP is also known to be produced in the placenta (Malek et al., 2006). The observed fall in plasma CRP in OW/OB women could alternatively represent a reduction in the placental production of CRP in healthy OW/OB pregnancies which may ameliorate any potential for placenta complications in this healthy OW/OB group.

During pregnancy IL6 plasma levels rose significantly (p<0.0001), and were higher in lean women. Previous authors have found IL6 to be significantly higher in obese women throughout pregnancy and compared to published data our OW/OB women had relatively low levels of IL6 (Stewart et al., 2007a) again indicating they were healthy OW/OB individuals. Interestingly, in the linear mixed model analysis there was a significant difference in the pattern of change

in IL6 concentrations between the lean and OW/OB group. This was because the lean women have a significant rise in IL6 concentrations between all gestational time point (15, 25 and 35 weeks) but the OW/OB group the increase was more gradual and the significant increase was only seen between 15 weeks and 35 weeks. On inspection of Figure 5.9, the actual concentrations of IL6 are very similar between the groups. In healthy pregnancies, there is significant remodelling of adipose tissue undergoing both hyperplasia, where pre-adipocytes are recruited to make new adipocytes, and a degree hypertrophy of existing adipocytes (McLaughlin et al., 2007) with a concurrent rise in serum inflammatory markers including IL6. The increase in adipokine secretion is considerably enhanced in obese individuals where excessive adipocyte hypertrophy takes place leading to hypoxic areas of adipose tissue, infiltration of macrophages and an inflammatory response. IL6 is also thought to play a part in the development of gestational physiological insulin resistance through the inhibition of the action of insulin at muscle, liver and adipocyte (Hutley and Prins, 2005). The fact that lean healthy women show an increase in IL6 suggests that IL6 may play a role in healthy pregnancy perhaps to generate the gestational insulin resistance, although little published data exists to support this.

The trend for lean women to have higher levels of NEFA and the decrease in concentration of NEFA in the OW/OB group was not expected. In the current lean cohort, measured NEFA levels were higher than in other studies (Meyer et al., 2013). Different methodologies were employed by different studies; the current analysis was performed by autoanalyser compared to the manual method employed by other authors (Meyer et al., 2013) which may account for the difference in concentrations.

Our observations on NEFA concentration were a 'snap shot' at different time points. Figure 5.18 illustrates the various sources of NEFA as well as the various outputs which contribute to plasma NEFA concentrations. The data presented here suggest that OW/OB women have either decreased production (lipolysis), or increased utilisation (placental transport) or increased storage either in adipose tissue or ectopically in liver or placenta by the third trimester. Placentae from obese mothers at term have been found to have significantly more lipid content

than those from lean women (Saben et al., 2014). As well as placental storage, the fetus requires NEFA for many aspects of development. Longitudinal studies have found that the offspring of obese mothers have greater percentage body fat levels compared to babies of lean mothers (Catalano et al., 2009) often referred to as the "overnutrition hypothesis". Increased deposition of fatty acids into each of these 'depots' may account for lower levels of plasma NEFA in OW/OB pregnancies.
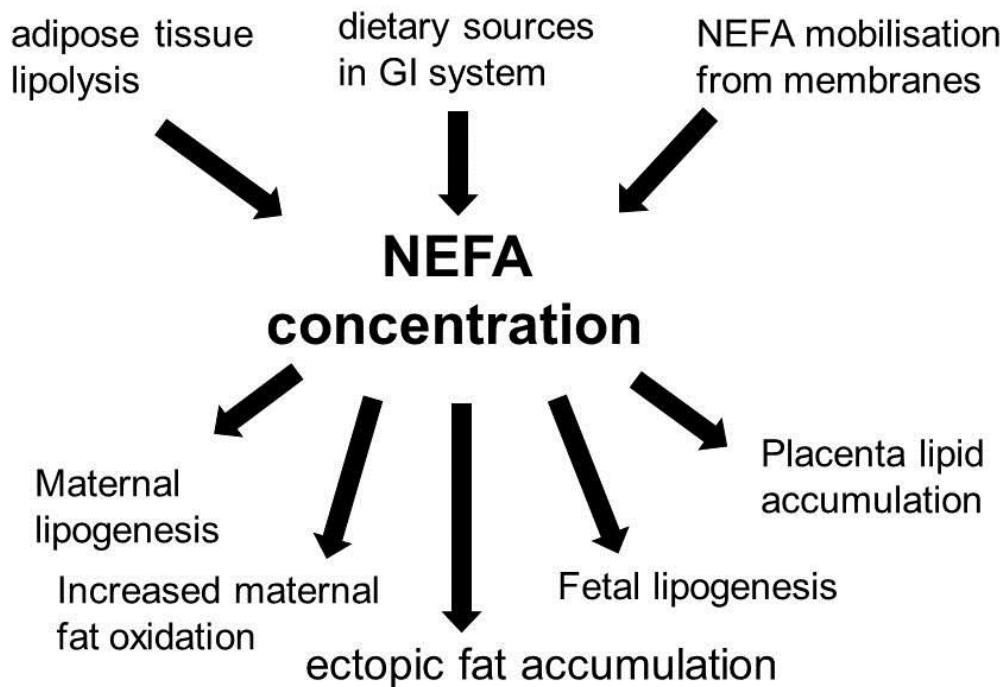
5.18 The inputs and outputs demand to the plasma NEFA compartment. The figure represents the various inputs and output pathways which can lead to a raised or decreased NEFA concentration in the maternal circulation, illustrating the difficulties in interpretation of steady state NEFA concentration during pregnancy.

The timing of sample collection may also have had an impact on the NEFA concentrations. All samples were collected from study participants after an overnight fast. OW/OB individuals have greater basal metabolic demands purely based on increased weight as shown in Chapter 4. Fat oxidation was on average 35.6% higher in OW/OB pregnancies (p type<0.0001, Chapter 4, section 4.4.4, Figure 4.4) and fat oxidation increased in OW/OB women as pregnancy progressed (Figure 4.4). These results may reflect the predominant utilisation of fat as an energy source by OW/OB women which would be more pronounced in

the fasting state leading to lower fasting NEFA levels than lean women which fall as pregnancy progresses.

As highlighted above, the amount of total fat accretion and site of subcutaneous fat storage has little impact on the gestational response of plasma biomarkers. In fact, healthy lean and OW/OB pregnancies show many similarities in this response. Both lean and OW/OB women have similar concentrations of IL6, total triglyceride and total cholesterol at the end of pregnancy despite the OW/OB group exhibiting more insulin resistance and some aspects of inflammation (CRP and TNFα). Lean women have higher levels of HDL. It is likely that our OW/OB women were relatively healthy leaving the groups too similar to tease out all metabolic differences associated with maternal obesity or their association with fat distribution in pregnancy. This may indicate that our OW/OB group is different to those studied by other authors as they are non-smokers, have higher socioeconomic scores and include some overweight rather than obese individuals. This suggestion is supported by the demographic differences between our OW/OB cohort and other obese populations. In Stewart et al, 40% of the obese women were smokers, no women in either lean or OW/OB group smoked in the present study. The OW/OB group presented here was well educated (88% of lean women and 75% of OW/OB women attended university) and came from high socioeconomic classes (73% of lean women and 44% of OW/OB women were in upper half of 2009 SMID [Scottish multiple index of deprivation] decile for social deprivation). The demographics of the Stewart et al cohort indicated that only 43% of the non-obese group and 41% of the obese group were in either affluent or intermediate DEPCAT deprivation score categories. Methodology for assessing social deprivation has changed between these two studies therefore direct comparison is difficult, however this may be a factor explaining the metabolic differences with previous data in obese women published from this laboratory. Plasma markers of inflammation (IL6 and CRP) have been found to be associated with poorer socioeconomic backgrounds (Packard et al., 2011). In addition, in the study published by Stewart et al, the obese group was heavier than our group with a mean BMI of 34.2 kg/m^2 (SD4.5) compared to a mean BMI of 31.5 kg/m^2 (SD2.7) in the present study.

The strength of the current study is a consistency in methodology across all gestational time points. A breadth of markers for carbohydrate, lipid and inflammatory profiles were studied which gave a comprehensive analysis. There were limitations to the study. Assessment of adiposity by skinfold thickness does not assess functionality of adipose tissue which may be more important than the actual size of the depot. The more atherogenic LDL-III that has been used to identify an atherogenic lipoprotein phenotype was not measured, although samples are available for future analysis. In addition, the OW/OB group recruited includes women who are apparently healthy and this may account for some of the unexpected data. Finally gestational changes were assessed as the absolute concentration at 35 weeks minus the concentration at 15 weeks. This may be too simple a measure of gestational adaptation and further analysis of the data could assess gestational changes using incremental area under curve to try and convey how these plasma markers change during pregnancy.

In conclusion, the majority of gestational carbohydrate, lipid and inflammatory profiles observed in this study support current published data. Some of the differences been between the lean and OW/OB women were anticipated with the OW/OB group were more insulin resistant and pro-inflammatory (CRP and TNFα) than lean women and lean women having higher concentration of plasma HDL. This suggests that these OW/OB women exhibit a degree of metabolic syndrome. However, in the lean group the higher levels of inflammation (IL6) which may reflect a physiological rather than pathological process. The observed differences in lean and OW/OB women in plasma NEFA concentration was difficult to explain and may be multifactorial. The presented data showed that in both lean and OW/OB pregnancies the studied subcutaneous adipose depots did not contribute to the gestational changes in markers of carbohydrate, lipid and inflammatory profiles, but that there is accepted limitations to the above cohort which may be the reason for the lack of associations. Further exploration of the data and inclusion of other depots may yield different results. Therefore the results of this chapter suggest that central subcutaneous fat depots may not be important in the development of adverse metabolic profiles. These findings therefore do not support the hypothesis that OW/OB women gain subcutaneous

fat in the central compartments and that this leads to the carbohydrate, lipid and inflammatory profiles observed in the gestational metabolic syndrome.

Chapter 6 - Gestational measures of microvascular function, oxidative stress and lipotoxicity in lean and OW/OB pregnancies

6.1 Introduction

Pregnancy is a time of immense physiological stress and involves adaptation of the maternal cardiovascular system. The main adaptation seen is peripheral vasodilatation thought to be mediated through endothelium-dependent factors upregulated by oestradiol and prostaglandins.

Published data have shown that, in the third trimester, obese women have a poorer microvascular response to ACH iontophoresis than lean women, indicating poorer endothelium-dependent vasodilatation (Ramsay et al., 2002a). It has been proposed that in maternal obesity the reduced improvement in endothelial function is related to a pathological pathway involving increased plasma lipids with the promotion of oxidative stress (Vincent and Taylor, 2005). There are a number of biomarkers which have been linked to endothelial dysfunction through the process of oxidative stress and these have been detailed in Chapter 1 including GGT, pro-inflammatory lipoproteins such as oxLDL, superoxide species and more novel markers such as urinary isoprostanes. Endothelial dysfunction can also be assessed by measuring *in vivo* markers of endothelial activation such as sVCAM-1 and sICAM-1 which have been found to be elevated in pregnancies complicated by pre-eclampsia (Chaiworapongsa et al., 2002) where endothelial damage is a hallmark of the disease. Interestingly, little research has looked at the gestational changes in these markers of lipotoxicity and their impact on microvascular function in pregnancy. The following chapter assesses microvascular function and lipotoxicity markers during pregnancy in lean and OW/OB women. The relationships of microvascular function and lipotoxicity to anatomical fat distribution and accumulation was also studied.

6.2 Research Questions

1. During pregnancy, are there changes in peripheral microvascular function and the concentration of plasma and urinary markers of lipotoxicity and oxidative stress?
2. Are there differences between lean women and OW/OB women in terms of microvascular function and plasma and urinary markers of lipotoxicity and oxidative stress?
3. Are there differences in the pattern of change in concentration in these markers during pregnancy between lean and OW/OB pregnancies?
4. Does anatomical distribution of subcutaneous fat during pregnancy have an impact on the change in peripheral microvascular function and the change in concentrations of plasma and urinary markers of lipotoxicity and oxidative stress in lean and OW/OB pregnancies?

6.3 Methods

Peripheral vascular function assessment and plasma collection and methodology have been detailed in section 2.4 and 2.5 respectively of the General Methods Chapter 2. Further information regarding specific analytic techniques has been detailed in each section of this chapter. Throughout pregnancy blood volume changes. Before analysis was performed on this data, a haematocrit and haemoglobin (Quantichrom, BioAssay Systems, Universal Biologicals Ltd Cambridge, UK) were measured for each sample and the blood volume was corrected as per the method used by Dill and Costill (Dill and Costill, 1974).

6.4 Gestational changes in microvascular function

6.4.1 Endothelium-dependent microvascular function

The gestational response in endothelium-dependent microvascular function (EDMVF) was assessed across time and between groups (Figure 6.1). During pregnancy there was a significant improvement in EDMVF, 'p time'=0.010; mean visit 1 log perfusion response was 2.78 PU MΩ/min (standard error of the mean 0.46) and mean visit 3 log perfusion response was 4.61 PU MΩ/min (0.57).

There was no difference between the groups ('p type') or in the pattern of change in EDMVF response between the two groups, shown as the interaction term ('p time x group').
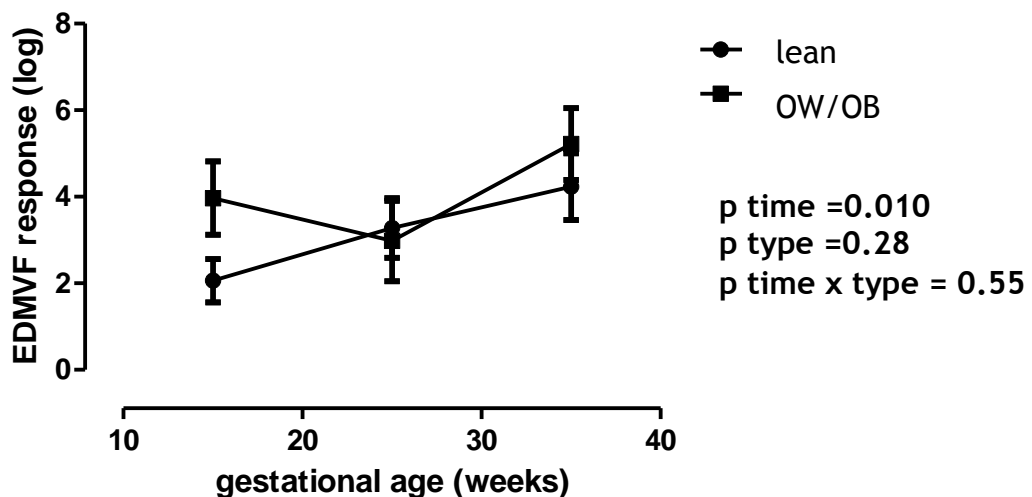
Figure 6.1 EDMVF response at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean EDMVF response (logged data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

6.4.2 Endothelium independent microvascular function

The gestational response seen in endothelium independent microvascular function (EIMVF) was assessed across time and between groups (Figure 6.2). There was no significant change in EIMVF response during pregnancy ('p time') and there was no difference observed between lean and OW/OB women ('p type'). The pattern of change in EIMVF response was not different between the groups ('p time x type').
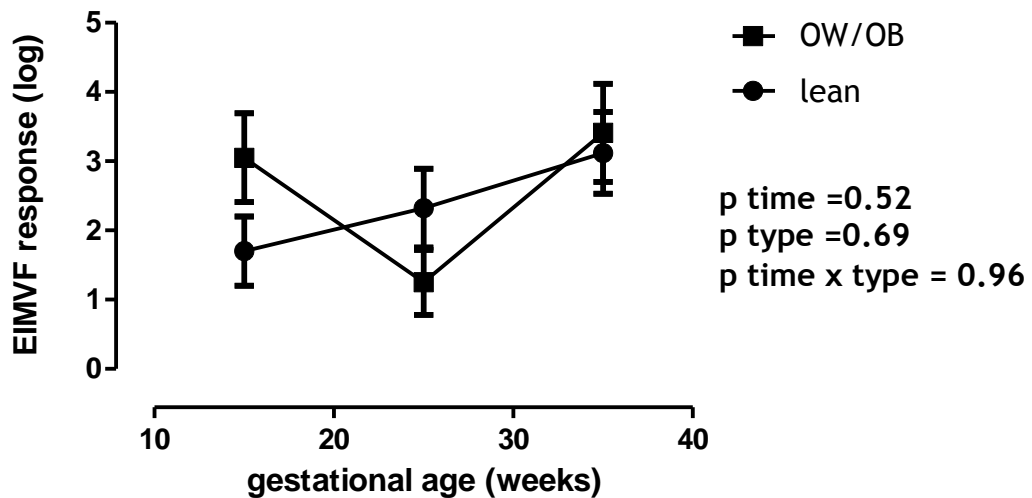
Figure 6.2 EIMVF response at each gestational time point in lean and OW/OB pregnancy.
Illustrated is the mean EIMVF response (logged data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

6.5 Plasma markers of vascular function and lipotoxicity

6.5.1 Soluble intercellular adhesion molecule-1

The change in plasma sICAM-1 was assessed across time and between the groups (Figure 6.3). Over the course of pregnancy sICAM-1 levels did not change ('p time'=0.57). There was a no difference between the two groups ('p type'=0.94). The pattern of change was not different between the two groups ('p time x type'=0.82).
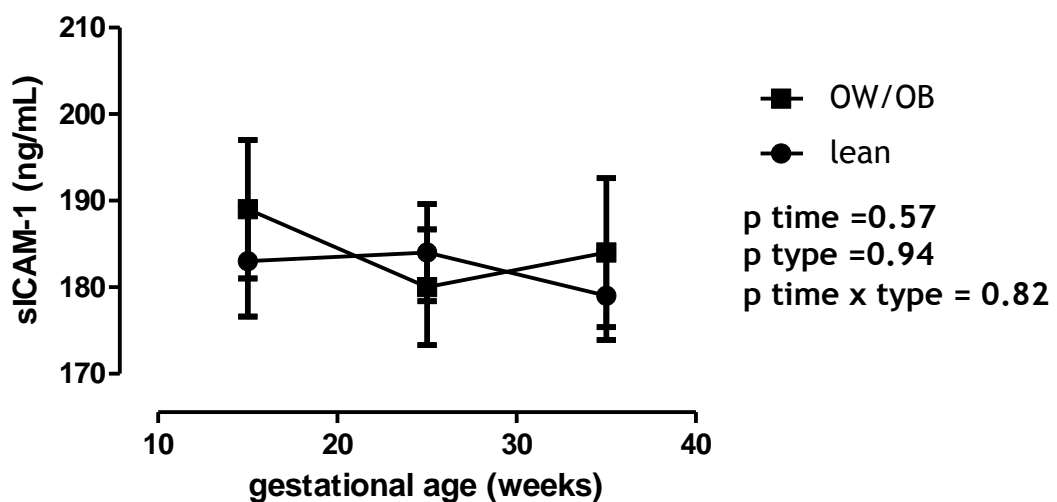
Figure 6.3 sICAM-1 at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean sICAM-1 concentration (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

6.5.2 Soluble vascular cell adhesion molecule-1

Using the linear mixed model plasma sVCAM-1 was assessed across time and between groups (Figure 6.4).

The concentration of sVCAM-1 increased significantly across gestation ('p time'<0.0001); mean visit 1 concentration was 641ng/mL (SEM 20) compared with mean visit 3 concentration 757ng/mL (29).

There was a trend for lean women to have higher concentrations of sVCAM-1 during pregnancy ('p type'=0.028), mean lean concentration 694ng/mL versus OW/OB 629ng/mL. There was no difference pattern of change ('p time x group') in sVCAM-1 levels.
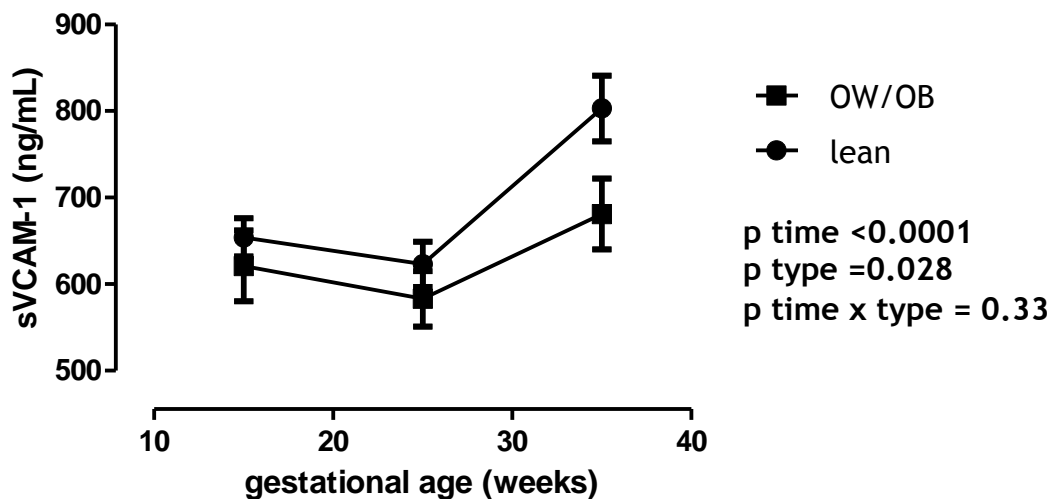
Figure 6.4 sVCAM-1 at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean sVCAM-1 concentration (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

6.5.3 Oxidised low density lipoprotein

The concentration of oxLDL was assessed across time and between the groups (Figure 6.5).

There was a significant rise in oxLDL during pregnancy ('p time'<0.0001); mean visit 1 concentration was 58.6U/L (SEM 2.6) and visit 3 concentration was 82.7U/L (3.5). However there were no significant differences between groups or in the pattern of change.
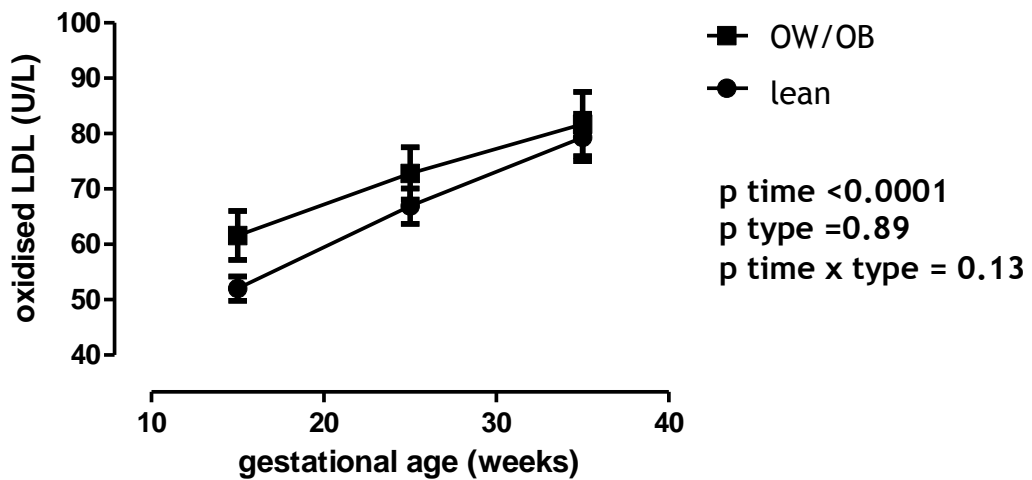
Figure 6.5 oxLDL at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean oxLDL concentration (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

6.5.4 Gamma-glutamyl transferase

The change in GGT concentration was assessed across time and between the groups (figure 6.6). The levels of GGT did not change significantly over the course of pregnancy (p time=0.98). There was no differences between the groups (p time=0.37) or in the pattern of change in GGT (p time x type=0.093).
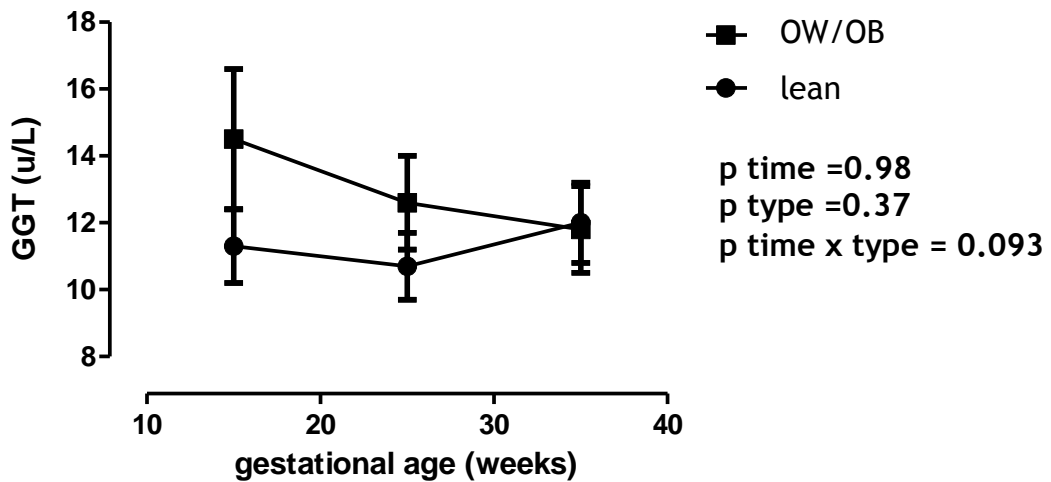
Figure 6.6 GGT at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean GGT concentration (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

6.5.5 Urinary isoprostanes

Concentration of urinary isoprostanes was assessed across time and between the groups (Figure 6.7).

There was a trend for the concentration of urinary isoprostanes to rise during pregnancy; mean visit 1 concentration 8.2ug/mmol (SEM 0.9) versus mean visit 3 concentration 9.5ug/mmol (0.7), but this did not reached significance ('p time'=0.029).

There was no significant difference between the lean and OW/OB women and the pattern of change in the level of this urinary metabolite, was similar ('p time x type'=0.97).
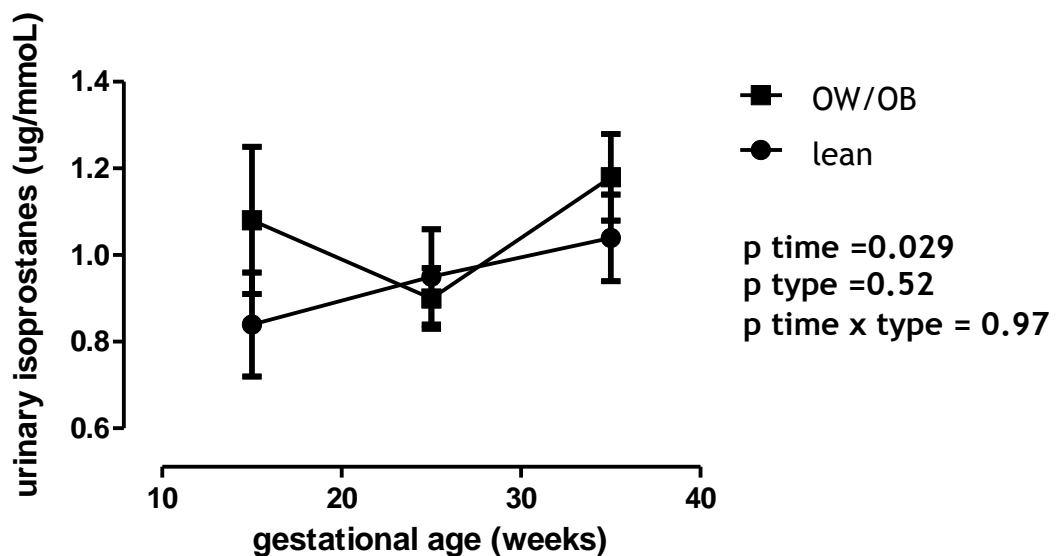
Figure 6.7 Urinary isoprostanes at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean urinary isoprostanes concentration (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

6.5.6 Plasma superoxide concentration

Plasma superoxide concentrations were measured in whole blood. Data was corrected for changes in blood volume as per the method used by Dill and Costill (Dill and Costill, 1974).

Plasma superoxide concentration was assessed across time and between the groups (figure 6.8). There was no significant change in superoxide levels during pregnancy ('p time'=0.48).There was no significant difference between the lean and OW/OB women and the pattern of change in the level of this plasma metabolite was similar.
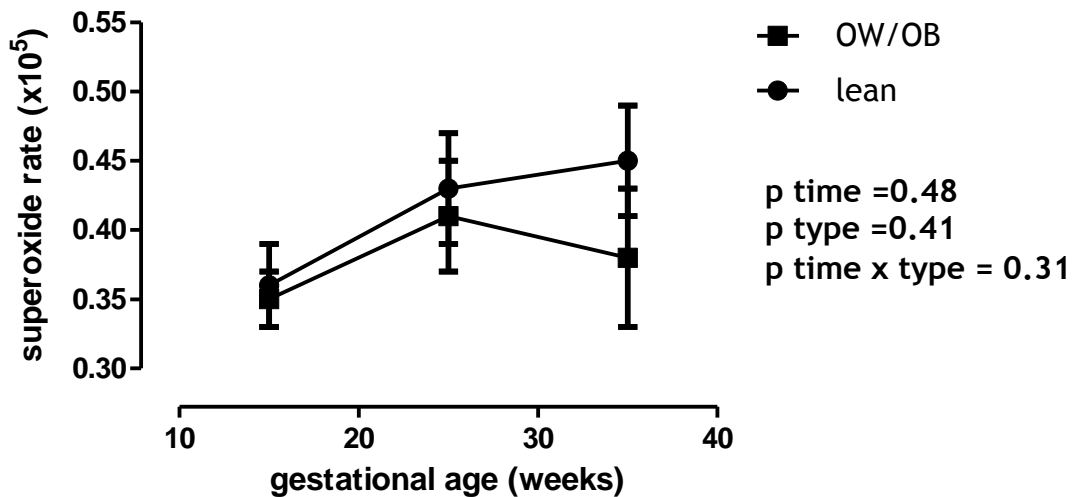
Figure 6.8 Plasma superoxide concentration at each gestational time point in lean and OW/OB pregnancy. Illustrated is the mean plasma superoxide concentration (raw data) and the standard error of the mean at each gestational time point in lean and OW/OB pregnancy. Differences across gestation were expressed as 'p time', between the lean and OW/OB groups as 'p type' and pattern of change in the variable between the groups as 'p time x type'.

6.6 Summary of gestational changes seen in microvascular function and markers of lipotoxicity
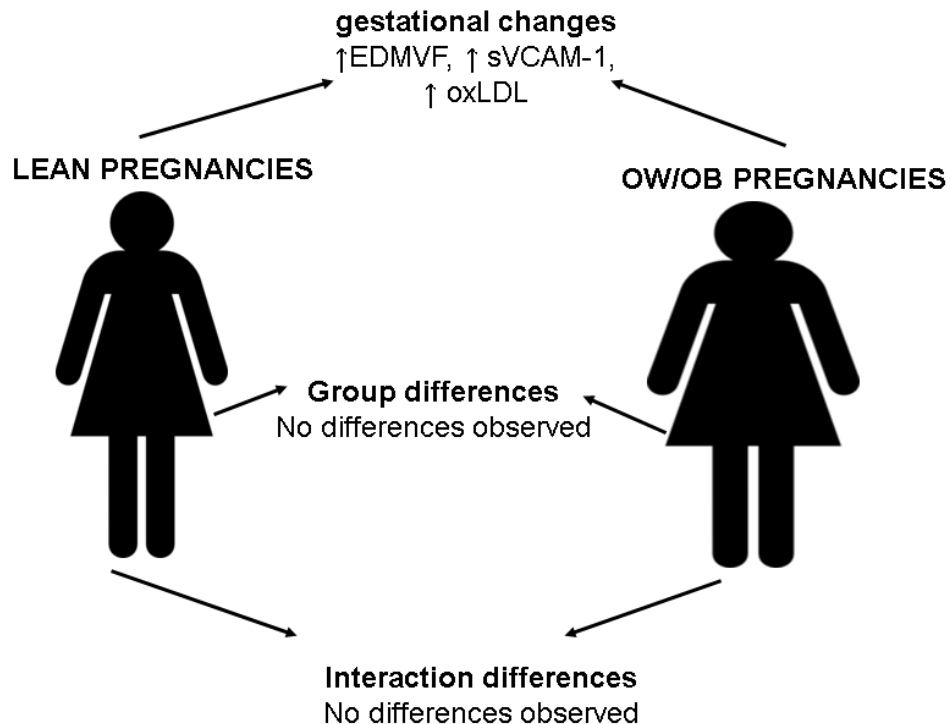
Figure 6.9 Summary of evidence found for endothelial function, activation and lipotoxicity in lean and OW/OB pregnancy. The above figure indicates that there are only gestational changes in vascular function and lipotoxic markers during pregnancy but there are no differences observed between the groups or any differences in the pattern of changes between the groups (interaction differences) for these markers.

During pregnancy there were gestational changes which were the same for both lean and OW/OB pregnancy: both show an improvement in EDMVF and an increase in sVCAM-1 and oxidised LDL concentrations. There were no significant differences between lean and OW/OB women in microvascular function (EDMVF or EIMVF) or any of the markers of lipotoxicity measured. From the above analysis there was no difference in the pattern of change seen in endothelial function and markers of lipotoxicity in the lean and OW/OB pregnancies studied.

6.7 The relationship between anatomical fat distribution, endothelial function and markers of lipotoxicity in healthy pregnancy

From the above analysis, no differences were found between lean and OW/OB pregnancies in terms of endothelial function or markers of lipotoxicity. Therefore further analysis on the impact of anatomical fat distribution was performed on the entire cohort, with and without BMI as a covariate in the analysis.

Further analysis looked at EDMVF response, but not EIMVF response as there was no change in this across pregnancy. Plasma oxLDL, sVCAM-1 increased during pregnancy and were assessed. There was a trend for urinary isoprostanes to increase during pregnancy and this was included in the analysis.

Univariate analysis was performed using Pearson's correlation, and significance was $p \leq 0.01$. If significant results were found multivariate analysis was performed using the General Linear Model (significance $p \leq 0.05$). The anthropometric measures included in this analysis were fat mass, total body skinfolds, upper body peripheral skinfolds and lower body peripheral skinfolds.

6.7.1 EDMVF response

Univariate analysis was performed using Pearson correlation to assess the relationship between the change in EDMVF response and change in fat mass, total body, upper body peripheral and lower body skinfolds (Table 6.1). There were no significant correlations found in this analysis.

Table 6.1 Univariate analysis of the associations between the gestational change in EDMVF response and the gestational change seen in anatomical and total fat mass in entire cohort. Analysis was performed using Pearson's correlation, significant result if $p<0.01$. Total body skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as LBS.

	fat mass V1-V3	TBS V1-V3	UBPS V1-V3	LBS V1-V3
V1-V3 EDMVF response				
Pearson correlation	-0.233	-0.187	-0.161	-0.167
P value	0.15	0.25	0.32	0.30

6.7.2 Oxidised LDL

Univariate analysis was performed using Pearson correlation to assess the relationship between the change in oxLDL and change in fat mass, total body, upper body peripheral and lower body skinfolds (Table 6.2). There were no significant correlations found in this analysis.

Table 6.2 Univariate analysis of the associations between the gestational change in oxLDL and the gestational change seen in anatomical and total fat mass in entire cohort. Analysis was performed using Pearson's correlation, significant result if $p<0.01$. Total body skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as LBS.

	fat mass V1-V3	TBS V1-V3	UBPS V1-V3	LBS V1-V3
V1-V3 oxLDL				
Pearson correlation	0.003	-0.089	0.064	-0.252
P value	0.99	0.59	0.70	0.12

6.7.3 sVCAM-1

Univariate analysis was performed using Pearson correlation to assess the
relationship between the change in sVCAM-1 and change in fat mass, total body,
upper body peripheral and lower body skinfolds (Table 6.3). There were no
significant correlations found in this analysis.

**Table 6.3 Univariate analysis of the associations between the gestational change in
sVCAM-1 and the gestational change seen in anatomical and total fat mass in entire cohort.**
Analysis was performed using Pearson's correlation, significant result if p<0.01. Total body
skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as
LBS.

	fat mass V1-V3	TBS V1-V3	UBPS V1-V3	LBS V1-V3
V1-V3 sVCAM-1				
Pearson correlation	-0.111	-0.270	0.181	-0.281
P value	0.50	0.092	0.27	0.079

6.7.4 Urinary Isoprostanes

Univariate analysis was performed using Pearson correlation to assess the
relationship between the change in urinary isoprostanes and change in fat mass,
total body, upper body peripheral and lower body skinfolds (Table 6.4). There
were no significant correlations found in this analysis.

**Table 6.4 Univariate analysis of the associations between the gestational change in urinary
isoprostanes and the gestational change seen in anatomical and total fat mass in entire
cohort.** Analysis was performed using Pearson's correlation, significant result if p<0.01. Total body
skinfolds shown as TBS, upper body peripheral skinfolds as UPBS and lower body skinfolds as
LBS.

	fat mass V1-V3	TBS (V1-V3)	UBPS V1-V3	LBS V1-V3
V1-V3 urinary isoprostanes				
Pearson correlation	0.106	0.168	0.204	0.139
P value	0.53	0.31	0.21	0.40

6.8 EDMVF and other biomarkers

There was no significant impact of the change in fat mass or anatomical fat distribution on EDMVF or biomarkers of lipotoxicity. However, further analysis was performed to assess the impact of energy metabolism, carbohydrate, lipid and inflammatory profiles and markers of lipotoxicity on EDMVF response. This was performed using univariate analysis.

6.8.1 EDMVF response and parameters of energy metabolism

Univariate analysis was performed using Pearson correlation to assess the relationship between the change in EDMVF response and change in energy metabolism parameters (Table 6.5). There were no significant correlations found in this analysis.

Table 6.5 Univariate analysis of the change in EDMVF response and the changes in parameters of energy metabolism

Energy parameter V1-V3	EDMVF response V1-V3
BMR	
Pearson correlation	-0.250
P value	0.12
BMR/kg	
Pearson correlation	-0.249
P value	0.13
NPRER	
Pearson correlation	0.055
P value	0.74
Fat oxidation	
Pearson correlation	-0.118
P value	0.48
Carbohydrate oxidation	
Pearson correlation	0.005
P value	0.98
Sedentary activity	
Pearson correlation	0.097
P value	0.62
Light activity	
Pearson correlation	-0.041
P value	0.83
MVPA	
Pearson correlation	-0.204
P value	0.29
Daily energy intake	
Pearson correlation	-0.213
P value	0.19
Fat intake	
Pearson correlation	-0.190
P value	0.25
Carbohydrate intake	
Pearson correlation	-0.131
P value	0.43
Protein intake	
Pearson correlation	0.15
P value	0.38

6.8.2 EDMVF response and carbohydrate, lipid and inflammatory profiles

Univariate analysis was performed using Pearson correlation to assess the relationship between the change in EDMVF response and the change in the plasma concentrations of markers of carbohydrate, lipid and inflammatory profiles (Table 6.6).There was a trend for IL6 to be positively associated with the improvement seen in EDMVF response (p=0.047).

Table 6.6 Univariate analysis of the change in EDMVF response and the change in plasma concentrations of markers of carbohydrate, lipid and inflammatory profiles

Plasma marker V1-V3	EDMVF response V1-V3
Fasting glucose	
Pearson correlation	-0.020
P value	0.90
Insulin	
Pearson correlation	-0.088
P value	0.60
HOMA	
Pearson correlation	-0.084
P value	0.62
Total cholesterol	
Pearson correlation	-0.140
P value	0.40
Total triglycerides	
Pearson correlation	-0.034
P value	0.84
HDL	
Pearson correlation	-0.18
P value	0.29
NEFA	
Pearson correlation	0.013
P value	0.94
CRP	
Pearson correlation	-0.010
P value	0.95
IL6	
Pearson correlation	0.324
P value	0.047
TNFα	
Pearson correlation	0.021
P value	0.90

6.8.3 EDMVF response and markers of lipotoxicity

Univariate analysis was performed using Pearson correlation to assess the relationship between the change in EDMVF response and change in markers of lipotoxicity (Table 6.7). There were no significant correlations found in this analysis.

Table 6.7 Univariate analysis of the change in EDMVF response and the change in markers of lipotoxicity

Plasma marker V1-V3	EDMVF response V1-V3
sICAM-1	
Pearson correlation	-0.120
P value	0.48
sVCAM-1	
Pearson correlation	0.075
P value	0.66
oxLDL	
Pearson correlation	-0.050
P value	0.77
GGT	
Pearson correlation	-0.006
P value	0.97
Urinary isoprostanes	
Pearson correlation	-0.184
P value	0.28
superoxides	
Pearson correlation	-0.093
P value	0.62

6.9 Discussion

As pregnancy progressed there was a significant improvement in the endothelium dependent microvascular function (EDMVF) response and increases in plasma concentrations of sVCAM-1 and oxidised LDL. These gestational changes were similar in both the lean and the OW/OB group. Previous studies have indicated that although all women exhibit an improvement in EDMVF during pregnancy, obese women show less improvement than lean women (Stewart et al., 2007a).This was not found in the current study. However, as discussed in chapter 5, this cohort was overall healthier and more affluent than previous populations which may account for the different findings. Higher deprivation scores have been linked to higher risk of cardiovascular mortality in both diabetic and non-diabetic populations (Jackson et al., 2012). In addition, markers of chronic inflammation and endothelial activation (CRP, IL6 and sICAM-1) have been found to be significantly associated with poorer childhood living conditions and paternal manual occupations (Packard et al., 2011). There is also the obvious impact that smoking has on endothelial function (Ambrose and Barua, 2004), and in this study none of the participants smoked compared to previous study cohorts (Stewart et al., 2007a). The demographic similarities between the lean and OW/OB groups in our cohort may account for why no difference was seen in vascular function between our lean and OW/OB groups.

In healthy pregnancy, sVCAM-1 has been found to increase significantly as pregnancy progresses (Beckmann et al., 1997). In the present study sVCAM-1 increased significantly during gestation and these findings are consistent with published data in both lean and obese women (Stewart et al., 2007a). In the comprehensive study performed by Stewart et al, sVCAM-1 concentrations in both lean and OW/OB women were lower than our findings throughout pregnancy. In addition, Stewart et al did not find any differences between lean and obese women in each trimester in sVCAM-1. There are no reference standards so it is difficult to compare our findings to previous published data.

In the present study plasma IL6 concentration was higher in lean women compared to OW/OB participants. This may be a result of up-regulation of IL6 production by adipocytes in lean pregnancy if their adipose tissue has a greater

potential to undergo differentiation from pre-adipocytes to functional adipocytes compared to the adipose tissue of OW/OB individuals. In the mouse model, it has been suggested that IL6 plays a role in pulmonary vascular remodelling in pulmonary hypertension (Savale et al., 2009). Although this is an example of a pathological process, it does provide a potential explanation of higher IL6 levels as gestation advanced in this study. There does not appear to be any published data examining the role of IL6 in vascular remodelling in pregnancy. However, in our study, there was a trend for the increase in IL6 to be positively correlated with the improvement in EDMVF response (r=0.324, p=0.047).

Previous authors have shown that plasma oxLDL levels are higher in pregnant women than in non-pregnant controls (Makedou et al., 2011). In healthy human pregnancy, gestational changes in LDL profile favours smaller species, which is thought to be a result of the increase in plasma triglyceride concentrations. This preference for smaller species of LDL is associated with an increased level of oxidised LDL (Belo et al., 2004). Lean and OW/OB women showed a significant increase in oxLDL during pregnancy in the present study, but there were no difference between the groups. Other authors have shown that although LDL-III concentration is higher in OW/OB pregnant women, only a proportion of the obese subjects exhibited oxLDL-III in a high enough concentration to have an atherogenic lipoprotein phenotype (Meyer et al., 2013), which is an indicator of metabolic pathology (Anber et al., 1996). In the current study, the OW/OB mothers may not be in this subset of high risk women who develop an atherogenic lipoprotein phenotype which would lead to a larger amount of small dense LDL that could be easily oxidised. If this is true then the current OW/OB cohort could be considered low risk as they exhibit a similar improvement in their EDMVF response compared to the lean group. The absolute change in oxidised LDL was not significantly correlated with the improvement in the EDMVF response for the entire cohort (Pearson's correlation -0.050. p=0.77), which supports the theory that the OW/OB cohort did not include women with an atherogenic lipoprotein profile. In Meyer et al, the mean BMI of the obese group was $33.7 kg/m^2$ (SD 4.2), and in the current OW/OB group this was $31.5 kg/m^2$ (SD2.7). As the current OW/OB group had a lower BMI than the Meyer et al study, this may explain the lack of differences in oxLDL as well as in EDMVF

response and the other markers of lipotoxicity. Therefore, in order to see an effect of oxLDL concentration on EDMVF response, inclusion of women with higher BMI or perhaps greater central obesity may be required.

Isoprostanes are markers of oxidative stress, which are produced as a result of the non-enzymatic peroxidation of arachidonic acid (Vincent et al., 2007). Previous authors have noted that both urinary and plasma measurements of these compounds can be used as biomarkers in the estimation of lipid peroxidation (Cracowski et al., 2002). Published data have shown that in women, BMI significantly correlated with levels of urinary isoprostanes (8-iso-PGF$_{2\alpha}$) (Il'yasova et al., 2007, Keaney et al., 2003), and that in pregnancy women have elevated levels of both plasma and urinary isoprostanes (Ishihara et al., 2004). In the current analysis, there was some evidence that urinary isoprostanes rose during pregnancy (p=0.029) but there was no difference seen between lean and OW/OB women. Current literature suggests that plasma levels of isoprostanes are elevated in pre-menopausal women with increased central obesity (Crist et al., 2009). In the current study anthropometric differences were observed, but these findings related to upper body peripheral subcutaneous fat depots rather than either groups having preponderance for centrally accumulated subcutaneous adipose tissue. None the less, assessing plasma isoprostanes levels in both groups or stratifying the data based on waist hip ratio may be useful to assess the impact of lipid peroxidation and oxidative stress during pregnancy. Currently there is ongoing analysis of plasma samples from this cohort to ascertain the concentration of plasma isoprostanes.

The initial analysis of microvascular function and markers of lipotoxicity did not find any differences between lean and OW/OB women in this cohort. For this reason, further analysis of the impact of anatomical fat depots on the EDMVF response and lipotoxic biomarkers were performed on the entire cohort. In this univariate analysis no significant associations were found. Therefore, for this cohort during pregnancy there was no impact of an increase in either total fat mass or anatomical fat depots on the gestational change in endothelial function or markers of lipotoxicity. This result may also indicate that these specific subcutaneous fat depots do not have a detrimental impact on endothelial function or promote lipotoxicity during healthy pregnancy.

Further analysis explored associations between the improvement in EDMVF response and gestational changes in direct measurements of energy metabolism, plasma markers of carbohydrate, lipid and inflammatory profiles and markers of lipotoxicity. There were no significant associations found in this analysis.

A key strength of this present study was the use of laser Doppler imaging to assess microvascular function during pregnancy. The laboratory where the study was performed had experience of the technique (Ramsay et al., 2002a, Stewart et al., 2007a) and expertise in the methodology which was utilised by the researcher during the study (Ramsay et al., 2002b). The technique used to assess superoxide in whole blood, electron paramagnetic resonance (EPR), is an advanced objective technique performed by investigators experienced with the measurement. This analysis has included a comprehensive panel of vascular function and markers of lipotoxicity and all data was rigorously corrected prior to analysis in order to account for changes in blood volume and glomerular filtration rate during pregnancy.

The limitations of our study are that our OW/OB group had a lower mean BMI and appeared to be a particularly healthy cohort. It is likely that the selection protocol resulted in OW/OB women being recruited who were extremely healthy and not at risk of developing an adverse obstetric outcome. Therefore we have missed out on recruiting women within the 30% of the obese maternal population who are at risk of pregnancy complications (Figure 1.2).

In conclusion, in the current study both lean and OW/OB women exhibited similar endothelial microvascular function improvement and increases in lipotoxic markers. Analysis of this data has shown that in healthy lean and OW/OB pregnancy, although there was an increase in markers of lipotoxicity, this had no detrimental effect on the improvement seen in EDMVF. In addition, the increase in the studied subcutaneous fat depots, changes in direct measurements of energy metabolism and plasma markers of carbohydrate, lipid and inflammation do not have an impact on the improvement seen in EDMVF. Therefore in OW/OB pregnancy there appear to be some women for whom their

obesity could be thought of as 'benign' as they exhibit the same gestational vascular improvement in pregnancy as lean women.
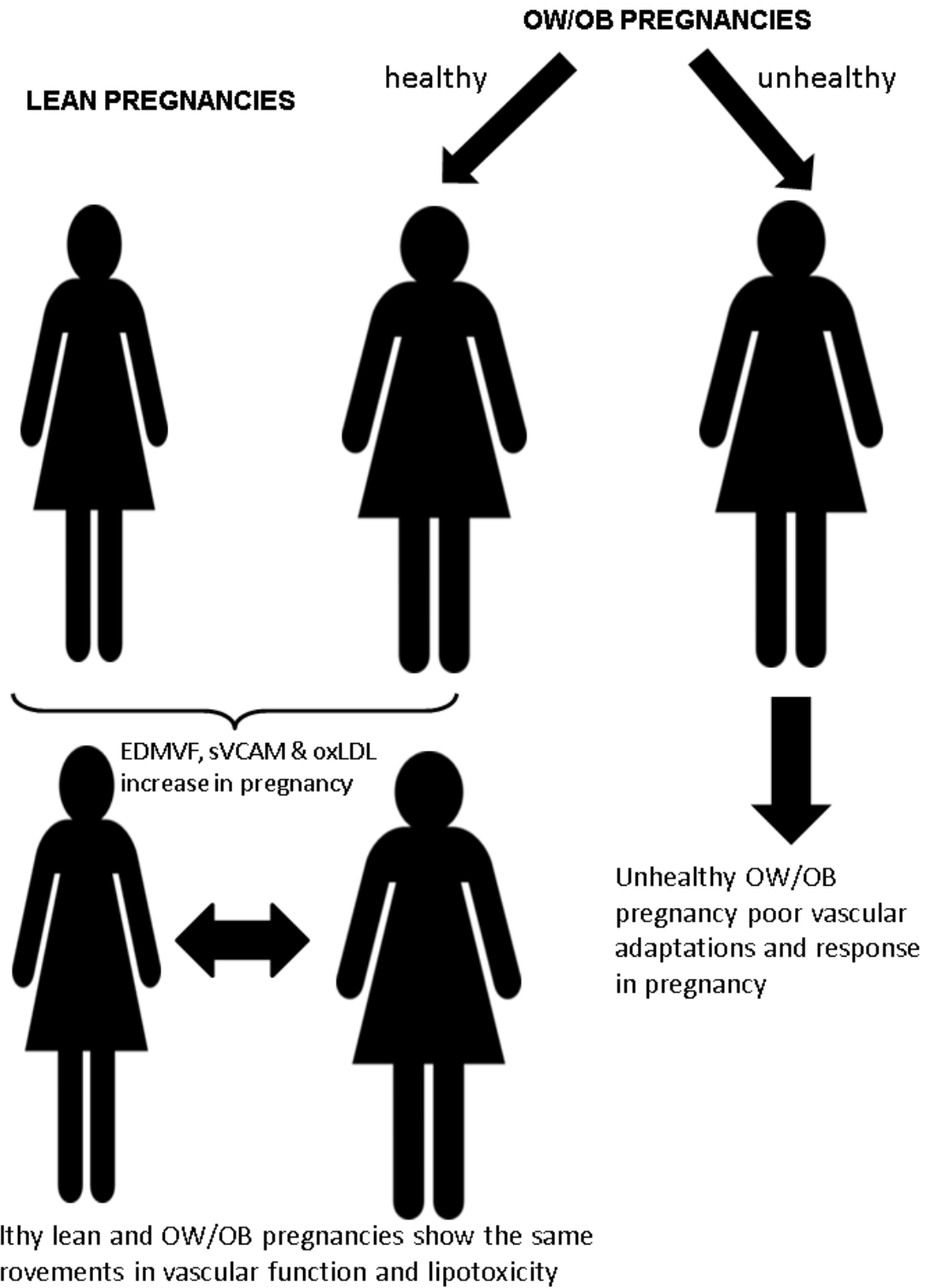
Figure 6.10 Summary of finding of chapter 6. In the cohort studied, lean and OW/OB women both exhibited an improvement in vascular function and increased endothelial activation and increased lipotoxicity (as measured by oxLDL). Findings suggest that OW/OB women could be considered as either metabolically healthy therefore responding to pregnancy in a similar way to lean women or unhealthy where they have poorer vascular improvement in pregnancy.

Chapter 7 General Discussion & Future Research

7.1 General Discussion

In the wider non-pregnant population Jensen et al (Jensen, 2008) have described in detail how different subcutaneous adipose tissue depots differ in terms of their function and their role in whole body fatty acid metabolism. Previous studies in pregnancy have suggested that there are differences in the distribution of adipose tissue accumulated during pregnancy, with obese women tending to have a propensity to central subcutaneous fat accretion, while lean women, tend to gain fat in the lower body compartment (Taggart et al., 1967, Soltani and Fraser, 2000). The work presented in this thesis was designed to test the hypothesis that the increased risk of adverse pregnancy outcomes for obese women was the result of the inadequate adipose tissue storage of fatty acids and the subsequent lipotoxicity leading to reduced vascular function during pregnancy (Jarvie et al., 2010).

In this thesis, the idea of functional adipose tissue depots in pregnancy was explored. One objective of this thesis was to ascertain whether OW/OB women gained gestational fat in the central subcutaneous fat compartment (measured using suprailiac and costal skinfolds), while lean women gained gestational fat in the lower body subcutaneous compartments (measured by midthigh and suprapatellar skinfolds). In this study, it was observed that lean and OW/OB women gain similar amounts of total body weight and fat mass during pregnancy, and all fat depots increased during pregnancy. The findings in this thesis were that in OW/OB pregnancy there was no preference in anatomical location of adipose tissue accumulation during pregnancy. In contrast lean women did have an anatomical depot-specific deposition of subcutaneous adipose tissue during pregnancy however this was not in the lower compartment. The primary location for gestational fat deposition in lean women was the upper peripheral subcutaneous fat stores (measured by biceps and triceps skinfolds) but this specific to gestational age. This preferential accumulation appeared only to increase in the second trimester (from 15 to 25 weeks). This may suggest that lean women are able to switch easily from a lipogenic to a lipolytic profile in later pregnancy when the fetal energy demands are greatest and the body is also

preparing for labour and breastfeeding energy demands. In contrast the finding that the OW/OB group did not show a gestational time or anatomical site preference for subcutaneous fat deposition throughout pregnancy may indicate a lack of metabolic flexibility due to a continued lipogenic profile within the adipose tissue in OW/OB pregnancy.

In this study OW/OB women were not of a particularly extreme BMI and in fact, as stated earlier, the OW/OB women did include some women who were classed as overweight. Thus it is not possible to conclude the pattern of subcutaneous fat accumulation observed in the OW/OB group under study here is the same as in obese pregnancies of much higher BMI. This makes it difficult to relate the present findings to obese women 'at risk' of an adverse outcome. Such women of extreme obese phenotype may show a propensity to central adipose tissue accretion. Furthermore, the visceral compartment was not measured and it is possible that in the OW/OB group in the current study, fat was accumulated in that depot initially. Once this storage site is replete then fat accumulated in subcutaneous sites, without preference for location as all grouped (upper body truncal, upper body peripheral and lower body) skinfolds increased throughout pregnancy. However, the present observations do give a comprehensive picture of how subcutaneous fat depots change in a group of healthy pregnant women (both lean and obese). This is important because, as highlighted above, in the non-pregnant population, location of fat stores is associated with increased inflammatory profiles and insulin resistance.

The next aim was to explore whether any differences in gestational fat accumulation between lean and OW/OB women could be explained by direct measurements of energy metabolism. These included basal metabolic rate, physical activity and dietary energy intake and observations were related to gestational fat gain and anatomical location of its storage. These were important variables to assess longitudinally as they have a major impact on total body and fat mass gain in the general adult population, and therefore gestational changes in energy metabolism could impact on gestational fat accumulation. In this study both groups responded to pregnancy similarly with a gestational rise in BMR, a decline in MVPA and a similar change in their macronutrient intake. These findings support current published data in lean pregnancy populations (mean BMI 24), where basal metabolic rate increased during pregnancy and physical activity

levels were maintained until 32 weeks where after they decreased (Lof and Forsum, 2006).

The results of this study observed some expected differences between lean and OW/OB women; OW/OB women had higher BMR, related to their higher body mass, and were less active than lean women throughout pregnancy. BMR is related to body mass, and in the non-pregnant obese population, BMR has been found to rise with increased levels of obesity (Elbelt et al., 2010). Thus because body mass was very different between lean and OW/OB women, BMR was corrected for this using BMR/kg. However, in the present study even when corrected for this there was no relationship between the change in BMR/kg and the increase in gestational fat mass. The lean and OW/OB group gained similar amounts of fat mass during pregnancy, which would indicate that they have a similar overall energy intake/energy expenditure balance. Published data has indicated that physical activity (both non-exercise and activity induced) plays an important part in basal metabolic rate and total energy expenditure (Westerterp, 2008). During pregnancy, increased MVPA was directly correlated with an increase in BMR/kg. Therefore the overall higher BMR/kg and physical activity in lean women compared to OW/OB women and the higher absolute rates of BMR in the OW/OB group could explain why, despite different activity levels and similar diets, both groups gain similar amounts of weight and fat.

In this study, there were no gestational differences observed in diet of the entire cohort during pregnancy or between the lean and OW/OB groups. In a more in depth analysis of the dietary data performed on the present study population (but not reported in this thesis) it was found that both lean and OW/OB women were not consuming enough vitamin D and folic acid, and although this did improve with vitamin supplementation, 20% of OW/OB women were still not achieving their recommended intake of vitamin D. In current published literature there is interest in the role of vitamin D deficiency and its impact on pregnancy and birth outcomes (Aghajafari et al., 2013, Rodriguez et al., 2014), although the association at this time remains contentious.

Strategies during pregnancy to try and improve pregnancy outcomes in obese women have focused on diet and exercise. Recent data indicates that while both

diet and activity modification can reduce the risk of PET and GDM compared to controls, dietary changes are most effective at reducing gestational weight gain (Thangaratinam et al., 2012). Other data suggests that a combination of dietary and activity advice did not improve maternal outcomes including GDM and PET in obese and overweight pregnancies (Dodd et al., 2014a), although antenatal advice did improve maternal activity levels and diet (Dodd et al., 2014b). Pilot data from the UPBEAT trial has indicated that there is more scope for changing diet than physical activity during obese pregnancies (Poston et al., 2013). The UPBEAT trial is currently being undertaken to further evaluate the impact of these interventions on maternal outcome in obese pregnancies (Briley et al., 2014). The different conclusions reported in the literature highlights the complex interactions of diet and activity on weight gain and maternal outcomes.

These findings have important implications on adapting behaviour during pregnancy. In this Scottish population, trying to change the dietary habits of the OW/OB group may be difficult when it appears there is no difference between lean and OW/OB diets during the antenatal period. Physical activity in pregnancy would also be difficult to maintain if, as these results suggest, all women become less active during gestation. Any intervention that attempts to improve maternal health is extremely important. Any positive change would be beneficial to both mother and the fetus (either via fetal programming or by reducing gestational over-nutrition), because it will reduce the metabolic risk for the next generation. An important question is when is the most effective time to intervene? As discussed above the results of the UPBEAT trial will help to answer how diet and exercise modifications during pregnancy can improve perinatal outcomes. Targeting obese women before conception may be another possibility. However targeting the pre-conceptual population would require a much wider public health approach and cannot be addressed by obstetrics alone. The focus for trying to reduce the risk of adverse metabolic and vascular complications needs to be two fold, with pre-pregnancy (and inter-pregnancy) counselling and optimising of healthy lifestyle in obese women prior to conception and during pregnancy providing additional support by means of an intervention strategy to improve physical activity and diet.

Across the entire cohort, increases in subcutaneous adipose tissue depots were not related to any changes in the measured variables of energy metabolism. This

suggests that in the specific depots assessed in the current study, their gestational increase is not driven by changes seen in energy metabolism and may be hormonally driven. Unfortunately, the inability to assess the visceral fat compartment does not allow one to gain a complete picture of the impact of energy parameters on gestational fat gain.

The third aim was to explore whether anatomical location of gestationally-acquired fat influenced the maternal metabolic adaptation to pregnancy building on the work of Jensen *et al* which showed that the location of storage of fat in the non-pregnant population influences metabolic profile. Plasma markers of lipid and carbohydrate metabolism and inflammation were examined to determine whether any differences in fat distribution was associated with the adverse metabolic profile. In the current study the changes in gestational carbohydrate, lipid and inflammatory profiles observed are broadly similar to current published data. There were some unexpected observations in biomarkers of inflammation and adipocyte fatty acid metabolism. Lean women had higher concentrations of IL6 and NEFA during pregnancy than OW/OB women, and OW/OB women had a greater gestational decrease in CRP than lean women. These findings were not anticipated and are difficult to explain.

Previous authors have commented that even in healthy pregnancy there is a rise in plasma IL6 levels (Freeman et al., 2004), suggesting that an increase in this inflammatory marker may reflect a physiological rather than pathological process. Two possible explanations could be suggested. Firstly a gestational increase in IL6 could be the result of increased secretion from adipocytes secondary to hyperplasia of functional preadipocytes in lean pregnancy. In obese pregnancy adipocytes may either not mature or there may be a hypertrophic rather than hyperplasic fat cell response (McLaughlin et al., 2007).

Alternatively there may be a role for IL6 in vascular remodelling as has been examined in animal models. It has been shown that under hypoxic conditions, IL6 receptor levels are increased and may play a role in pulmonary vascular remodelling in mice (Savale et al., 2009). It was also observed that the sVCAM-1 levels were higher in the lean group than the OW/OB group. As mentioned in chapter 6, higher sVCAM-1 levels have been found during physiological vascular

remodelling in endometrial spiral arterioles (Craven et al., 1998) and during in vitro small vessel inflammation experiments (Ohanian et al., 2012). If plasma increases in IL6 and sVCAM-1 reflect an increased vascular remodelling process, this would suggest that lean pregnant women have a better remodelling potential than OW/OB women and may explain a better vascular response in pregnancy. This observation may reflect not just an ability to adapt during pregnancy but the remodelling potential in the post natal period and subsequent pregnancies.

The final aim was to look for evidence of development of lipotoxicity using plasma and urine biomarkers in both lean and OW/OB pregnancy and to relate this to endothelial function. The hypothesis was that a gestational subcutaneous fat distribution could explain poorer maternal vascular response seen in obese pregnancies (Stewart et al., 2007a, Ramsay et al., 2002a). In the current study, no differences in endothelial function and lipotoxicity between lean and OW/OB women were observed. Both groups showed a significant improvement in EDMVF response as pregnancy progressed. In addition, no differences in the concentrations of markers of lipotoxicity were noted between the lean and OW/OB group. When the entire cohort was assessed, the gestational increases in the studied anatomical subcutaneous fat depots were not related to endothelial function or markers of lipotoxicity. Therefore the initial hypothesis that pregnant OW/OB women gain fat during pregnancy in the central subcutaneous adipose tissue depots leading to NEFA overflow with subsequent lipotoxicity and endothelial dysfunction was not supported.

There are a number of reasons why there was no evidence of lipotoxicity in the OW/OB women. In this study it was observed that the lean women had the metabolic and vascular response to pregnancy which was anticipated from previous literature. However, as discussed previously, while there was some evidence that the OW/OB group exhibited some of the abnormal metabolic adaptations this was not as pronounced as expected. When the current OW/OB cohort was compared to previous studies (Stewart et al., 2007a, Ramsay et al., 2002a), it became evident that the OW/OB cohort recruited here was relatively healthy and their metabolic response to pregnancy was more similar to that of lean women than that of previously studied obese groups. When the

demographic characteristics of the present OW/OB cohort were compared to those of a previous obese cohort that had a poorer metabolic and vascular response to pregnancy (Stewart et al., 2007a) it became obvious that the current group were all non-smokers (compared with 40% smokers), were of higher socioeconomic status and had a lower mean BMI. Thus it would appear that the current OW/OB cohort have 'benign' obesity. Therefore it is not surprising that there were no significant differences in vascular response and markers of lipotoxicity between these lean and OW/OB women.

In the non-pregnant population, the concept of 'metabolically healthy obesity' or 'benign obesity' is a current area of interest, and it has been suggested that this group may account for up to thirty percent of obese adults (Primeau et al., 2011). There is no current consensus on the definition of 'metabolically healthy obesity', but some authors have indicated that this group includes those obese individuals who are insulin sensitive and do not display abnormal lipid profiles, clinical hypertension or type 2 diabetes (Boonchaya-anant and Apovian, 2014). Other authors have included obesity with a favourable metabolic profile which includes low ectopic liver fat, low triglycerides, low inflammation, high HDL, low intima-media thickness, high adiponectin and low apolipoprotein B (ApoB) (Primeau et al., 2011, Stefan et al., 2008). In addition, data in non-pregnant adults show that obesity associated with visceral fat was associated with dyslipidaemia, atherosclerosis and insulin resistance but abdominal subcutaneous fat was not (Neeland et al., 2013).

In a population of obese postmenopausal women the definition of 'metabolically healthy obese' was based on insulin sensitivity. Brochu *et al,* showed that those women described as 'metabolically healthy' obese also displayed smaller adipocyte cell size and less visceral fat (Brochu et al., 2001). In addition, obese individuals found to be metabolically healthy, again based on insulin resistance, had higher levels of the nuclear receptor subtype peroxisome proliferator-activated receptor γ (PPAR-γ), which is important in stimulating adipogenesis (McLaughlin et al., 2007) and could lead to a hyperplasic rather than hypertrophic expansion of adipose tissue. In the current study, the OW/OB women recruited may be individuals who, although they exhibit increased insulin resistance compared to the lean group, are more insulin sensitive than other studied obese pregnant populations (Stewart et al., 2007a) and thus may still fit

into the 'metabolically healthy obese' category. If so they might be expected to exhibit similar gestational vascular improvement in pregnancy as lean women. On this basis, this study has not recruited women who are 'at risk' of obesity related obstetric complications.

In this thesis, much useful data which has bearing on current ongoing research in maternal obesity and clinical practice was collected. The study adds evidence to the existing non-pregnant data showing that obesity is a heterogeneous condition, and that many obese women remain healthy during their pregnancies. This study has produced a variety of data showing that many healthy OW/OB women respond to pregnancy in much the same way as healthy lean women in terms of metabolic and vascular response. Although the OW/OB women exhibit a subtle degree of metabolic disturbance, this had no impact on either vascular function or biomarkers of lipotoxicity during pregnancy. In conclusion whilst there is a significant proportion of obese women who may display a lipotoxic phenotype resulting in poor vascular adaptation during pregnancy, there are also many OW/OB women for whom pregnancy remains a period of health and should not be considered, at least in terms of vascular and metabolic function as high risk in the clinical setting.

7.2 Strengths and Limitations

There are significant strengths to this study. This study has provided extensive phenotyping that has been performed at both a physiological and biochemical level. The strengths and expertise of the methodologies employed has been discussed at length in the relevant chapters. In the current literature, there are limited data available on metabolically healthy obese pregnancies. Although this data set does not contain at risk OW/OB women, it offers detailed observations of the healthy OW/OB pregnant response during pregnancy. The design of the study, (all women were observed longitudinally by the same researcher) adds statistical power to the findings in the current data set. By performing multiple measures of subcutaneous fat stores during pregnancy the current study has shown how these fat depots change over pregnancy in lean and potentially 'metabolically healthy' OW/OB pregnant women; at the time of submission of

this thesis there were few published data available on this topic. In addition, not only has the current study extensively phenotyped a healthy cohort of women during pregnancy but the same measurements have been collected three months postnatally as well. In addition, there are stored samples for placentae, umbilical cord and cord blood which will be used in future studies.

The limitations of the current study should be considered. The main limitation is that the obese women most at risk of vascular complications were not recruited to the study. This was despite continued publicity and recruitment drives by the researcher. This may have been because obese women with extreme BMI do not considered themselves at risk, have no interest in being involved with a study about body shape or because they are self conscious about their weight and therefore also do not wish to participate in such a study. One potential OW/OB recruit reported to the researcher that she felt victimised for being asked to participate because she was being recruited to the OW/OB group. The converse was true about the lean group, it did not take long to recruit these women and again this may be self selection – those interested in how their body works and responds during pregnancy also are probably motivated to exercise outwith pregnancy and maintain a healthy weight. The reasons why obese women may or may not take part in this type of study are multifactorial and probably not only based on self image but also perceived 'ill-health' by being approached to participate in a study based on BMI. On reflection, prior to commencement of recruitment to this study it may have been advantageous to consult with patient groups or member of the general public to review the study protocol. Such a review may have helped gauge opinion on whether the intensity of the study visits was deemed appropriate and help to streamline the visit protocol. This may have helped improve either recruitment or retention to the study. In addition, because we did not achieve the number of study participants (n=30), which have been detailed in section 2.6, the sample size and number of gestation time points is a limiting factor in the results we have obtained.

Another limitation is that the visceral fat compartment was not assessed. Although in this study there is certainly a suggestion of site-specific accumulation of subcutaneous fat in lean pregnancies, unless there is information on the visceral fat compartment accumulation it is impossible to link

the impact of gestational fat gain to metabolic adaptations and lipotoxicity in pregnancy.

The analysis performed in this study looked at simple summary measures of the data such as change over time. Prior to analysis, biomedical statisticians provided advice on building a statistical model that would address the hypothesis and the aims of this study. The statistical analysis presented in this thesis using this model does address the key research questions from the outset therefore for the aims of this thesis the analysis was fit for purpose. However there are other approaches to analyse the collected data (such as area under the curve) any further analysis should take these approaches into consideration.

Dietary data are notoriously difficult to record and, the gold standard technique of four day weighed dietary intake (Holmes et al., 2008) was not feasible in this study. Therefore, recall bias may have resulted in the lack of differences between the lean and OW/OB dietary data. One of the most consistent problems in dietary analysis is participant's unintentional under-reporting of dietary intake. The dietary data was extensively studied for the occurrence of under reporting using the Goldberg cut-off value (Black, 2000)(not reported in this thesis). No differences were found in the rates of underreporting between the lean and OW/OB groups.

Although there was comprehensive collection of data throughout pregnancy and three months' postnatal (the latter not presented in the thesis) to compare lean and OW/OB women, the study did not have any data from either early pregnancy (less than 12 weeks) or non-pregnant women. Having a prospective data set from lean and OW/OB women preconception that were subsequently followed up through pregnancy and postnatally would be ideal, but recruitment and retention in this type of study would be extremely difficult. In the current study there was also no adipose tissue sampling. If, as in the non pregnant population, there are functional differences in depots (abdominal and lower body) then exploring this in *ex vivo* sample from pregnant women would be very informative. However it is difficult to anticipate when pregnant recruits will deliver their babies and collection of adipose tissue would only be appropriate at an elective caesarean section delivery. One could collect samples from a cross

section of healthy lean and OW/OB women undergoing uncomplicated caesarean delivery but you would lack the antenatal data in this case. Another option could be collection of needle biopsies of subcutaneous fat at different gestation but this is unlikely to be acceptable to all participants.

7.3 Clinical implications

This thesis provided support for the theory that not all obese women should be considered 'high risk' for an adverse pregnancy outcome based solely on their BMI. Thus current clinical practice needs to be reviewed. BMI is currently used to risk assess for GDM and PET at her initial antenatal appointment. It is also used to assess the need for thromboprophylaxis during pregnancy and referral for anaesthetic review. Although the latter risk assessment is still very valid, if an obese woman is metabolically healthy her risk of the former conditions may not be as high as previously thought. However, although many more obese than lean women develop antenatal complications, BMI may still be too unsophisticated a measure to accurately identify the 30% of obese women who do develop obstetric complications (see Table 1.2). By categorising every woman with a BMI of $\geq 30 \text{kg/m}^2$ as high risk, many of these women are being unnecessarily medicalised during pregnancy. Resources within the NHS are limited, and the escalation of healthy obese women's antenatal care to involve additional hospital appointments with consultant Obstetricians and specialist testing (such as glucose tolerance testing [GTT]), is not a good use of resources. In addition, with reference to figure 1.3, using BMI as a measure of risk means that the approximately 15% of lean women who are at increased risk of similar obstetric complications are not identified.

Subsequently any new screening method of ascertaining metabolic risk requires to be simple and quick and to be able to perform in the busy antenatal clinic environment. If visceral fat proves to be a discriminating marker that can separate benign and metabolic obesity then it could be amenable to simple assessment at least for screening purposes. Abdominal waist circumference has been used as a measure of central and therefore visceral fat in the past. In pregnancy abdominal waist circumference has been assessed at 16 weeks' gestation and larger waist circumferences (>80cm) have been associated with an increased risk of hypertensive disorders in pregnancy (Sattar et al., 2001). Newer techniques to measure visceral fat are currently being assessed in the non-pregnant adult population. One technique which looks promising and is conveniently quick to perform is the use of bioimpedance assessment (Khalil et al., 2014), which can now be used to identify the visceral fat compartment, and

highly correlates with measurements obtained by computed tomography (Yamakage et al., 2014). Most of these scales also measure total body mass which is part of the routine assessment of a woman at her initial antenatal appointment so this would not require additional time in the clinic.

In 2012, the Scottish Government introduced the HEAT targets (Health Improvement, Efficiency, Access to Services and Treatment) antenatal access target which aims to have at least 80% of pregnant women from each SMID deprivation quintile booked for antenatal care by the 12th week of pregnancy to improve breastfeeding rates and other poor pregnancy outcomes (NHS, 2012a). The aim is to have this target met by March 2015. If this target is met and a screening tool to measure visceral fat then this fat depot could be measured at this earlier gestation. Its' impact on metabolic profiles in early pregnancy could be could be assessed before gestational fat increases. In addition the measurement of visceral fat at 12 weeks' gestation was predictive of adverse metabolically-related pregnancy outcome, then it could also work as a triage tool for further assessment of women. For instance if visceral fat was found to be in the upper tertile then an early measurement of fasting insulin or a GTT could be used to further select those women, possibly both lean and obese, with a higher risk of metabolic complications in pregnancy. Present policy is to have all women with BMI>30 to be screened for gestational diabetes (NICE, 2010), if another screening tool could be implemented it may reduced unnecessary tests and improve costings within the NHS.

7.4 Future Research

7.4.1 Further potential analysis of existing data

As there were no differences in vascular adaptation between the lean and OW/OB groups, the current cohort could be regrouped based on a functional assessment of central obesity such as insulin resistance or on vascular improvement in pregnancy. The hypothesis of this thesis was that lipotoxicity is the mechanism which results poorer vascular adaptation to pregnancy, then grouping women with 'good' versus 'poor' function who allow phenotyping of these women. This could also apply to classifying women based on insulin resistance, as this metabolic biomarker has been used to categorise 'metabolically healthy obese' women.

Alternatively the cohort could be assessed as one whole group and the changes assessed across the tertiles. In this analysis, absolute values of the variables described could be analysed rather than looking at simple changes between gestational time points. This would be helpful in confirming or refuting the lack of differences noted between the lean and OW/OB groups. This could be done for all values or perhaps using visit 3 to gain a cross sectional analysis of the data.

There are alternative ways of analysing the data which could be employed using the entire cohort. These would include using incremental area under the curve in the total population with the addition of BMI as a covariate in the model. In addition, the possibility of multiple regression analysis could be explored to ascertain any differences in the absolute values as discussed above.

7.4.2 Future analysis of stored samples and data

In this study the dietary data was only assessed in terms of macronutrients (total fat, carbohydrate and protein intake). As mentioned above there has been more work performed on this data examining micronutrient intake (not included in

this thesis). There is also the potential to look at the essential fatty acid intake to include LC-PUFA, as well as comparing simple sugars and more complex carbohydrates (primarily starch) which could be correlated with maternal insulin resistance and anatomical adiposity.

As yet, the postnatal data has to be analysed, which covers all of the same parameters described in each of the results chapters. Information on breast feeding rates and duration were collected and the impact of this on any loss of subcutaneous fat distribution and vascular function in the postnatal period warrants further investigation.

Some tissue samples were obtained from our participants at delivery and included placental, umbilical cord and umbilical blood samples. Again assessing lipotoxicity markers in the umbilical cord blood and relating this to maternal markers of lipotoxicity and fatty acid metabolism would be informative.

In addition it would have been interesting to measure LDL size to see if this explained the lack of differences between the groups in terms of oxidised LDL. Further assays on other markers of lipotoxicity such as plasma isoprostanes and oxysterols concentrations are currently being performed to look for evidence of lipotoxicity which will add more information to the comprehensive panel of markers we have already assessed.

In order to try and establish whether obese women can also be considered healthy in terms of endothelial adaptation and lipotoxicity during pregnancy further assessment of CD36 expression and oxysterols would be important. Plasma sCD36, can be used as a marker of insulin resistance and ectopic liver fat accumulation (Handberg et al., 2012). If the recruited OW/OB group in this study are 'metabolically healthy obese', then lower concentration of sCD36 would support this theory. At the time of writing this thesis funding was not available to assess these markers although aliquots had been obtained for any further analysis.

7.4.3 Future research directions

This thesis has shown that there is further research which needs to be performed into how to clinically categorise metabolic 'at risk' pregnancies. One of the most important avenues of future research that this thesis has indicated is the need to recruit women who exhibit a metabolically abnormal phenotype. A similar longitudinal study should be performed to include obese women who are with in this group. In turn they could be compared to the existing data collected in this study. This would allow the idea of obesity heterogeneity which exists in non-pregnant adults, to be evaluated in the pregnant population. A future study should also be performed to include lean women who are considered 'metabolically unhealthy' (i.e. insulin resistant lean individuals) and this would allow an even more extensive phenotyping of both lean and obese women who may be at risk of metabolic related obstetric complications. To classify those women in either 'metabolically healthy' or 'metabolically unhealthy' HOMA or visceral fat thickness could potentially be used as classification criteria.

It would be vital that this future study assessed the visceral adipose tissue compartment measured throughout gestation as that was one of the limitations discussed in this thesis. Measuring this depot would yield information as to whether the size of this depot is important in early pregnancy and if it is an important site of gestational fat deposition. Further comparison of how these depots change in pregnancies complicated by gestational diabetes or pre-eclampsia would be important in completing the lipotoxic phenotype.

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Appendices

8.1 Appendix I

PATIENT INFORMATION LEAFLET

BODY SHAPE IN PREGNANCY STUDY

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.

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?
Congratulations on your pregnancy!
During pregnancy, a woman's body goes through many normal changes both inside and out. We wish to study how a woman's body shape changes during pregnancy, for instance where she gains weight. We think that where a woman gains her pregnancy weight may have an effect on her metabolic health.

2. Why have I been chosen?
We wish to study healthy pregnant women, like you, who have no current medical conditions and have fallen pregnant naturally. We are recruiting first time mums of different ages and weights.

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 decide to take part in this study you will have three additional appointments during your pregnancy and one after you have delivered your baby. These will be with the research doctor, Dr Eleanor Jarvie. She is an obstetric doctor and once enrolled in the study you will be able to contact her directly regarding your appointments and your pregnancy. These appointments will be at approximately 15 weeks, 25 weeks and 35 weeks of your pregnancy and when your baby is approximately 3 months old.

The tests require you to be fasted. We would ask that you not have anything to eat after 10pm the previous night. You can drink water. If possible you should not drink caffeinated drinks (tea and coffee) or fizzy drinks. Please avoid any over the counter medications and try not to smoke. The appointments will be at 8:00 or 9:30 in the morning. We will provide breakfast for you. We anticipate that the appointments will take 90 minutes.

In addition we would like to obtain tissue samples at the time of your delivery and gain information about your baby just after birth.

5. What measurements are being recorded during and after my pregnancy?

All tests are completely harmless to you and your baby.

(A) We will measure your height, weight and blood pressure.

(B) We will take a sample of blood (40ml which is about 3 tablespoons) and urine at each visit.

(C) *Body composition tests*
These tests are carried out to assess where in your body weight is put on during pregnancy. The first test is skin fold thickness measurements. The second test is a measure of percentage fat mass and percentage lean mass measured by the way your body displaces air around it. This measurement is carried out seated in a person-sized chamber (or capsule) with a window while you are wearing a swimsuit or similar which you will need to bring with you. Private changing facilities are available. Dr Jarvie and a research nurse will be present.

(D) *Resting metabolic rate*
This is measured by assessing the air that you breathe in and out through a special clear plastic hood.

(E) *Endothelial function tests*
This test looks at blood vessel function. We assess how well the cells which line the blood vessels (endothelial cells) are working. The assessment of skin blood flow is not painful. We have used this technique with pregnant ladies before and it is safe in pregnancy.

(F) *Dietary analysis*
We will be asking you about your diet and what you usually eat and drink.

(G) *Activity assessment*
At each visit we will give you an accelerometer (activity monitor) to take away with you. This is a small device (about the size of a mobile phone) that can clip onto a waistband and record the daily amount of activity of the wearer. We will ask you to wear this every day for 7 days. The accelerometer can then be posted back to Eleanor Jarvie in the pre-paid envelope provided.

6. Samples at the time of your delivery
(A) Fat cell samples
We are interested in how fat cells work in different sites of the body. We would like to obtain samples (a biopsy) of fat cells at your delivery but only if you happen to have a caesarean section delivery where there is a cut in the tummy already. We would not take samples of fat cells at natural or vaginal deliveries. **The decision to have a Caesarean delivery is made by your own obstetrician in charge of your clinical care and not by the research team.**

We would take two samples. The first is from just under the skin after the cut has been made. The second is from fat cells within the abdominal cavity after the womb had been stitched closed, but before the skin is stitched closed. This is an additional procedure that takes approximately 1-2 minutes. It will not significantly lengthen the time of your operation.

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 these samples.

(B)Samples from the placenta (afterbirth)
We would like to gain information about how the placenta (afterbirth) functions during pregnancy. 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 it will be passed onto the laboratory where it will be studied.

7. What do I have to do?
You will be asked to attend four additional appointments (three during your pregnancy and one after when your baby is about three months old). These appointments will be held in the Metabolic Suite at the University of Glasgow, which is next to the Western Infirmary of Glasgow. Dr Jarvie can meet you at the entrance of the University (on University Avenue) and accompany you to the Metabolic Suite.

So that we can collect tissue when you deliver, we would ask that you inform Dr Jarvie when you go into labour, or ask the midwife looking after you to inform her. There is a contact number at the end of this sheet.

8. Expenses and payment
You would not receive explicit payment for this study but we will reimburse you a nominal amount for your travel expenses or if you prefer we will organise a taxi to take you to and from your study appointment.
9. What are the possible disadvantages and risks of taking part?
There are no disadvantages or risks to you or your baby by taking part in this study. These appointments **do not** replace your antenatal care and you will need to see your midwife as planned.

10. 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.

11. 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 number is provided in 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.

12. 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 before making any decision.

PART 2
13. What if relevant new information becomes available?
Sometimes we get new information about the conditions being studied. If this happens, your research doctor will tell you and discuss whether you should continue in the study. If you or your research doctor decides not to carry on, we will inform your midwife or obstetrician. If you decide to continue in the study he may ask you to sign an updated consent form.

If the study is stopped for any other reason, we will tell you and inform your midwife or obstetrician.

14. 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.

15. 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 number is provided in 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.

16. 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.

17. 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.

18. 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.

19. Who is organising and funding the research?
This research will be funded by the Wellbeing of Women (RCOG) with support from the Chief Scientist's Office.

20. Who has reviewed the study?
All research in the NHS is looked at by 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 National Research Ethics Committee.

21. Further Information
Further information about this study may be obtained from Dr Eleanor Jarvie (e.jarvie@clinmed.gla.ac.uk)
Dr. Eleanor Jarvie MBChB MRCOG
Clinical Research Fellow
Department of Reproductive & Maternal Medicine
University of Glasgow
Level 3, McGregor Building Western Infirmary of Glasgow
Tel: 0141 211 2327 (office), study mobile 07790217442

8.2 Appendix II

LIPOTOXICITY IN PREGNANCY STUDY
VISIT PROTOCOLS

BOOKING/RECRUITMENT VISIT – antenatal clinic (12-14 weeks' gestation)

Trained midwives or other clinical members of the study team will ask if pregnant women agree to take part in the study at the booking visit identified by the criteria listed below (tables 1 and 2). Women who are interested in the study will have a consultation with the research doctor (Eleanor Jarvie EMKJ), to ensure their wellbeing, and to give them the opportunity to ask any questions. The research doctor will explain the additional appointments of the study, supply the participant with patient information leaflets, contact telephone numbers and first appointment time and location.

Table 1 Inclusion criteria

Criteria	Rationale
Primigravid	No influence of previous pregnancy on index pregnancy outcome
	Adverse outcomes will be excluded from analysis
Age 16-40	Women are of age to give consent and are within the range of normal healthy pregnancy
Three groups lean BMI<25, obese BMI≥30 kg/m^2	Established poorer outcome in obese pregnant population (CEMACH, 2007) WHO criteria for obesity (WHO, 2004) Predominant ethnic group in Glasgow
Caucasian	

Table 2 Exclusion criteria

Known metabolic disease (diabetes, thyroid disease, PCOS) Cardiovascular disease Parous women Assisted conception pregnancies Multiple pregnancies Previous loss >12 weeks' gestation Co-existing conditions that require treatment Women with a complicated pregnancy outcome (excluded from analysis)

VISIT 1 – 15 weeks' gestation
- Starting time 08:00
- Subjects fasted for at least 10 hours overnight
- Subjects to be met at the Faculty of Biomedical and Life Sciences (West medical building, University of Glasgow) entrance by research doctor (Dr Jarvie)
- Informed written consent will be obtained (copy to remain with patient, with investigators and in medical notes)
- Following consent, the demographic questionnaire will be completed which will highlight the maternal age and date of birth to check that the participant is suitable for the study before any investigations are commenced.
- Actual dietary intake (24 hour recall) will be performed at this point in the appointment or at the time of the Food Frequency Questionnaire.

Table 3 - Demographic data

Data	Details
Date of visit Age PMHx OBHx FHx including OBHx	DOB, and age noted CVD/DM Infertility/PCOS/misc/TOP/AC CVD/DM/PET/GDM
Social Hx	Alcohol, drugs, smoking Medication esp contraception at PN visit employment postcode

1) ENERGY METABOLISM/EXPENDITURE

- Participant will then have indirect calorimetry performed. (in total 20-25mins)

Table 4 – procedure for indirect calorimetry

- Ventilation hood and circuit must first be calibrated – 5 mins
- The subject lies supine at 45 degree angle
- Once the hood is in position they are asked to relax but not to fall asleep
- Once their ventilation rate has reached equilibrium then the recording starts
- Normally the first 5-10 mins will not be counted as subject will not be at a steady ventilation rate
- Measurements – VO_2 (ml/min) and VCO_2 (ml/min) are recorded onto hard drive of computer, ratio of VO_2/VCO_2 and energy expenditure are also collected. Measurements need to be converted into l/min for calculations.

2) ENDOTHELIAL FUNCTION

Non-invasive peripheral endothelial function Laser Doppler Iontophoresis (LDI) will be performed immediately after the indirect calorimetry as the subject will be rested. Skin blood flow will be assessed using LDI combined with constant current iontophoresis of vasoactive agents to assess vascular reactivity and the role of the endothelium.

Table 5 procedure for laser Doppler iontophoresis

- Measurements conducted in quiet, temperature controlled room
- Subject lies semirecumbent position
- Laser scanned over the areas to be assessed
- Iontophoresis chambers attached to the flexor aspect of the forearms (avoiding broken skin, hair and superficial veins)
- 1% solution of Acetylcholine (dissolved in 0.5% saline) placed in the anode chamber
- 1% sodium nitroprusside (dissolved in 0.5% saline), placed in the cathode chamber
- Skin perfusion is measured before, during and after an incremental current (baseline scan, four scans at $5\mu A$, four at $10\mu A$, four at $15\mu A$, and two at $20\mu A$, and five recovery scans)
- Laser doppler imaging (red laser) is used to record the changes in endothelial reactivity

Changes in response to these drugs are quantifiable as the area under the curve (AUC) with repeated scans. This method is highly reproducible (Jadhav et al., 2007) and has been previously used in a pregnant cohort(Stewart et al., 2007a). (in total 20mins)

3) BLOOD & URINE SAMPLE COLLECTION

Fasting blood (20ml in EDTA) and urine (25mL) will be collected. Blood and urine will be handed to technician for immediate processing. (5 mins)

Table 8 – Sample processing

Blood:
- Whole blood Electron Spin Resonance
- Freeze aliquots of whole blood
- Separation of rest of whole blood by Histopaque into plasma, white cells and erythrocytes
- FACS analysis of white cells (Scott Claire)
- Fresh plasma for lipoprotein fractionation (see Dorothy)
- Freeze aliquots of plasma, white cells and erthyrocytes

Urine:
- Freeze aliquots of urine

4) ANTHROPOMETRIC TESTS/BODY COMPOSITION
- Patient to change into swimming costume/underwear and observed in BODPOD)
- Height (cm) and weight (kg) to be measured to 0.5 cm, and recorded

Table 6 – procedure for BODPOD assessment

- They are also required to wear a swimming cap – provided – to prevent air being trapped in hair.
- The machine is calibrated with the door open and an empty canister inside it to predict lung volume
- The subject is then asked to sit in the BODPOD and rests her hands on her lap, and asked to remain still
- The door is closed and recording starts – there is normally three separate recording and these take approximately 2mins each. The door is opened in between measurements.
- The data collected is stored on the hard drive of the computer, serial measurements for each subject will be kept i.e. longitudinal data can be compared.

Following BODPOD measurements participant will have anthropometric data measurements performed. Waist, Hip and thigh circumferences will also be measured in metric units. Skinfold thickness will be performed with the participant in their underwear and covered by a hospital gown. Based on previous studies by Catalano(Okereke et al., 2004, Presley et al., 2000) the skinfold thickness measurements shown in Table 5 will be used. Our group has previously used the following criteria for waist and hip measurements(Stewart et al., 2007b). Thigh circumference has been publish with respect to being a risk factor for cardiovascular disease (Heitmann and Frederiksen, 2009).

Table 7 Sites for skin fold measurements

- Triceps (midway between lateral projection of acromion and olecranon)
- Biceps (anterior aspect of arm, midway between acromion and antecubital fossa)
- Subscapular (at a 45 degree angle just below inferior angle of scapula)
- Subcostal (midaxiliary line at the level of lowest rib)
- Suprailiac (skinfold at the mid point between superior anterior iliac crest and lowest rib)
- Mid thigh (at the midpoint between inguinal crease and proximal patella – anterior aspect of leg)
- Suprapatellar (anterior aspect of the thigh just above the patella)

Waist circumference level of umbilicus
Hip circumference widest point over buttocks
Thigh circumference (directly below the gluteal fold of the right thigh)
Measure twice, to the nearest 0.5cm and mean
If difference >2cm take a third measurement and mean
Waist Hip Ratio (WHR) waist circumference divided by the hip circumference

5) DIETARY ASSESSMENT

- Participant will be served breakfast
- The participant will have usual (food frequency questionnaire) dietary intake assessed. This will be performed using questionnaires which the participant will go through with the research doctor (EMKJ) (15mins)

Table 9 Confounding variables in dietary assessment

Information	Details
Hyperemesis	Yes/no/hospitalisation
Food frequency questionnaire	Socioeconomic status may affect this. If asking subject to fill in a questionnaire prior to appointment levels of literacy will affect reporting
Vitamin supplementation	Pregaday/pregacare/ferrous sulphate
Smoking status	Y/N current – how many
Socioeconomic status	Ex – how long/years smoked
Breast feeding status	

6) ACCELEROMETERS

- Study participants will be supplied with an accelerometer, at each visit, which they will be asked to wear for one week (7 days). This is to assess actual activity. (5 mins)
- The accelerometers will be collected by the research doctor.

(TOTAL VISIT TIME 90 MINUTES)
VISIT 2 25 weeks' gestation
VISIT 3 35 weeks' gestation
Visit 2 and visit 3 will have same protocol as visit 1.

DELIVERY DATA

Delivery data will be easier to obtain when women have elective delivery dates. Study participants have contact details for the research doctor and asked to inform if admitted in labour. In addition, stickers which indicate that the labouring woman is involved in our study will be on the hand held pregnancy notes, and the attending midwife or doctor can then contact the research doctor on the dedicated study mobile phone. A lot of the following data is normally recorded in delivery summary so could be collected retrospectively from maternity notes.

Table 10 Delivery data to be collected

Record:
- Birth weight
- APGAR scores
- placental weight
- mode of delivery
- labour yes/no
- gestation at delivery
- sex of baby

Collect (where feasible):
- placental tissue (Full thickness biopsy sections of third trimester placentae will be obtained [approximately five grams] at time of delivery from four separate pre-determined areas on each placenta, distinct from the umbilical cord insertion, and then samples will be randomised. Three biopsies will be immediately snap frozen in liquid nitrogen and stored at -70°C, one biopsy will be washed in PBS and fixed in 10% neutral buffered formalin and paraffin-embedded for immunocytochemistry.
- cord blood from the umbilical vein
- endothelial cells from cord – collagenase digestion
- maternal adipose tissue (subcutaneous and visceral) approximately $1cm^3$ will be collected from the incision site at time of Caesarean section, and washed in phosphate buffered saline. One half sample will be fixed in zinc formalin. The other half biopsy will be immediately snap frozen in liquid nitrogen and stored at -70°C.

Visit 4 – postnatal data (12 weeks following delivery)

The postnatal visit for the mother would be similar to visit 1-3. Additional information regarding breastfeeding status and contraception use is required (see Tables 8 and 9).

STUDY IDENTIFICATION
NUMBER_____

LIPOTOXICITY IN PREGNANCY STUDY (LIPS)

Your Consent	Please initial box
I confirm that I have read and understand the patient information sheet dated May 2011 (version 5) 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 blood samples at each visit and tissue samples at delivery. These samples will be retained by the section of Reproductive and Maternal Medicine 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   Date   Signature
obtaining consent

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

DEMOGRAPHIC QUESTIONNAIRE – LIPOTOXICITY IN PREGNANCY STUDY
(this questionnaire should be completed on the first visit – please see 'update' page for visits 2 and 3 and postnatal section for visit 4)

Date of visit

Study identification number	
Maternal date of birth	
Maternal age	
Current gestation (weeks)	
Estimated date of delivery	
Postcode for social deprivation score (use postcode minus last 2 letters to avoid identification of street where they live)	
Highest level of education	
Current employment	

Personal Past medical history

condition	yes	no
Cardiovascular disease , angina, essential hypertension		
Diabetes Type 1		
Diabetes Type 2		
Thyroid disease		
Asthma or breathing problems		
bowel conditions (irritable bowel)		
kidney disease		
Musculoskeletal e.g. arthritis		
Previous operations		

Obstetric History

	yes	no
Hospital admissions in this pregnancy	*If yes please detail*	
Hyperemesis/severe morning sickness, if so what treatment		
Miscarriage/Termination of pregnancy		
Assisted conception		
Polycystic ovarian syndrome		
Problems conceiving		

Family medical history

Condition	yes	no
Cardiovascular disease in any relatives	*1st degree* *Relative* ☐ *2nd degree* *Relative* ☐ *Relationship to subject* _____	
Diabetes any relatives Type 1 ☐ Type 2 ☐ Gestational diabetes mellitus ☐	*1st degree* *Relative* ☐ *2nd degree* *Relative* ☐ *Relationship to subject* _____	
Pre-eclampsia in female relatives		
Intrauterine growth restriction Stillbirth or recurrent miscarriage in your family members		

Drug & Medicines History

	yes	no
Are you currently taking any regular prescribed medications	*If yes details of meds*	
Are you taking vitamins supplements? If so what brand		
Are you taking any fish oil capsules		
Have you recently used or are you using over the counter medications? i.e. gaviscon		

Social history

	yes	no
Alcohol (a unit of alcohol is one 25ml single measure of whisky (ABV 40%), or a third of a pint of beer (ABV 5-6%) or half a standard (175ml) glass of red wine (ABV 12%).	Current Y☐ N☐ No of unit/wk___ Prior to preg Y☐ N☐ No of unit.wk___	
smoking	Current Y☐ N☐ No of cig.day___ Prior to preg Y☐ N☐ No of cig/day___	If ex smoker, when did they stop, how long did they smoke for and how many a day
Recreational drug use Please specify type and pattern of use		

Update section: (for visits 2 and 3)
Since your last visit have there been any changes in your health including stopping/starting smoking

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Since your last visit have you started to take any prescribed medications/vitamins or fish oils (please specifiy)

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Since your last visit, has anything changed in your pregnancy that you think is important (additional trips to hospital/admissions to hospital/moved house)

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Postnatal details only

Date of delivery	
Place of delivery	
Method of contraception currently used	

Initial infant feeding method *Tick as appropriate and detail*	Bottle fed	Breast fed *If yes for how long*
		Top up feeding?
Current Infant feeding status	Bottle fed	Breast fed
		Top up feeding?
Fish oil capsules		
Vitamins		
Prescribed medications		

LIPOTOXICITY IN PREGNANCY STUDY - RESTING METABOLIC RATE ASSESSMENT

SUBJECT ID _____   DATE _____

TIME _____   GESTATION _____
FASTING   YES/NO
IF POSTNATAL BREAST FEEDING   YES/NO
FIRST TEN MINUTES ACCLIMITISATION PERIOD

TIME (x)	DRIFT VO2	VO2 (ml/min)	DRIFT VCO2	VCO2 (ml/min)	RER	EE	CHO	FAT	PRO
1.00									
2.00									
3.00									
4.00									
5.00									
6.00									
7.00									
8.00									
9.00									
10.00									
11.00 HOOD JUST ON									
12.00									
13.00									
14.00									
15.00									
16.00									
17.00									
18.00									
19.00									
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30.00									
31.00									
32.00									
33.00									
34.00									
35.00									
36.00									
37.00 HOOD JUST OFF									
38.00									
39.00									
40.00									
41.00									
42.00									
43.00									
44.00									
45.00									
46.00									
47.00									

LIPOTOXICITY IN PREGNANCY STUDY – BODPOD BODY COMPOSITION ASSESSMENT

SUBJECT ID _____ height_____

TIME _____ weight_____

Measurement 1: GESTATION _____ DATE _____
FASTING YES/NO

Total body mass (kg)	
Total fat mass (kg)	
Total fat free mass (kg)	
Percentage body fat (%)	

Measurement 2: GESTATION _____ DATE _____
FASTING YES/NO

Total body mass (kg)	
Total fat mass (kg)	
Total fat free mass (kg)	
Percentage body fat (%)	

Measurement 3: GESTATION _____ DATE _____
FASTING YES/NO

Total body mass (kg)	
Total fat mass (kg)	
Total fat free mass (kg)	
Percentage body fat (%)	

Measurement 4: GESTATION _____ DATE _____
IF POSTNATAL BREAST FEEDING YES/NO
FASTING YES/NO

Total body mass (kg)	
Total fat mass (kg)	
Total fat free mass (kg)	
Percentage body fat (%)	

24 HOUR DIETARY RECALL QUESTIONNAIRE
LIPOTOXICITY IN PREGNANCY STUDY

I would like you to tell me everything that you had to eat and drink yesterday.
By yesterday I mean, from midnight to midnight, (or from the moment you got up until you went to bed yesterday).

Include everything that you had to eat and drink at home and away from home, including snacks, tea, coffee, sweets and soft drinks.

First we'll make a list of the foods you ate and drank all day yesterday (DAY).

Next I'll ask you about the foods including amounts and then I'll ask you a few questions.

It may help you to remember what you ate by thinking about where you were, whom you were with, or what you were doing yesterday; like going to work, eating out or watching television.

Feel free to keep these activities in mind and say them aloud if it helps you.

If you would like to start at midnight at the beginning of (DAY).

There are some foods that people often forget. In addition to what you have

already told me about, did you have any:

☐ Coffee, tea, soft drinks or milk

☐ Alcoholic drinks

☐ Biscuits, cakes, sweets, chocolate bars or other confectionery

☐ Crisps, peanuts or other snacks

☐ Sauces, dressings,

☐ Anything you have not already told me about?

Type of food or drink

• How was it bought – fresh, canned, frozen, dehydrated etc?

• Was it home-made – if so – what was in it? Don't forget to record any recipes on the Recipe Pages.

• How was it cooked – boiled, grilled, fried etc?

• If it was cooked in fat, or fat was used in pastry or cakes or any other dish, what sort of fat or oil was used?

• If it was a dried / dehydrated product, was it reconstituted using water, milk or both?

• Was the item coated before cooking – if so – was it flour, batter, egg, breadcrumbs etc?

• Was it unsweetened, sweetened with sugar/honey, or artificially sweetened?

• Was it low fat / low calorie?

Measurement	Imperial	Metric
Volumes 1 cup	0.568 pint	250 ml
	I mug	~300ml
Weight	Tsp	5ml (3.5ml)
	Tbsp	15ml or 30g (14.2ml)
	ounce	28.3g
	1.76 oz	50g
	3.52 oz	100g

Routine foods		
	Yoghurt pot standard	125g
	Med bowl cereal	45g
	Slice bread med	30g
	Slice bread thick	45g

Time	Description of food or drink	Amount	Brand	Leftovers y/n amount
		Slices_____ Tsp_____ Tbsp_____ Cup_____ Mug_____ Wgt if known _____ Bowl size _____ Plate size _____ Added fat? _____		
		Slices_____ Tsp_____ Tbsp_____ Cup_____ Mug_____ Wgt if known _____ Bowl size _____ Plate size _____ Added fat? _____		

FOOD FREQUENCY QUESTIONNAIRE LIPOTOXICITY IN PREGNANCY STUDY

DATE	
STUDY IDENTIFICATION NUMBER	
VISIT NUMBER/GESTATION	

1. What kind of bread do you usually eat
   - white
   - brown, granary, wheatmeal
   - wholemeal
   - other kind (please specify)
   - no usual type
   - do not know
   - do not eat bread

2. What do you usually spread on bread?
   - butter
   - hard/block margarine
   - soft margarine
   - reduced fat spread
   - low fat spread
   - no usual type
   - do not know
   - do not spread fat on bread

3. How much do you usually **eat in a day?**

	less than 1	1	2-3	4-5	6+
- slices of bread/rolls					
- biscuits (including chocolate biscuits)					
- cakes, scones, sweet pies and pastries					

4. What kind of milk do you usually use for drinks in tea or coffee and on cereals etc?
   - whole milk
   - semi-skimmed
   - skimmed
   - other kind (please specify)
   - no usual type
   - do not know
   - do not drink milk

5. Do you usually take sugar in:

	YES	NO
(a) tea		
(b) coffee		
DO NOT DRINK TEA/COFFEE		

6. At table do you:

	YES	NO
- generally add salt to food without tasting first		
- taste food and then generally add salt		
- taste food but only occasionally add salt		
- rarely or never add salt at table		

7. Which type of breakfast cereal do you normally eat?
- high fibre (eg All Bran, Branflakes, Shredded
  Wheat, Muesli, Porridge, Weetabix
- other (eg Cornflakes, Rice Krispies, Special K
  Sugar Puffs, Honey Snacks
- no usual type
- do not eat breakfast cereal

8. How often do you eat these foods **only fill in one box for each food**

	Per day (times)				Per week			Per month	
	6+	4-5	2-3	once	5-6	2-4	once	1-3	Less than once
Breakfast cereal									
Fresh fruit									
Cooked green vegetables (fresh or frozen)									
Cooked root vegetables (fresh or frozen)									
Raw vegetables or salad (including tomatoes)									
Chips									
Potatoes, pasta, rice									
Meat									
Meat products									
Poultry									
White fish									
Oil rich fish									
Cheese									
Beans or pulses									
Sweets, chocolates									
Ice cream									
Crisps, savoury snacks									
Fruit juice (NOT squash)									
Soft/fizzy drinks									
Cakes, scones, sweet pies or pastries									

biscuits

9. In summary:
   (a) how many times do you eat fruit and vegetables or pure fruit juice

   ☐ per day OR ☐ per week OR ☐ per month

   (b) how many times do you eat oil rich fish

   ☐ per day OR ☐ per week OR ☐ per month

   (c) how many times do you eat sweets, chocolates, cakes, scones, sweet pies, pastries or biscuits

   ☐ per day OR ☐ per week OR ☐ per month

Recipe pages:
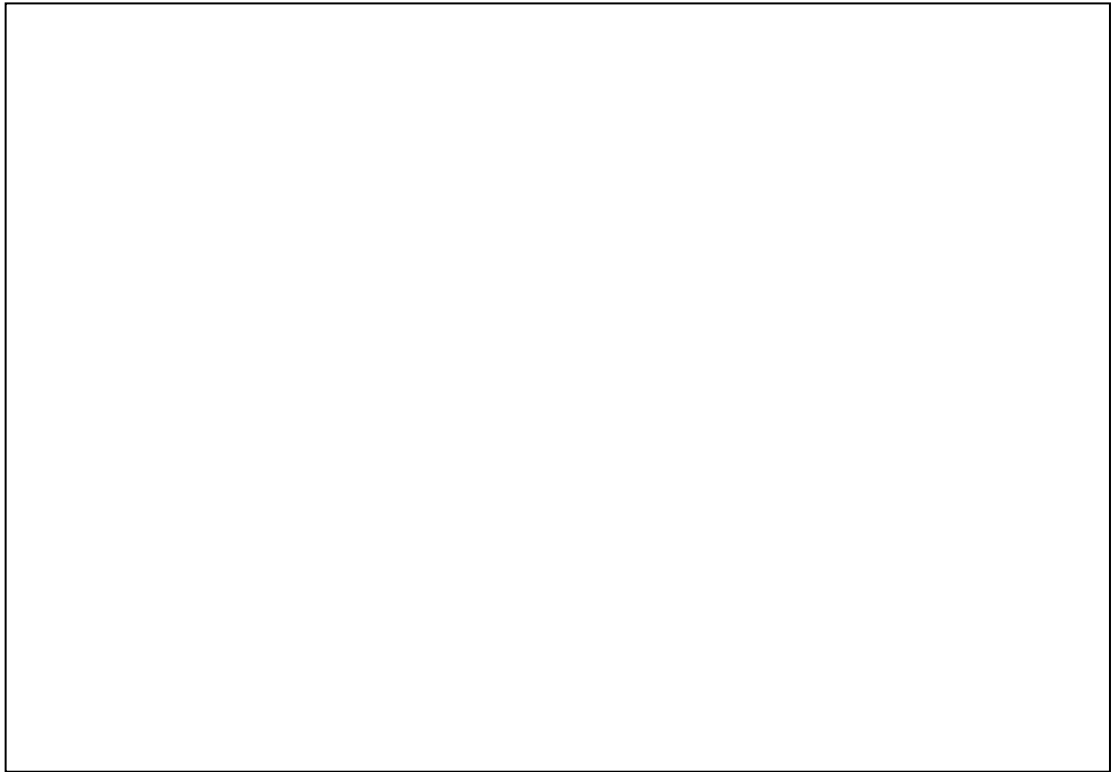
Recipe pages:
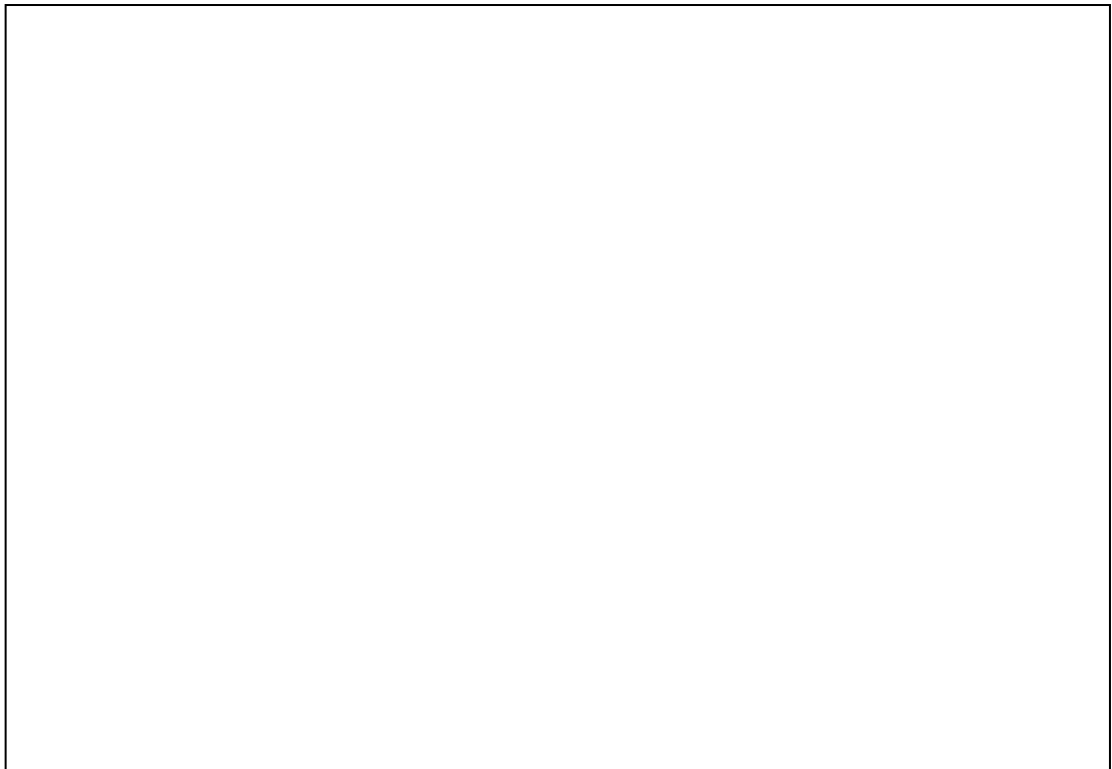
Physical activity monitor diary

This activity monitor is only to be worn when you are up and about. You don't need to wear it in bed or when having a shower or swimming. Please note down the times that you got up and went to bed so that we can discount this data from analysis.

ACTIVITY MONITOR
BEGINS _____
FINISHES _____

After the final day could you please send the activity monitor back to me in the pre-paid envelope as soon as is convenient.

Many thanks
Ellie

	Get up	Bedtime
Saturday 7/7/12		
Sunday 8/7/12		
Monday 9/7/12		
Tuesday 10/7/12		
Wednesday 11/7/12		
Thursday 12/7/12		
Friday 13/7/12		
Saturday 14/7/12		

8.3 Appendix III

physical activity parameter	visit (gestation wk)	lean mean (SD) [n]	OW/OB mean (SD) [n]	2 sample t-test p value
total wear time (mins/day)	V1 (15)	803.5 (67.8) [22]	810.0 (60.6) [14]	0.77
	V2 (25)	810.7 (54.4) [23]	813.6 (83.2) [14]	0.91
	V3 (35)	785.2 (68.9) [22]	796.8 (85.2) [12]	0.69
sedentary activity wear time (mins/day)	V1	550.8 (77.0)	571.3 (47.0)	0.29
	V2	541.7 (64.8)	565.4 (82.3)	0.38
	V3	526.6 (68.7)	553.8 (86.7)	0.37
light activity wear time (mins/day)	V1	216.9 (72.3)	214.2 (69.4)	0.91
	V2	238.2 (66.4)	228.6 (73.1)	0.66
	V3	235.7 (33.4)	226.3 (66.5)	0.55
MVPA wear time (mins/day)	V1	34.3 (20.0)	21.6 (11.5)	0.03
	V2	30.1 (23.8)	19.4 (10.4)	0.16
	V3	20.5 (17.4)	14.0 (10.7)	0.25
ratio sedentary:total wear time	V1	0.69 (0.08)	0.71 (0.07)	0.39
	V2	0.71 (0.07)	0.70 (0.08)	0.30
	V3	0.67 (0.05)	0.70 (0.08)	0.31
ratio light:total wear time	V1	0.27 (0.08)	0.27 (0.07)	0.77
	V2	0.29 (0.08)	0.28 (0.07)	0.61
	V3	0.30 (0.05)	0.28 (0.07)	0.45
ratio MVPA:total wear time	V1	0.04 (0.02)	0.03 (0.01)	0.02
	V2	0.04 (0.03)	0.02 (0.01)	0.08
	V3	0.03 (0.02)	0.02 (0.01)	0.14

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Accompanying Material

Distribution and accumulation of body fat in pregnancy has an effect on peripheral microvascular function

Eleanor Jarvie, William Ferrell, Jason Gill, Dilys Freeman

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INTRODUCTION

Maternal obesity is associated with vascular complications of pregnancy including pre-eclampsia. The exact anatomical location of fat accumulation during pregnancy is unknown, although data suggest that obese mothers gain weight with a central body distribution. Central adiposity in the non-pregnant is linked to excessive non-esterified fatty acid (NEFA) flux and ectopic fat accumulation leading to metabolic dysregulation. A similar mechanism may provoke the endothelial dysfunction associated with obesity-linked complications of pregnancy.

RESEARCH QUESTION

Is the site of subcutaneous fat storage in early pregnancy and the site of accumulation of subcutaneous fat during pregnancy associated with maternal microvascular function and the promotion of a lipotoxic environment.

RECRUITMENT

We are currently undertaking a longitudinal analysis of subcutaneous adipose tissue distribution and accumulation throughout pregnancy and concomitant measures of peripheral microvascular function (laser Doppler iontophoresis) and measurements of plasma lipotoxicity (oxysterols, oxidised LDL, superoxides). Sixty women will be recruited from antenatal clinics within Greater Glasgow & Clyde NHS Trust. Study visits are at 15, 25 and 35 weeks gestation and at 12 weeks postpartum. For this interim analysis we have baseline data on n=28 and completed data on n=16 mothers.

METHODS
Laser Doppler Iontophoresis (LDI)

LDI is a non-invasive tool for the assessment of peripheral (skin) microvascular function

Skinfold Thickness Measurements EJ was trained to Level 1 of the International Society for the Advancement of Kinanthropometry (ISAK).

Plasma Metabolic Markers Total triglycerides, total cholesterol, high density lipoprotein, non-esterified fatty acids, fasting glucose, gamma glutamyl transferase and C-reactive protein were measured by routine methodology at the Department of Biochemistry, Glasgow Royal Infirmary.

Statistical analysis Data was transformed if necessary to attain a normal distribution. Univariate analysis was performed using Pearson's correlation and multivariate analysis using the General Linear Model (Minitab vs15).

RESULTS

Table 1: Cohort demographics

Variable	Mean (range)
Maternal age (yrs)	29 (16-35)
Maternal weight (kg)	66.5 (48.5-99.6)
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Percentage body fat at booking (%)	20.0 (13.8-47.5)

Metabolic markers were assessed at baseline, as total area under the curve (AUC) and incremental AUC. Total AUC represented the total exposure of a particular marker throughout the gestational period. The incremental AUC metabolic response to pregnancy.
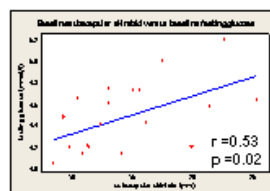

Figure 1: At baseline, subscapular skinfold thickness and fasting glucose where correlated (r=0.53, p=0.02), and remained independent of maternal age and smoking status. There was no other significant correlations with another trunkal skinfold and this metabolic marker.
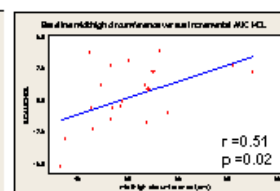
Figure 2: A larger midthigh circumference in early pregnancy was associated with an increased incremental change in HDL. This was not independent of maternal age and smoking status. Midthigh circumference did account for 18.6% of the variation in HDL on multivariate analysis.
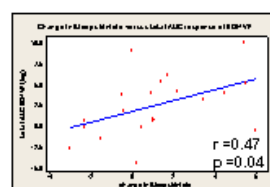
Figure 3: During pregnancy, the change in biceps skinfold was associated with improvement in EDMVF and was independent maternal age and smoking status but not BMI (p=0.05, 18.8% of the variation in EDMVF on multivariate analysis).
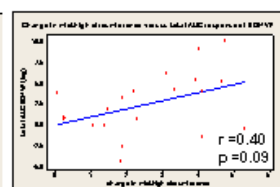
Figure 4: During pregnancy, the change in midthigh circumference is associated with improvement in EDMVF but this was not statistically significant.
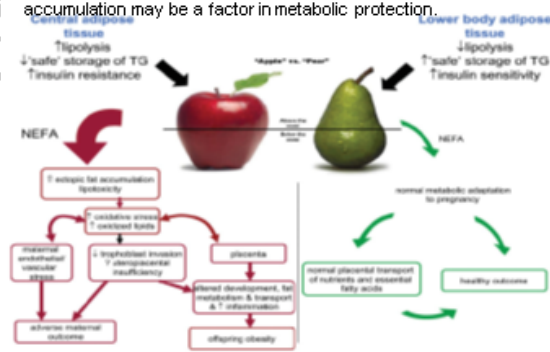
DISCUSSION

We observed a trend that accumulation of fat in upper peripheral (biceps, triceps) was directly associated with improvement in EDMVF during pregnancy (r=0.40, p=0.09) and this was significant for the change in biceps fat accumulation (r=0.47, p=0.04). No association was seen between lower peripheral (midthigh and suprapatella) or trunkal (subscapular, costal and suprailiac) skinfolds and EDMVF.

Baseline midthigh circumference and incremental HDL were directly correlated, suggesting that storage of fat in the lower body depots (i.e. gynoid distribution) confers metabolic protection during pregnancy.

Interestingly our data shows that subscapular adipose tissue is directly correlated with fasting glucose suggesting storage of fat in other trunkal sites may also convey metabolic risk.

CONCLUSION

This analysis suggests that during pregnancy, upper peripheral fat may have a protective effect on endothelial function and lower body fat accumulation may be a factor in metabolic protection.

Jarvie et al, Clin Sci 2010;119(3):123-9

Longitudinal assessment of energy metabolism: Comparison between lean and obese pregnancies in a Scottish Population

E Jarvie[1], J Gill[1], J Lovegrove[2], B Meyer[3], D Freeman[1]

[1]ICAMS University of Glasgow, [2]University of Reading, UK & [3]University of Wollongong NSW, Australia

INTRODUCTION

A longitudinal analysis of healthy lean (n=26) and obese (n=16) Scottish pregnant women indicated they gained similar total body mass (lean 9.4kg versus obese 10.4kg, p=0.48) and fat mass (4.3 versus 4.0kg, p=0.80). Ehrenberg et al also demonstrated a similar increase in body fat mass between lean and obese women, 4.7kg versus 4.2kg (p=0.58) respectively.

RESEARCH QUESTION

Our aim was to compare different elements of energy metabolism throughout pregnancy to establish whether these variables had different contributions to energy balance in lean and obese pregnancy.

RECRUITMENT

Healthy lean and obese pregnant women were recruited from their initial antenatal clinics (Greater Glasgow & Clyde NHS Trust) to take part in a longitudinal study assessing distribution and accumulation of subcutaneous adipose tissue, basal metabolic rate, substrate utilisation, physical activity and diet throughout pregnancy. Lean n=26 and obese n=16 unless otherwise stated.
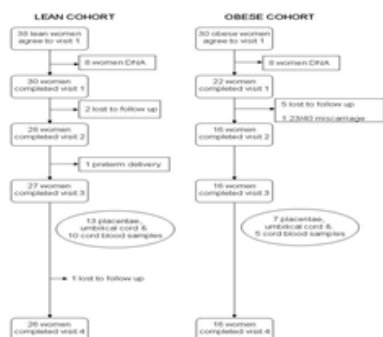

METHODS

Basal metabolic rate (BMR), fat and carbohydrate oxidation were measured at 15, 25 and 35 weeks' gestation by indirect calorimetry using an Oxycon ventilated hood system (Jaegar Oxycon Pro, Germany). Physical activity was objectively recorded using Actigraph GT3X accelerometers (ActiGraph, USA). Energy intake was assessed using a 24 hour recall questionnaire. Data were analysed using linear mixed modelling (IBM SPSS 19). Correlation analysis and t-test was assessed using Minitab version 16.

RESULTS

Baseline demographics for the cohort are displayed in table 1. At this stage in terms of energy metabolism, the obese group have a higher BMR. Lean women enter pregnancy more active than their obese counterparts.

Table 1: Baseline demographics of energy metabolism

| Indices at visit 1 (15 weeks)	Lean	Obese	p value
weight (kg)	60.7 (7.2)	87.0 (10.2)	<0.0001
fat mass (kg)	16.4 (4.4)	35.7 (7.3)	<0.0001
BMR (kJ/day)	5157 (716)	6114 (892)	0.001
CHO (g/day)	105.0 (100)	101.0 (62.9)	0.49
Fat (g/day)	56.8 (50.3)	68.5 (33.7)	0.56
MPVA (mins/day)	34.3 (20.0)	21.6 (11.6)	0.03
Total energy intake (kJ/day)	8183 (1406)	9512 (3044)	0.20
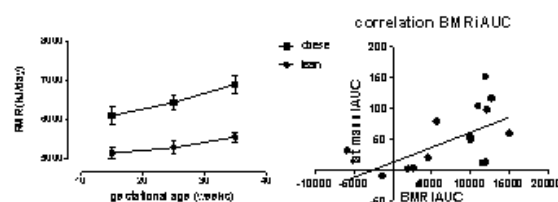
BMR increased over pregnancy in all women (5530kJ/day to 6080kJ/day, p<0.001) and was higher at all times in obese women (6492 versus 5336kJ/day, p<0.001). In obese, but not lean pregnancy, incremental area under the curve (iAUC) BMR correlated positively with iAUC fat mass (r=0.57, p=0.021).
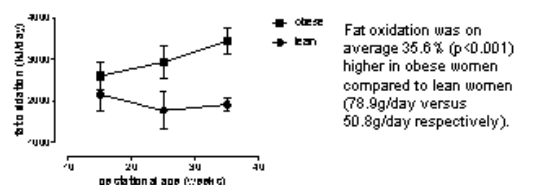
Fat oxidation was on average 35.6% (p<0.001) higher in obese women compared to lean women (78.9g/day versus 50.8g/day respectively).
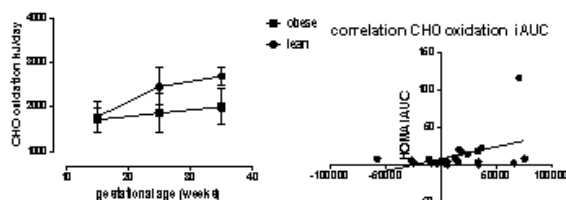
Carbohydrate oxidation was 20.0% (p<0.001) higher in lean women compared to obese women (137.0g/day versus 109.6g/day respectively). In lean pregnancy iAUC carbohydrate oxidation correlated positively with iAUC HOMA (r2=0.43, p=0.04).
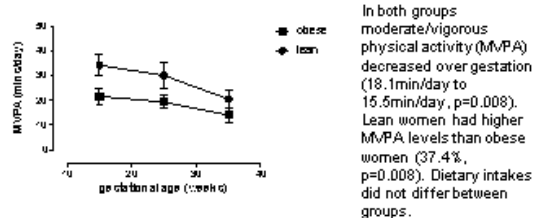
In both groups moderate/vigorous physical activity (MVPA) decreased over gestation (18.1min/day to 15.5min/day, p=0.008). Lean women had higher MVPA levels than obese women (37.4%, p=0.008). Dietary intakes did not differ between groups.

DISCUSSION

In conclusion, higher BMR in obese women may limit gestational fat gain. During pregnancy, lean women are more active than obese women and this may limit gestational weight gain. The contributions of the different elements of energy metabolism in lean and obese pregnancy differ suggesting that a "one size fits all" lifestyle intervention may not be appropriate for preventing excessive weight gain during pregnancy.

FURTHER WORK

As part of the overarching longitudinal study being performed a comprehensive assessment of metabolic markers (lipid profiles, inflammation, vascular activation and lipotoxicity) and peripheral microvascular function has been performed. Further analysis will focus on the impact of anatomical subcutaneous fat accumulation on these parameters and whether differences exist between lean and obese pregnancies

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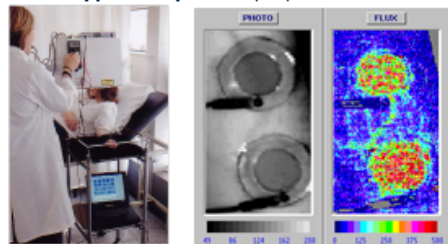
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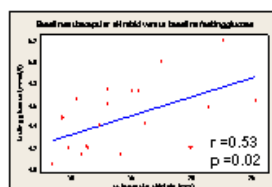

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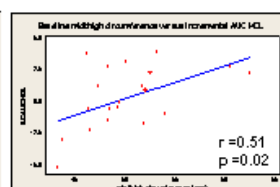
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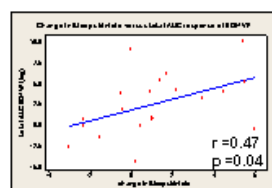
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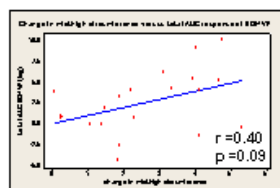Figure 4: During pregnancy, the change in midthigh circumference was associated with improvement in EDMVF but this was not statistically significant.
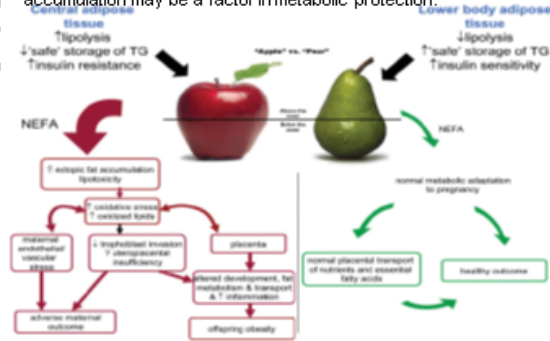
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Anatomical Adiposity & Metabolic Response in Lean and Non-Lean Pregnancies

Maternal obesity is a risk factor for GDM. Adipocyte dysregulation is thought to be a possible pathway for this pregnancy complication. This longitudinal study assessed distribution of subcutaneous fat accumulation in lean pregnancies (LP) and non-lean pregnancies (NLP) and whether different anatomical sites had impact on metabolic markers (glucose, insulin, TG, NEFA). Adipose tissue accumulation was measured by skinfold thickness by the same trained operator. Plasma markers were measured by routine methodology. Statistical analysis was performed using paired t-test, and Pearson's correlations.

There was no significant difference in gestational weight gained by LP (n=27) compared to the NLP (n=8). In healthy LP there was a significant increase in abdominal skinfolds (costal, suprailiac) (28.9mm v 34.4mm p=<0.0001) and lower body peripheral (midthigh, suprapatellar) (35.0mm v 43.5mm p=<0.0001) across gestation. In NLP, upper body peripheral (biceps, triceps, subscapular) (73.2mm v 86.7mm p=0.012) and lower body peripheral skinfolds (61.4mm v 75.1mm p=0.002) were significantly increased over gestation.

In LP there was significant gestational increases in insulin (3.0mU/L v 6.7 p=<0.0001) and TG (1.2mmol/l v 2.5 p=<0.0001) but not Glucose or NEFA. In LP gestational changes in insulin were inversely correlated with deposition in upper body peripheral during pregnancy (r=-0.55, p=0.009).

In NLP there was significant gestational increases in TG (1.8mmol/l v 3.0 p=0.003) but not insulin, glucose or NEFA. In NLP upper body skinfolds were correlated with gestational change (GC) in fasting glucose (r=0.91, p=0.002) and GC in TG (r=0.97, p=0.002).

We found that LP has significant increase in central adiposity, but this does not drive metabolic response. Although NLP gain fat preferentially in lower body depots, this does not have an effect on the changes seen in TG and glucose. We continue to collect data on the NLP (n=16 in total) for validation of findings and investigate the effect of fat accumulation on endothelial function, oxidised LDL and superoxide formation in LP and NLP.