

# The impact of maternal obesity on vascular and metabolic function throughout pregnancy.

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# Abstract

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# List of Publications

## Publications

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**Stewart FM**, Caslake MJ, et al. Body fat distribution correlates directly to endothelial function. The pattern of endothelial cell function changes throughout pregnancy. *Journal of Obstetrics and Gynaecology*. 26 (1). Sp 1. S12 April 2006.

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**Stewart FM**, Caslake MJ, Rodie VA et al. Prospective examination of Novel cardiovascular Risk factors throughout pregnancy in Lean and Obese women. *Hypertension in Pregnancy*. 23 Sp 1. 12A 2004.

**Stewart FM**, Rodie VA, et al. Erythrocyte fatty Acid Composition in Healthy Pregnancies and Pregnancies Complicated by PET or IUGR. *Hypertension in Pregnancy*. 23 Sp 1. 25A 2004.

**Stewart FM**, Rodie VA, et al. Prospective Examination of Microvascular Function Throughout Pregnancy in Lean and Obese Women. *Hypertension in Pregnancy*. 23 Sp 1. 102A 2004.

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**Stewart FM**, Ramsay JE, Greer IA, et al. Microvascular Dysfunction: A link between pre-eclampsia and maternal coronary heart disease. *British Journal of Obstetrics and Gynaecology*. 110 (11): 1029-1031 Nov 2003

**Stewart FM**, Ramsay JE, Caslake MJ, et al. Maternal Metabolic response to pregnancy determines newborn metabolic characteristics independent of both maternal and fetal adiposity. *Paediatric Research*. 53 (6): 14A-14A Part 2. Suppl. S. Jun 2003.

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Rodie VA, **Stewart FM**, Thomson AJ et al. Low Molecular weight Heparin for the treatment of venous thromboembolism in pregnancy: a case series. *British Journal of Obstetrics and Gynaecology*. 109: 1020-1024 Sep 2002.

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## **Presentations of work undertaken in this thesis to learned societies**

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Erythrocyte fatty Acid Composition in Healthy Pregnancies and Pregnancies Complicated by PET or IUGR.

- September 2004

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**Second World Congress on Fetal Origins of Adult Disease. Brighton UK.**

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## List of Abbreviations

ACh	acetyl choline
Apo B	apolipoprotein B
ANOVA	analysis of variance
AUC	area under the curve
BMI	body mass index
CHD	coronary heart disease
cAMP	cyclic guanosine-5-monophosphate
CRP	C-reactive protein
CI	confidence interval
CV	coefficient of variance
EDRF	endothelial derived relaxant factor
EDTA	ethylene diamine tetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
FAs	fatty acids
FFA	free fatty acids
GC	gas chromatography
GDM	gestational diabetes mellitus
GTP	guanosine triphosphate
HDL	high density lipoprotein
HERS	heart and oestrogen/ progestogen replacement study
HRT	hormone replacement therapy
ICAM	intracellular adhesion molecule
IHD	ischaemic heart disease
IL-6, -10, -1	interleukin -6, -10, -1
LDI	laser Doppler imaging
LDL	low density lipoprotein
MRNA	messenger ribonucleic acid
MUFA	mono unsaturated fatty acid
NaCl	sodium chloride
NEFAS	non esterified fatty acids
NICE	national institute of clinical excellence
NICU	neonatal intensive care unit
NIDDM	non-insulin dependent diabetes mellitus
NO	nitric oxide
NOS	nitric oxide synthase
NTD	neural tube defect
OCs	oral contraceptives
OR	odds ratio
PAI	plasminogen activator inhibitors
PCOS	polycystic ovarian syndrome
PET	pre-eclampsia
PGI <sub>2</sub>	prostacyclin
PIH	pregnancy induced hypertension
PU	perfusion units
RBC	red blood cell
RR	relative risk
SD	standard deviation
4S	Scandinavian simvastatin survival study

SHBG	sex hormone binding globulin
SIGN	Scottish intercollegiate guidelines network
SOGC	Society of obstetricians and gynaecologists of Canada
SNP	sodium nitroprusside
TC	total cholesterol
TG	triglyceride
TNF $\alpha$	tumour necrosis factor- $\alpha$
UK	United Kingdom
US	united States
VCAM	vascular cell adhesion molecule
VLDL	very low density lipoprotein
VTE	venous thromboembolism
vWF	von Willebrand s
WHR	waist hip ratio

## **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 composed by myself, and I have been responsible for patient recruitment, clinical investigation and data analysis, unless otherwise acknowledged.

Frances Stewart

June 2008

# **Chapter One**

## **INTRODUCTION**

**Obesity: The impact on women's health.**

***A review of present literature concerning obesity  
and its impact on the pathophysiology of  
vascular disease in pregnancy and later life.***

# 1 Introduction

The 2002-2004 Confidential Enquiries into Maternal and Child Health has highlighted obesity as a significant risk for maternal death. More than half of all the women who died from *Direct* or *Indirect* causes, were either overweight or obese.

For the mother maternal obesity increases the risk of obstetric complications during the antenatal, intrapartum and postnatal period as contributing to the technical difficulties of fetal assessment . The offspring of obese mothers also have a higher peri-natal morbidity and long term health problems

In 2000 the World Health Organisation estimated as many as 300 million people worldwide were clinically obese. This figure could double by the year 2025 if no action is taken against this threat (1). With obesity comes the increased risk of diabetes, cardiovascular disease, cancer and stroke. The influence of obesity on women's health is evident throughout both reproductive life and the menopause. Obesity increases the risks of gynaecological pathology such as infertility, menstrual disorders and endometrial cancer, making obese women more likely to attend the gynaecology clinic. In obstetrics, obesity increases the risk of antepartum, intrapartum and postpartum complications for the mother as well as contributing to the technical difficulties of fetal assessment. The offspring of obese mothers also have a higher perinatal morbidity and long term health problems.

## 1.1 Epidemiology of obesity

The prevalence of obesity, defined as a body mass index (BMI) of  $30 \text{ kg/m}^2$  or more has significantly increased during the last three decades, both in the United States (USA) and other first world countries. American trends are now seen among the European population as the Department of Health, for the United Kingdom (UK) Health survey for England reports that thirty two percent of 35- to 64-year-old women are overweight (BMI  $25\text{--}30 \text{ kg/m}^2$ ) and 21% obese (BMI  $> 30 \text{ kg/m}^2$ ) (2). In a British Women's Heart and Health Study, one quarter of the 4000 participants in England, Scotland and Wales were found to be clinically obese (3). One fifth of the women were inactive and two fifths did not eat a portion of fresh fruit a day. The department of Health has predicted that if current trends continue, by 2010 six million women will be obese in England (2). In line with this, a large Scottish maternity hospital has observed a twofold increase in the proportion of women with a booking body mass index (BMI)  $>30 \text{ kg/m}^2$  over the past decade (2) Obesity amongst women is of world-wide concern as shown in Australia by Callaway et al, in 2006 (5). They noted that thirty-five percent of Australian women between the ages of 25 and 35 years were overweight or obese. Obesity accounts for more than 280,000 deaths annually in the United States and will soon overtake smoking as the primary preventable cause of death if current trends continue (6).

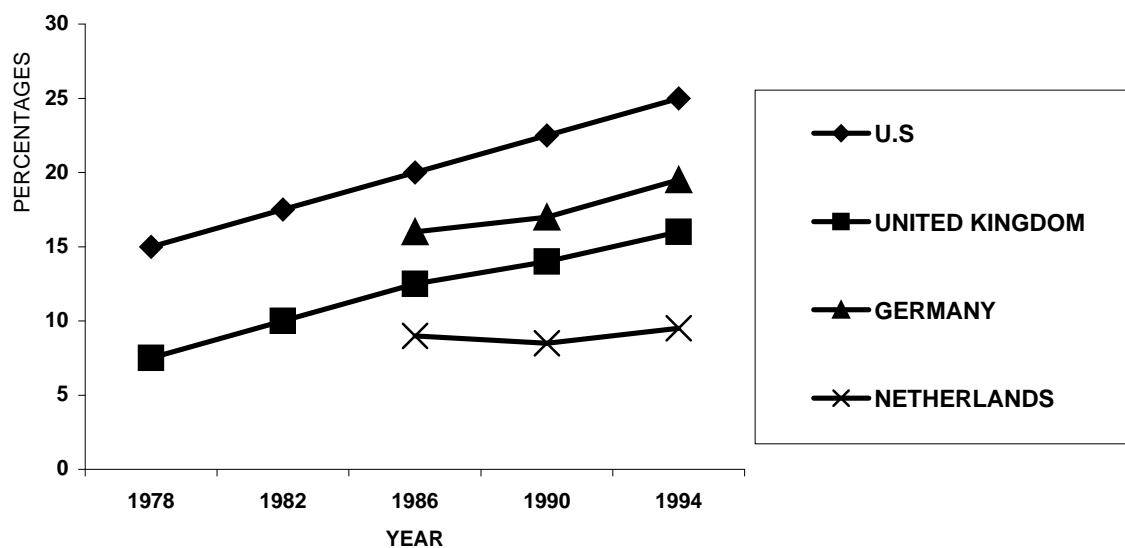
According to the Health Survey for England published by the Department of Health It is estimated that in 2010 around 6,658,953 men will be obese, increasing from

around 4,302,588 in 2003. For women, it is estimated that a further 1,230,573 women will be obese in 2010 than in 2003. Forecast projections showed that in 2010, a increase in the proportion and number of boys who are obese can be expected, rising from 746,662 in 2003 to 792,321 in 2010. The greatest increases are expected among girls, with around a six percentage point increase in obesity rates between 2003 and 2010. It is estimated that around 910,630 girls will be obese in 2010.

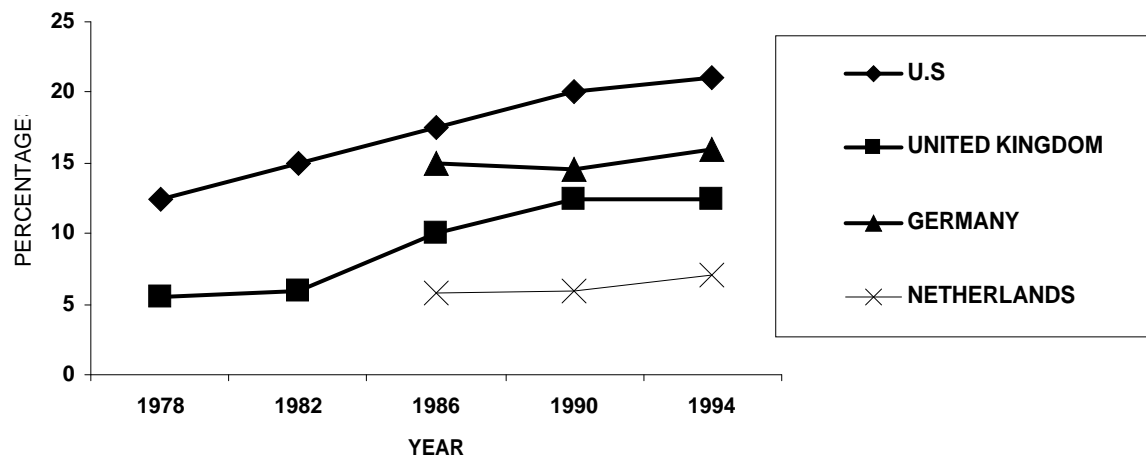
Obesity has now become pandemic in the U.S. where currently 2 in 3 American adults are classified as overweight or obese, compared with fewer than 1 in 4 in the early 1960s (3). Although still viewed more as a cosmetic rather than a health problem by the general public, excess weight is a major risk factor for premature mortality, cardiovascular disease, type 2 diabetes mellitus, osteoarthritis, certain cancers, and other medical conditions. Obesity accounts for more than 280,000 deaths annually in the U.S. and will soon overtake smoking as the primary preventable cause of death if current trends continue (3).

As shown in Figure 1-1, the prevalence of obesity varies widely among countries that are geographically not very distant (e.g., Netherlands and neighbouring Germany) and is increasing in most European countries as well as in the U.S.

# Women



# Men



**Figure 1-1. Time trends in the prevalence of obesity [BMI (in kg/m<sup>2</sup>) >30] in some European countries and in the U.S. Adapted from (4)**



In White populations living in the west and north of Europe, Australia, and the U.S. the prevalence of obesity is similarly high in men and women (5). Australia has a prosperous Western-style capitalist economy, and spends approximately 830 million dollars on the direct health care costs of obesity. For Australians, it is now more common to have a weight problem, with 48% of men and 30% of women being overweight and obesity affecting a further 19% of men and 22% of women (6). National survey data from the U.S. show that the prevalence of overweight and obesity among adults remained relatively constant over the 20-year period from 1960 to 1980 began to increase around the mid-1980s and has continued to increase (5). American trends are now being seen among the European population with 10% of children and 20% of adults in the UK now being classified as clinically obese (7).

Obesity and sedentary lifestyle is a health issue affecting large numbers of women. The British Women's Heart and Health Study, is a large study looking at lifestyle health issues among 4000 women in England, Scotland and Walse. One quarter of the 4000 participants were found to be clinically obese (8). One fifth of the women were inactive and two fifths did not eat a portion of fresh fruit a day.

In 1999 Reilly *et al* examined data on 2630 children, a nationally representative sample. He describes the alarming increase in prevalence of obesity among children (see table1-1) (7).

	BMI > 85 <sup>th</sup> percentile	BMI > 95 <sup>th</sup> percentile
AGED 6 YEARS	22%	10%
AGED 15 YEARS	31%	17%

**Table 1-1. Trends in childhood obesity;** Reilly J *et al.* Table showing the percentage children above the 85<sup>th</sup> and 95<sup>th</sup> centiles for BMI at age 6 years and 15 years (7).

Among populations ethnic variations exist in terms of obesity rates. One Norwegian study looking at 8,000 immigrants found high proportions of overweight and obese subjects from Pakistan and Turkey, but low proportions among those from Vietnam. Subjects from Sri Lanka and Pakistan had the highest waist hip ratio for any given value of BMI (9).

Obesity greatly increases risks for many serious and morbid conditions, including diabetes mellitus, hypertension, dyslipidemia, coronary artery disease, and some cancers.

The challenges of the epidemic are not limited to merely aesthetic concerns. The disabilities caused by obesity are not only physiological but also psychosocial. The increased waist to hip girth is associated with increased risk of cardiovascular disease, hyperlipidaemia, hypertension, and diabetes. Obesity also has been related directly to increased risk of sleep apnoea, cancer, gallbladder disease, musculoskeletal disorders, severe pancreatitis, diverticulitis, infertility, urinary incontinence, and idiopathic intracranial hypertension. The psychosocial factors concerning quality of life in the obese population have also been documented. Although there is some debate, the obese have

been found to be twice as likely to suffer from anxiety, impaired social interaction and depression when compared with the non obese population (10).

## **1.2 Influence of obesity on gynaecological issues**

Obesity in adolescence has increased significantly over the past 30-40 years and a recent international comparison study (13 European countries, Israel and the US) showed that the highest prevalence in adolescents was found in the US (12.6% in 13 year old boys, 10.8% in girls; 13.9% in 15 year old boys and 15.1% in 15 year old girls) and the lowest in Lithuania. This increase in adolescence is a public health concern, because most obese adolescents continue their obesity into adulthood with serious risk for chronic disease. Focus should therefore be on prevention programmes that increase healthier patterns of lifestyle and physical activity (11). Obesity in adolescence tends to persist into adulthood. Adolescent obesity is also connected to excess mortality. High BMI in adolescence seems to be predictive of both adult obesity and mortality (12). The obese teenager is becoming an increasingly common attendee at the gynaecology clinic. Presenting complaints include menstrual disorders, amenorrhoea, oligomenorrhoea and menorrhagia. Polycystic ovarian syndrome (PCOS), with its associated symptoms and signs including obesity, hirsutism and infertility are commonly seen among younger women (see table 1-2)

Percentage frequency of symptom or sign				
	Balen <i>et al.</i> (1995)	Franks (1989)	Goldzieher <i>et al.</i> (1981)	
	<i>n</i> = 1741 (%)	<i>n</i> = 300 (%)	<i>n</i> = 1079 (%)	No. of cases
<b>Menstrual cycle disturbance</b>				
Oligomenorrhoea	47	52	29	547
Amenorrhoea	19	28	51	640
Hirsutism	66.2	64	69	819
Obesity	38	35	41	600
Acne	35	27	–	–
Alopecia	6	3	–	–
Acanthosis nigricans	3	<1	–	–
<b>Infertility</b>				
(primary/secondary)	20	42	74	596

**Table 1-2. Clinical symptoms and signs in women with PCOS**, adapted from (13)

The effects of diet and adiposity have been implicated in disturbances of female reproductive function. Obesity complicates approximately 50% of all cases of PCOS and may underlie or contribute to ovulatory dysfunction by augmenting both the insulin resistance and the hyperandrogenaemia present in this disorder (14). An association independent of PCOS, however, has also been demonstrated between truncal obesity and irregular menses (15), suggesting that obesity itself may negatively impact on fertility. The Nurses' Health Study II, which examined prospectively collected data on adiposity for 830 cases of incident ovulatory infertility and 26,125 pregnancies, revealed an increased risk for ovulatory infertility with a BMI below 20.0 or above 24.0 kg/m<sup>2</sup> (16). Taken together, these findings demonstrate a U-shaped association between BMI and the relative risk for female infertility. Both smoking and obesity unfavourably affect the live birth rate after *in-vitro* fertilisation (IVF) (17). Incidentally, poor semen quality was found to be associated with sedentary lifestyle and obesity among the male population (18). Not only does the presence of obesity make a couple more likely to experience difficulties with regard to conception, it also infers a negative impact on efficacy of treatment. Weight reduction programmes and BMI limits prior to commencement of expensive assisted conception treatment programmes is a strategy now being used often by fertility centres throughout the United Kingdom. As there are a limited number of National Health Service funded treatment cycles available to them, pre treatment optimum BMI seems essential.

Obesity constitutes a relative contradiction for use of combined oral contraceptives (OCs) (19). Combined OCs may aggravate the obesity. Obesity on the other hand is a risk factor for cardiovascular incidents among OC users (19). The most serious risks to OC users who are over age 35 and smoke are deep vein thrombosis, pulmonary embolus, retinal thrombosis, or cardiovascular disease. Other risk factors for cardiovascular disease include obesity, diabetes, hypertension, increased serum cholesterol, and a family history of premature myocardial infarction. All users should have blood pressure checks 3 and 6 months after commencing pill use. OC usage does not increase the risk of developing breast cancer, but can stimulate the growth of breast cancer once it has occurred (20).

Obesity when present in the menopausal women poses problems with regard to treatment options. Clinical risk factors for venous thromboembolism (VTE) include obesity, smoking, lifestyle, and aging. These factors coupled with the inherent thrombotic risk of hormone replace therapy (HRT) can lead to a cumulative increased risk of VTE (21). The Heart and Oestrogen/progestin Replacement Study (HERS) looked at secondary prevention using HRT in women with established arteriosclerosis. No significant difference was observed between HRT and placebo, while a possible protection was postulated by year 4. The follow up HERS II study observed no sustained protection with longer term HRT use (22).

Obesity has also been described as a risk factor for development of breast and endometrial cancer (23). Therefore prescription of HRT, also recently suggested to increase the risk of both these conditions (24), must be carefully considered in the obese woman.

## 1.3 Influence of obesity on pregnancy

### 1.3.1 *Maternal health issues: Antenatal*

The Swedish Birth registry published data in the Lancet this year which found that compared with women whose BMI changed between -1.0 and 0.9 units, the adjusted odds ratios for adverse pregnancy outcomes for those who gained 3 or more units during an average 2 years were: pre-eclampsia, 1.78 (95% CI 1.52-2.08); gestational hypertension 1.76 (1.39-2.23); gestational diabetes 2.09 (1.68-2.61); caesarean delivery 1.32 (1.22-1.44); stillbirth 1.63 (1.20-2.21); and large-for-gestational-age birth 1.87 (1.72-2.04) (25). These findings support the concept of obesity as a causative factor in adverse pregnancy outcomes. Obesity is associated with an increased risk of first trimester and recurrent miscarriage. One UK case-control study compared first trimester miscarriage rates between 1,644 obese nulliparous (first pregnancy) (BMI >30 kg/m<sup>2</sup>) and 3,288 age matched control nulliparous women (BMI 19-24.9kg/m<sup>2</sup>). The prevalence of first trimester miscarriage and recurrent early miscarriage was significantly higher among the obese women (odds ratios 1.2 and 3.5, 95% CI 1.01-1.46 and 1.03-12.01, respectively; P = 0.04, for both] (26). Obesity is associated with poor pregnancy outcome and miscarriage in both women with PCOS, and in those with normal ovarian morphology. A meta-analysis of thirteen studies looking at gonadotrophin induced ovulation in women with normo-gonadotrophic anovulatory infertility, found obesity and insulin resistance to be the most clinically useful predictors for poor clinical outcome. The pooled odds ratio for obese versus non-obese women for spontaneous miscarriage rate was 3.05 (95% CI: 1.45-6.44) (27).

Several studies have shown an increased risk for neural tube defects (NTD) associated with pre-pregnancy maternal obesity. Those women with a higher BMI value have a higher risk of congenital malformations among their offspring (28). This association was confirmed by Watkins *et al* in a population-based case control study using information from the Atlanta Birth Defect Risk Factor Surveillance Study. The study concluded that for every incremental unit increase in BMI ( $\text{kg}/\text{m}^2$ ), the risk of NTD increased by 7% (29). Werler *et al* evaluated 604 cases of NTD identified within 6 months of delivery and compared them with 1,658 controls with other major malformations and 93 controls with no malformations (30). The women were classified according to their folate intake as above or below the recommended daily amount of 400  $\mu\text{g}$ . It was observed that this dose of folate was found to be protective against NTD in women whose absolute body weight was less than 70 kg and not protective in those greater than 70 kg. The risk of NTD was also found to increase with increasing maternal weight independent of folic acid intake (30). Data based on 56,857 children in an analysis from the National Institute of Neurological, Communicative Disorders and Stroke showed an increased incidence of major congenital malformations of 35% when mothers were overweight and of 37.5% when they were obese (31).

Obstetric ultrasound is widely practised throughout the developed world. Initially its major indications were dating of pregnancy, detection of multiple pregnancies and detection of intrauterine growth restriction, more recently with increasing resolution of modern machines, detection of fetal anomalies has become possible. The limitations of obstetric ultrasound as a screening test are dictated by the expertise of the clinician, the quality of the equipment and by the habitus of the patient. This should be clearly



understood in order to prevent unrealistic expectations. One American study examined the sensitivity of ultrasound to visualise fetal cardiac structures in obese gravid women (32). They demonstrated that despite the use of advanced ultrasound equipment, maternal obesity significantly limits visualisation of the fetal heart. Suboptimal ultrasound views of the fetal heart were more frequent in the obese women and this was not improved with higher specification equipment (32). By absorbing the associated energy adipose tissue can significantly attenuate the ultrasound signal. Therefore a high frequency, higher resolution signal would be more significantly absorbed at a lesser depth, sacrificing image quality and depth of field. A worrying consequence of maternal obesity is the risk of missing fetal anomalies when present.

Wolfe *et al* prospectively collected data on 1,622 ultrasounds performed in the second and third trimesters of women who delivered live, singleton infants (33). The average gestational age was 28.5 weeks at the time of ultrasound. Each ultrasound was classified as visualized or sub optimally visualized. In patients with BMI less than the 10th percentile visualization of the anatomic structures studied was reported as 90.2%. In patients with BMI in the 97.5th percentile, visualization decreased to 63%. The author also reported a 14.5% decrease in visualization of all organs systems in patients with a BMI greater than the 90th percentile compared with patients with normal BMIs. There was no improvement in examinations with increasing gestational age or length of time used to conduct the ultrasound examination in patients with higher BMIs (33). In diabetic mothers increased rates of fetal anomalies are well documented (reference), however the associated increased maternal habitus more prevalent among diabetic mothers results in a lower detection rate. An Australian study looking at fetal anomaly rates among diabetic

mothers found a fetal anomaly rate of 7.7% for major anomalies and 2.3% for minor anomalies in the fetuses of the diabetic women (34). Major fetal anomalies were more likely to be identified in obese mothers (78% vs. 37%;  $P < 0.05$ ). Compared to the 'low-risk' non-diabetic population from the same institution, the relative risk for a major congenital anomaly among the diabetic women was 5.9-fold higher (95% confidence interval, 2.9-11.9). Despite these statistics, the detection rate for major fetal anomalies was significantly lower for diabetic women (30% vs. 73%;  $P < 0.01$ ), and as expected the mean BMI for the diabetic group was significantly higher (29 vs. 23 kg/m<sup>2</sup>;  $P < 0.001$ ) (34).

Independent of pregnancy, hypertensive disorders are significantly more prevalent in obese women than in their lean counterparts. Elevated pre pregnancy BMI is an independent risk factor for development of pregnancy-induced hypertension (PIH) (35). Even when the degree of excess body fat is moderate, the occurrence of hypertension and pre eclampsia is significantly higher by comparison with control patients (31, 36-38). Maternal obesity, both in itself and as part of the insulin resistance syndrome, is an important risk factor for the development of pre eclampsia. O'Brien *et al* identified thirteen cohort studies, comprising nearly 1.4 million women. The risk of pre eclampsia typically doubled with each 5-7 kg/m<sup>2</sup> increase in pre pregnancy BMI. This relation persisted in studies that excluded women with chronic hypertension, diabetes mellitus or multiple gestations, or after adjustment for other confounders (39). In non pregnant subjects, hypertension is associated with upper-body rather than lower body fat. Body fat distribution is an important factor in the assessment of risk of cardiac disease. Similarly in pregnancy not only the pre pregnant BMI but also the body fat distribution may

provide a more sensitive risk estimate. One study looking at 22 patients with pre eclampsia and 126 controls, found that the ratio of upper to lower body fat was more accurately associated with the development of pre eclampsia rather than BMI (40). Sattar *et al* looked at early pregnancy waist hip ratio in 1,142 women. When the waist circumference was  $> 80\text{cm}$  the risk of pregnancy induced hypertension doubled (OR 1.8 95% CI 1.1-2.9) and the risk of pre-eclampsia was almost tripled (OR 2.7 95% CI 1.1-6.8) (41). This data reinforces the importance of central obesity as a predicting factor of adverse outcome as opposed to the less sensitive marker of BMI.

Gestational diabetes mellitus (GDM) affects about 5% of all pregnancies (42). Being overweight is a risk factor for impairment of carbohydrate tolerance both in the non-pregnant and pregnant state. Conversely half of the women who develop GDM have no identifiable risk factor (43). No firm definition of gestational diabetes is universally agreed. In 2001 the Scottish Intercollegiate Guidelines Network (SIGN) confirmed that as yet no consensus exists as regards the definition, management or treatment of GDM. Importantly this body felt that no clear criteria for screening were established. In 2002 the Society of Obstetricians and Gynaecologists of Canada (SOGC), published guidelines supporting target screening for those at higher risk of gestational diabetes. Obesity is included in their risk assessment. However in the UK in 2003 the National Institute of Clinical Excellence (NICE) stated that the available evidence did not support routine screening for GDM. The debate extends further as to the impact of treatment of GDM. Effective treatment does reduce the risk of macrosomia, however instrumental and operative delivery rates are increased even in the absence of fetal macrosomia (44). Crowther *et al* conducted a randomized clinical trial to determine whether treatment of

women with gestational diabetes mellitus reduced the risk of perinatal complications (see Table 1-3) (45).

**Table 2. Primary Clinical Outcomes among the Infants and Their Mothers.\***

Outcome	Intervention Group	Routine-Care Group	Unadjusted Relative Risk (95% CI)	Unadjusted P Value	Adjusted Relative Risk (95% CI)†‡	Adjusted P Value†‡	Step-Down Sidak P Value
	no. (%)						
Infants							
Total no.	506	524					
Any serious perinatal complication‡	7 (1)	23 (4)	0.32 (0.14–0.73)	0.004	0.33 (0.14–0.75)	0.01	0.04
Death	0	5 (1)		0.06		0.07	
Stillbirth	0	3 (1)§		0.25		0.26	
Neonatal death	0	2 (<1)		0.50		0.50	
Shoulder dystocia¶	7 (1)	16 (3)	0.45 (0.19–1.09)	0.07	0.46 (0.19–1.10)	0.08	
Bone fracture	0	1 (<1)		1.00		0.38	
Nerve palsy	0	3 (1)		0.25		0.11	
Admission to neonatal nursery <sup>***</sup>	357 (71)	321 (61)	1.15 (1.05–1.26)	0.002	1.13 (1.03–1.23)	0.01	0.04
Jaundice requiring phototherapy	44 (9)	48 (9)	0.95 (0.64–1.40)	0.79	0.93 (0.63–1.37)	0.72	0.98
Women							
Total no.	490	510					
Induction of labor††	189 (39)	150 (29)	1.31 (1.10–1.56)	0.002	1.36 (1.15–1.62)	<0.001	0.003
Cesarean delivery	152 (31)	164 (32)	0.96 (0.80–1.16)	0.70	0.97 (0.81–1.16)	0.73	0.98
Elective	72 (15)	61 (12)	1.23 (0.89–1.69)	0.20	1.17 (0.85–1.60)	0.33	
Emergency	80 (16)	103 (20)	0.81 (0.62–1.05)	0.11	0.87 (0.68–1.13)	0.31	

\* CI denotes confidence interval.

† Values were adjusted for maternal age, race or ethnic group, and parity.

‡ Serious perinatal complications were defined as one or more of the following: death, shoulder dystocia, bone fracture, and nerve palsy. The number needed to treat to benefit was 34 (95 percent confidence interval, 20 to 103).

§ Gestational ages at delivery for the three stillborn infants were 35, 37, and 40 weeks.

¶ Shoulder dystocia was recorded by the primary caregiver present at the birth.

|| One infant had both a fractured humerus and a radial-nerve palsy. One infant had both shoulder dystocia and Erb's palsy.

\*\* The number needed to treat to harm was 11 (95 percent confidence interval, 7 to 29).

†† Indications for the induction of labor in the intervention and routine-care groups were as follows: gestational diabetes in 23 percent and 4 percent, respectively; preeclampsia in 6 percent and 12 percent, respectively; past due dates in 8 percent of each group; fetal compromise in 5 percent and 2 percent, respectively; and other indications in 5 percent and 3 percent, respectively. The number needed to treat to harm was 11 (95 percent confidence interval, 7 to 31).

**Table 1-3. : Perinatal and maternal complications among women with gestational diabetes mellitus.**

Table taken from Crowther *et al* showing that the rate of serious perinatal complications was significantly lower among the infants of the women in the intervention group than among the infants of the women in the routine-care group (1% versus 4%,  $p=0.01$ ). However, more infants of women in the intervention group were admitted to the neonatal nursery (71 percent vs. 61 percent; adjusted relative risk, 1.13; 95 percent confidence interval, 1.03 to 1.23;  $P=0.01$ ). Women in the intervention group had a higher rate of induction of labour than the women in the routine-care group (39 percent vs. 29 percent; adjusted relative risk, 1.36; 95% CI, 1.15 to 1.62;  $P<0.001$ ), although the rates of caesarean delivery were similar (31 percent and 32 percent, respectively; adjusted relative risk, 0.97; 95 % CI, 0.81 to 1.16;  $P=0.73$ ) (45)

No epidemiological investigation of any significant size assessing the importance of body fat distribution as a risk factor for GDM has been undertaken as yet. However being overweight clearly increases the risk of carbohydrate intolerance during pregnancy. Even being moderately overweight (BMI 25-30 kg/m<sup>2</sup>) increases the risk of GDM 1.8-6.5 fold when compared with normal weight controls (31, 36-38). In obese women (BMI >30 kg/m<sup>2</sup>), the incidence of GDM is 1.4 to 20 fold higher than in normal weight controls (38, 46). If GDM is diagnosed, tight metabolic control should be achieved through diet and, when indicated, insulin therapy. Insulin therapy is required more often in obese women with GDM than in lean women with the condition (47). Treatment of the condition using insulin reduces maternal and fetal morbidity (45, 48, 49)

Women who suffered from impaired carbohydrate metabolism during pregnancy are more likely to develop diabetes later in their lives. One study investigating 200 women who had GDM diagnosed between 1980-1998 found that the risk of diabetes development was significantly higher (independently of the clinical type) in women who had GDM during their pregnancy (50). Mothers who develop gestational diabetes have a 50% greater risk of developing diabetes during their lifetime (50).

### **1.3.2 *Intra partum issues***

Obesity and diabetes are independently associated with adverse pregnancy outcomes. Rosenberg *et al* undertook a large population based study in which they collected data

from the 1999, 2000, and 2001 New York City birth files for 329,988 singleton births containing information on pre pregnancy weight and antenatal weight gain (51). Pre pregnancy and gestational diabetes were significant risks for a primary caesarean section and for preterm birth. (51). During pregnancy, obese women were at increased risk for several adverse perinatal outcomes, including anaesthetic, peri-operative, and other maternal and fetal complications. A population-based observational study carried out in the UK, looked at a total of 60,167 deliveries (Table 1-4) (52). All women were primigravid, more than 37 weeks' gestation and uncomplicated singleton pregnancies, height and weight were recorded at the booking visit. Risk of adverse clinical outcome among obese mothers is shown in table 1-4.

Adverse outcome	Obese Odds ratio, (95% Confidence intervals)
Induction of Labour	1.6 (1.3-1.9)
Caesarean Section	1.6 (1.4-2)
Macrosomia	2.1 (1.6-2.6)
Shoulder dystocia	2.9 (1.4-5.8)
Failed instrumental delivery	1.75 (1.1-2.9)
Increased maternal complications e.g. blood loss more than 500 mls	1.5 (1.2-1.8)

**Table 1-4. The impact of obesity on incidence of adverse intrapartum outcomes when compared to lean controls (52).**

Many reports have suggested increased rates of intrapartum, operative, and postpartum complications among obese mothers (38). Intrapartum complications include failed epidural, increased risk of aspiration during general anaesthesia, with difficulty of intubation being most commonly cited. Increased rates of caesarean delivery, macrosomia, shoulder dystocia, difficulty obtaining peripheral intravenous access, and inaccurate or difficult blood pressure monitoring are also reported for those with pre pregnancy obesity when compared with lean controls (53). Common operative complications include loss of landmarks making vascular access difficult and increased risk of respiratory complications such as aspiration and pneumonitis. Increased retention of lipid-soluble agents, increased drug distribution and more rapid desaturation have also been reported. In a prospective population-based cohort study, 3,480 women with morbid



obesity, defined as a BMI more than 40 kg/m<sup>2</sup>, and 12,698 women with a BMI between 35.1 and 40 kg/m<sup>2</sup> were compared with normal-weight women (BMI 19.8-26 kg/m<sup>2</sup>). The perinatal outcome of singletons born to these women was evaluated. Primary outcomes were, antenatally, the occurrence of preeclampsia, abruptio placenta, placenta previa, and stillbirths after 28 weeks of gestation among singleton pregnancies. Around-term variables evaluated were the rate of cesarean delivery, labor inductions, pre- and postterm delivery, instrumental delivery, anal sphincter injury, shoulder dystocia, postpartum hemorrhage, and epidural anesthesia. Small-for-gestational age infants were defined as those with birth weights more than 2 standard deviations below the mean birth weight for gestational age according to a Swedish reference curve, (54), and LGA infants were those with birth weight above 2 standard deviations. Estimated gestational age was in most cases based on second-trimester ultrasound screening.

In the group of morbidly obese mothers (BMI greater than 40 kg/m<sup>2</sup>) as compared with the normal-weight mothers, there was an increased risk of the following outcomes (adjusted odds ratio; 95% confidence interval): pre eclampsia (4.82; 4.04, 5.74), antepartum stillbirth (2.79; 1.94, 4.02), cesarean delivery (2.69; 2.49, 2.90), instrumental delivery (1.34; 1.16, 1.56), shoulder dystocia (3.14; 1.86, 5.31), meconium aspiration (2.85; 1.60, 5.07), fetal distress (2.52; 2.12, 2.99), early neonatal death (3.41; 2.07, 5.63), and large-for-gestational age (3.82; 3.50, 4.16). The associations were similar for women with BMIs between 35.1 and 40 kg/m<sup>2</sup> but to a lesser degree (55).

Each one unit increase in pre pregnancy BMI increases the risk of caesarean section by 7% (56). The association between maternal obesity and preterm delivery is disputed.

Studies report conflicting results from a lower incidence (57, 58), a similar incidence (36, 38, 46) to a higher incidence (31) Cnattinguis *et al* who carried out a cohort study in 167,750 women reported that among obese women an increased rate of preterm delivery (<32 weeks) is seen only in nulliparous women. Among parous women obesity is not a significant risk factor for pre-term delivery (59).

Perlow studied the impact of maternal obesity (>300 lbs) on perioperative morbidity among patients undergoing caesarean delivery (38). This case-control study compared 43 “massively” obese patients (weight >300 lbs) with 43 non-obese patients. In the obese group there was an increased rate of emergency caesarean delivery, prolonged delivery interval, increased total operative time, increased blood loss, multiple epidural placements, increased infection, and prolonged hospitalisation (38). Wolfe *et al* was among the first to compare the type of skin incision in the obese parturient with the risk of morbidity. In 107 obese women weighing greater than 200 lbs they reported that the type of incision was not related to operative morbidity (60).

The anaesthetist has a major role to play in preventing serious complications and must be aware of the many potential hazards. Extra vigilance is required to guide these patients safely through the perioperative period. In spite of this there is a lack of predictors specifically on the use of epidural and spinal anaesthesia in the obese patient. However many case reports exist on the difficulty of technique and the reduction in drug dosage requirements for such patients.

### **1.3.3 Post partum issues**

Obesity is characterized by a specific pattern of circulating concentrations of fat-cell products interleukin-6 (IL-6), leptin, and adiponectin. Circulating IL-6 and leptin levels are positively associated with BMI (61). It is now recognized that adipose tissue produces a variety of bioactive peptides, collectively termed "adipokines". Alteration of adipose tissue mass in obesity, affects the production of most adipose secreted factors. Several adipokines are increased in the obese state and have been implicated in hypertension (angiotensinogen), impaired fibrinolysis (PAI-1) and insulin resistance (ASP,  $\text{TNF}\alpha$ , IL-6, resistin) (62). Pregnancy is a prothrombotic state secondary to an increase in activity of coagulation factors XII, X and VIII, as well as fibrinogen (63). In the puerperium, deep venous thrombosis, endometritis, postpartum haemorrhage, prolonged hospitalization, wound infection, and dehiscence are also seen with increased frequency in the obese parturient (53).

The leading cause of maternal mortality remains venous thromboembolism (VTE) and the postpartum period is the time of greatest risk secondary to vascular damage of childbirth. The coagulation cascade is activated by inflammatory cytokines causing endothelial activation and obviously this is increased with obesity. Impaired fibrinolysis is a common finding in obese humans (64). This condition is now considered as an established risk factor for thromboembolic complications. Several studies have shown that obese patients have higher plasma concentrations of all pro-thrombotic factors (fibrinogen, vonWillebrand factor (vWF), and factor VII), as compared to non-obese controls, with a positive association with central fat (64-66). It has been proposed that the secretion of IL-

6 by adipose tissue, combined with the actions of adipose tissue-expressed TNF $\alpha$  in obesity, could underlie the association of insulin resistance with endothelial dysfunction, coagulopathy, and coronary heart disease (66).

## **1.4 Implications for the fetus of the obese mother**

### **1.4.1 Short term**

Lower Apgar scores have been reported amongst neonates of obese mothers when compared to those of lean mothers (67). However Sheiner *et al* did not observe this finding (68). Pre pregnancy BMI is a strong predictor of birth weight, with obese mothers having an increased incidence of delivering large-for-dates babies. Various studies have reported that the obese mother is 1.4-18 (O.R.) times more likely to deliver a large-for-dates infant (46, 58, 69). Macrosomia increases the risk of shoulder dystocia, birth injury, incidence of low Apgar scores and perinatal death. A recent three year retrospective analysis examined mode of delivery of infants weighing > 4kg. The incidence of caesarean section among the macrosomic group was 25.8% as compared with 13.1% ( $p<0.0001$ ) in the general population. The incidence of shoulder dystocia was 7.6% as compared with a background incidence of 0.48% ( $p<0.0001$ ). There were 7 cases of Erb's palsy all occurring among the macrosomic group (70).

Infants born of obese mothers are more likely to require admission to neonatal intensive care (NICU). In a retrospective study performed in France, the percentage of infants

requiring admission to a NICU was 3.5 times higher when maternal obesity was present (71). In addition the higher incidence of diabetes mellitus and gestational diabetes in the obese population may contribute to this effect with an increased risk of neonatal admission to the NICU for glycaemic control. The increased incidence of macrosomia and consequent birth trauma may also contribute to increased rates of perinatal morbidity in the offspring of obese mothers. (72-74)

One cohort study carried out in Denmark examined the relationship between maternal pre-pregnancy (BMI) and the risk of stillbirth and neonatal death (75). 24,505 singleton pregnancies were analysed and women were divided into 4 groups, underweight (BMI <18.5 kg/m<sup>2</sup>), normal weight (BMI 18.5-24.9 kg/m<sup>2</sup>), overweight (BMI 25-29.9 kg/m<sup>2</sup>) and obese (BMI >30 kg/m<sup>2</sup>) Maternal obesity was associated with a more than doubled risk of stillbirth (OR 2.8, 95% CI: 1.5-5.3) and neonatal death (OR 2.6, 95% CI: 1.2-5.8) compared with women of normal weight. This was not attenuated by adjustment for factors such as smoking, alcohol and caffeine intake, maternal age, height, parity, gender of the child, years of schooling, working status and cohabitation with partner, nor did exclusion of women with hypertensive disorders or diabetes mellitus. No single cause of neonatal death explained the higher mortality in children of obese women, but more stillbirths were caused by unexplained intrauterine death and fetoplacental dysfunction among obese women compared with normal weight women (75).

### **1.4.2 Long term**

Barker's hypothesis describes a theory of intra-uterine programming which suggests that babies who are small at birth or during infancy, secondary to maternal starvation during pregnancy, have increased rates of cardiovascular disease and non-insulin-dependent diabetes (NIDDM) in adult life (76). More recently Hattersley *et al* has provided another explanation for the association between low birth weight and insulin resistance, hypertension, coronary-artery disease, and non-insulin-dependent diabetes (NIDDM) in adult life (77). The proposal is that genetically determined insulin resistance results in impaired insulin-mediated growth in the fetus as well as insulin resistance in adult life and that low birth weight, measures of insulin resistance in life, and ultimately glucose intolerance, diabetes, and hypertension could all be phenotypes of the same insulin-resistant genotype. Insulin secreted by the fetal pancreas in response to maternal glucose concentrations is a key growth factor (77). Forsen *et al* showed that men whose mothers had a high BMI in pregnancy had an increased risk of coronary heart disease (78). The highest death rates were observed among men who were thin at birth with a low placental weight but whose mothers had a high BMI during pregnancy (78). While under nutrition is uncommon in the developed world, obesity and cardiovascular disease are common. The direct effects of maternal obesity on fetal programming are under researched. The concept of fetal programming being related to the quality of nutrition rather than the quantity needs to be addressed. One may hypothesise that in addition to maternal malnutrition secondary to famine, malnutrition secondary to an inappropriate diet of highly processed, high fat food, lacking in essential vitamins and nutrients, i.e. the

modern western diet, may contribute to long term adult health of offspring through fetal programming.

A recent retrospective cohort study by Whitaker (79) in over 8400 children in the USA in the early 1990s reported that children who were born to obese mothers (based on BMI in the first trimester) were twice as likely to be obese by 2 years of age. If a women had a BMI  $\geq 30$  in the first trimester, the prevalence of childhood obesity (BMI  $> 95$ th percentile based on Center for Disease Control criteria) at ages 2, 3 and 4 years was 15.1, 20.6 and 24.1%, respectively. This was between 2.4 and 2.7 times the prevalence of obesity observed in children of mothers whose BMI was in the normal range (18.5–24.9). This effect was only slightly modified by birthweight.

There is an independent effect of maternal pregravid weight and diabetes not only on birthweight but also on the adolescent risk of obesity. Langer *et al.* (74) reported that in obese women with GDM, whose glucose was well controlled on diet alone, the odds of fetal macrosomia (birthweight  $> 4000$  g) was significantly increased (OR 2.12) compared with those in women having a well-controlled (diet only) GDM with normal BMI.

Dabelea et al carried out work with the PIMA Indians who live in an area of today's southern Arizona and northwestern Mexico (80). These Native Americans successfully adapted to the desert life by elaborating an irrigation system and by hunting and gathering to supplement their cultivated crops. Diversion of their water supply by the increasing population of the area with people of European origin by the end of the 19th century disrupted their traditional agriculture and led to important changes in their way of life

(81). Today the Pimas suffer from several chronic diseases, mainly Type 2 diabetes and obesity, which may be conditions of relatively recent origin. The Pima Indians now have the world's highest prevalence and incidence of Type 2 diabetes (82), with about half of all individuals over 35 years of age having the disease (82). The prevalence and incidence of Type 2 diabetes in the Pimas has been increasing during the last century, and it is higher in recent years than it was 30 years ago. Moreover, the disorder is now common even among Pima children (83). Because this disease occurs at young ages, 10–15% of pregnancies are complicated by diabetes (84). A longitudinal study of diabetes conducted in this population for the past 30 years identified women who developed diabetes before or during pregnancy, women who had normal glucose tolerance during pregnancy but who subsequently developed diabetes (prediabetic women, diagnosed in retrospect), as well as women who never developed diabetes. Data on diabetes are available from offspring of all three groups of women. In the 5–9 and 10–14 year age groups there is little diabetes, but when present, it is almost exclusively found among offspring of diabetic mothers. At older ages as well, the prevalence of diabetes is much higher in the offspring of women who had diabetes in pregnancy (that is, those who were exposed to the diabetic intrauterine environment) than in the offspring of women who developed diabetes only after the pregnancy or in offspring of women who remained nondiabetic. These findings indicate that exposure to the diabetic intrauterine environment is an important determinant of Type 2 diabetes, in addition to the genetic predisposition. Dabelea et al describe a vicious vicious cycle. The woman with diabetes, whether diagnosed before or during pregnancy, has a high-risk pregnancy with potential complications for herself and her offspring. The effects on the offspring extend well



beyond the neonatal period. The infant of the woman with diabetes is at high risk of becoming obese and of developing Type 2 diabetes at a young age. The young woman whose mother had diabetes during pregnancy is at risk of perpetuating the cycle by becoming obese and developing diabetes before or during her childbearing years.

## **1.5 Cost implications with the obese patient**

A retrospective study carried out in 1995 found that the cost of antenatal care in overweight women was 5.4-16.2 fold higher when compared with normal weight controls (71). In 2000 researchers carried out a prospective analysis of cost of care in 435 women seen consecutively within their obstetric service. The average cost in terms of antenatal care and duration of hospital stay was 5 times higher for women who had a higher pre pregnancy BMI when compared to normal weight controls. In addition duration of hospital stay was 3.9-6.2 fold higher among the overweight mothers and a women with a pre pregnancy BMI > 29 kg/m<sup>2</sup> stayed in the hospital an average of 4.43 more days than her lean counterpart (85).

The overall financial burden of obesity generally on the National Health Service is difficult to determine. The implications of the increased incidence of maternal obesity for maternity services are that of more medicalisation of labour and delivery, more pressure on neonatal services, antenatal and postnatal beds and ultimately increased financial burden.

## **1.6 Obesity and the influence on general health**

### ***1.6.1 Cardiovascular disease***

Coronary heart disease (CHD) is the leading cause of mortality within the developed world. The association between obesity and in particular central obesity and elevated risk of cardiovascular disease is now well established (86, 87). One potential mechanism involves the relationship between insulin sensitivity and blood pressure. Abdominal fat rather than being inert tissue is an active source of free fatty acids (FFAs) and inflammatory cytokines which promote vascular inflammation and endothelial dysfunction. This pathophysiological mechanism has been suggested to predispose to impaired insulin sensitivity and microvascular disease. Therefore, cardiovascular morbidity and mortality is not only predetermined genetically, but may be influenced by lifestyle alterations such as weight loss and exercise. The Nurses Health Study claimed that women who maintain a desirable body weight, consume a healthy diet, exercise regularly, avoid smoking and drink alcohol in moderation reduce their risk of cardiovascular disease by 84% (88). Interventional studies have demonstrated an adjustment in cardiovascular disease risk profile with reduction in BMI and lifestyle changes. One study looking at a group of pre menopausal women who lost weight over a period of one year demonstrated a significant reduction in inflammatory cytokine concentrations as well as a reduction in adhesion molecule concentrations including ICAM-1, identified as an independent risk factor for coronary heart disease. Conversely these patients demonstrated a significant improvement in endothelial function (89). A randomised trial looking at 3000 patients with raised fasting plasma glucose

concentrations allocated patients to either lifestyle changes or medical therapy. The aim of intervention was to reduce the incidence of progression in these high risk patients to clinical disease, type 2 diabetes. The results showed that modification in lifestyle, i.e. weight reduction and exercise, was more effective than metformin in reducing the progression to type 2 diabetes (90).

### **1.6.2 Diabetes**

Obesity is a known risk factor for type 2 diabetes. The prevalence of obesity in the U.S. has increased from 12% in 1991 to 18% in 1998 and to 20% in 2000 (91). A parallel increase in diabetes is also reported from 4.9% in 1990 to 7.3% and 7.9% in 2000 and 2001 respectively (91). Until recently diabetes of onset in adolescence has been almost exclusively the consequence of type 1 diabetes, while type 2 diabetes has been the domain of adults. However, in the past few years several reports, mainly from North America, have documented the emergence of type 2 diabetes in young people that has paralleled an increased prevalence of obesity in this age group (92, 93). An increase in plasma free fatty acid (FFA) concentrations plays a key role in the pathogenesis of insulin resistance through specific actions that block insulin signal transduction. An increase in plasma FFA concentrations in normal subjects to levels comparable to those in the obese also results in the induction of oxidative stress, inflammation, and vascular dysfunction, in addition to causing insulin resistance (94). Because resistance to insulin also results in the relative non-suppression of adipocyte hormone-sensitive lipase, there is further

enhancement of lipolysis and increase in FFA concentration. Thus, a vicious circle of lipolysis, increased FFA, insulin resistance, and inflammation occurs.

### **1.6.3 Cancer**

A large population-based cohort study including over 145,000 Austrian adults over a period of 9.9 years looked at the relationship between cancer and obesity (23). The incidence of cancer was recorded; adjustment was made for smoking and occupation. A linear correlation between BMI and occurrence of many cancers including colon, pancreatic, breast and uterine was observed consistently throughout the study (23). When thinking about cancer risk we do not primarily think of BMI as a risk factor, certainly not as much as we do about smoking, genetic influences or exposure to toxins. In many cases the explanation for an obesity- cancer connection may not be the weight *per se*, but the things people do that result in obesity such as eating a poor diet or not getting much exercise. Calle *et al* carried out a prospective study looking at a population of over 900,000 American adults. All participants were cancer free at time of enrolment and each was followed up for 16 years. A linear correlation between BMI and deaths due to cancer was observed for the study period. Calle, in conclusion, postulated that 20% of all deaths from cancer among US women in age 50 years or over could be accounted for by the co existence of being overweight or obesity (95).

As previously stated, rather than being an inert tissue, adipose tissue is an active organ manufacturing hormones, such as oestrogen and growth factors which promote cell

division. This promotion of cell division may provide greater opportunities for mutations to occur and the development of cancer. Overweight women are known to have an increased risk of endometrial and breast cancer when post menopausal due to the increased levels of circulating oestrogen (95, 96). Obesity has been consistently associated with higher risk of colorectal cancer in men (Relative Risk (RR) 1.5-2.0) and women (RR 1.2- 1.5) in both case control and cohort studies (97). The risk of renal cell cancer is 1.5 to 2.5 fold higher in overweight and obese individuals when compared to normal weight controls (97). Obesity is associated with a 2-3 fold increase in the incidence of oesophageal adenocarcinoma (98, 99). The explanation for the association between obesity and oesophageal adenocarcinoma is the increased occurrence of gastric reflux among the obese.

## **1.7 Obesity and health implications: potential pathophysiological mechanisms.**

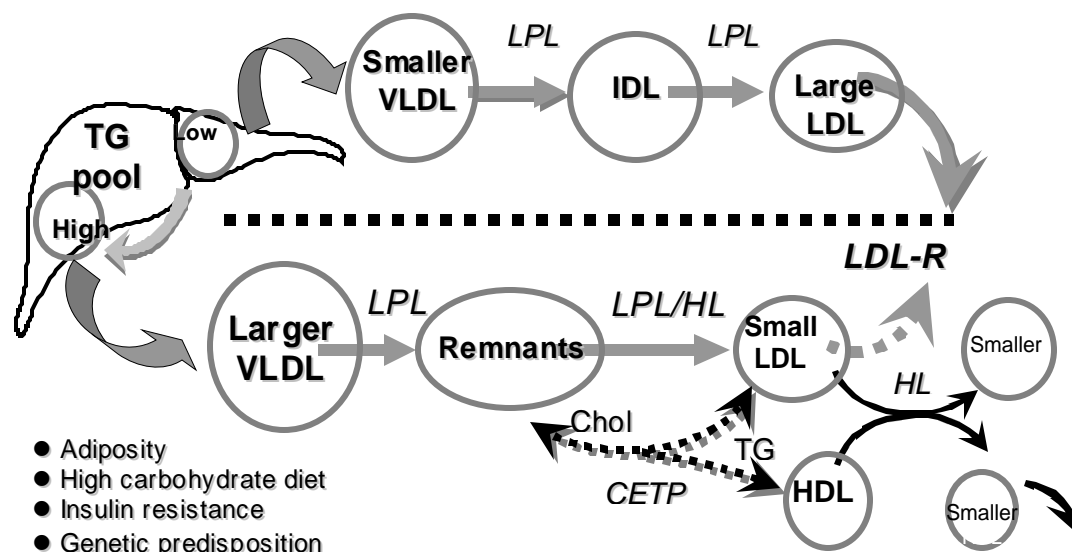
### **1.7.1 Lipids**

Obesity and dyslipidaemia are interlinked (100, 101) Various lipid/lipoprotein abnormalities have been observed in obese individuals, including elevated cholesterol, triglycerides, apolipoprotein B (apoB), and lower high-density lipoprotein (HDL) cholesterol levels. Of these indicators, changes in triglyceride and HDL cholesterol levels are most consistent and pronounced (102, 103). Some studies have demonstrated that central obesity is more strongly related to lipid/lipoprotein abnormalities than is general obesity (104-106). Hu *et al* examined data on 1500 adults including participants in the

American Strong Heart Study (107). Total obesity was assessed using BMI and also an estimation of central obesity using waist circumference. Lipoprotein concentrations were measured. Waist circumference was positively related to triglycerides ( $r = 0.14$ ,  $p < 0.001$ ) and negatively related to HDL cholesterol ( $r = -0.23$ ,  $p < 0.001$ ). BMI was positively correlated with triglycerides ( $r = 0.30$ ,  $p < 0.001$ ) and negatively correlated with HDL cholesterol ( $r = -0.35$ ,  $p < 0.001$ ) (108). Hypercholesterolaemia is a predictor of coronary heart disease. The West of Scotland Coronary Prevention Study (WOSCOPS) has shown a relative risk reduction with pravastatin treatment, a lipid lowering drug, of 29% (95% CI, 15% to 40%) for coronary events and 33% (1% to 55%) for coronary deaths (109). The Scandinavian Simvastatin Survival Study (4S) has shown that simvastatin treatment reduced coronary morbidity by 34% (25% to 41%) and coronary mortality by 42% (27% to 54%) (110).

### **1.7.1.1 Lipoprotein metabolism**

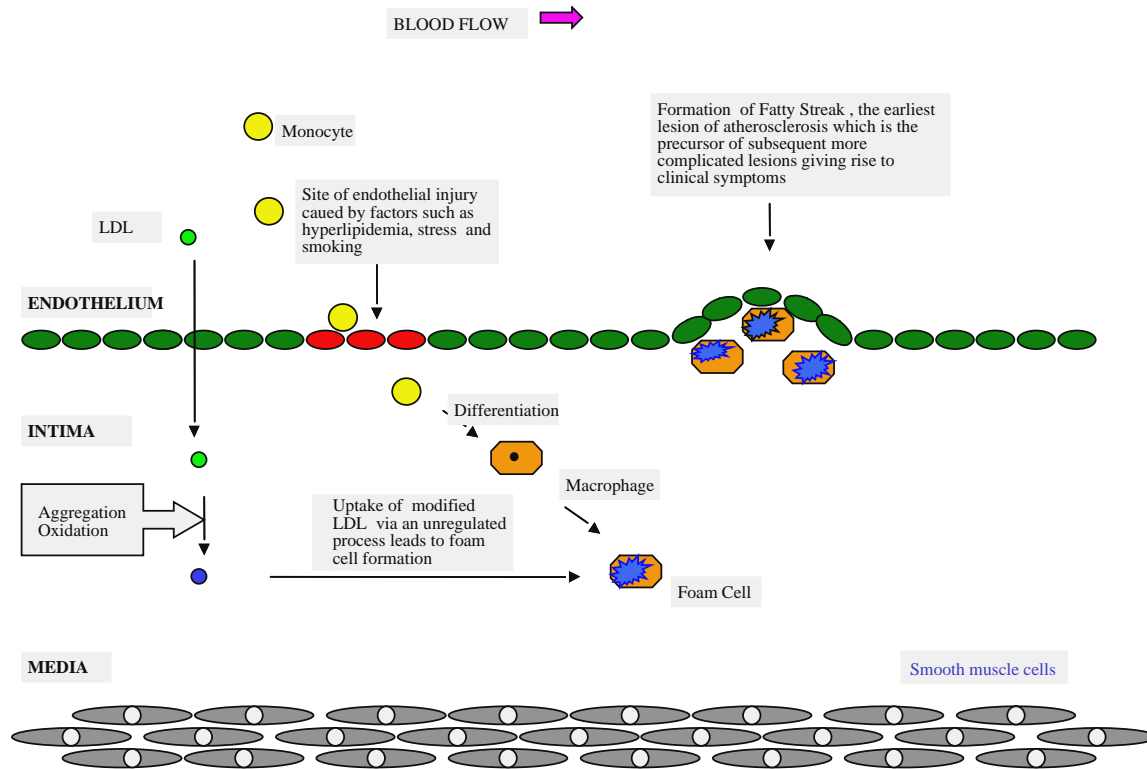
Lipoprotein particles are not homogeneous but contain discrete subfractions differing in structure, physicochemical properties, kinetic behaviour, and origin. The hypertriglyceridaemia seen with abdominal obesity and insulin resistance is related to the over secretion of triglyceride-rich VLDL particles, see figure 1-2.



**Figure 1-2: Lipoprotein metabolism.** CETP = cholesteryl ester transfer protein;  
 Chol = cholesterol;  
 HDL = high-density lipoprotein;  
 HL = hepatic lipase;  
 IDL = intermediate-density lipoprotein;  
 LDL = low-density lipoprotein;  
 LDL-R= LDL receptor;  
 TG, =triglycerides;  
 VLDL =very-low-density lipoprotein.

An increased rate of hepatic FFA uptake stimulates the secretion of apo B-100; leading to increase in numbers of apo B-containing particles (111). Apo B is the structural protein of atherogenic lipoproteins, including VLDL, intermediate density lipoproteins (IDL), and LDL. Each of these lipoproteins contains one apo B molecule, and the plasma apo B level reflects the total number of atherogenic particles in the blood. VLDL particles are exposed to lipoprotein lipase in the peripheral circulation, which hydrolyzes the triglyceride in VLDL particles, generating FFA. Under normal conditions, these FFA are taken up by muscle and adipose tissue for energy use or storage. The resultant remnant particles are then processed by the liver to form Low density lipoprotein (LDL). LDL

incorporates a spectrum of lipoproteins of differing atherogenic potential. Small, dense LDL (known as LDL-III), as distinct from the larger, more buoyant LDL-I and LDL-II particles, exhibit enhanced oxidation potential and reduced receptor binding. Once oxidized, these particles are believed to be highly atherogenic, promoting foam cell

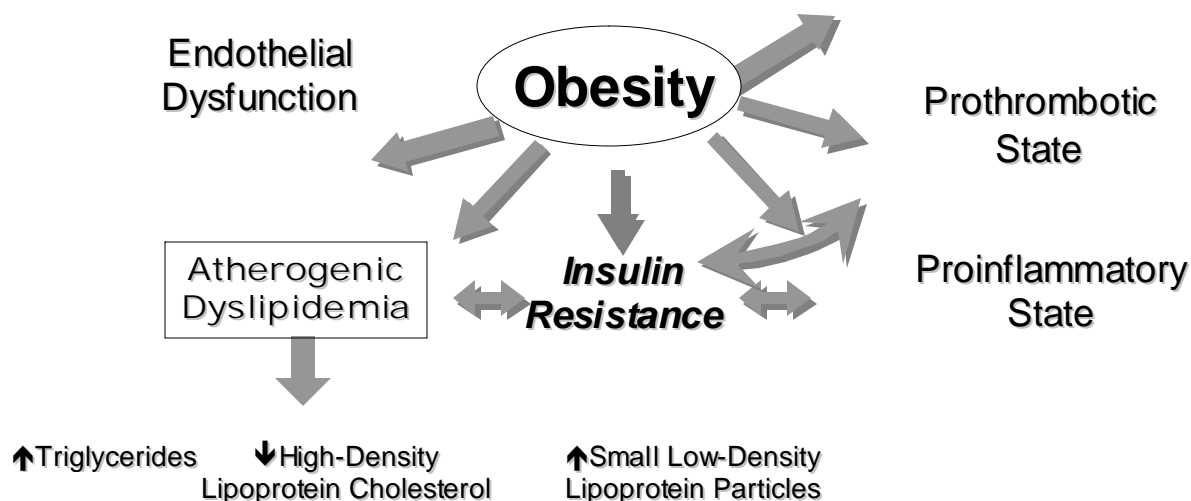


formation and initiating endothelial dysfunction (112) (see figure 1-3)

**Figure 1-3: Foam cell formation.** Diagram showing the formation of foam cells utilising oxidised LDL and the formation of atherosclerotic lesions.



The role of small LDL as a risk factor for coronary heart disease is now well established. Plasma triglyceride is the major determinant of small dense LDL, accounting for 40-60% of any variation in this fraction in the plasma (113). At normal triglyceride levels there is a positive correlation between LDL-II (the major LDL sub fraction) concentrations and that of plasma triglyceride (113). However cross sectional studies report a threshold effect, in that, above a certain triglyceride level, namely 1.5mmol/L in men, LDL-II concentrations correlate negatively with triglyceride levels. On the other hand the highly atherogenic LDL-III concentration which is at fairly constant levels below the threshold level starts to correlate positively with plasma triglyceride concentrations (113, 114). The dyslipidaemia of obesity characterized by low levels of high-density lipoprotein; increased triglycerides; increased sub fractions of small, dense LDL; and increased levels of apolipoprotein B-100 is now well recognised (115). The dyslipidemia associated with obesity is multi-factorial, and is frequently associated with a cluster of interrelated cardiovascular disease risk factors that has been designated the metabolic syndrome. Obesity is a critical determinant of this dyslipidemia, operating through a number of metabolic influences that include reduced insulin sensitivity and changes in fatty acid metabolism that are described subsequently. Variations in the nature and magnitude of the dyslipidemia are due to the interaction of genetic factors with environmental influences, most notably diet and physical activity, and possibly stress, see figure 1-4.



**Figure 1-4: The clinical influences of the dyslipidaemia of obesity.**

The metabolic syndrome or Syndrome X is a phenotype encompassing central adiposity, hyperinsulinaemia, hypertryglyceridaemia, reduced HDL concentrations and hypertension (87). Abdominal (visceral) adipose tissue is metabolically active and is largely responsible for the atherogenic dyslipidaemia, hyperinsulinaemia, hypertension, chronic inflammatory state, and prothrombotic state that constitute the metabolic syndrome, and the subsequent increased risk for cardiovascular disease and acute coronary events(115).

## **1.7.2 Pregnancy and lipids**

### **1.7.2.1 Normal pregnancy**

During the course of normal pregnancy, plasma triglyceride concentrations rise by 200-400% while plasma cholesterol levels rise by 25-50% (116, 117). Only a few studies have examined lipoprotein concentrations in detail as pregnancy advances. In the first Fahraeus *et al* investigated 19 healthy women in whom the levels of plasma lipoprotein fractions were determined before conception, at exact gestational ages every 6 to 8 weeks during pregnancy, and eight weeks after delivery. The HDL-cholesterol level was elevated in the 14th week and showed a maximum rise by 41% in the 28th week of pregnancy. The LDL level decreased in early pregnancy but then increased continuously. The VLDL triglyceride concentration showed a continuous increase from week 14, and in week 36, it was three times higher than before pregnancy. During lactation, up to eight weeks after delivery, LDL cholesterol remained elevated, whereas the other lipoproteins had returned to pre pregnancy levels (116). Sattar *et al* undertook a longitudinal examination of plasma lipoprotein sub fraction concentrations and compositions in uncomplicated pregnancy (118). Sattar *et al* hypothesised that as pregnancy advanced and plasma triglyceride concentrations increased, the LDL subfraction profile would be altered with the appearance of smaller, denser particles. Secondly they hypothesised a triglyceride concentration threshold would exist, above which LDL-III concentration would escalate (118). Median concentrations of VLDL-1, VLDL-2 and IDL increased in parallel, maximum increase being five fold, with increasing triglyceride concentrations with advancing gestation. This rise in VLDL-2 and IDL are of note as they do not occur

in the non pregnant state with increased plasma triglyceride concentrations (118). Moreover and particularly relevant to our study, the higher the booking fasting triglyceride concentration or the steeper the gradient of triglyceride concentration with advancing gestation the more likely the alteration of LDL profile to the smaller denser species. This LDL alteration toward smaller denser profiles exhibited a threshold effect, with a different threshold and timing of reaching that threshold for different women (118). This effect has been observed by others. Belo *et al* concluded after their longitudinal study of changes in levels of oxidised LDL with advancing pregnancy that during human gestation the change in LDL profile was towards smaller species (119).

Dyslipidaemia occurs in all pregnancies but not all women develop cardiovascular disease. It is known that oestradiol supplementation is associated with enhanced arterial endothelial function (120). As with advancing gestation oestradiol levels rise, one could hypothesise a protective influence upon the endothelium in response to the increasing dyslipidaemia.

### **1.7.2.2 Complicated pregnancy**

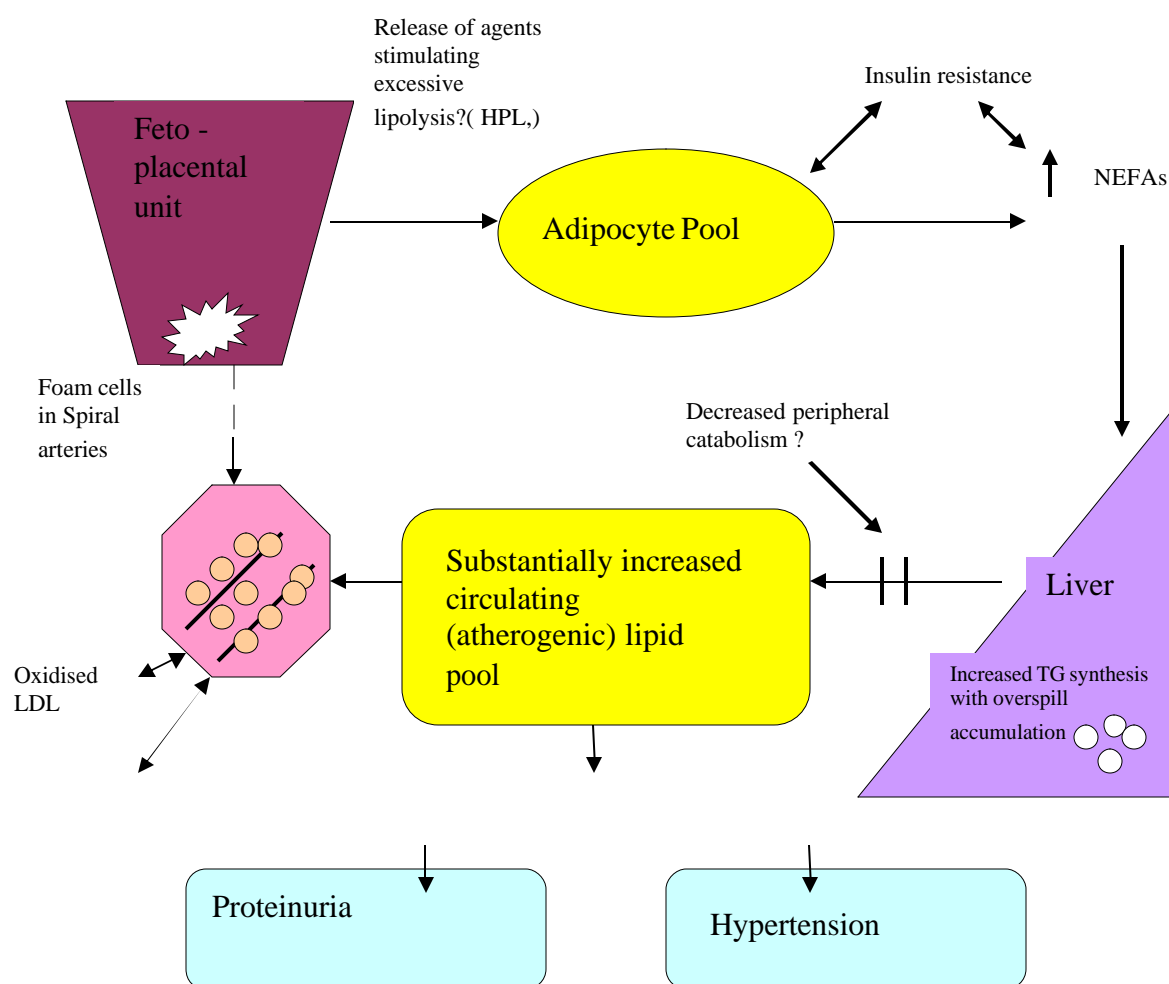
Pre-eclampsia (PE) is a pregnancy exclusive multi-system disorder characterised by widespread endothelial dysfunction. Characteristic clinical manifestations result, namely hypertension due to vaso-constriction, proteinuria due to glomerular damage and oedema due to increased vascular permeability. A complex pathological process underlies these clinical manifestations with the activation of the coagulation system, platelets and leukocytes, along with disturbances in metabolism which integrate to cause endothelial

damage and dysfunction (121). This resultant damage only serves to activate further circulating leukocytes and coagulation system; hence a vicious circle of vascular injury evolves (121). The precursor for this sequence of events is sourced within the placenta, confirmed by the incidence of pre-eclampsia among molar pregnancy when no fetal tissue develops. Various theories for the mechanism of activation of these events by the placenta have been proposed, including fragments of syncytiotrophoblast villous membranes (122) or cytokine released from immune cells within the placenta which in turn trigger endothelial dysfunction directly by activating leukocytes (121).

One unifying feature within the placenta of the pre-eclamptic patient is acute atherosclerosis. Accumulation of lipid laden macrophages surrounded by areas of fibrinoid necrosis in the spiral arteries are features similar to those observed in the non-pregnant atherosclerotic vascular bed (121). Furthermore the classic lesion within the glomerulus is glomerular endotheliosis, with the accumulation of lipids within the glomerular cells. The accumulation of lipids at key sites with the pre eclamptic patient emphasises the potential role of lipids and lipoprotein disturbance in PE (123). (See figure 1-5).

Gestational diabetes is characterised by the development of insulin resistance with advancing gestation. Insulin sensitivity is reduced as much as 80% during pregnancy (42). Placental secretion of hormones such as progesterone, cortisol, placental lactogen, prolactin and growth hormone are major contributors to the insulin resistant state seen in pregnancy. The insulin resistance which develops plays a key role in ensuring the fetus has an adequate supply of glucose by changing the maternal energy metabolism from

carbohydrates to lipids (124). The enhanced adipocyte pool and increased release of NEFAs only serves to enhance the insulin resistance, which may account for those women who present clinically (see figure 1-5).



**Figure 1-5: Dyslipidaemia of pregnancy.** Diagram adapted from Sattar *et al* (121).

Human placental lactogen (HPL), cortisol, and sex steroids, which are also at elevated concentrations in pregnancy stimulates FFA release from adipose tissue (125). Normal pregnancy is characterised by progressive increases in triglyceride (300%) and cholesterol (50%) concentrations. In the late second trimester increased amounts of FFAs

are released into the circulation through combined stimulation of hormone-sensitive lipase by HPL and by increased insulin resistance (121). Oestrogen increases hepatic output of VLDL, and decreases hepatic lipase activity promoting accumulation of triglycerides in lipoproteins of densities higher than that of VLDL (126). Decreased adipose tissue LPL activity and decreased hepatic lipase activity impair the removal of triglyceride-rich lipoproteins from the circulation (126), thus leading to higher serum triglyceride concentrations.

Summarized in figure 1-5 is the potential role of the dyslipidaemia of pregnancy in the pathogenesis of pre-eclampsia (PE). The placenta releases factor(s), such as human placental lactogen (hPL), which enhance peripheral lipolysis, which is already stimulated in normal pregnancy. The result is an increased flux to the liver of non-esterified fatty acids (NEFAs). This is then channelled into hepatic triglyceride synthesis so production of triglyceride rich lipoproteins (VLDL<sub>1</sub>) is increased over that of normal pregnancy. When the pathway is saturated accumulation of triglyceride within the hepatocyte occurs. The increased circulating levels of VLDL<sub>1</sub> promote the production of an atherogenic lipid profile by stimulating the production of small dense LDL (LDL III) and lowering HDL-cholesterol. This atherogenic lipid profile may contribute to endothelial dysfunction and the clinical manifestations of PE in the mother. This pathway plays a role in the formation of lipid laden macrophages (foam cells) in the spiral arteries of the deciduas basalis, and as a result, may be involved in the enhanced production by the placenta of inflammatory mediators in PE.

The regulation of lipolysis, the process for mobilization of free fatty acids (FFAs) through hydrolyzation of stored triglycerides, is important for the understanding of underlying factors behind metabolic events and disorders like obesity, diabetes, and insulin resistance. Activation of the sympathetic nervous system is said to activate lipolysis in subcutaneous adipocytes (127). This is a complex humoral response.

## 1.8 Inflammation

Adipose tissue has been proposed as a direct source of inflammatory markers collectively known as adipocytokines. Obesity, especially visceral obesity causes abnormalities of adipocytokine secretion (128). Adipocytes have been shown to be endocrine cells that secrete a variety of bioactive substances. These adipocytokines include, tumour necrosis factor alpha (TNF- $\alpha$ ), plasminogen activator inhibitor 1 (PAI-1), and heparin-binding epidermal growth factor-like growth factor (128). The levels of several pro-inflammatory cytokines, including TNF- $\alpha$ , interleukin-6 (IL-6) and leptin are significantly higher in the plasma of obese patients (129). Visfatin is a very recently discovered visceral fat-specific protein that may be related to the development of obesity-related diseases such as diabetes mellitus and cardiovascular disease (128). In contrast to these adipocytokines, adiponectin, also a new-found adipose tissue-specific collagen-like protein, has been noted recently as an important anti-atherogenic as well as anti-diabetic protein. Visceral fat accumulation causes dysfunction of adipocytes including over secretion of (TNF- $\alpha$ ), (PAI-1), and heparin-binding epidermal growth factor-like growth factor, as well as



hypo-secretion of adiponectin, which results in the development of a variety of metabolic and circulatory disease (128).

Atherosclerosis is now believed to be a disease of inflammation (130). Serum concentrations of inflammatory markers have been demonstrated to be predictive of coronary events. Ridker *et al* measured plasma C-reactive protein (CRP), a marker for systemic inflammation, in 543 apparently healthy men participating in the Physicians' Health Study in whom myocardial infarction, stroke, or venous thrombosis subsequently developed, and in 543 study participants who did not report vascular disease during a follow-up period exceeding eight years (131). The men in the quartile with the highest levels of CRP values had three times the risk of myocardial infarction (relative risk, 2.9;  $P < 0.001$ ) and two times the risk of ischemic stroke (relative risk, 1.9;  $P = 0.02$ ) when compared with the men in the lowest quartile (131). IL-6 has also been shown to be independently predictive of myocardial infarction risk despite adjustment for CRP levels (132). Leptin plays an important role in regulating adipose tissue mass. Leptin is a peptide hormone that is primarily synthesized and secreted by adipocytes (133). One of the major functions of this hormone is the control of energy balance by binding to receptors in the hypothalamus, leading to reduction in food intake, elevation in temperature and energy expenditure (133). Consistently elevated levels of leptin are found among obese individuals and significant correlations are found between serum leptin concentration and BMI, percentage body fat and abdominal subcutaneous adipose tissue (134). Furthermore, a resistance to circulating leptin in its ability control energy balance seems to be apparent among those who are obese (135). Leptin resistance is a hallmark of obesity, but its aetiology is unknown.

## **1.8.1 Pregnancy and inflammation**

### **1.8.1.1 Normal pregnancy**

The behaviour of inflammatory markers during pregnancy is under-investigated. However any work that has been done on normal pregnancy consistently reports pregnancy to be a pro inflammatory state (136). With the aim of determining physiological levels of pro inflammatory markers during normal pregnancy a longitudinal study carried out in Greece found IL-6, TNF- CRP to rise with advancing gestation (137). Other cross sectional studies show that IL-6 and IL-1 levels correlate positively with advancing gestation (138). Ramsay *et al* observed the concentrations of IL-6 and CRP in obese pregnant women to be elevated when compared with non-obese pregnant controls. IL-6 was found to be 50% higher while CRP was almost double when compared with lean controls (139).

### **1.8.1.2 Complicated pregnancy**

Substantial evidence now exists to support the theory that pre-eclampsia is connected to abnormalities within the inflammatory process with advancing gestation. Granulocytes and monocytes are activated (140-142) resulting in increased release of pro-inflammatory cytokines such as TNF , IL-6 and soluble phospholipase A2 (143, 144). An increase in

cell adhesion molecules, VCAM-1 and ICAM-1, has been demonstrated in women with pre-eclampsia also suggesting a state of inflammation and endothelial activation. Greer *et al* have provided further evidence supporting the link between inflammation and endothelial activation (145). A significant correlation between IL-6 and VCAM-1 ( $r=0.539$ ,  $p<0.005$ ) among patients with pre-eclampsia was observed by this group (145). This evidence supports the key role of the pro-inflammatory cytokine IL-6 as being pivotal in the pathophysiology of endothelial dysfunction and the development of pre-eclampsia.

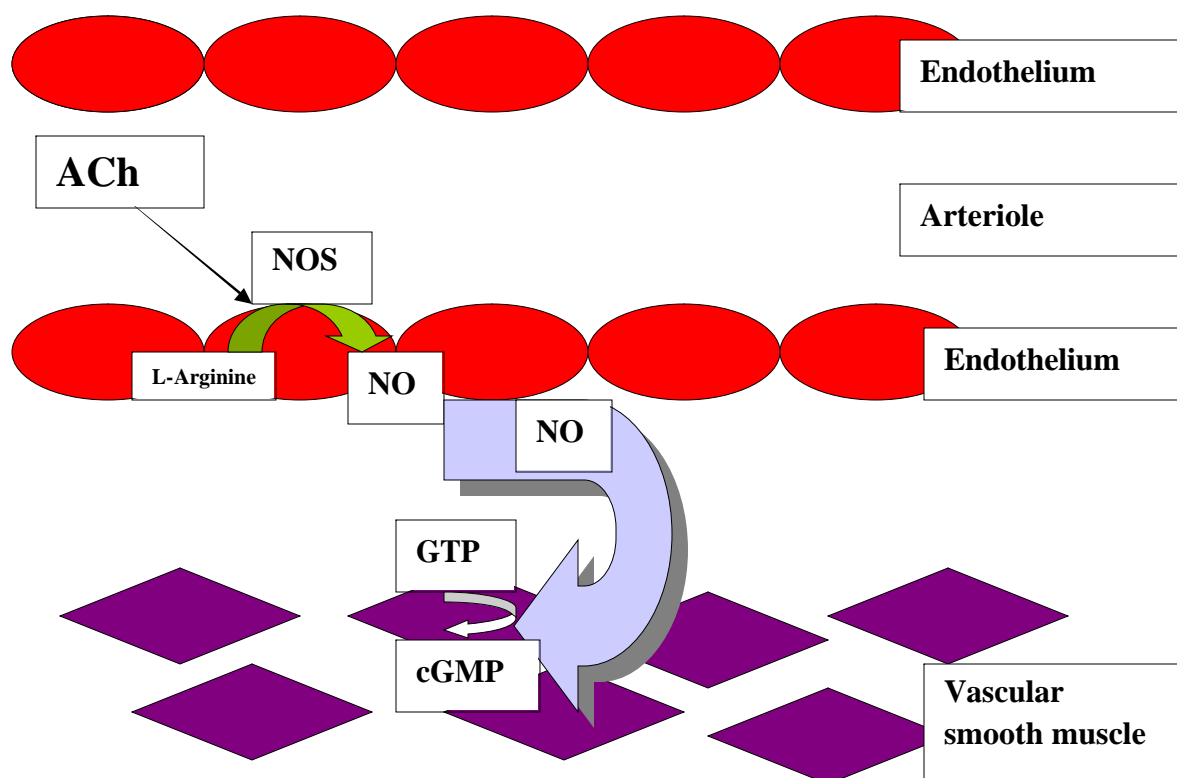
## 1.9 Microvascular function

The human vasculature rather than being an inert transport system is a complex and vital organ that can regulate its own tone and structure via numerous cellular mechanisms (146). The endothelium plays the role of gatekeeper in this process, sensing and responding to stimuli and activating various vasoactive systems that function as mediators. Locally generated vasoactive substances such as angiotensin II and nitric oxide (NO) appear to be important determinants of vessel function and structure.

Vasoactive substances generated within the endothelium influence cell proliferation and cell death in a complex interplay that, when disturbed, can result in structural alteration known as vascular remodelling. Normal vascular homeostasis is maintained by a balance between vasoconstrictors such as angiotensin II and vasodilators such as nitric oxide. Endothelial dysfunction involves an imbalance between vasoactive substances such that perturbations in the regulation of tone, haemostasis, and vessel structure result in the

development of cardiovascular diseases, such as hypertension, atherosclerosis, and heart failure (147).

An animal study in 1980 by Furchgott *et al* demonstrated that the isolated arteries relaxed in response to acetylcholine (ACh) but only in the presence of an intact endothelium (148). They went on to propose that, ACh, acting on muscarinic receptors endothelial cells, stimulates release of a substance(s) that causes relaxation of the vascular smooth muscle. This substance became referred to as endothelium derived relaxant factor (EDRF). Further work identified this factor to be Nitric Oxide (149). NO is derived from the terminal guanidine atom of L-arginine, a reaction catalysed by the calcium dependant enzyme nitric oxide synthase (NOS). The products of this reaction are NO and L-citruline. The NO diffuses into the adjacent smooth muscle activating guanylate cyclase, which increases cyclic guanosine 5-monophosphate (cAMP) stimulating the outward transport of calcium and therefore relaxation of vascular smooth muscle (see figure 1-6).



**Figure 1-6: The pathway to endothelium dependent vasodilation via the nitric oxide system.**

(ACh= Acetylcholine, NOS = Nitric Oxide Synthase, NO = Nitric Oxide, GTP = Guanosine triphosphate, cGMP= cyclical guanosine 5-monophosphate).

NO has other properties apart from vasodilation. It also inhibits platelet aggregation, smooth muscle cell migration and proliferation, adhesion molecule expression and therefore monocyte adhesion. Therefore NO plays a crucial role in protecting the vessel wall against the development of atherosclerosis and thrombosis (150).

Clinical studies report that the presence of endothelial dysfunction is a significant predictor of future cardiovascular events (151, 152). The vast majority of evidence to date indicates that microvascular endothelial function is impaired in the early stages of atherosclerosis and it is this endothelial dysfunction which is an important factor leading

to atherosclerosis and its complications. The vascular endothelium is often the target for pathogenic injury. Impairment of endothelium derived NO in atherosclerotic leads to vasoconstriction or rather lack of vasodilation activity. Platelet aggregation, thrombus formation, increased smooth muscle proliferation and enhanced leukocyte adhesion to and invasion through the endothelium are features which are postulated to follow. All of these changes are observed during the course of atherosclerotic disease. Activation of endothelial cells is another important factor in the pathological pathway of atherosclerosis (153, 154). Activated endothelial cells express adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemo attractant protein-1. Expression of these molecules promotes the platelet aggregation process and subsequent thrombus formation (155, 156).

### ***1.9.1 Lipids and vascular function***

An increasing number of studies have demonstrated the correlation between dyslipidaemia and vascular dysfunction (157). Therapeutic lowering of serum cholesterol, LDL and oxidised LDL has been shown to result in an improvement in endothelium dependent vasodilation in the forearm of patients with hypercholesterolaemia (157). Clinical trials such as The West of Scotland Coronary Prevention Study (WOSCOPS) (109) and The Scandinavian Simvastatin Survival Study (4S) (110) have also shown that the treatment of serum lipids can improve clinical outcomes. Others have demonstrated the improvement of endothelial dysfunction in atherosclerotic arteries in 6 to 12 months among treatment groups (158, 159). These findings have important clinical implication in the treatment of high risk patients.

Native LDL has little effect on essential function of cells within the arterial wall. When LDL is metabolized by the endothelial cells, however, the normal component antioxidants (e.g.  $\alpha$ -tocopherols and  $\beta$ -carotene) are exhausted, the polyunsaturated phospholipids are converted into reactive hydroxyfatty acids, lysophosphatidylcholine is formed, and the proteins in the apolipoprotein-B 100 moiety undergo modification and fragmentation so that the ligand can no longer bind to the classic LDL receptor.

Eventually, this “oxidised” LDL molecule becomes more negatively charged, and binds to alternative sites e.g. the scavenger receptor, and becomes capable of a wide variety of toxic effects, leading to cell dysfunction and hence vessel wall dysfunction (160).

Consistently in animal models these characteristic cell dysfunctions have been shown to be associated with the development of atherosclerosis in large arteries (112).

Oxidised LDL can rapidly impair endothelium-dependent dilation. This probably occurs through a number of mechanisms, including direct inactivation of NO by excess production of oxygen-derived free radicals, reduced transcription of nitric oxide synthase mRNA, and post-transcriptional destabilisation of mRNA (161). The decrease in availability of NO is accompanied by many other important vascular cell dysfunctions. There is an increased platelet adhesion, stimulation of plasminogen activator inhibitor, inhibition of plasminogen activator, induction of procoagulant tissue factor mRNA, inhibition of mRNA transcription of thrombomodulin caused by degradation by lysosomes, and finally stereochemical alterations in heparin sulphate proteoglycans (160). These changes impair the anti-platelet and anticoagulant properties of the endothelium and initiate thrombus formation (162). Upregulation of adhesion molecules, activation of protein kinase C, and diminished available NO are all likely to play a role in

proinflammatory changes that lead to accumulation of monocyte, macrophages and T-lymphocytes.

Evidence supports the fact that increased levels of oxidised LDL or greater susceptibility of LDL to oxidation is related to the severity of coronary atherosclerosis (163). Clinical investigations have shown that increases in serum cholesterol and/or LDL or decreases in HDL are associated with loss of endothelium-dependent dilation to acetylcholine in forearm vessels (164). Furthermore, recent evidence reveals small, dense LDL particles are more susceptible to oxidation and are particularly efficient at inducing endothelial dysfunction (157). Inhibition of oxygen free radicals can lead to significant improvement in endothelium-dependent vasodilation (157). The diminished levels of NO contribute to inflammation within the atherosclerotic plaques due to the increased adherence and migration of monocytes, formation of lipid-laden macrophages, and increased expression of plasminogen activator inhibitor and tissue factor, especially in the areas surrounding a lipid core. These factors add to the fragility of the plaque, increasing risk of rupture and release of thrombogenic core initiating intravascular thrombus formation (165).

HDL has been demonstrated to protect against abnormal responses to ACh (166) and, in isolated strips of rabbit aorta, HDL reversed LDL induced endothelial dysfunction (167).

Serum triglyceride has been demonstrated to have a significant and independent relationship with the incidence of major coronary events as shown by the eight year follow up data from the PROCAM study (168). In a large meta-analysis of eight population-based prospective studies, a 1 mmol/l increase in serum triglyceride concentration was equivalent to a 14% and 37% increase in relative risk of coronary heart



disease in men and women respectively (169). The mechanism by which triglycerides exert their influence on the development of atherogenesis may be variable, including effects on endothelial cell function, macrophage loading, thrombogenesis and determination of LDL size, i.e. defining the smaller denser atherogenic particle size (170).

### **1.9.2 Inflammation and vascular function**

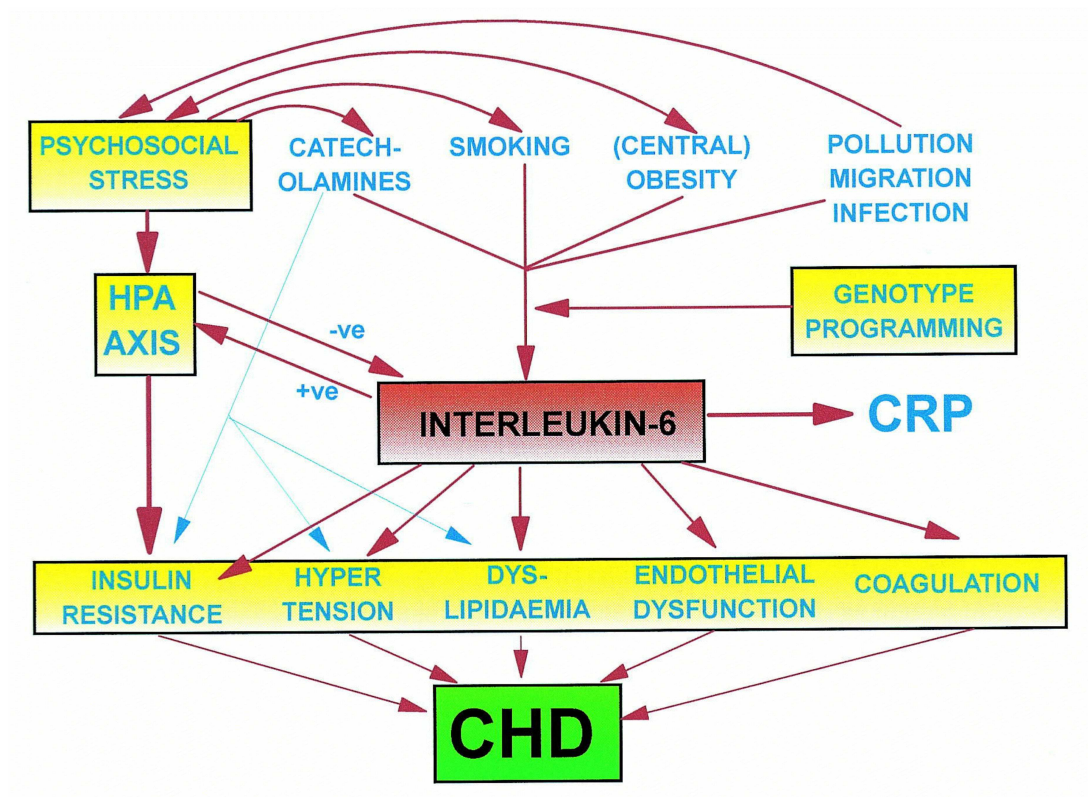
Some of the most commonly measured inflammatory markers, such as C-reactive protein, fibrinogen and serum amyloid A, originate in the liver, and their production is stimulated by systemic cytokines such as IL-1, IL-6 and TNF- $\alpha$ . Cytokines are produced at several extra hepatic sites, such as the heart, vessel walls, macrophages, and adipose tissue. Other types of inflammatory markers are produced at these sites. The heart secretes troponin T and I and creatinine kinase MB in response to injury. The atherosclerotic vessel wall produces soluble adhesion molecules, such as ICAM-1 and VCAM-1. Macrophages secrete phospholipases in response to inflammation.

Atherosclerosis is now considered an inflammatory disease (171). For the last 20 years evidence has been accumulating that serum levels of inflammatory markers can be used to predict cardiovascular events (131). Packard *et al* in the West of Scotland Coronary prevention Study evaluated whether the levels of certain markers are predictive of the risk of coronary events among men with hypercholesterolaemia (172). Levels of C-reactive protein and fibrinogen were both predictive of the risk of coronary events;

however, the strength of association was significantly reduced when other, traditional cardiovascular risk factors were considered (172).

Interleukin-6 levels even after adjustment for C-reactive protein levels have been shown to be predictive for risk of myocardial infarction (132). A recent report from the Rural Health Study has shown that elevated concentrations of IL-6 predict total and cardiovascular mortality over a 5 year period, the association being independent of prevalent vascular disease, smoking and traditional risk factors, and stronger than, but additive to, that for CRP (173).

As previously stated, adipose tissue is a source of cytokines such as IL-6, IL-1 and TNF-. Studies consistently show a strong correlation between inflammatory marker levels and the levels of body fat, markers of insulin resistance and other indicators of the dysmetabolic state including central obesity, hypertension and insulin resistance (Figure 1-7) (165, 174).



**Figure 1-7. The roles and regulation of IL-6. (From (165))**

The proposed link between inflammation and increased risk of coronary disease is endothelial dysfunction. As mentioned previously the cell wall itself produces cell adhesion molecules, ICAM-1 and VCAM-1. These cell surface molecules are expressed and up-regulated in response to stimulation by cytokines. Their soluble component can actually be quantified in the blood. Their purpose is recruitment of circulating inflammatory cells and the eventual transmigration of the inflammatory cell through the endothelium, an action thought to be the initiating step in the development of atheromatous deposits. It has been demonstrated that ICAM-1 levels are an independent predictor of adverse cardiovascular events (175).

### **1.9.3 Pregnancy and vascular function**

Maternal vascular resistance and blood pressure are normally reduced significantly during pregnancy, reaching a nadir by around 20 weeks' gestation. Both factors then rise again towards term (176, 177). Blood pressure falls during pregnancy despite a 50% rise in blood volume and 40% rise in cardiac output. This is almost certainly due to reduced peripheral vascular resistance. Maternal circulation has been shown to be relatively unresponsive to angiotensin-II, a vasoconstrictor (178). This impaired response is further confirmed by the improvement of responsiveness to angiotensin-II after the administration of prostaglandin synthetase inhibitors such as indomethacin and aspirin (179). This finding implies that pregnancy is associated with a down-regulation in response to a number of vasoconstrictors. Pregnancy has also been demonstrated to be associated with an increased synthesis of endothelium-derived vasodilators. Prostacyclin ( $\text{PGI}_2$ ) has been proposed to result in increased peripheral vasodilation and reduced constrictor response (180).  $\text{PGI}_2$  synthesis during pregnancy is most certainly increased; however this increased production appears to be confined to particular vascular beds, particularly the uterine circulation (181). Interestingly patients who develop pregnancy induced hypertension exhibit a lesser rise in prostacyclin biosynthesis when compared with healthy controls (182).

There is considerable evidence that nitric oxide (NO) synthesis is increased in animal pregnancies. NO may be released in response to receptor-mediated stimuli such as ACh or to physical forces such as shear stress on the vessel wall. Pregnant guinea pigs demonstrate increased calcium dependant nitric oxide synthase (NOS) activity in a

variety of tissues, and isolated carotid and uterine arteries from these animals showed enhanced relaxation to the endothelium dependant vasodilator, acetylcholine (183). Another possible mechanism for up-regulation of NO production in pregnancy is its increased synthesis by oestrogen (184). Weiner *et al* demonstrated NOS activity to be reduced by tamoxifen, and oestrogen receptor antagonist, while treatment with oestradiol increased NOS activity (184). Few studies on this subject involve human subjects however one study has demonstrated an increased in excretion of cGMP, the second messenger responsible for mediating NO-induced vasodilation, among pregnant healthy women (185). Furthermore increased nitrate turnover has been demonstrated among healthy pregnant women on a tightly controlled nitrate intake (186). This data adds to the suggestion that there is an increase in NO synthesis during healthy pregnancy.

McCarthy *et al* dissected arteries from biopsy specimens of subcutaneous fat obtained from pregnant women during caesarean section and from non pregnant women undergoing gynaecological surgery (187). This study failed to demonstrate any difference in acetylcholine-mediated relaxation in arteries from pregnant women as compared with non-pregnant (187). However previous work had suggested that increased receptor-mediated, endothelium-dependent vasodilatation may play a role in reducing pressor sensitivity and increasing vasodilatation in animal pregnancy (188). Enhanced relaxation has been demonstrated in response to bradykinin and shear stress, but not to acetylcholine among pregnant rats (189). Knock *et al* investigated bradykinin-mediated vasodilator function in small arteries from normotensive pregnant and non-pregnant women (190). Arteries from normotensive pregnant women demonstrated enhanced relaxation to bradykinin compared with those from non-pregnant women ( $p < 0.05$ ). This

study provides evidence for an increase in bradykinin-mediated nitric oxide synthesis from the vascular endothelium of small arteries from the peripheral circulation of normotensive pregnant women which may account for increased vasodilation during pregnancy.

Underlying all of this conflicting evidence one must accept that endothelium dependant vasodilators are likely to play a central role in the maternal cardiovascular response. Some consideration should however be given possible structural alteration of the non-contractile protein matrix of the artery (191). Alterations in structure may add further to improvements in arterial wall distensibility (192).

Additional insight into the importance of the endothelial response has also been gained from animal experiments in which shear stress has been acutely or chronically altered. Increasing shear stress in the rat by surgical construction of an aortocaval shunt results in increased cyclic guanosine 3'5'-monophosphate (presumably as a result of increased nitric oxide release), (193) and elevated shear increased endothelial nitric oxide synthase (eNOS) messenger RNA (mRNA), protein, and activity in high-shear stressed aortas compared with sham-operated controls (194). These increases were followed by vessel structural expansion (193). This structural increase in vascular lumen to normalize shear was prevented in the rat model by inhibition of nitric oxide synthase (NOS) with N-[omega]-nitro-l-arginine-methyl ester (195) The central role of eNOS in shear-mediated structural remodeling was confirmed by Rudic et al (196) when, in wild-type mice, the common carotid artery responded to surgically induced decrease in flow by reducing caliber to normalize shear stress to its preoperative level, whereas it failed to do so in

mutant mice that lacked the gene for eNOS (196). In a baboon polytetrafluoroethylene graft fistula model, elevated shear stress was associated with increased expression of eNOS, a lower degree of neointimal and smooth muscle proliferation, and even induced regression of previously established neointima (197). In contrast with their high-shear counterparts, low-shear grafts exhibited greater smooth muscle cell proliferation and higher levels of platelet-derived growth factor- $\alpha$  protein and mRNA (198). The connection between high shear stress and low intimal proliferation has been further clarified in rodent experiments showing that focal increases in shear stress in the aorta resulted in corresponding decreases in angiotensin-converting enzyme activity (199).

Shear stress has also been associated with the endothelial proliferative state in animal studies. Endothelial cell proliferation increased 18-fold within 48 hours of reduction in shear stress (200). Decreasing shear stress was followed by endothelial cell loss and desquamation, altered morphology with decreased elongation, decrease in actin stress fibers, greater monocyte attachment to and migration across the endothelial layer, (201) and increased endothelial surface expression of vascular cell adhesion molecule 1 (202). The increased endothelial cell loss in response to decreased shear has recently been suggested to be the result of apoptosis, which remains unabated until the shear normalization has been restored (203). These in vivo experiments obtained in various species using different methods to alter hemodynamics help establish a framework to understand the propensity for intimal hyperplasia and atherosclerosis initiation in areas of low shear stress and the protective effect of elevated shear stress in sheltered regions of the vasculature.

## 1.10 Insulin resistance

Insulin resistance is an impairment of insulin signalling, arising from post-receptor defects in propagation of the message evoked by the binding of insulin to the receptor (204). The term insulin resistance refers to the inability of circulating insulin to maintain normal homeostasis of glucose. Higher levels of insulin are circulated in order to maintain normoglycaemia, resulting in a hyperinsulinaemia. Insulin is an important metabolic hormone synthesized by pancreatic  $\beta$  cells, which stimulates glucose uptake in various organs, particularly muscle, adipose tissue, and the liver, and inhibits lipolysis in adipose tissue. The initiating step in the insulin-signalling cascade is the binding of insulin to the insulin receptor on the plasma membrane of insulin-sensitive tissues. Insulin evokes a broad array of metabolic responses and, accordingly, insulin resistance can be defined in a myriad of ways. The most logical starting point when defining insulin resistance is in relation to altered patterns of glucose metabolism. However disorders of lipid and fatty acid metabolism, altered patterns of protein and amino acid metabolism, blunted haemodynamic responses, changes in electrolyte homeostasis, and alterations of growth at a cellular level are all interlinked in the complex influences of insulin. The insulin resistance syndrome describes a collection of abnormalities of metabolism with insulin resistance as the primary abnormality giving rise to dyslipidaemia, essential hypertension, impaired glucose intolerance and type 2 diabetes.

Himsworth *et al* were among the first to recognise insulin resistance by observing the ability of insulin to blunt the rise in plasma glucose following glucose infusion (205). The



group noticed very varied responses to insulin. Among lean, young adult patients with diabetes, insulin substantially blunted the rise in glucose following glucose administration; yet in obese, older patients, insulin injections had only a marginal impact. In retrospect, it is clear that Himsworth made a fundamental observation as to the differences between type 1 diabetes, a disease of insulin deficiency, and type 2 diabetes, a disease characterised by insulin resistance.

In type 2 diabetes, insulin resistance and resultant hyperinsulinaemia is proposed as a mechanism by which cardiovascular complications such as hypertension arise (206). Essential hypertension is prevalent among older individuals, and approximately 50% of persons with hypertension can be considered to have insulin resistance and hyperinsulinaemia (207). It appears likely that insulin resistance and hyperinsulinaemia predispose to, rather than result from, hypertension. Insulin resistance is associated with abnormalities in lipoprotein metabolism, hypercoagulability, and endothelial function, which probably account in part for the increased cardiovascular risk among hypertensive patients. Others suggest the mechanism by which insulin resistance exerts its cardiovascular risk is due to increased blood glucose concentrations. The increased glycation of the glucose results in products which quench NO and therefore impair vascular function (208). This theory is however disputed by animal studies which report impaired vascular function only at supra-physiological glucose levels (209). Human studies have revealed not only no correlation between HbA1c values and vascular function (210), but also no vascular function impairment at moderate levels of hyperglycaemia (211).

The direct mechanism linking insulin resistance and increased cardiovascular risk remains uncertain. Serne *et al* demonstrated an inverse relationship between blood pressure and insulin sensitivity as well as a strong positive relationship between microvascular dysfunction and insulin insensitivity (212). They propose that adipose tissue is the primary link between all of these factors.

Adipose tissue contributes non-esterified fatty acids (NEFAs), which impair insulin action in both the liver and skeletal muscle (213). Added to this the inhibitory effects of NEFAs on nitric oxide synthase may contribute to the impaired vascular function observed in obese patients (214). The accumulation of fat in the abdominal region is thought to be a critical determinant of whole-body insulin resistance (215). It is widely accepted that the accumulation of fatty acids in insulin-sensitive non-adipose tissues, such as muscle and liver, can impair insulin-mediated glucose uptake in these tissues. Besides acting as a source of fatty acids, fat cells produce hormones that modulate insulin action. There is evidence suggesting that TNF- and resistin may impair insulin action *in vitro*, although whether these or other cytokines are more relevant to humans is not known (216).

Adiponectin is a large, abundant plasma protein (present in concentrations of 5–30 nmol in humans) that circulates in higher concentrations in females than in males (217). Plasma adiponectin concentrations are directly associated with insulin sensitivity and inversely with visceral or intra-abdominal, obesity. Adiponectin plasma levels are lower in patients with type 2 diabetes and atherothrombotic cardiovascular disease than in healthy controls (217–219). Despite the fact that adiponectin is secreted from adipose tissue, circulating

adiponectin levels are *lower* in obese individuals than in lean individuals, due to its strong association with insulin sensitivity (217, 218). Consistent with this finding, adiponectin levels have been shown to *increase* with weight loss. Adiponectin appears to improve insulin sensitivity by increasing fat oxidation and lowering lipid levels in muscle and liver.

Resistin is another adipocyte-derived hormone, the circulating levels of which are increased in genetic and diet-induced obesity in mice (216). This study found that treatment with recombinant resistin impaired insulin action in wild-type mice and that neutralizing anti-resistin antibodies improved insulin sensitivity and blood glucose levels in diabetic obese mice (216). Although it has been proposed that resistin may be the link between obesity and diabetes (216), its role in human insulin resistance remains controversial.

### **1.10.1      *Pregnancy and insulin resistance***

Alterations in maternal metabolism during pregnancy are required for supply of adequate nutrition for the fetus. Thus, in normal pregnancy, insulin resistance develops and assists in the provision of energy substrate for the baby. This insulin resistance leads to higher levels of glucose and free fatty-acids, constrained by increased secretion of maternal insulin. Pregnancy is associated with decreased phosphorylation of insulin-receptor-substrate-1 (220, 221). Insulin action changes over the course of pregnancy. At 12–14 weeks' gestation, insulin sensitivity is slightly increased but then declines for the rest of

the pregnancy, with insulin resistance being highest late in the third trimester (222). Insulin sensitivity improves with delivery of the placenta. Thus gestational diabetes, when the level of insulin resistance is too high to accommodate glucose metabolism within normal limits, typically appears late in the second trimester and resolves immediately postpartum. The time course for changes in insulin action suggests a hormonal cause for insulin resistance. In the past, human placental lactogen, human placental growth hormone, progesterone, cortisol, and prolactin were the prime suspects. Certainly, all rise in late pregnancy, causing insulin resistance, and return to pre-pregnancy values promptly postpartum.

### **1.10.2      *Pregnancy and Free Fatty Acids***

Stewart et al described fatty acid composition throughout pregnancy and in the post partum (223). Fatty acids are an important component of cell membrane and therefore are essential to tissue development (reviewed in (224)). The fetus is reliant on placental transfer of fatty acids from the mother to support growth (225, 226). The essential fatty acids 18:2n-6 (linoleic acid) and 18:3n-3 (alpha linoleic acid) undergo elongation and desaturation to form long chain polyunsaturated fatty acids (LCPUFA), which are commonly found in membranes, particularly in the brain. In order for the fetus to accrue the LCPUFA's for tissue formation the mother must mobilise and make available key fatty acids. Maternal LCPUFA status is critical in determining essential fatty acid status in their newborn (225, 227, 228).

Palmitoleic acid 16:1 $n$ 7 to be increased with advancing gestation and reduced in the post partum (223). However interestingly we noted the change in 16:1 $n$ 7 with advancing gestation to be negatively correlated to maternal booking body mass index (BMI) and waist circumference. 16:1 $n$ 7 is synthesised from 16:0 by stearyl CoA desaturase (SCD) (229). The activity of SCD is down regulated by leptin (230). Leptin, a polypeptide hormone produced by adipose and other endocrine tissues activity has a clear and well established association with obesity (231, 232). As obesity is associated with increased levels of leptin one would assume our data to be in conflict with the literature, however a degree of leptin resistance in pregnancy has been suggested, possibly mediated from the soluble isoform of the leptin receptor from the placenta, resulting in an increase in the amount of bound and therefore unavailable leptin within the maternal circulation with advancing gestation. this potential for leptin resistance in pregnancy may account for the unexpected inverse relationship between 16:1 $n$ 7 and obesity in pregnancy.

Ramsay et al have examined metabolism in the third trimester of pregnancy among both lean and obese mothers (139). Obesity in pregnancy has implications for morbidity and mortality in both mother and baby. The incidence maternal complications, such as pregnancy-induced hypertension or preeclampsia, are significantly greater if the mother is overweight, defined by BMI in early pregnancy, or centrally obese, as assessed by waist circumference (41, 233). Maternal obesity also increases the risk of metabolic complications, such as gestational diabetes. Furthermore, some data suggest that adiposity in the mother may critically influence the programming of metabolic pathways of her fetus and its risk for diabetes and cardiovascular disease in later life (78). Our

group reported a dyslipidemic pattern consistent with the metabolic syndrome among obese mothers in the third trimester of pregnancy (139). We therefore wanted to test whether there was a difference in third trimester maternal erythrocyte fatty acid composition.

## **1.11 Hypothesis and aims**

### **HYPOTHESIS**

1) Microvascular endothelial dependent vasodilatation will increase in pregnancy with advancing gestation, but to a lesser degree in women with pre-pregnancy obesity as

defined by an increased BMI. A difference in vascular function between lean and obese women will exist in early pregnancy but this will become less dramatic with advancing gestation suggesting a beneficial effect of pregnancy to vascular function.

2) The levels of circulating lipids and inflammatory cytokines will increase throughout pregnancy and negatively correlate with vascular function. This will provide some insight into potential mechanisms underlying vascular dysfunction.

3) Fatty acid concentrations, particularly long chain polyunsaturated fatty acids (PUFA) would decrease throughout pregnancy in favour of shorter more saturated fatty acids and again this would happen earlier and more dramatically in obese women.

## **AIMS**

My aim was to use this unique opportunity of extreme metabolic and vascular stress (i.e. pregnancy) to study potential pathophysiological processes associated with vascular disease in obese women. Obesity has significant correlations with hyperinsulinaemia, hypertriglyceridaemia, decreased high density lipoprotein and elevated blood pressure.

Advances in laser Doppler imaging technology in the non-invasive assessment of microvascular function allow us to objectively quantify vascular function within these women. Previous work has examined these factors in the third trimester but as yet no group has identified if there is a pregnancy specific effect in obese patients. Therefore longitudinal data is required. Therefore we aim to perform a longitudinal study

throughout pregnancy and the postnatal period in lean and obese women as defined by BMI. The following will be measured:

- Microvascular function using laser Doppler perfusion imaging.
- Fasting cholesterol, triglyceride, VLDL, LDL, sub fractions and Erythrocyte membrane fatty acid status
- Inflammatory markers including IL-6, CRP, IL-1, TNF- as well as ICAM-1, VCAM-1, PAI-1 and PAI-2.
- Adipocyte derived proteins such as leptin and adiponectin.

## **Chapter Two**

### **Materials and Methods**



## **2 Materials and Methods**

### **2.1 Subjects**

Women (n=60) were recruited from the antenatal clinic at booking visit in the first trimester of pregnancy. Women were selected on the basis of a negative medical or complicated obstetric history. Thirty women with a maternal booking BMI of less than 30 kg/m<sup>2</sup> were randomly recruited from the clinic at booking. While a further thirty women with a maternal booking BMI of greater than or equal to 30 kg/m<sup>2</sup>, were randomly recruited to the study. The women who declined from taking part were those women who could not commit to extra hospital visits to take part. All women booked for antenatal care at The Princess Royal Maternity Hospital, Glasgow. In order to be eligible for

inclusion in the study none of the women had a positive medical history including cardiac and metabolic disease. If any of the women developed pre eclampsia or any other complication of pregnancy she was excluded from the study. None of the women recruited developed such complications. Ethical approval for this study was granted by the Glasgow Royal Infirmary Research Ethics Committee. All subjects gave informed consent to participate.

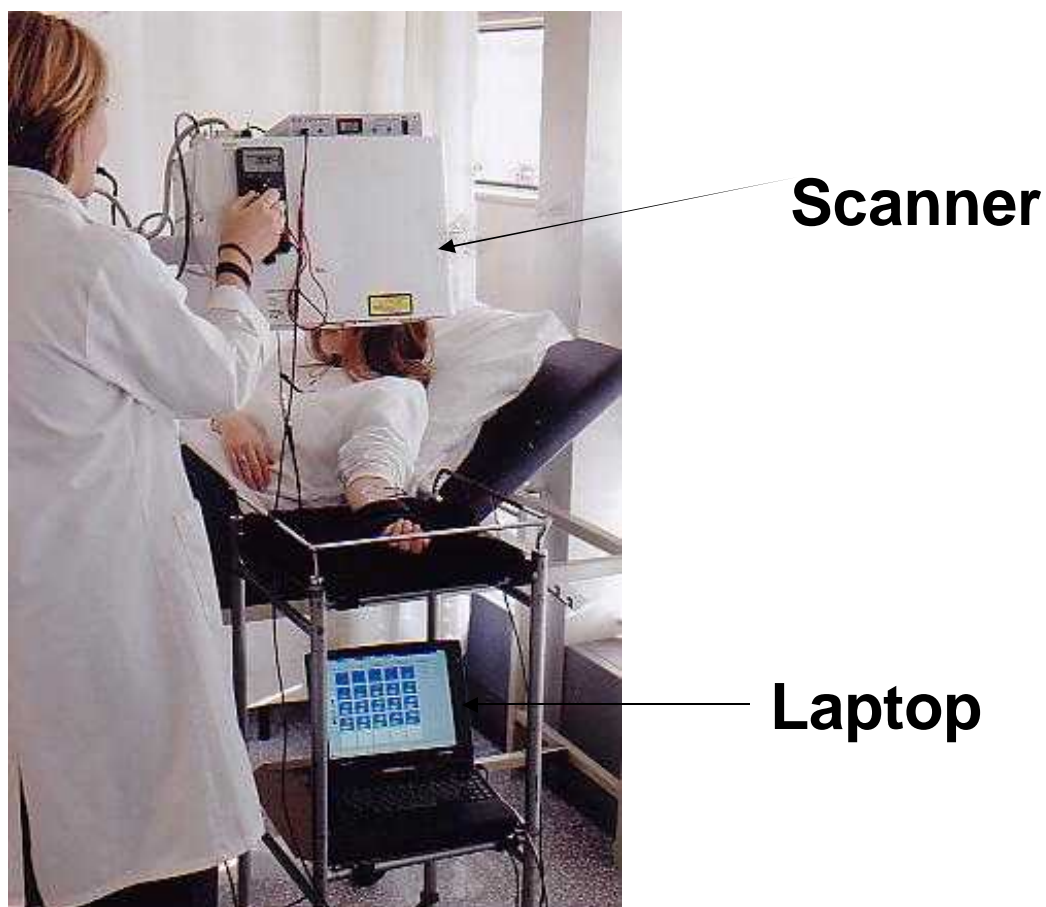
## 2.2 Experimental design

The women were asked to attend for their first “study visit” during the first trimester. All women attended for participation after an overnight fast (>10 hours) and underwent testing between 0900 and 1100 hours. Patient characteristics were recorded at this visit from patient notes. Fasting bloods were collected. Patient height, weight, waist circumference and hip circumference were measured. Waist circumference was measured at the level of the umbilicus. Hip circumference was measured at the widest point over the buttocks. Waist and hip circumference were measured in duplicate to the nearest 0.5 cm. If the difference between the two measurements was greater than 2 cm, a third measurement was taken and the mean of the two closest measurements was calculated. WHR was defined as waist circumference divided by hip circumference. All measurements were taken by the same examiner. Body mass index (BMI) was calculated as booking weight (kg), divided by height (m) squared. Deprivation (DEPCAT score), a measure of socio-economic status (234), was assigned using the Scottish Area Deprivation Index for Scottish postcode sectors, 1998. At this visit further arrangements

were made for the patient to attend in the second trimester and then again in the third trimester. At time of delivery all patients were seen by the lead examiner and arrangements made for a postnatal visit at three months. Details of gestation of delivery, mode of delivery, fetal sex, birth weight and placental weight were recorded from the patients' notes. Birth weights of offspring were normalised by gestation at birth, fetal sex and maternal parity (235) with a greater centile indicating a larger normalised birth weight. At each time point maternal venous blood was collected in EDTA, citrate, serum, lithium heparin and fluoride oxalate tubes and .sampled into 1mg/ml EDTA and packed blood cell collected by low speed centrifugation

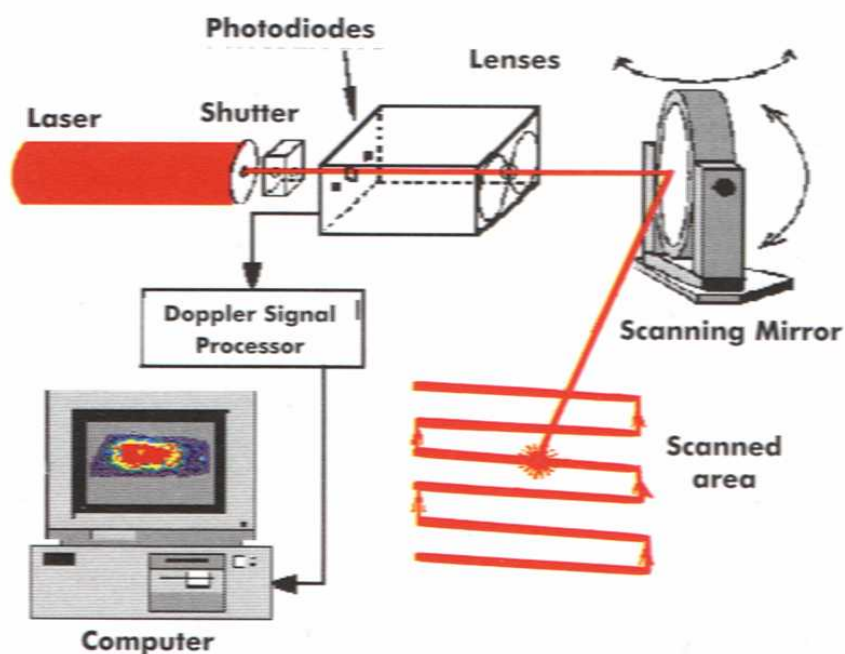
## **2.3 Iontophoresis**

Experiments were performed with subjects supine in a quiet room with an ambient temperature of  $22 \pm 1^\circ\text{C}$ . (See figure 2-1). I performed all of the Iontophoresis scanning. No history of peripheral vascular abnormalities such as Raynauds syndrome, dermatological diseases or systemic disease processes such as diabetes mellitus.



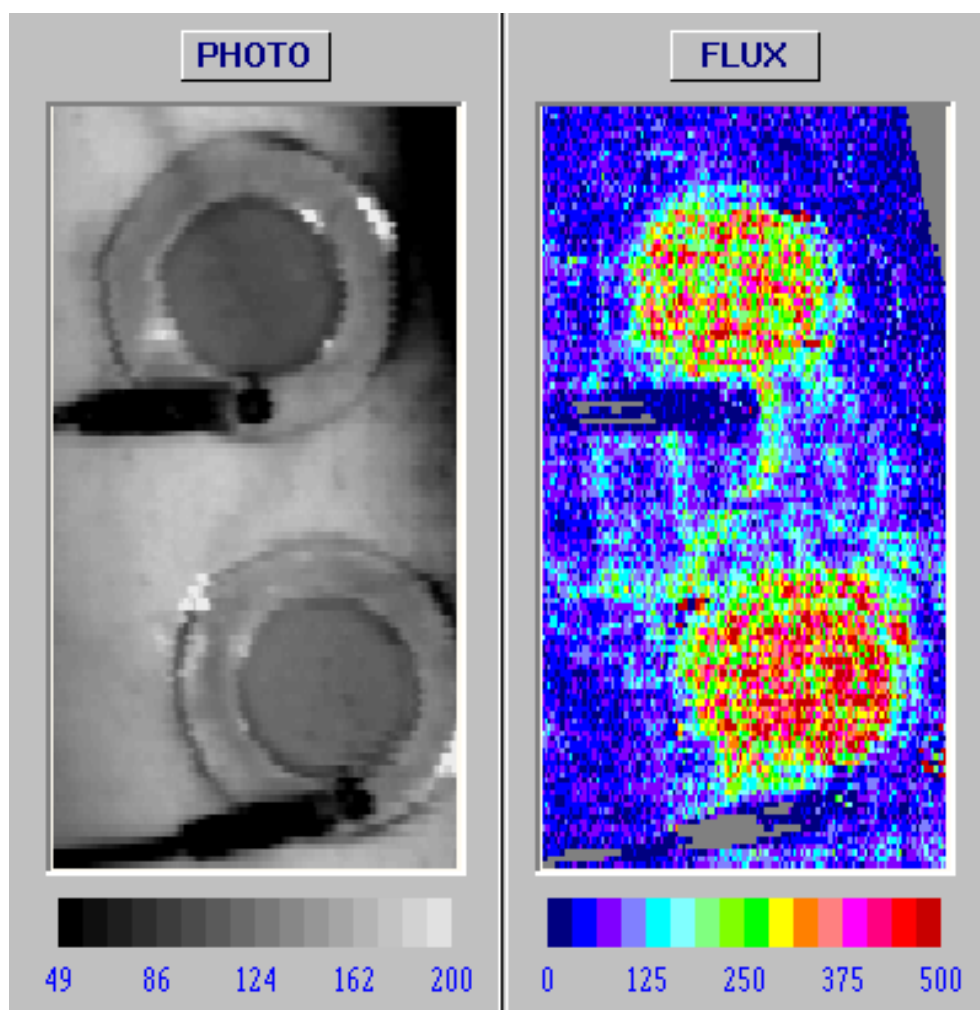
**Figure 2-1. Experimental set up of the Laser Doppler imager.**

This technique is based on the principle that a charged molecule migrates across the skin under the influence of an applied electrical field. ( $\text{Current} \times \text{time} = \text{charge, in Coulombs}$ ). Iontophoresis of acetylcholine (ACh) examines endothelial function. Because binding to muscarinic receptors, with subsequent generation of nitric oxide (NO), requires intact endothelial cells responses to ACh are therefore said to be endothelium-dependent. Vasodilatation is ultimately mediated by action of NO on vascular smooth muscle (*via* the cyclic GMP pathway); and so, iontophoresis of sodium nitroprusside (SNP), an NO donor, is used as an endothelium-independent control.



**Figure 2-2. Laser Doppler imager methodology.** The scanner as shown emits a laser beam onto the volar aspect of the forearm. The greater the degree of vasodilation imparted by the applied agent the greater the velocity of the red blood cells flowing through the microvasculature. The higher the velocity of the red blood cells the greater the degree of displacement of the laser beam.

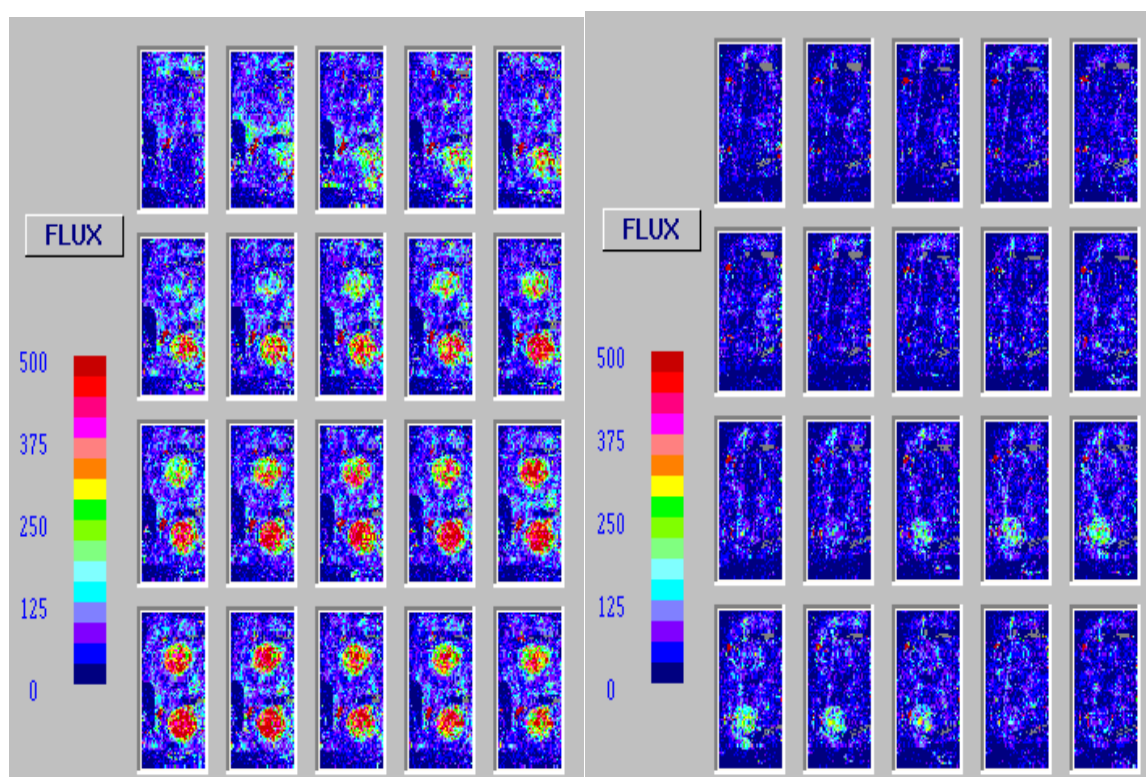
Drug delivery was achieved using a battery-powered constant current iontophoresis controller (Moor Instruments Ltd, MIC-1e). The chambers used for iontophoresis (Moor Instruments Ltd, ION 6) were constructed of Perspex (internal diameter, 22 mm; area 3.8 cm<sup>2</sup>) with an internal platinum wire electrode. Two chambers were attached to the skin of the volar aspect of the forearm, avoiding hair, broken skin, and superficial veins (see figure 2-3).



**Figure 2-3. Iontophoresis methodology.** Chambers containing Acetylcholine and sodium nitroprusside are placed on the forearm in contact with the skin. The chambers are connected to a positive and negative charge which “push” the positively charged acetylcholine and negatively charged sodium nitroprusside into the microvascular space.

The protocol involved incremental current delivery, with a baseline scan followed by four scans at 5  $\mu\text{A}$ , four at 10  $\mu\text{A}$ , four at 15  $\mu\text{A}$ , and two at 20  $\mu\text{A}$  giving a total charge of 8 milliCoulombs, followed by five recovery scans. Two and a half millilitres of 1% ACh (Sigma, Poole, UK) was introduced into the anodal chamber while 2.5 ml of 1% SNP (Sigma) was placed in the cathodal chamber. The vehicle for these drugs was 0.5% NaCl in deionized water. Responses were also observed with the vehicle alone as a control

experiment. Based on the raw perfusion-time integrals, the mean ( $\pm$  SD) between-day CV for the ACh response, measured in four subjects on 2 separate days, was  $6.4 \pm 3.3\%$ ; whereas the within-day, between-site CV, measured in both forearms on the same morning in four subjects, was  $8.9 \pm 5.3\%$  (139). (see figure 2-3).



**Figure 2-4. Vascular responses among lean and obese.** The distortion of the laser beam imparted by the moving red blood cells through the microvasculature is detected by photosensors within the scanner and interpreted by the software contained within the laptop, producing a series of scans. The software interprets these scans to give a value of microvascular function represented as area under the curve.

Perfusion measurements being performed after an overnight fast ensured no caffeine-containing drinks had been consumed before testing. Also, no over-the-counter medications were taken by any of the participants for at least 48 h before testing. Before

testing, a 10-min period of acclimatization was enforced, in a temperature-controlled room. The women were asked to lie in a semi recumbent position, with the flexor aspect of the forearm exposed on an armrest. Non-invasive measurement of skin perfusion was performed by means of a laser Doppler imaging (LDI) scanner (Moor Instruments Ltd, Axminster, UK) equipped with a red laser (wavelength, 633 nm; power, 1mW; beam diameter, 1 mm). The laser is scanned over the area to be examined, and backscattered light is collected by photodetectors and converted into a signal proportional to perfusion, in arbitrary perfusion units (PU). Twenty repetitive scans with an incremental iontophoretic current protocol were taken the first being a control (pre current administration) followed by incremental current protocol described above, of 14 scans, followed by a further 5 scans with no current administration. Measurement of response to drugs was obtained by taking raw values but an assessment of the overall response was defined as the area under the perfusion .time curve (AUC). As previously described by our group (236), correction for individual variation in skin resistance was performed, by dividing by the integral of conductance (the reciprocal of resistance) over time or more simply by multiplying the individual perfusion values by the integral of resistance over time, thereby normalising responses.

## 2.4 Lipid analysis

Plasma total cholesterol, triglyceride and HDL-cholesterol were determined by the standard Lipid Research Clinics Protocol (237). Very low density lipoprotein<sub>1</sub> (VLDL<sub>1</sub>) (Sf 60–400), very low density lipoprotein<sub>2</sub> (VLDL<sub>2</sub>) (Sf 20–60), intermediate density lipoprotein (IDL) (Sf 12–20), and LDL (Sf 0–12) were prepared from plasma by a



modification of the cumulative gradient ultracentrifugation technique described by Lindgren (238). The cholesteryl ester, triglyceride, free cholesterol, phospholipid, and proteins of the lipoprotein were assayed as described (239) and concentrations were calculated as the sum of the components (expressed as mg/dL plasma). Isolation of LDL subfractions from fasting plasma was achieved by density gradient ultracentrifugation using a discontinuous salt gradient (240). The LDL subfractions were displaced from the tube by upward displacement and identified by continuous monitoring of eluate at 280 nm. In all subjects, as in the normal population, three peaks of LDL were present, LDL-I, LDL-II, and LDL-III. The individual subfraction areas beneath the LDL profiles were quantified using Beckman Data Graphics software (Data Graphics; Beckman Industries, Fullerton, CA). Integrated areas were subsequently adjusted by specific extinction coefficients calculated previously for LDL-I, -II, and -III to give percentage abundance (% LDL). The total LDL mass (all protein and lipid components) of sequentially prepared LDL ( $d = 1.019\text{--}1.063$  g/mL) was subdivided in proportion to the percentage abundance values to give plasma concentration of the LDL subfractions. A proportion of the subfractions were carried out by me, ensuring laboratory experience. 80% of the subfractions were carried out by laboratory staff within the pathological Biochemistry laboratory.

## **2.5 Erythrocyte membrane fatty acid extraction**

Preparation of a total fatty acid extract from erythrocyte membranes was performed by a modified Folch extraction (16;17). Packed red blood cells (400 $\mu$ L) were suspended in

10mM Tris buffer pH 7.0, and incubated at room temperature for 30 minutes.

Suspensions were centrifuged in a Beckman L8-60M Ultracentrifuge, Type 50.4 rotor, at 49,000rpm, at 4 °C for 30 minutes. The erythrocyte pellet was re-suspended in 200µL of distilled H<sub>2</sub>O and 150µL was transferred to a clean glass screw top tube. Methanol: toluene (4:1, 2mL) containing heneicosanoic acid internal standard (0.2mg C<sub>21</sub>H<sub>42</sub>O<sub>2</sub>/ml toluene), was added followed by 200µL of 100% acetyl chloride while mixing. Tubes were capped and sealed with Teflon tape before heating at 100 °C for one hour. After cooling, 10% K<sub>2</sub>CO<sub>3</sub> (3mL) was slowly added to each tube followed by 100µL toluene. After centrifugation at 3000rpm for 8 minutes at 5 °C, the upper toluene phase was transferred to gas chromatography (GC) vials, and stored at -20 °C until ready for injection. This work was carried out by my research colleague Barbara Meyer School of Health Sciences, University of Wollongong. Northfields Ave Wollongong Australia.

### ***2.5.1 Gas chromatography of fatty acids***

Methyl fatty acids (FAs) were separated (1µL injection volume), identified and quantitated on a Shimadzu GC 17A gas chromatograph with flame ionisation detection and Class VP software. A 30m x 0.25mm-mm DB-23 fused silica capillary column (J&W Scientific, Folsom, CA) with a film thickness of 0.25 µm was used in conjunction with a Hewlett-Packard 7673B on-column auto-injector. Ultra-high purity hydrogen and air were used as carrier gases at a flow rate of 2mL/min. A temperature gradient programme was used with an initial temperature of 150°C, increasing at 20°C/min until 190°C, then at 5°C/min until 210°C, then at 2°C/min until 230°C and then at 4°C/min until 240°C (final time 18.5 min) and with an equilibration time of 1 minute. The total

programme time was 22 minutes. Identification of fatty acid methyl esters was made by comparison with the retention times of authentic standard mixtures (Fatty acid methyl ester mixture #189-19, product no. L9405, Sigma, Sweden). This work was carried out by my research colleague Barbara Meyer School of Health Sciences, University of Wollongong. Northfields Ave Wollongong Australia.

## **2.6 Analysis of inflammatory markers**

Plasma sensitive CRP was quantified using a double antibody sandwich ELISA with rabbit anti-human CRP and peroxidase conjugated rabbit anti-human CRP: DAKO A/S, DK-2600 Glostrup, Denmark. Plasma IL-6, IL-10 and TNF- were measured in citrated plasma stored at -80°C using high-sensitivity commercial ELISA kits (R&D Systems). Intra-cellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) were measured in citrated plasma stored at -80°C using high-sensitivity commercial ELISA kits (R&D Systems). PAI-1 and PAI-2 were assayed in citrated plasma using commercial ELISA according to manufacturers' instructions (TintElize PAI-1 supplied by Alpha Laboratories and Imubind PAI-2 [American Diagnostica] - supplied by Axis Shield). I carried out some of this work to ensure familiarity with the laboratory techniques. Laboratory staff carried out the majority of the testing.

## 2.7 Statistical analysis

Values for biochemical variables are given as mean  $\pm$  standard deviation unless otherwise stated. Normality testing was carried out using the Ryan-Joiner test in Minitab (vs13.0).

### 2.7.1 Lipoprotein analysis

Triglyceride and VLDL<sup>1</sup> levels were log transformed to achieve normality. A general linear model was used based on the following formula:

$$\text{OUTCOME} = \quad + \quad t * \text{BMI} + V_t$$

Where,  $\quad$  is a constant,  $t$  is the slope, which can be different for different time points and  $t = 1, 2, 3$ . BMI is the maternal body mass index, and  $V_t$  is the effect of visit  $t$ . When appropriate and  $\quad$  was constant for all visits, model estimates were pooled and a single slope was used. Associations between BMI and triglyceride levels were made by calculating the Pearson correlation coefficient ( $r$ ) and associated  $p$ - values. T tests were used for comparison of all parameters. The SPSS (version 11.5) statistical package was used. I carried out the Statistical analysis with the help of my supervisors.

### 2.7.2 Endothelial function and inflammatory markers

Data were analysed as  $\log_{10}$  or square root transformed data as the raw values were not normally distributed. Dose response curves were expressed as mean  $\pm$  SEM and were compared using two-way ANOVA. *Post hoc* analysis using Bonferroni correction was utilised. Analysis was also performed using the area under the perfusion time curve (AUC). Two sample  $t$  tests were used to assess any significant differences between the

lean and obese groups. Differences in endothelial function and inflammatory markers over time were tested using a repeated measures analysis in the general linear model (Minitab Vs 13.32). For multivariate analysis, the general linear model was used to examine the independent effects of time of sampling, obesity and plasma markers of inflammation and endothelial function on microvascular function, PAI-1/PAI-2 ratio, PAI-1 and PAI-2. Percent contribution to the variation (adjusted means squared, divided by the total means squared, expressed as a percentage) is quoted along with the level of significance.

### ***2.7.3 Erythrocyte fatty acid composition and concentration analysis***

Values for biochemical variables are given as mean  $\pm$  standard deviation unless otherwise stated. Normality testing was carried out using the Ryan-Joiner test in Minitab (vs13.0). Percent 14:0, 16:0, 17:0, 18:0, 20:0, 24:0, 14:1n7, 16:1n7, 17:1n7, 18:1n9, 20:1n9, 20:2n6, 22:2n6, 22:5 n6, 20:5n3, and 22:3n3 were transformed to log values to achieve normality before significance testing. Absolute 12:0, 14:0, 17:0, 20:0, 20:1n9, 22:2n6 and 22:3n3 were transformed to log values and absolute 17:1n7, 18:2n6 and 20:5n3 were transformed to square root values to achieve normal distribution. For summary measures, i.e. total of values at different gestations, % MUFA, total n-9, n6/n3 ratio and 20:4n6/22:6n3 were transformed to log values and total n-7 transformed to square root values to achieve a normal distribution. Statistical analyses were performed using JMP statistical analysis program (Version 5.1, SAS Institute, Cary, NC, USA). Each of the erythrocyte fatty acids were assessed for differences between the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimester

by using One way Analysis of Variance with comparison for all pairs using Tukey-Kramer HSD. Paired Student's t-tests were used for the comparison between the first trimester and the post-partum values. Linear relationships between variables were estimated using Pearson's product moment correlation coefficients. Statistical significance was set at  $\alpha = 0.005$  for all analyses unless otherwise stated.

## **Chapter Three**

### **Lipoprotein changes in healthy pregnancy and their relationship to maternal BMI.**

## **3 Lipoprotein changes in healthy pregnancy and their relationship to maternal BMI.**

### **3.1 Introduction**

Obesity has become pandemic in the U.S. with currently two in three American adults being classified as overweight or obese (3). Similar trends are now being seen among the European population with 10% of children and 20% of adults in the UK now being classified as clinically obese (7). In line with this, our maternity hospital has observed a greater than three fold increase in the proportion of women with a booking body mass index (BMI)  $>30\text{kg/m}^2$  over the past decade (2). With obesity comes an increased risk of miscarriage (26), an increased risk of neural tube defects and other congenital abnormalities (28). Metabolic complications of pregnancy such as gestational diabetes, pregnancy induced hypertension and pre eclampsia are also more prevalent among obese mothers (42) (35) (39).

In healthy pregnancy plasma triglyceride concentrations rise by 200-400%, while plasma cholesterol levels rise by 25-50% with advancing gestation. This physiological and temporary dyslipidaemia occurs presumably to accommodate the raw materials required for fetal development and growth. Many groups have established the pattern of dyslipidaemia in pregnancy [Fahraeus, 1985 #1; Piechota, 1992 #2], however fewer have analysed this phenomenon longitudinally (118, 119) or considered a detailed analysis of lipoprotein subclasses and, to our knowledge, none have correlated the degree of change to maternal BMI. Lipoprotein particles, rather than being homogenous, contain discrete



subfractions differing in structure, physiochemical properties, kinetic behaviour and origin (118). Low density lipoprotein (LDL), for example, incorporates a spectrum of lipoproteins of differing atherogenic potential. Small dense LDL (known as LDL-III) is more susceptible to oxidation than the larger and more buoyant subfractions (LDL-I and LDL-II particles) (112). Once oxidized these LDL III particles are believed to be highly atherogenic (112). Plasma triglyceride is the major determinant of small dense LDL, accounting for 40- 60% of the variability of this fraction (113) (241) (242).

In the non-pregnant state, obesity is associated with a triglyceride rich dyslipidaemia characterised by an increase in small dense LDL particles (243). These particles are readily taken up by macrophages to form foam cells and atheromatous plaques (244). LDL III, being more susceptible to oxidation, provokes the release of chemotactic and prothrombotic factors more readily. The dyslipidaemia of obesity characterised by low levels of HDL; increased triglycerides; increased subfractions of small, dense LDL; and increased levels of apolipoprotein B-100 is now well recognised (115). Studies have demonstrated that central obesity is more strongly related to lipid/lipoprotein abnormalities than is general obesity (104-106). These adverse lipid/lipoprotein profiles in obese individuals are important, because they may be responsible for their increased risk for cardiovascular disease (CVD).

By examination of the detailed dyslipidaemic response to pregnancy and its correlation to maternal BMI we hope to further define the link between obesity and the metabolic complications of pregnancy. The aim of this study is to examine changes in lipoprotein subclasses throughout pregnancy and to study the influence of maternal BMI over a wide

range (18-46 kg/m<sup>2</sup>). The study of these physiological modifications throughout pregnancy may provide better understanding into the proposed mechanisms of pregnancy related disorders such as pre eclampsia or pregnancy induced hypertension, which are more prevalent among obese mothers. Furthermore we hope to add insight to the concept of an adverse *in utero* environment which may promote cycling of vascular risk factors through generations (245).

## 3.2 Results

### 3.2.1 *Maternal characteristics*

Table 3-1 summarises the baseline first trimester clinical characteristics of all subjects within the study. All 60 women were seen in the first trimester of pregnancy. One woman miscarried at 15 weeks' gestation, while another woman moved her antenatal care to another unit at 18 weeks' gestation. 54 women attended for the second trimester visit, while 49 attended for the third trimester visit, hence 49 women attended all three trimester antenatal visits for examination.

Characteristic (N= 60)	Mean ( Standard deviation)
Maternal Age (years)	28.7 (5.3)
Smokers n (%)	23 (38.3%)
Booking maternal BMI (kg/m <sup>2</sup> )	29.1 (6.4)
Waist circumference (cm)	92.5 (18.1)
Waist hip ratio	0.83 (0.13)
Primigravidae n (%)	28 (46.7%)
Gestation at visit 1 (weeks)	12.4 (1.5)
Gestation at visit 2 (weeks)	26.1 (1.3)
Gestation at visit 3 (weeks)	35.5 (1.3)
Gestation at delivery (days)	284 (7)
Birth weight centile	56.9 (35.9)
Placenta weight (g)	741 (188)

**Table 3-1: Patient baseline, delivery and fetal characteristics.**

### ***3.2.2 Gestational changes in maternal plasma lipids and lipoproteins***

There were significant increases in total cholesterol, triglyceride, VLDL cholesterol and LDL cholesterol over the three trimesters (Table 3-2). For these lipids and lipoproteins the greatest increase took place between T1 and T2 (all  $P < 0.001$ ) and there were no significant differences between T2 and T3 on *post hoc* testing. Plasma HDL levels did not change with advancing gestation (table 3-2).

<b>Lipid</b>	<b>First Trimester (T1)</b>	<b>Second Trimester (T2)</b>	<b>Third Trimester (T3)</b>	<b>P Value (For T1 versus T3)</b>
	<b>Mean (SE)</b>	<b>Mean (SE)</b>	<b>Mean (SE)</b>	
Total Cholesterol (mmol/L)	4.7 (0.11)	6.0 (0.14)	6.0 (0.14)	<b>&lt;0.001</b>
Triglyceride (mmol/L)	1.4 (0.02)	2.5 (0.02)	2.9 (0.02)	<b>&lt;0.001</b>
VLDL cholesterol (mmol/L)	0.4 (0.03)	0.7 (0.04)	0.8 (0.04)	<b>&lt;0.001</b>
LDL cholesterol (mmol/L)	2.9 (0.11)	3.9 (0.13)	3.9 (0.14)	<b>&lt;0.001</b>
HDL cholesterol (mmol/L)	1.4 (0.04)	1.4 (0.04)	1.4 (0.04)	<b>1</b>

**Table 3-2: Plasma lipid and lipoprotein levels (mean [standard errors]) with advancing gestation.**

### **3.2.3 Gestational changes in LDL subfractions**

LDL mass increased between the first and second trimesters by 25.7 % ( $p < 0.001$ ) and then fell slightly with advancement into the third trimester but significance was not reached (Table 3-3). The rise in LDL mass was predominantly due to the significant rise in LDLIII mass (Table 3-3), which increased by 53% ( $P = 0.002$ ) between the first and second trimester. There was no significant alteration in mean percentages of LDL I with advancing pregnancy (Table 3-3). LDL II percentage decreased from 57.6% in the first trimester to 50.4% ( $P = 0.006$ ) in the second trimester. Conversely the proportion of LDL III rose markedly from 27.8% in the first trimester to 37.2% in the second trimester

( $P=0.005$ ). The mean proportions of LDL I, II and III did not change between the second and third trimester.

	First Trimester (T1) Mean (SE)	Second Trimester (T2) Mean (SE)	Third Trimester (T3) Mean (SE)	P Value (For T1 versus T3)
<b>LDL Subfractions</b>				
Total LDL mass (mg/dL)	132.4 (7.54)	166.5 (7.26)	153.1 (5.75)	<b>0.039</b>
LDL I mass	18.5 (1.64)	21.6 (2.12)	19.1 (1.97)	<b>0.823</b>
LDL II mass	75.6 (4.20)	83.5 (5.73)	81.1 (4.97)	<b>0.398</b>
LDL III mass	39.3 (4.51)	60.1 (4.97)	52.2 (4.00)	<b>0.044</b>
% LDL I	14.6 (1.19)	12.5 (0.93)	12.1 (0.92)	<b>0.128</b>
% LDL II	57.6 (1.53)	50.4 (2.15)	52.8 (2.38)	<b>0.080</b>
% LDL III	27.8 (1.87)	37.2 (2.76)	35.1 (2.72)	<b>0.024</b>

**Table 3-3: Changes in LDL subfractions with advancing gestation.**

### ***3.2.4 VLDL subfraction and IDL composition and concentration changes with advancing gestation.***

The large, triglyceride-rich VLDL<sub>1</sub> more than doubles in concentration ( $P<0.001$ ) between the first and second trimester and does not rise significantly between the second

and third trimester (Table 3-4). The cholesterol rich VLDL<sub>2</sub> also nearly doubles between the first trimester and the second trimester ( $P < 0.001$ ) with no significant further change in the third trimester (Table 3-4). IDL concentration rises significantly between the first and second trimester from a mean of 78.3mg/dL to 128.9mg/dL ( $p < 0.001$ ). There is a then a further increase in IDL concentration in the third trimester to 153.4mg/dL.

	First Trimester (T1) Mean (SE)	Second Trimester (T2) Mean (SE)	Third Trimester (T3) Mean (SE)	P Value (For T1 versus T3)
<b>VLDL Subfractions</b>				
<b>and IDL: <u>mg/dL</u></b>				
VLDL 1	22.0 (1.76)	48.4 (3.52)	50.1 (3.84)	<b>&lt;0.001</b>
VLDL 2	74.5 (6.21)	142.3 (9.66)	159.4 (11.6)	<b>&lt;0.001</b>
IDL	78.3 (6.58)	128.9 (6.32)	153.4 (11.5)	<b>&lt;0.001</b>
LDL	86.2 (4.49)	121.3 (6.04)	109.5 (6.24)	<b>&lt;0.001</b>

**Table 3-4: changes in VLDL and IDL subfractions with advancing gestation**

VLDL<sub>1</sub> composition is altered with advancing gestation. Percentage triglyceride rises with advancing gestation however the most significant rise occurring from 40.9% in the first trimester to 51.1% in the second ( $p < 0.001$ ) (table 3-5). Percentage esterified cholesterol within VLDL<sub>1</sub>, falls steadily with advancing gestation from 21.2% in the first trimester to 14.9% in the second trimester and 10.6% in the third trimester (T1 versus T3  $p < 0.001$ ) (table 3-5). The composition of VLDL<sub>2</sub> also changes with advancing gestation in that percentage triglyceride again rises with advancing gestation however not to the same extent as that seen in VLDL<sub>1</sub> and steadily across all three trimesters (Table 3-5). Percentage esterified cholesterol falls steadily with advancing gestation across all three

time points (Table 3-5). IDL composition changes again show an increase in percentage triglyceride from 14.7% in the first trimester to 18.5% in the third trimester ( $p<0.001$ ), at the expense of falling esterified cholesterol from 33.8% in the first trimester to 30.8% in the third trimester ( $p<0.0001$ ) (Table 3-5).

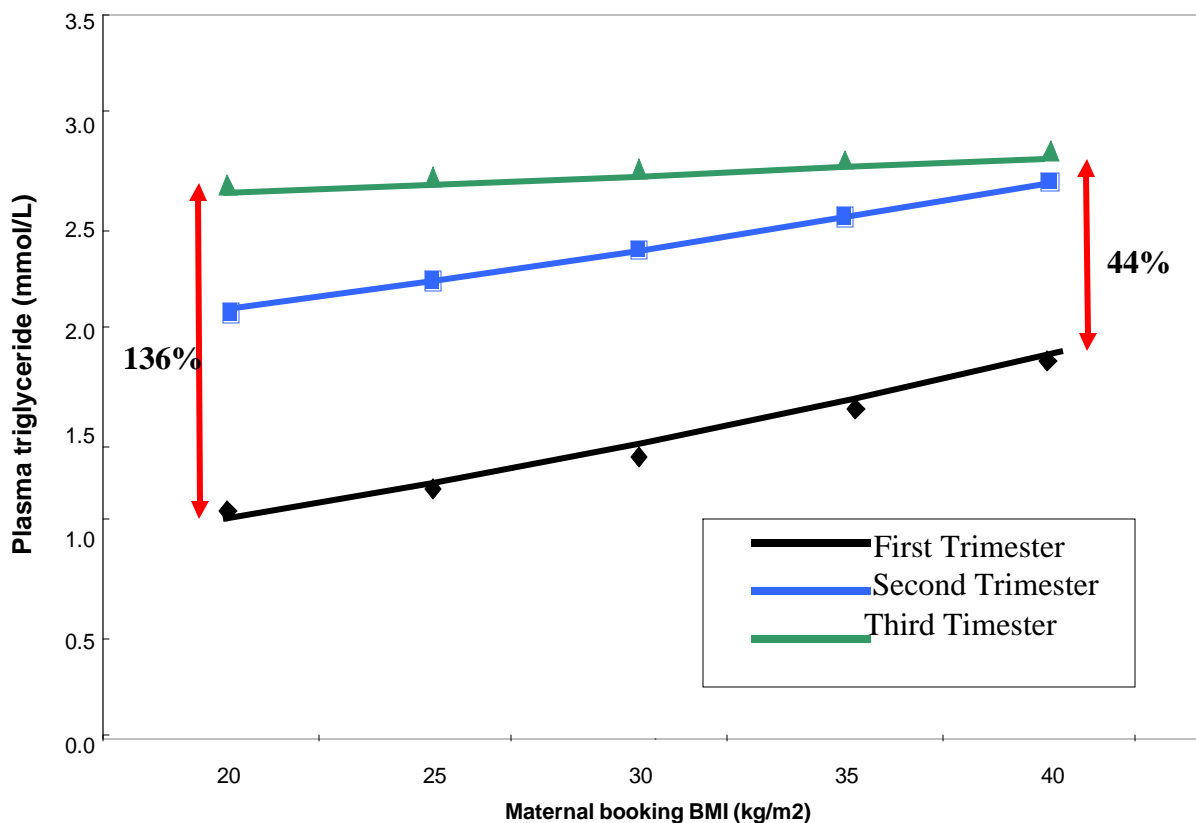
Lipid	First Trimester (T1)	Second Trimester (T2)	Third Trimester(T3)	P value T1 vs. T3
	<u>% Protein</u>			
VLDL 1	13.9 (0.65)	13.2 (0.58)	14.8 (0.47)	<b>&lt;0.001</b>
VLDL 2	19.3 (0.57)	17.4 (0.47)	19.1 (0.78)	<b>&lt;0.001</b>
IDL	22.2 (0.39)	21.4 (0.32)	22.4 (0.44)	<b>&lt;0.001</b>
LDL	26.2 (0.29)	26.1 (0.27)	26.6 (0.27)	<b>&lt;0.001</b>
	<u>% Free Cholesterol</u>			
VLDL 1	6.9 (0.36)	6.1 (0.15)	6.0 (0.11)	<b>0.033</b>
VLDL 2	7.2 (0.13)	7.2 (0.11)	7.0 (0.11)	
IDL	7.9 (0.14)	7.9 (0.09)	7.8 (0.11)	
LDL	7.8 (0.11)	7.4 (0.09)	7.1 (0.11)	
	<u>% Esterified Cholesterol</u>			
VLDL 1	21.2 (0.98)	14.9 (0.69)	10.6 (0.59)	<b>&lt;0.001</b>
VLDL 2	25.4 (0.66)	22.8 (0.52)	19.7 (0.54)	<b>&lt;0.001</b>
IDL	33.8 (0.48)	32.8 (0.44)	30.8 (0.48)	<b>&lt;0.001</b>
LDL	38.5 (0.29)	37.9 (0.34)	36.8 (0.40)	
	<u>% Triglyceride</u>			
VLDL 1	40.9 (1.38)	51.1 (1.03)	54.3 (0.77)	<b>&lt;0.001</b>
VLDL 2	28.2 (0.94)	33.6 (0.65)	35.3 (0.73)	<b>&lt;0.001</b>
IDL	14.7 (0.40)	17.4 (0.46)	18.5 (0.42)	<b>&lt;0.001</b>
LDL	6.7 (0.19)	8.8 (0.32)	9.9 (0.33)	
	<u>% Phospholipid</u>			
VLDL 1	17.1 (0.41)	14.7 (0.22)	14.3 (0.19)	<b>&lt;0.001</b>
VLDL 2	19.9 (0.24)	18.9 (0.18)	18.8 (0.21)	<b>0.002</b>
IDL	21.3 (0.23)	20.4 (0.14)	20.5 (0.16)	<b>0.005</b>
LDL	20.8 (0.12)	19.7 (0.13)	19.5 (0.14)	

**Table 3-5: Table showing changes in composition of VLDL<sup>1</sup>, VLDL<sup>2</sup>, IDL and LDL with advancing gestation**

### ***3.2.5 Influence of maternal booking BMI on lipids with advancing gestation.***

Triglyceride was positively correlated to booking BMI in the first trimester, ( $r = 0.31$ ;  $P = 0.016$ ) however this relationship is lost in the second and third trimester. The degree of change in plasma triglyceride was negatively correlated to maternal booking BMI. The women who booked for antenatal care with higher BMI values started pregnancy with high triglyceride levels which rose by 44% with advancing gestation. However those women who booked for antenatal care with lower BMI values started pregnancy with lower plasma triglyceride concentrations which rose by 136% with advancing gestation (Figure 3-1).

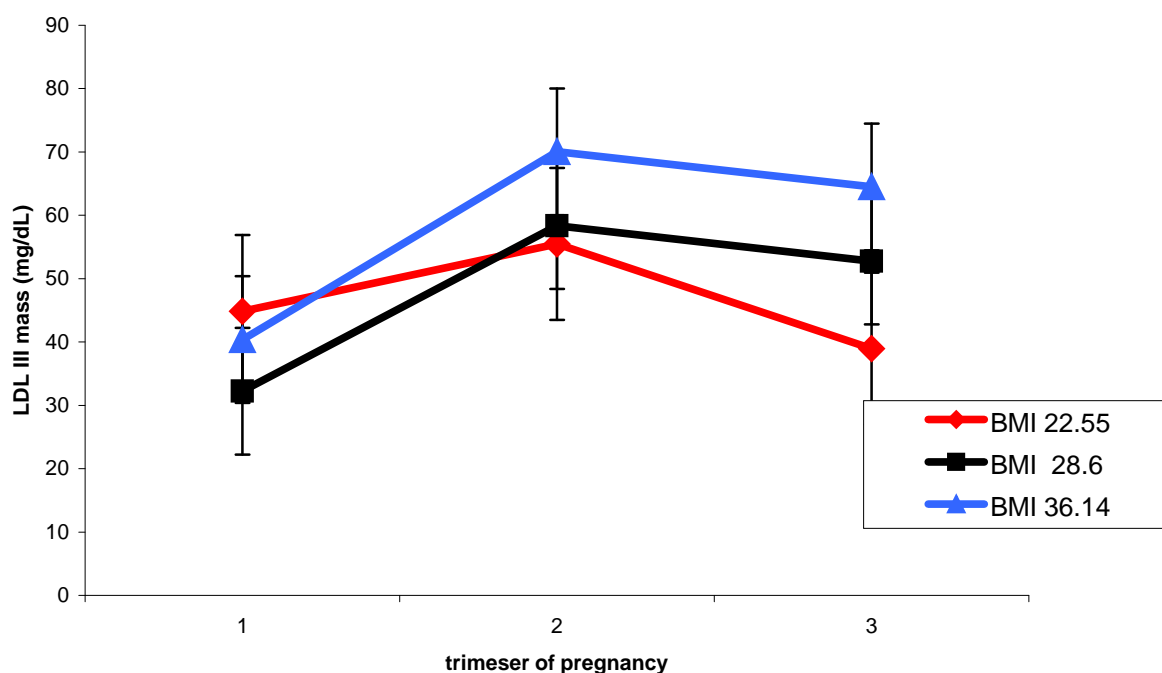




**Figure 3-1: Changes in plasma triglyceride with advancing gestation as influenced by maternal booking BMI.** Note the reduced degree of change among those with higher booking maternal BMIs as compared with those with lower maternal booking BMIs as demonstrated by the vertical arrows.

The influence of maternal BMI and length of gestation on LDL mass was investigated using a General Linear Model. BMI values were divided into tertiles of 18-26kg/m², 26-32kg/m² and 31-46.3kg/m². A mean BMI for each tertile was calculated. The pattern of change in LDL III mass with advancing gestation was plotted for each mean tertile maternal booking BMI (Figure 3-2). All women experience a significant rise in LDL III mass between the first and second trimesters. By the third trimester the influence of maternal booking BMI becomes evident. As shown in figure 3-2 women with a high

booking BMI (mean 36.1 kg/m<sup>2</sup>) continue to maintain their LDL III mass concentration achieved in the second trimester while those with a lower booking BMI (mean 22.6kg/m<sup>2</sup>) tend to reduce LDL III mass by the third trimester to near first trimester levels or lower.



**Figure 3-2: Changes in plasma LDL III mass with advancing gestation dependent on booking maternal BMI.** Three mean maternal BMIs are represented. Note the difference in behaviour of LDL III mass concentration as dictated by maternal booking BMI in the third trimester.

### 3.3 Discussion

Various lipid/lipoprotein abnormalities have been observed in obese individuals, including elevated cholesterol, triglycerides, apolipoprotein B (apoB), and lower high-density lipoprotein (HDL) cholesterol levels. Of these indicators, changes in triglyceride and HDL cholesterol levels are most consistent and pronounced (102, 103). The aim of the present study was to define the pattern of dyslipidaemia experienced with advancing gestation of pregnancy. By recruiting women with a large range of maternal booking BMI (18-46 kg/m<sup>2</sup>) we were able to study the influence of pre pregnancy obesity on the metabolic lipid response to pregnancy.

Plasma triglyceride (100% rise), total cholesterol (30% rise), VLDL cholesterol (100% increase) and LDL cholesterol (36% increase) levels increased significantly with advancing gestation. We demonstrated that the significant rise occurred by the second trimester and that the levels tended to plateau until the end of the third trimester. HDL cholesterol levels did not change with advancing gestation. Sattar et al reported a more than 200% rise in plasma triglyceride between the first and third trimesters of pregnancy and the increase occurred uniformly throughout pregnancy. This group also reported a 70% rise in plasma cholesterol between the first trimester and the third trimester however the majority of this increase had occurred by 25 weeks gestation (118). Sattar et al also observed no significant change in plasma HDL with advancing gestation in keeping with our findings (118). This investigation included 10 women and the range of BMI was 20-28kg/m<sup>2</sup>, therefore the differences between our observations could be due to the inclusion of women with a high BMI in the current study. As our data shows pre pregnancy

maternal BMI has a direct influence on the dyslipidaemia of pregnancy. A cross sectional study carried out by Piechota et al reported a 2.7 fold increase in plasma triglyceride by the third trimester however the majority of this rise had occurred by the second trimester. This group also reported a 43% rise in total cholesterol with 80 % of this rise having been achieved by the second trimester; these are findings comparable to ours. Piechota also reported a 36% rise in plasma LDL cholesterol of 36% by the third trimester, agreeing with our findings (117). Ninety percent of the LDL cholesterol increase was achieved by the second trimester (117).

As pregnancy advanced, the mass of the triglyceride-rich lipoproteins VLDL<sub>1</sub> and VLDL<sub>2</sub> increased in parallel, with the biggest changes observed between first and second trimester. The parallel increase in VLDL<sub>1</sub> and VLDL<sub>2</sub> are in agreement with findings of two previous studies (118, 246). These findings further enhance the uniqueness of the dyslipidaemia of pregnancy. In the non pregnant state a rising plasma triglyceride is associated with a preferential rise in VLDL<sub>1</sub> over VLDL<sub>2</sub> (242). In the general population increased concentrations of VLDL<sub>1</sub> are associated with insulin resistance and increased risk of coronary heart disease. The ratio of VLDL<sub>1</sub> to VLDL<sub>2</sub> with the increased triglyceride concentrations of the third trimester would be predicted from studies in non-pregnant subjects to be as high as 2 to 1 (242). However with advancing gestation and rising triglyceride levels the ratio remains the same, possibly a protective mechanism exclusive to pregnancy. The percentage triglyceride content of these lipoprotein subfractions increased at the expense of a decreased contribution to mass from cholesteryl esters and, to a lesser extent, phospholipid. These data suggest that the large amount of triglyceride mobilised during pregnancy is carried by all the main triglyceride-

rich lipoprotein fractions and is not due to an up-regulation of the production of one specific VLDL fraction. One previous study has demonstrated that the administration of potent oestrogens promotes a balance between VLDL subclasses (247). Pregnancy is a state of high oestradiol levels that may therefore contribute to the unique pattern of VLDL subclasses in response to increasing triglyceride levels.

The compositional analysis of VLDL<sub>1</sub> indicated that lean women exhibited a greater increase in the triglyceride to cholesteryl ester ratio of these particles. This would be consistent with lean women having less triglyceride in the VLDL<sub>1</sub> fraction at baseline and hence more capacity to increase the particle triglyceride content. Changes in LDL subfractions were also observed. There was a shift in the LDL subfraction profile towards smaller, denser LDL III as might be expected with the increase in circulating plasma triglyceride concentration. An increase in the triglyceride content of LDL in the presence of hepatic lipase leads to hydrolysis of the triglyceride in the core of the particle and a shrinkage in particle size (248) this effect has been observed by others. Belo et al concluded after their longitudinal study of changes in levels of oxidised LDL with advancing pregnancy that during human gestation the change in LDL profile was towards smaller species (119).

Plasma cholesterol was not found to correlate with maternal booking BMI at any time point. Plasma triglyceride correlated positively and plasma HDL correlated negatively with maternal booking BMI in keeping with previous reports in the literature regarding non-pregnant individuals (249). The relationship between plasma triglyceride and booking BMI was gradually lost with advancing gestation. During pregnancy it was

observed that women with a lower booking BMI started pregnancy with lower plasma triglyceride and as pregnancy advanced their triglyceride levels increased by a large amount; women with a BMI of  $20\text{kg/m}^2$  having a predicted 157% increase in plasma triglycerides (Figure 3-1). Women with a higher booking BMI had higher triglyceride levels in the first trimester increasing to about the same levels as those seen in lean women by the third trimester. Women with a BMI of  $40\text{kg/m}^2$  had a predicted rise in triglycerides of 75% (Fig 3-1). Since mobilisation of maternal fat stores is a key step in providing nutrient for the growing fetus, the lesser response to the physiological demands of pregnancy observed in those with higher maternal booking BMI values may indicate a decreased ability to metabolically adapt to pregnancy. On the other hand lean women show a greater metabolic flexibility with lower starting levels of plasma triglycerides but a larger response to pregnancy. Since maximum triglyceride levels attained during pregnancy are similar in lean and obese women these data would indicate that any adverse metabolic risk associated with obese pregnancy is more likely related to the quality of the increased lipid rather than the quantity.

When the pattern of change in LDL III mass was examined according to maternal BMI, LDL III mass levels behaved differently with advancing gestation dependent on maternal booking BMI. All women experienced a rise in plasma LDL III mass between the first and second trimester. With advancement into the third trimester however, those with a higher maternal booking BMI continued to experience a rise in LDL III mass while those with lower booking maternal BMI values experienced a fall in LDL III mass levels. Since, in the third trimester, both lean and obese women experience an increase in plasma triglyceride levels to above the threshold of  $1.5\text{mmol/L}$  (113) at which LDL III becomes

the dominant subfraction, an alternative explanation must be sought for the increased LDL III levels observed in obese women. In a study of identical twins, discordant for obesity, hepatic lipase activity was found to be higher in the obese twin (250). Thus higher hepatic lipase activity in obese pregnancy might explain the increased formation of LDL III. A link between LDL III levels and hepatic lipase activity would be consistent with the special case in pregnancy of pre eclampsia. This condition is characterised by a maternal dyslipidaemia more exaggerated than that of obesity (251). Women with pre eclampsia have significantly higher LDL III and hepatic lipase activity compared to weight-matched controls (251). A study of hepatic lipase activity in lean women indicated that in these women hepatic lipase activity declines throughout gestation and was strongly negatively correlated to oestradiol levels (126). Hepatic lipase is known to be regulated by sex hormones: native or alkylated oestrogens decrease its activity and progestagens with androgenic properties are known to increase it (252). It is therefore possible that differences in the reproductive steroid hormone environment between lean and obese women may influence hepatic lipase activity and hence LDL III levels.

LDL III is easily oxidised and once oxidised it is highly atherogenic. Oxidised LDL may lead to endothelial dysfunction by a combination of inducing adhesion molecule expression on the artery wall and by infiltrating the artery wall where it can be ingested by macrophages leading to foam cell formation. This mechanism may explain the link between obese pregnancy and endothelial dysfunction (139). A higher proportion of small, dense LDL in obese women also supports our hypothesis that obese women have a poorer “quality” of lipid in the face of gestational hypertriglyceridaemia. The delivery of poorer quality lipids to the fetus via the placenta may contribute to an adverse *in utero*

environment and go some way to explaining the cycling of vascular risk factors between generations that has been proposed for obese pregnancy (253).

We have demonstrated that the obese mother has an altered dyslipidaemia during pregnancy when compared to the lean woman. Increased LDL III mass in the third trimester may alter the *in utero* lipid environment for the fetus of the obese mother. Much previous work alluding to the concept of fetal programming refers to the adverse *in utero* effects of under-nutrition. Our work adds to the debate regarding the issue of quality of fetal nutrition *in utero* as opposed to the quantity. The fetus which receives fat transported from a maternal lipid pool including easily oxidised highly atherogenic LDL III particles in the third trimester may be exposed to higher levels of oxidised fats which could contribute to poor future endothelial function in the offspring. The abnormal blood flow as a result of the atherogenic environment may have detrimental long term effects on the developing child. Low birth weight babies are known to have a deficit in the number of nephrons, therefore there may be a cellular effect on many structures including vasculature. low birth weight (LBW) occurs more frequently in disadvantaged communities among whom there is often a disproportionately high incidence of adult cardiovascular disease, hypertension, diabetes mellitus, and kidney disease. Indeed, many epidemiologic studies have found an inverse association between LBW and higher blood pressures in infancy and childhood, and overt hypertension in adulthood. Multiple animal models have demonstrated the association of LBW with later hypertension, mediated, at least in part, by an associated congenital nephron deficit. Although no direct correlation has been shown between nephron number and birth weight in humans with hypertension, nephron numbers were found to be lower in adults with essential hypertension, and



glomeruli tend to be larger in humans of lower birth weight. We know that oxidative stress has the ability to alter DNA. This may then predispose to the development of disease in later life.

## **Chapter Four**

**Longitudinal assessment of erythrocyte fatty  
acid composition and concentration  
throughout pregnancy and in the post partum  
period.**

## **4 Longitudinal assessment of erythrocyte fatty acid composition and concentration throughout pregnancy and in the post partum period.**

### **4.1 Introduction**

Fatty acids are important constituents of cell membranes and therefore essential for tissue formation (reviewed in (224)). The long chain polyunsaturated fatty acids (LCPUFA) commonly found in membranes, especially in the brain, are derived via elongation and desaturation from the essential fatty acids 18:2n-6 (linoleic acid) and 18:3n-3 (alpha-linolenic acid) (reviewed in (225)). In pregnancy the fetus is reliant on placental transfer of fatty acids from the mother to support growth (225, 226). The mother must therefore mobilise key fatty acids and make them available for accretion by the fetus (254). Maternal LCPUFA status during pregnancy is critical in determining essential fatty acid status in the newborn (225, 227, 228).

Al *et al* have assessed the fatty acid status of mothers longitudinally throughout pregnancy by analysing plasma phospholipid fatty acid content. Focussing mainly on the essential fatty acids and their long chain derivatives, they observed that the percentage of 18:2n-6 did not change during pregnancy whereas the percentage of 20:4n-6 (arachidonic acid) decreased (255). Maternal plasma phospholipid 22:6n-3 (docosahexaenoic acid) percentage rose until 18 weeks and thereafter declined during pregnancy concomitant with an increase in umbilical plasma phospholipid 22:6n-3 content (255). These observations were consistent across populations selected from five different countries despite the variation in maternal essential fatty acid status between countries (256). Similar changes in total plasma

fatty acid compositions were observed longitudinally in pregnancy by Montgomery *et al* (257). It has been suggested (228, 258) that, in the populations studied and under their prevailing dietary conditions, the decline in plasma 22:6n-3 in late gestation indicates that the mother may be unable to meet the fetal demand for essential long chain polyunsaturated fatty acids.

While plasma phospholipid fatty acid status has been used to indicate fatty acid mobilisation, these fatty acids will be derived mainly from plasma lipoproteins and their measurement is open to confounding by maternal fasting status. Erythrocyte fatty acid composition is most likely to represent an integrative measure of the fatty acid status over the preceding three months (the half life of an erythrocyte is 120 days). Indeed it has been suggested that erythrocytes may act as a potential storage vehicle for 20:4n-6 and 22:6n-3 (259). Montgomery (257) observed similar changes in erythrocyte fatty acids during pregnancy to those seen for plasma fatty acids. In a cross-sectional study, Ashby *et al* (260) observed a 2<sup>nd</sup> trimester increase in the erythrocyte content of the essential fatty acids 22:6n-3, 20:5n-3 (eicosapentaenoic acid), 20:4n-6 and 18:2n-6 followed by a decline in the 3<sup>rd</sup> trimester in the absence of any significant changes in plasma fatty acids. Maternal plasma and erythrocyte 22:6n-3 and 22:5n-6 (docosapentaenoic acid) declined between 24 weeks' gestation and three months post partum in African-American women while 20:5n-3 and 20:4n-6 increased over the same period (261).

Most of longitudinal studies of changes in maternal fatty acid status in pregnancy have focussed primarily on the LCPUFA changes during gestation and have not emphasised changes in shorter chain and saturated fatty acids nor have changes in maternal fatty acid status across all trimesters been compared with the post-partum period. In this study we

report a full maternal erythrocyte fatty acid profile assessed at each trimester throughout pregnancy and at 4 months post-partum.

## **4.2 Results**

### ***4.2.1 Patient Characteristics***

Baseline, first trimester, characteristics of the pregnant women studied are shown in Table 5-1. The characteristics of the women studied here are fairly typical for the Glasgow population when compared with booking characteristics of women enrolled in the Glasgow Outcome APCR and Lipid (GOAL) Pregnancy Study (262) (Table 5-1). However the women studied here have a 3.5kg/m<sup>2</sup> higher body mass index (BMI) than the mean for the Glasgow population.

Characteristic	Total n=47	GOAL (18) n = 4218
Age (years)	28.7 (5.0)	28.4 (5.8)
Smokers n (%)	17 (36.2)	1701 (40.3)
Deprivation index n (%)		
Affluent (DEPCAT score(14) 1-2)	5 (10.6)	445 (10.6)
Intermediate (3-5)	17 (36.2)	1788 (42.4)
Deprived (6-7)	24 (51.1)	1754 (41.6)
Not known	1 (2.1)	231 (5.5)
Parity n (%)		1
0	24 (51.1)	842 (43.7)
1-2	20 (42.6)	1352 (32.1)
>2	3 (6.4)	289 (6.9)
not known	0	735 (17.4)
BMI (kg/m <sup>2</sup> )	28.4 (6.1)	24.9 (4.7)
Waist (cm)	90.0 (15.5)	80.1 (10.5)
Waist hip ratio	0.83 (0.11)	Not recorded
Gestation at delivery (days)	279 (10)	274 (15)
Mode of delivery n (%)		
Vaginal delivery	29 (61.7)	31 (45.2)
Assisted delivery	8 (17.0)	6 (13.0)
Elective Caesarean section	4 (8.5)	5 (5.5)
Emergency Caesarean section	6 (12.8)	5(5.5)
Fetal Sex n (%)		
Male	27 (57.4)	Not recorded
Female	20 (42.6)	
Birth weight centile	56.8 (33.2)	51.1 (29.6)
Placental weight (g)	770 (185)	674 (202)

**Table 4-1 Patient baseline and delivery characteristics for the current study and for women recruited to the Glasgow Outcome, APCR and LIPID (GOAL) Pregnancy Study (262).**

### **4.2.2 Gestational changes in maternal erythrocyte fatty acid composition**

Maternal erythrocyte fatty acid profiles are shown for each trimester of pregnancy expressed both as percentage total fatty acids (Table 5-2) and as absolute concentration (Table 5-3). There was a significant decrease in maternal 18:0 during pregnancy expressed as percentage total fatty acids however this was not reflected in a change in the absolute amount of this fatty acid. The significant increases in percentage of total fatty acids for 22:5n6, 18:3n3 and 22:6n3 were reflected in increases in the absolute amounts of these fatty acids of 25% ( $p=0.0003$ ), 41% ( $p=0.0007$ ) and 20% ( $p=0.0005$ ) respectively. Furthermore increases in the absolute amount of 16:1n7 (22%,  $p=0.0005$ ) and 24:1n9 (13%,  $p=0.0032$ ) were observed in the absence of a significant change in their percentage contribution to total fatty acids. The rise in all these fatty acids took place mostly between the first and second trimester with second and third trimester differences being non-significant on *post hoc* testing.

**Table 5-2 Mean (SD) erythrocyte fatty acid composition (% total fatty acids) in each trimester of pregnancy and 4 months postpartum. Gestation (mean [SD]) at sampling: first trimester 12.5 (1.3) weeks; second trimester 26.1 (1.3) weeks; third trimester 35.3 (1.3) weeks; post partum 18.1 (3.0) weeks post delivery.**

<b>Fatty Acid (%)</b>	<b>Trimester 1 n=46</b>	<b>Trimester 2 n=47</b>	<b>Trimester 3 n=47</b>	<b>P*</b>	<b>Post partum n=38</b>	<b>P** T1 vs post partum</b>
<b>SAFA</b>						
12:0	0.00 (0.00)	0.02 (0.09)	0.02 (0.08)	0.75	0.06 (0.18)	0.043
14:0	0.61 (0.27)	0.63 (0.30)	0.62 (0.26)	0.95	0.43 (0.21)	0.0002
16:0	21.9 (2.2)	22.0 (2.4)	22.2 (1.4)	0.70	20.3 (0.90)	<0.0001
17:0	0.35 (0.19)	0.31 (0.22)	0.33 (0.21)	0.81	0.40 (0.20)	0.98
18:0	15.7 (1.44)	14.8 (1.50)	14.5 (1.30)	<0.0001	15.3 (0.62)	0.08
20:0	0.72 (0.14)	0.70 (0.18)	0.63 (0.15)	0.0059	0.70 (0.18)	0.45
22:0	1.72 (0.41)	1.56 (0.36)	1.49 (0.37)	0.012	1.94 (0.28)	0.0061
24:0	3.20 (0.71)	3.30 (0.54)	3.29 (0.54)	0.54	3.64 (0.51)	0.0010
<b>MUFA</b>						
14:1 n7	0.00 (0.03)	0.02 (0.10)	0.01 (0.05)	1.00	0.03 (0.10)	0.67
16:1 n7	0.86 (0.23)	1.01 (0.27)	1.00 (0.23)	0.0057	0.74 (0.32)	0.0113
17:1 n7	0.04 (0.11)	0.04 (0.11)	0.06 (0.13)	0.65	0.14 (0.23)	0.320
18:1 n9	14.5 (1.7)	14.8 (2.0)	14.8 (1.7)	0.60	13.1 (1.1)	<0.0001
20:1 n9	0.60 (0.13)	0.64 (0.23)	0.56 (0.12)	0.0088	0.55 (0.20)	0.0397
22:1 n9	0.17 (0.24)	0.14 (0.23)	0.17 (0.23)	0.80	0.22 (0.24)	0.34
24:1 n9	4.93 (0.75)	5.13 (1.00)	5.22 (0.80)	0.29	4.59 (0.58)	0.0275
<b>PUFA</b>						
<b>n6</b>						
18:2 n6	8.60 (1.05)	8.91 (1.12)	8.90 (0.93)	0.27	9.75 (0.97)	<0.0001
18:3 n6	0.17 (0.17)	0.11 (0.15)	0.11 (0.14)	0.16	0.08 (0.16)	0.023
20:2 n6	0.38 (0.16)	0.41 (0.21)	0.41 (0.17)	0.36	0.29 (0.25)	0.47
20:3 n6	1.83 (0.41)	2.03 (0.51)	1.95 (0.43)	0.12	1.97 (0.43)	0.13
20:4 n6	13.7 (2.5)	12.7 (2.2)	12.9 (1.4)	0.054	15.4 (1.0)	0.0001



22:2 n6	0.04 (0.15)	0.01 (0.07)	0.01 (0.05)	0.65	0.04 (0.13)	0.43
22:4 n6	2.70 (0.75)	2.68 (0.79)	2.84 (0.70)	0.51	3.08 (0.47)	0.0088
22:5 n6	0.54 (0.22)	0.67 (0.21)	0.68 (0.20)	0.0024	0.54 (0.22)	0.82
PUFA n3						
18:3 n3	0.19 (0.15)	0.33 (0.20)	0.29 (0.18)	0.0009	0.23 (0.23)	0.31
20:5 n3	0.80 (0.45)	0.85 (0.46)	0.71 (0.41)	0.32	0.87 (0.40)	0.21
22:3 n3	0.37 (0.32)	0.38 (0.27)	0.41 (0.33)	0.73	0.62 (0.79)	0.098
22:5 n3	2.01 (0.41)	2.01 (0.49)	2.08 (0.31)	0.65	2.23 (0.45)	0.021
22:6 n3	3.29 (0.92)	3.79 (0.98)	3.85 (0.70)	0.0043	2.79 (0.68)	0.0060

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\* One way Analysis of Variance, across trimesters 1, 2 & 3, with comparison for all pairs using Tukey-Kramer HSD. \*\* t-test  
SAFA, saturated fatty acids, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids.

Table 5-3 Mean (SD) erythrocyte fatty acid concentration (pmol/ml blood) in each trimester of pregnancy and 4 months postpartum. Gestation (mean [SD]) at sampling: first trimester 12.5 (1.3) weeks; second trimester 26.1 (1.3) weeks; third trimester 35.3 (1.3) weeks; post partum 18.1 (3.0) weeks post delivery.

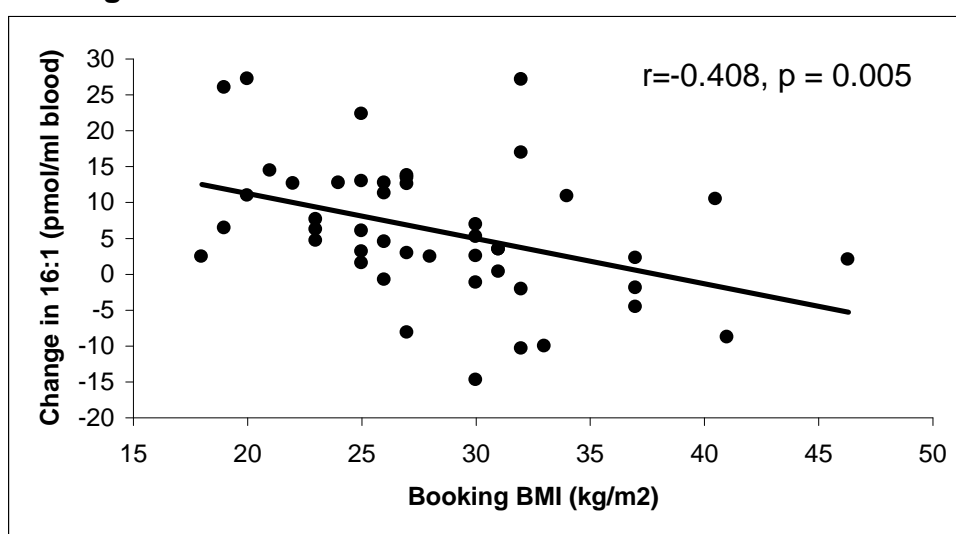
<b>Fatty Acid (pmol/ml)</b>	<b>Trimester 1 n=46</b>	<b>Trimester 2 n=47</b>	<b>Trimester 3 n=47</b>	<b>P*</b>	<b>Post partum n=38</b>	<b>P** T1 vs post partum</b>
<b>SAFA</b>						
12:0	0.0 (0.0)	0.8 (3.1)	0.7 (2.9)	0.81	2.0 (6.6)	0.048
14:0	16.8 (7.4)	17.6 (8.6)	19.1 (9.1)	0.64	12.0 (6.4)	0.0002
16:0	541.7 (90.5)	552.9 (94.2)	599.0 (114.1)	0.016	507.5 (77.7)	0.070
17:0	8.2 (4.5)	7.3 (5.4)	8.6 (5.8)	0.39	9.5 (5.0)	0.93
18:0	353.1 (69.0)	334.3 (58.8)	351.7 (63.0)	0.28	344.8 (53.7)	0.55
20:0	14.6 (3.5)	14.5 (4.5)	13.9 (3.7)	0.61	14.5 (4.3)	0.75
22:0	32.2 (9.5)	29.7 (8.0)	29.9 (7.8)	0.30	36.7 (7.2)	0.020
24:0	54.6 (10.6)	57.5 (11.0)	61.1 (11.5)	0.020	64.2 (14.9)	0.0009
<b>MUFA</b>						
14:1 n7	0.1 (1.0)	0.7 (3.3)	0.4 (1.8)	0.55	0.8 (2.8)	0.17
16:1 n7	21.3 (5.8)	25.5 (7.6)	27.2 (8.4)	0.0005	18.2 (8.3)	0.047
17:1 n7	1.0 (2.8)	1.0 (2.8)	1.8 (3.7)	0.48	3.3 (5.4)	0.015
18:1 n9	329.2 (78.3)	339.1 (72.1)	365.0 (80.5)	0.072	294.3 (47.1)	0.018
20:1 n9	12.3 (3.4)	13.4 (4.8)	12.5 (3.5)	0.58	11.3 (4.8)	0.087
22:1 n9	3.5 (4.7)	2.7 (4.3)	3.4 (4.6)	0.63	4.5 (4.9)	0.58
24:1 n9	85.0 (15.7)	89.7 (20.0)	98.0 (19.0)	0.0032	80.6 (16.6)	0.22
<b>PUFA n6</b>						
18:2 n6	196.2 (42.2)	206.6 (49.6)	219.7 (42.4)	0.041	222.6 (41.9)	0.0054
18:3 n6	3.7 (3.8)	2.6 (3.3)	2.7 (3.7)	0.30	1.9 (3.8)	0.038
20:2 n6	8.0 (3.4)	8.6 (4.5)	8.9 (4.1)	0.54	6.3 (5.3)	0.072
20:3 n6	37.9 (9.5)	43.0 (13.1)	44.1 (10.9)	0.020	41.3 (11.1)	0.13
20:4 n6	291.1 (81.4)	272.1 (65.5)	290.9 (53.8)	0.30	326.4 (57.2)	0.027
22:2 n6	0.9 (2.9)	0.2 (1.4)	0.2 (1.0)	0.54	0.8 (2.5)	0.23
22:4 n6	52.5 (17.9)	52.2 (16.5)	58.5 (15.7)	0.12	60.2 (12.7)	0.028
22:5 n6	10.4 (4.3)	13.0 (4.5)	13.9 (3.7)	0.0003	10.8 (4.8)	0.70
<b>PUFA n3</b>						
18:3 n3	4.4 (3.5)	7.7 (5.0)	7.4 (4.8)	0.0007	5.1 (5.4)	0.44
20:5 n3	16.8 (9.9)	18.2 (10.1)	16.1 (9.1)	0.61	18.3 (9.0)	0.37
22:3 n3	7.3 (6.6)	7.4 (5.1)	8.1 (6.7)	0.61	9.8 (5.5)	0.28
22:5 n3	39.0 (10.6)	39.5 (10.9)	43.4 (8.7)	0.073	43.7 (11.1)	0.048
22:6 n3	64.0 (19.7)	74.5 (21.0)	80.5 (19.4)	0.0005	54.6 (16.0)	0.021

\* One way Analysis of Variance, across trimesters 1, 2 & 3, with comparison for all pairs using Tukey-Kramer HSD. \*\* t-test SAFA, saturated fatty acids, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids.

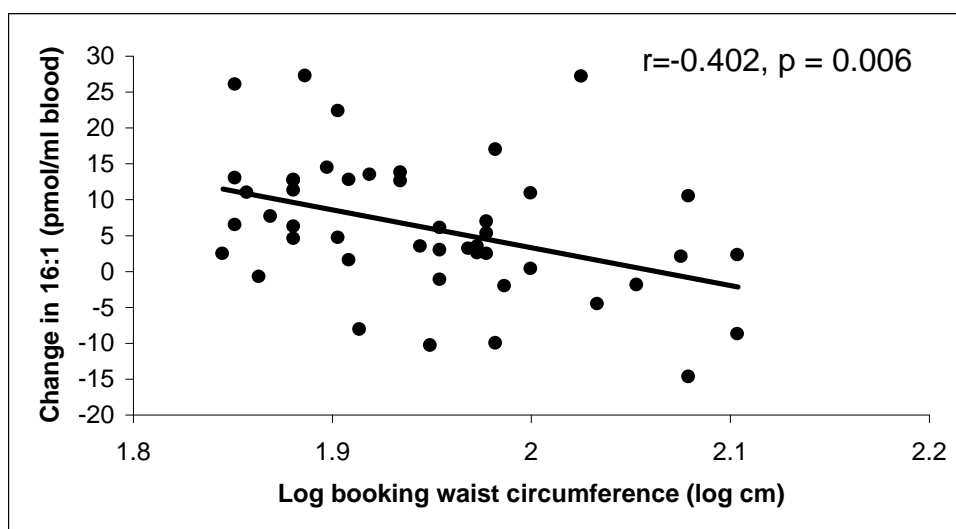
### 4.2.3 Correlation of gestational changes in maternal erythrocyte fatty acids with maternal characteristics

The change in absolute amount of 16:1n7 between the 1<sup>st</sup> and 3<sup>rd</sup> trimester was inversely correlated with booking BMI ( $r=-0.41$ ,  $p=0.005$ ) (Figure 5-1) and booking waist circumference ( $r=-0.40$ ,  $p=0.006$ ) (Fig 5-2).

**Figure 4-1: Correlation between the absolute concentration of 16:1n7 and booking BMI between the first and third trimester**

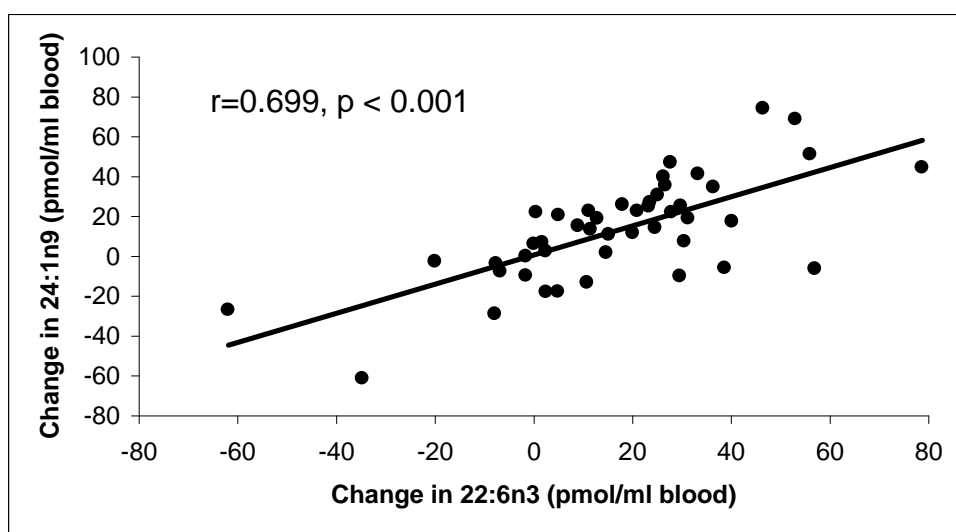


**Figure 4-2 Correlation between the absolute concentration of 16:1n7 and waist circumference between the first and third trimester.**

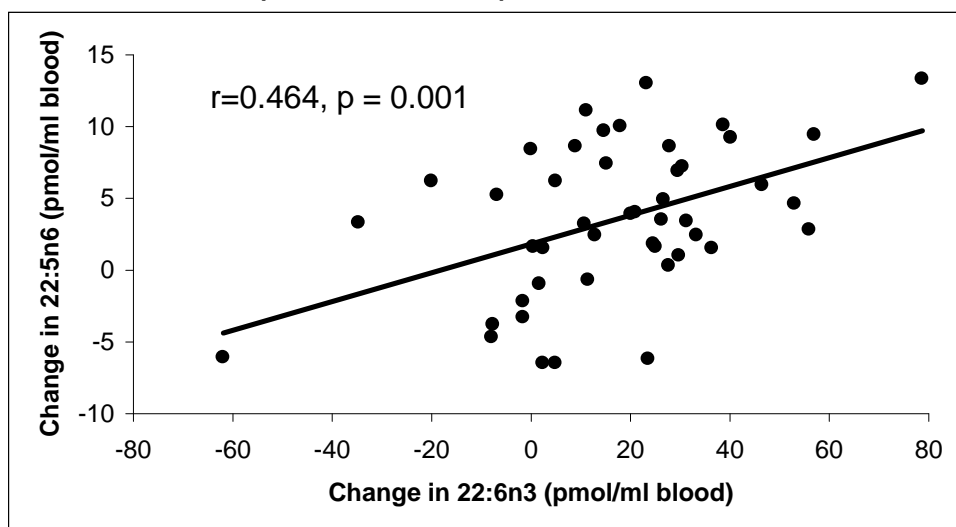


Thus there appears to be a link between the accumulation of 16:1n7 and maternal central obesity. Change in 16:1n7 concentration was also weakly correlated with accumulation of 18:3n3 ( $r=0.37$ ,  $p=0.012$ ). There were weak correlations between change in 24:1n9 and 22:6n3 concentrations and placental weight ( $r=0.33$ ,  $p=0.024$  and  $r=0.35$ ,  $p=0.017$  respectively). Finally change in absolute amount of 22:6n3 was strongly associated with changes in absolute amounts of 24:1n9 (fig 5-3) and 22:5n6 (Fig 5-4) ( $r=0.70$ ,  $p<0.001$  and  $r=0.46$ ,  $p=0.001$  respectively) between the 1<sup>st</sup> and 3<sup>rd</sup> trimester.

**Figure 4-3: Relationship between change in 22:6n3 concentration (pmol/mL blood) and changes in 24:1n9 concentration (pmol/mL blood) between the 1<sup>st</sup> and 3<sup>rd</sup> trimester (3<sup>rd</sup>-1<sup>st</sup> trimester).**



**Figure 4-4: Relationship between change in 22:6n3 concentration (pmol/mL blood) and changes in 22:5n6 concentration (pmol/mL blood) between the 1<sup>st</sup> and 3<sup>rd</sup> trimester (3<sup>rd</sup>-1<sup>st</sup> trimester).**



There were no relationships between changes in these fatty acids and maternal age, smoking status, deprivation category, parity, mode of delivery, gestation at delivery, fetal sex or birth weight centile.

#### ***4.2.4 Comparison of first trimester and post-partum maternal erythrocyte fatty acid composition***

In order to investigate whether fatty acids returned to baseline (trimester 1) pregnancy levels within 4 months after delivery, first trimester fatty acid composition was compared with erythrocyte fatty acid composition in samples collected a mean of 18 weeks after delivery (Tables 5-2 and 5-3). The percentage contribution to total fatty acids was lower post partum than in trimester 1 for 14:0, 16:0, 18:1n9 and 22:6n3 and higher post partum for 24:0, 18:2n6 and 20:4n6. However a reduction in absolute amounts was only seen for 14:0. Four months post partum, 14:0 was 29% lower ( $p=0.0002$ ) than during the 1<sup>st</sup>

trimester in pregnancy. An increase in absolute amount was only observed for 24:0 which was 15% higher ( $p=0.0009$ ) than during the 1<sup>st</sup> trimester in pregnancy.

#### ***4.2.5 Comparison of summary fatty acid measures during pregnancy and post-partum***

Table 5-4 shows the summary measures during pregnancy and post-partum period. During pregnancy, the n6/n3 ratio decreased by 11% ( $p=0.0019$ ) and the 20:4n6/22:6n3 ratio decreased by 23% ( $p<0.0001$ ) but of these two ratios, only the 20:4n6/22:6n3 ratio increased by 24% in the post-partum period ( $p<0.0001$ ). The percent MUFA of total fatty acids decreased by 8% in the post-partum period ( $p<0.0001$ ), which is reflected in the total n9 fatty acids (9%,  $p<0.0001$ ). Conversely, the percent PUFA of total fatty acids increased by 9% in the post-partum period ( $p<0.0001$ ), which is reflected in an increase in the n6 PUFA (11%,  $p<0.0001$ ). The unsaturated index ( $p=0.0013$ ), the average chain length ( $p=0.0006$ ) and the C20-22 ( $p=0.0005$ ) were all significantly higher in the post-partum period.

**Table 5- 4 Summary measures for % fatty acids**

<b>Fatty Acids</b>	<b>Trimester 1 n=46</b>	<b>Trimester 2 n=47</b>	<b>Trimester 3 n=47</b>	<b>P*</b>	<b>Post partum n=38</b>	<b>P** T1 vs post partum</b>
% saturated	46.6 (3.7)	45.6 (3.0)	45.4 (2.4)	0.16	44.8 (1.1)	0.0077
% unsaturated	53.4 (3.7)	54.4 (3.0)	54.6 (2.4)	0.16	55.2 (1.1)	0.0077
% MUFA	20.5 (1.7)	21.2 (1.8)	21.2 (1.7)	0.07	18.8 (1.1)	<0.0001
% PUFA	33.0 (4.3)	33.2 (3.9)	33.4 (2.5)	0.85	36.3 (1.5)	<0.0001
Total n-9	19.5 (1.6)	19.9 (1.7)	20.0 (1.5)	0.21	17.8 (1.0)	<0.0001
Total n-7	1.03 (0.29)	1.22 (0.38)	1.21 (0.32)	0.0062	1.03 (0.54)	0.63
Total n-6	27.0 (3.7)	26.7 (3.0)	26.9 (2.1)	0.82	30.3 (1.3)	<0.0001
Total n-3	6.0 (1.2)	6.6 (1.3)	6.6 (0.9)	0.0127	6.1 (1.0)	0.70
n6/n3 ratio	4.7 (1.0)	4.2 (0.8)	4.2 (0.6)	0.0019	5.1 (0.9)	0.0212
Unsaturated index	147.7 (16.8)	148.7 (16.4)	149.3 (10.8)	0.86	157.1 (5.7)	0.0013
Average chain length	18.6 (0.2)	18.6 (0.2)	18.6 (0.2)	0.86	18.7 (0.1)	0.0006
C20 –22	29.31 (4.2)	28.9 (4.5)	28.9 (3.2)	0.96	31.8 (2.0)	0.0005
20:4n6/22:6n3	4.4 (1.4)	3.6 (1.0)	3.4 (0.7)	<0.0001	5.8 (1.4)	<0.0001

\* One way Analysis of Variance, across trimesters 1, 2 & 3, with comparison for all pairs using Tukey-Kramer HSD. \*\* t-test SAFA, saturated fatty acids, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids.

## 4.3 Discussion

This paper describes clearly the changes in fatty acids that occur throughout pregnancy (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimester) and during the post-partum period (4 months post-partum). Comparison of our selected group with the large cohort of Glasgow women (n=4218) indicates that the women sampled were typical of the general Glasgow population apart from being more obese (Table 5-1). The fatty acids that increased significantly during pregnancy were 18:3n3, 22:5n6, 22:6n3, 16:1n7 and 24:1n9 (Tables 5-2 and 5-3). Because these changes were observed predominately between the 1<sup>st</sup> and 2<sup>nd</sup> trimester they must be initiated soon after implantation, especially if one considers that changes in erythrocyte composition integrate changes in fatty acid metabolism over the preceding 120 days.

Our data on changes in 22:6n3 are broadly consistent with previous observations that 22:6n3 is mobilised from maternal stores in order to supply the growing fetus (255-257). However we did not observe a decline in 22:6n3 levels between the 2<sup>nd</sup> and 3<sup>rd</sup> trimester which may indicate that our pregnant population is not deficient in 22:6n3 as has been suggested for other populations (228, 258). This would be supported by the significant decline in the n6/n3 ratio during pregnancy indicating an enrichment of maternal erythrocyte LC n3-PUFA. An increased ratio of 22:5n6/22:6n3 ratio has been reported to reflect an omega-3 or 22:6n3 deficiency (263). The calculated mol/mol 22:5n6/22:6n3 ratios in our population (1<sup>st</sup> trimester 0.16, 2<sup>nd</sup> trimester 0.17, 3<sup>rd</sup> trimester 0.17 and postpartum 0.20 mol/mol) were normal according to the cut-off values of >0.22mol/mol for 22:6n3 marginality and >0.48mol/mol for 22:6n3 deficiency suggested by Fokkema *et al* (263).



The 41% ( $p=0.0007$ ) increase in maternal erythrocyte 18:3n3 during pregnancy that we observed is most likely due to its role as a precursor for 22:6n3 synthesis. This large increase in 18:3n3 during pregnancy has either been reported, but not commented on (228, 256, 261) because the focus of research was on LCPUFA, or was not detected (257) or reported (255). 18:3n3 can be derived from green vegetables in the diet however it is unlikely that dietary changes explain the large accumulation seen in the women we studied since the population served by our hospital consumes a habitually poor diet low in fruit and vegetable content (264). However in the absence of detailed dietary intake information this explanation cannot be discounted. An alternative explanation might be increased mobilisation of 18:3n3 from maternal stores in order to provide substrate for LC n3-PUFA synthesis. Women tend to partition less 18:3n3 to  $\beta$ -oxidation than men (265) thus increasing availability for conversion to LCPUFAs. 18:3n3 can be elongated and desaturated to 20:5n3, 22:5n3 and 22:6n3 in women of child-bearing age (266) but only to 20:5n3 and 22:5n3 in men (267). Women taking synthetic oestrogens had an increased capacity to convert 18:3n3 to 22:6n3 (266). This gender difference underlines the potential importance of the capacity to synthesise 22:6n3 during pregnancy and lactation and it is possible that the rise in oestrogen during pregnancy regulates the conversion of 18:3n3 to LCPUFA (265). Fetal liver (268) can convert 18:3n3 to 22:6n3 but there is little evidence that placenta does so (269). It is possible that increased maternal 18:3n3 levels may provide substrate to the fetal liver synthetic pathways. The physiological relevance of the conversion from 18:3n3 can be questioned as 18:3n3 supplementation during pregnancy did not result in increased maternal or fetal DHA levels (270). However it is possible that if fetal 22:6n3 utilisation is efficient then additional 22:6n3 flux may not be detectable as changes in steady state levels in cord blood. Also all the women in the study were

Caucasian, and differences in deprivation scoring using DEPCAT were not significant between the two groups.

Nervonic acid (24:1n9) was also increased during pregnancy (Table 5-3) and it is well documented that 24:1n9 is important in neural development, particularly for myelination (271-273). The increased levels of 24:1n9 could be explained by an increased mobilisation of this fatty acid for fetal neural development from mid gestation and particularly during the post-partum period (272). 22:6n3 is also important for proper neural development (reviewed in (274, 275)) and is also increased during pregnancy (Table 5-3). The strong correlation between 22:6n3 and 24:1n9 ( $r=0.70$ ,  $p<0.001$ ) (Figure 5-3) suggests that the synthesis and/or transport of these two fatty acids may be coordinated. Interestingly the change in concentrations of these fatty acids during pregnancy was correlated to placental weight.

16:1n7 (palmitoleic acid) was also increased during pregnancy and is reduced in the post-partum period (Table 5-3). 16:1n7 was negatively associated with booking BMI and waist circumference (Fig 5-1 and Fig 5-2) indicating a relationship between levels of this fatty acid and central obesity. This relationship may be apparent in our study due to the wide range of booking BMI ( $18.0 - 46.3 \text{ kg/m}^2$ , Fig 1). 16:1n7 is synthesised from 16:0 by stearyl CoA desaturase (SCD) (232) an enzyme whose expression is downregulated by leptin (230) and whose activity is associated with obesity (231, 232). Our observation in pregnancy of an inverse association between the product of SCD and obesity is at odds with these published data. This discrepancy could be explained if 16:1n7 were not a good

index of SCD activity in pregnancy or if leptin metabolism were different in pregnancy. It has been suggested that maternal leptin resistance may occur in pregnancy (276, 277).

There is a well-recognised increase in maternal leptin in pregnancy (276, 278) which is even higher in obese pregnant women (139). Since leptin levels are increasing at a time when fat stores are accumulating this is in contradiction to leptin's role as a satiety signal.

It has been proposed that an increase in the amount of a soluble isoform of the leptin receptor derived from the placenta results in an increased proportion of bound, and hence unavailable, leptin in the maternal circulation with advancing gestation (277, 279). This potential for leptin resistance in pregnancy may account for the counterintuitive inverse association between 16:1n7 and obesity in pregnancy observed using the two independent measures of BMI and waist circumference. The association between fatty acid metabolism in pregnancy and obesity warrants further investigation especially in light of the increased booking BMI observed over the last decade in our hospital (2).

## **Chapter Five**

**Longitudinal assessment of maternal endothelial function and markers of inflammation & placental function throughout pregnancy in lean and obese mothers.**

## **5 Longitudinal assessment of maternal endothelial function and markers of endothelial and placental function throughout pregnancy in lean and obese mothers.**

### **5.1 Introduction**

Pre-pregnancy obesity is becoming a common occurrence in obstetric management.

Changes in the treatment of obesity-related infertility have resulted in more of these women achieving a pregnancy. Furthermore, as estimated by the World Health Organisation in 2000, 300 million people worldwide are clinically obese. In the U.S. the incidence of obesity is reported to have risen from 13% to 27% between 1980 and 1999 (280).

American trends are now being seen among the European population with 10% of children and 20% of adults in the UK being classified as clinically obese (7). In line with this, our maternity hospital has observed a greater than three fold increase in the proportion of women with a booking body mass index (BMI)  $>30\text{kg/m}^2$  over the past decade (2).

Increasing evidence relates impaired endothelial vasomotor function to coronary heart disease (170). Endothelial function can be assessed in the peripheral circulation and this has been shown to correlate with endothelial function within the coronary circulation (158, 281). Our group previously demonstrated that microvascular function in the third trimester of pregnancy (139) was better in lean compared to obese women. The study design

however did not permit identification of the dynamics of this phenomenon, i.e. whether it was a pregnancy-specific effect.

In healthy pregnancy several components of the metabolic syndrome are acquired; insulin resistance, hypertriglyceridaemia, an upregulation of the inflammatory cascade and an increase in coagulation factors. These changes impact substantially not only on carbohydrate and lipid control pathways, but also on vascular endothelial function. Several soluble markers produced by the endothelium, once activated, can be measured in peripheral blood. These soluble markers of endothelial activation include the adhesion molecules ICAM-1 and VCAM-1 (282), and the haemostatic factors vWF (283) and PAI-1 (284). The inflammatory response observed in pregnancy shifts from a Th1, mostly cellular, response to a Th2, mostly humoral response (285) and successful pregnancy is the result of a balance between the two responses. Some cytokines are more indicative of the Th1 response (TNF ) and some of the Th2 (IL-6) response. The influence of obesity on these factors throughout pregnancy and in the post-natal period has not been assessed longitudinally.

Khan et al have shown that by 6 months of age, offspring of dams fed a lard-rich diet in pregnancy and suckling develop a phenotype that is similar to that of human metabolic syndrome: hypertension, dyslipidaemia, insulin resistance obesity and blunted endothelium-dependent vasodilatation (286) (287, 288). Armitage et al went further to demonstrate that adult OHF animals exhibit reduced aortic endothelium-dependent vasodilatation and increased aortic stiffness (289). This was accompanied by reduced smooth muscle cell number and endothelial cell volume.

This finding of increased aortic stiffness in OHF supports the hypothesis that disturbances to maternal nutrition altered aortic function in offspring. A study of adult humans of low birthweight were shown to have reduced conduit artery compliance in the trunk and legs, as assessed by pulse wave velocity (290).

Armitage et al also observed reduced endothelium-dependent vasodilatation in the aorta from OHF (289). It is proposed that endothelial dysfunction may initiate many of the abnormalities clustering in the metabolic syndrome (291) and in the larger vessels this deficit is intimately involved in atherogenesis (292). The present data also showed a reduction in endothelial cell layer volume, evidence of altered endothelial cell morphology.

We hypothesise that microvascular endothelial function is up-regulated in both lean and obese women with advancing gestation; however the degree of improvement will be less in obese women when compared to lean counterparts. The aim of this Chapter was to use a non-invasive measure of microvascular endothelial function, laser Doppler imaging (LDI), in lean and obese subjects at intervals throughout their pregnancy. We examined the relationship between changes in endothelial function with advancing gestation and BMI. In addition, we assessed plasma markers of endothelial function, inflammation and placental function (PAI-1/PAI-2 ratio) and their association with microvascular function.

## 5.2 Results

### *Patient characteristics*

The demographics of the subject groups are shown in Table 3-1. The groups were well matched for age, parity and smoking status. Of note both first trimester diastolic and systolic blood pressures, although remaining within the normal range, were significantly higher in the obese women. First trimester BMI as well as waist circumference were significantly different between the two groups. There was no significant difference in gestation, or number of weeks *post partum*, at each visit between the groups. Babies born to obese mothers had a significantly greater birth weight centile when compared to those born to lean mothers. None of the women developed clinical evidence of hypertension or other metabolic complications of pregnancy.

**Table 5-1; Baseline characteristics of study subjects.**

Parameters	Lean (n=30)	Obese (n=30)	P value
------------	-------------	--------------	---------



	Mean $\pm$ S.D.	Mean $\pm$ S.D.	
Age (years)	28.5 $\pm$ 5.1	28.7 $\pm$ 1.1	0.87
Smokers (%)	36.7%	40.0%	0.88
Primigravidae (%)	40%	40%	1
Booking BMI (kg/m <sup>2</sup> )	24.0 $\pm$ 2.9	34.2 $\pm$ 4.5	<0.001
Waist circumference (cm)	79.4 $\pm$ 7.3	105.6 $\pm$ 16.1	<0.001
Booking Blood pressure (mmHg)			
Systolic	112.7 $\pm$ 11.4	123.7 $\pm$ 10.7	0.001
Diastolic	65.5 $\pm$ 7.7	72.7 $\pm$ 9.7	0.002
Gestation at Visit 1	12.2 $\pm$ 1.8	12.6 $\pm$ 1.0	0.33
( weeks) Visit 2	26.2 $\pm$ 1.2	26.0 $\pm$ 1.4	0.65
Visit 3	35.5 $\pm$ 1.4	35.5 $\pm$ 1.3	0.91
Number of weeks post-natal.	18.2 $\pm$ 3.0	15.9 $\pm$ 1.9	0.06
Birth weight centile	52.3 $\pm$ 34.7	74.1 $\pm$ 20.5	0.003
Vaginal deliveries (%)	83.3%	63.3%	0.059

### **5.2.1 Endothelial dependent vasodilator response**

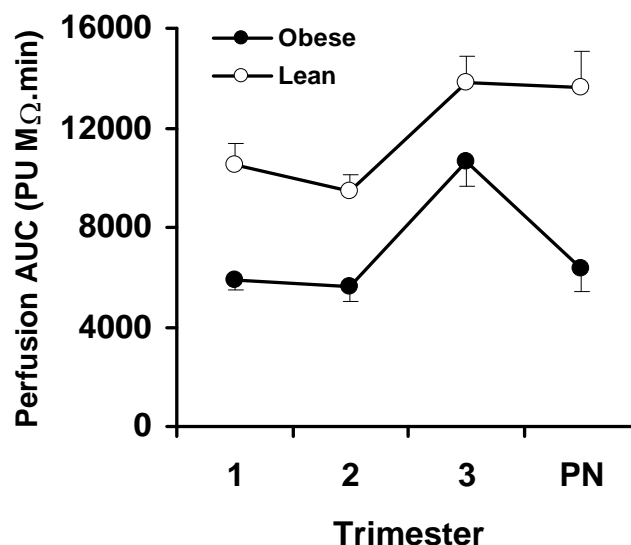
The response to ACh (expressed as the AUC) showed a highly significant difference (P<0.001, 2-way ANOVA) between the lean and obese groups (Fig 3-1). *Post hoc* analysis (Bonferroni correction) revealed that the response of the obese group was significantly

lower ( $P < 0.05$ ) at each trimester (51%, 41% and 39% respectively) when compared to the lean group and when tested at 4 months *post partum* (115% lower;  $P < 0.01$ ). Two-way ANOVA revealed that the ACh response differed significantly ( $P < 0.001$ ) between the different time points, with the third trimester differing from the other time points ( $P < 0.05$ , Bonferroni). Within the obese group, the third trimester response differed significantly from all other time points tested but within the lean group the third trimester response only differed significantly from second trimester response ( $P = 0.04$ ). *Post partum*, the ACh response did not decline significantly for the lean women but there was a significantly reduced response among the obese women ( $P < 0.001$ ).

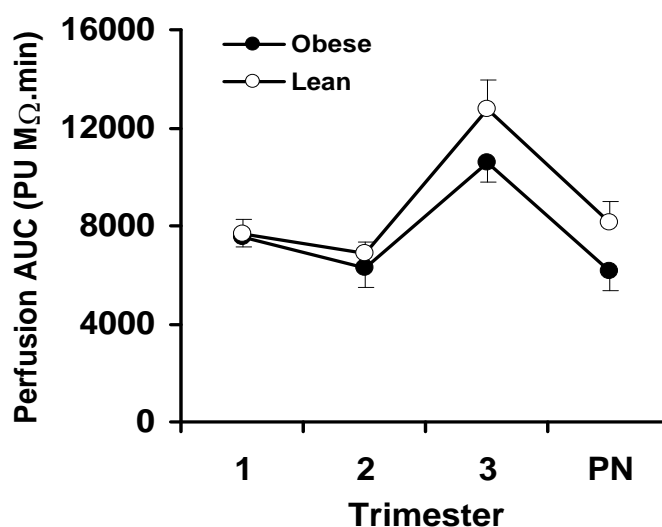
### **5.2.2 Endothelial independent vasodilator response**

Analysis of SNP data revealed a similar pattern with time as the ACh response (Fig 3-2) with a highly significant difference between the time points tested ( $P < 0.001$ , 2-way ANOVA). However, within the lean group the third trimester response differed significantly from all other time points while among the obese women third trimester response differed only significantly from the second trimester and post-natal. There is also a small but significant difference between the two groups, lean response being greater than obese ( $p = 0.021$ ). Interestingly the SNP response post-natal declined significantly for both groups ( $P < 0.001$ ).

**Figure 5-1: Endothelial dependent vasodilation in response to iontophoresis of Acetylcholine (ACh) in lean women (n=30) versus obese women (n=30), measured longitudinally with advancing gestation.**



**Figure 5-2: Endothelial independent vasodilation in response to iontophoresis of sodium nitroprusside (SNP) in lean women (n=30) versus obese women (n=30), measured longitudinally with advancing gestation. Means  $\pm$  SEM.**



### **5.2.3 Soluble plasma markers of inflammation and endothelial activation**

All of the measured inflammatory markers changed throughout pregnancy and the post-natal period apart from IL-10 in both groups and IL-6 in the obese (Table 2). For TNF ,

IL-10, sVCAM-1 and vWF there were no differences at any time point between the lean and obese groups. Only plasma CRP levels differed between lean and obese women at all time points tested with plasma CRP levels being significantly higher in the obese. Plasma IL-6 and sICAM-1 levels were significantly higher in obese women in early pregnancy (first and second trimester), but not in late pregnancy or the post-natal period (Table 3-2). PAI-1 levels were significantly lower in the obese in the first trimester and higher in the obese women in the post-natal period. PAI-2 levels were significantly lower in the obese in the first trimester.

**Table5-2: Changes in plasma concentrations of soluble markers of inflammation and endothelial function with advancing gestation in lean and obese pregnant women.** Median and interquartile ranges are shown. \* Difference testing was carried out using two sample t-test on transformed data. # Differences between time points was carried out on transformed data using repeated measures ANOVA in a general linear model.

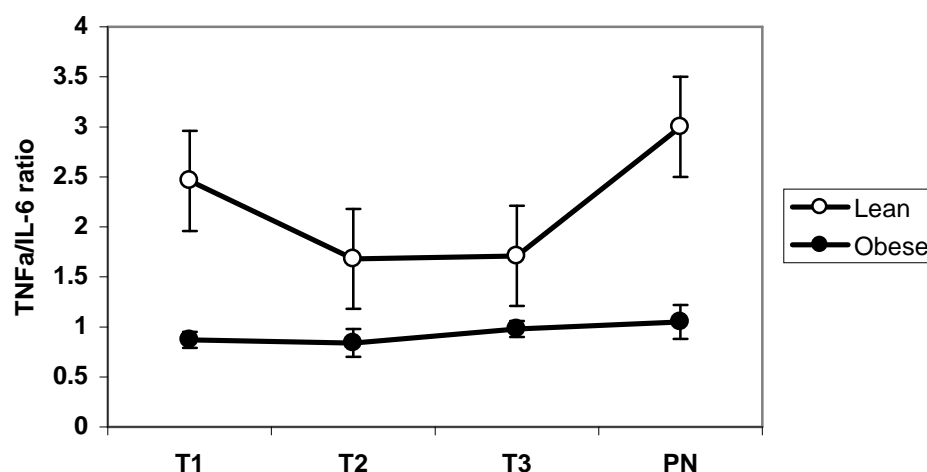
Inflammatory Marker	Lean Median (IQ range)	Obese Median (IQ range)	P value Lean vs. obese*
IL-6 (pg/ml)	<0.001#	0.36#	

	1 <sup>st</sup> Trimester	0.80 (0.50-0.93)	1.55 (1.10-2.65)	<0.001
	2 <sup>nd</sup> Trimester	1.00 (0.70-1.60)	1.80 (1.10-3.13)	0.001
	3 <sup>rd</sup> Trimester	1.60 (1.10-2.00)	2.00 (1.38-3.05)	0.074
	Post-natal	0.85 (0.60-1.38)	1.55 (1.05-3.18)	0.110
TNF (pg/ ml)		0.003#	<0.001#	
	1 <sup>st</sup> Trimester	1.25 (1.00-1.80)	1.20 (0.98-1.60)	0.36
	2 <sup>nd</sup> Trimester	1.50 (1.05-1.90)	1.20 (0.98-1.50)	0.39
	3 <sup>rd</sup> Trimester	2.00 (1.38-2.48)	1.70 (1.40-2.48)	0.70
	Post-natal	1.60 (1.10-2.40)	1.40 (1.20-2.00)	0.38
CRP (mg/L)		<0.001#	<0.001#	
	1 <sup>st</sup> Trimester	3.25 (1.45-4.99)	8.43(3.87-13.1)	0.001
	2 <sup>nd</sup> Trimester	3.38 (1.47-5.61)	8.58 (4.23-12.6)	0.002
	3 <sup>rd</sup> Trimester	3.11(1.54-4.16)	4.80 (3.01-12.6)	0.028
	Post-natal	1.02 (0.69-2.26)	4.76 (1.48-11.2)	0.003
IL-10 (pg/ml)		0.51#	0.33#	
	1 <sup>st</sup> Trimester	1.10(0.80-1.53)	1.00 (0.83-1.10)	0.19
	2 <sup>nd</sup> Trimester	1.10 (0.90-1.45)	0.95 (0.83-1.20)	0.055
	3 <sup>rd</sup> Trimester	1.10 (0.98-1.60)	1.00 (0.80-1.20)	0.86
	Post-natal	1.20 (0.80-1.60)	1.10 (1.00-1.50)	0.69
sICAM-1 (ng/ml)		<0.001#	0.029#	
	1 <sup>st</sup> Trimester	147 (125-164)	176 (143-206)	0.003
	2 <sup>nd</sup> Trimester	178 (151-228)	204 (175-244)	0.020
	3 <sup>rd</sup> Trimester	170 (138-193)	174(151-207)	0.20
	Post-natal	159 (138-203)	205 (146-223)	0.085
sVCAM-1 (ng/ml)		<0.001#	<0.001#	
	1 <sup>st</sup> Trimester	281 (230-314)	318 (260-354)	0.10
	2 <sup>nd</sup> Trimester	347 (323-387)	355 (334-386)	0.54
	3 <sup>rd</sup> Trimester	371 (342-394)	377 (334-428)	0.71
	Post-natal	307 (253-381)	305 (262-354)	0.46
vWF (mU/ml)		<0.001#	<0.001#	
	1 <sup>st</sup> Trimester	852 (658-1187)	769 (502-1319)	0.84
	2 <sup>nd</sup> Trimester	1407 (854-1982)	1615 (1053-2012)	0.27
	3 <sup>rd</sup> Trimester	2309 (1744-3009)	2497 (1877-3209)	0.79
	Post-natal	667 (511-840)	602 (504-930)	0.82
PAI-1 (ng/ml)		<0.001#	<0.001#	
	1 <sup>st</sup> Trimester	12.2 (8.5-17.6)	5.9 (13.1-17.3)	0.010
	2 <sup>nd</sup> Trimester	36.9 (32.4-73.1)	34.5 (26.8-40.0)	0.15

	3 <sup>rd</sup> Trimester	67.0 (52.3-103.1)	72.5 (51.3-123.6)	0.59
	Post-natal	8.6 (6.0-10.1)	11.5 (6.2-18.3)	0.049
PAI-2 (ng/ml)		<0.001#	<0.001#	
	1 <sup>st</sup> Trimester	36.5 (23.5-72.3)	18.5 (13.8-33.7)	0.001
	2 <sup>nd</sup> Trimester	312 (232-417)	314 (269-445)	0.79
	3 <sup>rd</sup> Trimester	491 (369-710)	450 (346-648)	0.48
	Post-natal	1.6 (0.5-4.1)	1.0 (0.4-3.7)	0.65

TNF is a cytokine indicative of the Th1 response whereas IL-6 is a cytokine indicative of the Th2 response. A ratio of TNF /IL-6 was calculated to indicate the extent of the switch from the Th1 response to the Th2 response in pregnancy (Figure 3-2). Lean women had a higher TNF /IL-6 ratio in the first trimester which declined in trimesters two and three and then rose in the post-natal period. In obese women the TNF /IL-6 ratio did not change throughout pregnancy or in the post-natal period ( $p=0.26$ ) and was significantly lower in obese than lean women at all time points apart from trimester three.

**Figure 5-3. Plasma TNF /IL-6 ratio throughout pregnancy and in the post natal period.** Means and SE are shown. Ratios were significantly higher in the lean compared to the obese at all time points apart from trimester 3.



#### ***5.2.4 Relationships between endothelial function and markers of inflammation.***

In a multivariate analysis using the general linear model, time of sampling and categorisation as lean or obese contributed to both endothelial-dependent and endothelial-independent microvascular function. However time of sampling had most impact on endothelial-independent function (18.5% [adjusted sum of squares expressed as a percentage of total means squared],  $p < 0.001$  for SNP response; 9.8%,  $p < 0.001$  for ACh response) and obesity had most impact on endothelial dependent microvascular function (1.7%,  $p = 0.046$  for SNP response; 19.3%,  $p < 0.001$  for ACh response). The association between inflammatory and soluble markers of endothelial function, independent of trimester, was tested in a general linear model. None of the markers listed in Table 3-2 predicted variability in endothelial-independent microvascular function. However endothelial-dependent microvascular function was predicted by IL-6 (10.7%,  $p < 0.001$ ), CRP (7.1%,  $p < 0.001$ ), IL-10 (2.0%,  $p = 0.043$ ) and sICAM-1 (5.0%,  $p = 0.001$ ). When these markers were entered into a multivariate model together with time of sampling, time of sampling (11.2%,  $p < 0.001$ ), IL-6 (4.0%,  $p = 0.002$ ) and IL-10 (2.4%,  $p = 0.018$ ) were

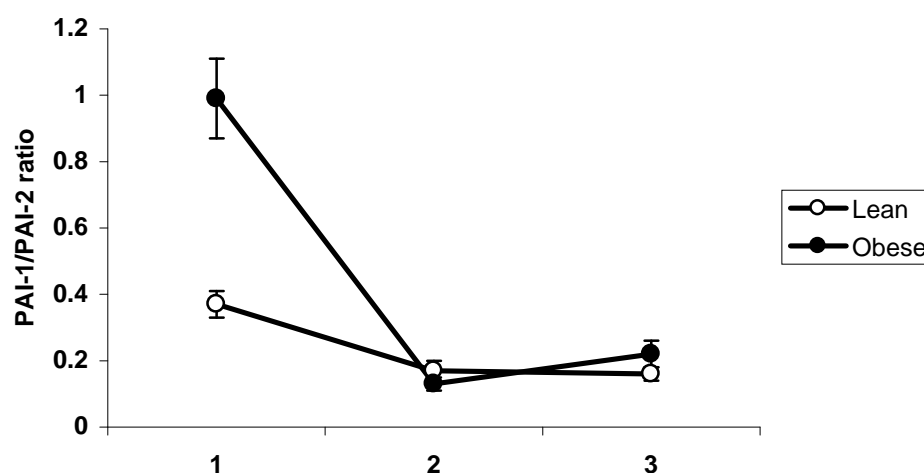
significant independent contributors to variation in endothelial-dependent microvascular function. To test whether these inflammatory markers might explain the effect of obesity on endothelial-independent function, whether subjects were lean or obese was also entered in the multivariate model. IL-6 and IL-10 were no longer significant and obesity explained 6.8% ( $p < 0.001$ ) of the variability in endothelial-dependent microvascular function.

### ***5.2.5 Index of placental function***

PAI-1/PAI-2 ratio has been proposed as an index of placental function (293, 294). PAI-1/PAI-2 ratios during pregnancy are shown in Figure 3-3 for lean and obese women. In the first trimester obese women had a significantly higher PAI-1/PAI-2 ratio indicating poorer placental function at this early time point (obese median [IQ range] 0.87 (0.54 – 1.21) vs. lean 0.30 [0.21 – 0.47],  $P < 0.001$ ). There were no differences in PAI-1/PAI-2 ratio in the second and third trimester. The higher ratios seen in obese women in the first trimester predominately reflect the lower PAI-2 levels in trimester one rather than a higher PAI-1 level (Table 3-2). Factors contributing to PAI-1/PAI-2 ratio, PAI-1 and PAI-2 variability in the first trimester were analysed in a multivariate model including first trimester vWF, sICAM-1, sVCAM-1, IL-10, IL-6, TNF and CRP. First trimester body mass index (7.6%,  $p = 0.012$ ), first trimester IL-10 (8.2%,  $p < 0.001$ ) and first trimester sVCAM-1 (0.73%,  $p = 0.007$ ) contributed to first trimester PAI-1/PAI-2 ratio. When consider separately, PAI-1 levels were explained partly by first trimester BMI (11.8%,  $p = 0.012$ ) and first trimester vWF (8.9%,  $p = 0.028$ ) whereas PAI-2 levels were explained by IL-10 alone (16.9%,  $p = 0.001$ ).



**Figure 5-4 PAI-1/PAI-2 ratios with advancing gestation.** Means (standard error) are shown. Ratios were significantly higher in the obese compared to the lean in the first trimester only.



## 5.3 Discussion

We examined both *in vivo* microvascular function and markers of endothelial activation prospectively throughout pregnancy in healthy lean and obese women. Microvascular endothelial-dependent vasodilatory responses were found to be lower at all time points in obese subjects compared with lean counterparts and obesity had more impact on endothelial-dependent than endothelial-independent microvascular function in multivariate analysis. These observations provide evidence of the detrimental effect of obesity on endothelial-dependent microvascular function, and that the effect is sustained throughout

pregnancy. Impaired endothelial-dependent microvascular function was demonstrated using a well tolerated non-invasive, *in vivo* method of assessment, suitable for use in pregnant patients. Although laser Doppler imaging combined with iontophoresis assesses the cutaneous microcirculation, in this case the forearm, the technique has been demonstrated to be a valid surrogate for evaluating function in other vascular beds. Impaired cutaneous responses to iontophoretically administered ACh are observed in patients with hypercholesterolaemia (295) and diabetes mellitus (210, 296). Assessment of endothelial function in peripheral vessels has also been shown to correlate directly to dysfunction within the coronary vasculature (281). The current findings expand our earlier observations of third trimester microvascular endothelial dysfunction in obese individuals (139). Microvascular endothelial dysfunction has also been demonstrated in type 2 diabetes, a condition strongly linked with obesity (296, 297).

Endothelium-independent vasodilatation was also significantly less in the obese women but the difference was of small magnitude. These data support the claim that obesity imparts vascular dysfunction by impacting not only directly upon the endothelium but also further downstream at the level of the smooth muscle. However, the smaller magnitude of difference between the two groups in SNP response in comparison to ACh response may suggest endothelial-dependent dysfunction to be the more important consequence of obesity. Multivariate analysis indicated that time of sampling during pregnancy had a large impact on endothelial-independent microvascular function. This observation might suggest that there are key changes in vascular smooth muscle cell biology associated with pregnancy *per se*.

During pregnancy, maternal peripheral blood lymphocytes secrete more Th2 cytokines and less Th1 cytokines than non-pregnant individuals (298). Furthermore trophoblast, decidua, chorionic and amniotic membranes can all act as a source of Th2 cytokines (285). Overall this leads to a change in the balance from Th1 to Th2 response that is typical of pregnancy (285). We have previously shown that obese women have higher third trimester plasma levels of the Th2 cytokine IL-6 and of CRP, an inflammatory mediator whose production is induced by IL-6, in obese compared to lean pregnant women (139). We expand this observation in the current study and show that plasma CRP levels are higher in obese compared to lean pregnant women at all time points measured and that plasma IL-6 and sICAM-1 levels are higher in early pregnancy in the obese. First trimester CRP was significantly higher than *post-natal* levels ( $P=0.001$ ) indicating a very early increase of CRP levels associated with pregnancy, consistent with the report of elevated CRP levels at 4 weeks' gestation (299).

We used TNF /IL-6 ratio as a guide to the relative contributions of the Th1 and Th2 response during pregnancy in the lean and obese individuals. In lean pregnant women we clearly see a high ratio in trimester 1, indicating more contribution from Th1 response. The ratio declines in the second and third trimester indicating a switch to a greater contribution from the Th2 response (Fig 2). In the post-natal period the ratio switches back to a greater Th1 contribution. Interestingly the obese women have a lower ratio than the lean women at all time points, apart from the third trimester, and the response to pregnancy is level. This suggests that the obese women are already predominately showing a Th-2 response and that there is no "Th1/Th2 switch" in pregnancy. It has been proposed, at least for preeclampsia, that an increase in maternal inflammatory response might provoke endothelial dysfunction (300). Using a multivariate model we found that none of the measured markers of

inflammation or endothelial activation explained the endothelial-independent microvascular response. However endothelial-dependent microvascular function was predicted by IL-6 and IL-10 levels, an effect which was lost if presence of obesity was added into the model. This suggests that the excessive Th2 response observed in obese pregnant women is linked to their endothelial-dependent vascular dysfunction.

To our knowledge we have demonstrated for the first time the dynamics in PAI-1/ PAI-2 ratio with advancing gestation among lean and obese women. PAI-1/PAI-2 ratios were similar in later pregnancy (second and third trimester) in lean and obese women. However the PAI-1/PAI-2 ratio was hugely elevated (2.7 fold,  $p=0.001$ ) in obese women in the first trimester. This was predominately due to the approximately two-fold lower first trimester PAI-2 levels in the obese women in the presence of higher first trimester PAI-1 levels in the lean. This raises the interesting possibility that, in the first trimester, placental growth/function is impaired in the obese pregnant woman but that a recovery in growth/function is achieved by the second trimester. In order to investigate possible mediators of this effect we carried out multivariate analysis of potential predictors of first trimester PAI-1 and PAI-2 levels. First trimester IL-10 levels predicted PAI-2 levels independent of BMI although this may merely indicate that placenta is a quantitatively important site of IL-10 production. It is possible that first trimester expression of a predominately Th2 response in obese individuals has adverse consequences for establishment of the placenta. An abnormally raised PAI-1/PAI-2 ratio also predates the onset of preeclampsia (301) a condition characterised by abnormal placentation and widespread endothelial dysfunction (187, 190, 302). Obesity is a significant risk factor for preeclampsia. Perhaps in preeclampsia, this early compromised placental growth/function

demonstrated in obese women persists among those who develop preeclampsia, while it recovers in those women who do not.

In conclusion we have employed a well-tolerated, non-invasive technique for the assessment of endothelial function longitudinally throughout pregnancy. We have demonstrated reduced endothelium-dependent and -independent vasodilation among obese mothers when compared to lean counterparts. We observed a significantly higher PAI-1/PAI-2 ratio in the first trimester in obese women; however these women went on during pregnancy to improve their PAI-1/PAI-2 ratio to levels comparable to their lean counterparts. We provide evidence that the increased risk of preeclampsia associated with obesity can be explained in part by chronic pre-existing endothelial activation and impairment of endothelial function secondary to increased production of inflammatory Th-2 cytokines. We also hypothesise that early impaired placental growth/function is characteristic of obese pregnancy and if not recovered may further explain the elevated risk of hypertension and preeclampsia among obese mothers.

## **Chapter Six**

## **Conclusion.**

## **6 Conclusion**

### **6.1 Obesity in pregnancy**

The mechanisms by which obesity influences pregnancy and its outcomes are poorly understood. Examining the physiological changes experienced during pregnancy and how these changes are influenced by maternal obesity will provide further insight into the mechanism by which obesity imparts an increased risk of adverse maternal and fetal outcome.

### **6.2 Obesity and metabolic syndrome**

The metabolic syndrome is a spectrum of metabolic abnormalities, commonly observed in the centrally obese, associated with insulin resistance. It is manifest as relative hyperglycaemia, hyperlipidaemia, and disturbance of coagulation. In the non-pregnant, central obesity and the metabolic syndrome are key factors underlying cardiovascular disease and type 2 diabetes (86, 87). An increase in plasma FFA concentrations in normal subjects, to levels comparable to those in the obese, also results in the induction of oxidative stress, inflammation, and vascular dysfunction, in addition to causing insulin resistance (94). Markers of inflammation such as C-reactive protein, interleukin-6, and raised white cell count have also been found to be independent predictors of cardiovascular events and diabetes.

## 6.3 Metabolic changes in pregnancy

The normal physiological response to pregnancy represents a time when several components of the metabolic syndrome are acquired: a relative degree of insulin resistance, a gestational hyperlipidaemia, and increased coagulability. Normal pregnancy also involves up-regulation of the inflammatory cascade and an increase in white cell count. Thus pregnancy provides a unique opportunity to look at temporary metabolic and inflammatory disturbances. There is a lack of knowledge within the literature with regard to the direct influence of obesity on metabolism during pregnancy. Most studies look at a limited number of metabolic parameters or involve the third trimester only. We hypothesised that the type of metabolic change experienced during pregnancy is linked to adverse outcome. If this is the case then the influence of obesity on the extent of metabolic adaptation during pregnancy may explain the impact of obesity on adverse pregnancy outcome. Furthermore the timing of alterations in particular metabolic changes may be related to whether adverse outcomes are manifest in early or late pregnancy.

This thesis attempts to address the lack of detailed knowledge on the impact of obesity on pregnancy metabolism. A longitudinal study is presented to establish the timing and degree of perturbation of a wide range of metabolic and physiological parameters. We wished to identify whether there are early changes in metabolism that may link with early adverse events and later changes in metabolism that may relate to late adverse outcomes. It was necessary to not only study the degree of metabolic change, but also the timing of such changes.



## 6.4 Metabolic changes and vascular function

Successful adaptation of vascular function both at the level of the maternal systemic circulation and at the placenta is imperative for healthy pregnancy. In early pregnancy placentation and the establishment of a healthy maternal-fetal circulation is necessary in order to avoid early miscarriage. Suboptimal invasion of trophoblasts and insufficient remodelling of the maternal spiral arteries leads to a dysfunctioning vascular network within the placenta resulting in poor placental perfusion and intrauterine growth restriction (IUGR). The poor placental perfusion can evoke the release of factors which, on exposure to the maternal circulation, give rise to preeclampsia. Preeclampsia is a multisystem disorder, exclusive to pregnancy, in which systemic endothelial dysfunction, platelet aggregation and reduced placental perfusion is the underlying pathology. The origins of preeclampsia are thought to lie at least partly within the placentation process a view supported by the prompt recovery post partum and the increased incidence among those with hydatidiform moles or multiple pregnancies. Thus the establishment of the placental circulation can also influence fetal and maternal outcome in later pregnancy.

Maternal vascular endothelial function is known to improve with advancing gestation. This improvement in endothelial function is necessary to accommodate the increased vascular volume as well as the increased coagulability present during pregnancy. The functional adaptation of the maternal cardiovascular system to meet the requirements of the mother and the growing fetus is a prerequisite for a successful pregnancy. Endothelial dysfunction is thought to contribute to maternal thrombosis a condition which is among one of the leading causes of maternal mortality in the UK. Adverse events such as these occur among women of all BMI values. By examining the degree of metabolic change among obese women when compared to lean, and the resultant effect on maternal endothelial function

with advancing gestation, further insight into the mechanisms by which these women are more susceptible to pathology may become evident.

Clinical studies report that the presence of vascular dysfunction is a significant predictor of future cardiovascular events (151, 152). The vast majority of evidence to date indicates that microvascular endothelial function is impaired in the early stages of atherosclerosis and it is this endothelial dysfunction which is an important factor leading to atherosclerosis and its complications. An increasing number of studies have demonstrated the correlation between dyslipidaemia, inflammation and vascular dysfunction (157). (171). By drawing parallels with the field of cardiovascular research we hypothesised that the metabolic changes experienced by the obese mother may adversely affect vascular function in pregnancy and this could constitute a mechanism by which obesity is associated with adverse pregnancy outcome.

## ***6.5 Metabolic changes in pregnancy***

### ***6.5.1 Early Gestation***

This thesis describes the metabolic changes that occur in pregnancy and relates the degree of change to obesity. With advancing gestation we observed that some metabolic changes occur early in pregnancy while others occur late. Specifically we observed that CRP was elevated in the first trimester as compared to the post partum period. We also found CRP levels to be significantly higher among obese mothers as opposed to lean at all time points. Our data and those of others suggest that there is indeed an inflammatory response present early in the first trimester. From as early as 6 weeks' gestation, there are increased numbers

of circulating monocytes (303), while in first trimester decidua there is a massive influx of pro-inflammatory macrophages and natural killer (NK) cells (304). In the first trimester, raised CRP levels have been reported (305) and Sacks *et al* provided evidence of a mild systemic maternal inflammatory response from as early as 4 weeks' gestation (299). The interaction between the invading trophoblast and maternal inflammatory cells is a key stage of early pregnancy and may be a factor in some cases of miscarriage. This is interesting in the context of our observation that CRP levels are higher in obese mothers who are more prone to early miscarriage. It is possible that an exaggerated inflammatory response as early as four weeks' gestation could indicate a failing pregnancy.

It is important to recognize that adipocytes and myofibers produce proinflammatory cytokines,  $\text{TNF}\alpha$  and IL-6, in response to antigen challenge. When the immune system is challenged, an important flow of energy is required. As a result, cytokines TNF are recruited to induce lipolysis [1] and to mobilize energy. Conversely, when adipose tissue stores are depleted, energy sparing becomes a priority, even at the cost of imposing a decrease in the level of immunologic surveillance. As stated, these cytokines have a notable history of inducing insulin- and growth hormone-resistance in adipose tissue and skeletal muscle. Both adipocytes and macrophages within fat secrete numerous hormones and cytokines that may contribute to the characteristic pathophysiologic changes seen in the metabolic syndrome, and local inflammation within adipose tissue may be the sentinel event that causes systemic insulin resistance and systemic inflammation, two of the cardinal features of the metabolic syndrome. Pregnancy as described throughout this thesis physiologically resembles the metabolic syndrome. In the obese mother the excess adipocyte secretion of inflammatory markers may account for the development of obstetric complications.

### **6.5.2 Late gestation**

Most evidence regarding the inflammatory response is from the third trimester, and investigators have postulated that an abnormal inflammatory response causes pregnancy complications such as pre-eclampsia (306) or preterm labour (307). CRP levels are raised in women with pre-eclampsia (308), in women with ruptured membranes complicated by chorioamnionitis (309) and in women who develop preterm labour (307). Women with higher CRP levels at 9–13 weeks are more likely to develop gestational diabetes mellitus (310) and pre-eclampsia (311). Higher CRP levels are associated with increasing BMI (312) an observation consistent with the present study. Furthermore we observed that endothelial function is influenced by changes in type 2 inflammatory cytokines in our study women. Thus the greater degree of inflammatory response observed in obese women may explain the higher risk of preeclampsia, GDM and other late pregnancy complications in obese pregnancy.

Obesity was associated with late gestation LDL III concentrations in our study. LDL III is an easily oxidised lipoprotein that is highly atherogenic and may provoke endothelial dysfunction. We observed LDL III levels to rise in all individuals between the first and second trimester. However with advancement into the third trimester maternal BMI had a direct influence on the behaviour of LDL III concentrations. Those mothers with BMI values in the lowest tertile experienced a fall in LDL III concentration in the third trimester while those mothers with BMI values in the middle tertile have LDL III concentrations that plateau in the third trimester. Most strikingly, those women with BMI values in the highest tertile experienced a further significant increase in LDL III concentration in the third trimester of pregnancy. It is possible that this elevated LDL III concentration in the

maternal circulation of the obese mother contributes to the increased susceptibility of late complications of pregnancy such as pre eclampsia and gestational diabetes.

## **6.6 The impact of obesity on metabolism throughout pregnancy**

When comparing lean and obese not only did we observe a difference in the relative concentrations of lipoproteins such as LDLIII, with advancing gestation we also found that maternal BMI had a direct influence on the degree of total changes in lipid concentration as pregnancy advanced. We observed that obese women started pregnancy with already elevated lipids such as triglyceride and cholesterol and as pregnancy advanced their lipids increased. Interestingly we found that lean women experienced a much more extreme change in lipid profile, from a lower baseline, with advancing gestation. Lean women started pregnancy with lower lipid levels than their obese counterparts but by the end of pregnancy their lipid levels had increased dramatically to levels similar to those of the obese women. Thus lean women exhibited large dynamic response to pregnancy while the response among the obese women was somewhat attenuated largely because they start from a higher baseline value.

Human metabolism needs to be highly adaptable in order to cope with the impact of the physiological changes associated with pregnancy. This adaptability requires a clear ability to utilize lipids and carbohydrate fuels and to switch from one energy source to another(313). This cellular capacity is termed “metabolic flexibility” and is impaired in obesity(314).. In obese individuals this relative metabolic inflexibility is manifest in a range of metabolic pathways and tissues including the impairment of early phase post

prandial insulin secretion coupled with failure to suppress hepatic glucose production and NEFA efflux from adipose tissue. Among the obese there is a failure of skeletal muscle to appropriately move between use of lipid in the fasting state and use of carbohydrate in the insulin stimulated post prandial state. Impaired transition from fatty acid efflux to storage in response to a meal is also evident among obese (314). It has become increasingly clear that reduced capacity for fuel usage in, for example, skeletal muscle, is characteristic of the metabolic syndrome and a fundamental component of metabolic inflexibility. We have observed an analagous phenomenon in pregnancy whereby lean women are highly adaptable to the metabolic changes required for pregnancy whereas obese women have a much reduced ability to respond. In a similar way to lean individuals being metabolically flexible in response to the stimulus of a meal we observed that lean mothers are more flexible in their response to pregnancy. One might speculate that the obese mother is less able to mobilise fats from stores in order to provide nutrition to her developing fetus.

It is also interesting to note that in our study lean mothers demonstrated a clear Th1/Th2 switch in response to pregnancy whereas obese mothers did not. We used TNF /IL-6 ratio as a guide to the relative contributions of the Th1 and Th2 response during pregnancy in the lean and obese individuals. In lean pregnant women we clearly saw a high ratio in the first trimester, indicating more contribution from Th1 response. The ratio declined in the second and third trimester indicating a switch to a greater contribution from the Th2 response. In the post-natal period the ratio switched back to a greater Th1 contribution. It is tempting to speculate that the flexibility of physiological adaptation to pregnancy may extend beyond metabolic responses to inflammatory response. Furthermore endothelial-dependent microvascular function was predicted by IL-6 and IL-10 levels in a multivariate statistical model, an effect which was lost if presence of obesity was added into the model. This suggests that the excessive Th2 response observed in obese pregnant women is linked

to their endothelial-dependent vascular dysfunction. Thus metabolic inflexibility in obese pregnancy may extend to inflexibility of the inflammatory adaptive response and to an inadequate vascular adaptation. As discussed earlier, a failure to make adequate vascular adaptations to pregnancy could result in both early (miscarriage, IUGR) and late gestation (preeclampsia, GDM) adverse pregnancy outcomes.

## **6.7 The impact of obesity on placental function**

To our knowledge we have demonstrated for the first time the dynamics in PAI-1/ PAI-2 ratio with advancing gestation among lean and obese women. PAI-1/PAI-2 ratio was hugely elevated in obese women in the first trimester, predominately due to lower PAI-2 levels. PAI-1/PAI-2 ratios were similar in later pregnancy in lean and obese women. This raises the interesting possibility that, in the first trimester, placental growth/function is impaired in the obese pregnant woman (as indicated by low PAI-2 levels) but that a recovery in growth/function is achieved by the second trimester. It is possible that first trimester expression of a predominately Th2 response in obese individuals has adverse consequences for establishment of the placenta. An abnormally raised PAI-1/PAI-2 ratio is also known to predate the onset of preeclampsia (301) and obesity is a significant risk factor for preeclampsia. Perhaps in preeclampsia, this early compromised placental growth/function demonstrated in obese women persists among those who develop preeclampsia, while it recovers in those women who do not. Whether the impact of obesity relates to vascular development in the placenta or to trophoblast invasion is worthy of further investigation.

## 6.8 Consequence of obese pregnancy for the offspring

Metabolic changes experienced during pregnancy are now known from this study to be quite different throughout the pregnancy for lean women as compared to obese. For example the area under the lipid.gestational time curve is higher in obese women; 3<sup>rd</sup> trimester LDLIII levels are high in obese women and in general the maternal metabolic response to pregnancy is less flexible in obese mothers. Since there are differences in both the concentrations and the quality of metabolites circulating in maternal blood we would hypothesize that the nutrient mixture delivered to the fetus via the placenta would also be affected. Hence we propose that the offspring of lean and obese pregnancies experience different *in utero* environments.

The issue of fetal programming is an area widely debated. Pettit's long-term follow-up studies of the Pima Indians showed that a diagnosis of diabetes during gestation significantly increases the risk of both adolescent obesity and glucose intolerance, in contrast with that of children of the same woman when her glucose tolerance was normal during gestation (93). Montgomery et al looked at health scores of 7 to 16 year olds (315). The data was correlated to rates of exposure to cigarette smoking in utero. Montgomery concluded in utero exposure due to smoking during pregnancy may increase the risk of both diabetes and obesity through programming, resulting in lifelong metabolic dysregulation, possibly due to fetal malnutrition or toxicity. Smoking during pregnancy may represent another important determinant of metabolic dysregulation and type 2 diabetes in offspring (315).

One of the most interesting clinical observations in the past decade has been the association between low birthweight and hypertension, coronary-artery disease, and non-insulin-dependent diabetes (NIDDM) in adult life (316-318). Hattersley et al propose that the



association between low birthweight and adult insulin resistance is principally genetically mediated. Genetically determined insulin resistance could result in low insulin-mediated fetal growth in utero as well as insulin resistance in childhood and adulthood. Low birthweight, measures of insulin resistance in life, and ultimately glucose intolerance, diabetes, and hypertension, would all be phenotypes of the same insulin-resistant genotype. Central to this fetal insulin hypothesis is the concept that insulin-mediated fetal growth will be affected by fetal genetic factors that regulate either fetal insulin secretion or the sensitivity of fetal tissues to the effects of insulin. The fetal insulin hypothesis offers an alternative explanation for the consistent association between impaired fetal growth and insulin resistance during life and the link with hypertension and vascular disease. Both genetics and the fetal environment are likely to be important in determining fetal growth, in the same way as both genetic and environmental influences are important in adult disease susceptibility.

Barker et al suggest undernutrition at different stages of pregnancy leads to phenotypes characterised by low birthweight, or low birthweight relative to placental weight, or thinness at birth, or shortness at birth with subsequent failure of infant growth. Each of these phenotypes is associated with a particular pattern of metabolic abnormalities in adult life. The abnormalities may depend on the different timing of the undernutrition and its different effects on organs and tissues according to their stage of development. This group went further to suggest that adaptation by the fetus exposed to periods of under nutrition change the body's physiology, structure, and metabolism, they may lead to cardiovascular disease in later life.

Catalano et al question whether or not obesity begets obesity (245). We have reported an increased CRP concentration among obese women at all time points. Pregravid obesity

represents a state of chronic inflammation. On the basis of these observations, one could hypothesize that increased maternal pregravid obesity is a state of increased insulin resistance and inflammation as estimated by CRP. During gestation, obese women make available increased nutrients to the fetus, resulting in increased placental growth in early gestation and increased somatic growth, particularly fetal adipose tissue in late gestation. The worldwide epidemic of adolescent and adult obesity may not only be a result of our lifestyle of inadequate activity and poor diet; it may also be propagated and enhanced at a much earlier stage in life because of an abnormal metabolic milieu *in utero* during gestation

In pregnancy the fetus is reliant on placental transfer of fatty acids from the mother to support growth. These fatty acids are used both as a source of energy and as building blocks for membranes. In chapter five we examined a full maternal erythrocyte fatty acid profile assessed at each trimester throughout pregnancy and at 4 months post-partum. Many short chain and long chain fatty acids were shown in our study to increase with advancing gestation. The 41% increase in maternal erythrocyte 18:3n3 during pregnancy observed by our group is most likely due to its role as a precursor for 22:6n3 synthesis. DHA (22:6n3) and nervonic acid (24:1n9) are important in neural development, as a structural component of membranes and for myelination respectively. Increased mobilisation of these fatty acids for fetal development was correlated indicating the importance of the fetal fatty acid *in utero* environment for the development of the brain.

Palmitoleic acid, 16:1n7 was also increased during pregnancy and concentrations of this fatty acid correlated negatively to booking BMI and waist circumference. This introduces the concept that the quality of fatty acids delivered to the placenta for transfer to the fetus can be influenced by obesity. Essential fatty acids are important for membrane synthesis and

membrane composition can influence membrane functions such as insulin signalling. Enrichment of cell membranes with LC n-3 PUFA can improve insulin sensitivity via a number of mechanisms. An increase in unsaturation index of cell membranes, particularly in muscle, has been associated with insulin sensitivity (319) (320) probably by affecting molecular insulin signalling. Changes in fatty acid composition may also affect insulin resistance by acting as ligands for peroxisomal proliferator activated receptors (PPARs). PPARs regulate expression of a wide array of genes involved in lipid metabolism and inflammation (321). LC n-3 PUFAs also have important roles in inflammation. EPA can displace arachidonic acid, a precursor of prostaglandins and leukotrienes, from cell membranes reducing inflammatory response. EPA can also be metabolised to anti-inflammatory mediators (322). Maternal insulin resistance has been related to membrane phospholipid fatty acid composition in breast-fed children, suggesting that maternal metabolism may impact on the degree of insulin resistance in the offspring (323). Furthermore, growth-restricted animals have a composition of 22:6 n-3 in skeletal muscle membrane very close to that of Pima Indians, supporting the relevance of membrane composition in programming (324).

## 6.9 Future Research

We have raised a lot of questions which pave the way for future research. Firstly we plan to examine metabolic differences between lean and obese pre pregnancy and in early gestation. Recruitment of women from an assisted conception programme would facilitate very early gestation blood sampling. The impact of these factors could then be correlated to both early and late pregnancy clinical outcomes for both the mother and the fetus. By establishment of the pre pregnancy factors critical to improvement of clinical outcome for

pregnancy education and pre treatment criteria could be developed to ensure the maximum chance of success in treatment and the avoidance of both maternal and fetal pathology in alter pregnancy. Intervention studies looking at the impact of pre pregnancy weight loss or lifestyle changes as well as the impact of lifestyle changes or dietary improvement during pregnancy would expand further our knowledge of the reversibility of the adverse influences of obesity on both maternal and fetal health.

A further aspect of our work will involve the examination of PAI 1/PAI 2 ratios, along with other markers of placental function (hCG and placental size), from day zero of pregnancy. Placental function will be assessed in those who have a continuing pregnancy and among those who miscarry thus providing a further insight into the mechanisms of first trimester miscarriage. A detailed comparison of erythrocyte fatty acid composition among lean and obese women, particularly in early gestation, is the next stage for our work in this field. As the fetus is reliant on the mother for placental transfer of fatty acids for growth and development by examining the influence of maternal BMI on red blood cell membrane composition we can perhaps add further dimension to the maternal issues involved in fetal programming.

We have collected adipose tissue from the abdominal fat of women who during the study underwent caesarean section for obstetric reasons. We also collected placental tissue from a high percentage of women involved in the study at delivery. Fetal blood was collected from the umbilical cord. This collection allows us to explore the molecular mechanisms, such as gene expression in both maternal adipose tissue and placental tissue, in lean and obese women. By establishing the placental gene expression for fatty acid transfer proteins for example, we would be able to ascertain molecular influences of maternal obesity on placental function. The maternal influence on the fetal biochemical environment will also

be examined in order to take further the issue of fetal programming. The long term follow up of the children from these pregnancies would also be an extremely interesting study and allow us to establish further the influences of the *in utero* environment on future health.

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