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The Impact of Maternal Obesity and Gestational Diabetes Mellitus on Adipose Tissue and Placental Derived Adipocytokines

Dr. Kavitha Sivakumar

Submitted to the University of Warwick

Faculty of Medicine

For the Degree of Doctor of Medicine

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

Declarations

I declare that all the work presented in this thesis, except where specifically stated, and was original research performed by myself under the supervision of Dr Manu Vatish & Dr Harpal Randeva. None of this work has been previously submitted for any other degree. All sources of information have been acknowledged by means of reference. Excerpts from papers on which I am first author are included within this thesis. These are reproduced with kind permission from The Journal of Clinical Endocrinology & Metabolism K.Sivakumar et al 2013 Elevated Fetal Adipsin/Acylation-Stimulating Protein (ASP) in Obese Pregnancy: Novel Placental Secretion via Hofbauer Cells. J Clin Endocrinal Metab 2013 Oct; 98 (10): 4113-22. Also excerpts from papers on which I am joint first author are included within this thesis. These are also reproduced with kind permission from PLoS One; Kavitha Sivakumar et al 2013 Lower Cerebrospinal Fluid/ Plasma Fibroblast Growth Factor 21 (FGF21) Ratios and Placental FGF21 Production in Gestational diabetes. PLoS One.2013 Jun 3; 8(6):e65254

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Abstract

Pregnancy; a natural insulin resistant state; becomes exaggerated when complicated by obesity and gestational diabetes (GDM). Both obesity and GDM are associated with severe maternal and fetal complications as well as with increased risks of obesity in the offspring later in life. Little work has been performed on the levels of adipokines in lean, obese and diabetic pregnancy. This study aimed to explore the roles of three adipokines; namely, Adipsin, Acylation stimulating protein and Fibroblast Growth Factor-21, all of which are involved in insulin resistant and dysmetabolic states such as obesity and type 2 DM. We hypothesized that these adipokines might play a role in pregnancy. A cohort of Caucasian pregnant women undergoing elective caesarean section was studied. Clinical parameters were assayed as well as circulating maternal and fetal levels of adipsin, ASP and FGF21. Paired samples of fat and placental tissue were taken for explant studies to measure secreted Adipsin, ASP and FGF21 levels. Cord levels of adipsin and ASP were significantly elevated in the offspring of obese and diabetic mothers compared to their lean controls. Plasma FGF21 levels were significantly higher in GDM compared to lean controls. FGF21 levels in cerebrospinal fluid (CSF) were also measured and a CSF/Plasma ratio calculated.

I have identified the human placenta as a source of adipsin, ASP and FGF21. More specifically, I have shown that placental Hofbauer cells (macrophages) produce adipsin and ASP. This is the first time secretion of adipsin and ASP by Hofbauer cells has been demonstrated. I conjecture a role of these macrophages in lipid metabolism at the materno-fetal interface. Also, I describe that GDM mothers have higher CSF FGF21 as compared to controls but the CSF:plasma ratio of FGF21 was lower in GDM mothers, potentially suggesting an alternative reason for and contributing to hyperglycaemia in GDM.

Abbreviations

Commonly used abbreviations and those not defined when they first appear within the text are listed below.

Complement components:

Nomenclature for human complement components is based on,

Human Complement components are previously reported in Complement nomenclature (World Health Organisation, 1968) and

alternative pathway nomenclature (World Health Organisation, 1981).

AT	Adipose Tissue
ASP	Acylation Stimulating Protein
BMI	Body Mass Index
BSA	Bovine Serum Albumin
BBB	Blood Brain Barrier
CHD	Coronary Heart Disease
CSF	Cerebrospinal fluid
C3adesArg	Complement 3a desarginase
cDNA	complementary DNA
CTs	cytotrophoblasts
DNA	Deoxyribonucleic acid
DGAT2	Diacylglycerol acyltransferase -2
EIA	Enzyme Immuno Assay
ELISA	Enzyme Linked Immuno Sorbent Assay
Factor D	Adipsin
FGF	Fibroblast Growth Factor
EFA	Essential Fatty Acids
FFM	Fat-Free Mass
FFA	Free Fatty Acid
GDM	Gestational Diabetes Mellitus
GLUT	Glucose transporter translocation
HDL-C	High Density Lipoprotein Cholesterol
HSL	Hormone Sensitive Lipoprotein lipase

HBCs	Hofbauer cells
HOMA-IR	Homeostasis Model Assessment- Insulin Resistance
IADPSG	International Association of Diabetes and Pregnancy study Groups
ICAM-1	Intracellular adhesion molecule
IDDM	Insulin Dependent Diabetes Mellitus
IL-6	Interleukin-6
IL-1Ra	Interleukin-1 Receptor antagonist
IR	Insulin resistance
IVF In	Vitro fertilization
IUGR	Intrauterine Growth Restriction/Retardation
kDa	KiloDalton
LDL-C	Low density Lipoprotein Cholesterol
LOD	Limit of detectable dose
LPL	Lipoprotein lipase
mRNA	messenger Ribonucleic Acid
MCP-1	Monocyte Chemotactic Protein
MDD	Minimum detectable dose
NEFA	Non Esterified Fatty Acid
NIDDM	Non Insulin dependent Diabetes mellitus
NTD	Neural Tube Defects
NHLBI	The National Heart, Lung and Blood Institute
OGTT	Oral Glucose Tolerance Test
PAI-1	Plasminogen Activator Inhibitor-1
PBS	Phosphate Buffered Saline
TNF-α	Tumor Necrosis Factor-α
Type 2 DM	Type 2 diabetes mellitus
TG	Triacylglycerol
TBS	Tris Buffered Saline
TGL	Triglycerides
WAT	White Adipose Tissue
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1. Introduction

Obese pregnancy is evolving as a major risk for both mother and child. Obesity has been noted as a significant risk factor for maternal death as more than fifty percent of all women who died reported as being obese according to the Confidential Enquiry into Maternal and Child Health., 2009 (Confidential Enquiry into Maternal and Child Health., 2009).

The adverse outcomes from maternal obesity and maternal diabetes impact both the early and long-term fetal health and have been documented by numerous studies. A principal complication on offspring from obese pregnancy and maternal diabetes is fetal macrosomia. Many reports have showed that intrauterine exposure either to hyperglycemia or excessive nutrients might increase the risk and thus tend the offspring to develop obesity and/or else diabetes at the later life. Gestational diabetes and maternal obesity are the two main factors have been associated with insulin resistance as well as inflammation which are regulated by several adipokines and cytokines (Ategbo et al., 2006). In general, obesity is linked with hyperlipidemia and increased adiposity (Dandona et al., 2004). Low grade inflammation has been reported to have an association with insulin resistance, obesity and type 2 diabetes mellitus (Dandona et al., 2004). Accordingly, it suggests that inflammation may possibly adapt insulin resistance in gestational diabetes.

1.2 Definition of Obesity

Obesity is a state of imbalance between calories ingested versus calories expended which can lead to excessive or abnormal fat accumulation (Morton, 2005). The most universally used technique of body fat is Body mass index (BMI) which is defined as weight in kilograms (Kgs), divided by the square of height in meters (m^2).

1.3 Classification of Obesity

Obesity is generally classified based on Body Mass Index. BMI between 25 and 30 kg/m^2 is overweight; BMI greater than or equal to 30 kg/m^2 is obesity and BMI greater or equal to 40 kg/m^2 is extreme obesity. World Health Organization (WHO) and The National Heart, Lung and Blood Institute (NHLBI), Classified the body mass index (World Health Organization. Division of Noncommunicable Diseases. and World Health Organization. Programme of Nutrition Family and Reproductive Health., 1998), as

Underweight	<18.5
Normal	18.5–24.9
Overweight	25.0–29.9
Obesity class I	30.0–34.9
Obesity class II	35.0–39.9
Obesity class III	40+

1.4 Prevalence

In the last 20 years, obesity rates have increased dramatically all over the world. The World Health Organization in 2005, reported that as a minimum of 400 million adults were found to be obese of BMI >30 with a predictable rise to above 700 million by 2015 (World Health Organization. Office of Health Communications and Public Relations., 2006). In developed countries, obesity has become a major public health issue and recently, over 1.1 billion of the population in the world were classified as obese (World Health Organization. Office of Health Communications and Public Relations., 2006, World Health Organization., 2009). In UK, it has been reported that nearly 24% of all women are obese and 34% are overweight

(Confidential Enquiry into Maternal and Child Health., 2009). In UK, there are about 50 % are found to be overweight or obese in women of reproductive age group and for about 15 years from 1990 to 2004, there has been an increase of 60% of obesity in pregnancy (Heslehurst et al., 2007). In Ireland, about one in five pregnant women who registered in antenatal clinics is found to be obese (Fattah et al., 2009). Around one-third of women of reproductive age were classified as obese in the USA (World Health Organization., 2009) and similarly in the UK, 32% of women aged between 35and 64 years were found to be overweight (BMI 25–30) and 21% were obese (Prescott-Clarke et al., 1998). In the developing world, obesity is dramatically increasing particularly in urban (World Health Organization. Office of Health Communications and Public Relations., 2006).

There are inadequate statistics in pregnant women regarding the incidence of obesity. The cohort studies in the UK have reported that there are increased levels of obesity comparable to the population as a whole (Kanagalingam et al., 2005, Usha Kiran et al., 2005, Heslehurst et al., 2007, Kim et al., 2007). The underlying aetiology of obesity is multi factorial with more calories consumed than exhausted which results in energy imbalance. Obesity is linked with an extensive range of medical complications like diabetes (Vazquez et al., 2007), cardiovascular disease (Romero-Corral et al., 2006), dyslipidaemia and hypertension (Brown et al., 2000), cancer (Wolk et al., 2001) and osteoarthritis (Lievense et al., 2002). In UK and other Mediterranean countries, the prevalence of overweight among children age between 5-11 years has increased significantly from steady level to nearly 30% (1980-2000) (Lobstein and Frelut, 2003, Stamatakis et al., 2005, Wang and Lobstein, 2006).

1.5 Pro-inflammatory state of Obesity

Obesity are linked with the tissue expansion of adipose tissue due to the lipids accumulation into adipocytes (Weyer et al., 2002, Sartipy and Loskutoff, 2003, Samad et al., 1996, Samad and Loskutoff, 1996, De Pergola and Pannacciulli, 2002). These expanded adipose tissues produce cytokines such as IL-6, resistin, TNF-α, PAI-1 and MCP-1, which are considered to be proinflammatory (Weisberg et al., 2003, Xu et al., 2003). These cytokines has effects on the endothelium locally which leads to the synthesis of intracellular and vascular cell adhesion molecule which in turn increases vascular permeability of monocytes, and chemokines like MCP-1 (Cinti et al., 2005). A state of local and systemic insulin resistance is induced by the production of proinflammatory cytokines by the inflammatory signal which is improved by crosstalk among endothelial cells, resident macrophages and adipocytes (Bruun et al., 2003). The inflammatory signal in adipose tissue is intiated by the lipids accumulation in adipocytes as well as by resident macrophages.

1.6 Obesity and insulin resistance

Obesity is defined as a chronic low grade inflammation which has an important relationship between obesity and insulin resistance. For many years, adipocyte and metabolic disturbances has been linked between insulin resistance and obesity. Initially, adipose tissue was considered as a storage organ of excessive triglycerides. But recently, this view has altered and adipose tissue has revealed to produce a wide variety of cytokines which influences both metabolic and physiological functions (Halberg et al., 2008). Due to excessive triglycerides storage in obesity which leads to adipogenesis in turn results in hypertrophy as well as hyperplasia of adipocytes (Avram et al., 2007), which in turn results in cellular dysfunction leading to

adipokines dysregulation, increased FFA release and inflammation. The excessive FFA leads to fat accumulation in different areas like liver, heart, pancreatic cells and skeletal muscle (de Ferranti and Mozaffarian, 2008). Excessive ectopic fat deposition in skeletal muscle increases peripheral insulin resistance by reduced insulin mediated glucose uptake (Boden, 2006). The elevated FFA in liver leads to reduced extraction of insulin, which leads to systemic hyperinsulinaemia and altered gluconeogenesis (Boden, 2006). The excessive FFA in pancreatic cells contributes still further to the relative insulin deficiency state (Lupi et al., 2002). Obesity in pregnancy has definite effects on glucose metabolism with a decrease in fasting glucose in early gestation and with considerable increase in hepatic insulin resistance and in peripheral tissues (Mills et al., 1998, Sivan et al., 1997). Moreover, women with obesity are liable to risk of 4 times to have gestational diabetes and morbidly obese about 9 times than lean women (Chu et al., 2007a). These increased risks are due to multifactorial mechanisms, and has effects on insulin signaling similar to non-pregnant women with high BMI (Catalano et al., 2002).

1.7 Effects on adipocytes in obesity

The adipocytes enlarge in obesity, with adipose tissue consecutively undergoing cellular and molecular alterations that result in altered systemic metabolism. The fasting free fatty acid and release of glycerol from adipocytes is found to be higher in obese women compared with women with normal BMI which induces insulin resistance (Horowitz and Klein, 2000). Increased release of free fatty acids are shown to induce insulin resistance in muscle (Shulman, 2000) and this is due to alterations in the expression of key phosphoproteins, perilipins. Perilipins are found in adipocytes on the surface of triacylglycerol droplets which acts as gatekeepers and

prevents the lipases from hydrolyzing triacylglycerol to allow FFAs release (Zhang et al., 2003). Even though the fat cells are larger in obesity, they were to found to be perilipins deficient and their basal rate of lipolysis are increased (Wang et al., 2003). The numbers of macrophages in adipose tissue also increase with obesity (Weisberg et al., 2003). Macrophages are found to be responsible for the production of most cytokines in adipose tissues of obese individuals (Weisberg et al., 2003, Xu et al., 2003) and with increase in obesity these macrophages are found to scavenge moribund adipocytes (Cinti et al., 2005).

1.8 Pregnancy

Pregnancy is a natural inflammatory state with leucocytes activation and increased concentration of cytokines and acute phase reactants, systemically (Klover et al., 2003). When it is complicated by preeclampsia and gestational diabetes, there is an exaggeration of physiological inflammatory state, returning to baseline levels after delivery (Pradhan et al., 2001, Ridker et al., 2000, Wake and Walker, 2004). This exaggerated physiological inflammatory state results in an increased release of the inflammatory products by the placenta and thus these micro particles are released into the systemic circulation which are detaching from the syncytial surface of the placenta (Dandona et al., 2004, Kern et al., 2001, Dandona et al., 1998). The inflammatory changes in pregnancy are mediated mainly in the placenta which regulates both adaptive and innate immune responses.

The macrophages which are produced from hematopoietic cells in fetus are identified in the placenta in the very early stage of development (Vozarova et al., 2001, Wakabayashi, 1998) which exhibit cytokines and immunoregulatory molecular

profile to macrophages in adipose tissue; however, their precise role in the placenta is not well understood.

1.9 Pregnancy and insulin resistance

Pregnancy is normally linked with distinct changes in metabolism of glucose and insulin resistance (IR) to aid stipulation of fuel substrate for the growing fetus. There is an increase in the secretion of insulin in early pregnancy, whilst insulin sensitivity is stable or even more slightly enhanced (Catalano et al., 1993, Catalano et al., 1999). The insulin mediated utilisation of glucose decreases by 40-60% and increases in insulin secretion as pregnancy progresses to facilitate euglycaemic state in mother (Catalano et al., 1991, Catalano et al., 1992). The primary glucose disposal site is considered to be the skeletal muscle, but in the latter half of gestation, there is severely insulin resistant in the adipose tissue. During late gestation, the insulin decreases lipolysis, which leads to larger postprandial increases in free fatty acids, amplified gluconeogenesis and insulin resistance (IR) (Homko et al., 1999). Pregnancy is physiologically associated with insulin resistance and hyperinsulinaemia, which predispose vulnerable women to develop diabetes in pregnancy. This insulin resistance is predominantly prominent in the second half of gestation, with increased insulin resistance subsequently contributing to the development of gestational diabetes (GDM).

1.10 Role of lipids in pregnancy

Adaptations of maternal lipid metabolism throughout the pregnancy plays a major role in the growth of fetus. The altered maternal lipid metabolism which occurs normally during the gestational period are the lipids accumulation in maternal tissues (Hytten and Robertson, 1971, Villar et al., 1992) and the development of maternal hyperlipidemia (Alvarez et al., 1996, Montelongo et al., 1992). The common conditions which are known to alter these two manifestations by impairing maternal fat deposition such as diabetes or hypothyrodism during first trimester have been shown to affect growth of fetus at late trimester even with the treatment during the second trimester (Bonet and Herrera, 1991, Martin and Herrera, 1991). The development of fetus are maintained by metabolites crossing the placenta from those nutrients present in maternal circulation. The most important nutrient which crosses the placenta is glucose, subsequently amino acids and fetal developmental directly depends on their availability (Herrera et al., 1985, Hay, 1994, Sibley et al., 1997). Lipids also play an important role in the fetal development but knowledge about lipid transfer to placenta are very inadequate (Herrera, 2002). The altered availability of lipid components are known to affect the fetal development (Herrera, 2002). These findings have proven that the most important role of lipid metabolism in pregnancy is on fetal growth and outcome, inspite of the lack of clear knowledge about lipid transfer across the placenta.

1.11 Maternal Hyperlipidemia

Maternal hypertriglyceridemia is an important distinguishing feature during late gestation (Knopp et al., 1992). The maximum increase in triglycerides in plasma directly corresponds to VLDL triglycerides, which are synthesized in the liver

(Alvarez et al., 1996). In addition to their enhanced production in the liver (Wasfi et al., 1980), and their removal from the circulation is decreased, as a result of decreased activity of LPL in adipose tissue further contributes to their increase levels in plasma (Martin-Hidalgo et al., 1994). The increases in both plasma estrogen levels and insulin resistance occuring during late gestation are two main hormonal factors responsible for these changes in metabolism that results in the development of maternal hypertriglyceridemia. The insulin resistance which is normally present during late gestation contributes to enhanced lipolytic activity of adipose tissue, which results in the production of NEFA as well as glycerol to the liver and their conversion into triglycerides which are then released back into the circulation as VLDL (Ramos and Herrera, 1995). The increase in estrogen levels throughout gestation contributes to maternal hypertriglyceridemia (De Hertogh et al., 1975).

1.12 Role of Maternal hypertriglyceridemia in fetus

Pregnancy is characterized by an increase in the concentration of lipids which present throughout pregnancy. The concentrations of plasma triglycerides and cholesterol increase by 30-50% and 200-400% respectively. In maternal obesity, the hyperlipidemia is increased further with higher triglycerides and VLDL concentrations, lower HDL cholesterol, LDL and total cholesterol concentrations in the serum appear similar than lean pregnant women (Merzouk et al., 2000, Ramsay et al., 2002). Triglycerides do not crosses the placental barrier directly (Herrera, 2002), although the essential fatty acids which are obtained from diet intake by mothers are transported as triglycerides rich lipoproteins in maternal plasma (Herrera, 2002). The plasma lipoproteins in maternal triglycerides are then hydrolysed and carried up by the placenta and then reesterified as fatty acids

(Coleman and Haynes, 1987). After hydrolysis of glycerides, the fatty acids are diffusely released to fetal plasma, where they are bind to α-fetoprotein, a specific oncofetal protein (Benassayag et al., 1997). These fatty acids are then rapidly transported to liver in fetus and reesterified, released back as triglycerides into fetal circulation. The maternal hypertriglyceridemia plays an important role in the availability of esssential fatty acids (EFA) to the fetus. Kitajima et al (Kitajima et al., 2001) has found that there exists a direct association between maternal triglycerides in plasma and newborn bodyweight. Moreover, a reduction in maternal hypertriglyceridemia could be achieved with the treatment of hypolipidemic drugs which in turn resulted in negative drawbacks in the development of fetus (Hrab et al., 1994, Soria et al., 2002).

1.13 Maternal obesity and inflammation

Pregnancy and obesity are related with changes in insulin resistance and inflammation (Hotamisligil and Spiegelman, 1994). Due to obesity, the placenta has exaggerated inflammatory response because of the structurally similar inflammatory transcriptomes of the adipose tissue and placenta (Kern et al., 2001). Obesity is a low grade inflammatory condition in nonpregnant women which increases the pro-inflammatory production from the macrophages in the adipose tissue (Kern et al., 2001, Zhang et al., 2002). When the pregnancy is complicated by maternal obesity and GDM, the placenta releases cytokines as well as adipocytokines (Bruun et al., 2003, Hotamisligil and Spiegelman, 1994, Kern et al., 2001, Zhang et al., 2002, Mohamed-Ali et al., 1997).

There are some changes in both biochemical markers and inflammatory cells in maternal obesity, which is considered to be a chronic as well as sub-acute inflammation state result in diseases like preeclampsia and gestational diabetes. There is a continuous undesirable stimulus in maternal obesity due to dysregulation of inflammatory, vascular and metabolic pathways by increased circulation of inflammatory molecules. There arises a question whether obesity in pregnancy induced inflammation in the placenta might play a pathogenic role in the development of pregnancy linked diseases. The characteristic of the inflammation arising in placenta is related to maternal obesity, which still needs further research.

1.14 Clinical Complications of Maternal Obesity in pregnancy

1.14.1 Early pregnancy

Maternal obesity has increased risk for spontaneous abortion (miscarriage) for both spontaneous and through assisted reproductive conceptions like in vitro fertilization (IVF). Maternal obesity is also related to an increased risk factor for congenital anomalies such as neural tube defects (NTD), spina bifida, congenital heart disease and omphalocele in the offspring (Catalano and Ehrenberg, 2006, Waller et al., 1994, Watkins et al., 2003, Rasmussen et al., 2008).

1.14.2 Late pregnancy

Maternal obesity is linked with an increased risk of developing pregnancy induced complications like hypertensive disorders of pregnancy, including preeclampsia (gestational proteinuric hypertension) (Weiss et al., 2004). The increase in risk of developing preeclampsia is two-fold with increase in BMI of 5-7 kg/m^2 (O'Brien et al., 2003). Maternal obesity has an increase risk with intrauterine fetal demise (stillbirth). Several studies has reported that pregnant women with obesity have an

increased jeopardy of stillbirth almost doubled than pregnant women with normal BMI (Chu et al., 2007b). Maternal obesity is highly related with high risk of developing both pre-gestational diabetes and gestational diabetes mellitus (Hedderson et al., 2008, Chu et al., 2007a).

1.14.3 Peripartum

Maternal obesity has an increased risk of developing complications during delivery and labour. As maternal BMI increases, the rate of vaginal delivery decreases progressively (Chu et al., 2007c). The rate of cesarean delivery for women with normal BMI was 18%, whereas it was 39.6% in morbidly obese women (Ehrenberg et al., 2002). Carroll et al study has revealed that attempted vaginal birth after cesarean (VBAC) success rate was 81.8% in women who weighed less than 200 lb versus with 57.15 in women who weighed 200-300 lb and it was 13.3% in women with more than 300 lb (Carroll et al., 2003).

There is also an increased risk of operative delivery for obese women which results in intraoperative complications like increased wound infections and thromboembolism (Soens et al., 2008). The cause of the increased rate of cesarean delivery in obese pregnant women is not clearly understood. However, Vahratian et al found that this increase in cesarean rate in obese pregnant women might be due to dysfunctional labour (Vahratian et al., 2004). Maternal obesity in first trimester were associated with increased risk of reduced rate of spontaneous labour at term which results in post term pregnancy and intrapartum complications (Denison et al., 2008)

1.15 Implications of maternal obesity on fetal/neonatal complications

1.15.1 Fetal Macrosomia-Adverse outcome of maternal obesity

Maternal obesity is highly associated with abnormal growth in the fetus. The fetal macrosomia which is a major outcome in maternal obesity, which is distinct with estimated fetal weight equal to or more than 4500g and found to be increased to two-three fold in pregnant women with high body mass index (Ehrenberg et al., 2004). Chu et al, in their meta-analysis reported that prevalence of fetal macrosomia were 14.6% and 13.6% for morbidly obese and obese women respectively versus 8.3% for normal weight pregnant women (Chu et al., 2007b). The babies of GDM mothers have increased fetal fat mass (Sparks, 1984, Catalano et al., 2003b), as do the babies of women who are obese but not diabetic (Chu et al., 2009, Sewell et al., 2006) suggesting that hyperglycaemia, might be an important driver for intrauterine growth.

There are however, some caveats to the hypothesis that hyperglycaemia alone acts as a driver for intrauterine growth. A large proportion of women with diabetes, who maintain excellent glycaemic control, continue to have a macrosomic fetus of about 3.5 times higher risk than the general population (Evers et al., 2002, Murphy et al., 2008), which appears to be a function of the pregravid BMI (Langer et al., 2005). This finding is highlighted when considering that an overweight woman with well controlled GDM has a 50% higher chance of having a macrosomic baby compared to a lean woman with well-controlled GDM.

Obesity therefore, independently of diabetes, predisposes to fetal macrosomia (doubling the risk without diabetes) (Langer et al., 2005). While it is known that the increase in fetal macrosomia represents an increase in fetal fat mass, how the maternal environment enhances this lipid deposition in fetal tissues, either in the absence of diabetes (as in obesity) or in well-controlled diabetes, is not completely

understood. Sparks et al (Sparks, 1984) has hypothesized that genetic factors is related to fat-free mass (FFM), whereas in utero environment may also related to fetal fat mass, in agreement with the fetal origins of disease hypothesis, the offspring of both GDM and obese women have increased risks of obesity and type 2 diabetes (Stothard et al., 2009, Garcia-Vargas et al., 2012).

1.16 Literature Review for Offspring

1.16.1 Barker hypothesis

In utero fetal programming hypothesis or Barker hypothesis (Barker, 1990), the birth size is related to developing disease in later life. The Barker hypothesis was originally studied on low birth weight. There are some studies revealed that high birth size may also have complications later in life. A relationship between maternal obesity during the first trimester and childhood obesity has been reported. Whitaker (Whitaker, 2004) reported that high risk of developing childhood obesity has been related with maternal obesity in the first trimester of pregnancy was 2.0 at 2 years, 2.3 at 3 years, and 2.3 at 4 years. There has been a direct association between the birth weight and body mass index in later life (Oken and Gillman, 2003).

When there is an insufficiency or surplus of nutrients supply to the placenta, the acquired adaptations of the fetuses may reflect change in physiology and metabolism permanently (de Boo and Harding, 2006). The root cause of various diseases that occur in later life, including hypertension, heart disease and non–insulin dependent diabetes might be due to these changes in fetal programming. Obesity may become a significant long term problem due to fetal programming. Daughters of obese mothers may be susceptible to obesity and further liable to have children with obesity who have again this susceptibility creating a vicious cycle.

1.16.2 "Metabolic Memory"

According to the fetal origin hypothesis, during pregnancy the fetal malnutrition affects fetal growth which leads to thinness at birth, metabolic syndrome and type 2 DM (Hales and Barker, 2001). Hales and Barker hypothesis has suggested that during the process of fetal development, there might be some long term impacts on fetus due to a stimulus, which was described previously as "fetal programming" (Hales and Barker, 2001), is now defined as "metabolic memory". In an intrauterine diabetic environment, the fetal programming is termed as "metabolic memory". Obesity in pregnancy has a relative high risk of developing obesity and diabetes in the fetus in adulthood. Moreover, pregnant women with gestational diabetes could possibly show evidence of greater fetal macrosomia than pregnant women with obesity (Cox, 1994). Both maternal obesity and GDM have their own obstetric complications.

This metabolic memory seems to cause physiological anomalies during gestational period which are liable for the diseases in fetus in later life such as obesity and type 2 DM allied with metabolic syndrome (Dorner and Plagemann, 1994). According to this metabolic memory phenomenon, Palinski and Napoli (Palinski and Napoli, 2002) have shown that maternal hypercholesterolemia is linked with increase in the formation of fatty streak in fetal arteries which enhances the childhood atherosclerosis (Palinski and Napoli, 2002). There exists a relationship between maternal and fetal cholesterol levels in human plasma in 5-6 month fetuses (Napoli et al., 1997, Vogel et al., 1997). Furthermore, maternal hyperglycemia might lead to fetal hyperglycemia has been found to stimulate pancreatic islets cells in fetus to produce fetal hyperinsulinemia (Schwartz and Teramo, 2000).

1.17 Studies on Maternal Obesity

During pregnancy, the association between obesity and inflammation has been detailed by a number of studies. Challier et al (Challier et al., 2008) found higher circulating pro-inflammatory cytokines, secondary to production of peripheral mononuclear cells in maternal blood, which has revealed higher production of TNF and IL6 mRNA expression. Ramsay et al. 2002 (Ramsay et al., 2002), reported that the leptin, and IL6 levels in serum were elevated in obese women compared with lean women. A recent study has found that the maternal obesity was associated with infiltration of macrophage into adipose tissue (Farley et al., 2009). Challier et al (Challier et al., 2008) studied that there is an increase in proinflammatory cytokines in peripheral blood mononuclear cells in pregnancy. Boomsma et al (Boomsma et al., 2006) study has revealed that there is a link between obesity in pregnancy and adverse pregnancy outcome and also found that intolerance in glucose and insulin resistance both would mediate adverse pregnancy outcome due to obesity. A study by Kirwan et al (Kirwan et al., 2002), reported that TNF was an interpreter of insulin resistance in lean pregnant women in late gestation, when compared to non-obese women with gestational diabetes.

Stewart et al studied that higher ICAM-1 and lower plasminogen activator inhibitor in serum of obese women compared with lean pregnant women (Stewart et al., 2007). Metzger et al (Metzger, 1991) has studied that women with polycystic ovarian syndrome has intolerance in glucose and insulin resistance. Ramsay et al and Martin et al (Ramsay et al., 2002, Martin et al., 2009), has studied that abdominal obesity are linked with glucose intolerance and insulin resistance, which results in gestational diabetes which is more common in pregnant women with high

BMI. Colomiere et al has identified the defects in the insulin-signaling cascade in obese pregnant women with normal glucose tolerance in both skeletal muscle and adipose tissue (Colomiere et al., 2009).

1.18 Studies on maternal obesity outcomes

There are numerous studies on maternal obesity outcome, which has long term implication on the offspring. Maternal BMI is positively correlated with childhood obesity (Jensen et al., 2003, Singhal and Lucas, 2004, Oddy et al., 2006). Danielzik et al (Danielzik et al., 2002) has reported the relationship between offspring BMI and paternal BMI has proved that it does not shown to be as strong as the maternal association. Burdette et al (Burdette et al., 2006) has shown that offspring of obese mothers have 0.54 kg more fat mass than offspring of non-obese mothers. Knight et al (Knight et al., 2007) showed that maternal BMI is related with offspring weight at birth, 1 year and 2 years and also shown that prepregnancy BMI correlated with offspring obesity during the first 2 years of life and after 1 year post-birth offspring obesity has been also correlated with paternal BMI. O'Callaghan et al (O'Callaghan et al., 1997) reported that pre-pregnancy maternal obesity and paternal obesity are self-determining predictors of severe obesity at 5 years.

Parson et al (Parsons et al., 2001) reported that pre-pregnancy maternal obesity might explain the relationship between birth weight and adulthood obesity. Whitaker et al (Whitaker, 2004) showed that the childhood obesity has more risk at 4 years was linked with maternal obesity during first trimester. Salsberry et al (Salsberry and Reagan, 2005, Li et al., 2005) has shown that pre-pregnancy BMI is related with an increased overweight odds ratio in the offspring. Blair et al (Blair et al., 2007) has also shown that maternal obesity results in offspring obesity. Lawlor et al (Lawlor et

al., 2007) has studied that pre-pregnancy maternal BMI has proved to be a high risk for offspring obesity than paternal BMI. Danielzik et al (Danielzik et al., 2002) has reported that childhood BMI has been significantly related with parental BMI, though a closer correlation was observed between maternal than paternal BMI.

Koupil and Toivanen (Koupil and Toivanen, 2008) also reported that maternal pre-pregnancy BMI was proved to be the strongest predictor of offspring obesity. The maternal IDDM which has diagnosed after has significantly high risk of developing childhood obesity and IDDM compared to the siblings born before the diagnosis of maternal IDDM (Dabelea et al., 2000). Similarly, the pattern of growth of fetus and infant adiposity mass at birth, 6 weeks and 6 months post partum was studied by Ay et al (Ay et al., 2009) and suggested that the obesity risk is established in fetal life in later life. Children of mothers with obesity are susceptible of developing obesity even if they are born with normal birthweight. Dabelea et al (Dabelea et al., 2000) has reported that obese women who delivers children of normal birth weight will become as overweight, obese or morbidly obese (88%) compared to children born to lean women (13%). These studies have suggested that pre-pregnancy BMI and gestational obesity might be a initial key step in a multifactorial process which extends from mother to the child.

1.19 Gestational diabetes (GDM)

Gestational diabetes can be defined as any degree of glucose intolerance with the first onset or recognition during pregnancy (Hillier et al., 2008, Callaway et al., 2006). Clinically, GDM manifests, when about 70% of fetal growth occurs in late gestation. Based on the screening and diagnostic criteria, the prevalence of GDM ranges from 1.3% to 19.9% (Simmons, 2011). An increase in maternal obesity is a

well-known risk factor for gestational diabetes. There is an increased risk of developing GDM in obese pregnant women than lean pregnant. An obese woman has three to ten times of higher rates of preexisting gestational diabetes mellitus and hypertension (Sebire et al., 2001, Kumari, 2001). In a meta-analysis study has shown that the risk of increasing gestational diabetes was 2.14-fold greater in pregnant women who were overweight, 3.56-fold greater in obese pregnant women and 9-fold greater in pregnant women with grade 3 obesity compared to lean pregnant women (Sebire et al., 2001).

The incidence between gestational diabetes and obesity has strong association. It is predictable that in the absence of obesity, the occurrence of gestational diabetes may fall by 50% approximately (Jensen et al., 2003). The risk of increasing gestational diabetes is strongly predisposed to preexisting maternal BMI (Weiss et al., 2004, Bo et al., 2004). In obese pregnant women, the fasting levels of insulin are found to be higher than non obese pregnant women (Catalano et al., 2003a). Insulin resistance is already higher in obese women before conception without any clinical symptoms (Catalano et al., 2003a). In obese pregnant women, insulin levels are increased in an attempt to maintain normoglycemia. However, this is not possible, as the usual increase in insulin resistance during pregnancy is overstated in obese women.

The risk of developing GDM in overweight women is 6.5 times higher than non-obese women. However, the incidence of GDM in obese women is 1.4–20 times to percentage between 6-11% (Galtier-Dereure et al., 2000, Glazer et al., 2004). The incidence of gestational diabetes in morbidly obese women is 9.5 % compared to 2.3% in nonobese pregnant women (Weiss et al., 2004). Similarly, the incidence of GDM is 24.7% in morbid obese women (Kumari, 2001).

In early pregnancy, the glucose levels in fasting state cannot be used to predict GDM (Bhattacharya, 2004). However, by measuring the fasting glucose concentrations in plasma in the second half of pregnancy, it is possible to identify GDM in 70% of women (Perucchini et al., 1999). Moreover, it is worthwhile to screen obese women in antenatal clinic during first visit for subclinical hyperglycemia. There is an increased jeopardy of developing preeclampsia in women with GDM. In this relationship, obesity is considered as a major contributing feature (Ostlund et al., 2004, van Hoorn et al., 2002).

A new criteria for the diagnosis of GDM based on Adverse Pregnancy outcomes (HAPO) and hyperglycemia study has been proposed by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) (Metzger et al., 2010). The diagnostic criteria for GDM is without prior glucose challenge, use of 75g oral glucose tolerance test (OGTT), when the glucose at fasting state is ≥5.1 mmol/L and /or when 1 hr postload glucose is ≥10.0 mmol/L and /or when 2 hrs postload glucose is ≥8.5 mmol/L (Metzger et al., 2010). The diagnostic criteria of WHO for GDM are glucose at fasting state is ≥7 mmol/l or 2 hr glucose ≥ 7.8 mmol/l (Alberti and Zimmet, 1998). However, to diagnose GDM, 1-hr postprandial plasma glucose test is considered as a perfect screening method with high sensitivity and specificity (Hidar et al., 2001). In obese and overweight women with gestational diabetes or impaired glucose tolerance, the outcomes in both maternal and fetal were not only poor but also in obese pregnant women, who have good criteria for normoglycemia following a 75 g oral glucose tolerance test (OGTT) has also poor outcomes (Jensen et al., 2003, Owens et al., 2010).

Gestational diabetes mellitus has considered carrying health risks for both the mother and the fetus (Coustan and Carpenter, 1998). Gestational diabetic women will return

to normoglycemic level after delivery and it is well recognized that glucose intolerance detected during pregnancy is a definite predictive of developing maternal type 2 DM in later life (Metzger, 1991, Kjos and Buchanan, 1999). GDM mothers have an increased possibility of developing Type 2 Diabetes mellitus in later life (Henry and Beischer, 1991).

1.20 Pedersen Hypothesis

The Pedersen Hypothesis proposes that in the poorly controlled maternal diabetes mellitus, both over nutrition as well as fetal hyperglycaemia will cause poor fetal outcomes, suggested that the extreme mobility of carbohydrate across the placenta would increase relative hyperglycaemia and in turns the compensatory hyperinsulinaemia in the fetus (Pedersen, 1971). It is now recognized to produce a twofold increase in developing macrosomia due to fetal hyperinsulinaemia (Pedersen and Molsted-Pedersen, 1978).

1.21 Fetal Macrosomia-main adverse outcome of GDM

Diabetes in pregnancy is well characterized by an increased glucose transport and other essential nutrients to the placenta from mother to the fetus which results in fetal macrosomia (Van Assche et al., 2001). Several studies have reported that either type 1 DM and type 2 DM (preexisting diabetes) or gestational diabetes (GDM) is related with fetal macrosomia (Evers et al., 2004, Giordano, 1990, Ategbo et al., 2006, Mitanchez, 2010, Dorner et al., 1987, Pettitt et al., 1988). Gestational diabetes and Type 2 DM are also linked with fetal macrosomia and diabetes in offspring (Dorner et al., 1987). The risk of developing diabetes in fetus is significantly higher when maternal type 2 diabetes is present rather than paternal diabetes (Dorner et al., 1987,

Pettitt et al., 1988). However, the risk of developing insulin resistance is much higher in children of GDM mothers than in children of mothers who develop diabetes after pregnancy (Pettitt et al., 1988).

The adverse outcome of maternal diabetes which is commonly seen is fetal macrosomia (Mitanchez, 2010). About 43% of GDM mothers will have a macrosomic history has been estimated by several human studies (Ategbo et al., 2006, (Grissa et al., 2007).

1.22 Alteration in lipid metabolism in GDM

In accordance with WHO (World Health Organisation) criteria, GDM could be detected by the oral glucose tolerance test and several human studies have revealed diabetes during the second or third trimester of pregnancy (Grissa et al., 2007). Several studies have revealed that both control pregnant women and GDM mothers had hypertriglyceridemia and hypercholesterolemia during pregnancy (Ategbo et al., 2006, (Grissa et al., 2007). Moreover, macrosomic babies had higher levels of serum total and free cholesterol as well as triglyceride compared with control babies (Ategbo et al., 2006, (Grissa et al., 2007, Toescu et al., 2004). These studies have revealed that diabetes in pregnancy and macrosomia will provoke an alteration in the metabolism of lipids.

1.23 Common adverse effects associated with maternal obesity and GDM

Obesity epidemic is common among children, adolescents, young adults and elderly adults (Guelinckx et al., 2008, Caballero, 2005). Subsequently, the number of pregnant women with high BMI is also increasing. The epidemic of obesity is explained by the high consumption of energy rich foods and a lack of physical

activity. Besides, there are many other factors which might explain the flare-up in the prevalence of obesity (McAllister et al., 2009). These additional factors work through genetic factors, reproductive behaviours and intrauterine environment, which is important for maternal obesity and gestational diabetes (McAllister et al., 2009). It is well understood that obesity is directly connected to high adiposity and hyperlipidemia (Dandona et al., 2004). However, besides peripheral adiposity, central fat is more linked to insulin resistance and is a predisposing factor to gestational diabetes (Carey et al., 1997, Knopp et al., 1992).

Recently, several studies have found that increase in weight gain during pregnancy in turn increases the birth weight of offspring, thus increases the possibility of developing obesity later in life. This is independent of genetic factors (Ludwig and Currie, 2010). Furthermore, maternal obesity was significantly related to complications on both mother and baby (Roman et al., 2011) and leads to gestational diabetes, pregnancy related hypertension and cesarean delivery and increase risks of developing stillbirth, macrosomia, hypoglycemia, shoulder dystocia, and jaundice (Roman et al., 2011). Moreover, recent studies have strongly supported occurrence of fetal macrosomia due to GDM (Mitanchez, 2010) and despite the consequences of diabetes status, obesity is an additional risk factor for further complications in pregnancy (Mitanchez, 2010). There are still some differences between GDM and maternal obesity, as GDM mothers have greater incidence of having fetal macrosomia even after treatment and greater risk of developing jaundice and neonatal hypoglycemia in the babies of GDM mothers than obese mothers, besides some women may have undiagnosed preexisting diabetes mellitus (Simmons, 2011).

1.24 Scientific background

1.24.1 Biology of Adipose tissue

Lipid is important as a nutritional depot, having hormonal role, and in providing structural support. Adipose tissue is the body's main fat depots, and is a highly active organ and contains adipocytes. The adipocytes are cells particularly adapted for fat storage, which help to avoid the negative metabolic effects of excess deposition of lipid in organs such as muscle, liver, and heart. The adipose tissue contains a connective tissue matrix, with numerous fibroblasts, mast cells, macrophages, and leukocytes (Fain, 2006). Numerous peptides and steroid hormones as well as the cytokines and chemokines, synthesized and secreted by adipose and non fat cells, which influence local and systemic physiology (Fain, 2006, Kershaw and Flier, 2004). Additionally, it functions as an endocrine organ (Kershaw and Flier, 2004) that causes pathology linked to obesity. It also stores and secretes preformed steroid hormones, which induce the conversion of biologically active hormones from their precursors, and also convert the active hormones to inactive metabolites. They also secrete numerous enzymes vital to steroid hormones (Kershaw and Flier, 2004).

1.24.2 Types of adipose tissues

In White adipose tissue (WAT), the triglyceride is stored; and lipids are mobilized for the other tissues for the systemic consumption for energy (Weisberg et al., 2003). WAT commonly presents as subcutaneous and abdominal depots; they are distinct in their physiologies, and actions in pathology (Xu et al., 2003). In contrast, brown adipose tissue, is thought to be involved in the process of heat production through the uncoupling of oxidative phosphorylation, called non-shivering thermogenesis. An integral membrane protein, named the uncoupling protein-1, located in the

mitochondrial inner membrane, regulates the thermogenic proton leak in brown adipose tissue (Krauss et al., 2005).

1.24.3 Cytokines

A large number of peptides have been secreted by white adipose tissue (WAT). They were initially well-defined as metabolic regulators, and were later found to adapt inflammatory processes, for example interleukin (IL)-6, IL-1 and its receptor antagonist (IL-1Ra), and leptin, adiponectin and tumour necrosis factor (TNF)-α (Hansson, 2005). These immuno-modulatory factors which are adipose derived, in which both the cytokines and chemokines play an important role in chronic vascular inflammatory process (Hansson, 2005). Consequently, the various peptides which are secreted by adipose tissue have acknowledged extensive attention.

More than 100 different cytokines are well recognized at this current period. Cytokines are identified to be implicated in many biological processes, together with growth, differentiation, immunity, inflammation, cell division and apoptosis which are classified as chemokines, haematopoietic factors, interleukins, interferons, and growth factors (Hansson, 2005). They are identified to be bioactive even at very low concentrations, for instance even 10% of receptor is adequate to activate a response (Oppenheim, 2001).

1.24.4 Adipokines

A large variety of proteins identified as adipokines are secreted in adipose tissue which controls diverse metabolic functions through various pathways like auto, para, or endocrine. Out of more than 50 adipokines identified, leptin and adiponectin have been extensively researched (Gavrilova et al., 2000).

1.25 ADIPSIN

1.25.1 Identification of Adipsin

Adipsin (ADIPocyte-trypSIN) was originally identified as a 28-kDa adipocyte secreted protease with homologous to complement D in human. Adipsin was first identified by Spiegelman et al (Spiegelman et al., 1983) in the mouse cultured adipocyte cell line (3T3-F442A) as mRNA on the basis of differentiation-dependent expression. Cook et al (Cook et al., 1985) identified adipsin as a novel serine protease. Adipsin, a serine protease or complement factor D, was described first as an adipocyte derived endocrine factor in 1986 (Min and Spiegelman, 1986). Later, it has been identified in murine 3T3-F442A adipocytes together with complement C3, D and B (all three important components of the alternative pathway) (Esterbauer et al., 1999, Peake et al., 1997). Adipsin protein was subsequently found in adipose tissue and reported as serine protease (Min and Spiegelman, 1986). It was subsequently found to be homologous to complement D, which plays as a key enzyme in the regulation of the alternative complement pathway (Rosen et al., 1989). Choy and Spiegelman et al have explained that there might be an association between the adipose tissue metabolism and activation of the alternative complement pathway (Choy et al., 1992, Choy and Spiegelman, 1996). Adipsin was first described as an adipokine (Cook et al., 1987) before being identified in muscle (Zhu et al., 1994), lung, peripheral nerves (White et al., 1992) and more recently in the murine placenta (Takeshita et al., 2010).

1.25.2 Role of Adipsin

The precise function of adipsin in adipose tissue remains still uncertain. Flier et al (Flier et al., 1987) that adipsin protein expression was severely impaired and also circulating adipsin protein was decreased in both acquired and genetic models of obesity in rodents. But in these "cafeteria models" of rats (which obtained obesity by overfeeding), the expression of adipsin showed only a little change (Flier et al., 1987) and suggested a possible role of adipsin in metabolic disorders like obesity. Moreover, the study about drug induced obesity and genetic (db/db,fa/fa and ob/ob) revealed that mRNA adipsin levels in adipose tissue as well as adipsin concentrations in plasma were found to be reduced (Choy et al., 1992). In contrast, in humans, adipsin levels were increased in a positive correlation with BMI. It is therefore possible that adipsin may play a role in adipose tissue lipid metabolism indirectly (Maslowska et al., 1999). There were several other adipose tissue derived complement components, which have considered previously unsuspected links between immunity and energy balance in addition to adipsin. Factor b as well as Complement C2, C3, C4, C7 has increased expression in omental than subcutaneous adipose tissue. Moreover, adipsin and components of classical pathway C1QB, C1R and C1S were expressed in both adipose tissues (Gabrielsson et al., 2003).

Napolitano et al has measured adipsin concentration in serum of both lean and obese humans, and found it to be elevated in circulating levels in serum of obese than lean individuals (Napolitano et al., 1994). The alternative complement pathway is activated which results in the interaction of complement C3 and adipsin, with activated factor B, resulting in the protein C3 cleavage into C3a and C3b and results finally in the desaggregation of C3a to produce C3adesArg- Acylation stimulating protein (ASP) (Cianflone et al., 1994). Interestingly, adipsin is the rate limiting enzyme in the formation of Acylation stimulating protein (ASP) (Baldo et al., 1993).

ASP is an adipose tissue secreted factor that manipulates the rate limiting step in synthesis of triacylglycerol (TG) in adipose tissue (Cianflone et al., 1994). In addition to this, ASP increases transport of glucose through translocation of glucose transporters (GLUT-1, GLUT-3 and GLUT-4) (Maslowska et al., 1999).

1.26 Acylation stimulating Protein

There has been an increasing interest in Acylation stimulating Protein (ASP), which is 76-amino acid protein, and found to be one of the controlling factors in adipose tissue function in modern years. ASP has been determined to be the complement C3 derivative and was first identified in the human plasma (Cianflone et al., 1989).

Complement C3 has been related to insulin resistance, obesity, dyslipidaemia and CHD (coronary heart disease). Complement C3 is cleaved to form ASP or Acylation stimulating protein or C3adesArg by a cascade of enzymes, which is mostly regulated by adipsin. ASP is a strong stimulator in the synthesis of triglyceride in adipocytes (Cianflone et al., 1994).

1.26.1 Identification of ASP

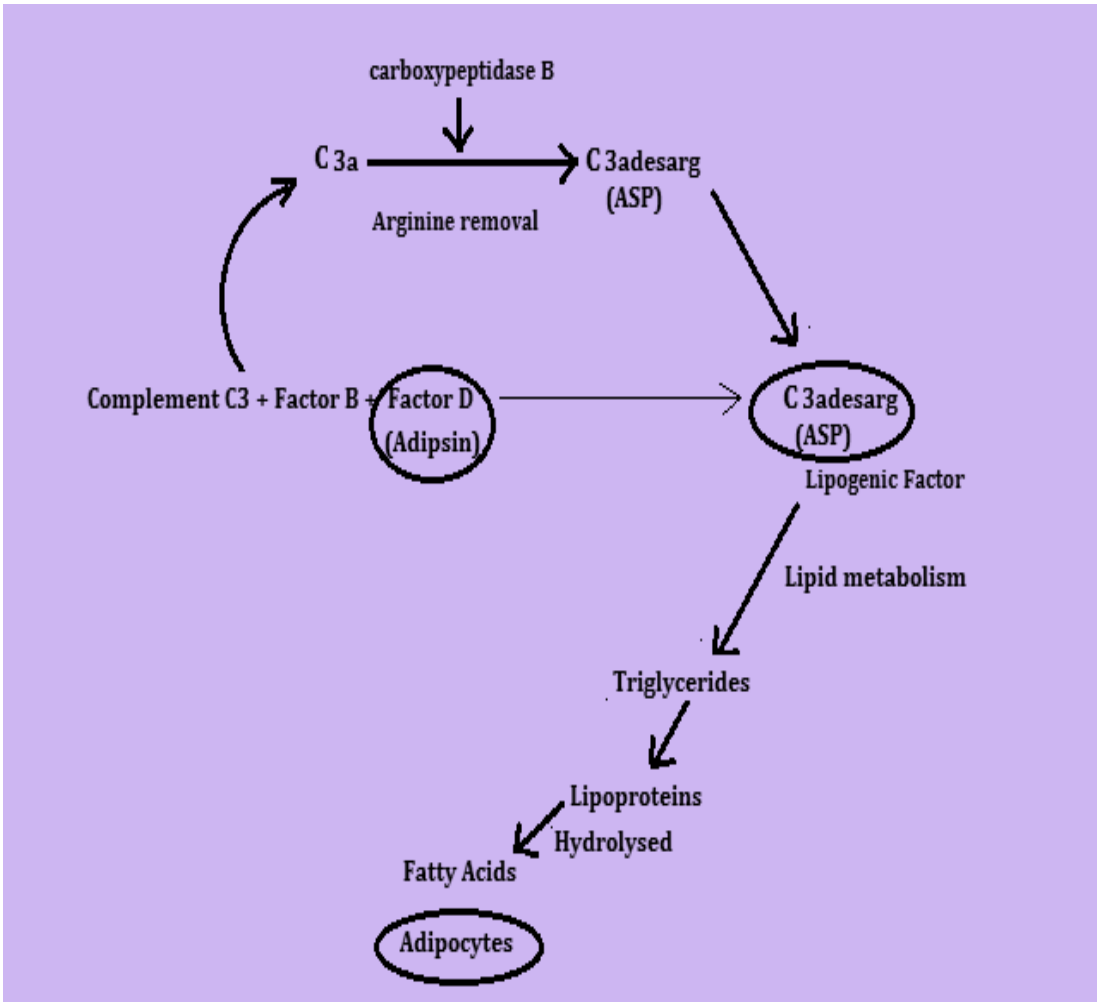
Acylation stimulating protein is 8.9 kDa hormone which is formed through an interaction of complement C3 in which arginine removal from activated C3 (C3a) by carboxypeptidase, C3a together with factor B and factor D (Adipsin) which forms C3adesArg in the adiocytes (Cianflone et al., 1994). ASP is formed through the interaction of complement C3 with factor B and factor D (adipsin) through a two steps (Fig 1.1)

1) Cleavage of complement C3 (parent protein) to produce C3a
2) Carboxyl terminus is desarginased to produce ASP (C3adesArg)

Several studies recently have reported that human fat cells have mRNA for complement factor D, a serine protease enzyme (Adipsin), cofactor B and C3, all of these components are necessary to generate C3a which is a precursor for ASP. In numerous studies, it has been studied that adipocytes express and also secrete mRNA for all three factors in a differentiation dependent manner (Cianflone et al., 1995b, Choy and Spiegelman, 1996, Peake et al., 1997). It has also been demonstrated that basal ASP is produced in the cultured adipocytes (Cianflone et al., 1995b, Choy and Spiegelman, 1996, Peake et al., 1997) with an increase in the ASP production after 7 days of adipocyte differentiation.

ASP is also produced in the alternate complement pathway by the interaction of complement C3, factor B and factor D (adipsin) and can also be produced by the classical complement pathway as well as the lectin pathway (Sniderman and Cianflone, 1994). Carboxypeptidase B is present in plasma at high levels and produced in many cells including fibroblasts. Complement C3a receptor has been identified recently (Ames et al., 1996, Crass et al., 1996) and cloned in human (Ames et al., 1996), mouse (Crass et al., 1996, Tornetta et al., 1997) and rat (Fukuoka et al., 1998). ASP is known to bind saturably specific to omental and subcutaneous adipocytes (Saleh et al., 1999), preadipocytes (Murray et al., 1997) and fibroblasts (Zhang et al., 1998) in murine and human cells despite a lack of C3a receptor protein or mRNA in adipose tissue (Saleh et al., 1999).

Fig.1.1 Formation of ASP (Acylation Stimulating Protein)



1.26.2 Actions of ASP

ASP has been identified to enhance the synthesis of triglyceride and storage in adipocyte in association with diacylglycerol acyltransferase-2 (DGAT2) stimulation and glucose uptake and inhibition of hormone sensitive lipoprotein lipase (HSL) through phosphodiesterase and released into the circulation (Cianflone et al., 1999, Sniderman et al., 2000, Van Harmelen et al., 1999) and thus ASP contributes to energy storage as lipids (Fig 1.1). This was confirmed in a study by Murray et al (Murray et al., 1999a) who showed that the administration of ASP to mice increases clearance of triglycerides from the plasma. It has also been confirmed that ASP deficient mice (C3-/-) have delayed clearance of lipids postprandially and have reduced depots in adipose tissue which are obesity resistant provoked by high fat diet (Murray et al., 1999b, Murray et al., 2000). While ASP increases lipogenesis in the adipose tissue through the activation of DGAT2, it also reduces lipoprotein lipase activity, thereby decreasing triglyceride breakdown (Yasruel et al., 1991, Van Harmelen et al., 1999), which results in ASP-driven enhancement of lipid storage in the adipose tissue (Cianflone et al., 1994, Millar et al., 2000). The concentrations of ASP in the human plasma in normal adults range from 10-58 nM which no related gender differences (Maslowska et al., 1999).

ASP release in adipose tissue increases at 4-5 hrs postprandially after intake of meal, but the circulating levels stay constant (Saleh et al., 1998). Studies have revealed that the ASP levels in plasma are increased in obesity (Weyer and Pratley, 1999) and reduced in fasting (Cianflone et al., 1995a) and in post-obese women (after bariatric surgery) (Faraj et al., 2001).

In human adipocytes and skin fibroblasts, ASP increases the synthesis of triglyceride by inducing the incorporation of fatty acid into adipose triglyceride (Cianflone et al., 1999). The triglyceride synthesis stimulation by ASP is more effective than insulin

and synergistic with the combined effect of insulin and ASP with re-esterification and fatty acid storage (Cianflone et al., 1994).

1.26.3 Identification of ASP receptor

Recently, the ASP receptor, C5L2, has been identified in ASP responsive cells (Kalant et al., 2005). Since the identification of the ASP receptor, the physiological role of ASP has drawn more interest on its research. Marcil et al (Marcil et al., 2006) has studied a French Canadian family and shown that a variant of the ASP receptor was associated with increased total cholesterol, low density lipoprotein-cholesterol (LDL-C), triglycerides, apoprotein B and acylation stimulating protein. These study subjects also shown a reduction of about 50% ASP stimulated triglyceride synthesis, glucose transport and significant reduction in ASP binding compared with the controls which suggested malfunction of receptor (Marcil et al., 2006). This hyperlipidemic milieu created in these dyslipidemic subjects suggested the hypothesis of ASP resistance might possibly due to receptor down regulation.

1.26.4 Role of ASP in Obesity

A study by Cianflone et al has demonstrated morbidly obese subjects have higher ASP in the fasting state but in contrast, the ASP levels increase more post prandially than in lean controls (Cianflone et al., 1995a). ASP levels decreased due to weight loss together with a decrease in ASP precursors (complement C3, factor B and adipsin) with weight loss (Muscari et al., 1990). Obese humans have 60-400% increase in ASP above normal and there is a reduction of ASP to normal levels in these obese subjects after a 4 week diet state (Kalant et al., 2000). This suggests that higher circulating ASP levels in obese might increase the storage of triglycerides,

while adipocytes in the obese remain as sensitive to ASP effects as in lean controls. However, ASP induced triglyceride synthesis is not only more effective than triggered by insulin, but also appears to be independent and synergistic with insulin action (Germinario et al., 1993). Interestingly, plasma adipsin and ASP levels are increased in states of insulin resistance and ASP levels are significantly increased in obese individuals (Cianflone et al., 2003, Weyer et al., 2000, Koistinen et al., 2001). These contribute to enhance storage of triglycerides in insulin impairment conditions (Zimmet et al., 1999, Faraj et al., 2008). Insulin resistance is a normal state in pregnancy and is linked with increase in plasma ASP concentrations in late gestation (Saleh et al., 2007).

1.26.5 Role of ASP in Diabetes

Maslowska et al has studied that the ASP synthesis enhances with increases in insulin and both ASP and insulin suppress lipolysis in fatty tissue (Maslowska et al., 2006). ASP also stimulates the uptake of glucose in adipocyte significantly *in vitro* studies (Maslowska et al., 2005, Cui et al., 2007). ASP secretion increased after a fatty meal in humans but the concentration of ASP remained constant after an oral administration of glucose. ASP levels were increased in obesity, nephrotic syndrome and in metabolic disorders with insulin resistance and dyslipidemia (Saleh et al., 1998). Obesity could be a bewildering factor in type 2 diabetes, as in these subjects there has been an increase in the levels of ASP, factor B, adipsin and complement C3 than non-diabetic controls of matched weight (Koistinen et al., 2001). In non-diabetic controls, ASP levels correlated with blood glucose and lipids but there was no such correlation in diabetic subjects might be due to a dysregulation in the production or metabolism of ASP (Koistinen et al., 2001).

1.26.6 Role of ASP in other metabolic disorders

Cianflone et al studied HyperapoB subjects and demonstrated that there has been a dysfunction of the ASP pathway and also has higher levels of ASP in plasma of those subjects (Cianflone et al., 1990). This might be probably due to decreased responsiveness to ASP at the adipose tissue resulting in a compensatory increase in ASP levels. The levels of C3 mRNA correlate with plasma NEFA, triglycerides and leptin and these factors might up-regulate the production of ASP in the insulin resistant state (Maslowska et al., 1997). There was little or no effect on the production of ASP with glucose, FFA, VLDL, LDL, and HDL, while insulin increases ASP production in a dose dependant manner (Maslowska et al., 1997).

1.27 Studies on Adipsin, C3, factor B and ASP in obesity and diabetes

There are numerous studies on Adipsin, complement C3, factor B and ASP which have examined the association between the precursor proteins like complement C3a, factor B and adipsin (factor D) and ASP in plasma. There are many interesting studies about ASP and C3, but there are only few studies on adipsin and factor B. Each of these proteins was related to be increased in obesity in many studies, even though not in all studies. For instance, the increase in ASP ranges from 58-400% in normal (Koistinen et al., 2001, Maslowska et al., 1999, Weyer and Pratley, 1999, Cianflone et al., 1995a), whereas the increases in C3 ranges from 11-25% normal (Pomeroy et al., 1997, Muscari et al., 2000, Scriba et al., 1979), the increases in adipsin ranges from 14-64% normal (Napolitano et al., 1994, Mavri et al., 1999, Maslowska et al., 1999, Alessi et al., 1995). These studies confirmed that there were significant increases in adipsin and C3 with the increases in ASP tending to exist higher. This is reliable with the hypothesis suggested that ASP represents an

important bioactive molecule and any changes in adipsin and C3 might reflect on the production of ASP. The link between obesity and complement C3 is not so new (Scriba et al., 1979). In obese Caucasians, ASP was increased more than in obese Pima Indians, besides BMI were compared between these two subjects (Weyer and Pratley et al., 1999). Plasma triglycerides and cholesterol was lower in obese Pima Indians, but insulin was higher (Weyer and Pratley et al., 1999) as was adipsin (Napolitano et al., 1994). The fasting complement C3 concentration , insulin and especially insulin sensitivity were found to be related to adiposity, insulin resistance and atherosclerosis (Weyer et al., 2000).

The complement C3, factor B, ASP and adipsin (factor D) have reported to be increased significantly in NIDDM (Type 2 DM) and IDDM (Type 1 DM). Obesity may be a confounding factor which is often associated with diabetes. When compared to non-diabetic groups of matched weight, ASP has been found to be increased (Koistinen et al., 2001(Ozata et al., 2001). The plasma C3 levels are increased in non-obese Type 1 diabetes and this is exacerbated by hypertension (Mantov and Raev, 1996). In nondiabetics, ASP has been linked with glucose and lipid metabolism but not in diabetics (Koistinen et al., 2001), which has been proved that in diabetes, metabolic disturbances has overcame the role of ASP which would explain the loss of association (Koistinen et al., 2001).

In obese subjects, ASP decreased with loss of weight (Muscari et al., 1990) and post obese women with normal weight had normal ASP levels (Faraj et al., 2001). Plasma C3 also decreased with loss of weight (Pomeroy et al., 1997, Pasquali et al., 1987, Fisler et al., 1982) and in anorexia nervosa (Pomeroy et al., 1997). Factor B and factor D (adipsin) have been found to be decreased with weight loss (Pomeroy et al., 1997, Mavri et al., 1999) and in anorexia nervosa (Napolitano et al., 1994,

Pomeroy et al., 1997) and improved to normal levels with weight gain in those subjects.

Despite the evidence that the adipsin-ASP pathway could contribute to increasing fetal fat mass (Saleh et al., 2008) neither adipsin and ASP function nor their levels in mothers and developing fetus have been investigated in the context of obese pregnancy and GDM pregnancy.

1.28 Leptin

1.28.1 Identification of Leptin

Leptin was the first adipocyte hormone identified which has a direct consequence on the hypothalamus and influences food intake (Halaas et al., 1995, Lee et al., 1996). Leptin is cytokine of 16-kDa peptide, which is produced by adipose tissue and a circulating factor performing both peripherally and centrally, whose effects are mediated through its OB-Rb receptor, also regulate metabolic and inflammation functions (Tartaglia et al., 1995). Leptin, meaning thin from the Greek leptos, is a product of the cytokine obese gene (ob), which is produced by both white and brown adipocytes. It is a pleiotropic protein that belongs to the cytokine family with long-helical chain which has similar structure with interleukins like IL-6, Il-12, Il-15, granulocyte colony stimulating factor and oncostatin M. Leptin has an extensive variety of biological actions including regulation of hypothalamic-pituitary-adrenal axis, reproduction, metabolism of glucose and insulin, lipolysis, immune and inflammatory response, sympathetic nerve activity, hematopoiesis, angiogenesis, blood pressure, bone formation and wound healing (Ahima and Flier, 2000, Friedman, 1998, Fruhbeck and Gomez-Ambrosi, 2001, Otero et al., 2005, Frank et al., 2000, Murad et al., 2003).

1.28.2 Role of leptin

Leptin concentrations in plasma are extremely linked with body mass index (BMI) both in rodents and humans (Maffei et al., 1995). An obese phenotype has been induced by mutation of leptin receptor in mice (db/db) and in rats (fa/fa) (Frederich et al., 1995, White and Martin, 1997). Leptin is found to control appetite as well as energy outflow through hypothalamic pathways and it is produced in equivalent to the body adiposity (Friedman and Halaas, 1998). Leptin is known to be a modulator of various inflammatory and immune responses along its metabolic and endocrine functions (Juge-Aubry and Meier, 2002, Fantuzzi and Faggioni, 2000). Leptin inhibits the immune response also; food deprivation decrease leptinaemia and lymphoid tissue are atrophied in both humans and rodents (Chandra, 1991, Ahima et al., 1996). Furthermore, the serum level of leptin and the leukocyte count were strongly correlated (Juge-Aubry and Meier, 2002).

1.28.3 Role of Leptin in Obesity

Leptin stimulate the process of lipolysis through direct effects on both adipose tissue and on pancreatic cells (Siegrist-Kaiser et al., 1997, Maedler et al., 2004, Seufert, 2004). It has identified that the lack of gene coding for leptin in mice (ob/ob) are found to be very obese and diabetic (Halaas et al., 1995). When treated with leptin injections in ob/ob mice, they reduce their food intakes which in turn increase their metabolic rate and results in loss of weight (Halaas et al., 1995, Pelleymounter et al., 1995). Genetic mutation in both mice and rats, which affects the receptor of leptin in the hypothalamus, reveals the same phenotype to ob/ob mice (Lee et al., 1996, Chua et al., 1996).

The function of leptin in regulating the body weight has converted to more multifaceted in both animals and humans who become obese. There are a few cases where a mutation, which affects the gene coding for the leptin receptor, has been found with a high incidence of morbid obesity (Montague et al., 1997, Farooqi et al., 2001, Clement et al., 1998). However, leptin therapy has an effect in children with leptin deficiency (Farooqi et al., 2002, Farooqi et al., 1999).

The leptin concentrations are very high due to increased levels of leptin secreting adipose tissue in obese individuals (Farooqi et al., 1999). However, with increase in leptin concentrations, this in turn triggers the objective cells to become resistant. The effect of leptin in the mouse with congenital lipodystrophy on peripheral metabolism resulting in insulin resistance, hyperglycemia, hyperinsulinemia and enlarged fatty liver and therapy with leptin upturned insulin resistance and diabetes (Shimomura et al., 1999). In lipotrophic diabetes disorder (little or no fat mass) in humans, there is reduced leptin in serum and increases in serum triacylglycerol concentrations. This leads to an inflamed fatty liver, which results in severe liver disease. Thus, leptin is an important hormone acts on energy balance and metabolism on both central and peripheral effects.

Leptin leads to a considerable reduction of fat mass, has an effective role on lipid metabolism within several days of administration (Pelleymounter et al., 1995). Leptin increases glucose metabolism in spite of its reducing action in bodyweight, proving its range of activity is not only on lipid metabolism and besides on glucose metabolism (Kamohara et al., 1997).

1.28.4 Role of Leptin in Obese and GDM Pregnancy

Several studies have discussed the leptin's role in reproductive function. The significance of sufficient nutrition, for the maintenance of reproductive function has also been well studied. However, inadequate nutrition delays which prevents the puberty onset but relating nutrition to the reproductive system has not been fully studied (Aubert and Sizonenko, 1996). Severe lipodystrophy is distinguished by low leptin levels, caused by destruction of adipose tissues (Oral et al., 2002). Other abnormalities include hypertriglyceridemia and severe insulin resistance which is generally associated with diabetes mellitus (Van Gaal et al., 2003). There are several studies on genetically engineered animal models as well as in humans in several genetic and acquired forms of lipodystrophy has demonstrated the metabolic abnormalities as a result of loss of fat (Gavrilova et al., 2000, Savage and O'Rahilly, 2002). Leptin has a definite role in preventing the insulin resistance, and hypertriglyceridemia of lipodystrophy. Of interest, replacement therapy with leptin reaches the physiological levels to a remarkable enhancement in insulin resistance, hyperglycemia, hypertriglyceridemia and hepatic steatosis in a mouse model of lipodystrophy (Shimomura et al., 1999). Hyperleptinemia corrects steatosis in diverse organs that act as sites of lipid accumulation, such as liver, islet cells and heart in diet-induced obesity (Lee et al., 2001).

Leptin plays an important role in transmitting signals for energy status to the central nervous system and it crosses the blood-brain barrier (BBB) to act on the arcuate nucleus (Banks and Farrell, 2003). Failure of the leptin transporter is considered as a key supplier to the development of leptin resistance in obesity. The BBB transporter for leptin is mediated by epinephrine, insulin, glucose and triglycerides and by starvation too (Banks, 2004).

Leptin is directly correlated with obesity (Havel, 2002). Leptin resistance has been replicated by hyperleptinemia is considered to associate obesity to impaired glucose metabolism, hypertension and pro-atherogenic status (Correia and Rahmouni, 2006). Leptin signaling is also liable for modulation of food intake and expenditure of energy (Correia and Rahmouni, 2006, Havel, 2002). Pregnancies complicated by GDM are associated with hyperleptinaemia (Kautzky-Willer et al., 2001). Insulin influences leptin expression on glucose metabolism (Wellhoener et al., 2000). Leptin also mimics some of insulin's actions in the adipose tissue, liver and muscle (Berti et al., 1997, Berti and Gammeltoft, 1999).

1.29 Non Esterified Fatty Acids (NEFA)

The metabolism in normal pregnancy is distinguished by low normal glycaemia and physiological hyperlipidemia (Herrera, 2000, Nolan et al., 1995). Pregnant women with higher levels of non-esterified fatty acids (NEFA) in the third trimester are more possible to be obese or overweight (Chen and Scholl, 2008). Obese women in pregnancy also have abnormal lipid proteins (Ramsay et al., 2002). However, type 2 DM, gestational diabetes and poorly controlled type 1 diabetes are regularly related with higher levels of plasma lipids than in normal pregnancy (Nolan et al., 1995, (Koukkou et al., 1996, Hollingsworth and Grundy, 1982). Increased NEFA levels have been shown to be associated with intrauterine growth retardation (IUGR), preterm delivery (Chen and Scholl, 2008), preeclampsia with IUGR (Alvino et al., 2008) and fetal adiposity (Schaefer-Graf et al., 2008). In fetal growth, the role of maternal hyperlipidemia is not yet clearly defined as maternal triglycerides do not cross the placenta and transfer of fatty acids, even though stimulated during late gestation, is very poor compared to glucose diffusion across the placenta

(Magnusson et al., 2004). The transport of fatty acid into the fetal environment would be essential for the establishment of endogenous factors to increase trapping of fatty acid and fat storage which is enhanced during late gestation (Magnusson et al., 2004). During early gestation, the fat content in fetal compartment increases about 0.5% of body weight to 16% at term which increases in fetal birth weight. This increase in birth weight is due to fatty acids transfer from the mother to the fetus as maternal lipid levels in serum, which increases the lipogenesis during late gestation (Saleh et al., 2007). Magnusson-Olsson et al (Magnusson-Olsson et al., 2006) has reported that fatty acids crosses the placenta from lipolysis of maternal triglycerides by lipoprotein lipase found to be dynamic in the placenta at late gestation. However, the mechanisms regulating this are not completely understood.

1.30 Fibroblast Growth Factor 21-FGF21

Fibroblast growth factor (FGF family) has 22 members which have a extensive variety of biological effects (Ornitz and Itoh, 2001) including regulation of cell growth and differentiation (Gospodarowicz, 1974). FGF polypeptides were named initially by their ability to stimulate the proliferation of fibroblast. Among FGF family, FGF19, FGF21 and FGF23, which are all members belonging to the same FGF subfamily, have significant metabolic functions (Beenken and Mohammadi, 2009). Fibroblast growth factor 21 is a novel member of FGF family. FGF21 is principally produced by liver and adipose tissues (Badman et al., 2009), and plays a significant role in control of glucose metabolism and energy balance (Badman et al., 2009). In contrast, FGF19 is produced in the intestinal epithelium, which has been found to regulate the synthesis of cholesterol and bile acid (Inagaki et al., 2005). FGF23, in contrast, is predominately produced in the bone and regulates absorption

of phosphate and biosynthesis of vitamin D through the kidneys (2000). However, an exact knowledge of bioactivity of FGF21 and its mode of action have been missing to date.

Recently, FGF21 has been found to regulate metabolism and as a hormonal mediator of adaptive starvation. FGF21 was found to reduce the concentration of glucose and triglyceride in the plasma of db/db and ob/ob mice and activates uptake of glucose in adipocytes, in turn protecting against diet induced obesity in transgenic mice; moreover, FGF21 improves lipoprotein when administered to diabetic rhesus monkeys (Kharitonenkov et al., 2005, Kharitonenkov et al., 2007, Wente et al., 2006). FGF21 has been found to be a novel pharmacological target for the obesity treatment and other related metabolic disorders (Kliewer and Mangelsdorf, 2010). FGF21 treatment in obese rodents has been found to reduce body fat content and improve glucose tolerance, insulin sensitivity and hepatic and circulating lipid parameters (Coskun et al., 2008, Xu et al., 2009, Berglund et al., 2009). FGF21 mediated weight loss has been found to be involved in an increase in fat oxidation and metabolic rate with no change of intake of food (Coskun et al., 2008). Interestingly, the insulin-sensitizing effects of FGF21 are reliant on reduced body fat and largely attribute to improved insulin action in the liver (Potthoff et al., 2009). This proposed that FGF21 might regulate hepatic substrate metabolism through a mechanism on hepatocytes that cannot be enlighten by a direct effect (Potthoff et al., 2009). FGF21 levels are positively associated with metabolic syndrome, obesity and type 2 DM (Kharitonenkov et al., 2005,(Zhang et al., 2008, Li et al., 2008, Lin et al., 2011). The hypothalamus has an abundance of neuropeptides that regulate satiety (Enriori et al., 2007). Of note, these neuropeptides, such as NPY, orexin, CRH are also present in adipose tissue. Interestingly, on the flip side, Hsuchou et al

(Hsuchou et al., 2007) has shown, in mice, that FGF21(an adipokine and hepatokine) crosses the blood-brain barrier (BBB) and hence that FGF21 from peripheral could reach the brain directly and coordinate its central effects (Hsuchou et al., 2007, Sarruf et al., 2010).

Recently, Tan et al (Tan et al., 2011) investigated whether this observation extended to humans. They sought to determine the FGF21 existence in human cerebrospinal fluid (CSF) and its relation to metabolic parameters. They established significantly elevated circulating FGF21 levels in CSF in obese compared with lean subjects, and that fat mass was the only influential factor of both circulating and CSF FGF21 levels. However, there were negative correlations between the CSF to plasma FGF21 ratio and BMI and fat mass, and the authors suggest the competence of FGF21 uptake into the CSF is reduced in obese subjects.

1.31 The Placenta

The placenta can produce and also respond to multiplicity of inflammatory stimuli. The placental resident macrophages (Hofbauer cells), syncytiotrophoblast and cytotrophoblast cells produces the cytokines. Nevertheless, the accurate physiologic role of placental cytokines, with progression of pregnancy, is still indistinct.

1.31.1 Placental Macrophages

Macrophages are the main cell type in the uteroplacental unit and are particularly profuse in the fibrous tissues of the placenta and in the decidua (Bulmer and Johnson, 1984, Lessin et al., 1988). Macrophages are found clustered near the implantation site in rodents (Tachi et al., 1981). Macrophages are forbidden from colonizing the decidua basalis as pregnancy progresses and are dispersed throughout the

myometrium and stroma of endometrium of mice (Redline et al., 1988) and in rats (Hunt et al., 1989). Macrophages has been identified by the isolation of placental cells in human tissues (Moskalewski et al., 1975) and in the mesenchymal stroma of the placenta (Wood et al., 1978, Wood and King, 1982). Fetal macrophages accumulate in the mesenchymal stroma between the extraplacental membranes, amnion and chorion (Bulmer and Johnson, 1984, Lessin et al., 1988) and in the yolk sac membranes (Wood, 1980).

The placental villus in human is composed of syncytiotrophoblast, the superficial cell layer lining the intervillous space, which is in direct contact with the maternal circulation and adjacent to fetal capillaries, as well as stromal cells which consists of fibroblast and Hofbauer cells (Castellucci et al., 2000, Georgiades et al., 2002). More than 100 years ago, Hofbauer cells (HBCs) were identified in the placental villus. Hofbauer cells were found to be large pleomorphic cells of about 10-30 µm and highly vacuolated with a granular cytoplasm (Georgiades et al., 2002, Castellucci et al., 2000).

Hofbauer cells are first visible on the 18th day of gestational period and found until term gestation (Castellucci et al., 1980). In the mid trimester, the placental villous stroma makes their identification difficult due to compression; this has provoked the use of antibodies raised against macrophage proteins like CD68 and CD163 using immunocytochemistry (Fox, 1967, Kim et al., 2008). Several researchers have identified Hofbauer cells might play a role in early placental development by influencing vasculogenesis (Seval et al., 2007), angiogenesis (Khan et al., 2000), and mesenchymal maturation (Ingman et al., 2010).

Placenta and the obesity have been less researched. Obesity is linked with larger placental size and accumulation of placental macrophages and inflammation

(Challier et al., 2008). In GDM and type 1 DM, placentae were shown to have altered genes in lipid metabolism in placenta (Radaelli et al., 2009). There is only limited information about fatty acid metabolism in pregnancies of obese women and gestational diabetes in human placenta (Szabo and Szabo, 1974, Lindegaard et al., 2006).

1.31.2 Placental Cytokines

The cell types identified almost in the uteroplacental tissues contribute in the production of cytokines (Hunt, 1989). Human placenta has been shown to express more cytokines. However, their chronological prototype of expression is still moderately understood (Bowen et al., 2002). The placental resident macrophages (Hofbauer cells), syncytiotrophoblast and cytotrophoblast cells produces the cytokines. All these three cell types allocate similarities with respect to the receptor expression, the ligand production and in their mechanism of action (Guilbert et al., 1993).

Most of the cytokines produced by the placenta are similar to that secreted by adipose tissues. During the third trimester, these might contribute to the low grade systemic inflammation (Lepercq et al., 1998, Kirwan et al., 2002, Radaelli et al., 2003). The reported cytokines in placenta are TNF α, leptin, and IL-6 (Guilbert et al., 1993, Lepercq et al., 2001, Malek et al., 2001), though their role is not understandable. Accordingly it may be significance in studying the placental cytokines in the third trimester.

The production of TNF-α by the Hofbauer and the trophoblast cells (Chen et al., 1991, Phillips et al., 2001) has not been estimated. On the contrary, syncytiotrophoblast cells are the main sites of synthesis of leptin and IL-6 (Phillips

et al., 2001). There is much facts that the combination of placental and T cell derived cytokines is involved in pregnancy (Kirwan et al., 2002).

The physiological role of placental cytokines in pregnancy becomes uncertain. One established hypothesis is that numerous placental cytokines released into the maternal systemic circulation contribute to metabolic changes to regulate increased energy needs of the fetus during the third trimester of pregnancy (Lepercq et al., 2001, Kirwan et al., 2002, Malek et al., 2001).

Due to increased local production of cytokine release into the maternal circulation is related with gestational complications like diabetes and pre-eclampsia (Lepercq et al., 1998, Benyo et al., 2001, Coughlan et al., 2001). As a result of increased release of cytokine might lead to the activation of particular inflammatory pathways, in turn induces maternal insulin resistance, which is necessary for the normal gestational sequence (Redman and Sargent, 2003, Radaelli et al., 2003).

Thesis Aims

Pregnancy which demands significant nutritional requirements on the mother and placenta have found to release cytokines and more recently some adipokines. While it is now established that increased maternal adiposity directly affects the physiology of the developing fetus, the mechanisms by which these processes occur are incompletely understood. However, comparatively little work has been performed in humans examining the role of adipose tissue derived factors in obese and GDM pregnancies.

With the aforementioned in mind, this thesis has hypothesized that alterations in adipose tissue derived factors such as adipsin and its downstream effector Acylation stimulating Protein (ASP) might underpin an altered nutritional state in pregnancy. Adipsin/ASP might represent a mechanism by which the weight gain in the fetus of obese mothers might be explained.

Therefore, this thesis aims to investigate and establish the following points:

Aim-1

Can the generation of a well characterized cohort of patients allow the dissection of molecular mechanisms of adipsin/ASP action?

Many studies have failed to determine important differences because of variation in clinical samples. Hence I focused on patients undergoing cesarean section because a) they are all delivered at the same gestation (39 weeks), b) they are all fasted (permitting meaningful interpretation of parameters such as HOMA-IR and c) access to tissues (adipose, placenta) is more straight forward.

Aim- 2

Do circulating concentrations of adipsin and ASP in both fetal and maternal establish a marked difference in obese pregnancies as well in diabetic pregnancies compared to lean pregnancies?

Adipsin and ASP are well known to play significant roles in triglyceride storage and are increased in circulating levels in obesity. The Adipsin levels in human pregnancy, an insulin resistant state, have not previously been studied, but the ASP levels have been described, with the evidence of increased maternal ASP at late gestation and increased fetal ASP in conjunction with the elevated maternal triglycerides. The lipid transfers are regulated in the normal placenta from maternal to fetal blood through storage and release of FFA, which is essential for energy source for the growing embryo. However, no previous reports have examined adipsin and ASP in both obese pregnancy and diabetic pregnancy. Therefore this thesis aims to investigate the circulating concentrations of maternal and fetal adipsin and ASP in lean, obese and GDM pregnancies and their corresponding cord bloods.

Aim-3

Does the placenta release Adipsin and ASP?

Increased fetal concentrations of adipsin and ASP in both obese and GDM pregnancy were observed. This has speculated whether the placenta might be a source of these molecules. Moreover, a very important thrust has aimed to investigate the release of adipsin and ASP from placenta and adipose tissue.

Aim-4

Where is the localization of adipsin and ASP in the placental tissues?

The secretion of both adipsin and ASP in the placenta has been identified, for the first time. This novel identification has intended to discover the cell types within the placenta that were responsible for the synthesis and secretion of these molecules.

Aim-5

Are there any differences in protein binding of FGF21 in lean, obese and GDM Pregnancies ?

Having described FGF21, as a regulator of metabolism and were positively associated with obesity and type 2 DM and has been reported to be produced in the brain. Therefore this work was aimed further to investigate the FGF21concentrations in CSF corresponding to plasma levels within these cohorts (lean, obese and GDM pregnant) to describe the differences.

CHAPTER 2

MATERIALS AND METHODS

2.1 SUBJECTS AND ETHICS

All study participants were pregnant women scheduled for elective caesarean section delivering at 39-40 weeks of gestation, and was identified from the operating theatre lists generated in the University Hospitals Coventry & Warwickshire Maternity department. The study protocol was approved by The Coventry Local Research Ethics Committee for Human Experimentation of the Medical University of Warwick (Research Ethics Committees 07/H1210/141). All the study participants filled out written informed consent forms.

After the evaluation of body weight and height, the body mass index (BMI) was determined using the formula-BMI=body mass in kilograms/height in square meters.

Accordingly, the study participants were divided into 3 groups:

1. Control group - group of thirty-five pregnant lean women in whom pre-pregnancy body mass index was determined within the range of 18.9-24.9 (lean pregnant-BMI 19-25, mean age 32 years; range 18-44),

2. Test group 1 (Obese) - group of thirty-nine obese pregnant women, in whom pre-gravid body mass index was determined above 30.0 (obese pregnant-BMI>30, mean age 32.49 years; range 22-44) and

3. Test group 2 (GDM) – group of eighteen pregnant women with gestational diabetes in whom pre-pregnancy body mass index was determined above 30.0 (GDM-BMI-31.16; range 23-46, mean age 34.4 years; range 26-47) were recruited.

All the patients filled in a questionnaire which consisted of questions concerning maternal age, gestational age, parity, pre pregnancy weight, height, obstetric history, previous caesarean sections, illnesses, smoking, alcohol consumption and indications for the caesarean section. Subjects with multiple pregnancies, cardiovascular diseases, preeclampsia and other metabolic disorders were excluded from the study.

Oral glucose tolerance tests (OGTTs) at 26-28 weeks gestation in pregnancy or in the preceding 6 months for non-pregnant controls had been performed in all participants to characterize glucose metabolism and exclude diabetes.

2.2 ANALYSIS

Paired maternal venous and cord blood samples were collected at the time of caesarean section and were centrifuged (spun at 3000g-Beckman Coulter DS-9623C) immediately. The supernatant were collected (serum and plasma) and stored immediately at -80˚C until analysis. Cerebrospinal fluid (CSF) was also collected during spinal anaesthesia and immediately frozen in liquid nitrogen and preserved at -80˚C until analysis. Venous blood samples were collected in plain tubes without anticoagulant for glucose, insulin and the measurements of lipid profile and EDTA tubes for Adipsin and ASP measurements. All samples were analysed for the fasting glucose, lipid profile, including TGs, total cholesterol, high-density lipoprotein cholesterol (HDL) and low -density lipoprotein cholesterol (LDL). Plasma glucose levels quantification and lipid profile analysis were performed using an automated clinical chemistry analyzer Roche modular system (Roche Diagnostics Scandinavian, Bromma, Sweden). The score of insulin resistance by Homeostasis Model Assessment (HOMA-IR) was calculated as previously described (Matthews et al., 1985). All other chemicals and reagents were from Sigma-Aldrich (Gillingham, UK) unless otherwise stated.

2.3 HUMAN INSULIN ELISA

The technique was performed according to the protocol of manufacturer (Invitrogen, Camarillo, CA, USA). It is an immunoenzymatic assay for the quantitative measurement of human insulin in human serum. Briefly, 50µL of standards, controls and serum samples were added to antibody coated micro well plate, simultaneously 50µL of anti-Insulin-HRP conjugate were added into all wells, and incubated for 30 minutes at room temperature with continuous shaking. Following this, the wells were aspirated and washed 3 times with wash solution. After the wash, 100 µL of stabilized chromogen was added within 15 min of washing and incubated in the dark for 15 minutes at room temperature. Finally, 100 µL of stop solution was added and absorbance was recorded at 450 nm using an ELISA plate reader (EL800, Bio-Tek Instruments, Inc., Winooski, VT, USA). The minimum detection limit for the assay is 0.17µIU/ml. Graph pad prism4 and Graph pad Instat (www.graphpad.com) were used to generate the standard curve. The concentrations for unknown samples and controls from the standard curve were determined from this curve.

2.4 HUMAN ADIPSIN ELISA

This protocol was performed according to the manufacturer's instructions (Adipobioscience, Cambridge, UK). Briefly, all the working standards and reagents were prepared and plasma samples were diluted (1:4000). Then 100 µL of standard, samples, positive controls were added to antibody coated micro-well plate and incubated for 2 hours on the plate shaker at room temperature. Followed this, the wells were washed for 4 times with wash solution. Then 100 µL of Detection Antibody was added to each well and then incubated for 2 hours on the plate shaker at room temperature followed by wash for 4 times. Then 100 µL of Streptavidin

HRP conjugate was added to each well and incubated for 45 minutes on the plate shaker at room temperature followed by wash for 4 times. 100 µL of Substrate was added to each well and incubated for 5-10 minutes in the dark. Then 100 µL of Stop Solution was added to each well. The absorbance was recorded at 450 nm (reference wavelength at 540nm) using an ELISA plate reader (EL800, Bio-Tek Instruments, Inc., Winooski, VT, USA). The minimum detection limit for the assay is 15.6 pg /ml. Graph pad prism4 and Graph pad Instat was used to generate the standard curve. The concentrations for unknown samples and controls from the standard curve were determined from this curve.

2.5 TISSUE EXPLANT CULTURE

Paired explants experiments were performed by using placentae and subcutaneous adipose tissue from lean (n=4), obese (n=4) and GDM pregnant patients (n=4) undergoing elective caesarean section at term. Paired placental and subcutaneous explants from lean, obese and GDM pregnant patients were prepared as previously described (Gould et al., 2010, Tan et al., 2007). The procedures were done under sterile condition. The fresh tissues of placenta and subcutaneous fat were collected in PBS, and samples were washed in PBS for 4 times. Areas of each placenta were randomly sampled and villous tissue was cultured on Costar Net well plates (Corning, NY). Adipose tissue explants (identical tissue weight for lean, obese and GDM pregnant) were cultured in 6-well plate. Briefly, to make up-to 1 litre of medium, 100mls of Fetal Calf Serum (GIBCO 10108-157) was incubated in 37ºC to thaw. Then 100ml of CMRL-1066 (GIBCO 21540 10x concentrate) culture medium concentrate was added to 800ml of MilliQ water and 2.2g $NaHCO_3$ was added. Following this, 100mg streptomycin sulphate (SIGMA S-1277), 60mg penicillin

G(SIGMA penicillin G 1,000,000 units P-3032), 100µg hydrocortisone (water soluble hydrocortisone SIGMA H-0396), 1mg of Insulin (SIGMA I-6634), 100µg retinol acetate (SIGMA R-0635;retinol acetate water soluble), 100mg of L-glutamine were added. Then pH of the culture medium was adjusted to 7.2 by adding a few drops of 10 M NaOH. About 800ml of MilliQ water was taken in 1 litre beaker and the prepared medium was added and then filtered through a 0.22µm filter.

1.5ml of prepared culture medium to each well was used in all sterile culture plates (net well plates were used for placental tissue) and placental as well as adipose explants were cultured in the medium. Both the explants were treated without and with insulin (2.5 µl or 0.00875 mg of insulin to 1.5 ml of medium to each well). The plates were incubated in the incubator for 2 days under 5% CO_2 and 95% air. Media aliquots were collected after 24 hours and 48 hours of incubation, centrifuged at 1000 rpm (46g) for 5 min and supernatants were stored in –80ºC until assayed. Simultaneously, the tissue explants were then weighed and stored in –80ºC.

2.6 IMMUNO-CYTOCHEMISTRY STAINING

The technique was performed using ImmPress Universal Reagent, Anti-Mouse/rabbit IgG (Vector Laboratories, Inc., Peterborough, UK). It is based on a new staining method of polymerization of enzymes and attaching these polymers to antibodies. About 8 µm frozen placental sections were obtained using a cryostat (Leica Microsystems, Milton Keynes, UK) and air dried. Immuno histochemistry analyses were performed on serial sections of placenta as described previously (Gould et al., 2010). Immediately before staining, sections were fixed with 75% ethanol for 5 min and the slides were transferred into water. Then 2.5 ml of 0.3% H_2O_2 in 22.5ml of methanol were added to the sections to lessen the risk of antigen destruction or tissue

loss and also for quenching of endogenous peroxidise for 30 minutes, followed by 3 washes in water for 5 minutes. The sections were incubated for 30 minutes with 2.5% normal horse blocking serum. Following this, the sections were incubated with mouse antihuman adipsin antibody (Santa Cruz Biotechnology, Inc., UK) diluted (1:100) in appropriate diluents solution (PBS) for 1 hour. The slides were then washed in water for 3 times for 5 minutes. Subsequently, the slides were incubated with Immpress reagent for 30 minutes and washed 3 times in water for 5 minutes. After this, the sections were incubated in peroxidase substrate solution for 5 minutes until the desired stain intensity was developed. Haematoxylin was added to the sections for 1 minute and washed with PBS. Then it was rinsed in75% ethanol for about 1 minute. Following this, the sections were immersed in 100% ethanol and Xylene. The slides were then counterstained, cleared and mounted and adipsin positive cells were detected.

2.7 HUMAN EIA ASP (C3adesArg) DETERMINATION

This protocol was performed according to the manufacturer's protocol (ENZO life sciences, Exeter, UK). This EIA human complement C3adesArg kit is a competitive immunoassay for the quantitative determination of C3adesArg in plasma. All plasma samples were treated with the reagents provided to precipitate whole protein from plasma, as whole protein in the sample competes with the complement in the assay. About 112.5µl volumes of plasma was added to 112.5µl of complement reagent A and vortex thoroughly. Then 25µl of 10.0 N Hcl was added to it, vortexed and incubated at room temperature for 1 hour. Assay buffer 10 was prepared, by diluting 10ml of 1x of supplied concentrate with 90ml deionised water. Then the samples were spun at 10, 000 rpm in a microcentrifuge for 5 minutes at room

temperature. 90 µl of the supernatant was transferred to a clean tube. To this supernatant, 10µl of 9.0 N NaOH was added and vortexed. Then 300µl of complement reagent B was added to the supernatant and vortexed thoroughly. Then 5.35 µl of Assay Buffer 10 was added to the supernatant and vortexed. Then 50 µl of this sample was diluted with 950 µl of assay buffer 10. Thus 1:200 dilutions of all plasma samples were prepared.

In NSB and Bo wells, about 100 µl of Assay Buffer 10 was pipetted. Then 100 µl of standards and 1:200 diluted samples were pipetted into the wells. Then 50 µl of assay buffer 10 was added into the NSB wells. 50 µl of antibody were added into each wells, expect total activity and blank wells. Then the plate was incubated at room temperature on a plate shaker at 500rpm for 2 hours. The plate was washed with the wash buffer for 3 washes. After the final wash, 5 µl of the 1:10 dilution of conjugate was added to total activity wells. 200 µl of the p-Npp substrate solution was added to every well. At the end of the assay, 50 µl of stop solution was added, which stopped the reaction and the plate was read immediately on a plate reader at optical density 405nm.

This kit uses a polyclonal antibody to human C3a des Arg to bind in a competitive manner. The intensity of the bound yellow colour is inversely proportional to the concentration of C3a des Arg in either standards or samples. The measured optical density was used to calculate the concentration of human C3a des Arg. The minimum detection limit for the assay is 0.120ng/ml. Graph pad prism4 and Graph pad Instat was used to generate the standard curve. The concentrations for unknown samples and controls from the standard curve were determined from this curve Intra-assay coefficient of variation for this EIA was 8.7% and inter-assay coefficient of variation was 5.7%.

2.8 HUMAN LEPTIN IMMUNOASSAY

This protocol was performed according to the manufacturer's instructions (Quantikine, R&D Systems, Inc. Abingdon, UK). Briefly, all the working standards and reagents were prepared and the plasma samples were diluted (1:100). Then about 100 µl of Assay Diluents RD1-19 to each well were added. About100 µl of standard, control and samples were added to each well and incubated for 2 hours at room temperature, after which each well were aspirated and washed, this process were repeated for three times for a total of four washes with wash buffer (400 µl). Then 200 µl of Leptin Conjugate was added to each well and incubated for 1 hour at room temperature. The plate was then washed for four washes with wash buffer. Then 200 µl of substrate solution was added to each well and incubated for 30 minutes at room temperature and protected from light. Finally, 50 µl of stop solution was added to each well. The colour in the wells changed from blue to yellow. The optical density of each well was determined using a micro plate reader set to 450nm with wavelength correction of 540 nm or 570 nm. The minimum detectable dose (MDD) of Leptin for the assay is less than7.8 pg/ml. Graph pad prism4 and Graph pad Instat was used to generate the standard curve. The concentration for unknown samples and controls from the standard curve was determined from this curve. Intra-assay coefficient of variation for this EIA was 3.2% and inter-assay coefficient of variation was 5.4%.

2.9 NON ESTERIFIED FREE FATTY ACID QUANTIFICATION

This protocol was performed using Bio vision's free Fatty Acid quantification kit (Bio Vision Research Products, USA). It is a sensitive enzyme based method for detecting long-chain free fatty acids in plasma. The protocol was assayed in 100 µl

per micro plate well. For the colorimetric assay, 0, 2, 4, 6, 8, 10 µl palmitic acid standards was added into 96 well plates individually. Assay buffer was added and the volume was adjusted to 50 µl/well to generate 0, 2, 4, 6, 8, 10 nmol/well of the fatty acid standard. Then 30 µl of samples was diluted with 20 µl of assay buffer, to bring up the volume to 50µl/well. Then 2 µl of Acyl-CoA Reagent was added into all standard and sample wells, mixed well and incubated at room temperature for 30 minutes. Then a total 50 µl reaction mix was prepared containing 44 µl Assay Buffer, 2 µl fatty acid probe, 2 µl enzyme mix and 2 µl enhancer for each well. 50 µl of the reaction mix was added to each well and incubated for 30 minutes at room temperature and protected from light. The optical density was determined at 570 nm for colorimetric assay in a micro plate reader. NEFA concentration was measured using graph pad prism and InStat software. The minimum detection limit of the assay using this method is 2µm free fatty acid.

2.9 HUMAN FGF-21 IMMUNOASSAY

This protocol was performed according to the manufacturer's instructions (Biovendor Research and Diagnostic products, Karasek, Czech Republic). Briefly, 100 µl of standards, reconstituted Quality controls and diluted samples (125 µl of sample +125 µl of dilution buffer for duplicates) were pipetted into the appropriate wells and the plate was incubated at room temperature for 1 hour, shaking at 300 rpm on an orbital micro plate shaker. The wells were washed with wash solution, 0.35 ml per well 3 times. After the final wash, the plate was inverted and tapped against paper towel. Then 100 µl of Biotin labelled Antibody was pipette into each well and the plate was incubated at room temperature for 1 hour, shaking at 300 rpm on an orbital micro plate shaker. After this, the wells were washed with wash solution 3

times. Then 100 µl of Streptavidin –HRP Conjugate was pipetted into each well and incubated at room temperature for 30 minutes, shaking at 300 rpm on an orbital micro plate shaker. The wells were washed 3 times with wash solution. After the wash, 100 µl of Substrate solution was added to each well and the micro-titer plate was protected from direct sunlight and the plate was incubated for 15 minutes at room temperature without shaking. The colour development was stopped by adding 100 µl of stop solution and the absorbance of each well was determined using a micro-plate reader set to 450 nm, with the reference wavelength set to 630 nm. The minimum limit of detectable dose (LOD) of FGF-21 was typically 7pg /ml. Graph pad prism4 and Graph pad Instat was used to generate the standard curve. The concentrations for unknown samples and controls from the standard curve were determined from this standard curve. Intra-assay coefficient of variation for this EIA was 3.0% and inter-assay coefficient of variation was 3.9%.

2.10 WESTERN BLOTTING

Sample preparation

Proteins were harvested in RIPA buffer (Sigma) with protease inhibitor cocktail added at 0.1% v/v (Cell Signaling, UK) and following treatment, proteins were centrifuged at 13,000 rpm for 3 minutes and stored at -20º C until use.

Western blotting

Protein samples of 20 micrograms of each sample per well were separated on a 15% reducing polyacrylamide gel and electro blotted onto a nitrocellulose membranes (Millipore, Bedford, MA, USA). The nitrocellulose membrane was then blocked with 2.5% of non-fat milk in 1M Trizma/base, 1.54M NaCl, 0.05% Tween 20 (Tris buffered solution plus Tween 20, TBST, pH 7.4) for 2 hours at room temperature,

and then incubated overnight at 4 °C to TBST containing primary antibodies [Monoclonal mouse-antihuman adipsin antibody-Santa Cruz, UK] (dilution 1:500) (Santacruz, UK)] in the blocking buffer (2.5% non-fat milk in Tris-buffered saline). The immunoblots were then washed thoroughly three times with TBST 0.1% Tween and incubated with appropriate horseradish peroxidise-conjugated secondary anti mouse antibodies (1:5000) (Vector labs, UK) for one hour at room temperature. Membranes were washed in TBST and antigen-antibody complexes were visualized by chemiluminescence kit using a SuperSignal West Pico kit (Thermo Scientific Pierce Protein biology Products, Rockford, Illinois, USA). Signal was quantified on a Chemigenius bioimaging system using the genetools program (Syngene, Cambridge, UK). Then, for standardization, the same membranes were then stripped by submersion in stripping buffer [10% SDS (Bio-Rad Laboratories, Hemel Hempstead, UK), 1M Tris-HCl (Sigma-Aldrich, Gwellingham, UK), and pH 6.8, β-mercaptoethanol (Sigma-Aldrich, Gwellingham, UK) for 30 minutes with gentle agitation. After washing with TBS-0.1% Tween at room temperature for 10 minutes, the membrane were then blocked by incubation in 2.5% non-fat milk in 1M Trisma/base, 1.54M NaCl, 0.05% Tween 20 (Tris buffered solution plus Tween 20, TBST, pH 7.4) for about one hour. Following this the membrane was re-probed with the mouse anti human β-actin antibody (Abcam Cell Signalling Technology Inc., USA; 1 in 5,000 dilutions).

2.11 ISOLATION OF PLACENTAL CYTOTROPHOBLAST, FIBROBLAST AND HOFBAUER CELLS

Placental cells were isolated as previously described by (Tang et al., 2011b). Briefly, isolation of the different cell types comprising placenta was initiated through

protocols previously employed to obtain cytotrophoblasts (CTs) using trypsin/DNase I digestion and discontinuous Percoll gradient fractionation (Kliman et al., 1986). CTs (>95% purity) were generated following enzymatic digestion of placenta with trypsin, centrifugation on Percoll gradients, and negative immuno-selection by simultaneous incubation with anti-CD45 and anti-CD9 antibodies. HBC were isolated using collagenase digestion of the trypsin-treated tissue, followed by centrifugation on Percoll gradients and negative immuno-selection by sequential incubation with anti-EGFR and then with anti-CD10 antibodies. Cultures of FIBs (>95% purity) were obtained from cells attached to magnetic beads containing CD9 and CD45 antibodies from CT isolations and those attached to anti-CD10 beads from HBC preparations. HBC were isolated with 98-99% purity and a yield of 130-200 x 10(6) cells/80-100 g of tissue.

2.12 IMMUNOFLUORESCENCE

Placental sections were obtained as described previously in immuno-histochemistry methods. These were fixed for 10 minutes in 100% cold acetone at room temperature. After extensive washing in PBS, the slides were incubated for additional 30 min at room temperature in PBS supplemented with 1% BSA. After washing three times in PBS, the slides were incubated overnight with either a rabbit polyclonal antibody directed against rabbit anti-human adipsin (1:500) or mouse anti-human CD206 (1:150) for HBC or a mouse anti-human CK7 (1:100) for trophoblast or a mixture of the above antibodies. After extensive rinsing with PBS, the slides were incubated for 1 h with either anti-mouse coupled to Alexa Fluor 488 (1:200) or anti-rabbit coupled to Dylight Fluor 633 (1:200) or a mixture of both of these secondary antibodies (Thermo Scientific Pierce Protein Biology Products,

Rockford, Illinois, USA). Slides were washed three times with PBS, mounted and examined using a Zeiss LSM-510 confocal microscope (Zeiss, Jena, Germany) (Zhu et al., 1994). Acquisition parameters were kept constant and below background signal (as determined by samples incubated without primary antibody) to allow comparison between different samples.

CHAPTER 3

RESULTS

Systematic Investigations between Lean and Obese pregnancies

RESULTS:

3.1 Anthropometric and biochemical characteristics: (Lean and Obese pregnant)

The anthropometric and metabolic characteristics between lean pregnant and obese pregnant groups are presented in the Table 3.1.The characteristics of maternal age in years, booking BMI in kg/m^2, mean gestational age at delivery in weeks, fasting status and OGTT in 26-28 wks between lean pregnant and obese pregnant are tabulated. The booking BMI of obese pregnant ($33.723kg/m^2$) were significantly higher than the lean pregnant ($23.477kg/m^2$). The birth-weight of obese off springs (3.61kg±0.03) was significantly higher than the babies of lean pregnant (3.51kg±0.04). The HOMA-IR values were calculated which were found to be elevated in obese pregnant women and their fetuses as measured in cord blood compared to lean pregnant women and their fetuses.

3.2 Maternal and fetal biochemical profiles (Lean and obese pregnant women)

The maternal and fetal biochemical profiles between lean and obese are summarized in Table 3.2.There were no statistically differences in the glucose and HDL cholesterol levels between lean pregnant and obese pregnant women or their corresponding fetuses. However, statistically significant differences ($p<0.05$) were found in the levels of total cholesterol, triglycerides and LDL which were all elevated in obese pregnant mothers as well as their corresponding fetal cord bloods compared to the lean pregnant mothers and their fetuses. The Insulin levels were significantly ($p=0.008$) elevated in obese pregnant compared to lean pregnant women. The leptin levels were significantly ($p<0.0001$) elevated in obese mothers as

well as their corresponding fetal cord bloods ($p<0.0007$) compared to lean pregnant women and their fetuses.

3.3 Maternal and Fetal HOMA-IR (lean and obese pregnant)

The HOMA-IR values were calculated, which were found to be elevated in obese pregnant women (2.436±0.21 compared to lean pregnant women (1.64±0.283). Similarly, the HOMA-IR values were also elevated in the off spring of obese pregnant as measured in cord blood (2.171±0.288) compared to fetuses of lean pregnant women (1.165±0.18) (Fig.3.1)

3.4 Maternal Glucose and lipid concentration (lean and obese pregnant)

Maternal glucose levels quantification and lipid profile analysis in serum between lean and obese pregnant women were performed using automated chemical analyser. The glucose levels (4.28mmol/l) were similar between both lean and obese pregnant women. The total cholesterol of obese pregnant (6.743 mmol/l±0.194) were significantly higher than the lean pregnant (6.086mmol/l±0.235). Similarly, the triglycerides (mmol/l) of obese pregnant (2.887±0.14) were significantly higher than the lean pregnant (2.494±0.12). Similarly, the LDL (mmol/l) of obese pregnant (4.011±0.152) were significantly higher than the lean pregnant (3.488±0.175) .However the HDL (mmol/l) of lean pregnant (1.744±0.069) were higher than the obese pregnant (1.671±0.07) but were not statistically significant (Fig.3.2).

3.5 Fetal Glucose and lipid concentration (lean and obese pregnant)

Fetal glucose levels quantification and lipid profile analysis in the cord serum of lean and obese pregnant women were analysed using automated chemical analyser (Fig 3.3) .The glucose levels (mmol/l) of fetuses of obese pregnant women (3.869±0.072)

were slightly elevated compared to fetuses of lean pregnant women (3.732±0.067) and are not significant. The total cholesterol levels of fetuses of obese pregnant (1.734 mmol/l±0.059) were significantly higher than that of the lean pregnant (1.562mmol/l±0.059). Similarly, the triglycerides (mmol/l) of fetuses of obese pregnant (0.269±0.02) were significantly higher than that of the lean pregnant (0.209±0.013). Similarly, the LDL (mmol/l) of fetuses of obese pregnant (0.879±0.044) was significantly higher than that of the lean pregnant (0.722±0.028). However the HDL (mmol/l) levels of fetuses of lean pregnant (0.813±0.042) were higher than the fetuses obese pregnant (0.732±0.039) and are not statistically significant.

3.6 Maternal and Fetal Insulin concentration (lean and obese pregnant)

The Insulin levels (µIU/ml) were measured in the serum using ELISA were significantly ($p=0.008$) elevated in obese pregnant (17.815±1.782) compared to lean pregnant women (11.871±1.267). However, the insulin levels were not statistically different in the cord bloods of babies born to obese pregnant women (11.4±2.099) compared to the lean pregnant women (8.976±0.763) (Fig.3.4).

3.7 Maternal and Fetal Leptin concentration (lean and obese pregnant)

The Leptin levels (ng/ml) were measured in the plasma using ELISA were significantly ($p<0.0001$) elevated in obese pregnant (48.6031±2.801) compared to lean pregnant women (23.933±2.66). Similarly, the leptin levels were statistically different ($p<0.0007$) in the cord bloods of babies born to obese pregnant women (4.862 ±5.408) compared to babies of the lean pregnant women (2.674±2.143) (Fig.3.5).

3.8 Maternal and Fetal NEFA concentration (lean and obese pregnant)

The NEFA levels (nmol/µl or mM) were measured in the plasma were significantly ($p<0.05$) elevated in obese pregnant women (1.348±0.068) compared to lean pregnant women (1.088±0.12). Similarly, the NEFA levels were statistically different ($p<0.05$) in the cord bloods of babies born to obese pregnant women (0.194 ±0.025) compared to the babies of lean pregnant women (0.123±0.012) (Fig.3.6).

3.9 Plasma adipsin is significantly elevated in obese pregnancy:

The adipsin levels were measured in the plasma (pg/ml) using ELISA were found to be significantly ($p <0.05$) elevated in obese pregnant women (843.42pg/ml ± 33.14) compared to lean pregnant women (720.63pg/ml ± 33.13). However, cord blood samples revealed significantly higher levels of adipsin than maternal samples. Furthermore babies of obese pregnant women were significantly($p<0.05$) higher levels of adipsin (1663.78 ± 52.76 pg/ml) in the cord bloods as compared to babies of lean pregnant women (1354.37 ± 33.82 pg/ml) (Fig.3.7).

3.10 Secretion of adipsin by human placenta- lean and obese pregnant

Adipsin was found to be secreted from the adipose tissue explants as expected, with tissues from obese patients producing significantly($p<0.05$) more adipsin per mg weight (790.27 ± 64.60 pg/ml/mg) as compared to adipose tissue from lean mothers (504.74 ± 31.94 pg/ml/mg) (Fig.3.8). Surprisingly, in addition to the secretion of adipsin in the adipose tissue explants, adipsin secretion from placental explants was identified, which was significantly($p < 0.04$) greater in placental explants from obese pregnant women (546.0 ± 44.38 pg/ml/mg) than from placental explants from lean pregnant women (284.56 ± 43 pg/ml/mg) (Fig.3.8).

3.11 Plasma ASP is significantly elevated in obese pregnancy.

The ASP levels in plasma using ELISA were found to be significantly ($p < 0.05$) elevated in obese pregnant women (608.70 ± 67.15ng/ml) compared to lean pregnant women (420.30 ± 39.98ng/ml) (Fig.3.9). In contrast to adipsin, the ASP levels in the cord blood samples were within a similar range to the maternal samples, but offspring of obese pregnant women had significantly ($p < 0.05$) greater levels of cord blood ASP (354.48 ± 12.12ng/ml) than the offspring of lean pregnant women (302.63 ± 14.98ng/ml).

3.12 Secretion of ASP by human placenta- lean and obese pregnant

Similar analyses utilizing obese and lean pregnant explants of placenta and adipose tissue were performed to identify, whether the human placenta was also producing ASP. ASP secretion was identified from both adipose and placental explants. Moreover, ASP secretion was significantly greater from placental tissues as compared to the adipose tissue explants. The ASP levels in the medium of placental tissues of obese pregnant women (5485.74 ± 163.32ng/ml/mg) were significantly ($p<0.05$) higher than those of lean pregnant women (2399.16 ± 181.83ng/ml/mg) (Fig.3.10). Although to a much lesser extent, ASP secretion from adipose tissues of obese pregnant women (1508.40± 659.65ng/ml/mg) was higher than ASP secretion from adipose tissues of lean pregnant women (807.21 ± 254.8ng/ml/mg).

Table 3.1 Anthropometric and biochemical characteristics between Normal (Lean) and obese pregnant women.

Characteristics	**Normal (Lean) pregnant women(n=35)**	**Obese pregnant women (n=39)**
Maternal age (years)	32 (18-44)	32.487 (22-47)
Booking BMI (Kg/m2)	23.477 (20 - 24.9)	33.723 (30 - 46)
Mean Gestational Age at delivery (weeks)	39+2	39+2
Fasting status	35/35	39/39
OGTT (26-28 weeks)	Normal 35/35	Normal 39/39
Birth weight	3.51Kg ± 0.04	3.61Kg ± 0.03
HOMA-IR Mother/Baby	1.84 ± 0.283/1.365 ± 0.08	2.436± 0.21/2.171 ± 0.288

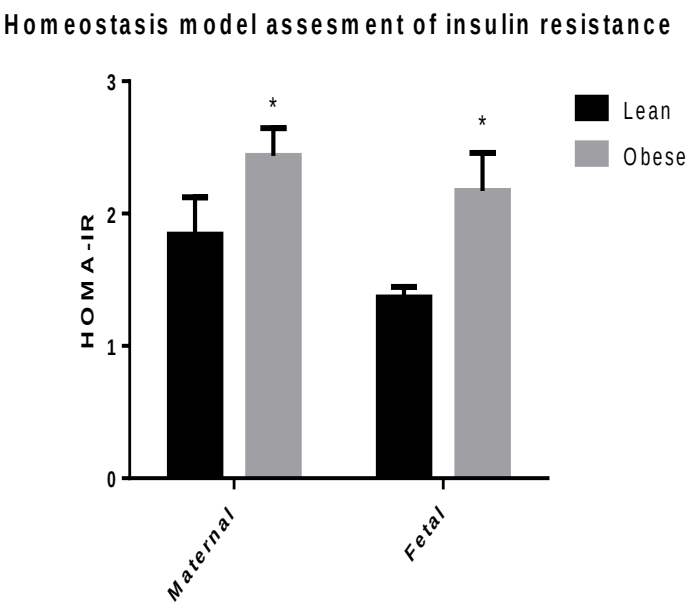
Body Mass Index (BMI), Oral Glucose Tolerance Test (OGTT), Homeostatic Model Assessment-Insulin resistance (HOMA-IR), Number of patients (n).Data are presented as mean ± range.

Table 3.2 Maternal and fetal biochemical profiles between Lean and obese pregnant women.

	Maternal			**Fetal**		
Biochemical parameters	**Lean (n=34)**	**Obese (n=39)**	**p-value**	**Lean (n=34)**	**Obese (n=39)**	**p-value**
Glucose (mmol/l)	4.288 ± 0.05	4.279 ± 0.098	0.938	3.732 ±0.07	3.87±0.07	0.168
TGL (mmol/l)	**2.494 ± 0.12**	**2.887 ± 0.14**	**0.037***	**0.209±0.1**	**0.26 ± 0.02**	**0.014***
Total cholesterol (mmol/l)	**6.086 ± 0.24**	**6.743 ± 0.194**	**0.036***	**1.562 ±0.06**	**1.734± 0.06**	**0.043***
HDL (mmol/l)	1.74 ± 0.069	1.671 ± 0.07	0.459	0.81±0.042	0.732 ±0.04	0.163
LDL (mmol/l)	**3.49 ± 0.175**	**4.011 ± 0.152**	**0.029***	**0.72 ±0.028**	**0.879 ±0.04**	**0.003***
Insulin (µIU/ml)	**11.87 ± 1.27**	**17.82 ± 1.782**	**0.008***	8.98 ±0.763	11.4 ±2.099	0.283
Leptin (ng/ml)	**23.933±2.66**	**48.6031±2.80**	**0.0001***	**2.674±2.14**	**4.862 ±5.41**	**0.0007***

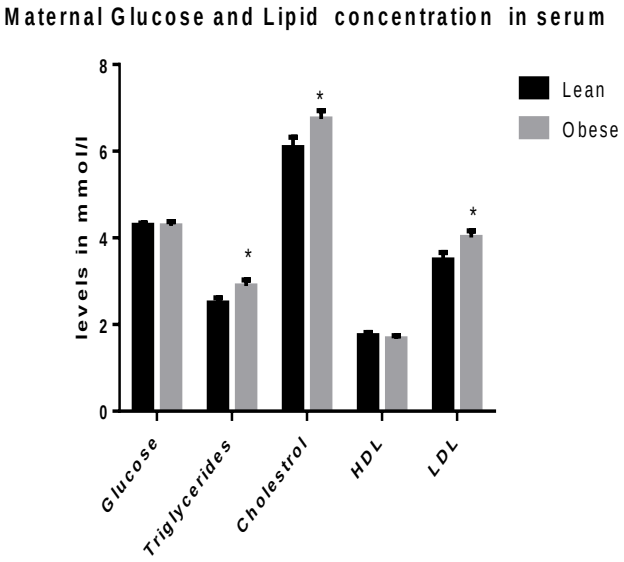
Triglycerides (TGL), High Density Lipoprotein cholesterol (HDL), Low Density Lipoprotein cholesterol (LDL), Lean-Pregnant women with normal BMI, Obese-Pregnant women with high BMI. Data are presented as mean ± SEM.*$p<0.05$ significant compared to lean pregnant women.

Figure 3.1



HOMA-IR(Homeostasis assesment of insulin resistance) in maternal and fetal samples of lean and obese .Data are shown in mean and standard error of mean (SEM)
*p<0.05 compared with lean.

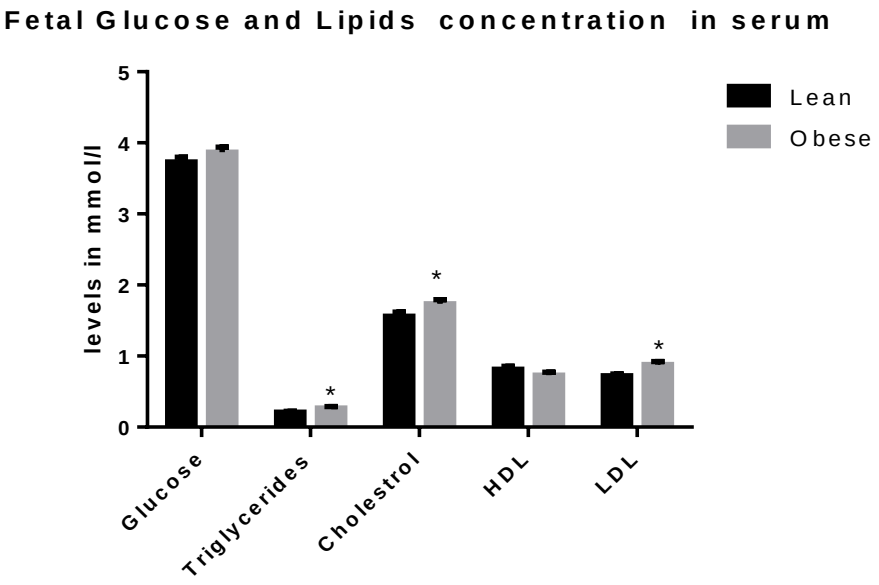
Figure 3.2



Maternal Glucose and Lipid Concentrations in serum of lean and obese groups.Data are shown as mean and standard error of mean(SEM)

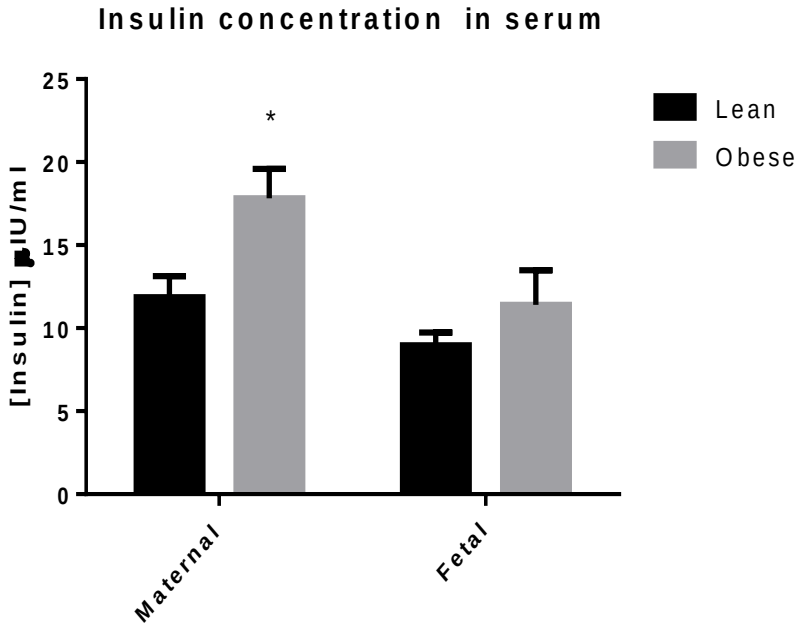
* $p<0.05$ compared with lean

Figure 3.3



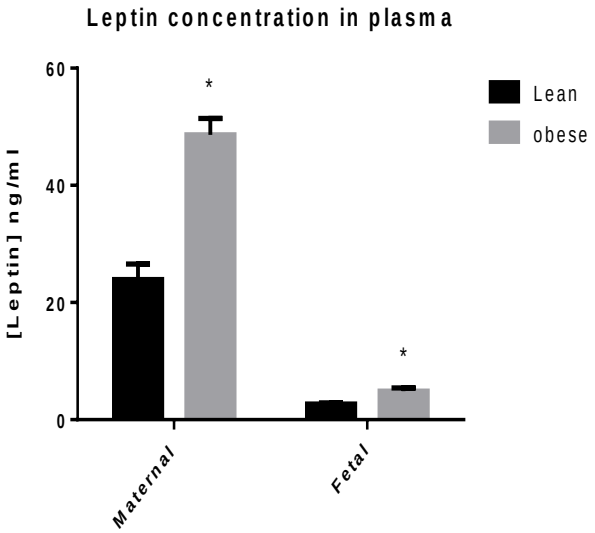
Fetal Glucose and Lipid Concentrations in serum of lean and obese groups.Data are shown as mean and standard error of mean(SEM)
*** p<0.05 compared with lean**

Figure 3.4



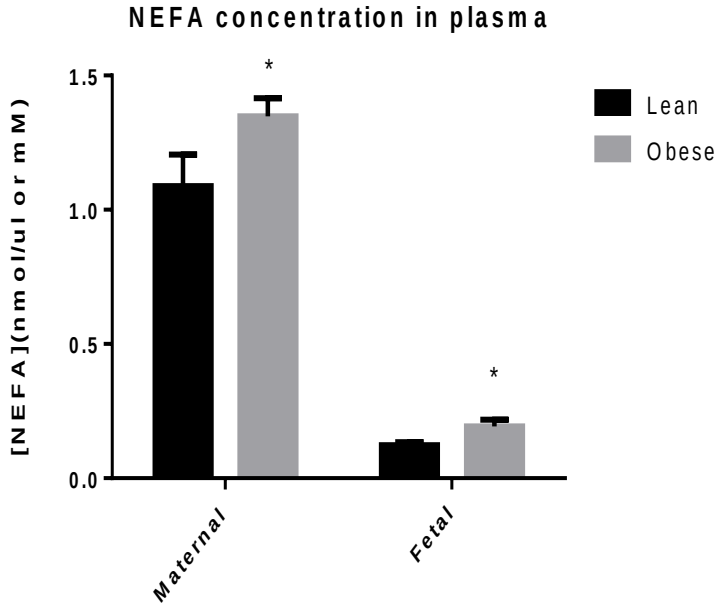
Maternal and Fetal insulin concentration in lean and obese groups using ELISA.Data are shown as mean and standard error of mean(SEM).*p<0.05 compared with lean

Figure 3.5



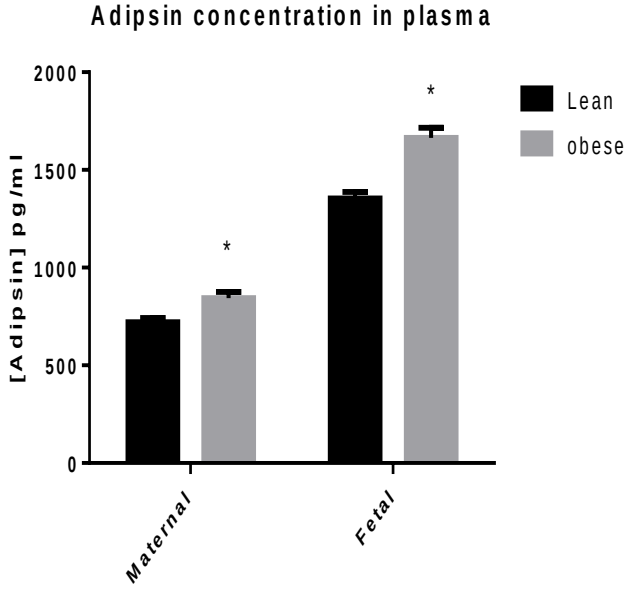
Leptin concentration in maternal and fetal samples of lean and obese .Data are shown in mean and standard error of mean (SEM) *p<0.05 compared with lean.

Figure 3.6



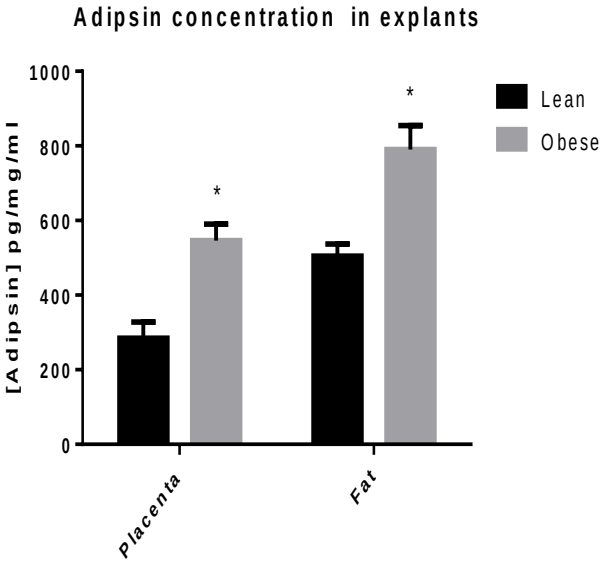
NEFA(Non Esterified free fatty acid) concentration in maternal and fetal samples of lean and obese .Data are shown in mean and standard error of mean (SEM) *p<0.05 compared with lean.

Figure 3.7



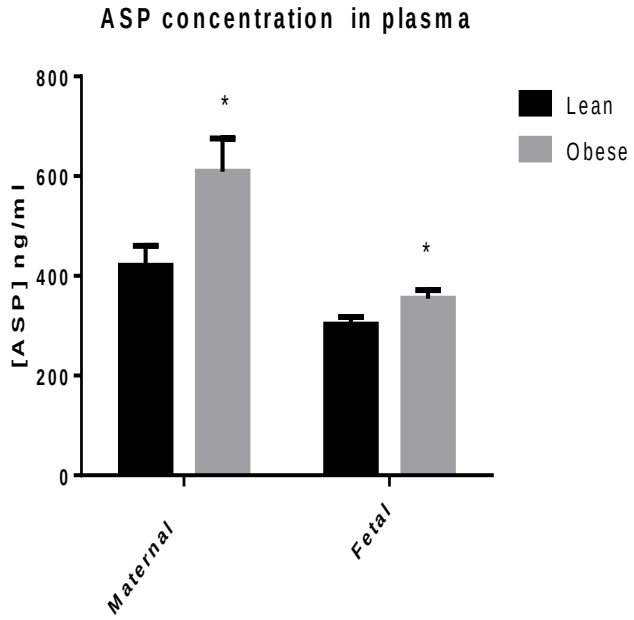
Plasma adipsin concentration in maternal and fetal samples of lean and obese using ELISA.Data shown as mean and standard error of mean(SEM) *p<0.05 compared with lean pregnant samples.

Figure 3.8



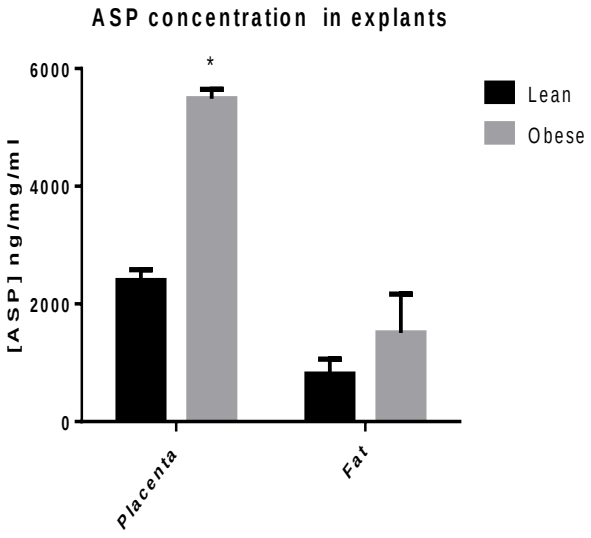
Adipsin Concentration in tissue explants of placenta & fat in pregnant women with lean & obese using ELISA.Data are shown as mean and standard error of mean(SEM). *p<0.05 compared with lean.

Figure 3.9



Plasma ASP(c3a des arg) concentration in maternal and fetal samples of lean & obese using ELISA.Data are shown in mean and standard error of mean (SEM) *p<0.05 compared with lean

Figure 3.10



ASP Concentration in tissue explants of placenta & fat in pregnant women with normal BMI & highBMI using ELISA

Data are shown as mean and standard error of mean(SEM). *p<0.05 compared with pregnant women with normal BMI.Day2-after 48 hrs of incubation of the conditioned medium.

CHAPTER 4

RESULTS

Systematic Investigations between lean and GDM Pregnancies

4.1 Anthropometric and biochemical characteristics: (Lean pregnant and GDM pregnant)

The anthropometric and metabolic characteristics between lean pregnant and GDM pregnant are presented in the Table 4.1.The characteristics of maternal age in years, booking BMI in kg/m^2, mean gestational age at delivery in weeks, fasting status and OGTT in 26-28 wks between lean pregnant and GDM pregnant are tabulated. The booking BMI of GDM pregnant (34.4 kg/m^2) were significantly higher than the lean pregnant (23.477kg/m^2). The birth-weight of GDM offspring (3.86 $\pm$ 0.08) were significantly higher than the babies of lean pregnant (3.51kg±0.04).The HOMA-IR values were calculated which were found to be significantly elevated in GDM pregnant women and their fetuses as measured in cord blood compared to lean pregnant women and their fetuses.

4.2 Maternal and fetal biochemical profiles (Lean and GDM pregnant women)

The maternal and fetal biochemical profiles between lean and GDM are summarized in Table 4.2. There were statistically significant differences ($p<0.05$) found in the levels of glucose, triglycerides, HDL and LDL which were all elevated in GDM mothers as well as their corresponding fetal cord bloods compared to the lean pregnant mothers and their babies. However, no statistically significant differences were found in the levels of total cholesterol, in GDM pregnant mothers but it was found to be significantly elevated in their corresponding fetal cord bloods compared to the lean pregnant mothers and their babies. The Insulin levels were significantly ($p<0.04$) elevated in GDM mothers as well as their corresponding fetal cord bloods compared to lean pregnant women and their babies. The Leptin levels were

significantly ($p<0.0004$) elevated in GDM mothers as well as their corresponding fetal cord bloods ($p<0.0002$) compared to lean pregnant women and their babies.

4.3 Maternal and Fetal HOMA-IR (lean and GDM pregnant)

The HOMA-IR values were calculated, which were found to be elevated in GDM pregnant women (3.5541 ±0.184) compared to lean pregnant women (1.84±0.283) (Fig.4.1). Similarly, the HOMA-IR values were also elevated in the offspring of GDM pregnant as measured in cord blood (2.39 ±0.16) compared to babies of lean pregnant women (1.365±0.08).

4.4 Maternal Glucose and lipid concentration (lean and GDM pregnant)

Maternal glucose levels quantification and lipid profile analysis in serum between lean and GDM pregnant women were performed using automated chemical analyser (Fig.4.2). The glucose of GDM (4.783 mmol/l±0.265) were significantly higher than the lean pregnant (4.288mmol/l±0.055). Similarly, the triglycerides (mmol/l) of GDM (2.837±0.21) were significantly higher than the lean pregnant (2.494±0.12). Similarly, the LDL (mmol/l) levels of GDM (3.585±0.3) were significantly higher than the lean pregnant (3.488±0.175). Likewise, the HDL (mmol/l) of lean pregnant (1.744 ± 0.069) was significantly higher than the GDM pregnant (1.281 ± 0.1). Similarly, the total cholesterol (mmol/l) of GDM (6.393± 0.343) were higher than the lean pregnant (6.086±0.235) but were not significant (Fig.4.2).

4.5 Fetal Glucose and lipid concentration (lean and GDM pregnant)

Fetal glucose levels quantification and lipid profile analysis in the cord serum of lean and GDM pregnant women were analysed using automated chemical analyser (Fig.4.3) .The glucose levels (mmol/l) of babies of GDM pregnant women (4.478mmlo/l±0.357) were significantly ($p<0.05$*)elevated compared to babies of lean pregnant women (3.732±0.067). The total cholesterol levels of babies of GDM pregnant (1.653 ± 0.124) were higher than that of the lean pregnant (1.562mmol/l±0.059) and are significant ($p<0.05$). Similarly, the triglycerides (mmol/l) of babies of GDM pregnant (0.2412±0.0169) were significantly ($p<0.012$) higher than that of the lean pregnant (0.209±0.013). Similarly, the LDL (mmol/l) of babies of GDM pregnant (0.852±0.06) were significantly ($p<0.03$*) higher than that of the lean pregnant (0.722±0.028). However, the HDL (mmol/l) levels of babies of lean pregnant (0.813±0.042) were significantly higher than the babies GDM pregnant (0.631±0.036; $p<0.002$) (Fig.4.3).

4.6 Maternal and Fetal Insulin concentration (lean and GDM pregnant)

The Insulin levels (µIU/ml) were measured in the serum were significantly ($p<0.04$) elevated in GDM mothers (20.013±3.463) compared to lean pregnant women (11.871±1.267) (Fig.4.4). Similarly, the insulin levels were statistically different ($p<0.05$) in the cord bloods of babies born to GDM pregnant women (12.79±2.282) compared to the lean pregnant women (8.976±0.763).

4.7 Maternal and Fetal Leptin concentration (lean and GDM pregnant)

The Leptin levels (ng/ml) were measured in the plasma were significantly ($p<0.0004$) elevated in GDM pregnant women (42.24±2.621) compared to lean pregnant women (23.933±2.66) (Fig.4.5). Similarly, the leptin levels were statistically different ($p<0.0002$) in the cord bloods of babies born to GDM pregnant women (4.133 ±0.255) compared to the lean pregnant women (2.674±2.143).

4.8 Maternal and Fetal NEFA concentration (lean and GDM pregnant)

The NEFA levels (nmol/µl or mM) were measured in the plasma were significantly ($p<0.0001$) elevated in GDM pregnant women (2.1505±0.2004) compared to lean pregnant women (1.088±0.12) (Fig.4.6). Similarly, the NEFA levels were statistically different ($p<0.0078$) in the cord bloods of babies born to GDM pregnant women (0.311±0.1104) compared to the babies of lean pregnant women (0.123±0.012) (Fig.4.6).

4.9 Plasma adipsin is significantly elevated in GDM pregnancy:

The adipsin levels in plasma (pg/ml) were found to be significantly ($p <0.05$) elevated in GDM pregnant women (865.59 ± 56.304) compared to lean pregnant women (720.63 ± 33.13) (Fig.4.7). Cord blood samples revealed significantly higher levels of adipsin than maternal samples. Furthermore babies born to GDM pregnant women showed significantly($p<0.05$) higher levels of adipsin (1576.99 ± 90.888 pg/ml) in the cord bloods as compared to babies of lean pregnant women (1354.37 ± 33.82 pg/ml) (Fig.4.7).

4.10 Secretion of adipsin by human placenta-lean and GDM pregnant

As expected, adipsin was found to be secreted from the adipose tissue, with tissues from GDM patients producing significantly($p<0.05$) more adipsin per mg weight (691.393 ± 17.462 pg/ml/mg) as compared to adipose tissue from lean mothers (504.74 ± 31.94 pg/ml/mg) (Fig.4.8).

Surprisingly, adipsin secretion from placental explants was identified, which was significantly ($p < 0.04$) greater in placentae from GDM patients (441.654 ± 38.1206 pg/ml/mg) than from placentae from lean patients (284.56 ± 43 pg/ml/mg) (Fig.4.8).

4.11 Plasma ASP is elevated in GDM pregnancy.

The ASP levels in plasma using ELISA were found to be elevated in GDM pregnant women (458.389 ± 20.982 ng/ml) compared to lean pregnant women (420.30 ± 39.98 ng/ml) and were not significant (Fig.4.9). In contrast to adipsin, the ASP levels in the cord blood samples were within a similar range to the maternal samples, but offspring of GDM pregnant women had greater levels of cord blood ASP (336.528 ± 29.754 ng/ml) than the offspring of lean pregnant women (302.63 ± 14.98 ng/ml) and were not significant (Fig.4.9).

4.12 Secretion of ASP by human placenta-lean and GDM pregnant

Similar analyses utilizing obese and lean pregnant explants of placenta and adipose tissue were performed to identify whether the GDM placenta was also producing ASP (Fig.4.10). ASP secretion was identified from both adipose and placental explants. Moreover, ASP secretion was significantly greater from placental tissues as compared to the adipose tissue explants. The ASP levels in the medium of placental tissues of GDM pregnant women (4813.69 ± 153.76 ng/ml/mg) were significantly

($p<0.05$) higher than those of lean pregnant women (2399.16 ± 181.83 ng/ml/mg) (Fig.4.10). Although ASP secretion from adipose tissues of lean pregnant women was higher (807.21 ± 254.8 ng/ml/mg) than ASP secretion from adipose tissues of GDM pregnant women (644.145 ± 105.78 ng/ml/mg) but was not significant.

Table 4.1 Anthropometric and biochemical characteristics between Normal (Lean) and GDM pregnant women.

Characteristics	**Normal (Mean + Range) n=35**	**GDM (Mean + Range) n=18**
Maternal age (yrs)	32 (18-44)	34.4 (26-47)
Booking BMI (Kg/m2)	23.477 (20 - 24.9)	31.16 (23-46)
Mean Gestational Age at delivery (wks)	39+2	39+2
Fasting status	35/35	18/18
OGTT (26-28 weeks)	Normal 35/35	Abnormal 18/18
Birth weight	3.51Kg ± 0.04	3.86 Kg ± 0.08
HOMA-IR Mother/Baby	1.84 ± 0.283/1.365 ± 0.08	3.5541 ±0.184/2.39 ±0.166

Body Mass Index (BMI), Oral Glucose Tolerance Test(OGTT), Homeostasis Model Assessment-Insulin resistance (HOMA-IR),Number of patients(n).Data are presented as mean+range.

Table 4.2 Maternal and fetal biochemical profiles between Lean and GDM pregnant women

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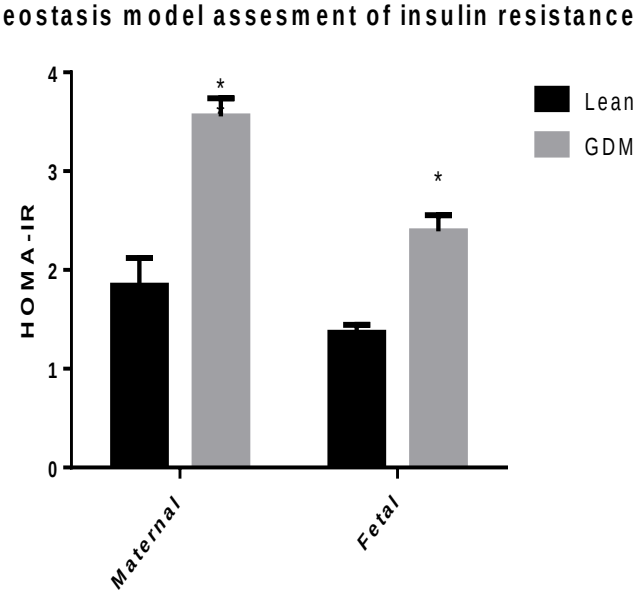
	Maternal			**Fetal**		
Biochemical parameters	**Lean (n=34)**	**GDM (n=18)**	**p-value**	**Lean (n=34)**	**GDM (n=18)**	**p-value**
Glucose (mmol/l)	4.288 ± 0.055	4.783 ± 0.265	0.04*	3.732 ± 0.067	4.478 ± 0.357	0.05*
TGL (mmol/l)	**2.494 ± 0.12**	**2.837 ± 0.21**	**0.037***	**0.209 ± 0.013**	**0.241 ± 0.02**	**0.012***
Total cholesterol (mmol/l)	**6.086 ± 0.235**	**6.393 ± 0.343**	**0.202**	**1.562 ± 0.059**	**1.653 ± 0.124**	**0.05***
HDL (mmol/l)	1.744 ± 0.069	1.281 ± 0.1	0.0004*	0.813 ± 0.042	0.631 ± 0.036	0.002*
LDL (mmol/l)	**3.488 ± 0.175**	**3.585 ± 0.3**	**0.03***	**0.722 ± 0.028**	**0.852 ± 0.061**	**0.03***
Insulin (µIU/ml)	**11.871 ± 1.267**	**20.013 ± 3.463**	0.04*	8.976 ± 0.763	12.79 ± 2.282	0.05*
Leptin (ng/ml)	23.933±2.66	42.24±2.621	**0.0004***	2.674±2.143	4.133 ±0.255	0.0002*

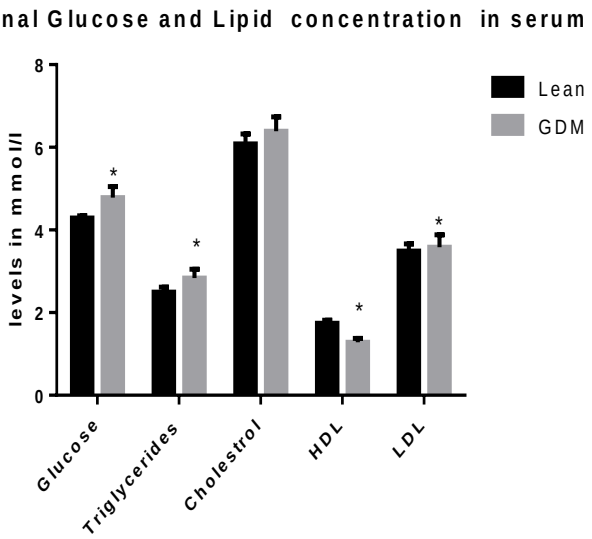
Triglycerides(TGL),High Density Lipoprotein cholesterol(HDL),Low Density Lipoprotein cholesterol(LDL),Lean-Pregnant women with normal BMI,GDM-Pregnant women with gestational Diabetes. Data are presented as mean±SEM.*$p<0.05$ significant compared to lean pregnant women.

Figure 4.1



HOMA-IR(Homeostasis assesment of insulin resistance) in maternal and fetal samples of lean and GDM .Data are shown in mean and standard error of mean (SEM)
*p<0.05 compared with lean.

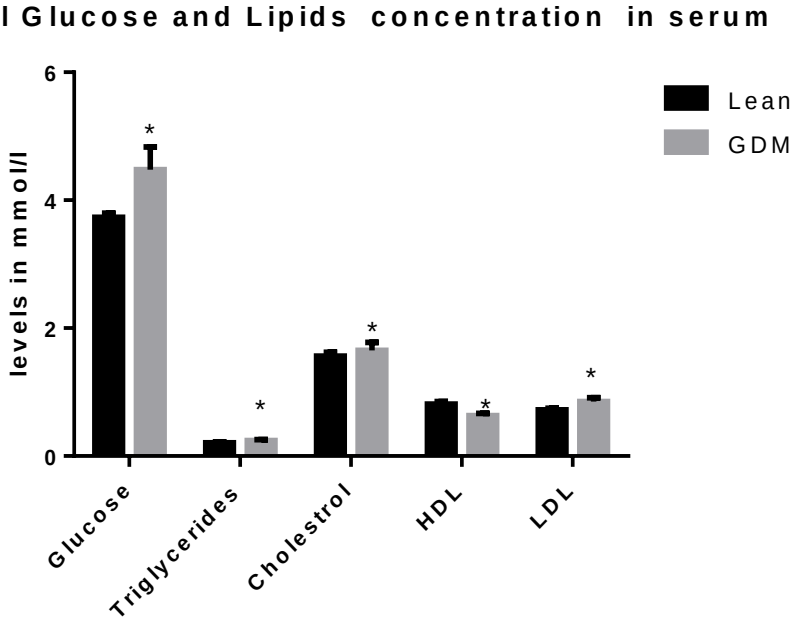
Figure 4.2



Maternal Glucose and Lipid Concentrations in serum of Lean and GDM groups.Data are shown as mean and standard error of mean(SEM)

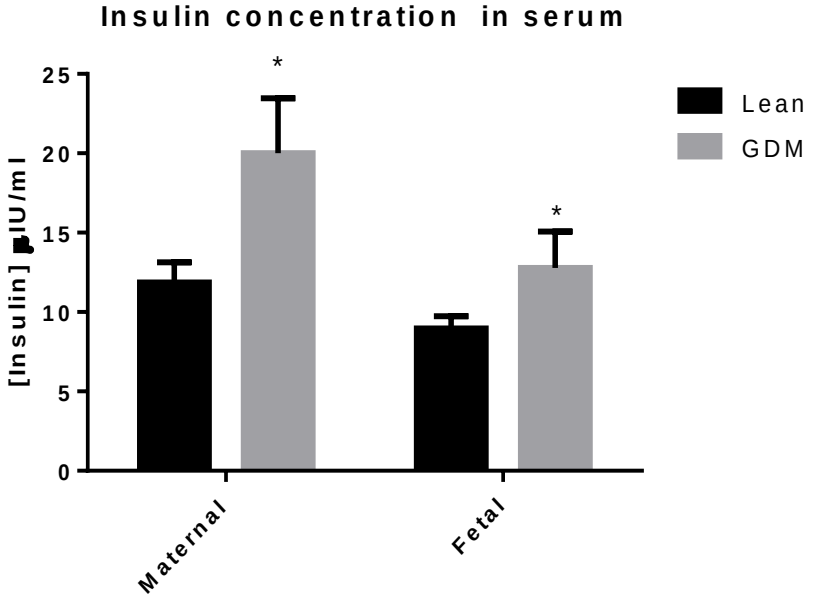
*** p<0.05 compared with lean**

Figure 4.3



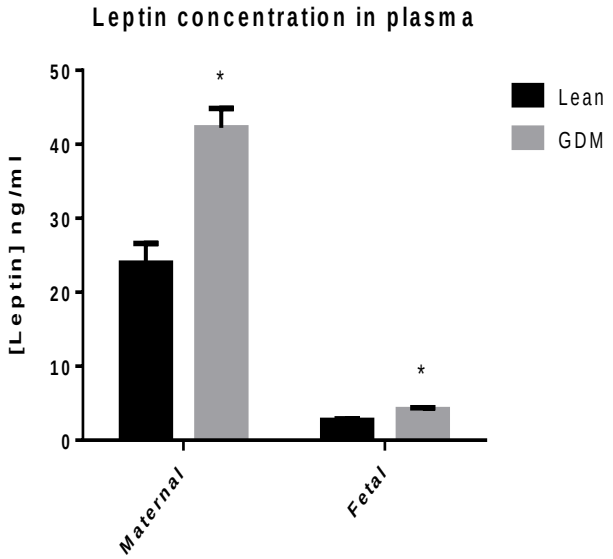
Fetal Glucose and Lipid Concentrations in serum of lean and GDM.Data are shown as mean and standard error of mean(SEM)
*** p<0.05 compared with lean**

Figure 4.4



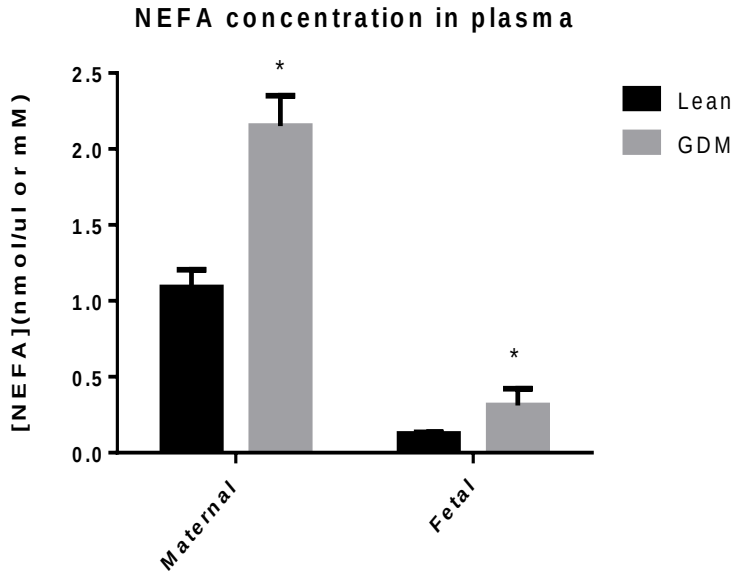
Maternal and Fetal insulin concentration in lean and GDM using ELISA.Data are shown as mean and standard error of mean(SEM).*p<0.05 compared with lean

Figure 4.5



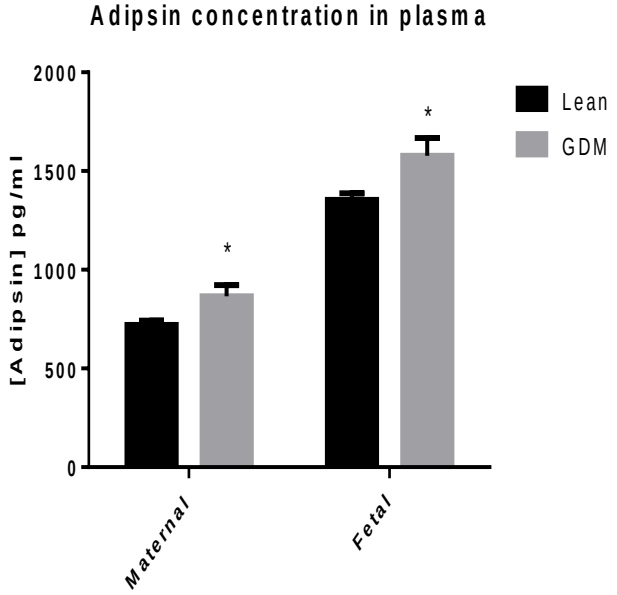
Leptin concentration in maternal and fetal samples of lean and GDM .Data are shown in mean and standard error of mean (SEM) *p<0.05 compared with lean.

Figure 4.6



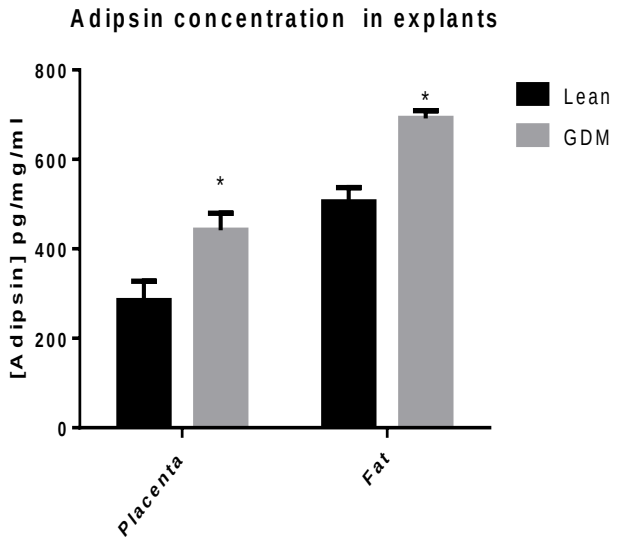
NEFA(Non Esterified free fatty acid) concentration in maternal and fetal samples of lean and GDM .Data are shown in mean and standard error of mean (SEM) *p<0.05 compared with lean.

Figure 4.7



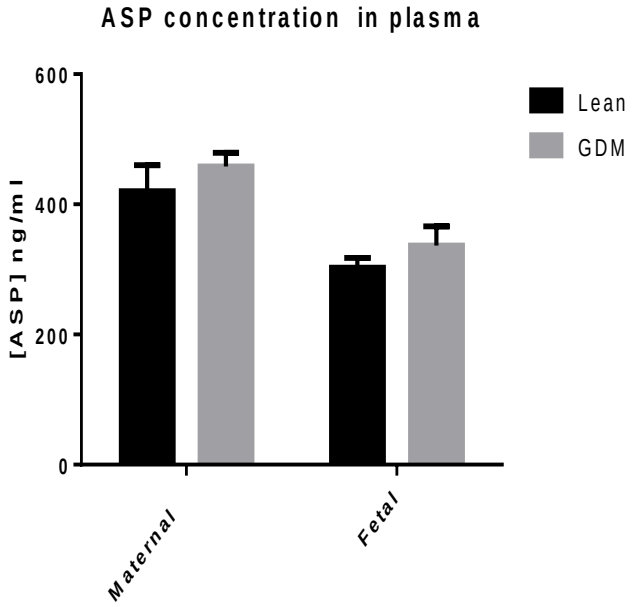
Plasma adipsin concentration in maternal and fetal samples of lean and GDM using ELISA.Data shown as mean and standard error of mean(SEM) *p<0.05 compared with lean.

Figure 4.8



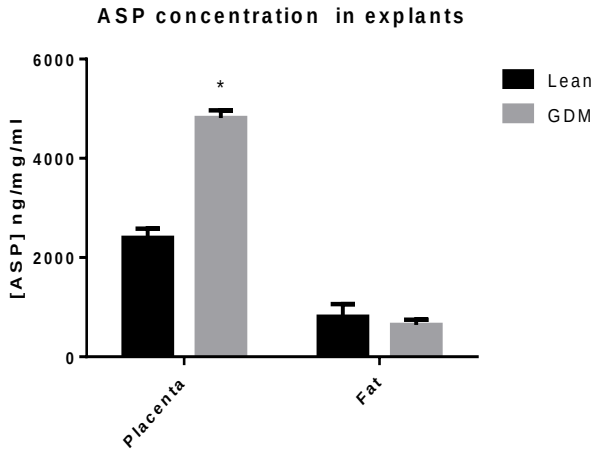
Adipsin Concentration in tissue explants of placenta & fat in pregnant women with normal BMI &GDM using ELISA.Data are shown as mean and standard error of mean(SEM).

Figure 4.9



Plasma ASP(c3a des arg) concentration in maternal and fetal samples of lean and GDM using ELISA.Data are shown in mean and standard error of mean (SEM)

Figure 4.10



ASP Concentration in tissue explants of placenta & fat in pregnant women with normal BMI &GDM using ELISA

Data are shown as mean and standard error of mean(SEM). *p<0.05 compared with pregnant women with normal BMI.Day2-after 48 hrs of incubation of the conditioned medium.

CHAPTER 5

RESULTS

Differences between lean, obese and GDM pregnancies

RESULTS:

5.1 Differences between lean, obese and GDM pregnant women

Differences between lean, obese and GDM pregnant women are tabulated in Table 5.1. The levels of glucose were similar between lean and obese pregnant, but it was elevated in GDM pregnant women. However, the insulin levels were elevated in both obese pregnant and GDM pregnant compared to lean pregnant. Similarly, the serum TGL level were found to be elevated in obese pregnant than lean and GDM pregnant women. Plasma adipsin levels were found to be elevated in obese pregnant (843.42pg/ml±33.14) compared to lean pregnant (720.63 pg/ml±21.77), but the levels were slightly elevated in GDM pregnant (865.59 pg/ml ±56.304) than obese pregnant women. Plasma ASP concentration were elevated in obese pregnant (608.70 ng/ml±67.15) than lean pregnant (420.30 ng/ml ±39.98) and GDM (458.39 ng/ml ±20.98) (Table 5.1).

5.2 Effect of insulin on adipsin release

Human placentas and subcutaneous fat were obtained from lean pregnant (n=4), obese pregnant (n=4) and GDM pregnant (n=4) were incubated in the presence of insulin as well as in the absence of insulin.

The adipsin release in adipose tissue as expected was found to be significantly more in obese pregnant (790.27 pg/mg/ml ± 64.6) than lean (504.74 pg/mg/ml ± 31.94**)** and GDM pregnant (691.39 pg/mg/ml ±17.43) (Fig.5.1&Table 5.2).However, when adipose explants were treated with insulin the release of adipsin were found to be elevated in obese pregnant (712 ±32.93pg/mg/ml) than lean pregnant (611.39pg/mg/ml ± 83.42), but the release were found to be lower in GDM (540.21pg/mg/ml ± 70.52) than obese pregnant and lean pregnant and were not

significant. Moreover, when the adipose explants of lean pregnant were treated with insulin the release of adipsin were increased than the release from non-insulin treated adipose explants, whereas the adipose explants of obese pregnant and GDM were treated with insulin, the release of adipsin were found to be decreased than the non-insulin treated adipose explants(Fig 5.1).

Similar analyses on placental explants were performed. The adipsin release in placental tissues were found to be significantly more in obese pregnant (546 ± 44.38pg/mg/ml) than lean (284.56pg/mg/ml ± 43**)** and GDM pregnant (441.65 pg/mg/ml ±38.121) (Fig 5.1&Table 5.2). However, when placental explants were treated with insulin the release of adipsin were found to be elevated in obese pregnant (689.53 ±28.23pg/mg/ml) than lean pregnant (393.19pg/mg/ml ± 24.18) , but the release were found to be lower in GDM (342 pg/mg/ml ± 10) than obese pregnant and lean pregnant and were not significant (Fig 5.1). Furthermore, when the placental explants of obese pregnant and lean pregnant were treated with insulin the release of adipsin were increased than the non-insulin treated placental tissues, whereas the placental explants of GDM treated with insulin, the release of adipsin were found to be decreased than the non-insulin treated GDM placental explants(Fig 5.1).

Table 5.1

Differences between lean, obese and GDM pregnant women

	Lean pregnant	**Obese pregnant**	**GDM**
Glucose (mmol/l)	4.288 ± 0.055	4.28±0.09	4.783 ± 0.265
Insulin (µIU/ml)	11.871 ± 1.267	17.82±1.782	20.013 ± 3.463
TGL (mmol/l)	2.494 ± 0.12	2.89 ±0.14	2.837 ± 0.21
[Adipsin] pg/ml	720.63±21.77	843.42±33.14	865.59±56.304
[ASP] ng/ml	**420.30**±39.98	**608.70**±67.15	**458.39**±20.98

TGL-Triglycerides, ASP-Acylation stimulating hormone. Data are expressed in mean ± SEM (standard error of mean).

Table 5.2

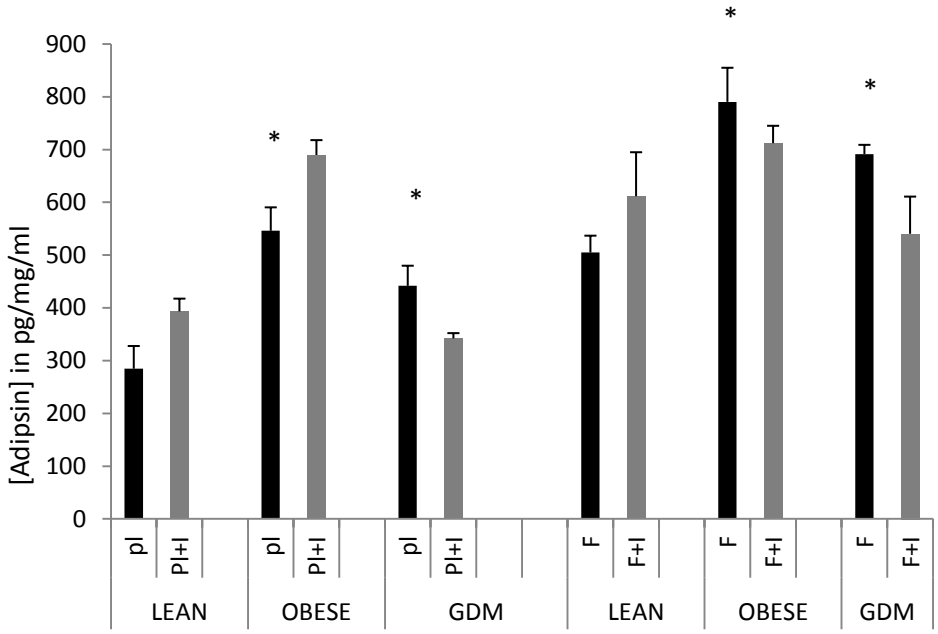
Adipsin secretion in tissue explants-placenta and subcutaneous fat-treated without and with Insulin

	Lean pregnant (n=4)	**Obese pregnant (n=4)**	**GDM (n=4)**
Adipsin secretion in placental explants (pg/mg/ml)	**284.56 ± 43**	**546 ± 44.38**	**441.65 ±38.121**
Adipsin secretion in placental explants treated with insulin (pg/mg/ml)	**393.19 ± 24.18**	**689.53 ± 28.23**	**342 ±10**
Adipsin secretion in fat explants (pg/mg/ml)	**504.74 ±31.94**	**790.27 ± 64.6**	**691.39 ± 17.43**
Adipsin secretion in fat explants treated with insulin (pg/mg/ml)	**611.39 ± 83.42**	**712 ±32.93**	**540.21 ± 70.52**

Data are expressed in mean ± SEM (standard error of mean).

Figure 5.1

Adipsin concentration in explants



Adipsin Concentration in tissue explants of placenta & fat in pregnant women with normal BMI,high BMI &GDM using ELISA.

Data are shown as mean and standard error of mean(SEM).

*p<0.05 compared with pregnant women with lean.Day2-after 48 hrs of incubation.

The conditioned medium was treated without & with Insulin(I).

pl-placenta,F-Fat,GDM-Gestational diabetes.

pl+I-placental explants treated with insulin.

F+I-fat explants treated with insulin.

CHAPTER 6

RESULTS

Localization of Adipsin and ASP in Human Placenta

And

Identification of Adipsin and ASP in Hofbauer cells

6.1 Immuno histochemistry -Adipsin staining in the human placenta

Utilizing immunohistochemistry, adipsin was localized in the placenta (fig.6.1a, 6.1b, 6.1c &6.1d). Adipsin positive areas (fig.6.1c) were found in specific cells dispersed throughout the human placenta. Surprisingly, marked staining were seen in the peri-vascular area (fig.6.1d), suggesting that there would be higher levels of adipsin in proximity to the fetal vasculature. The distribution of these cells suggested that they would be either fibroblast or Hofbauer cells.

6.2 Western Blotting:

Antibody specificity was then confirmed using western blotting, with human adipose tissue as positive control. Clear single band was visible at 28 kDa in adipose tissue as well as placental proteins (Fig.6.2).

6.3 Hofbauer cells (placental macrophages) secrete both adipsin and ASP

Having identified the human placenta as the one of the source of both adipsin and ASP, negative magnetic selection of cell types from the human placenta was performed as previously described in order to determine which cell type was responsible for the secretion of these molecules. To determine which cell types were positive for adipsin; individual cells were separated using magnetic beads.

The fibroblasts, Cytotrophoblasts and Hofbauer cells (placental macrophages) from four human placentas were then cultured and were only able to detect minimal ASP or adipsin in either fibroblasts or CTs. However, clear secretion of both adipsin (49.75 pg/ml/10^6 cells) (fig.6.3) and ASP (13.62 ng /ml/ 10^6 cells) (fig.6.4) from placental macrophage (HBCs) were detected.

6.4 Immunoflourescence: The dual staining of adipsin and CD206 on placental tissues showed co-localization (Fig6.5b). In contrast, no co-localization was observed for adipsin and CK7 (controls) (Fig6.5a).

Immuno histochemistry

Adipsin staining in the human placenta

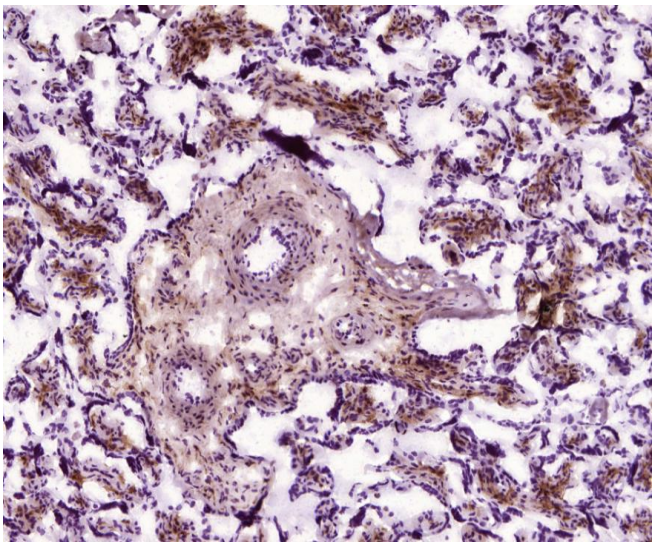


Figure 6.1a
Dispersion and positive areas of adipsin throughout the placenta

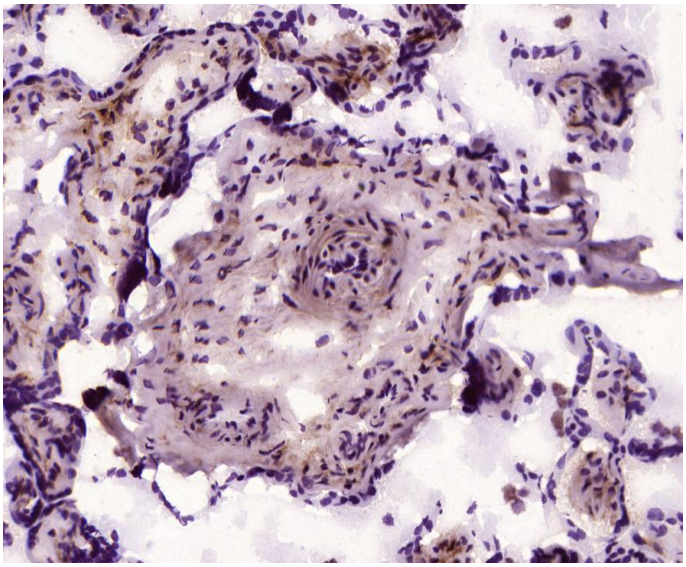


Figure 6.1b
Localization of Adipsin in placental tissue

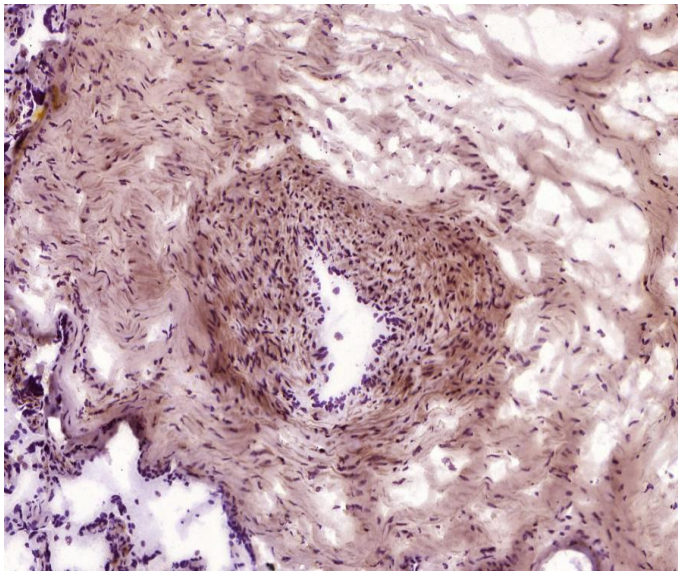


Figure 6.1c
Marked staining seen around peri-vascular area

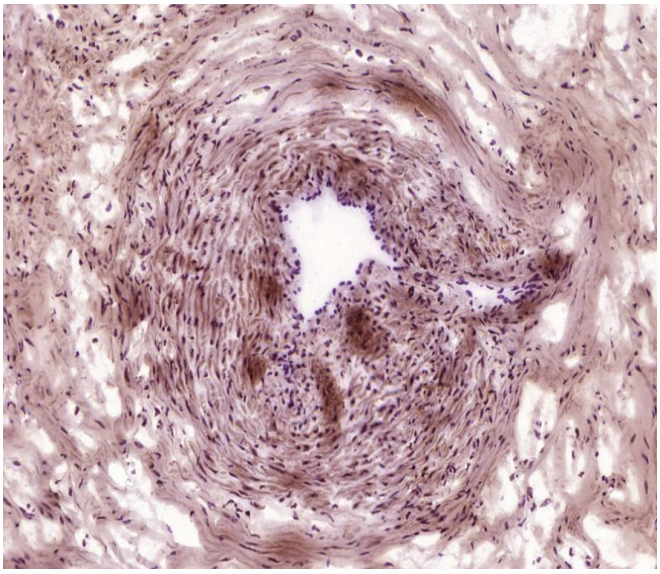
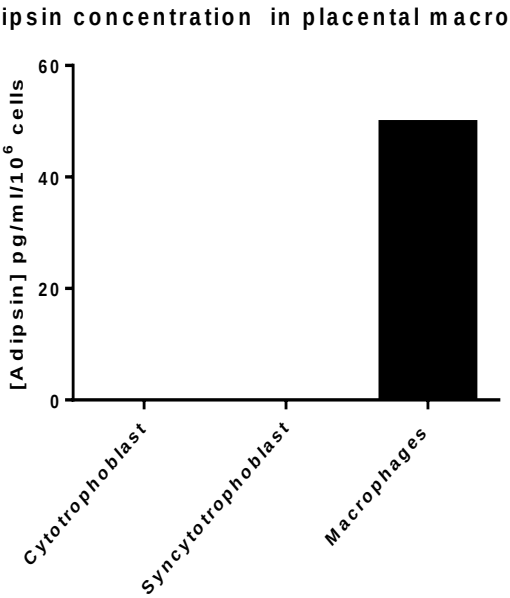


Figure 6.1d
Marked staining with Adipsin in the peri-vascular areas in placental tissue

Figure 6.2
Adipsin Western Blot

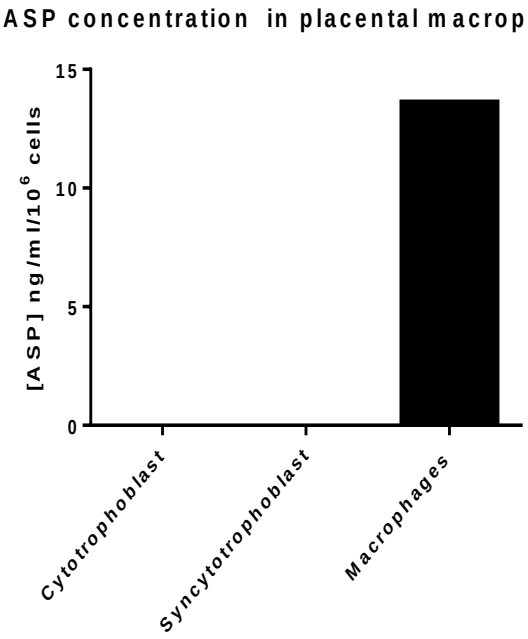
Adipose Placenta

Figure 6.3



Adipsin concentration in placental macrophages,cytotrophoblast and syncytotrophoblast using ELISA

Figure 6.4



ASP concentration in placental macrophages,cytotrophoblast and syncytotrophoblast using ELISA

Figure 6.5a: CK7 and Adipsin dual staining on placental frozen sections (Adipsin=Red, CK7=Green)

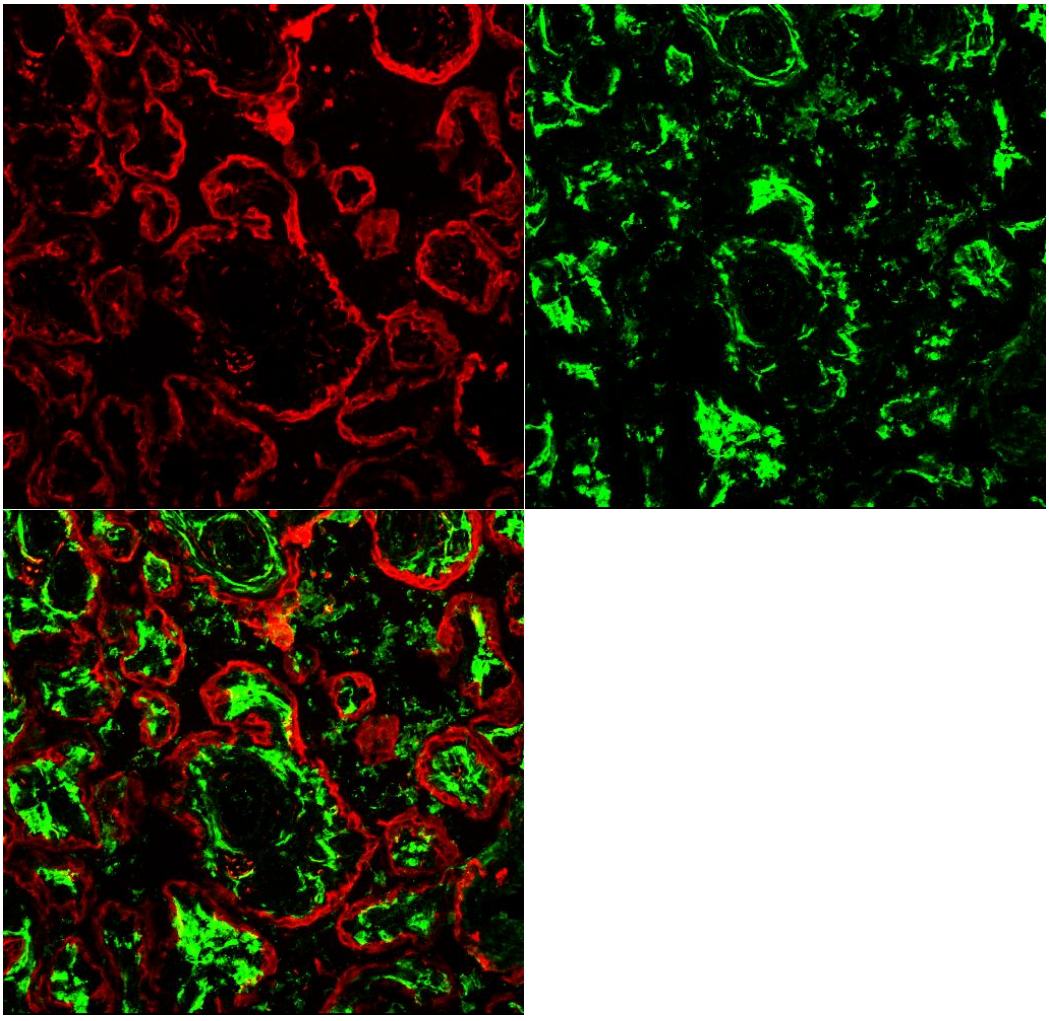
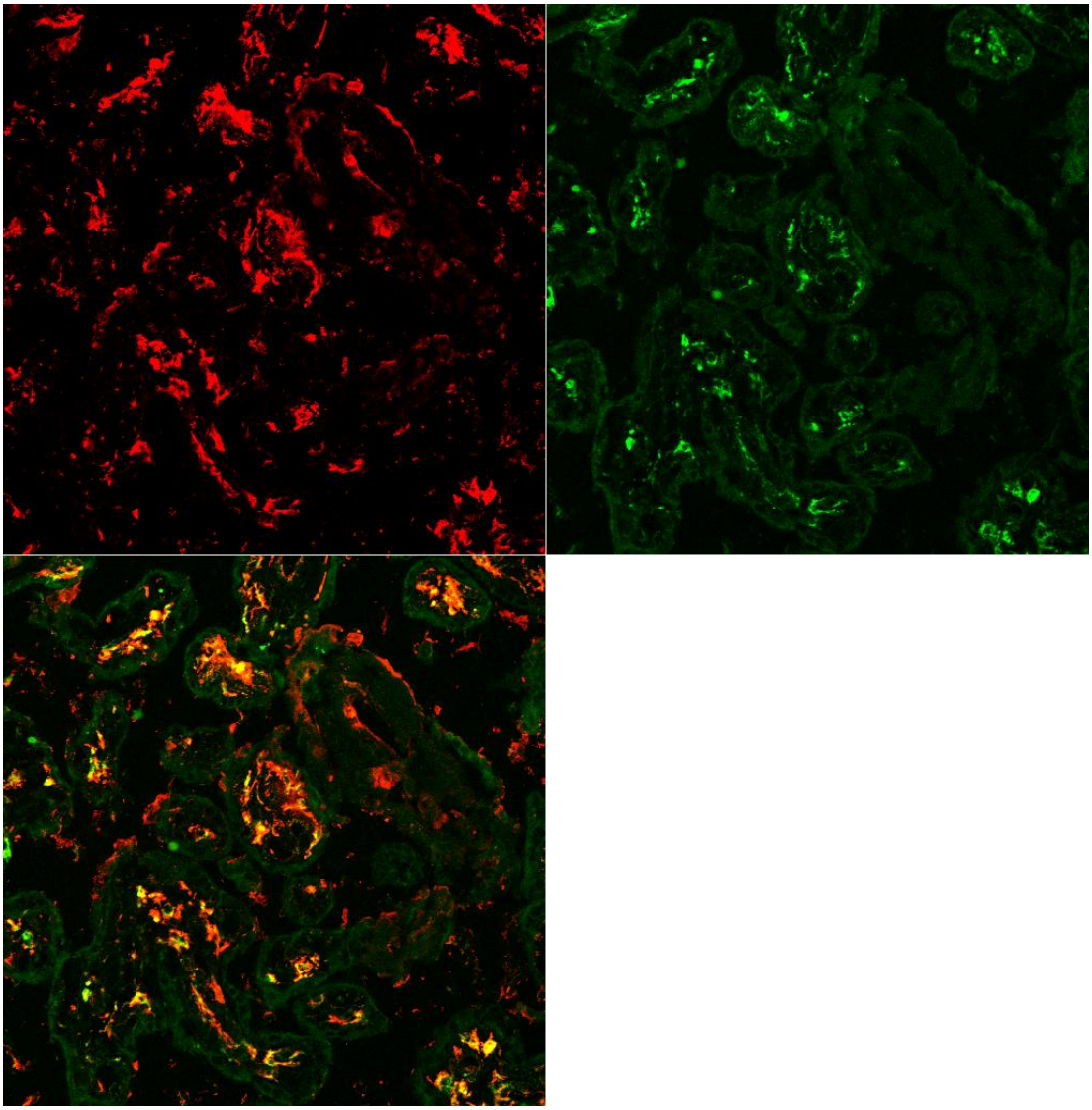


Figure 6.5b: CD206 and Adipsin dual staining on placental frozen sections (Adipsin=Red, CD206=Green)



CHAPTER 7

RESULTS

Systematic Investigations of FGF21 in lean, obese and GDM Pregnancies

RESULTS:

7.1 Plasma FGF21 is significantly elevated in GDM pregnancy.

Plasma FGF21 levels were found to be significantly elevated in GDM pregnant women (234.3pg/ml ± 30.698) compared to pregnant women with normal BMI (115.5pg/ml ± 31.768) ($p<0.05$) (Fig.7.1).

However, plasma FGF21 concentration in obese pregnant women (148.87 pg/ml ± 32.762) showed higher levels than the lean pregnant women and are not significant (Fig.7.1).

7.2 FGF21 concentration in CSF in lean, obese and GDM pregnant women

Having identified the levels of FGF21 in plasma in these three groups (lean, obese and GDM pregnant women), similar analyses was performed in their cerebrospinal fluids. Levels of FGF21 were found to be much lower in CSF than in maternal plasma (Fig .7.1&7.2)

CSF FGF21 concentration were found to be slightly elevated in GDM pregnant women (96.2 pg/ml ± 0.419) compared to lean pregnant women (93.1 pg/ml ± 0.173) and obese pregnancy (94.3 pg/ml ± 0.125) but these failed to reach significance (Fig.7.2).

7.3 CSF/Plasma FGF21 ratio in lean, obese and GDM pregnant women

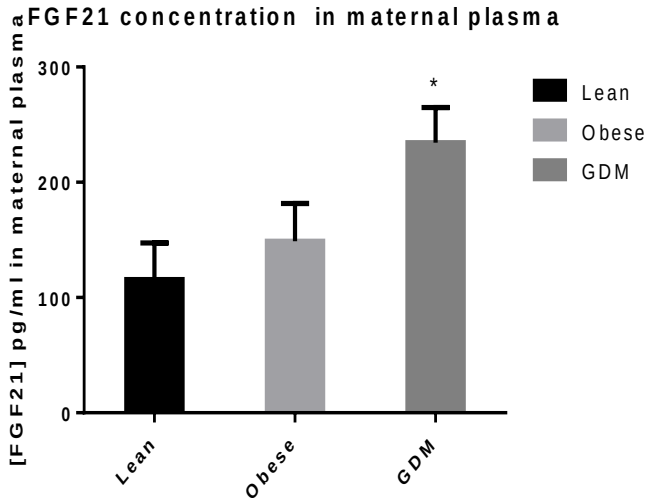
Having identified the levels of FGF21 in plasma and CSF in these three groups (lean, obese and GDM pregnant women), CSF/Plasma ratio was estimated. Interestingly, CSF/Plasma FGF21 ratio was significantly lower in women with GDM (0.4 ± 0.02) compared to lean pregnant (0.8 ± 0.01) and obese pregnant women (0.6 ± 0.03); ($p<0.05$) (Fig.7.3).

7.4 Secretion of FGF21 by human placenta

Analyses on explants media of both placental and adipose tissue were performed to identify if human placenta was also producing FGF21, as human placenta secretes many adipose tissue related proteins. As expected, adipose tissue (AT) secreted FGF21, with tissues from GDM patients producing slightly higher FGF21 per mg weight (0.215 pg/ml/mg ± 0.02) as compared to adipose tissue from lean mothers (0.1 pg/ml/mg ± 0.03) and obese pregnant patients (0.399 pg/ml /mg ± 0.01) and was not significant (Fig.7.4).

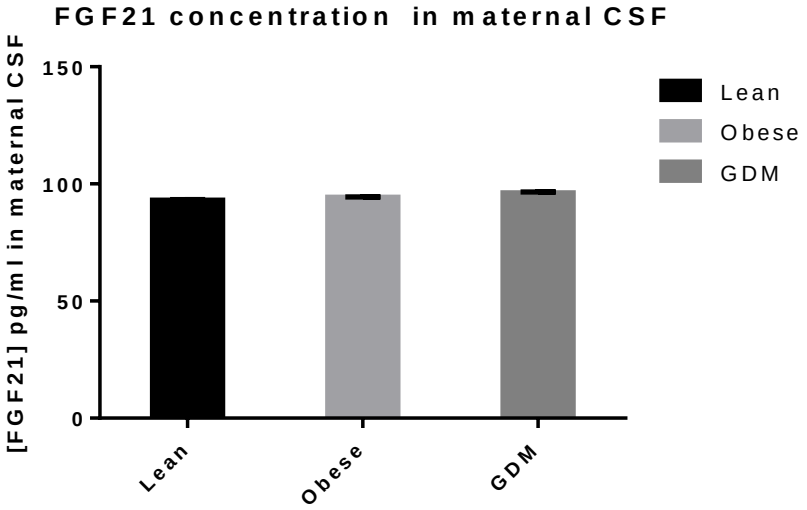
In addition, FGF21 secretion from placental explants, which were lower in placenta explants from GDM pregnancy (0.2 pg/ml/mg ± 0.02) than from placenta from lean pregnancy patients (0.28 pg/ml/mg ± 0.03) and placenta of obese pregnancy (0.48 pg/ml/mg ± 0.02) these differences were significant ($p<0.05$) (Fig.7.4).

Figure 7.1



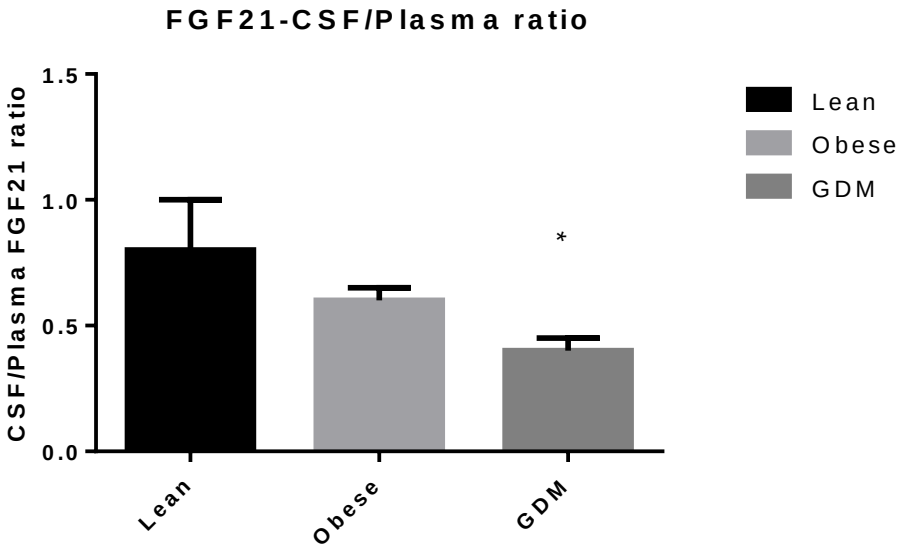
FGF21 concentration in maternal plasma in lean,obese and GDM using ELISA
Data are shown in mean and standard error of mean (SEM). *p<0.05 compared to lean controls

Figure 7.2



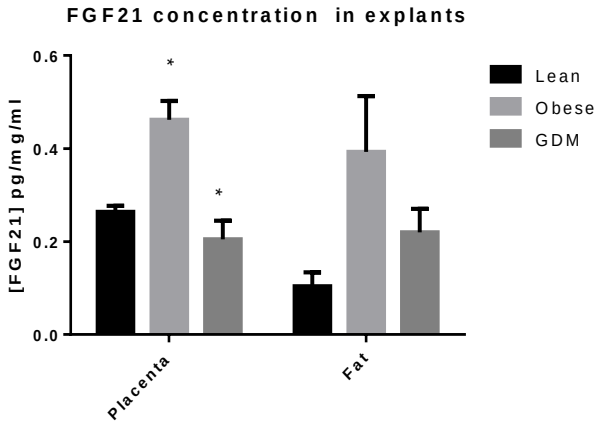
FGF21 concentration in maternal CSF in lean,obese and GDM using ELISA
Data are shown in mean and standard error of mean (SEM).
p=NS(non significant) compared to lean controls

Figure 7.3



FGF21-CSF/Plasma Ratio in maternal lean, obese and GDM.
***p<0.05 compared to lean controls**
CSF/Plasma FGF21 ratio was significantly lower in women with GDM

Figure 7.4



FGF21 Concentration in tissue explants of placenta & fat in pregnant women with normal BMI(lean), obese &GDM using ELISA
Data are shown as mean and standard error of mean(SEM). Day2-after 48 hrs of incubation of the conditioned medium.
***p<0.05 compared to lean controls.**

CHAPTER 8

GENERAL DISCUSSION

GENERAL DISCUSSION

A potential role for the adipsin/Acylation Stimulating Protein pathway and FGF21 in diabetic and obese pregnancy has been described. The adipsin/ASP pathway increases lipogenesis by increasing the activity of DGAT1, decreasing the activity of lipoprotein lipase (LPL) and stimulating the GLUT4 transporter at the adipocyte membrane. Adipsin has a physiological effect on the generation of ASP (also known as C3adesArg) together with C3 and factor B and this ASP has enzymatic activity in the synthesis and storage of triacylglycerol (Cianflone et al., 1994). Adipsin and ASP are well known to play significant roles in triglyceride storage, with elevations after high fat meals and both are known to be increased in circulating levels in association with obesity (Xia and Cianflone, 2003). Adipsin and ASP appears to be of particular importance in states of insulin resistance (Xia and Cianflone, 2003). Cianflone et al has described the adipsin/ASP pathway and shown that it raises triglyceride storage in adipocytes by increasing the activity of DGAT2 (Cianflone et al., 1994), decreasing the activity of hormone sensitive lipase (Yasruel et al., 1991), and increasing glucose transport (Cianflone et al., 2003)in the adipocytes.

As previously described in adipose tissue, my present data showed

a) That obese and GDM fetuses have higher levels of adipsin/ASP,

b) That the placenta is releasing adipsin/ASP and

c) That placental macrophages (Hofbauer cells) produce both components of this pathway.

More importantly, it has been shown that the receptor for ASP (C5L2) is expressed in the placenta (Cianflone et al., 1994) and circulating NEFA and TG levels in the fetus are significantly lower than in the mother suggesting that these molecules are having a biological effect. Having hypothesized that, in concert with hyperglycemia, the adipsin/ASP pathway provides a mechanism by which increased fetal fat mass seen in the clinical situations described above can be explained.

With regards to FGF21, a known insulin sensitizer; having identified lower CSF/plasma ratios of FGF21 in GDM pregnancy as well as having identified novel secretion of FGF21 from human placenta, with GDM explants producing less. These findings suggest a central mechanism of insulin resistance.

As discussed in the introduction, gestational diabetes and obesity are major obstetric problems. Despite clear differences between these two diseases there are a number of common themes, namely insulin resistance, dyslipidemia and fetal macrosomia. The later is particularly important since it predisposes to obesity and Type 2 diabetes later in life.

Fetal macrosomia, which is principally due to increased fetal fat mass (Sparks, 1984), occurs in 3-10% of all pregnancies (Martin et al., 2006) and is responsible for significant risks particularly in the offspring of diabetic mothers as well as those who are obese (but who retain normal glycaemic control). These include increased neonatal morbidity, neonatal injury, maternal injury and caesarean section (Spellacy et al., 1985). Equally importantly these babies have a greater risk of obesity and Type 2 diabetes in later life (Hillier et al., 2007, Catalano et al., 2009a). This issue is set to become more important as the incidence of maternal diabetes and obesity increases (Monte et al., 2011, Chen et al., 2012). In diabetes, this increase in fetal

fat mass has been described to the Pedersen hypothesis, which suggests elevated maternal glycemia results in fetal hyperglycemia, which then predisposes the fetus to produce more insulin, leading to an increase in fat.

However, there are significant clinical findings that that the Pedersen hypothesis is not able to completely explain the issues, where,

1. Those pregnancies with excellent glycemic control, and
2. Those obese pregnancies, (in the absence of hyperglycemia) are complicated by fetal macrosomia.

The babies of women with GDM have increased fetal fat mass (Sparks, 1984, Catalano et al., 2003b), as do the babies of women who are obese but not diabetic (Chu et al., 2009, Sewell et al., 2006) suggesting that hyperglycaemia, whilst important as a driver for intrauterine growth, may not wholly explain our common clinical observations.

As alluded to above, despite excellent glycaemic control, a significant number of diabetic women will have a macrosomic fetus of about 3.5 times higher risk than the general population (Evers et al., 2002, Murphy et al., 2008), which appears to be a function of the pregravid BMI (Langer et al., 2005). This finding is highlighted when considering that an overweight woman with well controlled GDM has a 50% higher chance of having a macrosomic baby compared to a lean woman with well-controlled GDM.

Obesity therefore, independently of diabetes, predisposes to fetal macrosomia (doubling the risk without diabetes) (Langer et al., 2005). While it is known that the increase in fetal macrosomia represents an increase in fetal fat mass, how the

maternal environment enhances this lipid deposition in fetal tissues, either in the absence of diabetes (as in obesity) or in well-controlled diabetes, is not completely understood. It has been (Sparks, 1984) hypothesized that genetic factors have a stronger relationship with fat-free mass (FFM), whereas the in utero environment may correlate better with fetal fat mass, in accordance with the fetal origins of disease hypothesis, and in agreement with this, the offspring of both GDM and obese women have increased risks of obesity and type 2 diabetes (Stothard et al., 2009, Garcia-Vargas et al., 2012). It is therefore important to identify common features between obese pregnancy and gestational diabetes (either well or poorly controlled) that might allow these discrepancies to be resolved, since these common features might impart some insight into a common mechanism.

A common feature between both GDM women and overweight women without diabetes is maternal insulin resistance and consequent dyslipidemia. Both GDM (Ramsay et al., 2002) and obese women without diabetes (Di Cianni et al., 2005) have higher circulating triglycerides (TG) and free fatty acids (FFA) than their respective lean controls. Circulating TG have been noted to positively correlate with birth weight, independently of obesity and glucose (Kitajima et al., 2001) and maternal TG in early pregnancy are significantly correlated with birth weight (Nolan et al., 1995). Maternal FFAs have been shown to correlate with fat mass at birth in well-controlled GDM populations (Schaefer-Graf et al., 2008) as well as in obese populations (Rao et al., 2012, Kitajima et al., 2001). However, whilst significant research into the role of glycemia has been performed, relatively little work has explored the potential roles of dyslipidemia in macrosomia.

Clearly, there is a requirement for the substrates for adipogenesis to be present in the maternal circulation and a mechanism by which these substrates can be transported across the placenta. As far as the former is concerned, all pregnancies are associated with a degree of hypertriglyceridemia as a result of the natural insulin resistance of pregnancy. This hypertriglyceridemia is more marked in diabetic and obese pregnancies, which would enhance substrate availability to the fetus.

This prompted me to explore alternate abnormal features of these pregnancies that are common and which might explain the fetal macrosomia seen in cases of diabetes with excellent glycemic control as well as obese pregnancies with normal glycemia.

I have therefore considered that, in order for adipogenesis in the fetus to occur, two principal requirements must be met:

1. The ability for the fetus to acquire these substrates in an insulin resistant environment.
2. The presence of substrates in the maternal compartment.

I have focussed on two mechanisms addressing each of these features. In terms of the mechanisms by which the fetus might access these substrates, I have investigated the role of the adipsin/ ASP pathway. In terms of the presence of substrates in the maternal compartment, I have assessed the role of FGF21.

There are some substantial evidence that the circulating triglycerides (TG) and free fatty acids (FFA) are elevated in both GDM (Ramsay et al., 2002) and obese pregnancy without diabetes (Di Cianni et al., 2005) than their respective lean controls.

The mechanism by which fat crosses the placenta is not completely understood, yet this would be crucial if fetal macrosomia is to be explained.

The previous paragraph has dealt with the evidence that these substrates are present, at higher levels, in the maternal compartment; however, little information is available about how the fetus acquires these substrates.

Whilst the fetus requires fatty acids for appropriate brain development, and in the case of macrosomia, for adipose tissue formation, there is no mechanism by which maternal lipoproteins directly transfer across the placenta. Indeed, the placenta possesses VLDL, LDL, HDL and LDL receptor related proteins, which allow these lipoproteins to be taken up by the placenta, where the actions of LPL, Phospholipase A2 and intracellular lipases on these allow fatty acids to be transferred to the fetus (Saleh et al., 2007, Contois et al., 1996).

The precise mechanisms by which these fatty acids are subsequently processed by the fetus are still unknown. Adipsin levels in human pregnancy, an insulin resistant state, have not previously been reported, but the levels of ASP have been described, with the evidence of maternal ASP levels increasing at late gestation (Saleh et al., 2007), increased maternal ASP with pregnancy weight gain (Sodowski et al., 2008), and increased fetal ASP in conjunction with the elevated maternal triglycerides (Saleh et al., 2008). The transfer of lipids are regulated in the normal placenta from maternal to fetal blood through storage and release of FFA, which are important for steroid hormone precursors as well as for energy source for the growing embryo (Shafrir and Barash, 1987). However, no previous reports have examined adipsin and ASP in obese pregnancy as well as in diabetic pregnancy.

This study has confirmed that both obese pregnant women and their fetuses in utero are insulin resistant as previously reported (Catalano et al., 2009b), as indicated by their elevated HOMA-IR values. In agreement with other studies (Harmon et al., 2011), significant differences in cholesterol, LDL and triglycerides with elevated levels are seen in obese mothers as well as in their corresponding cord bloods.

This study has also confirmed a marked difference in the concentration of fetal and maternal lipoproteins in pregnancies complicated by gestational diabetes as previously reported. Other studies have previously shown a significant increase in LDL cholesterol and decrease in HDL cholesterol in the cord blood of type 1 diabetes mellitus (Fordyce et al., 1983). The observations made in this study has a similar trend of these two variable cholesterols but statistical significance was apparent, also significant hypertriglyceridemia was noted in venous cord sera of gestational diabetes. The differences between these studies may be that Fordyce et al (Fordyce et al., 1983) did not analyze their data accounting for mode of delivery but in this study, all pregnant women were delivered in the morning by elective caesarean section under spinal anaesthesia after at least an eight hour fast. Thus several studies have shown that maternal diabetes might affect the metabolic components of the plasma in the circulation of fetal compartment. In this present study, both maternal obesity and maternal DM has been noted to exert an effect on lipoprotein concentration in fetal blood.

In GDM pregnancies, plasma levels of NEFA were increased 2-fold compared with levels in the plasma of non-diabetic mothers. Similarly, the cord blood levels of NEFA were lower compared with the levels in the diabetic mothers. The increased concentration of NEFA in cord blood of GDM pregnancies than the cord blood of non-diabetic lean mothers might probably be caused by increased delivery from the

maternal circulation. This could be explained from the previous reported studies that this increase in NEFA might be due to increased maternofetal gradient in diabetes (Thomas, 1987). This profuse supply of NEFA to the fetus might be an important factor stimulating synthesis of other lipoproteins.

In this study, the increase in maternal NEFA concentration in GDM could be explained by the relatively higher concentration of insulin in the peripheral blood circulation of gestational diabetes, as insulin induces the activity of lipoprotein lipase which increases the production of HDL precursors released during breakdown of triglycerides rich lipoproteins, which would explain this high concentration (Kissebah et al., 1976, Taskinen and Nikkila, 1979). Noteworthy was the observation that circulating triglyceride and NEFA levels were significantly lower in the fetal compartment than compared to maternal, which might suggest a physiological action of ASP at the placental barrier. To clarify this, further studies are needed in future.

Several studies have reported that in the human placenta, leptin is produced and is secreted into both the maternal and fetal circulation (Masuzaki et al., 1997). It has been reported that leptin levels in serum are generally related to adipose tissue mass and are correlated with body mass index and body fat mass in both pregnant (Highman et al., 1998), and non-pregnant adults (Considine et al., 1996). During normal pregnancy, the concentration of leptin in serum is doubled (Hardie et al., 1997, Highman et al., 1998).

The observation made in this thesis confirms that circulating concentrations of leptin are significantly doubled in both obese pregnant and GDM compared to their lean pregnant controls. Also the concentration of leptin was significantly higher in the cord bloods from obese pregnant as well as in the cord bloods from GDM. This

study has also confirmed that obese pregnant women have significantly higher plasma leptin concentrations than non obese lean pregnant women as previously reported (Misra and Trudeau, 2011), as indicated by their elevated leptin values.

A very important finding in this study was that circulating concentrations of adipsin in both obese pregnant and GDM mothers were increased compared to their lean pregnant controls. Of particular interest was the finding that adipsin was significantly higher in the cord bloods than in the maternal circulation, with a significant elevation in the cord bloods from obese mothers as well as in the cord bloods from GDM mothers.

In general, fasting ASP is strongly predictive of postprandial TG clearance in both men and women(Cianflone et al., 2004). It has been reported that ASP is increased in diabetes, as well as insulin resistance, obesity, hyperthyroidism, cardiovascular disease and polycystic ovary syndrome (Cianflone et al., 2003, Maslowska et al., 1999, Yang et al., 2006, Yu et al., 2006). The findings with ASP in the mother's blood also observations with higher levels noted in the obese pregnant groups than GDM pregnant groups.

The release of adipsin and ASP may be affected by a number of factors, including glucose, GDM, hormones and inflammatory stimuli which are known to affect insulin sensitivity. Due to the observed significantly increased fetal concentration of adipsin, together with elevated fetal concentration of ASP in both obese and GDM pregnancy has speculated whether the placenta might be a source of these molecules. I therefore also aimed to investigate the release of adipsin and ASP from placenta and adipose tissue.

Therefore paired explants experiments utilizing subcutaneous adipose tissue and placenta from lean pregnant, obese pregnant and GDM were carried out. Subcutaneous adipose tissue has previously been demonstrated to release similar amounts of adipsin than visceral tissue (Fain et al., 2007) and preliminary experiments confirmed these findings in our laboratory (data not shown).

Interestingly, it has been demonstrated that placental explants secrete both adipsin and ASP into the media. Of note, adipsin secretion from the placental explants was lower than that seen from the paired adipose tissue explants whilst ASP secretion from placental explants was substantially greater than that seen from adipose tissue. Whilst the secretion of adipsin from the placental explants was translated into higher levels of adipsin in cord blood, the extremely high levels of ASP released by placental explants did not translate into concomitantly higher levels of cord ASP, even though the cord bloods from obese mothers as well as GDM mothers did show a significant increase in ASP compared to lean pregnant controls. These findings suggest that either the explants environment stimulates ASP production, perhaps as a result of an altered oxygen tension in the culture media or the placenta utilizes ASP locally.

In addition to this, when the placental explants of both lean and obese pregnant are treated with insulin, it has been observed that there is an increase in the adipsin release than non–insulin treated placental explants, although this failed to reach significance. But in contrast, the adipsin release is found to be decreased in insulin treated GDM placental explants. Whilst the secretion of adipsin from adipose tissue explants of lean pregnant treated with insulin is higher than non-insulin adipose explants. Whereas, the adipsin release is observed to be decreased in both adipose explants of obese and GDM pregnant treated with insulin.

There are many possible reasons for these conflicting results between insulin and non-insulin treated tissue explants with respect to adipsin levels from lean, obese and GDM pregnant. As tissue explants are not only comprised of just one cell type and contain other paracrine mediators, such as immunocytes and vascular cells (non-trophoblast cells), these may be having an effect on adipsin release. Furthermore, several metabolic and hormonal changes that occur in insulin resistant women could contribute to this regulation of placental adipsin expression. To explore this further, future studies are needed.

Adipsin localization has been confirmed in muscle, lungs, adipose tissue and peripheral nerves (Chrast et al., 2004, White et al., 1992), also the localization in placenta has been reported in mouse placenta (Takeshita et al., 2010). However, there are only few fundamental data regarding the appearance and activity of adipsin in placenta.

Having demonstrated the novel secretion of both adipsin and ASP in the human placenta, we then intended to identify the cell types within the placenta that were responsible for the synthesis and secretion of these molecules. It has previously been demonstrated in adipose tissue that the non-adipocytes are responsible for the release of adipsin (Fain et al., 2007).

The placenta is a complex organ, comprised of trophoblast (cytotrophoblast and syncytio-trophoblast), endothelial cells, connective tissue, fibroblasts and Hofbauer cells. Hofbauer cells are of mesenchymal origin and are present throughout pregnancy. These phagocytic cells possess many of the cell surface markers associated with macrophages. Immuno-histochemistry revealed perivascular staining of adipsin, in a cell type that was neither endothelial nor trophoblast. Since positive

staining was localized to areas with both fibroblasts and Hofbauer cells, we further aimed to identify the responsible cell type using a negative immuno-selection technique that have previously used to isolate cell types from the placenta (Tang et al., 2011a). Use of this technique revealed that both adipsin and ASP were released from Hofbauer cells, with negligible release from any of the other cell types such as cytotrophoblast and fibroblasts. Increases in placental macrophage number in obese pregnancy have been demonstrated by a several other groups (Challier et al., 2008, Farley et al., 2009) and would explain the observed increased release of adipsin and ASP from placentae of obese women as well as from placentae of diabetic women. The increased release of adipsin and ASP from obese pregnant women, which would suggest a higher Hofbauer cell number, might be responsible for this increased secretion.

Immunoflourescence showed HBC and adipsin co-localization, and culturing separated cytotrophoblast, fibroblasts and HBC revealed that both adipsin and ASP were released from HBC, with negligible release from any of the other cell types seen. This novel finding indicates that Hofbauer cells release molecules associated with regulation of triglyceride metabolism.

After showing adipsin/ASP might play a role in increasing substrate transfer across the placenta, I then focused on mechanisms by which these adipogenic substrates might be increased in maternal disease. In terms of the presence of substrates in the maternal compartment, I have assessed the role of FGF21.

FGF21 is predominately a hepatokine, also known to be secreted by adipose tissue. Interestingly, human placenta secretes many adipose tissue related proteins and hence I sought to investigate whether the human placenta secreted FGF21. Unlike circulating levels, FGF21 secretion into conditioned media was significantly lower

in human placental explants from women with GDM compared to lean and obese pregnant women.

Recently, Fibroblast Growth Factor 21 (FGF21) has been described as a regulator of metabolism and were positively associated with obesity, metabolic syndrome and in type 2 diabetes mellitus (Zhang et al., 2008, Galman et al., 2008, Li et al., 2008, Li et al., 2009). It has been reported that FGF21 alleviated obesity in mice (Coskun et al., 2008). Recently, it has also been demonstrated that FGF21 crosses the blood-brain barrier (BBB) in mice (Hsuchou et al., 2007). Another study has examined the presence of FGF21 in human CSF and also the CSF concentrations of FGF21 in relation to corresponding plasma levels (Tan et al., 2011).

Given the above, CSF concentrations of FGF21 in relation to corresponding plasma levels within these same cohorts of subjects (lean, obese and GDM pregnant) have been studied in agreement with recent studies (Zhang et al., 2008, Li et al., 2008, Tan et al., 2011).

I present novel data of CSF and plasma FGF21 levels in women with GDM, obese pregnant and matched (age, BMI) controls. Circulating FGF21 levels were significantly higher in women with GDM compared to controls in agreement with the only study measuring circulating FGF21 levels in women with GDM (Stein et al., 2010). There were no significant differences in plasma FGF21 levels in obese pregnant women than the lean pregnant women. Additionally, there were no significant differences in CSF FGF21 levels in women with GDM, obese pregnant compared to lean controls. Importantly, the CSF/Plasma FGF21 ratio was significantly lower in women with GDM compared to control subjects.

Furthermore, it is possible that FGF21 has protein binding and these differences in binding of protein in lean, obese and GDM pregnant may explain these findings.

Moreover, it is possible that the uptake of FGF21 into CSF is reduced in obese pregnant women may be secondary to transporters saturation. However it has been reported that FGF21 crosses the blood brain barrier nonsaturably and through slow passive diffusion (Hsuchou et al., 2007), this could explain the above findings. To clarify these points, future studies are needed in future.

Recently, a landmark study by Sarruf et al. had shown that continuous intracerebroventricular infusion of FGF21 stimulated appetite and energy production in rats by improving insulin sensitivity via increased insulin induced inhibition of hepatic gluconeogenesis (Sarruf et al., 2010). It was proposed that FGF21 engages with fibroblast growth factor receptor-1, which is mainly expressed in the arcuate and ventromedial nuclei of the hypothalamus (areas of the hypothalamus that also mediate the central metabolic effects of insulin, leptin, oleate and glucose) and regulates gluconeogenesis (Sarruf et al., 2010).

In order for FGF21 to impose its central effects, circulating FGF21 should cross the blood-brain and/or blood-CSF barriers. In relation to this, Hsuchou et al. surmised that FGF21 traverses the BBB by simple diffusion in mice (Hsuchou et al., 2007). My study has found the novel finding of a significantly lower CSF/Plasma FGF21 ratio in women with GDM compared to obese pregnant as well as lean pregnant subjects implies either that there is a deficiency of FGF21 transport across the BBB or that discrepancies in the production and/or metabolism of FGF21 by the central nervous system may explain the differences in CSF/plasma FGF21 ratio between the GDM and obese pregnant as well as lean controls in this study.

In relation to this, Yamashita et al. had reported that FGF23 is produced in the brain, particularly, by the ventrolateral thalamic nuclei (Yamashita et al., 2000). It is thus possible that FGF21 may also be produced in the brain (Tan et al., 2011). Moreover,

it is probable that FGF21 has protein binding, and that differences in protein binding between GDM and obese pregnant as well as lean pregnant subjects may also explain these findings. Furthermore, it is potential that the efficiency of FGF21 uptake into the CSF is decreased in GDM subjects possibly secondary to saturation of transporters. When saying this, the evidence by Hsuchou et al. who had shown that FGF21 crosses the BBB non-saturably (Hsuchou et al., 2007) has been considered. The coexistence of non-saturable and saturable mechanisms is plausible given that other adipokines, for example, leptin, have been shown to cross the BBB through both non-saturable and saturable mechanisms (Nam et al., 2001).

Taken together, given that FGF21 crosses the BBB via a relatively slow passive diffusion and exhibits considerable degradation in the central nervous system (Hsuchou et al., 2007) as well as my findings of lower secretion of FGF21 by GDM placenta; aberrations in the production of FGF21 within the brain may be the main explanation of the lower CSF/FGF21 ratio observed in GDM subjects than the other two study subjects (obese pregnant and lean pregnant).

Given the recently reported central nervous system actions of FGF21 (Sarruf et al., 2010), it could be hypothesized that the relatively lower levels of FGF21 in CSF in GDM subjects may lead to dysfunctional FGF21 signaling in the brain and in turn contribute to the insulin resistance of GDM subjects in line with the report of Sarruf et al. This is plausible as dysfunctional central nervous system signaling of other growth factors and adipokines such as insulin and leptin, has been reported (Morton et al., 2006). Further studies are needed to clarify these points.

In addition, this study has reported for the first time the secretion of FGF21 by the placenta. It has been also found that the secretion of FGF21 from human placental explants of women with GDM was significantly lower compared to obese pregnant

as well as lean pregnant women. This might postulate an impact upon both maternal and fetal physiology and lead to the complications observed in women with GDM.

Strengths of this study include, the relatively large number of subjects investigated, the identification of placental HBC as the source of adipsin and ASP, and support of this findings from results in ex vivo explants.

Limitations include, the use of estimates of insulin sensitivity rather than measuring this parameter using euglycemic hyperinsulinemic clamps (which understandably would not receive ethical approval in our institution), and the lack of information about body fat distribution in the pregnant participants (although leptin was utilized as a surrogate marker of fat mass). Furthermore, since only Caucasian women were investigated, these results may not apply in members of other ethnicities. Finally, whether high adipsin levels in the fetus may be casually involved in driving up the birth weight and insulin resistance, or just reflect a phenomenon of higher permeability or diffusion for adipsin and ASP at the fetal end of the cord needs to be investigated in future studies.

Furthermore, limitation of the FGF21 study is that the presence of other members of the FGF family in human CSF has not been assessed. Moreover, the energy status in these study subjects has not been measured. Given that intracerebroventricular infusion of FGF21 increased appetite and energy production in rats (Sarruf et al., 2010), it would be of interest to establish the relationship between the levels of other members of the FGF family in human CSF with FGF21 and could have been correlated with the energy status of these study subjects. Finally, the role of FGF21 derived from the placenta on both mother and child has not been explored.

Conclusions

In conclusion, given the relative insulin resistance in pregnancy (particularly in obese pregnancy and GDM pregnancy), these molecules may play a role in triglyceride metabolism in the fetus. Having demonstrated the concentrations of adipsin were considerably higher in the cord blood than in the mothers and this was exacerbated in obese pregnancies than GDM pregnancies. It seems that adipsin has an influential effect in placental formation and for the development of growing embryo through the other effects such as lipid metabolism in placenta. Additionally, having identified the source of adipsin and ASP as coming from the Hofbauer cells (specialized placental macrophages) equally, these data place HBC as the source of these metabolically active molecules and implicates these cells in nutrient sensing at the materno-fetal interface. The central actions of FGF21 in GDM subjects may be pivotal in the pathogenesis of insulin resistance in GDM subjects. The significance of FGF21 produced by the placenta remains by and large unexplored. Future research should aim to reveal these points.

This study has aimed to improve the understanding the effects of obesity in pregnancy as well as diabetic pregnancy on changes in physiological and metabolic factors that might influence intrauterine environment for fetus and pregnancy outcomes in particular fetal macrosomia, since it predisposes to obesity and Type 2 diabetes mellitus later in life.

CHAPTER 9

REFERENCES

2000. Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. *Nat Genet,* 26**,** 345-8.
AHIMA, R. S. & FLIER, J. S. 2000. Leptin. *Annu Rev Physiol,* 62**,** 413-37.
AHIMA, R. S., PRABAKARAN, D., MANTZOROS, C., QU, D., LOWELL, B., MARATOS-FLIER, E. & FLIER, J. S. 1996. Role of leptin in the neuroendocrine response to fasting. *Nature,* 382**,** 250-2.
ALBERTI, K. G. & ZIMMET, P. Z. 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med,* 15**,** 539-53.
ALESSI, M. C., PARROT, G., GUENOUN, E., SCELLES, V., VAGUE, P. & JUHAN-VAGUE, I. 1995. Relation between plasma PAI activity and adipsin levels. *Thromb Haemost,* 74**,** 1200-2.
ALVAREZ, J. J., MONTELONGO, A., IGLESIAS, A., LASUNCION, M. A. & HERRERA, E. 1996. Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. *J Lipid Res,* 37**,** 299-308.
ALVINO, G., COZZI, V., RADAELLI, T., ORTEGA, H., HERRERA, E. & CETIN, I. 2008. Maternal and fetal fatty acid profile in normal and intrauterine growth restriction pregnancies with and without preeclampsia. *Pediatr Res,* 64**,** 615-20.
AMES, R. S., LI, Y., SARAU, H. M., NUTHULAGANTI, P., FOLEY, J. J., ELLIS, C., ZENG, Z., SU, K., JUREWICZ, A. J., HERTZBERG, R. P., BERGSMA, D. J. & KUMAR, C. 1996. Molecular cloning and characterization of the human anaphylatoxin C3a receptor. *J Biol Chem,* 271**,** 20231-4.
ATEGBO, J. M., GRISSA, O., YESSOUFOU, A., HICHAMI, A., DRAMANE, K. L., MOUTAIROU, K., MILED, A., GRISSA, A., JERBI, M., TABKA, Z. & KHAN, N. A. 2006. Modulation of adipokines and cytokines in gestational diabetes and macrosomia. *J Clin Endocrinol Metab,* 91**,** 4137-43.
AUBERT, M. L. & SIZONENKO, P. C. 1996. Environmental factors and sexual maturation in rodents. *Acta Paediatr Suppl,* 417**,** 86-8.
AVRAM, M. M., AVRAM, A. S. & JAMES, W. D. 2007. Subcutaneous fat in normal and diseased states 3. Adipogenesis: from stem cell to fat cell. *J Am Acad Dermatol,* 56**,** 472-92.
AY, L., VAN HOUTEN, V. A., STEEGERS, E. A., HOFMAN, A., WITTEMAN, J. C., JADDOE, V. W. & HOKKEN-KOELEGA, A. C. 2009. Fetal and postnatal growth and body composition at 6 months of age. *J Clin Endocrinol Metab,* 94**,** 2023-30.
BADMAN, M. K., KOESTER, A., FLIER, J. S., KHARITONENKOV, A. & MARATOS-FLIER, E. 2009. Fibroblast growth factor 21-deficient mice demonstrate impaired adaptation to ketosis. *Endocrinology,* 150**,** 4931-40.
BALDO, A., SNIDERMAN, A. D., ST-LUCE, S., AVRAMOGLU, R. K., MASLOWSKA, M., HOANG, B., MONGE, J. C., BELL, A., MULAY, S. & CIANFLONE, K. 1993. The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. *J Clin Invest,* 92**,** 1543-7.
BANKS, W. A. 2004. The many lives of leptin. *Peptides,* 25**,** 331-8.
BANKS, W. A. & FARRELL, C. L. 2003. Impaired transport of leptin across the blood-brain barrier in obesity is acquired and reversible. *Am J Physiol Endocrinol Metab,* 285**,** E10-5.
BARKER, D. J. 1990. The fetal and infant origins of adult disease. *BMJ,* 301**,** 1111.

BEENKEN, A. & MOHAMMADI, M. 2009. The FGF family: biology, pathophysiology and therapy. *Nat Rev Drug Discov,* 8**,** 235-53.
BENASSAYAG, C., MIGNOT, T. M., HAOURIGUI, M., CIVEL, C., HASSID, J., CARBONNE, B., NUNEZ, E. A. & FERRE, F. 1997. High polyunsaturated fatty acid, thromboxane A2, and alpha-fetoprotein concentrations at the human feto-maternal interface. *J Lipid Res,* 38**,** 276-86.
BENYO, D. F., SMARASON, A., REDMAN, C. W., SIMS, C. & CONRAD, K. P. 2001. Expression of inflammatory cytokines in placentas from women with preeclampsia. *J Clin Endocrinol Metab,* 86**,** 2505-12.
BERGLUND, E. D., LI, C. Y., BINA, H. A., LYNES, S. E., MICHAEL, M. D., SHANAFELT, A. B., KHARITONENKOV, A. & WASSERMAN, D. H. 2009. Fibroblast growth factor 21 controls glycemia via regulation of hepatic glucose flux and insulin sensitivity. *Endocrinology,* 150**,** 4084-93.
BERTI, L. & GAMMELTOFT, S. 1999. Leptin stimulates glucose uptake in C2C12 muscle cells by activation of ERK2. *Mol Cell Endocrinol,* 157**,** 121-30.
BERTI, L., KELLERER, M., CAPP, E. & HARING, H. U. 1997. Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: evidence for a P13-kinase mediated effect. *Diabetologia,* 40**,** 606-9.
BHATTACHARYA, S. M. 2004. Fasting or two-hour postprandial plasma glucose levels in early months of pregnancy as screening tools for gestational diabetes mellitus developing in later months of pregnancy. *J Obstet Gynaecol Res,* 30**,** 333-6.
BLAIR, N. J., THOMPSON, J. M., BLACK, P. N., BECROFT, D. M., CLARK, P. M., HAN, D. Y., ROBINSON, E., WALDIE, K. E., WILD, C. J. & MITCHELL, E. A. 2007. Risk factors for obesity in 7-year-old European children: the Auckland Birthweight Collaborative Study. *Arch Dis Child,* 92**,** 866-71.
BO, S., MENATO, G., GALLO, M. L., BARDELLI, C., LEZO, A., SIGNORILE, A., GAMBINO, R., CASSADER, M., MASSOBRIO, M. & PAGANO, G. 2004. Mild gestational hyperglycemia, the metabolic syndrome and adverse neonatal outcomes. *Acta Obstet Gynecol Scand,* 83**,** 335-40.
BODEN, G. 2006. Fatty acid-induced inflammation and insulin resistance in skeletal muscle and liver. *Curr Diab Rep,* 6**,** 177-81.
BONET, B. & HERRERA, E. 1991. Maternal hypothyroidism during the first half of gestation compromises normal catabolic adaptations of late gestation in the rat. *Endocrinology,* 129**,** 210-6.
BOOMSMA, C. M., EIJKEMANS, M. J., HUGHES, E. G., VISSER, G. H., FAUSER, B. C. & MACKLON, N. S. 2006. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. *Hum Reprod Update,* 12**,** 673-83.
BOWEN, J. M., CHAMLEY, L., MITCHELL, M. D. & KEELAN, J. A. 2002. Cytokines of the placenta and extra-placental membranes: biosynthesis, secretion and roles in establishment of pregnancy in women. *Placenta,* 23**,** 239-56.
BROWN, C. D., HIGGINS, M., DONATO, K. A., ROHDE, F. C., GARRISON, R., OBARZANEK, E., ERNST, N. D. & HORAN, M. 2000. Body mass index and the prevalence of hypertension and dyslipidemia. *Obes Res,* 8**,** 605-19.
BRUUN, J. M., LIHN, A. S., VERDICH, C., PEDERSEN, S. B., TOUBRO, S., ASTRUP, A. & RICHELSEN, B. 2003. Regulation of adiponectin by adipose

tissue-derived cytokines: in vivo and in vitro investigations in humans. *Am J Physiol Endocrinol Metab,* 285**,** E527-33.
BULMER, J. N. & JOHNSON, P. M. 1984. Macrophage populations in the human placenta and amniochorion. *Clin Exp Immunol,* 57**,** 393-403.
BURDETTE, H. L., WHITAKER, R. C., HALL, W. C. & DANIELS, S. R. 2006. Maternal infant-feeding style and children's adiposity at 5 years of age. *Arch Pediatr Adolesc Med,* 160**,** 513-20.
CABALLERO, B. 2005. A nutrition paradox--underweight and obesity in developing countries. *N Engl J Med,* 352**,** 1514-6.
CALLAWAY, L. K., PRINS, J. B., CHANG, A. M. & MCINTYRE, H. D. 2006. The prevalence and impact of overweight and obesity in an Australian obstetric population. *Med J Aust,* 184**,** 56-9.
CAREY, V. J., WALTERS, E. E., COLDITZ, G. A., SOLOMON, C. G., WILLETT, W. C., ROSNER, B. A., SPEIZER, F. E. & MANSON, J. E. 1997. Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. *Am J Epidemiol,* 145**,** 614-9.
CARROLL, C. S., SR., MAGANN, E. F., CHAUHAN, S. P., KLAUSER, C. K. & MORRISON, J. C. 2003. Vaginal birth after cesarean section versus elective repeat cesarean delivery: Weight-based outcomes. *Am J Obstet Gynecol,* 188**,** 1516-20; discussion 1520-2.
CASTELLUCCI, M., KOSANKE, G., VERDENELLI, F., HUPPERTZ, B. & KAUFMANN, P. 2000. Villous sprouting: fundamental mechanisms of human placental development. *Hum Reprod Update,* 6**,** 485-94.
CASTELLUCCI, M., ZACCHEO, D. & PESCETTO, G. 1980. A three-dimensional study of the normal human placental villous core. I. The Hofbauer cells. *Cell Tissue Res,* 210**,** 235-47.
CATALANO, P. M. & EHRENBERG, H. M. 2006. The short- and long-term implications of maternal obesity on the mother and her offspring. *BJOG,* 113**,** 1126-33.
CATALANO, P. M., FARRELL, K., THOMAS, A., HUSTON-PRESLEY, L., MENCIN, P., DE MOUZON, S. H. & AMINI, S. B. 2009a. Perinatal risk factors for childhood obesity and metabolic dysregulation. *Am J Clin Nutr,* 90**,** 1303-13.
CATALANO, P. M., HUSTON, L., AMINI, S. B. & KALHAN, S. C. 1999. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. *Am J Obstet Gynecol,* 180**,** 903-16.
CATALANO, P. M., KIRWAN, J. P., HAUGEL-DE MOUZON, S. & KING, J. 2003a. Gestational diabetes and insulin resistance: role in short- and long-term implications for mother and fetus. *J Nutr,* 133**,** 1674S-1683S.
CATALANO, P. M., NIZIELSKI, S. E., SHAO, J., PRESTON, L., QIAO, L. & FRIEDMAN, J. E. 2002. Downregulated IRS-1 and PPARgamma in obese women with gestational diabetes: relationship to FFA during pregnancy. *Am J Physiol Endocrinol Metab,* 282**,** E522-33.
CATALANO, P. M., PRESLEY, L., MINIUM, J. & HAUGUEL-DE MOUZON, S. 2009b. Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care,* 32**,** 1076-80.
CATALANO, P. M., THOMAS, A., HUSTON-PRESLEY, L. & AMINI, S. B. 2003b. Increased fetal adiposity: a very sensitive marker of abnormal in utero development. *Am J Obstet Gynecol,* 189**,** 1698-704.

CATALANO, P. M., TYZBIR, E. D., ROMAN, N. M., AMINI, S. B. & SIMS, E. A. 1991. Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women. *Am J Obstet Gynecol,* 165**,** 1667-72.

CATALANO, P. M., TYZBIR, E. D., WOLFE, R. R., CALLES, J., ROMAN, N. M., AMINI, S. B. & SIMS, E. A. 1993. Carbohydrate metabolism during pregnancy in control subjects and women with gestational diabetes. *Am J Physiol,* 264**,** E60-7.

CATALANO, P. M., TYZBIR, E. D., WOLFE, R. R., ROMAN, N. M., AMINI, S. B. & SIMS, E. A. 1992. Longitudinal changes in basal hepatic glucose production and suppression during insulin infusion in normal pregnant women. *Am J Obstet Gynecol,* 167**,** 913-9.

CHALLIER, J. C., BASU, S., BINTEIN, T., MINIUM, J., HOTMIRE, K., CATALANO, P. M. & HAUGUEL-DE MOUZON, S. 2008. Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. *Placenta,* 29**,** 274-81.

CHANDRA, R. K. 1991. 1990 McCollum Award lecture. Nutrition and immunity: lessons from the past and new insights into the future. *Am J Clin Nutr,* 53**,** 1087-101.

CHEN, H. L., YANG, Y. P., HU, X. L., YELAVARTHI, K. K., FISHBACK, J. L. & HUNT, J. S. 1991. Tumor necrosis factor alpha mRNA and protein are present in human placental and uterine cells at early and late stages of gestation. *Am J Pathol,* 139**,** 327-35.

CHEN, L., MAGLIANO, D. J. & ZIMMET, P. Z. 2012. The worldwide epidemiology of type 2 diabetes mellitus--present and future perspectives. *Nat Rev Endocrinol,* 8**,** 228-36.

CHEN, X. & SCHOLL, T. O. 2008. Association of elevated free fatty acids during late pregnancy with preterm delivery. *Obstet Gynecol,* 112**,** 297-303.

CHOY, L. N., ROSEN, B. S. & SPIEGELMAN, B. M. 1992. Adipsin and an endogenous pathway of complement from adipose cells. *J Biol Chem,* 267**,** 12736-41.

CHOY, L. N. & SPIEGELMAN, B. M. 1996. Regulation of alternative pathway activation and C3a production by adipose cells. *Obes Res,* 4**,** 521-32.

CHRAST, R., VERHEIJEN, M. H. & LEMKE, G. 2004. Complement factors in adult peripheral nerve: a potential role in energy metabolism. *Neurochem Int,* 45**,** 353-9.

CHU, S. Y., CALLAGHAN, W. M., BISH, C. L. & D'ANGELO, D. 2009. Gestational weight gain by body mass index among US women delivering live births, 2004-2005: fueling future obesity. *Am J Obstet Gynecol,* 200**,** 271 e1-7.

CHU, S. Y., CALLAGHAN, W. M., KIM, S. Y., SCHMID, C. H., LAU, J., ENGLAND, L. J. & DIETZ, P. M. 2007a. Maternal obesity and risk of gestational diabetes mellitus. *Diabetes Care,* 30**,** 2070-6.

CHU, S. Y., KIM, S. Y., LAU, J., SCHMID, C. H., DIETZ, P. M., CALLAGHAN, W. M. & CURTIS, K. M. 2007b. Maternal obesity and risk of stillbirth: a metaanalysis. *Am J Obstet Gynecol,* 197**,** 223-8.

CHU, S. Y., KIM, S. Y., SCHMID, C. H., DIETZ, P. M., CALLAGHAN, W. M., LAU, J. & CURTIS, K. M. 2007c. Maternal obesity and risk of cesarean delivery: a meta-analysis. *Obes Rev,* 8**,** 385-94.

CHUA, S. C., JR., CHUNG, W. K., WU-PENG, X. S., ZHANG, Y., LIU, S. M., TARTAGLIA, L. & LEIBEL, R. L. 1996. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. *Science,* 271**,** 994-6.
CIANFLONE, K., KALANT, D., MARLISS, E. B., GOUGEON, R. & SNIDERMAN, A. D. 1995a. Response of plasma ASP to a prolonged fast. *Int J Obes Relat Metab Disord,* 19**,** 604-9.
CIANFLONE, K., MASLOWSKA, M. & SNIDERMAN, A. 1995b. The acylation stimulating protein-adipsin system. *Int J Obes Relat Metab Disord,* 19 Suppl 1**,** S34-8.
CIANFLONE, K., MASLOWSKA, M. & SNIDERMAN, A. D. 1999. Acylation stimulating protein (ASP), an adipocyte autocrine: new directions. *Semin Cell Dev Biol,* 10**,** 31-41.
CIANFLONE, K., RONCARI, D. A., MASLOWSKA, M., BALDO, A., FORDEN, J. & SNIDERMAN, A. D. 1994. Adipsin/acylation stimulating protein system in human adipocytes: regulation of triacylglycerol synthesis. *Biochemistry,* 33**,** 9489-95.
CIANFLONE, K., XIA, Z. & CHEN, L. Y. 2003. Critical review of acylation-stimulating protein physiology in humans and rodents. *Biochim Biophys Acta,* 1609**,** 127-43.
CIANFLONE, K., ZAKARIAN, R., COUILLARD, C., DELPLANQUE, B., DESPRES, J. P. & SNIDERMAN, A. 2004. Fasting acylation-stimulating protein is predictive of postprandial triglyceride clearance. *J Lipid Res,* 45**,** 124-31.
CIANFLONE, K. M., MASLOWSKA, M. H. & SNIDERMAN, A. D. 1990. Impaired response of fibroblasts from patients with hyperapobetalipoproteinemia to acylation-stimulating protein. *J Clin Invest,* 85**,** 722-30.
CIANFLONE, K. M., SNIDERMAN, A. D., WALSH, M. J., VU, H. T., GAGNON, J. & RODRIGUEZ, M. A. 1989. Purification and characterization of acylation stimulating protein. *J Biol Chem,* 264**,** 426-30.
CINTI, S., MITCHELL, G., BARBATELLI, G., MURANO, I., CERESI, E., FALOIA, E., WANG, S., FORTIER, M., GREENBERG, A. S. & OBIN, M. S. 2005. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res,* 46**,** 2347-55.
CLEMENT, K., VAISSE, C., LAHLOU, N., CABROL, S., PELLOUX, V., CASSUTO, D., GOURMELEN, M., DINA, C., CHAMBAZ, J., LACORTE, J. M., BASDEVANT, A., BOUGNERES, P., LEBOUC, Y., FROGUEL, P. & GUY-GRAND, B. 1998. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature,* 392**,** 398-401.
COLEMAN, R. A. & HAYNES, E. B. 1987. Synthesis and release of fatty acids by human trophoblast cells in culture. *J Lipid Res,* 28**,** 1335-41.
COLOMIERE, M., PERMEZEL, M., RILEY, C., DESOYE, G. & LAPPAS, M. 2009. Defective insulin signaling in placenta from pregnancies complicated by gestational diabetes mellitus. *Eur J Endocrinol,* 160**,** 567-78.
CONFIDENTIAL ENQUIRY INTO MATERNAL AND CHILD HEALTH. 2009. *Confidential enquiry into maternal and child health (CEMACH) : perinatal mortality 2007: United Kingdom,* London, CEMACH.
CONSIDINE, R. V., SINHA, M. K., HEIMAN, M. L., KRIAUCIUNAS, A., STEPHENS, T. W., NYCE, M. R., OHANNESIAN, J. P., MARCO, C. C., MCKEE, L. J., BAUER, T. L. & ET AL. 1996. Serum immunoreactive-leptin

concentrations in normal-weight and obese humans. *N Engl J Med,* 334**,** 292-5.

CONTOIS, J. H., LAMMI-KEEFE, C. J., VOGEL, S., MCNAMARA, J. R., WILSON, P. W., MASSOV, T. & SCHAEFER, E. J. 1996. Plasma lipoprotein(a) distribution in the Framingham Offspring Study as determined with a commercially available immunoturbidimetric assay. *Clin Chim Acta,* 253**,** 21-35.

COOK, K. S., GROVES, D. L., MIN, H. Y. & SPIEGELMAN, B. M. 1985. A developmentally regulated mRNA from 3T3 adipocytes encodes a novel serine protease homologue. *Proc Natl Acad Sci U S A,* 82**,** 6480-4.

COOK, K. S., MIN, H. Y., JOHNSON, D., CHAPLINSKY, R. J., FLIER, J. S., HUNT, C. R. & SPIEGELMAN, B. M. 1987. Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. *Science,* 237**,** 402-5.

CORREIA, M. L. & RAHMOUNI, K. 2006. Role of leptin in the cardiovascular and endocrine complications of metabolic syndrome. *Diabetes Obes Metab,* 8**,** 603-10.

COSKUN, T., BINA, H. A., SCHNEIDER, M. A., DUNBAR, J. D., HU, C. C., CHEN, Y., MOLLER, D. E. & KHARITONENKOV, A. 2008. Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology,* 149**,** 6018-27.

COUGHLAN, M. T., OLIVA, K., GEORGIOU, H. M., PERMEZEL, J. M. & RICE, G. E. 2001. Glucose-induced release of tumour necrosis factor-alpha from human placental and adipose tissues in gestational diabetes mellitus. *Diabet Med,* 18**,** 921-7.

COUSTAN, D. R. & CARPENTER, M. W. 1998. The diagnosis of gestational diabetes. *Diabetes Care,* 21 Suppl 2**,** B5-8.

COX, N. J. 1994. Maternal component in NIDDM transmission. How large an effect? *Diabetes,* 43**,** 166-8.

CRASS, T., RAFFETSEDER, U., MARTIN, U., GROVE, M., KLOS, A., KOHL, J. & BAUTSCH, W. 1996. Expression cloning of the human C3a anaphylatoxin receptor (C3aR) from differentiated U-937 cells. *Eur J Immunol,* 26**,** 1944-50.

CUI, W., PAGLIALUNGA, S., KALANT, D., LU, H., ROY, C., LAPLANTE, M., DESHAIES, Y. & CIANFLONE, K. 2007. Acylation-stimulating protein/C5L2-neutralizing antibodies alter triglyceride metabolism in vitro and in vivo. *Am J Physiol Endocrinol Metab,* 293**,** E1482-91.

DABELEA, D., HANSON, R. L., LINDSAY, R. S., PETTITT, D. J., IMPERATORE, G., GABIR, M. M., ROUMAIN, J., BENNETT, P. H. & KNOWLER, W. C. 2000. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. *Diabetes,* 49**,** 2208-11.

DANDONA, P., ALJADA, A. & BANDYOPADHYAY, A. 2004. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol,* 25**,** 4-7.

DANDONA, P., WEINSTOCK, R., THUSU, K., ABDEL-RAHMAN, E., ALJADA, A. & WADDEN, T. 1998. Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab,* 83**,** 2907-10.

DANIELZIK, S., LANGNASE, K., MAST, M., SPETHMANN, C. & MULLER, M. J. 2002. Impact of parental BMI on the manifestation of overweight 5-7 year old children. *Eur J Nutr,* 41**,** 132-8.

DE BOO, H. A. & HARDING, J. E. 2006. The developmental origins of adult disease (Barker) hypothesis. *Aust N Z J Obstet Gynaecol,* 46**,** 4-14.
DE FERRANTI, S. & MOZAFFARIAN, D. 2008. The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. *Clin Chem,* 54**,** 945-55.
DE HERTOGH, R., THOMAS, K., BIETLOT, Y., VANDERHEYDEN, I. & FERIN, J. 1975. Plasma levels of unconjugated estrone, estradiol and estriol and of HCS throughout pregnancy in normal women. *J Clin Endocrinol Metab,* 40**,** 93-101.
DE PERGOLA, G. & PANNACCIULLI, N. 2002. Coagulation and fibrinolysis abnormalities in obesity. *J Endocrinol Invest,* 25**,** 899-904.
DENISON, F. C., PRICE, J., GRAHAM, C., WILD, S. & LISTON, W. A. 2008. Maternal obesity, length of gestation, risk of postdates pregnancy and spontaneous onset of labour at term. *BJOG,* 115**,** 720-5.
DI CIANNI, G., MICCOLI, R., VOLPE, L., LENCIONI, C., GHIO, A., GIOVANNITTI, M. G., CUCCURU, I., PELLEGRINI, G., CHATZIANAGNOSTOU, K., BOLDRINI, A. & DEL PRATO, S. 2005. Maternal triglyceride levels and newborn weight in pregnant women with normal glucose tolerance. *Diabet Med,* 22**,** 21-5.
DORNER, G. & PLAGEMANN, A. 1994. Perinatal hyperinsulinism as possible predisposing factor for diabetes mellitus, obesity and enhanced cardiovascular risk in later life. *Horm Metab Res,* 26**,** 213-21.
DORNER, G., PLAGEMANN, A. & REINAGEL, H. 1987. Familial diabetes aggregation in type I diabetics: gestational diabetes an apparent risk factor for increased diabetes susceptibility in the offspring. *Exp Clin Endocrinol,* 89**,** 84-90.
EHRENBERG, H. M., DIERKER, L., MILLUZZI, C. & MERCER, B. M. 2002. Prevalence of maternal obesity in an urban center. *Am J Obstet Gynecol,* 187**,** 1189-93.
EHRENBERG, H. M., DURNWALD, C. P., CATALANO, P. & MERCER, B. M. 2004. The influence of obesity and diabetes on the risk of cesarean delivery. *Am J Obstet Gynecol,* 191**,** 969-74.
ENRIORI, P. J., EVANS, A. E., SINNAYAH, P., JOBST, E. E., TONELLI-LEMOS, L., BILLES, S. K., GLAVAS, M. M., GRAYSON, B. E., PERELLO, M., NILLNI, E. A., GROVE, K. L. & COWLEY, M. A. 2007. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. *Cell Metab,* 5**,** 181-94.
ESTERBAUER, H., KREMPLER, F., OBERKOFLER, H. & PATSCH, W. 1999. The complement system: a pathway linking host defence and adipocyte biology. *Eur J Clin Invest,* 29**,** 653-6.
EVERS, I. M., DE VALK, H. W., MOL, B. W., TER BRAAK, E. W. & VISSER, G. H. 2002. Macrosomia despite good glycaemic control in Type I diabetic pregnancy; results of a nationwide study in The Netherlands. *Diabetologia,* 45**,** 1484-9.
EVERS, I. M., DE VALK, H. W. & VISSER, G. H. 2004. Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in the Netherlands. *BMJ,* 328**,** 915.
FAIN, J. N. 2006. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. *Vitam Horm,* 74**,** 443-77.

FAIN, J. N., NESBIT, A. S., SUDLOW, F. F., CHEEMA, P., PEEPLES, J. M., MADAN, A. K. & TICHANSKY, D. S. 2007. Release in vitro of adipsin, vascular cell adhesion molecule 1, angiotensin 1-converting enzyme, and soluble tumor necrosis factor receptor 2 by human omental adipose tissue as well as by the nonfat cells and adipocytes. *Metabolism,* 56**,** 1583-90.

FANTUZZI, G. & FAGGIONI, R. 2000. Leptin in the regulation of immunity, inflammation, and hematopoiesis. *J Leukoc Biol,* 68**,** 437-46.

FARAJ, M., BEAUREGARD, G., TARDIF, A., LOIZON, E., GODBOUT, A., CIANFLONE, K., VIDAL, H. & RABASA-LHORET, R. 2008. Regulation of leptin, adiponectin and acylation-stimulating protein by hyperinsulinaemia and hyperglycaemia in vivo in healthy lean young men. *Diabetes Metab,* 34**,** 334-42.

FARAJ, M., JONES, P., SNIDERMAN, A. D. & CIANFLONE, K. 2001. Enhanced dietary fat clearance in postobese women. *J Lipid Res,* 42**,** 571-80.

FARLEY, D., TEJERO, M. E., COMUZZIE, A. G., HIGGINS, P. B., COX, L., WERNER, S. L., JENKINS, S. L., LI, C., CHOI, J., DICK, E. J., JR., HUBBARD, G. B., FROST, P., DUDLEY, D. J., BALLESTEROS, B., WU, G., NATHANIELSZ, P. W. & SCHLABRITZ-LOUTSEVITCH, N. E. 2009. Feto-placental adaptations to maternal obesity in the baboon. *Placenta,* 30**,** 752-60.

FAROOQI, I. S., JEBB, S. A., LANGMACK, G., LAWRENCE, E., CHEETHAM, C. H., PRENTICE, A. M., HUGHES, I. A., MCCAMISH, M. A. & O'RAHILLY, S. 1999. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med,* 341**,** 879-84.

FAROOQI, I. S., KEOGH, J. M., KAMATH, S., JONES, S., GIBSON, W. T., TRUSSELL, R., JEBB, S. A., LIP, G. Y. & O'RAHILLY, S. 2001. Partial leptin deficiency and human adiposity. *Nature,* 414**,** 34-5.

FAROOQI, I. S., MATARESE, G., LORD, G. M., KEOGH, J. M., LAWRENCE, E., AGWU, C., SANNA, V., JEBB, S. A., PERNA, F., FONTANA, S., LECHLER, R. I., DEPAOLI, A. M. & O'RAHILLY, S. 2002. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest,* 110**,** 1093-103.

FATTAH, C., FARAH, N., O'TOOLE, F., BARRY, S., STUART, B. & TURNER, M. J. 2009. Body Mass Index (BMI) in women booking for antenatal care: comparison between selfreported and digital measurements. *Eur J Obstet Gynecol Reprod Biol,* 144**,** 32-4.

FISLER, J. S., DRENICK, E. J., BLUMFIELD, D. E. & SWENDSEID, M. E. 1982. Nitrogen economy during very low calorie reducing diets: quality and quantity of dietary protein. *Am J Clin Nutr,* 35**,** 471-86.

FLIER, J. S., COOK, K. S., USHER, P. & SPIEGELMAN, B. M. 1987. Severely impaired adipsin expression in genetic and acquired obesity. *Science,* 237**,** 405-8.

FORDYCE, M. K., DUNCAN, R., CHAO, R., CHRISTAKIS, M., HSIA, S. L., ROBERTSON, E., KAFATOS, A. & CHRISTAKIS, G. 1983. Cord blood serum in newborns of diabetic mothers. *J Chronic Dis,* 36**,** 263-8.

FOX, H. 1967. The incidence and significance of Hofbauer cells in the mature human placenta. *J Pathol Bacteriol,* 93**,** 710-7.

FRANK, S., STALLMEYER, B., KAMPFER, H., KOLB, N. & PFEILSCHIFTER, J. 2000. Leptin enhances wound re-epithelialization and constitutes a direct function of leptin in skin repair. *J Clin Invest,* 106**,** 501-9.

FREDERICH, R. C., LOLLMANN, B., HAMANN, A., NAPOLITANO-ROSEN, A., KAHN, B. B., LOWELL, B. B. & FLIER, J. S. 1995. Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. *J Clin Invest,* 96**,** 1658-63.

FRIEDMAN, J. M. 1998. Leptin, leptin receptors, and the control of body weight. *Nutr Rev,* 56**,** s38-46; discussion s54-75.

FRIEDMAN, J. M. & HALAAS, J. L. 1998. Leptin and the regulation of body weight in mammals. *Nature,* 395**,** 763-70.

FRUHBECK, G. & GOMEZ-AMBROSI, J. 2001. Modulation of the leptin-induced white adipose tissue lipolysis by nitric oxide. *Cell Signal,* 13**,** 827-33.

FUKUOKA, Y., EMBER, J. A. & HUGLI, T. E. 1998. Cloning and characterization of rat C3a receptor: differential expression of rat C3a and C5a receptors by LPS stimulation. *Biochem Biophys Res Commun,* 242**,** 663-8.

GABRIELSSON, B. G., JOHANSSON, J. M., LONN, M., JERNAS, M., OLBERS, T., PELTONEN, M., LARSSON, I., LONN, L., SJOSTROM, L., CARLSSON, B. & CARLSSON, L. M. 2003. High expression of complement components in omental adipose tissue in obese men. *Obes Res,* 11**,** 699-708.

GALMAN, C., LUNDASEN, T., KHARITONENKOV, A., BINA, H. A., ERIKSSON, M., HAFSTROM, I., DAHLIN, M., AMARK, P., ANGELIN, B. & RUDLING, M. 2008. The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARalpha activation in man. *Cell Metab,* 8**,** 169-74.

GALTIER-DEREURE, F., BOEGNER, C. & BRINGER, J. 2000. Obesity and pregnancy: complications and cost. *Am J Clin Nutr,* 71**,** 1242S-8S.

GARCIA-VARGAS, L., ADDISON, S. S., NISTALA, R., KURUKULASURIYA, D. & SOWERS, J. R. 2012. Gestational Diabetes and the Offspring: Implications in the Development of the Cardiorenal Metabolic Syndrome in Offspring. *Cardiorenal Med,* 2**,** 134-142.

GAVRILOVA, O., MARCUS-SAMUELS, B., GRAHAM, D., KIM, J. K., SHULMAN, G. I., CASTLE, A. L., VINSON, C., ECKHAUS, M. & REITMAN, M. L. 2000. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest,* 105**,** 271-8.

GEORGIADES, P., FERGUSON-SMITH, A. C. & BURTON, G. J. 2002. Comparative developmental anatomy of the murine and human definitive placentae. *Placenta,* 23**,** 3-19.

GERMINARIO, R., SNIDERMAN, A. D., MANUEL, S., LEFEBVRE, S. P., BALDO, A. & CIANFLONE, K. 1993. Coordinate regulation of triacylglycerol synthesis and glucose transport by acylation-stimulating protein. *Metabolism,* 42**,** 574-80.

GIORDANO, C. 1990. Immunobiology of normal and diabetic pregnancy. *Immunol Today,* 11**,** 301-3.

GLAZER, N. L., HENDRICKSON, A. F., SCHELLENBAUM, G. D. & MUELLER, B. A. 2004. Weight change and the risk of gestational diabetes in obese women. *Epidemiology,* 15**,** 733-7.

GOSPODAROWICZ, D. 1974. Localisation of a fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth. *Nature,* 249**,** 123-7.

GOULD, P. S., GU, M., LIAO, J., AHMAD, S., CUDMORE, M. J., AHMED, A. & VATISH, M. 2010. Upregulation of urotensin II receptor in preeclampsia causes in vitro placental release of soluble vascular endothelial growth factor receptor 1 in hypoxia. *Hypertension,* 56**,** 172-8.

GRISSA, O., ATEGBO, J. M., YESSOUFOU, A., TABKA, Z., MILED, A., JERBI, M., DRAMANE, K. L., MOUTAIROU, K., PROST, J., HICHAMI, A. & KHAN, N. A. 2007. Antioxidant status and circulating lipids are altered in human gestational diabetes and macrosomia. *Transl Res,* 150**,** 164-71.

GUELINCKX, I., DEVLIEGER, R., BECKERS, K. & VANSANT, G. 2008. Maternal obesity: pregnancy complications, gestational weight gain and nutrition. *Obes Rev,* 9**,** 140-50.

GUILBERT, L., ROBERTSON, S. A. & WEGMANN, T. G. 1993. The trophoblast as an integral component of a macrophage-cytokine network. *Immunol Cell Biol,* 71 (Pt 1)**,** 49-57.

HALAAS, J. L., GAJIWALA, K. S., MAFFEI, M., COHEN, S. L., CHAIT, B. T., RABINOWITZ, D., LALLONE, R. L., BURLEY, S. K. & FRIEDMAN, J. M. 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science,* 269**,** 543-6.

HALBERG, N., WERNSTEDT-ASTERHOLM, I. & SCHERER, P. E. 2008. The adipocyte as an endocrine cell. *Endocrinol Metab Clin North Am,* 37**,** 753-68, x-xi.

HALES, C. N. & BARKER, D. J. 2001. The thrifty phenotype hypothesis. *Br Med Bull,* 60**,** 5-20.

HANSSON, G. K. 2005. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med,* 352**,** 1685-95.

HARDIE, L., TRAYHURN, P., ABRAMOVICH, D. & FOWLER, P. 1997. Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. *Clin Endocrinol (Oxf),* 47**,** 101-6.

HARMON, K. A., GERARD, L., JENSEN, D. R., KEALEY, E. H., HERNANDEZ, T. L., REECE, M. S., BARBOUR, L. A. & BESSESEN, D. H. 2011. Continuous glucose profiles in obese and normal-weight pregnant women on a controlled diet: metabolic determinants of fetal growth. *Diabetes Care,* 34**,** 2198-204.

HAVEL, P. J. 2002. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr Opin Lipidol,* 13**,** 51-9.

HAY, W. W., JR. 1994. Placental transport of nutrients to the fetus. *Horm Res,* 42**,** 215-22.

HEDDERSON, M. M., WILLIAMS, M. A., HOLT, V. L., WEISS, N. S. & FERRARA, A. 2008. Body mass index and weight gain prior to pregnancy and risk of gestational diabetes mellitus. *Am J Obstet Gynecol,* 198**,** 409 e1-7.

HENRY, O. A. & BEISCHER, N. A. 1991. Long-term implications of gestational diabetes for the mother. *Baillieres Clin Obstet Gynaecol,* 5**,** 461-83.

HERRERA, E. 2000. Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. *Eur J Clin Nutr,* 54 Suppl 1**,** S47-51.

HERRERA, E. 2002. Implications of dietary fatty acids during pregnancy on placental, fetal and postnatal development--a review. *Placenta,* 23 Suppl A**,** S9-19.

HERRERA, E., PALACIN, M., MARTIN, A. & LASUNCION, M. A. 1985. Relationship between maternal and fetal fuels and placental glucose transfer in rats with maternal diabetes of varying severity. *Diabetes,* 34 Suppl 2**,** 42-6.
HESLEHURST, N., ELLS, L. J., SIMPSON, H., BATTERHAM, A., WILKINSON, J. & SUMMERBELL, C. D. 2007. Trends in maternal obesity incidence rates, demographic predictors, and health inequalities in 36,821 women over a 15-year period. *BJOG,* 114**,** 187-94.
HIDAR, S., CHAIEB, A., BACCOUCHE, S., LARADI, S., FKIH, M., MILLED, A. & KHAIRI, H. 2001. [Post-prandial plasma glucose test as screening tool for gestational diabetes: A prospective randomized trial]. *J Gynecol Obstet Biol Reprod (Paris),* 30**,** 344-7.
HIGHMAN, T. J., FRIEDMAN, J. E., HUSTON, L. P., WONG, W. W. & CATALANO, P. M. 1998. Longitudinal changes in maternal serum leptin concentrations, body composition, and resting metabolic rate in pregnancy. *Am J Obstet Gynecol,* 178**,** 1010-5.
HILLIER, T. A., PEDULA, K. L., SCHMIDT, M. M., MULLEN, J. A., CHARLES, M. A. & PETTITT, D. J. 2007. Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. *Diabetes Care,* 30**,** 2287-92.
HILLIER, T. A., VESCO, K. K., PEDULA, K. L., BEIL, T. L., WHITLOCK, E. P. & PETTITT, D. J. 2008. Screening for gestational diabetes mellitus: a systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med,* 148**,** 766-75.
HOLLINGSWORTH, D. R. & GRUNDY, S. M. 1982. Pregnancy-associated hypertriglyceridemia in normal and diabetic women. Differences in insulin-dependent, non-insulin-dependent, and gestational diabetes. *Diabetes,* 31**,** 1092-7.
HOMKO, C. J., SIVAN, E., REECE, E. A. & BODEN, G. 1999. Fuel metabolism during pregnancy. *Semin Reprod Endocrinol,* 17**,** 119-25.
HOROWITZ, J. F. & KLEIN, S. 2000. Whole body and abdominal lipolytic sensitivity to epinephrine is suppressed in upper body obese women. *Am J Physiol Endocrinol Metab,* 278**,** E1144-52.
HOTAMISLIGIL, G. S. & SPIEGELMAN, B. M. 1994. Tumor necrosis factor alpha: a key component of the obesity-diabetes link. *Diabetes,* 43**,** 1271-8.
HRAB, R. V., HARTMAN, H. A. & COX, R. H., JR. 1994. Prevention of fluvastatin-induced toxicity, mortality, and cardiac myopathy in pregnant rats by mevalonic acid supplementation. *Teratology,* 50**,** 19-26.
HSUCHOU, H., PAN, W. & KASTIN, A. J. 2007. The fasting polypeptide FGF21 can enter brain from blood. *Peptides,* 28**,** 2382-6.
HUNT, J. S. 1989. Cytokine networks in the uteroplacental unit: macrophages as pivotal regulatory cells. *J Reprod Immunol,* 16**,** 1-17.
HUNT, J. S., SOARES, M. J., LEI, M. G., SMITH, R. N., WHEATON, D., ATHERTON, R. A. & MORRISON, D. C. 1989. Products of lipopolysaccharide-activated macrophages (tumor necrosis factor-alpha, transforming growth factor-beta) but not lipopolysaccharide modify DNA synthesis by rat trophoblast cells exhibiting the 80-kDa lipopolysaccharide-binding protein. *J Immunol,* 143**,** 1606-13.
HYTTEN, F. E. & ROBERTSON, E. G. 1971. Maternal water metabolism in pregnancy. *Proc R Soc Med,* 64**,** 1072.
INAGAKI, T., CHOI, M., MOSCHETTA, A., PENG, L., CUMMINS, C. L., MCDONALD, J. G., LUO, G., JONES, S. A., GOODWIN, B.,

RICHARDSON, J. A., GERARD, R. D., REPA, J. J., MANGELSDORF, D. J. & KLIEWER, S. A. 2005. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab,* 2**,** 217-25.

INGMAN, K., COOKSON, V. J., JONES, C. J. & APLIN, J. D. 2010. Characterisation of Hofbauer cells in first and second trimester placenta: incidence, phenotype, survival in vitro and motility. *Placenta,* 31**,** 535-44.

JENSEN, D. M., DAMM, P., SORENSEN, B., MOLSTED-PEDERSEN, L., WESTERGAARD, J. G., OVESEN, P. & BECK-NIELSEN, H. 2003. Pregnancy outcome and prepregnancy body mass index in 2459 glucose-tolerant Danish women. *Am J Obstet Gynecol,* 189**,** 239-44.

JUGE-AUBRY, C. E. & MEIER, C. A. 2002. Immunomodulatory actions of leptin. *Mol Cell Endocrinol,* 194**,** 1-7.

KALANT, D., MACLAREN, R., CUI, W., SAMANTA, R., MONK, P. N., LAPORTE, S. A. & CIANFLONE, K. 2005. C5L2 is a functional receptor for acylation-stimulating protein. *J Biol Chem,* 280**,** 23936-44.

KALANT, D., PHELIS, S., FIELDING, B. A., FRAYN, K. N., CIANFLONE, K. & SNIDERMAN, A. D. 2000. Increased postprandial fatty acid trapping in subcutaneous adipose tissue in obese women. *J Lipid Res,* 41**,** 1963-8.

KAMOHARA, S., BURCELIN, R., HALAAS, J. L., FRIEDMAN, J. M. & CHARRON, M. J. 1997. Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature,* 389**,** 374-7.

KANAGALINGAM, M. G., FOROUHI, N. G., GREER, I. A. & SATTAR, N. 2005. Changes in booking body mass index over a decade: retrospective analysis from a Glasgow Maternity Hospital. *BJOG,* 112**,** 1431-3.

KAUTZKY-WILLER, A., PACINI, G., TURA, A., BIEGLMAYER, C., SCHNEIDER, B., LUDVIK, B., PRAGER, R. & WALDHAUSL, W. 2001. Increased plasma leptin in gestational diabetes. *Diabetologia,* 44**,** 164-72.

KERN, P. A., RANGANATHAN, S., LI, C., WOOD, L. & RANGANATHAN, G. 2001. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab,* 280**,** E745-51.

KERSHAW, E. E. & FLIER, J. S. 2004. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab,* 89**,** 2548-56.

KHAN, S., KATABUCHI, H., ARAKI, M., NISHIMURA, R. & OKAMURA, H. 2000. Human villous macrophage-conditioned media enhance human trophoblast growth and differentiation in vitro. *Biol Reprod,* 62**,** 1075-83.

KHARITONENKOV, A., SHIYANOVA, T. L., KOESTER, A., FORD, A. M., MICANOVIC, R., GALBREATH, E. J., SANDUSKY, G. E., HAMMOND, L. J., MOYERS, J. S., OWENS, R. A., GROMADA, J., BROZINICK, J. T., HAWKINS, E. D., WROBLEWSKI, V. J., LI, D. S., MEHRBOD, F., JASKUNAS, S. R. & SHANAFELT, A. B. 2005. FGF-21 as a novel metabolic regulator. *J Clin Invest,* 115**,** 1627-35.

KHARITONENKOV, A., WROBLEWSKI, V. J., KOESTER, A., CHEN, Y. F., CLUTINGER, C. K., TIGNO, X. T., HANSEN, B. C., SHANAFELT, A. B. & ETGEN, G. J. 2007. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. *Endocrinology,* 148**,** 774-81.

KIM, J. S., ROMERO, R., KIM, M. R., KIM, Y. M., FRIEL, L., ESPINOZA, J. & KIM, C. J. 2008. Involvement of Hofbauer cells and maternal T cells in villitis of unknown aetiology. *Histopathology,* 52**,** 457-64.

KIM, S. Y., DIETZ, P. M., ENGLAND, L., MORROW, B. & CALLAGHAN, W. M. 2007. Trends in pre-pregnancy obesity in nine states, 1993-2003. *Obesity (Silver Spring),* 15**,** 986-93.

KIRWAN, J. P., HAUGUEL-DE MOUZON, S., LEPERCQ, J., CHALLIER, J. C., HUSTON-PRESLEY, L., FRIEDMAN, J. E., KALHAN, S. C. & CATALANO, P. M. 2002. TNF-alpha is a predictor of insulin resistance in human pregnancy. *Diabetes,* 51**,** 2207-13.

KISSEBAH, A. H., ALFARSI, S., ADAMS, P. W. & WYNN, V. 1976. Role of insulin resistance in adipose tissue and liver in the pathogenesis of endogenous hypertriglyceridaemia in man. *Diabetologia,* 12**,** 563-71.

KITAJIMA, M., OKA, S., YASUHI, I., FUKUDA, M., RII, Y. & ISHIMARU, T. 2001. Maternal serum triglyceride at 24--32 weeks' gestation and newborn weight in nondiabetic women with positive diabetic screens. *Obstet Gynecol,* 97**,** 776-80.

KJOS, S. L. & BUCHANAN, T. A. 1999. Gestational diabetes mellitus. *N Engl J Med,* 341**,** 1749-56.

KLIEWER, S. A. & MANGELSDORF, D. J. 2010. Fibroblast growth factor 21: from pharmacology to physiology. *Am J Clin Nutr,* 91**,** 254S-257S.

KLIMAN, H. J., NESTLER, J. E., SERMASI, E., SANGER, J. M. & STRAUSS, J. F., 3RD 1986. Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. *Endocrinology,* 118**,** 1567-82.

KLOVER, P. J., ZIMMERS, T. A., KONIARIS, L. G. & MOONEY, R. A. 2003. Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. *Diabetes,* 52**,** 2784-9.

KNIGHT, B., SHIELDS, B. M., HILL, A., POWELL, R. J., WRIGHT, D. & HATTERSLEY, A. T. 2007. The impact of maternal glycemia and obesity on early postnatal growth in a nondiabetic Caucasian population. *Diabetes Care,* 30**,** 777-83.

KNOPP, R. H., MAGEE, M. S., WALDEN, C. E., BONET, B. & BENEDETTI, T. J. 1992. Prediction of infant birth weight by GDM screening tests. Importance of plasma triglyceride. *Diabetes Care,* 15**,** 1605-13.

KOISTINEN, H. A., VIDAL, H., KARONEN, S. L., DUSSERRE, E., VALLIER, P., KOIVISTO, V. A. & EBELING, P. 2001. Plasma acylation stimulating protein concentration and subcutaneous adipose tissue C3 mRNA expression in nondiabetic and type 2 diabetic men. *Arterioscler Thromb Vasc Biol,* 21**,** 1034-9.

KOUKKOU, E., WATTS, G. F. & LOWY, C. 1996. Serum lipid, lipoprotein and apolipoprotein changes in gestational diabetes mellitus: a cross-sectional and prospective study. *J Clin Pathol,* 49**,** 634-7.

KOUPIL, I. & TOIVANEN, P. 2008. Social and early-life determinants of overweight and obesity in 18-year-old Swedish men. *Int J Obes (Lond),* 32**,** 73-81.

KRAUSS, S., ZHANG, C. Y. & LOWELL, B. B. 2005. The mitochondrial uncoupling-protein homologues. *Nat Rev Mol Cell Biol,* 6**,** 248-61.

KUMARI, A. S. 2001. Pregnancy outcome in women with morbid obesity. *Int J Gynaecol Obstet,* 73**,** 101-7.

LANGER, O., YOGEV, Y., XENAKIS, E. M. & BRUSTMAN, L. 2005. Overweight and obese in gestational diabetes: the impact on pregnancy outcome. *Am J Obstet Gynecol,* 192**,** 1768-76.

LAWLOR, D. A., MORTON, S., BATTY, G. D., MACINTYRE, S., CLARK, H. & SMITH, G. D. 2007. Obstetrician-assessed maternal health at pregnancy predicts offspring future health. *PLoS One,* 2**,** e666.
LEE, G. H., PROENCA, R., MONTEZ, J. M., CARROLL, K. M., DARVISHZADEH, J. G., LEE, J. I. & FRIEDMAN, J. M. 1996. Abnormal splicing of the leptin receptor in diabetic mice. *Nature,* 379**,** 632-5.
LEE, Y., WANG, M. Y., KAKUMA, T., WANG, Z. W., BABCOCK, E., MCCORKLE, K., HIGA, M., ZHOU, Y. T. & UNGER, R. H. 2001. Liporegulation in diet-induced obesity. The antisteatotic role of hyperleptinemia. *J Biol Chem,* 276**,** 5629-35.
LEPERCQ, J., CAUZAC, M., LAHLOU, N., TIMSIT, J., GIRARD, J., AUWERX, J. & HAUGUEL-DE MOUZON, S. 1998. Overexpression of placental leptin in diabetic pregnancy: a critical role for insulin. *Diabetes,* 47**,** 847-50.
LEPERCQ, J., CHALLIER, J. C., GUERRE-MILLO, M., CAUZAC, M., VIDAL, H. & HAUGUEL-DE MOUZON, S. 2001. Prenatal leptin production: evidence that fetal adipose tissue produces leptin. *J Clin Endocrinol Metab,* 86**,** 2409-13.
LESSIN, D. L., HUNT, J. S., KING, C. R. & WOOD, G. W. 1988. Antigen expression by cells near the maternal-fetal interface. *Am J Reprod Immunol Microbiol,* 16**,** 1-7.
LI, C., KAUR, H., CHOI, W. S., HUANG, T. T., LEE, R. E. & AHLUWALIA, J. S. 2005. Additive interactions of maternal prepregnancy BMI and breast-feeding on childhood overweight. *Obes Res,* 13**,** 362-71.
LI, K., LI, L., YANG, M., ZONG, H., LIU, H. & YANG, G. 2009. Effects of rosiglitazone on fasting plasma fibroblast growth factor-21 levels in patients with type 2 diabetes mellitus. *Eur J Endocrinol,* 161**,** 391-5.
LI, L., YANG, G., NING, H., YANG, M., LIU, H. & CHEN, W. 2008. Plasma FGF-21 levels in type 2 diabetic patients with ketosis. *Diabetes Res Clin Pract,* 82**,** 209-13.
LIEVENSE, A. M., BIERMA-ZEINSTRA, S. M., VERHAGEN, A. P., VAN BAAR, M. E., VERHAAR, J. A. & KOES, B. W. 2002. Influence of obesity on the development of osteoarthritis of the hip: a systematic review. *Rheumatology (Oxford),* 41**,** 1155-62.
LIN, Z., ZHOU, Z., LIU, Y., GONG, Q., YAN, X., XIAO, J., WANG, X., LIN, S., FENG, W. & LI, X. 2011. Circulating FGF21 levels are progressively increased from the early to end stages of chronic kidney diseases and are associated with renal function in Chinese. *PLoS One,* 6**,** e18398.
LINDEGAARD, M. L., DAMM, P., MATHIESEN, E. R. & NIELSEN, L. B. 2006. Placental triglyceride accumulation in maternal type 1 diabetes is associated with increased lipase gene expression. *J Lipid Res,* 47**,** 2581-8.
LOBSTEIN, T. & FRELUT, M. L. 2003. Prevalence of overweight among children in Europe. *Obes Rev,* 4**,** 195-200.
LUDWIG, D. S. & CURRIE, J. 2010. The association between pregnancy weight gain and birthweight: a within-family comparison. *Lancet,* 376**,** 984-90.
LUPI, R., DOTTA, F., MARSELLI, L., DEL GUERRA, S., MASINI, M., SANTANGELO, C., PATANE, G., BOGGI, U., PIRO, S., ANELLO, M., BERGAMINI, E., MOSCA, F., DI MARIO, U., DEL PRATO, S. & MARCHETTI, P. 2002. Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell

death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. *Diabetes,* 51**,** 1437-42.

MAEDLER, K., SERGEEV, P., EHSES, J. A., MATHE, Z., BOSCO, D., BERNEY, T., DAYER, J. M., REINECKE, M., HALBAN, P. A. & DONATH, M. Y. 2004. Leptin modulates beta cell expression of IL-1 receptor antagonist and release of IL-1beta in human islets. *Proc Natl Acad Sci U S A,* 101**,** 8138-43.

MAFFEI, M., HALAAS, J., RAVUSSIN, E., PRATLEY, R. E., LEE, G. H., ZHANG, Y., FEI, H., KIM, S., LALLONE, R., RANGANATHAN, S. & ET AL. 1995. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med,* 1**,** 1155-61.

MAGNUSSON-OLSSON, A. L., HAMARK, B., ERICSSON, A., WENNERGREN, M., JANSSON, T. & POWELL, T. L. 2006. Gestational and hormonal regulation of human placental lipoprotein lipase. *J Lipid Res,* 47**,** 2551-61.

MAGNUSSON, A. L., WATERMAN, I. J., WENNERGREN, M., JANSSON, T. & POWELL, T. L. 2004. Triglyceride hydrolase activities and expression of fatty acid binding proteins in the human placenta in pregnancies complicated by intrauterine growth restriction and diabetes. *J Clin Endocrinol Metab,* 89**,** 4607-14.

MALEK, A., SAGER, R. & SCHNEIDER, H. 2001. Effect of hypoxia, oxidative stress and lipopolysaccharides on the release of prostaglandins and cytokines from human term placental explants. *Placenta,* 22 Suppl A**,** S45-50.

MANTOV, S. & RAEV, D. 1996. Additive effect of diabetes and systemic hypertension on the immune mechanisms of atherosclerosis. *Int J Cardiol,* 56**,** 145-8.

MARCIL, M., VU, H., CUI, W., DASTANI, Z., ENGERT, J. C., GAUDET, D., CASTRO-CABEZAS, M., SNIDERMAN, A. D., GENEST, J., JR. & CIANFLONE, K. 2006. Identification of a novel C5L2 variant (S323I) in a French Canadian family with familial combined hyperlipemia. *Arterioscler Thromb Vasc Biol,* 26**,** 1619-25.

MARTIN-HIDALGO, A., HOLM, C., BELFRAGE, P., SCHOTZ, M. C. & HERRERA, E. 1994. Lipoprotein lipase and hormone-sensitive lipase activity and mRNA in rat adipose tissue during pregnancy. *Am J Physiol,* 266**,** E930-5.

MARTIN, A. & HERRERA, E. 1991. Different responses to maternal diabetes during the first and second half of gestation in the streptozotocin-treated rat. *Isr J Med Sci,* 27**,** 442-8.

MARTIN, A. M., BERGER, H., NISENBAUM, R., LAUSMAN, A. Y., MACGARVIE, S., CRERAR, C. & RAY, J. G. 2009. Abdominal visceral adiposity in the first trimester predicts glucose intolerance in later pregnancy. *Diabetes Care,* 32**,** 1308-10.

MARTIN, J. A., HAMILTON, B. E., SUTTON, P. D., VENTURA, S. J., MENACKER, F. & KIRMEYER, S. 2006. Births: final data for 2004. *Natl Vital Stat Rep,* 55**,** 1-101.

MASLOWSKA, M., LEGAKIS, H., ASSADI, F. & CIANFLONE, K. 2006. Targeting the signaling pathway of acylation stimulating protein. *J Lipid Res,* 47**,** 643-52.

MASLOWSKA, M., SCANTLEBURY, T., GERMINARIO, R. & CIANFLONE, K. 1997. Acute in vitro production of acylation stimulating protein in differentiated human adipocytes. *J Lipid Res,* 38**,** 1-11.

MASLOWSKA, M., VU, H., PHELIS, S., SNIDERMAN, A. D., RHODE, B. M., BLANK, D. & CIANFLONE, K. 1999. Plasma acylation stimulating protein, adipsin and lipids in non-obese and obese populations. *Eur J Clin Invest,* 29**,** 679-86.

MASLOWSKA, M., WANG, H. W. & CIANFLONE, K. 2005. Novel roles for acylation stimulating protein/C3adesArg: a review of recent in vitro and in vivo evidence. *Vitam Horm,* 70**,** 309-32.

MASUZAKI, H., OGAWA, Y., SAGAWA, N., HOSODA, K., MATSUMOTO, T., MISE, H., NISHIMURA, H., YOSHIMASA, Y., TANAKA, I., MORI, T. & NAKAO, K. 1997. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nat Med,* 3**,** 1029-33.

MATTHEWS, D. R., HOSKER, J. P., RUDENSKI, A. S., NAYLOR, B. A., TREACHER, D. F. & TURNER, R. C. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia,* 28**,** 412-9.

MAVRI, A., STEGNAR, M., KREBS, M., SENTOCNIK, J. T., GEIGER, M. & BINDER, B. R. 1999. Impact of adipose tissue on plasma plasminogen activator inhibitor-1 in dieting obese women. *Arterioscler Thromb Vasc Biol,* 19**,** 1582-7.

MCALLISTER, E. J., DHURANDHAR, N. V., KEITH, S. W., ARONNE, L. J., BARGER, J., BASKIN, M., BENCA, R. M., BIGGIO, J., BOGGIANO, M. M., EISENMANN, J. C., ELOBEID, M., FONTAINE, K. R., GLUCKMAN, P., HANLON, E. C., KATZMARZYK, P., PIETROBELLI, A., REDDEN, D. T., RUDEN, D. M., WANG, C., WATERLAND, R. A., WRIGHT, S. M. & ALLISON, D. B. 2009. Ten putative contributors to the obesity epidemic. *Crit Rev Food Sci Nutr,* 49**,** 868-913.

MERZOUK, H., MEGHELLI-BOUCHENAK, M., LOUKIDI, B., PROST, J. & BELLEVILLE, J. 2000. Impaired serum lipids and lipoproteins in fetal macrosomia related to maternal obesity. *Biol Neonate,* 77**,** 17-24.

METZGER, B. E. 1991. Summary and recommendations of the Third International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes,* 40 Suppl 2**,** 197-201.

METZGER, B. E., GABBE, S. G., PERSSON, B., BUCHANAN, T. A., CATALANO, P. A., DAMM, P., DYER, A. R., LEIVA, A., HOD, M., KITZMILER, J. L., LOWE, L. P., MCINTYRE, H. D., OATS, J. J., OMORI, Y. & SCHMIDT, M. I. 2010. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care,* 33**,** 676-82.

MILLAR, C. A., MEERLOO, T., MARTIN, S., HICKSON, G. R., SHIMWELL, N. J., WAKELAM, M. J., JAMES, D. E. & GOULD, G. W. 2000. Adipsin and the glucose transporter GLUT4 traffic to the cell surface via independent pathways in adipocytes. *Traffic,* 1**,** 141-51.

MILLS, J. L., JOVANOVIC, L., KNOPP, R., AARONS, J., CONLEY, M., PARK, E., LEE, Y. J., HOLMES, L., SIMPSON, J. L. & METZGER, B. 1998. Physiological reduction in fasting plasma glucose concentration in the first trimester of normal pregnancy: the diabetes in early pregnancy study. *Metabolism,* 47**,** 1140-4.

MIN, H. Y. & SPIEGELMAN, B. M. 1986. Adipsin, the adipocyte serine protease: gene structure and control of expression by tumor necrosis factor. *Nucleic Acids Res,* 14**,** 8879-92.

MISRA, V. K. & TRUDEAU, S. 2011. The influence of overweight and obesity on longitudinal trends in maternal serum leptin levels during pregnancy. *Obesity (Silver Spring),* 19**,** 416-21.
MITANCHEZ, D. 2010. Foetal and neonatal complications in gestational diabetes: perinatal mortality, congenital malformations, macrosomia, shoulder dystocia, birth injuries, neonatal complications. *Diabetes Metab,* 36**,** 617-27.
MOHAMED-ALI, V., GOODRICK, S., RAWESH, A., KATZ, D. R., MILES, J. M., YUDKIN, J. S., KLEIN, S. & COPPACK, S. W. 1997. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab,* 82**,** 4196-200.
MONTAGUE, C. T., FAROOQI, I. S., WHITEHEAD, J. P., SOOS, M. A., RAU, H., WAREHAM, N. J., SEWTER, C. P., DIGBY, J. E., MOHAMMED, S. N., HURST, J. A., CHEETHAM, C. H., EARLEY, A. R., BARNETT, A. H., PRINS, J. B. & O'RAHILLY, S. 1997. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature,* 387**,** 903-8.
MONTE, S., VALENTI, O., GIORGIO, E., RENDA, E., HYSENI, E., FARACI, M., DE DOMENICO, R. & DI PRIMA, F. A. 2011. Maternal weight gain during pregnancy and neonatal birth weight: a review of the literature. *J Prenat Med,* 5**,** 27-30.
MONTELONGO, A., LASUNCION, M. A., PALLARDO, L. F. & HERRERA, E. 1992. Longitudinal study of plasma lipoproteins and hormones during pregnancy in normal and diabetic women. *Diabetes,* 41**,** 1651-9.
MORTON, A. 2005. Obesity and pregnancy. *Acta Obstet Gynecol Scand,* 84**,** 709-10.
MORTON, G. J., CUMMINGS, D. E., BASKIN, D. G., BARSH, G. S. & SCHWARTZ, M. W. 2006. Central nervous system control of food intake and body weight. *Nature,* 443**,** 289-95.
MOSKALEWSKI, S., CZARNIK, Z. & PTAK, W. 1975. Demonstration of cells with igg receptor in human placenta. *Biol Neonate,* 26**,** 268-73.
MURAD, A., NATH, A. K., CHA, S. T., DEMIR, E., FLORES-RIVEROS, J. & SIERRA-HONIGMANN, M. R. 2003. Leptin is an autocrine/paracrine regulator of wound healing. *FASEB J,* 17**,** 1895-7.
MURPHY, H. R., RAYMAN, G., LEWIS, K., KELLY, S., JOHAL, B., DUFFIELD, K., FOWLER, D., CAMPBELL, P. J. & TEMPLE, R. C. 2008. Effectiveness of continuous glucose monitoring in pregnant women with diabetes: randomised clinical trial. *BMJ,* 337**,** a1680.
MURRAY, I., HAVEL, P. J., SNIDERMAN, A. D. & CIANFLONE, K. 2000. Reduced body weight, adipose tissue, and leptin levels despite increased energy intake in female mice lacking acylation-stimulating protein. *Endocrinology,* 141**,** 1041-9.
MURRAY, I., PARKER, R. A., KIRCHGESSNER, T. G., TRAN, J., ZHANG, Z. J., WESTERLUND, J. & CIANFLONE, K. 1997. Functional bioactive recombinant acylation stimulating protein is distinct from C3a anaphylatoxin. *J Lipid Res,* 38**,** 2492-501.
MURRAY, I., SNIDERMAN, A. D. & CIANFLONE, K. 1999a. Enhanced triglyceride clearance with intraperitoneal human acylation stimulating protein in C57BL/6 mice. *Am J Physiol,* 277**,** E474-80.
MURRAY, I., SNIDERMAN, A. D., HAVEL, P. J. & CIANFLONE, K. 1999b. Acylation stimulating protein (ASP) deficiency alters postprandial and adipose tissue metabolism in male mice. *J Biol Chem,* 274**,** 36219-25.

MUSCARI, A., BOZZOLI, C., PUDDU, G. M., ROVINETTI, C., FIORENTINI, G. P., ROVERSI, R. A. & PUDDU, P. 1990. Correlations between serum lipids and complement components in adults without demonstrated atherosclerotic disease. *Atherosclerosis,* 81**,** 111-8.

MUSCARI, A., MASSARELLI, G., BASTAGLI, L., POGGIOPOLLINI, G., TOMASSETTI, V., DRAGO, G., MARTIGNANI, C., PACILLI, P., BONI, P. & PUDDU, P. 2000. Relationship of serum C3 to fasting insulin, risk factors and previous ischaemic events in middle-aged men. *Eur Heart J,* 21**,** 1081-90.

NAM, S. Y., KRATZSCH, J., KIM, K. W., KIM, K. R., LIM, S. K. & MARCUS, C. 2001. Cerebrospinal fluid and plasma concentrations of leptin, NPY, and alpha-MSH in obese women and their relationship to negative energy balance. *J Clin Endocrinol Metab,* 86**,** 4849-53.

NAPOLI, C., D'ARMIENTO, F. P., MANCINI, F. P., POSTIGLIONE, A., WITZTUM, J. L., PALUMBO, G. & PALINSKI, W. 1997. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest,* 100**,** 2680-90.

NAPOLITANO, A., LOWELL, B. B., DAMM, D., LEIBEL, R. L., RAVUSSIN, E., JIMERSON, D. C., LESEM, M. D., VAN DYKE, D. C., DALY, P. A., CHATIS, P. & ET AL. 1994. Concentrations of adipsin in blood and rates of adipsin secretion by adipose tissue in humans with normal, elevated and diminished adipose tissue mass. *Int J Obes Relat Metab Disord,* 18**,** 213-8.

NOLAN, C. J., RILEY, S. F., SHEEDY, M. T., WALSTAB, J. E. & BEISCHER, N. A. 1995. Maternal serum triglyceride, glucose tolerance, and neonatal birth weight ratio in pregnancy. *Diabetes Care,* 18**,** 1550-6.

O'BRIEN, T. E., RAY, J. G. & CHAN, W. S. 2003. Maternal body mass index and the risk of preeclampsia: a systematic overview. *Epidemiology,* 14**,** 368-74.

O'CALLAGHAN, M. J., WILLIAMS, G. M., ANDERSEN, M. J., BOR, W. & NAJMAN, J. M. 1997. Prediction of obesity in children at 5 years: a cohort study. *J Paediatr Child Health,* 33**,** 311-6.

ODDY, W. H., LI, J., LANDSBOROUGH, L., KENDALL, G. E., HENDERSON, S. & DOWNIE, J. 2006. The association of maternal overweight and obesity with breastfeeding duration. *J Pediatr,* 149**,** 185-91.

OKEN, E. & GILLMAN, M. W. 2003. Fetal origins of obesity. *Obes Res,* 11**,** 496-506.

OPPENHEIM, J. J. 2001. Cytokines: past, present, and future. *Int J Hematol,* 74**,** 3-8.

ORAL, E. A., SIMHA, V., RUIZ, E., ANDEWELT, A., PREMKUMAR, A., SNELL, P., WAGNER, A. J., DEPAOLI, A. M., REITMAN, M. L., TAYLOR, S. I., GORDEN, P. & GARG, A. 2002. Leptin-replacement therapy for lipodystrophy. *N Engl J Med,* 346**,** 570-8.

ORNITZ, D. M. & ITOH, N. 2001. Fibroblast growth factors. *Genome Biol,* 2**,** REVIEWS3005.

OSTLUND, I., HAGLUND, B. & HANSON, U. 2004. Gestational diabetes and preeclampsia. *Eur J Obstet Gynecol Reprod Biol,* 113**,** 12-6.

OTERO, M., LAGO, R., LAGO, F., CASANUEVA, F. F., DIEGUEZ, C., GOMEZ-REINO, J. J. & GUALILLO, O. 2005. Leptin, from fat to inflammation: old questions and new insights. *FEBS Lett,* 579**,** 295-301.

OWENS, L. A., O'SULLIVAN, E. P., KIRWAN, B., AVALOS, G., GAFFNEY, G. & DUNNE, F. 2010. ATLANTIC DIP: the impact of obesity on pregnancy outcome in glucose-tolerant women. *Diabetes Care,* 33**,** 577-9.

OZATA, M., GUNGOR, D., TURAN, M., OZISIK, G., BINGOL, N., OZGURTAS, T. & OZDEMIR, I. C. 2001. Improved glycemic control increases fasting plasma acylation-stimulating protein and decreases leptin concentrations in type II diabetic subjects. *J Clin Endocrinol Metab,* 86**,** 3659-64.

PALINSKI, W. & NAPOLI, C. 2002. The fetal origins of atherosclerosis: maternal hypercholesterolemia, and cholesterol-lowering or antioxidant treatment during pregnancy influence in utero programming and postnatal susceptibility to atherogenesis. *FASEB J,* 16**,** 1348-60.

PARSONS, T. J., POWER, C. & MANOR, O. 2001. Fetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: longitudinal study. *BMJ,* 323**,** 1331-5.

PASQUALI, R., CASIMIRRI, F. & MELCHIONDA, N. 1987. Protein metabolism in obese patients during very low-calorie mixed diets containing different amounts of proteins and carbohydrates. *Metabolism,* 36**,** 1141-8.

PEAKE, P. W., O'GRADY, S., PUSSELL, B. A. & CHARLESWORTH, J. A. 1997. Detection and quantification of the control proteins of the alternative pathway of complement in 3T3-L1 adipocytes. *Eur J Clin Invest,* 27**,** 922-7.

PEDERSEN, J. 1971. [Pregnancy and diabetes mellitus]. *Tidsskr Nor Laegeforen,* 91**,** 1320-4.

PEDERSEN, J. & MOLSTED-PEDERSEN, L. M. 1978. Congenital malformations: the possible role of diabetes care outside pregnancy. *Ciba Found Symp***,** 265-71.

PELLEYMOUNTER, M. A., CULLEN, M. J., BAKER, M. B., HECHT, R., WINTERS, D., BOONE, T. & COLLINS, F. 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science,* 269**,** 540-3.

PERUCCHINI, D., FISCHER, U., SPINAS, G. A., HUCH, R., HUCH, A. & LEHMANN, R. 1999. Using fasting plasma glucose concentrations to screen for gestational diabetes mellitus: prospective population based study. *BMJ,* 319**,** 812-5.

PETTITT, D. J., ALECK, K. A., BAIRD, H. R., CARRAHER, M. J., BENNETT, P. H. & KNOWLER, W. C. 1988. Congenital susceptibility to NIDDM. Role of intrauterine environment. *Diabetes,* 37**,** 622-8.

PHILLIPS, T. A., NI, J. & HUNT, J. S. 2001. Death-inducing tumour necrosis factor (TNF) superfamily ligands and receptors are transcribed in human placentae, cytotrophoblasts, placental macrophages and placental cell lines. *Placenta,* 22**,** 663-72.

POMEROY, C., MITCHELL, J., ECKERT, E., RAYMOND, N., CROSBY, R. & DALMASSO, A. P. 1997. Effect of body weight and caloric restriction on serum complement proteins, including Factor D/adipsin: studies in anorexia nervosa and obesity. *Clin Exp Immunol,* 108**,** 507-15.

POTTHOFF, M. J., INAGAKI, T., SATAPATI, S., DING, X., HE, T., GOETZ, R., MOHAMMADI, M., FINCK, B. N., MANGELSDORF, D. J., KLIEWER, S. A. & BURGESS, S. C. 2009. FGF21 induces PGC-1alpha and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proc Natl Acad Sci U S A,* 106**,** 10853-8.

PRADHAN, A. D., MANSON, J. E., RIFAI, N., BURING, J. E. & RIDKER, P. M. 2001. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA,* 286**,** 327-34.

PRESCOTT-CLARKE, P., PRIMATESTA, P., GREAT BRITAIN. DEPT. OF HEALTH., JOINT HEALTH SURVEYS UNIT (GREAT BRITAIN) & UNIVERSITY COLLEGE LONDON. DEPT. OF EPIDEMIOLOGY AND PUBLIC HEALTH. 1998. *Health survey for England 1996 : a survey carried out on behalf of the Department of Health,* London, Stationery Office.

RADAELLI, T., LEPERCQ, J., VARASTEHPOUR, A., BASU, S., CATALANO, P. M. & HAUGUEL-DE MOUZON, S. 2009. Differential regulation of genes for fetoplacental lipid pathways in pregnancy with gestational and type 1 diabetes mellitus. *Am J Obstet Gynecol,* 201**,** 209 e1-209 e10.

RADAELLI, T., VARASTEHPOUR, A., CATALANO, P. & HAUGUEL-DE MOUZON, S. 2003. Gestational diabetes induces placental genes for chronic stress and inflammatory pathways. *Diabetes,* 52**,** 2951-8.

RAMOS, P. & HERRERA, E. 1995. Reversion of insulin resistance in the rat during late pregnancy by 72-h glucose infusion. *Am J Physiol,* 269**,** E858-63.

RAMSAY, J. E., FERRELL, W. R., CRAWFORD, L., WALLACE, A. M., GREER, I. A. & SATTAR, N. 2002. Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. *J Clin Endocrinol Metab,* 87**,** 4231-7.

RAO, K. R., PADMAVATHI, I. J. & RAGHUNATH, M. 2012. Maternal micronutrient restriction programs the body adiposity, adipocyte function and lipid metabolism in offspring: a review. *Rev Endocr Metab Disord,* 13**,** 103-8.

RASMUSSEN, S. A., CHU, S. Y., KIM, S. Y., SCHMID, C. H. & LAU, J. 2008. Maternal obesity and risk of neural tube defects: a metaanalysis. *Am J Obstet Gynecol,* 198**,** 611-9.

REDLINE, R. W., SHEA, C. M., PAPAIOANNOU, V. E. & LU, C. Y. 1988. Defective anti-listerial responses in deciduoma of pseudopregnant mice. *Am J Pathol,* 133**,** 485-97.

REDMAN, C. W. & SARGENT, I. L. 2003. Pre-eclampsia, the placenta and the maternal systemic inflammatory response--a review. *Placenta,* 24 Suppl A**,** S21-7.

RIDKER, P. M., RIFAI, N., STAMPFER, M. J. & HENNEKENS, C. H. 2000. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation,* 101**,** 1767-72.

ROMAN, A. S., REBARBER, A., FOX, N. S., KLAUSER, C. K., ISTWAN, N., RHEA, D. & SALTZMAN, D. 2011. The effect of maternal obesity on pregnancy outcomes in women with gestational diabetes. *J Matern Fetal Neonatal Med,* 24**,** 723-7.

ROMERO-CORRAL, A., MONTORI, V. M., SOMERS, V. K., KORINEK, J., THOMAS, R. J., ALLISON, T. G., MOOKADAM, F. & LOPEZ-JIMENEZ, F. 2006. Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: a systematic review of cohort studies. *Lancet,* 368**,** 666-78.

ROSEN, B. S., COOK, K. S., YAGLOM, J., GROVES, D. L., VOLANAKIS, J. E., DAMM, D., WHITE, T. & SPIEGELMAN, B. M. 1989. Adipsin and complement factor D activity: an immune-related defect in obesity. *Science,* 244**,** 1483-7.

SALEH, J., AL-RIYAMI, H. D., CHAUDHARY, T. A. & CIANFLONE, K. 2008. Cord blood ASP is predicted by maternal lipids and correlates with fetal birth weight. *Obesity (Silver Spring),* 16**,** 1193-8.

SALEH, J., CHRISTOU, N. & CIANFLONE, K. 1999. Regional specificity of ASP binding in human adipose tissue. *Am J Physiol,* 276**,** E815-21.

SALEH, J., CIANFLONE, K., CHAUDHARY, T., AL-RIYAMI, H., AL-ABRI, A. R. & BAYOUMI, R. 2007. Increased plasma acylation-stimulating protein correlates with hyperlipidemia at late gestation. *Obesity (Silver Spring),* 15**,** 646-52.

SALEH, J., SUMMERS, L. K., CIANFLONE, K., FIELDING, B. A., SNIDERMAN, A. D. & FRAYN, K. N. 1998. Coordinated release of acylation stimulating protein (ASP) and triacylglycerol clearance by human adipose tissue in vivo in the postprandial period. *J Lipid Res,* 39**,** 884-91.

SALSBERRY, P. J. & REAGAN, P. B. 2005. Dynamics of early childhood overweight. *Pediatrics,* 116**,** 1329-38.

SAMAD, F. & LOSKUTOFF, D. J. 1996. Tissue distribution and regulation of plasminogen activator inhibitor-1 in obese mice. *Mol Med,* 2**,** 568-82.

SAMAD, F., YAMAMOTO, K. & LOSKUTOFF, D. J. 1996. Distribution and regulation of plasminogen activator inhibitor-1 in murine adipose tissue in vivo. Induction by tumor necrosis factor-alpha and lipopolysaccharide. *J Clin Invest,* 97**,** 37-46.

SARRUF, D. A., THALER, J. P., MORTON, G. J., GERMAN, J., FISCHER, J. D., OGIMOTO, K. & SCHWARTZ, M. W. 2010. Fibroblast growth factor 21 action in the brain increases energy expenditure and insulin sensitivity in obese rats. *Diabetes,* 59**,** 1817-24.

SARTIPY, P. & LOSKUTOFF, D. J. 2003. Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proc Natl Acad Sci U S A,* 100**,** 7265-70.

SAVAGE, D. B. & O'RAHILLY, S. 2002. Leptin: a novel therapeutic role in lipodystrophy. *J Clin Invest,* 109**,** 1285-6.

SCHAEFER-GRAF, U. M., GRAF, K., KULBACKA, I., KJOS, S. L., DUDENHAUSEN, J., VETTER, K. & HERRERA, E. 2008. Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus. *Diabetes Care,* 31**,** 1858-63.

SCHWARTZ, R. & TERAMO, K. A. 2000. Effects of diabetic pregnancy on the fetus and newborn. *Semin Perinatol,* 24**,** 120-35.

SCRIBA, P. C., BAUER, M., EMMERT, D., FATEH-MOGHADAM, A., HOFMANN, G. G., HORN, K. & PICKARDT, C. R. 1979. Effects of obesity, total fasting and re-alimentation on L-thyroxine (T4), 3,5,3'-L-triiodothyronine (T3), 3,3',5'-L-triiodothyronine (rT3), thyroxine binding globulin (TBG), cortisol, thyrotrophin, cortisol binding globulin (CBG), transferrin, alpha 2-haptoglobin and complement C'3 in serum. *Acta Endocrinol (Copenh),* 91**,** 629-43.

SEBIRE, N. J., JOLLY, M., HARRIS, J. P., WADSWORTH, J., JOFFE, M., BEARD, R. W., REGAN, L. & ROBINSON, S. 2001. Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London. *Int J Obes Relat Metab Disord,* 25**,** 1175-82.

SEUFERT, J. 2004. Leptin effects on pancreatic beta-cell gene expression and function. *Diabetes,* 53 Suppl 1**,** S152-8.

SEVAL, Y., KORGUN, E. T. & DEMIR, R. 2007. Hofbauer cells in early human placenta: possible implications in vasculogenesis and angiogenesis. *Placenta,* 28**,** 841-5.
SEWELL, M. F., HUSTON-PRESLEY, L., SUPER, D. M. & CATALANO, P. 2006. Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. *Am J Obstet Gynecol,* 195**,** 1100-3.
SHAFRIR, E. & BARASH, V. 1987. Placental function in maternal-fetal fat transport in diabetes. *Biol Neonate,* 51**,** 102-12.
SHIMOMURA, I., HAMMER, R. E., IKEMOTO, S., BROWN, M. S. & GOLDSTEIN, J. L. 1999. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature,* 401**,** 73-6.
SHULMAN, G. I. 2000. Cellular mechanisms of insulin resistance. *J Clin Invest,* 106**,** 171-6.
SIBLEY, C., GLAZIER, J. & D'SOUZA, S. 1997. Placental transporter activity and expression in relation to fetal growth. *Exp Physiol,* 82**,** 389-402.
SIEGRIST-KAISER, C. A., PAULI, V., JUGE-AUBRY, C. E., BOSS, O., PERNIN, A., CHIN, W. W., CUSIN, I., ROHNER-JEANRENAUD, F., BURGER, A. G., ZAPF, J. & MEIER, C. A. 1997. Direct effects of leptin on brown and white adipose tissue. *J Clin Invest,* 100**,** 2858-64.
SIMMONS, D. 2011. Diabetes and obesity in pregnancy. *Best Pract Res Clin Obstet Gynaecol,* 25**,** 25-36.
SINGHAL, A. & LUCAS, A. 2004. Early origins of cardiovascular disease: is there a unifying hypothesis? *Lancet,* 363**,** 1642-5.
SIVAN, E., CHEN, X., HOMKO, C. J., REECE, E. A. & BODEN, G. 1997. Longitudinal study of carbohydrate metabolism in healthy obese pregnant women. *Diabetes Care,* 20**,** 1470-5.
SNIDERMAN, A. D. & CIANFLONE, K. 1994. The adipsin-ASP pathway and regulation of adipocyte function. *Ann Med,* 26**,** 388-93.
SNIDERMAN, A. D., MASLOWSKA, M. & CIANFLONE, K. 2000. Of mice and men (and women) and the acylation-stimulating protein pathway. *Curr Opin Lipidol,* 11**,** 291-6.
SODOWSKI, K., ZWIRSKA-KORCZALA, K., KUKA, D., KUKLA, M., BUDZISZEWSKA, P., ZEBATY, A., WENDER-OZEGOWSKA, E., BAUMERT, M. & WLOCH, A. 2008. Acylation stimulating protein is associated with pregnancy weight gain. *J Physiol Pharmacol,* 59 Suppl 4**,** 33-43.
SOENS, M. A., BIRNBACH, D. J., RANASINGHE, J. S. & VAN ZUNDERT, A. 2008. Obstetric anesthesia for the obese and morbidly obese patient: an ounce of prevention is worth more than a pound of treatment. *Acta Anaesthesiol Scand,* 52**,** 6-19.
SORIA, A., BOCOS, C. & HERRERA, E. 2002. Opposite metabolic response to fenofibrate treatment in pregnant and virgin rats. *J Lipid Res,* 43**,** 74-81.
SPARKS, J. W. 1984. Human intrauterine growth and nutrient accretion. *Semin Perinatol,* 8**,** 74-93.
SPELLACY, W. N., MILLER, S., WINEGAR, A. & PETERSON, P. Q. 1985. Macrosomia--maternal characteristics and infant complications. *Obstet Gynecol,* 66**,** 158-61.
SPIEGELMAN, B. M., FRANK, M. & GREEN, H. 1983. Molecular cloning of mRNA from 3T3 adipocytes. Regulation of mRNA content for

glycerophosphate dehydrogenase and other differentiation-dependent proteins during adipocyte development. *J Biol Chem,* 258**,** 10083-9.

STAMATAKIS, E., PRIMATESTA, P., CHINN, S., RONA, R. & FALASCHETI, E. 2005. Overweight and obesity trends from 1974 to 2003 in English children: what is the role of socioeconomic factors? *Arch Dis Child,* 90**,** 999-1004.

STEIN, S., STEPAN, H., KRATZSCH, J., VERLOHREN, M., VERLOHREN, H. J., DRYNDA, K., LOSSNER, U., BLUHER, M., STUMVOLL, M. & FASSHAUER, M. 2010. Serum fibroblast growth factor 21 levels in gestational diabetes mellitus in relation to insulin resistance and dyslipidemia. *Metabolism,* 59**,** 33-7.

STEWART, F. M., FREEMAN, D. J., RAMSAY, J. E., GREER, I. A., CASLAKE, M. & FERRELL, W. R. 2007. Longitudinal assessment of maternal endothelial function and markers of inflammation and placental function throughout pregnancy in lean and obese mothers. *J Clin Endocrinol Metab,* 92**,** 969-75.

STOTHARD, K. J., TENNANT, P. W., BELL, R. & RANKIN, J. 2009. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. *JAMA,* 301**,** 636-50.

SZABO, A. J. & SZABO, O. 1974. Placental free-fatty-acid transfer and fetal adipose-tissue development: an explantation of fetal adiposity in infants of diabetic mothers. *Lancet,* 2**,** 498-9.

TACHI, C., TACHI, S., KNYSZYNSKI, A. & LINDNER, H. R. 1981. Possible involvement of macrophages in embryo--maternal relationships during ovum implantation in the rat. *J Exp Zool,* 217**,** 81-92.

TAKESHITA, A., KONDO, T., OKADA, T. & KUSAKABE, K. T. 2010. Elevation of adipsin, a complement activating factor, in the mouse placenta during spontaneous abortion. *J Reprod Dev,* 56**,** 508-14.

TAN, B. K., CHEN, J., LEHNERT, H., KENNEDY, R. & RANDEVA, H. S. 2007. Raised serum, adipocyte, and adipose tissue retinol-binding protein 4 in overweight women with polycystic ovary syndrome: effects of gonadal and adrenal steroids. *J Clin Endocrinol Metab,* 92**,** 2764-72.

TAN, B. K., HALLSCHMID, M., ADYA, R., KERN, W., LEHNERT, H. & RANDEVA, H. S. 2011. Fibroblast growth factor 21 (FGF21) in human cerebrospinal fluid: relationship with plasma FGF21 and body adiposity. *Diabetes,* 60**,** 2758-62.

TANG, Z., TADESSE, S., NORWITZ, E., MOR, G., ABRAHAMS, V. M. & GULLER, S. 2011a. Isolation of hofbauer cells from human term placentas with high yield and purity. *American journal of reproductive immunology,* 66**,** 336-48.

TANG, Z., TADESSE, S., NORWITZ, E., MOR, G., ABRAHAMS, V. M. & GULLER, S. 2011b. Isolation of hofbauer cells from human term placentas with high yield and purity. *Am J Reprod Immunol,* 66**,** 336-48.

TARTAGLIA, L. A., DEMBSKI, M., WENG, X., DENG, N., CULPEPPER, J., DEVOS, R., RICHARDS, G. J., CAMPFIELD, L. A., CLARK, F. T., DEEDS, J., MUIR, C., SANKER, S., MORIARTY, A., MOORE, K. J., SMUTKO, J. S., MAYS, G. G., WOOL, E. A., MONROE, C. A. & TEPPER, R. I. 1995. Identification and expression cloning of a leptin receptor, OB-R. *Cell,* 83**,** 1263-71.

TASKINEN, M. R. & NIKKILA, E. A. 1979. Lipoprotein lipase activity of adipose tissue and skeletal muscle in insulin-deficient human diabetes. Relation to high-density and very-low-density lipoproteins and response to treatment. *Diabetologia,* 17**,** 351-6.

THOMAS, C. R. 1987. Placental transfer of non-esterified fatty acids in normal and diabetic pregnancy. *Biol Neonate,* 51**,** 94-101.

TOESCU, V., NUTTALL, S. L., MARTIN, U., NIGHTINGALE, P., KENDALL, M. J., BRYDON, P. & DUNNE, F. 2004. Changes in plasma lipids and markers of oxidative stress in normal pregnancy and pregnancies complicated by diabetes. *Clin Sci (Lond),* 106**,** 93-8.

TORNETTA, M. A., FOLEY, J. J., SARAU, H. M. & AMES, R. S. 1997. The mouse anaphylatoxin C3a receptor: molecular cloning, genomic organization, and functional expression. *J Immunol,* 158**,** 5277-82.

USHA KIRAN, T. S., HEMMADI, S., BETHEL, J. & EVANS, J. 2005. Outcome of pregnancy in a woman with an increased body mass index. *BJOG,* 112**,** 768-72.

VAHRATIAN, A., ZHANG, J., TROENDLE, J. F., SAVITZ, D. A. & SIEGA-RIZ, A. M. 2004. Maternal prepregnancy overweight and obesity and the pattern of labor progression in term nulliparous women. *Obstet Gynecol,* 104**,** 943-51.

VAN ASSCHE, F. A., HOLEMANS, K. & AERTS, L. 2001. Long-term consequences for offspring of diabetes during pregnancy. *Br Med Bull,* 60**,** 173-82.

VAN GAAL, L. F., MERTENS, I. L. & ABRAMS, P. J. 2003. Health risks of lipodystrophy and abdominal fat accumulation: therapeutic possibilities with leptin and human growth hormone. *Growth Horm IGF Res,* 13 Suppl A**,** S4-9.

VAN HARMELEN, V., REYNISDOTTIR, S., CIANFLONE, K., DEGERMAN, E., HOFFSTEDT, J., NILSELL, K., SNIDERMAN, A. & ARNER, P. 1999. Mechanisms involved in the regulation of free fatty acid release from isolated human fat cells by acylation-stimulating protein and insulin. *J Biol Chem,* 274**,** 18243-51.

VAN HOORN, J., DEKKER, G. & JEFFRIES, B. 2002. Gestational diabetes versus obesity as risk factors for pregnancy-induced hypertensive disorders and fetal macrosomia. *Aust N Z J Obstet Gynaecol,* 42**,** 29-34.

VAZQUEZ, G., DUVAL, S., JACOBS, D. R., JR. & SILVENTOINEN, K. 2007. Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a meta-analysis. *Epidemiol Rev,* 29**,** 115-28.

VILLAR, J., COGSWELL, M., KESTLER, E., CASTILLO, P., MENENDEZ, R. & REPKE, J. T. 1992. Effect of fat and fat-free mass deposition during pregnancy on birth weight. *Am J Obstet Gynecol,* 167**,** 1344-52.

VOGEL, R. A., CORRETTI, M. C. & PLOTNICK, G. D. 1997. Effect of a single high-fat meal on endothelial function in healthy subjects. *Am J Cardiol,* 79**,** 350-4.

VOZAROVA, B., WEYER, C., HANSON, K., TATARANNI, P. A., BOGARDUS, C. & PRATLEY, R. E. 2001. Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes Res,* 9**,** 414-7.

WAKABAYASHI, I. 1998. Age-related change in relationship between body-mass index, serum sialic acid, and atherogenic risk factors. *J Atheroscler Thromb,* 5**,** 60-5.

WAKE, D. J. & WALKER, B. R. 2004. 11 beta-hydroxysteroid dehydrogenase type 1 in obesity and the metabolic syndrome. *Mol Cell Endocrinol,* 215**,** 45-54.
WALLER, D. K., MILLS, J. L., SIMPSON, J. L., CUNNINGHAM, G. C., CONLEY, M. R., LASSMAN, M. R. & RHOADS, G. G. 1994. Are obese women at higher risk for producing malformed offspring? *Am J Obstet Gynecol,* 170**,** 541-8.
WANG, Y. & LOBSTEIN, T. 2006. Worldwide trends in childhood overweight and obesity. *Int J Pediatr Obes,* 1**,** 11-25.
WANG, Y., SULLIVAN, S., TRUJILLO, M., LEE, M. J., SCHNEIDER, S. H., BROLIN, R. E., KANG, Y. H., WERBER, Y., GREENBERG, A. S. & FRIED, S. K. 2003. Perilipin expression in human adipose tissues: effects of severe obesity, gender, and depot. *Obes Res,* 11**,** 930-6.
WASFI, I., WEINSTEIN, I. & HEIMBERG, M. 1980. Hepatic metabolism of [1-14C]oleate in pregnancy. *Biochim Biophys Acta,* 619**,** 471-81.
WATKINS, M. L., RASMUSSEN, S. A., HONEIN, M. A., BOTTO, L. D. & MOORE, C. A. 2003. Maternal obesity and risk for birth defects. *Pediatrics,* 111**,** 1152-8.
WEISBERG, S. P., MCCANN, D., DESAI, M., ROSENBAUM, M., LEIBEL, R. L. & FERRANTE, A. W., JR. 2003. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest,* 112**,** 1796-808.
WEISS, J. L., MALONE, F. D., EMIG, D., BALL, R. H., NYBERG, D. A., COMSTOCK, C. H., SAADE, G., EDDLEMAN, K., CARTER, S. M., CRAIGO, S. D., CARR, S. R. & D'ALTON, M. E. 2004. Obesity, obstetric complications and cesarean delivery rate--a population-based screening study. *Am J Obstet Gynecol,* 190**,** 1091-7.
WELLHOENER, P., FRUEHWALD-SCHULTES, B., KERN, W., DANTZ, D., KERNER, W., BORN, J., FEHM, H. L. & PETERS, A. 2000. Glucose metabolism rather than insulin is a main determinant of leptin secretion in humans. *J Clin Endocrinol Metab,* 85**,** 1267-71.
WENTE, W., EFANOV, A. M., BRENNER, M., KHARITONENKOV, A., KOSTER, A., SANDUSKY, G. E., SEWING, S., TREINIES, I., ZITZER, H. & GROMADA, J. 2006. Fibroblast growth factor-21 improves pancreatic beta-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. *Diabetes,* 55**,** 2470-8.
WEYER, C. & PRATLEY, R. E. 1999. Fasting and postprandial plasma concentrations of acylation-stimulation protein (ASP) in lean and obese Pima Indians compared to Caucasians. *Obes Res,* 7**,** 444-52.
WEYER, C., TATARANNI, P. A. & PRATLEY, R. E. 2000. Insulin action and insulinemia are closely related to the fasting complement C3, but not acylation stimulating protein concentration. *Diabetes Care,* 23**,** 779-85.
WEYER, C., YUDKIN, J. S., STEHOUWER, C. D., SCHALKWIJK, C. G., PRATLEY, R. E. & TATARANNI, P. A. 2002. Humoral markers of inflammation and endothelial dysfunction in relation to adiposity and in vivo insulin action in Pima Indians. *Atherosclerosis,* 161**,** 233-42.
WHITAKER, R. C. 2004. Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. *Pediatrics,* 114**,** e29-36.
WHITE, B. D. & MARTIN, R. J. 1997. Evidence for a central mechanism of obesity in the Zucker rat: role of neuropeptide Y and leptin. *Proc Soc Exp Biol Med,* 214**,** 222-32.

WHITE, R. T., DAMM, D., HANCOCK, N., ROSEN, B. S., LOWELL, B. B., USHER, P., FLIER, J. S. & SPIEGELMAN, B. M. 1992. Human adipsin is identical to complement factor D and is expressed at high levels in adipose tissue. *J Biol Chem,* 267**,** 9210-3.

WOLK, A., GRIDLEY, G., SVENSSON, M., NYREN, O., MCLAUGHLIN, J. K., FRAUMENI, J. F. & ADAM, H. O. 2001. A prospective study of obesity and cancer risk (Sweden). *Cancer Causes Control,* 12**,** 13-21.

WOOD, G., REYNARD, J., KRISHNAN, E. & RACELA, L. 1978. Immunobiology of the human placenta. II. Localization of macrophages, in vivo bound IgG and C3. *Cell Immunol,* 35**,** 205-16.

WOOD, G. W. 1980. Immunohistological identification of macrophages in murine placentae, yolk-sac membranes and pregnant uteri. *Placenta,* 1**,** 309-17.

WOOD, G. W. & KING, C. R., JR. 1982. Trapping antigen-antibody complexes within the human placenta. *Cell Immunol,* 69**,** 347-62.

WORLD HEALTH ORGANIZATION. 2009. *Global prevalence of vitamin A deficiency in populations at risk 1995-2005 : WHO global database on vitamin A deficiency,* Geneva, World Health Organization.

WORLD HEALTH ORGANIZATION. DIVISION OF NONCOMMUNICABLE DISEASES. & WORLD HEALTH ORGANIZATION. PROGRAMME OF NUTRITION FAMILY AND REPRODUCTIVE HEALTH. 1998. *Obesity : preventing and managing the global epidemic : report of a WHO Consultation on Obesity, Geneva, 3-5 June 1997,* Geneva, World Health Organization.

WORLD HEALTH ORGANIZATION. OFFICE OF HEALTH COMMUNICATIONS AND PUBLIC RELATIONS. 2006. *Obesity and overweight,* Geneva, World Health Organization.

XIA, Z. & CIANFLONE, K. 2003. Acylation-stimulating protein precursor proteins in adipose tissue in human obesity. *Metabolism,* 52**,** 1360-6.

XU, H., BARNES, G. T., YANG, Q., TAN, G., YANG, D., CHOU, C. J., SOLE, J., NICHOLS, A., ROSS, J. S., TARTAGLIA, L. A. & CHEN, H. 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest,* 112**,** 1821-30.

XU, J., LLOYD, D. J., HALE, C., STANISLAUS, S., CHEN, M., SIVITS, G., VONDERFECHT, S., HECHT, R., LI, Y. S., LINDBERG, R. A., CHEN, J. L., JUNG, D. Y., ZHANG, Z., KO, H. J., KIM, J. K. & VENIANT, M. M. 2009. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes,* 58**,** 250-9.

YAMASHITA, T., YOSHIOKA, M. & ITOH, N. 2000. Identification of a novel fibroblast growth factor, FGF-23, preferentially expressed in the ventrolateral thalamic nucleus of the brain. *Biochem Biophys Res Commun,* 277**,** 494-8.

YANG, Y., LU, H. L., ZHANG, J., YU, H. Y., WANG, H. W., ZHANG, M. X. & CIANFLONE, K. 2006. Relationships among acylation stimulating protein, adiponectin and complement C3 in lean vs obese type 2 diabetes. *Int J Obes (Lond),* 30**,** 439-46.

YASRUEL, Z., CIANFLONE, K., SNIDERMAN, A. D., ROSENBLOOM, M., WALSH, M. & RODRIGUEZ, M. A. 1991. Effect of acylation stimulating protein on the triacylglycerol synthetic pathway of human adipose tissue. *Lipids,* 26**,** 495-9.

YU, H., YANG, Y., ZHANG, M., LU, H., ZHANG, J., WANG, H. & CIANFLONE, K. 2006. Thyroid status influence on adiponectin, acylation stimulating protein (ASP) and complement C3 in hyperthyroid and hypothyroid subjects. *Nutr Metab (Lond)*, 3**,** 13.

ZHANG, H. H., HALBLEIB, M., AHMAD, F., MANGANIELLO, V. C. & GREENBERG, A. S. 2002. Tumor necrosis factor-alpha stimulates lipolysis in differentiated human adipocytes through activation of extracellular signal-related kinase and elevation of intracellular cAMP. *Diabetes,* 51**,** 2929-35.

ZHANG, H. H., SOUZA, S. C., MULIRO, K. V., KRAEMER, F. B., OBIN, M. S. & GREENBERG, A. S. 2003. Lipase-selective functional domains of perilipin A differentially regulate constitutive and protein kinase A-stimulated lipolysis. *J Biol Chem,* 278**,** 51535-42.

ZHANG, X., YEUNG, D. C., KARPISEK, M., STEJSKAL, D., ZHOU, Z. G., LIU, F., WONG, R. L., CHOW, W. S., TSO, A. W., LAM, K. S. & XU, A. 2008. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes,* 57**,** 1246-53.

ZHANG, X. J., CIANFLONE, K., GENEST, J. & SNIDERMAN, A. D. 1998. Plasma acylation stimulating protein (ASP) as a predictor of impaired cellular biological response to ASP in patients with hyperapoB. *Eur J Clin Invest,* 28**,** 730-9.

ZHU, L., WIGLE, D., HINEK, A., KOBAYASHI, J., YE, C., ZUKER, M., DODO, H., KEELEY, F. W. & RABINOVITCH, M. 1994. The endogenous vascular elastase that governs development and progression of monocrotaline-induced pulmonary hypertension in rats is a novel enzyme related to the serine proteinase adipsin. *J Clin Invest,* 94**,** 1163-71.

ZIMMET, P., BOYKO, E. J., COLLIER, G. R. & DE COURTEN, M. 1999. Etiology of the metabolic syndrome: potential role of insulin resistance, leptin resistance, and other players. *Ann N Y Acad Sci,* 892**,** 25-44.

APPENDIX