CRANFIELD UNIVERSITY

Dr JYOTI BALANI

OBESITY IN PREGNANCY: RISK OF GESTATIONAL DIABETES

School of Aerospace, Transport and Manufacturing

Doctor of Philosophy
Academic Year: 2012- 2018

Academic Supervisors: Professor Selim Cellek & Dr Fady Mohareb
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This thesis is submitted in partial fulfilment of the requirements for the degree of PhD

ABSTRACT

Background: Maternal obesity is a risk factor for gestational diabetes and other adverse pregnancy outcomes, but the body fat distribution may be a more important risk factor than body mass index. Pregnancy is an insulin resistant state and more so, in obese women. Metformin could be beneficial in obese pregnant women due to its insulin sensitizing action. The aims of this study are to investigate visceral fat mass as a risk factor for gestational diabetes (VFM study), to develop a mathematical model for the prediction of gestational diabetes in obese women (VFM study) and to examine the effect of metformin on pregnancy outcomes in obese non-diabetic women (MOP Trial).

Methods and Results:

VFM study: The body composition of 302 obese pregnant women was assessed using bioelectrical impedance. A mathematical model to predict gestational diabetes using machine learning was developed using visceral fat mass which is a novel risk factor in addition to conventional risk factors. 72 of the women developed gestational diabetes (GDM). These women had higher visceral fat mass. Women with a baseline visceral fat mass ≥ 75th percentile, had a 3-fold risk of subsequent gestational diabetes. The mathematical model predicted gestational diabetes with an average overall accuracy of 77.5% and predicted birth centile classes with an average accuracy of 68%. According to the decision tree developed, VFM emerged as the most important variable in determining the risk of GDM and a VFM < 210 was used as the first split in the decision tree.

MOP Trial: 133 obese pregnant women were randomised to either metformin or placebo. The pregnancy outcomes were compared in both groups. Insulin resistance was measured in all women. 118 women completed the trial. Metformin did not reduce the neonatal birth weight z-score, which was the primary outcome of the trial or the incidence of large for gestational age babies. However, metformin therapy significantly reduced gestational weight gain, reduced the pregnancy rise in visceral fat mass, and attenuated the expected

physiological rise in insulin resistance at 28 weeks gestation. However, this did not result in an overall significant reduction in the incidence of gestational diabetes. There was a trend towards a reduced incidence of gestational diabetes in women with high baseline insulin resistance randomised to metformin.

Conclusions: Visceral fat mass is a novel risk factor for gestational diabetes. The mathematical model successfully predicted gestational diabetes. Metformin reduced gestational weight gain and insulin resistance but did not lower the median neonatal birth weight or reduce the incidence of GDM.

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LIST OF ABBREVIATIONS

ACHOIS	Australian Carbohydrate Intolerance in Pregnancy Study
ACOG	American College of Obstetricians and Gynaecologists
ADA	American Diabetes Association
AGE	Advanced glycation end products
BIA	Bioelectrical impedance analysis
BME	Black and minority ethnic
BMI	Body mass index
BW	Birth weight
CDC	Centre for Disease Control and Prevention
CEMACH	Confidential Enquiry into Maternal and Child Health
CMACE	Centre for Maternal and Child Enquiries
CI	Confidence interval
CRP	C-reactive protein
CS	Caesarean section
CTA	Clinical Trial Authorisation
CUHREC	Cranfield University Health Research Ethics Committee
CVD	Cardiovascular disease
DEXA	Dual energy X-ray absorptiometry
DM	Diabetes mellitus
DSM-BIA	Direct segmental multi-frequency bioelectrical impedance analysis
EASD	European Association for the Study of Diabetes
ECF	Extracellular fluid
EMPOWaR	Effect of metformin on maternal and foetal outcomes
FDA	Food and Drug Administration
FPG	Fasting plasma glucose
FFA	Free Fatty Acids
GAD	Glutamic acid decarboxylase

GDM	Gestational diabetes mellitus
GLP-1	Glucagon like protein 1
GWG	Gestational weight gain
HAPO	Hyperglycaemia and Adverse Pregnancy Outcomes
HbA1c	Glycosylated haemoglobin
HDL	High density lipoproteins
HOMA	Homeostatic model of assessment
HPL	Human placental lactogen
HR	Hazard ratio
IAA	Insulin autoantibodies
IADPSG	International Association of Diabetes and Pregnancy Study Group
ICA	Islet cell autoantibodies
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IOM	Institute of Medicine
IQR	Interquartile range
IRS	Insulin Receptor Substrate
LDL	Low density lipoproteins
LGA	Large for gestational age
LADA	Latent Autoimmune Diabetes of the Adult
LiP	Lifestyle in pregnancy study
MHRA	Medicines and Health Regulatory Agency
MiG	Metformin in Gestational diabetes trial
MiG-TOFU	MiG- The offspring follow-up study
MOP	Metformin in Obese non-diabetic Pregnant women trial
MODY	Maturity Onset Diabetes of the Young
NHANES	National Health and Nutrition Examination Survey

NICE	National Institute for Health and Care Excellence
OGTT	Oral glucose tolerance test
OR	Odds ratio
PBF	Percentage body fat
PCA	Principal Component Analysis
PET	Pre-eclamptic toxaemia
P13K	Phosphoinositide 3’ Kinase
RF	Random Forest
SAE	Serious Adverse Event
SCBU	Special care baby unit
SEM	Standard error of the mean
SGA	Small for gestational age
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TBW	Total body water
TNF-α	Tumour Necrosis Factor- α
TOFU	The Offspring Follow-Up
UCL	University College of London
UPBEAT	UK pregnancies better eating and activity trial
UPSTIF	US Preventive Services Task Force
VBAC	Vaginal delivery after Caesarean section
VFM	Visceral fat mass
VLDL	Very low density lipoproteins
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist Hip Ratio
Zn T8	Zinc Transporter protein 8

1 INTRODUCTION

1.1 Diabetes Mellitus

1.1.1 Definition

Diabetes mellitus (DM) can be defined as a state of chronic hyperglycaemia sufficient to cause long-term damage to organs and tissues including retina, kidney, nerves and blood vessels [1]. It is thought to be due to a varying combination of insufficient production of insulin and /or resistance to the glucose lowering action of insulin [1]. The word diabetes is derived from a Greek word meaning a "*siphon*". The 2nd century AD Greek physician Aretus the Cappadocian, named this condition diabetes as he observed that these patients "*passed water like a siphon*", referring to polyuria observed in patients with untreated DM [2].

DM has been known since about 1500 BC, when Hindu scholars described a condition wherein a patient would present with symptoms of polydipsia, polyuria and production of sweet urine which would attract flies and ants. These patients would also have signs of wasting [3].

1.1.2 Aetiological classification of diabetes mellitus

DM is now classified depending on the pathologic process that leads to hyperglycaemia, as opposed to earlier when it was classified depending on age of onset and treatment required [4]. The two broad categories of diabetes are designated as type 1 (T1DM) and type 2 (T2DM) [4].

Type 1 diabetes mellitus

T1DM is caused by an autoimmune process which selectively causes pancreatic β cell destruction leading to deficiency of insulin [1]. T1DM has long been called "*juvenile diabetes*" because of the more frequent and straightforward diagnosis in children. However, a majority of the individuals with T1DM are adults [5].

Type 2 diabetes mellitus

T2DM is characterised by a combination of insulin resistance, impaired insulin secretion and increased glucose production in varying proportions [4]. Patients usually have a period of abnormal glucose tolerance which may be impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) for a few years before the diagnosis of T2DM [4].

Other specific types of diabetes mellitus

Genetic defects of β cell development

Maturity onset diabetes of the young (MODY) is the most common form of monogenic diabetes, accounting for 2-5% of diabetes. MODY is characterised by a primary defect in insulin secretion and hyperglycaemia, non-ketotic disease, monogenic autosomal dominant mode of inheritance, age at onset less than 25 years, and lack of auto-antibodies. The diagnosis can be suspected by careful clinical evaluation, but genetic analysis is essential for subtyping [6].

Genetic defects in insulin secretion

Mutations in the insulin receptor cause a group of rare disorders characterised by severe insulin resistance like type A insulin resistance and Lipodystrophy syndromes [4].

Diseases of the exocrine pancreas

DM secondary to pancreatic disease is commonly referred to as pancreatogenic diabetes or type 3c DM. The prevalence of this kind of diabetes is 5% to 10% among all diabetic subjects. In nearly 80% of all type 3c diabetes mellitus cases, chronic pancreatitis seem to be the underlying disease [7]. The other common causes are cystic fibrosis and hereditary hemochromatosis.

Endocrinopathies

Several hormones, such as adrenaline, glucagon, cortisol and growth hormone antagonise the actions of insulin. Endocrine abnormalities involving primary over-secretion of these hormones can result in overt diabetes including Cushing's syndrome, due to pituitary or adrenal disease or to exogenous

glucocorticoid administration, acromegaly, catecholamine excess in pheochromocytoma and glucagon secreting tumours (Glucagonomas) [4].

Drug or chemical induced diabetes mellitus

A large number of drugs can impair glucose tolerance by decreasing insulin secretion, increasing hepatic glucose production or causing resistance to the action of insulin. The common drugs implicated are glucocorticoids, oral contraceptives, beta blockers, thiazide diuretics, nicotinic acid, statins and drugs like tacrolimus, sirolimus and cyclosporine used to prevent transplant rejection [4].

Gestational diabetes

Gestational diabetes mellitus (GDM) is traditionally defined as “Carbohydrate intolerance of varying severity with onset or first recognition in pregnancy” [8]. The new definition “diabetes first recognised in the second or third trimester of pregnancy that is not clearly overt diabetes” reflects the changes in the approach to this condition [9]. It is described in detail later.

Latent autoimmune diabetes of the adult (LADA)

Individuals diagnosed with autoimmune diabetes when they are adults and who may not initially require insulin treatment have been classified as having latent autoimmune diabetes of the adult (LADA). [10]. It is also called slowly progressive insulin-dependent diabetes or type 1.5 diabetes [10].

1.1.3 Epidemiology of diabetes mellitus

The prevalence of DM has significantly increased in the last 20 years from 30 million cases in 1985 to 382 million in 2013 [4]. The Centre for Disease Control and Prevention (CDC) in its most recent estimate for the US (2012) estimated that 9.3% of the population had diabetes and 28% of the individuals with diabetes were undiagnosed [4].

The prevalence as well as the type of DM also varies in the different regions of the world due to ethnic, genetic and environmental influences. Scandinavia has the highest incidence of T1DM, and the lowest is in the Pacific Rim. Northern

Europe and the US have an intermediate incidence [4]. The prevalence of T2DM is highest in certain Pacific Islands and in the Middle East, and low in Russia and China [4]. In the United States, DM was listed as the seventh leading cause of death [4].

1.1.4 Pathophysiology of DM

Regulation of glucose homeostasis

Glucose homeostasis is maintained by a balance between hepatic glucose production and peripheral glucose uptake and utilisation. Insulin is initially synthesised as preproinsulin in the β cells of the pancreatic islets. Subsequent proteolytic processing removes the peptide giving rise to proinsulin [4]. Cleavage of an internal fragment from proinsulin generates the C peptide and the A (21 amino acids) and B (30 amino acids) chains of insulin, which are connected by disulphide bonds. The mature insulin molecule and C peptide are stored together and co-secreted from secretory granules in the β cells. C-peptide is cleared more slowly than insulin and is therefore a useful marker of insulin secretion [4].

Insulin secretion

Glucose is the key regulator on insulin secretion by the pancreatic β cell. Glucose levels greater than 3.9 mmol/L stimulate insulin release, primarily by enhancing protein translation and processing. It begins with the transport of glucose into the β cell. Glucose phosphorylation by glucokinase is the rate limiting step [4]. Further metabolism of glucose-6-phosphate via glycolysis generates ATP, which inhibits the activity of an ATP-sensitive K channel. Inhibition of the K channel induces β cell membrane depolarisation, which opens calcium channels and stimulates insulin secretion [4].

Insulin action

After insulin is secreted into the portal venous system, 50% is removed and degraded by the liver. Unmetabolised insulin enters the systemic circulation where it binds to receptors in target sites. The molecular signal initiating insulin action in humans involves activation of the insulin receptor tyrosine kinase,

resulting in phosphorylation of insulin receptor substrates (IRSs) on multiple tyrosine residues. These phosphotyrosine residues act as docking sites for proteins, including the p85 subunit of phosphoinositide 3' kinase (PI3K). Binding of the p110 subunit of PI3K to p85 activates the lipid kinase that promotes glucose transport [11]. The signals downstream of PI3K are unknown.

Pathogenesis of type 1 diabetes mellitus

It is believed that T1DM develops as a result of the additive effects of genetics, environmental and immunological factors ultimately causing destruction of pancreatic β cells and insulin deficiency [4]. These patients may either progress rapidly to clinical diabetes or evolve slowly. DM manifests when 70-80% of β cells are destroyed. The transition to frank diabetes may be seen during puberty, pregnancy or during infections which are associated with increased insulin requirements [4]. It is now known that most individuals with T1DM have the HLADR3 and/or DR4 haplotype. T1DM has a genetic predilection with a 15-fold increased risk among family members [5].

Pancreatic antibodies are characteristic of T1DM. Five autoantibodies have been detected- Glutamic acid decarboxylase (GADA) antibodies, islet cell antibodies (ICA), insulin autoantibodies (IAA), protein tyrosine phosphatase antibodies (ICA_{512} or IA_2A) and zinc transporter protein (ZnT8). Highly sensitive laboratory measurements can capture 98% of the individuals with auto antibodies at diagnosis. However, commercial laboratories do not have relatively sensitive or specific assays that measure all 5 auto antibodies. Therefore, it is inappropriate to report a patient as autoantibody negative. Also, testing far out from diagnosis may be a cause of "false negative" results as antibody titres diminish in time [5].

After the initial clinical presentation of T1DM, a "honeymoon" phase may ensue during which time glycaemic control may be achieved with small doses of insulin or, rarely insulin is not needed. Eventually, this fleeting phase of endogenous insulin production from residual beta cells disappears [4].

Pathogenesis of type 2 diabetes mellitus

T2DM has also been suggested to have a genetic component and there is an increased risk of DM in an individual if one of his parents has the disease; if both parents have the disease, the risk approaches 40%. The aetiology of T2DM is multifactorial and is contributed by genetic factors as well as lifestyle factors like obesity and decreased physical activity [4]. The pathophysiology of T2DM involves a varying combination of impaired insulin secretion, insulin resistance, excessive hepatic glucose production and abnormal lipids. Obesity, specially visceral obesity is seen in 80% or more of the patients with T2DM [4].

In patients with T2DM, insulin resistance develops years before they present with the diagnosis. This result in the liver, skeletal muscle and adipocytes all becoming less sensitive to the action of insulin resulting in a fasting and postprandial hyperinsulinemia. This compensates and counters the insulin resistance for some time. However, with time, the β cell fails to maintain the high rate of insulin secretion and the relative insulinopenia, relative to the degree of insulin resistance leads to the development of impaired glucose tolerance and ultimately overt diabetes mellitus.

Chronic hyperglycaemia can paradoxically impair islet function and this is known as "*glucose toxicity*" and leads to worsening of hyperglycaemia. In addition, elevation of free fatty acids known as "*lipotoxicity*" and dietary fat can also worsen islet function. Reduced GLP-1 action can also contribute to reduced insulin action [4].

Insulin resistance in T2DM involves both the liver and peripheral muscle tissues. Thus, hepatic glucose production fails to suppress normally and muscle uptake is diminished. The accelerated rate of hepatic glucose output is entirely due to augmented gluconeogenesis [11]. There is evidence that the loss of the first phase of insulin secretion is the earliest detectable abnormality in patients destined to develop DM. Defects at the level of the β cell, muscle or the liver can lead to the development of glucose intolerance. The full blown syndrome of T2DM requires the simultaneous presence of two major defects, insulin resistance and impaired β function [11].

Obesity, which commonly accompanies T2DM, particularly in a central or visceral location is thought to be part of the pathologic process. The increased adipocyte mass leads to increased levels of circulating free fatty acids and adipokines like tumour necrosis factor α (TNF α), leptin, resistin and adiponectin. In addition to regulating body weight, appetite and energy expenditure, adipokines also modulate insulin sensitivity. Increased production of adipokines may cause insulin resistance in skeletal muscle and liver [4]. Insulin resistance in adipose tissue causes increased lipolysis and free fatty acids flux from adipocytes leading to increased lipid synthesis in hepatocytes. Very low density lipoproteins (VLDL) and triglycerides are mainly synthesised. This leads to lipid storage or steatosis in the liver and may lead to non-alcoholic fatty liver disease and abnormal liver function tests. This is also responsible for the dyslipidaemia characterised by elevated triglycerides, low high density lipoprotein (HDL) levels and high low density lipoprotein (LDL) levels seen in T2DM.

1.1.5 Clinical presentation

Type 1 diabetes mellitus

In T1DM, the classical clinical presentation is polyuria, polydipsia and severe weight loss due to increased catabolism and hyperglycaemia [1]. Diuresis is caused mainly by the osmotic effect of glucose causing polyuria, nocturia and enuresis in children. Excessive thirst can occur and can be precipitated by sugary drinks. Severe weight loss occurs due to the loss of fat and muscle and dehydration. These may be accompanied by systemic symptoms like tiredness and lack of energy and blurred vision due to changes in the shape of the lens due to osmotic shifts [1]. Sometimes, they present with diabetic ketoacidosis with vomiting, acidotic breathing and altered consciousness and may progress to coma [1].

Type 2 diabetes mellitus

The common presenting features of T2DM are polyuria, polydipsia, blurred vision due to hyperglycaemia related refractive changes in the lens, infections like genital candidiasis and weight loss. Sometimes, patients may present in the

hyperosmolar non-ketotic state with confusion or coma, but they rarely have ketoacidosis. Sometimes, patients present with the complications of diabetes at the time of diagnosis of T2DM [1].

Chronic complications can be divided into vascular and nonvascular complications [1]. The vascular complications are further divided into microvascular comprising of retinopathy, neuropathy, and nephropathy and macrovascular complications including coronary heart disease, peripheral arterial disease, and cerebrovascular disease. Nonvascular complications include infections and dermopathy. Gastroparesis could be considered a microvascular complication with autonomic neuropathy [4].

1.1.6 Diagnosis

The international committee of experts comprising of members appointed by the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD) and the International Diabetes Federation (IDF) has issued diagnostic criteria for DM based on the two premises that the level of glycaemia leading to complications is more important than a deviation from a population based mean and the response to an oral glucose tolerance test (OGTT) differs among individuals [4].

The criteria for diagnosis of DM are symptoms of DM plus random blood glucose concentration > 11.1 mmol/L (200mg/dl) or fasting plasma glucose (FPG) > 7mmol/L (126mg/dl) or (glycated haemoglobin) HbA_{1c} > 6.5% or 2-hour plasma glucose value > 11.1 mmol/L (200mg/dl) during an OGTT [4]. Impaired Fasting Glucose (IFG) is defined as FPG of 5.6-6.9 mmol/L (100-125mg/dl). World Health Organisation (WHO) defines IFG as FPG of 6.1-6.9 mmol/L (110-125mg/dl). Plasma glucose levels between 7.8 and 11 mmol/L (140 and 199 mg/dl) following an oral glucose challenge, is termed as Impaired Glucose Tolerance (IGT) [4]. It is also known as "*pre-diabetes*", "*increased risk of diabetes*" (ADA) or intermediate hyperglycaemia (WHO) [4].

1.1.7 Principles of management

The goals of therapy in T1DM and T2DM are to reduce symptoms of hyperglycaemia, and decrease the long term microvascular and macrovascular complications of DM while allowing the patient to achieve as normal a lifestyle as possible [4].

The care of an individual with DM requires a multidisciplinary team including primary care provider and preferably a diabetologist, a diabetes educator and a nutritionist with the patients' participation and input at the centre of the care. In addition, experienced subspecialists including neurologists, nephrologists, vascular surgeons, cardiologists, ophthalmologists, urologists and podiatrists would be needed as required [4]. It is equally important to have a holistic view towards the management of DM and tackle other cardiovascular risk factors like hypertension, dyslipidaemia, obesity and smoking along with the hyperglycaemia. This would have a positive impact on the timing of onset and severity of the macrovascular complications. About 80% of patients with T2DM are obese as are at least 30% of those with T1DM. Obesity increases insulin resistance, dyslipidaemia and worsens hypertension, besides being an independent risk factor for heart disease. Therefore weight reduction should be given utmost importance in treating obese patients with DM [1].

In T2DM, when dietary therapy fails to achieve euglycaemia, glucose lowering oral agents like sulphonylureas, biguanides, and thiazolidinediones or insulin, either singly or in combination, is added. Newer anti-hyperglycaemic agents like Glucagon-like peptide-1 (GLP-1) receptor agonists and Sodium-glucose Co-transporter-2 (SGLT2) Inhibitors are also available for the treatment of T2DM. Insulin is the cornerstone for the treatment of T1DM and is usually given as the basal bolus regimen.

Biguanides

Historically, biguanides can be traced from the use of galega plant (Galega officinalis) also known as "*Professor Weed*" or "*French lilac*" for treating DM in medieval Europe. Biguanides are synthesised from guanidine, the active component of galega plant. The incidence of lactic acidosis with metformin in

therapeutic doses is rare [12]. Recently, a study was designed to determine whether the use of metformin in type 2 diabetic patients with various levels of renal insufficiency is associated with an increased risk of lactic acidosis [13]. The authors concluded that the overall incidence for lactic acidosis for patients on metformin in the study was within the range of rates reported in the literature for patients with T2DM, and no significant difference was observed in patients with normal, mildly reduced, moderately reduced or severely reduced renal function [13].

Metformin is a dimethylbiguanide that is freely soluble in water and does not undergo substantial metabolism. The clinical efficacy of metformin requires the presence of insulin and involves several therapeutic effects, some of which are mediated via increased insulin action and some are not directly insulin dependent [14]. Metformin's principal mode of action is suggested to be on the liver. Metformin suppresses hepatic gluconeogenesis by potentiating the effect of insulin, reducing the hepatic extraction of lactate and by opposing the effect of glucagon. Also, metformin reduces the rate of glycogenolysis and decreases the activity of hepatic glucose-6-phosphatase [14].

Metformin also enhances insulin stimulated glucose uptake in the skeletal muscle by increased movement of glucose transporters into the cell membrane. This is associated with increased glycogen synthetase activity and glycogen storage [14]. Metformin also causes an insulin- independent suppression of fatty acid oxidation and a reduction in hypertriglyceridemia, thus reducing the energy supply for gluconeogenesis. This is associated with decreased synthesis and increased clearance of VLDL. Reduction in triglyceride levels reduces insulin resistance [14].

Molecular action of metformin

Metformin has effects on the cell membrane and especially on the mitochondrial membranes [15]. The physiological function of the plasma membrane depends on the ability of its protein components to freely move in the phospholipid bilayer. In clinical and experimental diabetes, there is reduction in the membrane fluidity or increased membrane stiffness or viscosity. Metformin has

been shown to increase the fluidity of human plasma membrane [15]. Metformin was also associated with an 80% increase in the activity of AMP-activated protein kinase (AMP-kinase). The enzyme AMP-kinase is involved in multiple aspects of glycaemic regulation, including the regulation of GLUT4 glucose transporters and fatty acid oxidation [14]. More recently, metformin is also thought to be responsible for a reduction in the lactate and glycerol metabolism to glucose. Metformin exerts its anti-hyperglycaemic effect through inhibition of Complex 1 of the mitochondrial respiratory chain [15].

Several mechanisms have been suggested for a gut effect of metformin including delayed intestinal glucose absorption, augmented lactate production by enterocytes, and enhanced secretion of gastrointestinal hormones containing glucagon-like-peptide 1, bile acid metabolism and potential role of intestinal microbiota. Recently, one study has offered clinical evidence suggesting the primary effect of metformin resides in the human gut [16].

Long term hyperglycaemia leads to glycation of proteins within the vascular wall. The non-enzymatic reaction between sugars, such as glucose, and free amino groups of proteins is also called the Maillard reaction, glycation or glycoxidation. The Maillard reaction ultimately results in increased production of highly chemically reactive glucose and alpha-dicarbonyl compounds which lead to the production of a large number of complex chemically irreversible structures called advanced glycation end products (AGE). The accumulation of AGE within the vascular system may impair the structure and function of cardiovascular tissues and lead to the cardiovascular complications of diabetes. AGE promotes inflammation and oxidative stress. This may stimulate release of growth factors, cytokines and reactive oxygen species that are pro- atherogenic [17]. Metformin has been suggested to reduce the production of AGE indirectly, through reduction of hyperglycaemia and directly, via an insulin dependent mechanism. The chemical formation of AGE depends on the production of intermediates like glycoxal or methyl glyoxal. Metformin may act by detoxification of methylglycoxal to form Trizepinone and be cardioprotective [17].

1.2 Gestational diabetes

1.2.1 Introduction

DM in pregnancy may be pre-gestational, which is when a woman with established DM becomes pregnant, or gestational.

The International Association of Diabetes and Pregnancy Study Groups (IADPSG), the ADA and others have recently attempted to distinguish women with probable pre-existing DM that is first recognised during pregnancy (overt diabetes) from transient manifestation of pregnancy related insulin resistance (gestational diabetes) [18].

The prevalence of gestational diabetes (GDM) is increasing worldwide as pregnant population is becoming older and also as the prevalence of obesity is increasing [19][20]. Using the new IADPSG criteria proposed in 2010, the global prevalence of hyperglycaemia in pregnancy has been estimated at 17%, with regional estimates varying between 10% in North America and 25% in southeast Asia [21].

1.2.2 Pathogenesis

Glucose metabolism during normal pregnancy

Carbohydrate metabolism towards the second half of pregnancy is directed towards supplying glucose and amino acids to the growing fetus while providing extra free fatty acids, ketones and glycerol as maternal fuel. Normal pregnancy is characterised by hyperplasia of the insulin-secreting pancreatic β cells, increased insulin secretion and an early increase in insulin sensitivity followed by progressive insulin resistance. The lactogenic hormones prolactin and human placental lactogen (HPL) cause an increase in the number of pancreatic β cells in pregnancy, through unclear mechanisms [22].

Maternal insulin resistance in normal pregnancy begins in the second trimester and peaks in the third trimester. Several hormones that are elevated in the maternal circulation during pregnancy like progesterone, HPL, cortisol and prolactin are responsible for causing insulin resistance [1]. Progesterone

prohibits normal changes of the pancreatic β cell reserve during pregnancy and is hugely responsible for increased insulin resistance [1]. HPL causes a decrease in phosphorylation at insulin receptor substrate 1 and insulin resistance increases as the level of HPL rises in the second trimester [1]. HPL peaks at 30 weeks of gestation and plays a major role in maternal insulin resistance [23]. Additionally, cortisol and prolactin have an effect on insulin function and are also instrumental in increasing insulin resistance [1]. The serum leptin levels are significantly higher and the adiponectin levels lower in women with GDM compared to women without GDM and these may be contributory factors in altering carbohydrate-fat metabolism leading to development of GDM [1].

Pathogenesis of GDM

Studies using the hyperglycaemic-euglycaemic glucose clamp technique and intravenous-glucose-tolerance-test have indicated that insulin action in late normal pregnancy is 50-70% lower than in nonpregnant women. Metabolic adaptations do not fully compensate in GDM leading to glucose intolerance. GDM may reflect a predisposition to T2DM or may be an extreme manifestation of metabolic alterations that normally occur in pregnancy [22].

Buchanan et al studied insulin sensitivity in the third trimester and reported that, mild gestational diabetes is characterised by an impairment of β cell function rather than an exaggeration of the normal insulin resistance of late pregnancy [24].

1.2.3 Screening

GDM is usually diagnosed by an OGTT which is used as a screening test [18]. Sometimes, GDM is suspected when the scan shows a macrosomic baby or polyhydramnios or mother presents with significant and persistent glycosuria.

A systematic review was performed by the United States Preventive Services Task Force (USPSTF) on the accuracy of screening tests for GDM, the benefits

and harms of screening before and after 24 weeks of gestation, and the benefits and harms of treatment. They found good evidence to support universal screening after 24 weeks but not for universal screening earlier in pregnancy [25].

There is also no consensus regarding the diagnostic test criteria for gestational diabetes. O'Sullivan and Mahan formulated the diagnostic criteria depending on the future risk of T2DM in the mother and it was not necessarily to identify pregnancies with increased risk for adverse perinatal outcomes [8]. In the UK, most NHS hospitals used the WHO 1999 diagnostic criteria for the diagnosis of GDM, wherein an oral glucose tolerance test result of fasting plasma glucose of ≥ 6 mmol/l or a 2-hour plasma glucose level ≥ 7.8 mmol/l is considered diagnostic of GDM [26].These have been replaced in February 2015 by the criteria suggested by National Institute of Health and Care Excellence (NICE) guidance, wherein a diagnosis of GDM is made on the basis of fasting plasma glucose level ≥ 5.6 mmol/litre or a 2-hour plasma glucose level ≥ 7.8 mmol/litre [27].

The Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study has demonstrated a linear correlation between fasting glucose levels and post 75 g oral glucose tolerance test levels to maternal, perinatal and neonatal outcomes. These adverse outcomes were also seen at below the current accepted level. There appeared to be no apparent threshold. This would suggest that GDM and fetal macrosomia could be considered a metabolic complication of diabetes like macrovascular disease rather than a microvascular complication of diabetes. Macrovascular disease with its multiple metabolic predictors does not have a sharp cut off.

The IADSPG have now suggested new criteria for the screening of GDM on the basis of findings from the HAPO study [28]. A fasting glucose > 5.1mmol/l, 1-hour post OGTT value > 10 mmol/l or a 2-hour value > 8.5 mmol/l were considered abnormal. Only 1 abnormal value would be sufficient to make the diagnosis of GDM in the women [28]. A study was undertaken to determine whether adopting the IADPSG criteria would be cost-effective, compared with

the current standard of care. It concluded that the IADPSG recommendation for glucose screening in pregnancy is cost-effective [29]

1.2.4 Implications for mother and fetus

The complications of DM in pregnancy are mainly due to maternal hyperglycaemia resulting in fetal hyperinsulinemia [1]. According to the modified Pedersen hypothesis, fetal hyperinsulinemia may lead to chronic fetal hypoxia, stimulating extramedullary haematopoiesis, fetal polycythaemia and neonatal hyperbilirubinemia leading to increased admission to neonatal intensive care units [4]. As insulin is an anabolic hormone, the macrosomic baby is fat and plethoric with enlarged organs especially the liver, and a disproportionately increased abdominal circumference. Other adverse outcomes could be preeclampsia and hydramnios. Macrosomia can lead to birth trauma and maternal morbidity from operative delivery [30]. Risk of macrosomia is great, when GDM is not recognised or is treated casually. Infants with macrosomia are at risk of shoulder dystocia, which can result in an increased risk for fracture of the clavicle and, more seriously, brachial plexus palsy during delivery [25]. Infants of women with GDM can potentially have other neonatal morbidities like hypoglycaemia, hypocalcaemia and poor feeding. GDM with onset in mid-pregnancy or later pregnancy is not associated with an increased prevalence of congenital malformations. However, GDM diagnosed in early pregnancy with elevated fasting plasma glucose >6.7 mmol/l or HbA1c ≥ 7%, possibly represents pre-existing type 2 diabetes and is associated with a higher rate of congenital anomalies than found in the general obstetric population [30]. Macrosomia also possibly increases the risk of glucose intolerance and obesity in the offspring [30]. Identification and intensive management of GDM are associated with a decrease in neonatal morbidity and mortality and also a decrease in the likelihood of intrauterine deaths [30].

1.2.5 Management of gestational diabetes

A multidisciplinary team involving obstetricians, physicians, specialist dietitian, nurses and midwives experienced in the care of pregnant women with diabetes is essential in the management of these patients.

The benefit of treatment of GDM is clearer from recent landmark studies. The results of the Australian Carbohydrate Intolerance in Pregnancy Study (ACHOIS Study) showed that there were significantly fewer complications in the infants of mothers in the intervention group (n=490) as compared to the infants of mothers receiving routine care (n=510). They concluded that perinatal mortality can be reduced by treating GDM and it also improves women's quality of life [31].

Langer *et al.* studied 555 pregnant women with GDM diagnosed after 37 weeks in comparison with 1110 pregnant women treated for GDM and 1110 matched non diabetic pregnant women [32]. They concluded that lifestyle and dietary modifications and, when indicated, insulin therapy clearly improves outcome in GDM [32].

A systematic review and meta-analysis of randomised trials in 2013 for the USPSTF found that appropriate management of GDM with nutritional therapy, self-blood glucose monitoring and administration of insulin, if target blood glucose concentrations are not met with diet alone resulted in reductions in preeclampsia, birth weight > 4000 g and shoulder dystocia [33].

In the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study, obesity and GDM (diagnosed by IADPSG criteria) were independently predictive of fetal macrosomia, preeclampsia, primary Caesarean delivery and neonatal adiposity [34]. Macrosomia was more likely when GDM was present in the absence of obesity (Odds Ratio (OR) 2.2, 95% CI 1.9 to 2.5) than when obesity was present in the absence of GDM (OR 1.7, 95% CI 1.5 to 2.0) and the independent effects of GDM and obesity were additive. The odds ratio for birth weight > 90^{th} percentile when both GDM and obesity was present was 3.6 (3.0-4.3). Odds for birth weight >90^{th} percentile were progressively greater with both higher OGTT glucose and higher maternal BMI [34].

Nutritional therapy for gestational diabetes

The cornerstone of therapy for diabetes in pregnancy is diet [1]. Women are advised to reduce the proportion of carbohydrates to 35%-40% of the meals

and preferably use low glycaemic index carbohydrates which result in a slower and more even release of glucose [1]. The ADA recommends that nutrition therapy for GDM provide adequate nutrition to promote fetal and maternal health, achieve glycaemia with absence of ketones and provide adequate energy levels for appropriate weight gain in pregnancy [35].

A recent meta-analysis showed that an overall low glycaemic index diet in which carbohydrate are mainly sourced from fruits, vegetables and whole grains, with low consumption of flour based products and potatoes had a favourable effect on blood glucose and lowered the need for insulin therapy [36].

Exercise

The ADA encourages a program of moderate exercise as part of the treatment plan for women with GDM if no medical or obstetric contraindications to this level of physical activity [37].

Glucose monitoring and targets

The ADA recommends that women with GDM be asked to measure their blood glucose concentrations at least 4 times daily, pre-breakfast and one or two hours after the first bite of each meal. This multiple testing allows recognition of women who should begin an anti-hyperglycaemic agent. Results are recorded in a log book along with dietary information [38]. The ADA and the American College of Gynaecologists (ACOG) currently recommend the following upper limits for glucose levels, with insulin therapy initiated if they are exceeded-

Fasting glucose ≥ 5.3 mmol/l, 1-hour postprandial blood glucose ≥ 7.8 mmol/l, 2-hour postprandial glucose ≥ 6.7 mmol/l.

HbA_{1C} may be a helpful test in assessing glycaemic control but has been suggested to be unreliable in pregnancy.

Pharmacological therapy of GDM

Conventionally, women with gestational diabetes were treated with insulin when diet alone failed to achieve euglycaemia.

Insulin Therapy

Use of insulin preparations of low antigenicity may minimise the transplacental transport of insulin antibodies and hence human insulin which is the least immunogenic preparation is used [30]. The type of insulin used depends on the blood glucose profile of the patient. The newer insulin analogues, Lispro and Aspart have been investigated in pregnancy and shown to have acceptable safety profiles, minimal transfer across the placenta and no evidence of teratogenesis [30]. They both improve postprandial excursions compared to human regular insulin and are associated with lower risk of delayed postprandial hypoglycaemia. Insulin detemir is the only basal insulin analogue approved in pregnancy, Food & Drug Administration (FDA) category B classification [39].

Metformin

Metformin is now gaining more acceptance as a safe, effective and a rational oral option offering advantages over insulin [40]. Metformin is a biguanide, which reduces insulin resistance and hepatic gluconeogenesis and stimulates GLP-1 release [41]. Metformin crosses the placenta but no teratogenic effects have been reported so far [42].

The Metformin in Gestational Diabetes (MiG) trial concluded that in women with GDM, metformin alone or with supplemental insulin is not associated with increased perinatal morbidity as compared to insulin treatment and when women were asked what they would choose in next pregnancy, metformin was the preferred choice to insulin [40].

When the MiG investigators published their interim favourable safety data on metformin use in pregnancy, I, as part of the St Helier diabetes team, conducted a case control study in 2007 comparing the maternal and neonatal outcomes in women with GDM treated with either metformin or insulin [43]. In this previously published work, I concluded that the pregnancy outcomes in the 100 GDM women treated exclusively with metformin were comparable to a retrospective cohort of 100 insulin-treated GDM women attending the same clinic and managed by the same team. Women gained less weight from time of enrolment

to delivery in the metformin treated group as compared to the insulin group. I concluded that metformin, being an oral medication was more acceptable to patients, was less expensive and was also more cost effective as compared to insulin. In 2012, I, along with the St Helier diabetes team conducted another case-control study on pregnancy outcomes in women treated with metformin for GDM [44]. I compared the pregnancy outcomes in 324 metformin treated GDM women with 175 GDM women treated with diet alone and matched for age and ethnicity. In this second study, I again concluded that metformin treatment had a favourable impact on reducing the rates of Large for Gestational Age (LGA) babies despite more severe glucose intolerance at baseline [44]

Metformin therapy has some advantages and disadvantages compared with insulin therapy. A recent systematic review and meta-analysis in 2015, comparing different treatments for GDM showed that compared with insulin, use of metformin resulted in less gestational weight gain (mean difference = -1.1kg, 95% Confidence Interval (CI) -2.2 to -0.06kg) but lower gestational age at delivery and a higher risk of preterm birth [39]. There was no statistical difference between metformin and insulin users in mean birth weight or risk of macrosomia, but a trend towards a lower rate of any neonatal hypoglycaemia was noted in metformin users [39].

1.3 Obesity

1.3.1 Prevalence of obesity

The World Health Organisation (WHO) defines obesity as abnormal or excessive fat accumulation that may impair health [45]. Body mass index (BMI) is the measurement of the mass in the human body, in kilograms, divided by the height in meters squared (Equation 1-1).

Equation 1-1: Body mass index (BMI)

$$BMI = \frac{Weight\ (kg)}{Height^2\ (m^2)}$$

BMI is used as a rough estimate of obesity - a BMI > 25 kg/m2 is called as overweight and a BMI ≥ 30 kg/m2 is categorised as obesity. Obesity is further subdivided into class I (BMI 30-34.9 kg/m2, Class II (BMI 35-39.9 kg/m2) and class III (BMI ≥ 40 kg/m2) [45]. The BMI may not provide an accurate measure of obesity but it provides a very useful population level measure of overweight and obesity and there is no variation between sexes and at all ages in adults. However, it does not always correspond to the same degree of fat content in different individuals as the contribution of lean mass to body weight may differ in people [45].

The prevalence of obesity, worldwide, has more than doubled since 1980. In 2014, 39% of adults aged 18 years and more were overweight. Overall, about 13% of the world's adult population (11% of men and 15% of women) were obese in 2014 [45].

Overweight and obesity are estimated to be the fifth leading risk for global deaths. Besides that, obesity is also responsible for up to 44% of the diabetes burden, 23% of the heart disease burden and a large chunk of certain cancer burden [45].

It has been estimated worldwide that the proportion of adults with BMI > 25 kg/m^2 has increased between 1980 and 2013 from 28.8% to 36.9% in men and from 29.8% to 38% in women [46]. Increases were observed in both developed and developing countries. The reported prevalence rates of obesity included 20% of men and 21.7% of women in Belgium, 21% of men and women in the UK and 21% of men and 33% of women in Mexico [46].

1.3.2 Screening measures

Measuring BMI is the first step to determine the degree of overweight. The BMI is easy to measure, reliable and correlated with percentage body fat and body fat mass. However, the disadvantages of BMI are that it may overestimate the degree of fatness in individuals who are overweight but very muscular like professional athletes or bodybuilders and underestimate in older persons because of loss of muscle mass associated with aging.

Also, the definition of overweight and obesity varies with race and population. In some populations, the level of risks in terms of per cent body fat is reached at a much lower BMI (South Asian) and in others a higher BMI (Blacks) as compared with Whites. A study comparing South Asian and European subjects showed that the mean BMI associated with the development of an adverse metabolic profile, defined by markers of glucose and lipid metabolism was 21 kg/m^2 in South Asians and 30 kg/m^2in Europeans [47].

Waist Circumference

In addition to BMI, waist circumference (WC) is a useful tool in overweight and obese adults to assess abdominal obesity. A WC of ≥ 40 inches (102 cm) for men and ≥ 35 inches (88 cm) for women is considered significantly elevated and indicative of increased cardiometabolic risk [48]. There is evidence that patients with abdominal obesity (also called central adiposity, visceral, android, or male-type obesity) are at increased risk for heart disease, DM, hypertension, dyslipidaemia, and non-alcoholic fatty liver disease [49]. It has been found that there is ethnic variability in WC values that predict increased risk. As an example, it has been observed that Japanese-Americans and Indians from South Asia have more total fat and visceral fat and therefore may be at higher risk of developing T2DM for a given BMI than whites [50]. Consequently, in Asian females a WC > 80 cm and in Asian males a value > 90 cm are considered abnormal [50].

Data from the Third National Health and Nutrition Examination Survey (NHANES III) study suggest that normal-weight central obesity is associated with higher mortality than BMI-defined obesity, especially in individuals without central obesity [51]. A survey of over 15,000 individuals, men with a normal BMI but central obesity (Waist Hip Ratio (WHR) ≥ 0.9) had the highest total mortality risk when compared to men without central obesity who were normal weight, overweight or obese (HR 1.9, 2.2, and 2.4, respectively). Similarly, normal weight women with central obesity (WHR ≥ 0.85) had higher mortality risk compared to normal weight and obese women without central obesity (HR 1.5

and 1.3, respectively). A limitation of the study is that no quantitative imaging studies of adipose tissue were performed [51].

Body Composition Analysis

Body composition can be analysed by measuring body impedance using instruments such as Inbody 720^{R}. This instrument performs body composition analysis using Direct Segmental Multi-Frequency Bioelectrical Impedance Analysis Method (DSM- BIA Method). The InBody 720 gives a quantitative value for the various body compartments which equals the weight of each compartment, when added together they equal the person's weight. It measures BMI, WHR and various body compartments like lean body mass, total percentage body fat (PBF) and visceral fat mass (VFM). The bioelectrical impedance analysis (BIA) method is based on the electric resistance difference between the fat and components of other organs [52].

Validation of InBody 720

The InBody 720 has been validated and correlates well with intra-abdominal fat area assessed by CT scan [53] and DEXA [54]. Ogawa H *et al.* studied the efficacy of bioelectrical impedance analysis by InBody 720 as a new tool for measuring visceral fat area [53]. They concluded that visceral fat area values measured by InBody 720 significantly correlated with those by computed tomography (R = 0.8) [53]. Malavolti M *et al.* also compared Eight-polar bioelectrical impedance analysis (BIA) against dual-energy X-ray absorptiometry (DEXA) for the assessment of total and appendicular body composition in 110 healthy adults [54]. They concluded that Eight-polar BIA offers accurate estimates of total and appendicular body composition [54]. InBody 720 has also been used in studies of patients with obesity [55][56].

A study at Osaka University was conducted to assess the correlation of VFA (visceral fat area) by the BIA method with VFA determined by CT scan [52]. The usefulness of abdominal BIA on evaluating metabolic syndrome was also investigated. The best combination of sensitivity and specificity for detecting subjects with multiple risk factors was VFA ≥ 100 cm^2. The VFA by BIA correlated significantly with VFA determined by CT ($r=0.9$, $P<0.0001$). They

concluded that BIA is a simple, non-invasive, non-expensive method for estimation of visceral fat with excellent correlation with CT measurements and should be used in routine clinical practice [52].

It has been also been shown to be safe in the second and third trimesters of pregnancy and has also been validated against deuterium and hydrodensitometry techniques for body composition analysis [57][58]. Van Loan MD *et al.* examined the accuracy of bioelectrical impedance spectroscopy for estimating fluid volumes before, during and after pregnancy and concluded that bioimpedance may be useful in estimating volumes of extracellular fluid (ECF) and total body water (TBW) during pregnancy [57] McCarthy *et al* compared various methods of determining maternal body composition in pregnancy published between 1950 and 2004 [58]. They conclude that bioimpedance is a safe technique and uses simple equipment [58]. DSM-BIA is also an accurate technique for assessing body water distribution which changes during pregnancy [59].

1.3.3 Pathophysiology of obesity

Metabolic and socioeconomic factors associated with obesity have been identified. Among the former are a low metabolic rate, increased carbohydrate oxidation, insulin resistance, and low sympathetic activity. Among the latter are lower socioeconomic class, lower education level, and cessation of smoking [60]. Genetic factors play a permissive role and interact with environmental factors to produce obesity [61].

The fundamental problem in obesity is an imbalance of energy between calories consumed and calories expended. An increase in body fat requires that energy intake be increased persistently over energy expenditure. However, there is a feedback mechanism between energy intake and expenditure that tends to maintain body weight. Weight gain is associated with an increase in energy expenditure which retards further weight gain, whereas weight loss is associated with a decrease in total and resting energy expenditure, a change that makes further weight loss more difficult [62].

The feedback control system consists of the cellular processes for energy expenditure and for digestion and utilization as fuels. The central nervous system controller in the hypothalamus receives afferent signals from the periphery about deficits or surpluses of foods. The controller then initiates metabolic and cognitive responses according to whether food is needed and also initiates signals that alter metabolism of nutrients and the cognitive processes for food seeking.

The afferent signals are hormones like leptin. Gut hormones like glucagon-like peptide-1 (GLP-1), cholecystokinin, enterostatin, and polypeptide Y 3-36 reduce food intake. Ghrelin is produced in the stomach and duodenum, and has two major effects: it stimulates growth hormone secretion and increases food intake in humans. Serum concentrations of ghrelin increase in anticipation of a meal, and are suppressed by food ingestion [63].

Secondary causes of obesity though uncommon, should be considered and ruled out. The neuroendocrine causes of obesity are hypothalamic obesity, Cushing's syndrome, polycystic ovaries, hypogonadism, hypothyroidism, and psedohypoparathyroidism. Drugs like insulin, sulfonylureas, thiazolidinediones, antipsychotics, antidepressants and antiepileptics can cause weight gain [64].

Obesity and insulin resistance

The association of obesity and T2DM is now well recognised and the link is through insulin resistance. The mechanisms by which obesity causes systemic insulin resistance are unknown, but are thought to act through the adipo-insulin axis [65]. Insulin resistance with hyperinsulinemia is characteristic of obesity and is present before the onset of hyperglycaemia. After the onset of obesity, the first demonstrable changes are impairment in glucose removal and increased insulin resistance, which result in hyperinsulinemia. The hyperinsulinemia in turn increases hepatic very-low-density triglyceride synthesis, plasminogen activator inhibitor-1 synthesis, sympathetic nervous

system activity, and sodium reabsorption. These changes contribute to hyperlipidaemia and hypertension in obese subjects [66].

Adipocytes as endocrine cells

Adipocytes are energy storage depots for triglycerides releasing fuel as fatty acids and glycerol in time of fasting and starvation. An additional role of the adipocyte is of a secretory cell [65]. Adipocytes secrete numerous peptide hormones and cytokines including TNF-a; plasminogen-activator inhibitor-1, which helps maintain haemostasis, angiotensinogen which regulates vascular tone and leptin which plays a central role in regulating energy balance. Adipose tissue can also produce active steroid hormones, including oestrogen and cortisol. Through these secreted peptides, adipocytes can influence local adipocyte biology as well as systemic metabolism in diverse sites like the brain, liver, muscle, β cells, gonads, lymphoid organs and systemic vasculature [65].

TNF-α has many effects on adipocyte function including inhibiting lipogenesis and increasing lipolysis. TNF-α may be a mediator for insulin resistance. TNF-α signalling impairs insulin signalling and can reduce GLUT4 gene expression [65].

Leptin, the product of the *ob* gene may be another contributor to insulin resistance [65]. Leptin has profound effect on satiety, energy expenditure and neuroendocrine function. Leptin is viewed as being primarily involved in the starvation/feeding switch. The absence of leptin in both rodents and humans produces severe obesity which is cured by leptin. The paradox that absence or excess of adipose tissue causes insulin resistance highlights the complexity of the relation [65].

1.4 Obesity in pregnancy

1.4.1 Prevalence of obesity in pregnancy

Obesity is defined as pre-pregnancy BMI ≥ 30 kg/m^2 .The Confidential Enquiry into Maternal and Child Health (CEMACH) in its 2003-2005 triennia report has highlighted obesity in pregnancy as a major risk to mother and baby [67].

CEMACH found that approximately one-thirds of the women who died were obese and 30% of the mothers who had a stillbirth or a neonatal death were also obese [67].

Galtier-Dereure *et al.* studied the complications of obesity in pregnancy and the costs in management of these patients and estimated that there is almost a five-fold increase in antenatal care costs in obese women as they spend an average of 4.8 more days in hospital [68]. Similarly, there is an increase in costs of neonatal care also as there is a 3.5 fold increase in admissions to intensive care unit in babies born to obese mothers [68].

A national project by the Centre for Maternal and Child Enquiries (CMACE) on maternal obesity in the UK, 2010 highlighted the increasing prevalence of obesity and the important findings are given below [69]. The UK prevalence of women with a known BMI ≥ 35 in pregnancy is high at 4.9% = 38,478 maternities each year. 20% of the babies were LGA (twice as high as expected in the general population). Obese women with diabetes were more likely to have a LGA baby (40% versus 17%). Neonatal unit admissions were higher and correlated directly with maternal BMI. Caesarean sections rate was higher at 37% (versus 25% in general maternities) in England [69].

Heslehurst *et al* studied the trends in maternal obesity incidence rates in the UK. They reported that the proportion of obese women at the start of pregnancy has increased significantly over 19 years (1989 to 2007) from 9.9% to 16%. Predictors of maternal obesity are associated with health inequalities, particularly socio-economic disadvantage [70].

Sebire *et al* studied maternal obesity and pregnancy outcomes in 287,213 pregnancies in London. He reported that, compared to normal BMI, the following outcomes were more common in obese women: GDM (OR 3.6), PET (OR 2.1), LGA (2.36). Also, excessive weight gain in pregnancy is associated with an increased risk of complications [71].

1.4.2 Risks of Obesity in Pregnancy

The risks associated with obesity in pregnancy can be antepartum, intrapartum, postpartum and offspring risks [68]. The antepartum risks are pregnancy-induced hypertension/preeclampsia, spontaneous miscarriages, gestational diabetes, and increased risks of venous thromboembolism and ultrasound difficulties in fetal assessment. The intrapartum risks are increased Caesarean sections, and failed Vaginal delivery after Caesarean (VBAC, perineal trauma, shoulder dystocia, risks associated with anaesthesia and surgical difficulties. The postpartum risks are increased rates of puerperal infection and haemorrhage, decreased rates of breastfeeding initiation or continuation, postnatal depression and thrombo-embolic disorders. The offspring risks are higher risk for having congenital anomalies, stillbirths and increased risk of childhood and adult obesity [68].

A study by Sheiner *et al*. aimed to investigate the correlation between maternal obesity and incidence of Caesarean section (CS) while controlling for confounding effects of other variables associated with obesity like diabetes and hypertension [72]. They found higher rates (27.8%) of CS among obese women as compared to normal weight women (10.8%), (OR=3.2, 95% CI 2.9 to 3.5, P<0.001) and this association remained significant even after corrections for confounders [72]. Another study reported a 9.5 fold increased risk of wound infection after CS when obesity and diabetes were both present (95% CI, 4.5 to 19.2, P<0.01) [73].

Adverse outcomes are usually thought to be due to the increased prevalence of diabetes in obese women. However, non-diabetic obese women are also at greater risk of adverse outcome. Hence, other pathways are likely to play a role [74]. It may be due to adipose tissue-related dysregulation of metabolic, vascular, and inflammatory pathways, affecting many organ systems. Insulin resistance and abnormalities in inflammatory pathways have been linked to development of preeclampsia [75].

The HAPO study also demonstrated that increasing maternal BMI contributes to fetal size independent of variations in glycaemic exposure [76]. In addition to

the strong relationship with birth weight > 90th percentile, maternal BMI was also strongly related to fetal adiposity and hyperinsulinemia even after adjustment for maternal glycaemia. This suggests the potential importance of other nutrients including triglycerides, free fatty acids and amino acids and potentially of total caloric intake [76].

All these studies show that numerically, more macrosomic babies are born to obese mothers than mothers with GDM [74] [76]. Fetal macrosomia is an important adverse outcome as it can lead to birth injuries like shoulder dystocia, fracture of the clavicle and brachial plexus injury.

A 2011 systematic review showed that the odds of having ≥ 1 miscarriage were increased for obese women (OR 1.3, 95% CI 1.18 to 1.46) and overweight women (OR 1.1, 95% CI 1.0 to 1.2), when compared with women with normal BMI [77].

There is evidence of an association between obesity and hypertensive disorders during pregnancy. A large systematic review of 13 cohort studies found that the risk of preeclampsia doubled with each 5 to 7 kg/m^2 increase in pre pregnancy BMI [78].

The underlying mechanism suggested is that the pathophysiologic changes associated with obesity related cardiovascular risk such as insulin resistance, hyperlipidaemia and subclinical inflammation are also responsible for the preeclampsia [79]. A large study in 2014, aiming to validate clinical risk factors for preeclampsia, concluded that being overweight or obese was the most important risk factor for both preeclampsia and severe preeclampsia with an attributable risk per cent of 64.9% and 64.4%, respectively [80].

Pre pregnancy BMI and maternal weight gain are both determinants of infant birth weight and obesity increases the risk of delivering a LGA infant [81]. This relationship is independent of the increased prevalence of GDM in obese women.

Maternal obesity and long-term effects on the child

It has been proposed that the transmission of obesity risk from mother to the child can be explained by the “Developmental origins of disease” hypothesis, which suggests that elements of heritability can be transmitted in a non Mendelian way from one generation to the next [82]. However, it remains unproven whether these associations represent an intrauterine influence, or more simply reflect shared familial, genetic or lifestyle characteristics.

Recently, rodent models of diet induced obesity have reported that the offspring develop increased adiposity, insulin resistance and hypertension [83]. Rodent studies have also implicated a raised n-6:n-3 fatty acid ratio in premature adipocyte maturation and proliferation [84]. The mechanism whereby nutrient status in early life can permanently influence the metabolic phenotype of the offspring is likely to involve epigenetic modification of DNA and this may lead to permanent change in organ structure, cell number or metabolic function [85].

A human cohort study recently suggested that precocious development of neonatal fat depots, or persistently altered adipocyte metabolism in response to fetal metabolic and hormonal profile, may also contribute to obesity in later life [86]. A study showed a 3.6-fold greater risk of metabolic syndrome among LGA offspring of mothers with GDM as compared to appropriate-for-gestational age children [87].

1.4.3 Gestational weight gain in obese women

A large cohort study of 120,251 pregnant, obese women delivering full-term infants concluded that it is beneficial to limit weight gain during pregnancy in obese pregnant women [88].

Gestational Weight Gain (GWG) occurs to support the function of growth and development of the fetus [89]. It is related to changes in maternal and placental metabolism. The placenta acts as an endocrine organ and a barrier and transporter of substances between the two circulations [89]. Hence, changes in the maternal metabolism can alter fetal growth rate and, conversely, placental function can also change maternal metabolism through alterations in insulin

sensitivity [89]. The Institute of Medicine (IOM) has revised its guidelines for weight gain in pregnancy in 2009 and included specific pregnancy weight guidance for underweight, normal weight and overweight and obese women and adolescents and women carrying twins or higher order multiples [89].

A retrospective cohort study of 142 consecutive pregnancies in 28 women of normal weight, 39 overweight women and 75 obese women with T2DM was carried out to evaluate fetal growth in relation to GWG in women with T2DM [90]. The authors concluded that the infant birth weight was almost 0.5 kg higher in women with T2DM and excessive GWG than in women with non-excessive GWG [90].

Excessive GWG is primarily related to an excessive increase in maternal adiposity in the absence of pathological oedema. There are several biologic and genetic factors that affect fat metabolism in pregnancy. Increased progesterone levels are responsible for fat accumulation during the first and second trimesters, and for fat mobilization during the third trimester. Also, increased leptin levels during pregnancy correlate positively with body fat content and body mass index (BMI) and appear to play a direct role in GWG and postpartum weight retention [91].

Factors like maternal weight and gestational weight gain have been shown to impart greater risk for neonatal outcomes like macrosomia and shoulder dystocia, particularly in women diagnosed with GDM at lower glucose thresholds [92]. The study by Black *et al.* showed that the prevalence of LGA infants was significantly higher for overweight and obese women without GDM compared with their normal weight counterparts. They concluded that interventions that focus on obesity and gestational weight gain, regardless of GDM status, have the potential to reach far more women at risk of having a LGA infant [92].

1.4.4 Management of Obesity in Pregnancy

Dietary Approaches

A Cochrane review concluded that it may be more harmful than beneficial to restrict protein and energy in obese and overweight women [93]. However, studies have shown that cutting down on calories to 1600-1800 kcal/day restricts excessive weight gain in pregnancy without risking ketosis in the fetus and may be beneficial [94].

Physical Activity

The ACOG guidelines advocate moderate exercise for 30 minutes or more on most days of the week in obese pregnant mothers unless there are obstetric complications [95]. Though these recommendations are not evidence based, they are widely used. A Cochrane review on physical activity in pregnancy concluded that the current available data were inconclusive regarding risks or benefits to the mother or infant [96].

Behavioural Intervention

There have been several studies using behavioural intervention to limit weight gain in pregnancy in obese women. Claesson *et al.* used a 'motivational' talk approach in early pregnancy which was followed by an aqua-aerobics class and then followed weekly by a midwife. In this programme, obese women in the intervention group gaining only 8.7 kg which was significantly less than weight gained by the control group (11.3kg). Increased frequency of contact with the health care professional could have been a contributory factor [97]. Asbee *et al.* offered a single contact with the dietitian at the initial visit and this also reduced the weight gain in the intervention group (13 kg) versus the control group (16.1kg) [98]. However, none of these above lifestyle interventions brought any change to birth weight or any other pregnancy outcome.

As in nonpregnant individuals, exercise with or without a healthy diet helps to prevent excessive weight gain and should be recommended, unless there are contraindications to exercise [99]. Several systematic reviews and meta-analyses examining the effect of antenatal behavioural interventions for prevention of excessive weight gain during pregnancy, have found these interventions significantly decreased gestational weight gain compared with

usual care. However, there was no clear reduction in maternal complications or adverse neonatal outcomes [100] .

Bariatric Surgery

Two recent reviews of case control and cohort trials in patients undergoing bariatric surgery before pregnancy, have shown improved fertility rates and decrease in maternal and neonatal complications in obese pregnant women [101][102]. Sometimes the adverse outcomes are reduced to the frequency of adverse events in non-obese patients. Observational studies also consistently report a lower prevalence of GDM among women who have had bariatric surgery than among obese women who have not undergone this surgery [103].

1.4.5 Maternal obesity and risk of diabetes mellitus

Obese women are at increased risk of GDM compared with normal weight women. In a prospective study of more than 16,000 patients with BMI 30-40 kg/m^2, the odds ratio (OR) for GDM were 2.6 [95% CI 2.4 to 6.0] compared with women with BMI < 30 kg/m^2 [104]. A meta-analysis, including twenty studies was conducted to better estimate the risk of GDM in obese pregnant women [105]. The unadjusted ORs of developing GDM were 2.1 (95% CI 1.8 to 2.5) in overweight, 3.6 (3.1 to 4.2) in obese, and 8.6 (5.1 to 16.0) in severly obese compared with normal BMI women.They concluded that higher the maternal weight, higher is the risk for GDM [105].

More recently, a meta-analysis was conducted to quantify the risk for GDM depending on pre pregnancy BMI [106]. They found the OR for GDM was 1.9 (95% CI 1.8 to 2.2) for overweight, 3.0 [95% CI 2.3 to 3.9] for moderate obesity and 5.6 [95% CI 4.3 to 7.2] for women with morbid obesity (BMI > 40 kg/m^2) compared with normal weight women [106]. Also, for every 1 kg/m2 increase in BMI, the GDM prevalence went up by 0.9% (95% CI 0.7 to 1.1). They concluded that the risk of GDM is positively associated with pre-pregnancy BMI [106].

1.4.6 Visceral Obesity and Risk of Diabetes Mellitus

A strong association between measures of abdominal obesity (WC, waist:hip ratio, and CT-assessed intra-abdominal fat area) and the development of T2DM is well established: A meta-analysis of 15 cohorts from 10 longitudinal studies suggested there was a strong association between measures reflecting abdominal obesity and the incidence of T2DM, and the pooled odds ratio was 2.1 (95% CI 1.7 to 2.7; P<0.0001) [107]. Also, WC was at least as good as other measures in predicting outcome and reducing WC could reduce risk of T2DM [107]. Visceral fat assessed by CT remained a significant predictor of incident diabetes even after adjustment for BMI, total body fat and subcutaneous fat [107].

Neeland IJ *et al.* investigated the associations between adiposity phenotypes characterised by excess visceral fat and insulin resistance with the risk of incident pre-diabetes and diabetes in 732 obese non-diabetic subjects enrolled in the Dallas Heart Study [108]. They concluded that baseline visceral fat mass measured by DEXA and MRI imaging but not general adiposity was independently associated with risk of development of pre-diabetes and diabetes [108]. Kaess BM *et al* investigated the association of the ratio of visceral adipose tissue to subcutaneous adipose tissue with cardiometabolic traits in participants from the Framingham Heart study. They concluded that this ratio is a correlate of cardiometabolic risk factors reflecting blood pressure, dyslipidaemia and insulin resistance, above and beyond BMI [109]

The pathogenic mechanism linking visceral fat and the onset of diabetes is likely to be through the development of insulin resistance. A study in which 63 patients with T2DM underwent measurements of fat free mass, subcutaneous and visceral fat area by MRI and insulin sensitivity showed that visceral fat (VF) area was positively related to fasting hyperglycaemia (partial r=0.46; P=0.001) and HbA1c (partial r=0.5; P=0.0003) [110]. The interesting result was that insulin sensitivity was reciprocally related to VF independent of BMI (partial r=0.33; P=0.01). They concluded that, in patients with established T2DM,

visceral fat accumulation has a significant negative impact on glycaemic control through decreased insulin sensitivity [110].

The specific mechanisms by which fat in the visceral compartment confer greater risk than subcutaneous fat is unknown. It has been suggested that one or more moieties secreted by the visceral adipocyte might mediate insulin resistance-for example, free fatty acids (FFA) themselves (portal theory) or the adipose tissue related cytokines (adipokines) such as interleukin 1, interleukin 6, tumour necrosis factor-alpha, resistin, or a reduction in adiponectin. The unique anatomical position of the visceral fat depot, with effluent entering the liver is also an important consideration [111].

1.4.7 Lifestyle and other intervention studies in obese pregnant women

The Lifestyle in Pregnancy (LiP) study was a randomised controlled trial among 360 obese women allocated in early pregnancy to lifestyle interventions with diet counselling and physical activities or to the control group [112]. The intervention resulted in significantly lower GWG as compared with the control group (7.4 kg ± 4.6 versus 8.6 kg ± 4.4, P=0.01) but without improvement in rates of clinical pregnancy complications with respect to preeclampsia or pregnancy-induced hypertension, GDM, CS, LGA and admission to neonatal intensive care unit.

The UK Pregnancies Better Eating and Activity Trial (UPBEAT) trial concluded that a behavioural intervention addressing diet and physical activity in women with obesity during pregnancy is not adequate to prevent GDM, or to reduce the incidence of LGA babies [113].

Recently, the results of the “Effect of metformin on maternal and fetal outcomes in obese pregnant women (EMPOWaR)” trial were published [114]. The estimated effect size of metformin on the primary outcome was not significant [114]. There was no evidence of a reduction in the main secondary outcome of Homeostatic model for Mathematical Assessment (HOMA) – insulin resistance (HOMA-IR) at 36 weeks of gestation. Metformin did not seem to prevent GDM

as proportions of women fulfilling either IADPSG or WHO criteria for GDM were similar between the groups. Metformin also did not delay the onset of GDM [114].

1.4.8 C-reactive protein and obesity in pregnancy

CRP is a sensitive but not a specific marker of inflammation. High levels of CRP are associated with infection. Minor elevations of CRP (between 3 and 10 mg/L) are associated with low grade inflammation as in obesity and insulin resistance, cigarette smoking, diabetes mellitus, hypertension and uraemia. CRP can be elevated in obesity and this may at least be in part due to IL-6 secretion by the adipose tissue [115].

There have been several studies evaluating the associations of CRP with insulin resistance and the metabolic syndrome. Festa A *et al.* studied the relation of body fat mass and distribution to the markers of chronic inflammation and concluded that measures of body fat are strongly associated with circulating levels of CRP and fibrinogen [116]. Pannacciulli *et al.* also investigated whether CRP concentrations are influenced by body composition, insulin resistance and body fat distribution in healthy women and showed an independent relationship of central fat accumulation and insulin resistance with CRP plasma levels [117].

Several studies have been conducted to investigate the role of inflammatory markers as predictors of T2DM among healthy women. Hu FB *et al.* conducted a prospective case-control study of inflammatory markers as predictors of T2DM among healthy women and concluded that elevated CRP levels are a strong independent predictor of T2DM [118]. In a similar study in 1992 in healthy middle-aged women in the US, Pradhan AD *et al*. concluded that elevated levels of CRP and IL-6 predict the development of T2DM [119].

There have been a few studies to determine whether CRP is elevated in patients with GDM with contradictory results. In one such study by Retnakaran R *et al*., pre-pregnancy BMI emerged as a most important determinant of CRP

concentration, whereas glycaemic tolerance status was not a significant factor [120].

Leipold H *et al.* measured CRP concentrations longitudinally throughout pregnancy and concluded that, in women with GDM, the CRP concentration is primarily related to the degree of adiposity until the second trimester and that thereafter impaired glucose metabolism appears to be a predominant predictor of changes in CRP [121]. Wolf M *et al.* investigated the association between first trimester CRP levels with the subsequent development of GDM and found that first trimester CRP levels were significantly increased among women who subsequently developed GDM compared with control subjects. The risk of developing GDM among women in the highest CRP tertile was higher compared with the lower tertile. When BMI was included in the model, however, the association between increased CRP and GDM was attenuated [122]. A similar positive association between CRP and GDM risk was also seen in studies by Qui C *et al.* [123].

1.5 Safety data for the use of metformin in pregnancy

Metformin is classified by the FDA as a Category B drug in pregnancy *i.e*. no evidence for risk in humans [124] . Metformin is widely used in clinical practice in the management of DM in pregnancy in many centres of the world and its use in the UK is approved and recommended in the NICE guidance [125]. There is data from over 20 years of use of metformin in women with GDM or T2DM in pregnancy in South Africa [126]. Glueck *et al.* prospectively assessed growth and motor-social development during the first 18 months of life in 126 infants born to 109 mothers with polycystic ovaries who conceived on metformin and continued metformin throughout pregnancy [42]. They concluded that metformin reduced the risk of development of GDM in women with polycystic ovaries and that it was not teratogenic and did not adversely affect the length, weight, growth and motor-social development at 18 months of life [42].

The Metformin in Gestational Diabetes – The Offspring Follow Up study (MiG TOFU) assessed the body composition of 154 babies of mothers who were exposed to metformin in pregnancy, at 2 years of age. This was to assess the

potential effects on growth of the baby, as metformin is known to cross the placenta [127]. No difference was observed between the two groups for central fat measures, total fat mass or percentage body fat. They found that children exposed to metformin had larger measures of subcutaneous fat, but overall body fat was the same as in children whose mothers were treated with insulin alone [127]. Compared with the insulin group, the metformin group had larger upper arm circumferences and bigger biceps and sub scapular skin folds, indicating a more favourable pattern of fat distribution for children exposed to metformin. They suggested further follow-up is required to examine whether these findings persist into later life and whether children exposed to metformin will develop less visceral fat and be more insulin sensitive [127]. Also, blood pressure results obtained at 2 years in a large cohort of children exposed to metformin (170 children) in the MiG trial were comparable to published norms and were no different in those children whose mothers had received either metformin or insulin [128]. Reassuringly, there is no clinical evidence of abnormalities in growth or motor development in infants exposed to metformin in utero [129] .

Previous studies have demonstrated that in women with T2DM who take metformin in pregnancy, there is an increased metformin clearance due to enhanced renal elimination. Therefore, metformin doses may have to be increased by at least 20% in late pregnancy to maintain a therapeutic effect [130].

1.6 Aims and objectives of this study

Literature review has revealed that obesity in pregnancy contributes to increased morbidity and mortality for both mother and baby [67]. There is evidence in non-pregnant individuals that body fat distribution, especially visceral fat, is a more important risk factor for the development of T2DM than the BMI [107][108]. However, there are no such studies published in pregnancy.

1.6.1 Aims of the VFM study

1. To investigate whether a higher baseline VFM confers a higher risk of GDM in obese pregnant women.
2. To develop a mathematical model to predict GDM and LGA babies in obese pregnant women in early pregnancy using machine learning.

1.6.2 Objectives of the VFM study

1. To set up a clinical study in obese non-diabetic pregnant women attending an antenatal weight management clinic.
2. To measure the body composition of women at recruitment into the study, between 12 to 18 weeks of gestation.
3. To record the results of the OGTT conducted at 28 weeks of gestation.
4. To compare the baseline characteristics and body composition between women who developed GDM and those who did not.
5. To perform statistical analysis and investigate whether women with VFM ≥ 75^{th} percentile are at higher risk of GDM.
6. To develop a mathematical model to predict GDM and LGA babies incorporating VFM in early pregnancy in addition to classical risk factors.

Lifestyle intervention programmes in pregnancy in obese women have shown no evidence of benefit for the neonate. Since insulin resistance is increased in obesity and is strongly associated with birth weight, metformin is a rational choice to improve outcomes in this population.

1.6.3 Aims of the Metformin in Obese non-diabetic Pregnant women (MOP) Trial

1. To investigate whether metformin improves pregnancy outcomes (incidence of LGA (≥90% birth weight centile) babies, onset of maternal GDM, hypertension, preeclampsia, macrosomia, shoulder dystocia, admission to SCBU in obese non-diabetic women.

We aim to compare perinatal outcomes in women randomised to the two groups

Group 1: Standarised life style intervention and placebo

Group 2: Standarised life style intervention and metformin

2. To determine whether there is an association between baseline insulin resistance and adverse pregnancy outcomes such as gestational diabetes, pregnancy-induced hypertension and preeclampsia.

3. To investigate whether metformin will improve body fat distribution with particular emphasis on VFM during pregnancy

4. To examine the hypothesis that metformin is most effective in those patients with the highest baseline insulin resistance and treatment with metformin throughout pregnancy will reduce the risk of gestational diabetes in this group of women.

1.6.4 Objectives of the MOP Trial

1. To set up a randomised controlled trial in obese non-diabetic pregnant women with BMI ≥ 35 kg/m^2.
2. To obtain ethical approval and Clinical Trial Authorisation from Medicines and Health Regulatory Agency (MHRA).
3. To organise manufacture and packaging of placebo to match the metformin.
4. To obtain informed written consent of each participant.
5. To randomise recruited women to metformin or placebo.
6. To organise Oral glucose tolerance test (OGTT), fasting insulin and other blood tests at recruitment.
7. To measure the body composition of women at recruitment and repeat at 22 weeks, 28 weeks, 36 weeks and postnatal.
8. To record the results of the OGTT conducted at 28 weeks of gestation.
9. To record all adverse events in the participants.
10. To perform statistical analysis and compare the baseline characteristics, body composition, pregnancy and neonatal outcomes between the women randomised to metformin or placebo.

11. To calculate insulin resistance at booking and 28 weeks of gestation using the Homeostatic model assessment (HOMA) model.
12. To perform statistical analysis to compare the change of fasting insulin, insulin resistance, visceral fat and CRP at 28 weeks of gestation from baseline in the metformin and placebo groups.

2 VISCERAL FAT MASS STUDY

This chapter outlines the risks of obesity in pregnancy for the mother and the baby, the rationale and aims of the visceral fat mass (VFM) study, and discusses the methodology of this study and the results obtained. The body composition of 302 obese pregnant women, including VFM was assessed at 12-18 weeks of gestation. The maternal and neonatal outcomes were compared between the group of women who developed diabetes and those who did not. This chapter also discusses the importance of body fat distribution of the women, and in particular, VFM in early pregnancy and the risk of subsequent GDM.

This chapter also describes how a mathematical model was developed using machine learning, to predict GDM and birth centile classes in early pregnancy in obese women. VFM, which is a novel risk factor for GDM, was used in addition to conventional risk factors.

2.1 Introduction

There is substantial evidence that obesity in pregnancy contributes to increased complications for both mother and baby [67]. Obese women are at increased risk of GDM compared with normal weight women [104][105][106]. The complications of GDM are mainly due to maternal hyperglycaemia resulting in fetal hyperinsulinemia and macrosomia. This increases the risk for shoulder dystocia, fracture of the clavicle and more seriously, brachial plexus palsy during delivery of the baby [104]. Besides this, there is also a risk to the mother and offspring of diabetes and cardiovascular disease in later life [131]. A meta-analysis on studies conducted in patients with diabetes outside of pregnancy, suggested there was a strong association between measures reflecting abdominal obesity and the incidence of T2DM, and the pooled odds ratio was 2.1 (95% CI 1.7 to 2.7, P<0.0001) [107]. Similarly, as discussed in the literature

review, Neeland *et al.* concluded that baseline VFM measured by DEXA and MRI imaging but not general adiposity was independently associated with risk of development of pre-diabetes and diabetes [108].

Machine learning algorithms are being increasingly used in clinical medicine. In pregnancy, they are currently used to predict genetic disorders like Down's syndrome or trisomy 21 and Edward's syndrome or Trisomy 18. The first trimester screening for Downs syndrome uses an algorithm and detects 90% of the Down's syndrome fetuses prenatally with a 5% false positive rates [132].

2.2 Rationale

There is no published data on the possible association of VFM in pregnancy and the risk of GDM, despite these consistent results outside pregnancy. Hence, the aim of the study was to evaluate the relation of body fat distribution, particularly VFM and the risk of GDM in a cohort of obese women with no known diabetes. The study also investigated whether a higher VFM in early pregnancy would confer a higher risk of subsequent GDM.

This study also aimed to develop a mathematical model which could predict GDM and LGA babies in obese pregnant women using machine learning. It may be clinically useful to identify women at greatest risk of GDM or a LGA baby early in their pregnancy, as lifestyle and pharmacological interventions to improve baby outcomes could then be utilised in this high risk group [113]. Metformin, for example can be used to reduce the risk of GDM in women with polycystic ovaries [133].

The current method of GDM screening is based on risk factors like maternal age, BMI, history of polycystic ovarian syndrome, family history of diabetes, previous GDM, ethnicity and previous macrosomia. This method provides a detection rate of approximately 60% with a 40% false positive rate [125][114]. Currently, those women identified with even a single risk factor undergo an OGTT at 24-28 weeks gestation. Risk stratification for GDM early in pregnancy may reduce the need for OGTT in women at low risk resulting in savings in

costs and in burdensome diagnostic testing. Only 24% of obese [114] or very obese women [134] developed GDM in the control arms of two recent prospective trials investigating the possible beneficial effects of metformin in these women. Risk stratification in early pregnancy may help to avoid extra clinic visits and extra scans in low risk obese women.

2.3 Aims of the study

1. To investigate whether a higher baseline VFM confers a higher risk of GDM in obese pregnant women.
2. To develop a mathematical model to predict GDM and LGA babies in obese pregnant women in early pregnancy using machine learning.

2.4 Objectives of the study

1. To set up a clinical study in obese non-diabetic pregnant women attending an antenatal weight management clinic.
2. To measure the body composition of women at recruitment into the study, between 12 to 18 weeks of gestation.
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5. To perform statistical analysis and investigate whether women with VFM ≥ 75^{th} percentile are at higher risk of GDM.
6. To develop a mathematical model to predict GDM and LGA babies incorporating VFM in early pregnancy in addition to classical risk factors.

2.5 Material and Methods

2.5.1 Ethical Approval

The London-Surrey Borders Research Ethics committee advised us that ethical approval was not required for the study as all women would only undergo routine clinical investigations and management (See Appendix A.1). No study specific procedure was undertaken on any of the participants.

2.5.2 Inclusion criteria

- Obese pregnant women
- Gestation between 12 and 18 weeks

2.5.3 Exclusion criteria

- Pre-existing established diabetes
- Multiple fetuses
- Moving out of area for pregnancy management

2.5.4 Study design

This study was conducted at St Helier Hospital, Surrey in the UK (Figure 2-1). 302 women attending the antenatal clinic at St Helier Hospital and fulfilling the eligibility criteria were enrolled in this study. I recorded their demographic, medical and obstetric history. All women received standardised personal advice on healthy eating and carbohydrate content of food, emphasizing low glycaemic index foods. They were encouraged to undertake 30 minutes of physical activity at least 5 days in a week.

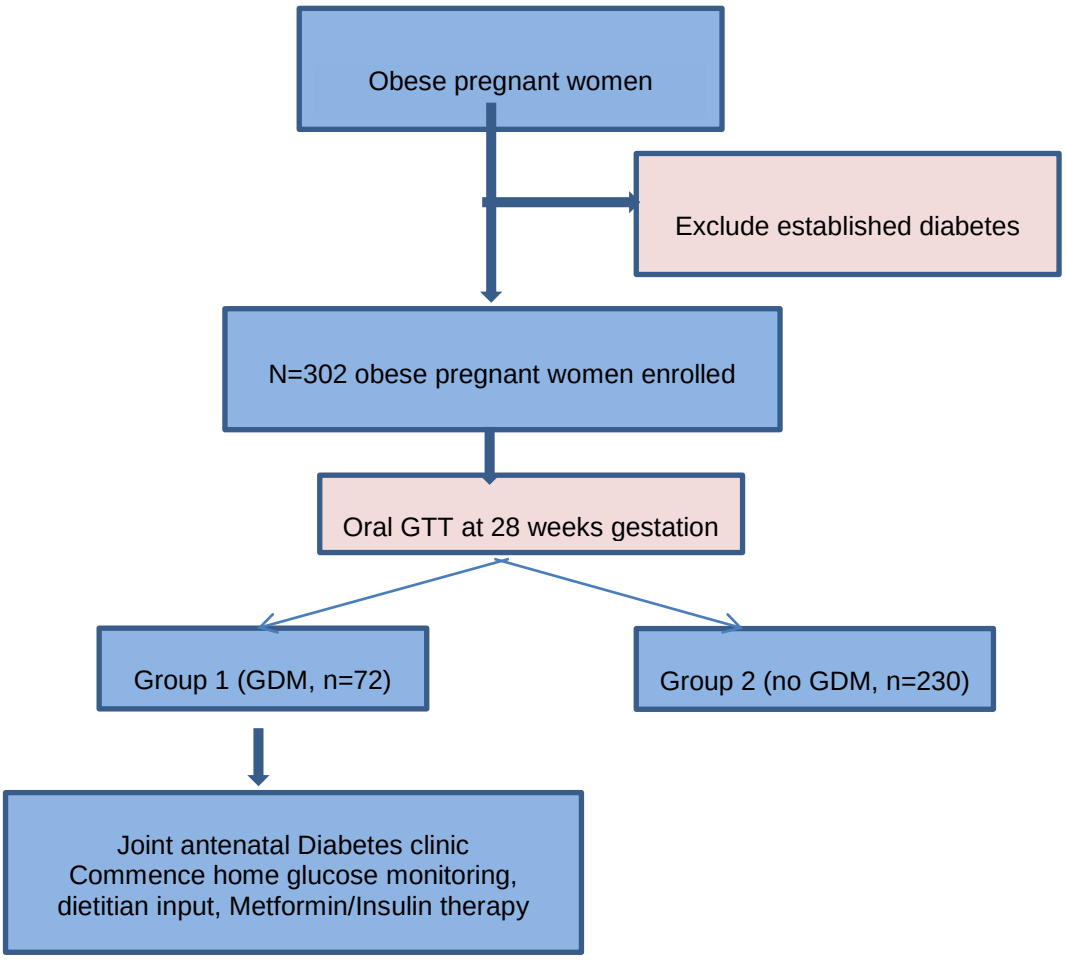


Figure 2-1: Flowchart for women participating in the Visceral Fat Mass study

The flowchart shows that 302 women were enrolled into the VFM study.

I performed the body composition analysis on all women using a machine called Inbody 720^{R} on enrolment into the study. The schedule of clinical assessments is shown in Table 2-1. I assessed all women clinically including weight and blood pressure. The midwife carried out the fetal assessment. They also had a 75g OGTT at 24-28 weeks of gestation, as per the hospital protocol for a pregnant woman with high BMI. The WHO 1999 criteria for the diagnosis of GDM was used [135].

Women with abnormal results of the OGTT were referred to the joint antenatal diabetes clinic and given dietitian input and advised to commence home glucose monitoring. If target blood glucose values were not achieved on diet

alone, metformin was started at a dose of 500mg twice a day and the dose was titrated up to a maximum of 2500 mg per day to achieve target home blood glucose monitoring values (Fasting glucose < 6mmol/l, 1 hour postprandial < 8mmol/l and 2 hour postprandial levels < 7mmol/l). Fasting was defined as an overnight fast of 10-12 hours. Insulin was added if hyperglycaemia persisted in spite of maximum doses of metformin or the patient was intolerant to metformin or did not wish to take it. Some women had a normal OGTT result at 28 weeks, but later developed GDM as diagnosed by glucose monitoring. They were advised glucose monitoring for persistent glycosuria or after the growth scan showed a macrosomic baby.

Table 2-1: Schedule of visits for participants of the visceral fat mass study

Visit No	**1** **Screening and recruit**	**2**	**3**	**4**
Week of gestation	**12-18**	**28 weeks**	**36 weeks**	**Term**
Inclusion/ Exclusion	**X**			
Medical and obstetric History	**X**			
Blood Pressure, Urine , fetal check	**X** **X**		**X** **X**	**X**
Body Composition	**X**			
OGTT		**X**		
Delivery and baby details				**X**

Onset of GDM, pregnancy induced hypertension (PIH), preeclampsia, deep venous thrombosis and mode of delivery were recorded for each participant. Birth weight, neonatal hypoglycaemia, neonatal hyperbilirubinemia, major

malformations, shoulder dystocia and admission to special care baby unit were also recorded. In patients who develop GDM, the OGTT was repeated 6 weeks postnatal to screen for persistent glucose intolerance.

2.5.5 Statistical Analysis

The statistical analysis was performed by using R. Each continuous variable was tested individually for normality using the skewness and kurtosis test. The maternal baseline characteristics, pregnancy and neonatal outcome in patients developing GDM (Group 1, n=72) were compared with those with normal glucose tolerance (Group 2, n=230). The normally distributed data were expressed as mean ± standard deviation. Parametric tests like Welch's t-test, which is an adaptation of Student's t-test was used to compare the means of two groups as it is more reliable when the two samples have unequal variances and unequal sample sizes These tests are also called as "unpaired" or "independent samples" t-tests as they are typically applied when the statistical units underlying the two samples are non-overlapping. Fisher's test was used to compare categorical variables and the level of significance is $P<0.05$. The association between variables in a normally distributed data was investigated with the Pearson's correlation coefficient.

Data which was not normally distributed has been expressed as median and interquartile range (IQR). The dataset was divided by 3 quartiles (Q1, Q2 and Q3) to four different parts. The data lying between the first and third quartile form the interquartile range. The non-parametric tests like Mann-Whitney U test was used to explore the difference between the variables in the two groups with and without GDM and the level of significance is $P<0.05$. The association between variables in a data not normally distributed was investigated with the Spearman's rank correlation.

Data mining and analysis

Principal Component Analysis (PCA) was used as part of the statistical analysis of the data. PCA is an unsupervised multivariate statistical technique for

identifying correlations between samples using a smaller number of uncorrelated variables called “Principal Components” from a large set of data. In PCA, the dataset is transformed from its original coordinate system to a new coordinate system. The new coordinate system is chosen by the data itself. The first new axis is chosen in the direction of the most variance in the data. The second axis is orthogonal to the first axis and in the direction of an orthogonal axis with the largest variance. This procedure is repeated for as many features as in the original data. The majority of the variance is contained in the first few axes and the rest of the axes can be ignored reducing the dimensionality of the data. PCA makes the data interpretation easier by reducing its dimensionality [136].

Machine learning is the method that turns data into information. Supervised learning asks the machine to learn from our data when we specify a target variable. The machines task is to divine some pattern from the input data to get the target variable [136]. We used various algorithms like Decision Trees and Random Forest to develop the model [137] [138].

Random Forest and Decision Tree Modelling

Random Forest (RF) is a family of algorithms for regression and classification that works by constructing a “forest” of decision trees and outputting the class that appeared most times. The general idea behind the algorithm is constructing many classification trees [139]. Each tree is given the same input vector and outputs predicted class. The final class prediction is the one that appeared as a result for most predictive trees. RF is an ensemble method based on bootstrap aggregation. This method constructs multiple versions of the training data by sampling with replacement (bootstrapping), creates a model and makes predictions for all of them and combines the predictions [136].

RF was implemented with 200 trees using the “randomForest” function from the “randomForest” package in R [140]. The performance of the developed model was validated using the Monte Carlo cross validation method [141]. For K=100, the samples from each dataset were randomly distributed into training and testing datasets in 100 different splits. Then, the performance was calculated as

an average of the performance of the 100 models. Firstly, the input dataset (n = 302) was randomly split over 100 iterations into a training dataset, which contained 70% of the samples (n = 227), and a testing dataset (n = 75) composed by the remaining samples. The training dataset was then used to build the model while the testing dataset was used to calculate the performance of such model. As the performance was calculated as a mean of 100 individually trained and optimised models, the outcome was less likely to suffer from optimistic prediction accuracy and/or over-fitting.

Decision trees are constructed by analysing a set of training examples for which the class labels are known. They are then applied to classify new examples. A decision tree classifies data items by asking a series of questions about the features associated with the items. Each question is contained in a node, and every internal node points to one child node for each positive answer to its question. There is a hierarchy in the questioning, encoded as a tree. In its simplest form, yes-or-no questions are asked, and each internal node has a “yes” child and a “no “child. An item is sorted into a class as it passes down from the topmost node, the root, to a node without children, a leaf, depending on the answers. The item is then assigned to the class that has been associated with the leaf it reaches. Decision trees are easy to interpret as they combine simple questions on the data in an understandable way [137].

2.6 Results

The distribution of the continuous variables was analysed by performing the skewness test. Most of the continuous variables like age, weight, HbA1c, percentage body fat, visceral fat area, 2-hour blood glucose values after OGTT and baby birth weight had a normal distribution. BMI, WHR and fasting glucose values at the OGTT did not have a normal distribution (Table 2-2).

Table 2-2: Test of normality for maternal and neonatal characteristics

Variable	Minimum	Quartile 1	Median	Mean	Quartile 3	Maximum	Skew	Normality
Age (years)	16	26	31	30.21	34	43	-0.07	Yes
Weight (kg)	73.5	95.4	104.2	105.1	112.4	157.2	0.47	Yes
HbA1c (mmol/mol)	28	33.2	37	37.7	42	50	0.29	Yes
Body Mass Index (Kg/m^2)	31.4	36.7	38.3	39.34	41.6	63.9	1.37	No
Percentage Body fat (%)	35.1	47.2	50	49.31	51.6	57.9	-0.61	Yes
Waist Hip Ratio	0.89	0.96	0.99	0.99	1.3	1.43	1.91	No
Visceral fat Mass (units)	113.9	164.3	182.8	187.5	207.7	351.7	0.88	Yes
Fasting blood glucose (mmol/l)	3.5	4.3	4.6	4.64	4.9	6.9	1.13	No
2-hour blood glucose (mmol/l)	3	4.8	5.4	5.57	6.2	11.8	0.87	Yes
Birth weight (grams)	1100	3141	3500	3494	3882	5040	-0.18	Yes

Most of the variables were distributed normally except body mass index, waist hip ratio and fasting blood glucose.

Maternal Baseline Characteristics

302 obese pregnant women were enrolled into the study. The median age of the group was 31 years (interquartile range 26-34 years) and the median BMI was 38.3 kg/m$^{2.}$ (interquartile range 36.7-41.6). Most the women were Caucasians (74.5%). Seventy-two patients of the 302 enrolled patients were diagnosed to have gestational diabetes (23.8%). Women who developed GDM were older (32.1 ± 5.5 years vs. 29.6 ± 5.8 years, P<0.05), had a higher median BMI (40.6 kg/m^2 [37.5-43.4] vs. 38 kg/m^2 [36.3-40.9], P<0.05) and greater

waist:hip ratio (1[0.99-1.04] vs. 0.98 [0.95-1.02], P<0.05] when compared with women who did not develop GDM ("no GDM" group). They also had a significantly greater visceral fat mass (199.2 ± 40.5 units vs. 183.8 ± 31.5 units, P<0.05). However, the total percentage body fat was similar in both groups (49.8 ± 3.5 % vs. 49.2 ± 3.6%, P=0.19) (Table 2-3).

Table 2-3: Maternal age and body composition: Statistical comparison between the GDM and no GDM groups

	GDM group n=72 Mean (± SD) [a] Median (IQR)[b]	no GDM group n=230 Mean (± SD) [a] Median (IQR)[b]	P value
Age (years)	32.1(± 5.5)	29.6 (± 5.8)	<0.05[a]
Weight in early pregnancy (kg)	107.3 (± 16.4)	103.3 (± 14.9)	0.07[a]
Percentage Body Fat (PBF) (%)	49.8 (± 3.5)	49.2 (± 3.6)	0.19[a]
Visceral Fat Mass [c] (units)	199.2 (± 40.5)	183.8 (± 31.5)	<0.05[a]
BMI (kg/m^2)	40.6 (37.5-43.4)	38 (36.3-40.9)	<0.05[b]
Waist-Hip ratio	1 (0.99-1.04)	0.98 (0.95-1.02)	<0.05[b]

[a] Independent two samples t-test, [b] Mann-Whitney U test, IQR- Interquartile range, [c] Normal value < 100 units. Women who developed GDM were significantly older, had a higher body mass index, waist-hip ratio and visceral fat area. However, weight in early pregnancy and percentage body fat was not significantly different in both the groups.

Figure 2-2 compares the age of women between the 2 groups. The boxplot depicting the age of women who developed GDM (GDM+) has a higher median than the boxplot of the age of women who did not develop GDM. It also shows the interquartile range.

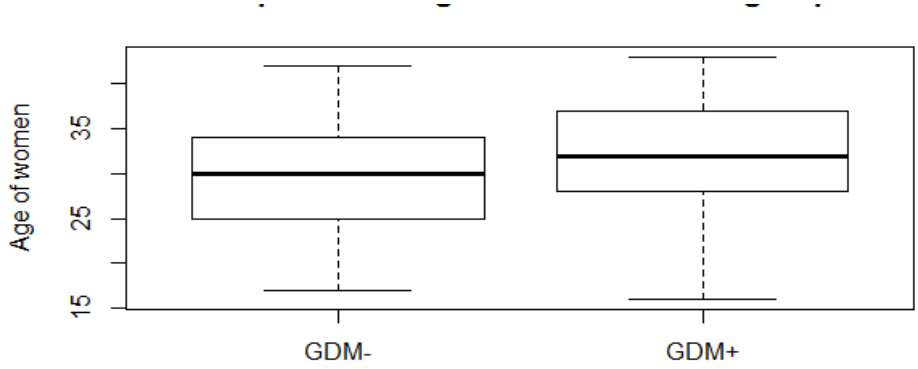


Figure 2-2: Comparison of the age of women - GDM and no-GDM groups

GDM+ is the group of women who developed GDM. GDM- is the group of women who did not develop GDM. The median depicting the age of women with GDM was higher than the median depicting the age of women without GDM.

Figure 2-3 compares the BMI of women between the 2 groups. The boxplot depicting the BMI of women who developed GDM (GDM+) has a higher median than the boxplot of the BMI of women who did not develop GDM (Figure 2-3).

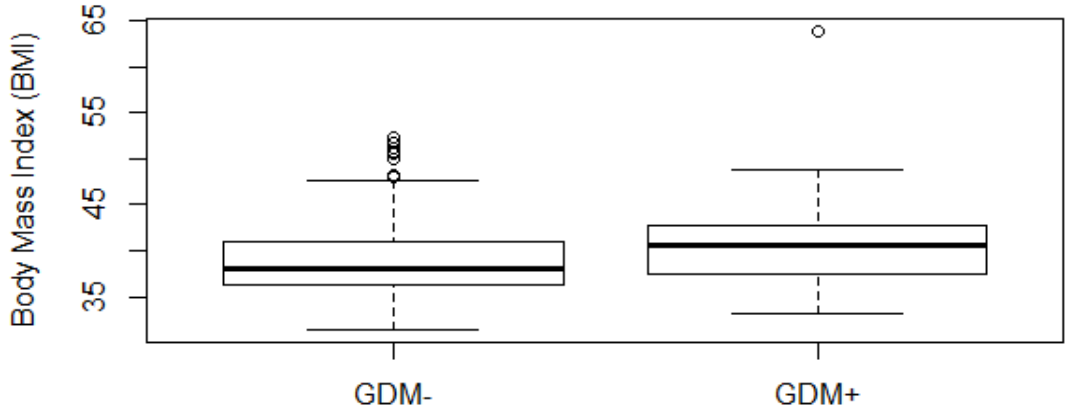


Figure 2-3: Comparison of the BMI of women - GDM and no-GDM groups

The median of the box plot depicting the BMI of women with GDM was higher than the median of the box plot depicting the BMI of women without GDM.

Figure 2-4 shows that the boxplot depicting the VFM of women who developed GDM (GDM+) has a higher median than the boxplot depicting the VFM of women who did not develop GDM.

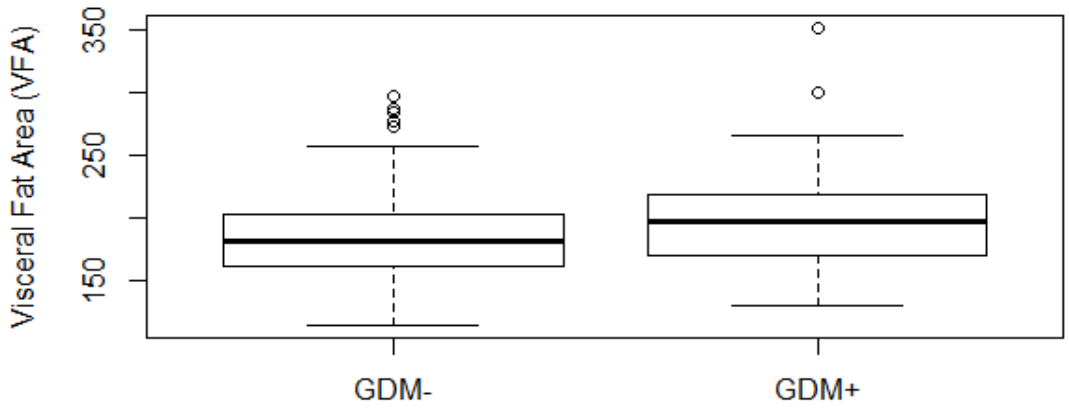


Figure 2-4: Comparison of the VFM between the GDM and no-GDM groups

The median of the box plot depicting the VFM of women with GDM was higher than the median of the box plot depicting the VFM of women without GDM.

The ethnic distribution of women in both groups was not very different (Table 2-4). Women with GDM were more likely to have a history previous GDM when compared with women without GDM. Women with history of GDM in any previous pregnancy were treated as having GDM in all subsequent pregnancies according to hospital guidelines.

Table 2-4: Maternal baseline characteristics: GDM and no GDM groups

	GDM group (n=72)	no GDM group (n=230)	P value [a]
Ethnicity n (%)			
Caucasian	60 (83.3)	165 (71.7)	0.06
Asian	7 (9.7)	30 (13)	0.54
Black	3 (4.2)	27 (11.7)	0.07
Other	2 (2.8)	8 (3.5)	0.8
Polycystic ovaries, n (%)	15 (20.8)	16(7)	<0.05
Family history of DM n (%)	41(56.9)	79(34.3)	<0.05
Previous GDM n (%)	12 (16.8)	0	<0.05
Smokers n (%)	4 (5.6)	16 (7)	0.79

[a] Fisher's exact test. Women who developed GDM were more likely to have polycystic ovaries, family history of diabetes and previous GDM.

The OGTT results showed, as expected, significantly higher median fasting glucose (4.8 mmol/l [4.6-5.3] vs. 4.6 mmol/l [4.3-4.8], P=0.0001) and higher mean 2-hour glucose (6.3 ± 2.9 mmol/l vs. 5.4 ± 1.3 mmol/l, P=0.0004) in women who developed GDM compared with women who did not (Table 2-5).

Table 2-5: OGTT results at 28 weeks' gestation: Statistical comparison between the GDM and no GDM groups

	GDM group (n=72) **Mean (± SD) [a]** **Median (IQR) [b]**	**no GDM group, n=230** **Mean (± SD) [a]** **Median (IQR) [b]**	P value
OGTT-Fasting glucose value (mmol/l)	4.8 (4.6-5.3)	4.6 (4.3-4.8)	<0.05[b]
OGTT-2 hour glucose value (mmol/l)	6.3 (± 2.9)	5.4 (± 1.3)	<0.05[a]

[a] Independent two samples t-test, [b] Mann-Whitney U test, IQR - interquartile range. The median fasting glucose and the mean 2-hour glucose were higher in the women who developed GDM as compared to the women who did not develop GDM.

Pregnancy induced hypertension, preeclampsia and thromboembolism were not significantly different between the two groups. Similarly, the rates of Caesarean section were also not significantly different between the groups. Women with GDM showed a trend towards more emergency Caesarean sections (30.6%) compared to women without GDM (23%). However, there was a trend towards lesser instrumental deliveries in the women with GDM (4.2%) as compared to the women without GDM (10%), and the trend persisted even after excluding women who had emergency Caesarean sections (Table 2-6).

Table 2-6: Maternal outcomes: Statistical comparison between the GDM and no GDM groups.

	GDM group n=72	no GDM group n=230	P value [a]
PIH, n (%)	8 (11.1)	21 (9.1)	0.6
Preeclampsia n (%)	1 (1.4)	2 (0.9)	0.6
Mode of Delivery: n (%) **Vaginal** **Instrumental** **Elective C/section** **Emergency C/section**	 34 (47.2) 3 (4.2) 13 (18.1) 22 (30.6)	 122 (53) 23 (10) 32 (13.9) 53 (23)	 0.4 0.2 0.5 0.2
Deep vein thrombosis n (%)	0	1 (0.4)	0.6

[a] Fisher's exact Test. Maternal outcomes like PIH, preeclampsia and DVT were not significantly different between groups. There was also no statistical difference in the number of women delivering by Caesarean section between the two groups.

The difference in mean birth weight between the groups was not significantly different (3452.8 ± 626 g vs. 3506.7 ± 564 g, P=0.5)**.** Similarly, the percentage of LGA babies was similar in both groups (18.3% vs. 18.1%, P=1). There was also no significant difference in the rate of admission to neonatal care units (11.2% vs. 9.6%, P=0.7), neonatal jaundice (4.2% vs. 0.8%, P=0.08) and shoulder dystocia (0% vs. 0.4%, P=1) between the groups. More babies in the GDM group had neonatal hypoglycaemia (4.2% vs. 0.4%) as compared to no GDM group and this difference reached statistical significance (P=0.04) (Table 2-7).

Table 2-7: Neonatal outcomes: Comparison between the groups

	GDM group (n=72) Mean (± SD) [a]	no GDM group (n=230) Mean (± SD) [a]	P value
Birth weight (g)	3452.8 ± 626	3506.7 ± 564	0.5[a]
Large for gestational age, n (%)	13 (18.3)	42 (18.1)	0.96[b]
Admissions to Neonatal Intensive Care Unit, n (%)	8 (11.2)	22 (9.6)	0.7[b]
Major malformations n (%)	0	0	1.00[b]
Neonatal hypoglycaemia [c], n (%)	3 (4.2)	1 (0.4)	0.04[b]
Neonatal hyperbilirubinemia n(%)	3 (4.2)	2 (0.8)	0.08[b]
Shoulder dystocia n (%)	0	1 (0.4)	0.57[b]

[a] Independent two samples t-test, [b] Fisher's exact test, [c] Capillary glucose <2.6mmol/l . There was no significant difference in any of the neonatal outcomes except neonatal hypoglycaemia. More babies of mothers who developed GDM had hypoglycaemia at birth

As discussed earlier, women diagnosed with GDM were referred to the specialist antenatal diabetes clinic where they were treated intensively with diet, metformin alone or in combination with insulin, until euglycaemia was achieved. This intensified treatment for gestational diabetes was responsible for the minimal differences seen in neonatal outcomes in women with and without GDM in my study. There were no still births in my study.

Correlations between variables

The correlation of VFM obtained at recruitment with the 28-week glucose values obtained during OGTT is shown in Table 2-8. A moderate positive correlation was seen between VFM and fasting glucose values in Group 1 (r=0.32; P=0.002) .There was a weak positive correlation between VFM and fasting glucose in the whole cohort.

Table 2-8: Correlation of VFM with OGTT test results

	Correlation coefficient Pearson's [a] Spearman's [b]	P value
VFM versus fasting glucose (whole)	0.18[b]	<0.05
VFM versus 2-hr glucose (whole)	0.09[a]	0.1
VFM versus fasting glucose (no GDM)	0.14 [b]	0.07
VFM versus 2-hr glucose (no GDM)	0.008[a]	0.9
VFM versus fasting glucose (GDM)	0.32[b]	<0.05
VFM versus 2-hr glucose (GDM 1)	-0.19[a]	0.1

[a] Pearson's correlation coefficient, [b] Spearman's rank correlation. A moderately positive correlation was seen between visceral fat mass and fasting glucose in GDM group.

There was a weak positive correlation between PBF and the fasting glucose in all groups. No such correlation was seen with the 2-hour glucose values and PBF in the whole group or each group individually (Table 2-9).

Table 2-9: Correlation of PBF with OGTT test results

	Correlation coefficient Pearson's [a] Spearman's [b]	P value
PBF versus fasting glucose (whole)	0.14[b]	0.01
PBF versus 2-hr glucose (whole)	0.04[a]	0.5
PBF versus fasting glucose (no GDM)	0.14[b]	0.03
PBF versus 2-hr glucose (no GDM)	0.04[a]	0.7
PBF versus fasting glucose (GDM)	0.24[b]	0.03
PBF versus 2-hr glucose (GDM)	0.13[a]	0.3

[a] Pearson's correlation coefficient, [b] Spearman's rank correlation. A weak positive correlation was seen between percentage body fat and fasting glucose in all groups.

There was a moderate positive correlation between maternal BMI and HbA_{1c} (r=0.39; P<0.05), and a strong positive correlation between VFM and HbA_{1c} (r=0.47; P<0.05 in women who developed GDM (Figure 2-5). However, no significant correlation was found between total PBF and HbA_{1c} (r=0.16; P= 0.2).

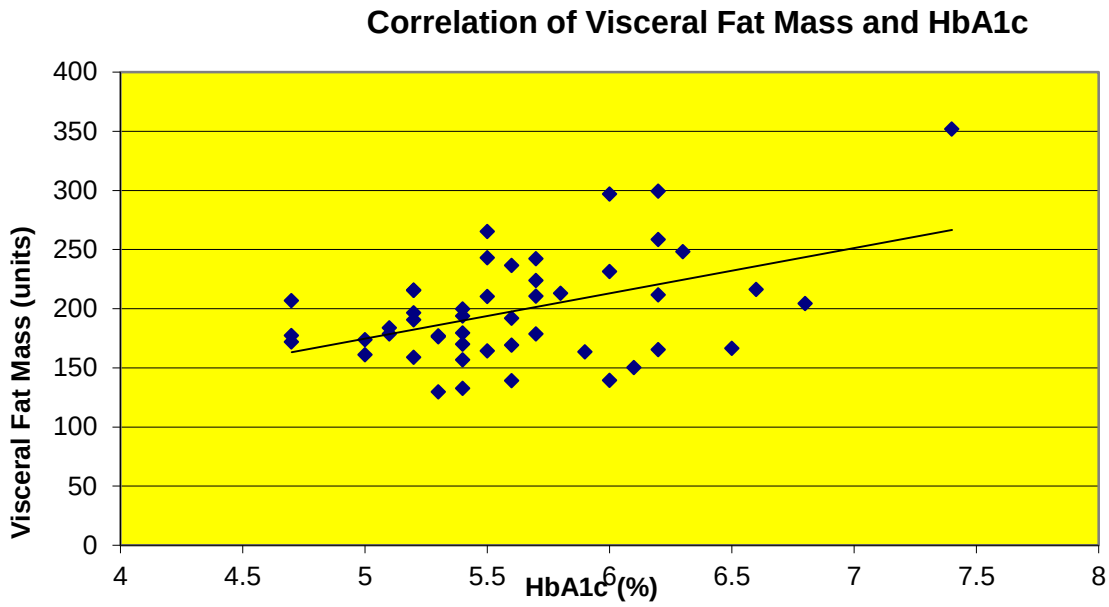


Figure 2-5: Correlation of HbA_{1c} and VFM in women who developed GDM

A strong positive correlation was seen between VFM and HbA1c in GDM group

A weak positive correlation was seen between maternal BMI and VFM at recruitment with birth weight, for the whole cohort and in the no GDM group. However, no such correlation was seen between maternal BMI and VFM at recruitment with birth weight in the group of women who developed GDM in pregnancy (Table 2-10).

Table 2-10: Correlation between BMI, PBF and VFM with the baby birth weight

	Correlation coefficient Pearson's [a] Spearman's [b]	P value
BMI versus birth weight (whole)	r=0.13[b]	0.03
PBF versus birth weight (whole)	r=0.02[a]	0.7
VFM versus birth weight (whole)	r=0.17[a]	<0.05
BMI versus birth weight, no GDM group	r=-0.13[b]	0.04
PBF versus birth weight, no GDM group	r=0.03[a]	0.65
VFM versus birth weight, no GDM group	r=0.18[a]	<0.05
BMI versus birth weight, GDM group	r=0.13[b]	0.2
PBF versus birth weight, GDM group	r=-0.02[a]	0.8
VFM versus birth weight, GDM group	r=0.19[a]	0.1

[a] Pearson's correlation coefficient, [b] Spearman's rank correlation. A weak positive correlation was seen between maternal BMI and VFM with birth weight, for the whole cohort and in the no GDM group

In the overall cohort, 72 of the 230 women developed GDM. Women who developed GDM were more likely to have a baseline VFM ≥ 75th percentile as compared to women who did not (43.1% vs. 19.5% [Odds ratio 3.1(1.8 to 5.5)], P=0.0001)(Table 2-11).

Table 2-11: Baseline VFM and subsequent GDM

Measure	GDM (n=72)	No GDM (n=230)	Odds ratio (95%CI)	P value
VFM ≥ 75th percentile, n (%)	31 (43.1)	45 (19.5)	3.1(1.8 to 5.5)	<0.05
VFM ≥ 90th percentile, n(%)	11 (15.3)	13 (5.6)	3.0(1.3 to 7)	0.01

More women in the GDM group had baseline VFM ≥ 75th percentile and VFM ≥ 90th percentile as compared to women who did not develop GDM.

A subgroup analysis was also performed based on ethnicity comparing BMI, WHR and VFM in Caucasians versus Asians in the group of women who developed GDM, in order to evaluate whether ethnicity influences the relation of body fat to GDM. We have reports in literature, outside pregnancy, that Asians develop type 2 diabetes at a lower BMI as compared to Caucasians. The VFM was significantly higher in Caucasians than in the Asians in this group (Table 2-12).

Table 2-12: Comparison of BMI, WHR and VFM in Caucasians and Asians who develop GDM

	Caucasians, n=60 Mean (± SD) [a] Median (IQR)[b]	Asians, n=7 Mean (± SD) [a] Median (IQR)[b]	P value
Body Mass Index (BMI)	41 (37.7-43.6)	37.5 (35.8-40.8)	0.16[b]
Waist-Hip Ratio	1 (0.98-1.04)	1 (0.98-1.04)	0.1[b]
Visceral Fat Mass (VFM)	202.3 ± 40.5	167.9 ± 36.4	0.03[a]

[a] Independent two samples t-test, [b] Mann-Whitney U test, IQR-interquartile range. The visceral fat mass was significantly higher in Caucasians than the Asians in the group who develop GDM

Results of Principal Component Analysis

Principal Component Analysis was carried out by reducing the variables into two Principal Components and plotting the variance. Maternal age, BMI, family history of diabetes, polycystic ovarian syndrome which are classical risk factors for GDM were combined with body composition measurements like PBF, skeletal muscle mass, WHR and VFM as input data. There were no distinct clusters of samples seen between GDM and no GDM and hence no evident separation in Principal Component 1 (PC1) versus Principal Component 2 (PC2), PC1 versus Principal Component 3 and PC2 versus PC3 (Figure 2-6, Figure 2-7 & Figure 2-88). 99.7% of the variance was captured in PC1, 0.2% of the variance was captured in PC2 and 0.1% in PC3.

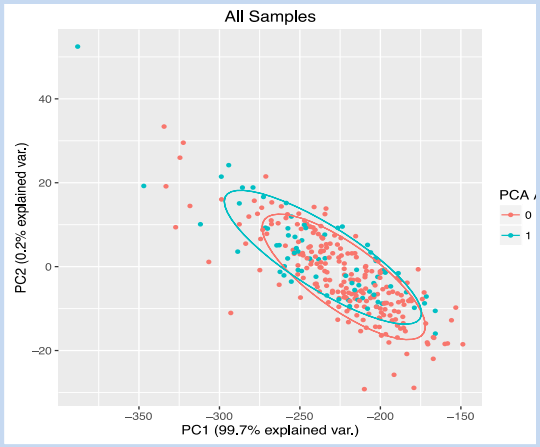


Figure 2-6: Principal Component Analysis (PC1 versus PC2) according to GDM

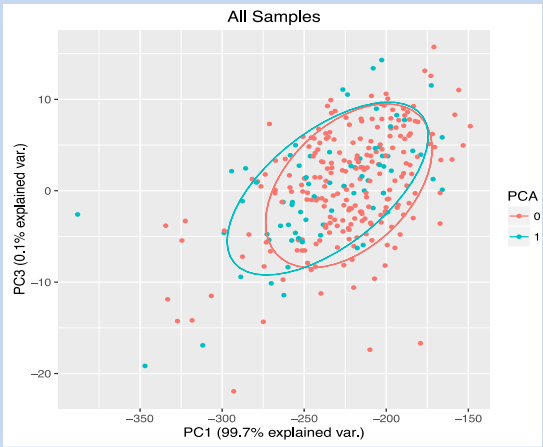


Figure 2-7: Principal Component Analysis (PC1 versus PC3) according to GDM

Figure 2-6 & Figure 2-7 : Principal Component Analysis according to GDM

The blue dots represent GDM and the red dots represent no GDM. There were no distinct clusters of samples seen between GDM and no GDM. 99.7% of the variance was captured in PC1, 0.2% of the variance was captured in PC2 and 0.1% of the variance was captured in PC3.

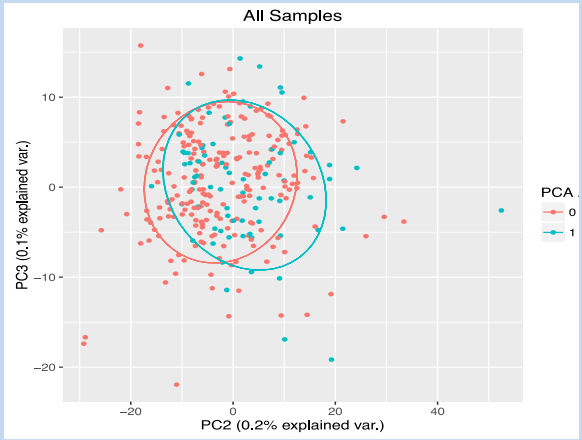


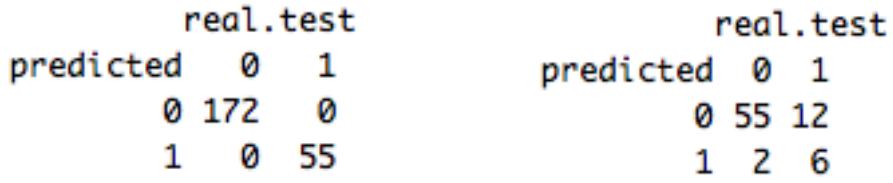
Figure 2-8: Principal Component Analysis (PC2 versus PC3) according to GDM

Figure 2-8: Principal Component Analysis according to GDM

The blue dots represent GDM and the red dots represent no GDM. There were no distinct clusters of samples seen between GDM and no GDM. 0.2% of the variance was captured in PC2 and 0.1% of the variance was captured in PC3.

A line could not be drawn between GDM and no GDM in any of the plots. Hence, supervised learning methods were applied to design and develop a GDM predictive model using random forest and decision tree modelling.

A GDM predictive model was developed using the Random Forest (RF) algorithm. RF is an ensemble learning method based on the combination of several decision trees in order to provide a more accurate prediction than the individual trees on their own [138]. RF was implemented using the "randomForest" function from the "randomForest" package [140]. The optimisation confusion matrix (Figure 2-9) indicates that for the model achieved 100% classification accuracy where all 227 training samples were correctly classified. The model validation achieved an initial prediction accuracy of 81.1%; where 61 out of 75 samples where correctly predicted. However, 14 patients were wrongly classified.



	real.test	
predicted	0	1
0	172	0
1	0	55

	real.test	
predicted	0	1
0	55	12
1	2	6

Figure 2-9 : Confusion Matrix for prediction of GDM

The model achieved 100% classification accuracy where all 227 training samples were correctly classified. The validation model predicted 61 testing samples correctly out of 75 giving an initial prediction accuracy of 81.1%.

Upon running a series of 200 iterations, while randomly reshuffling samples within the training and testing subsets, the model stabilised after 20 iterations as showed from the performance accumulative mean, achieving a mean performance of 77.5% (Figure 2-10).

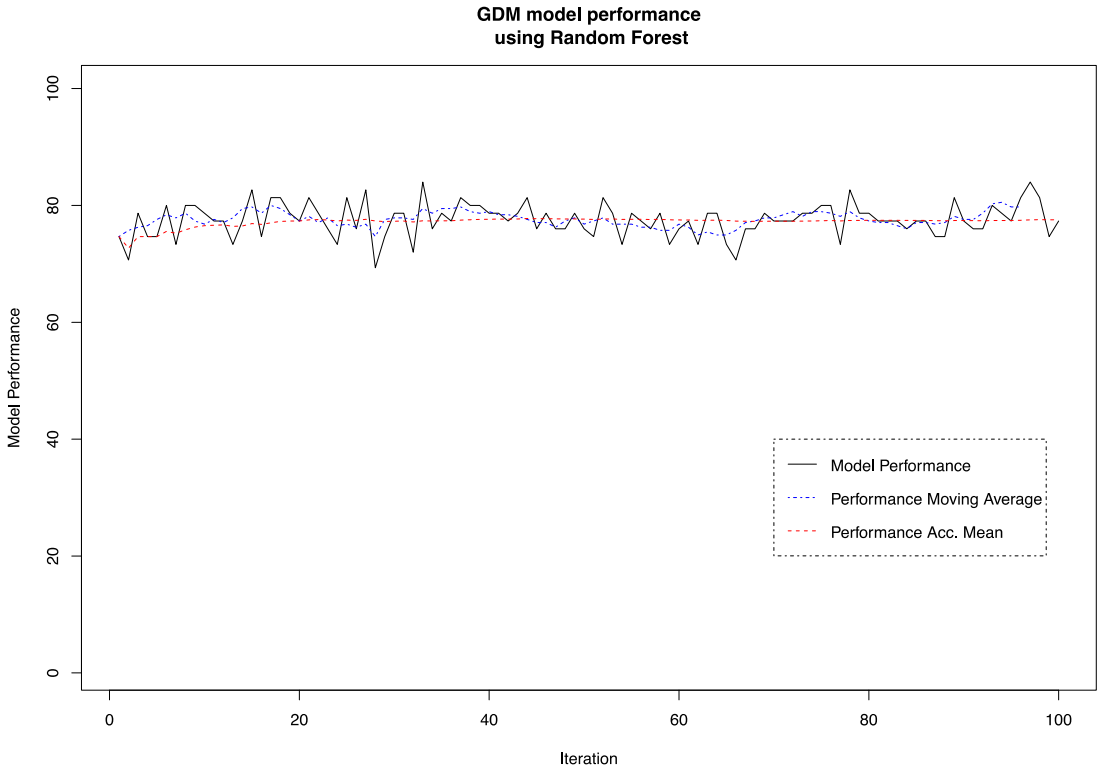


Figure 2-10: GDM model performance using Random Forest

The model's performance accumulative mean was 77.5%

Variable importance for predicting GDM, measured by RF, was measured using the "varImpPlot" function. The VFM came as the most important variable, followed by BMI, skeletal muscle mass (SMM), weight and PBF in that order. Less important variables included waist-Hip ratio, history of previous GDM and history of polycystic ovarian syndrome (PCOS) (Figure 2-11).

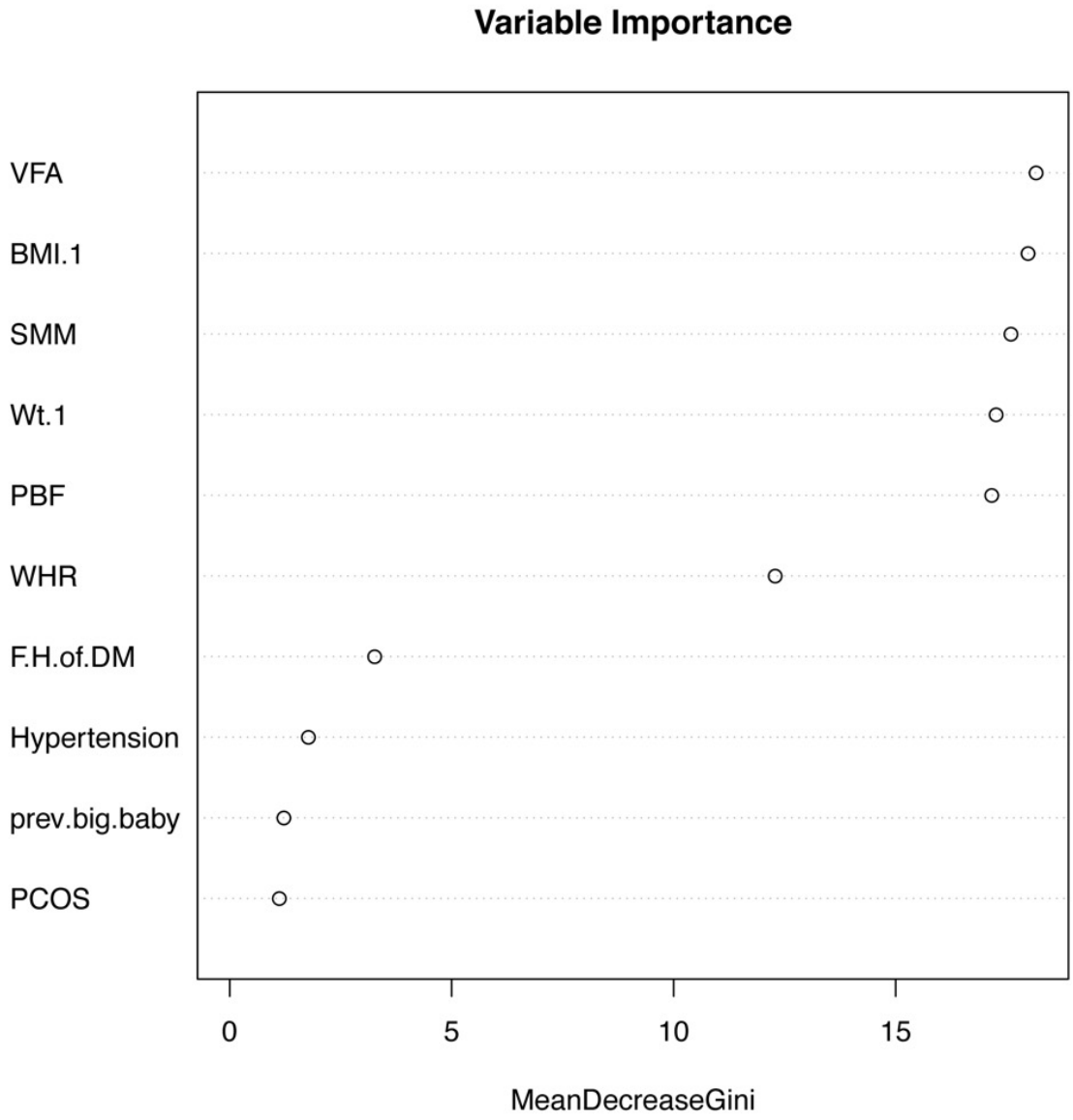


Figure 2-11: Ranking of variables as predictors of GDM

Visceral fat mass was the most important variable followed by BMI, SMM, weight and percentage body fat.

The decision tree used a value of VFM < 210 as the first split, which was the most important split in any decision tree (Figure 2-12).

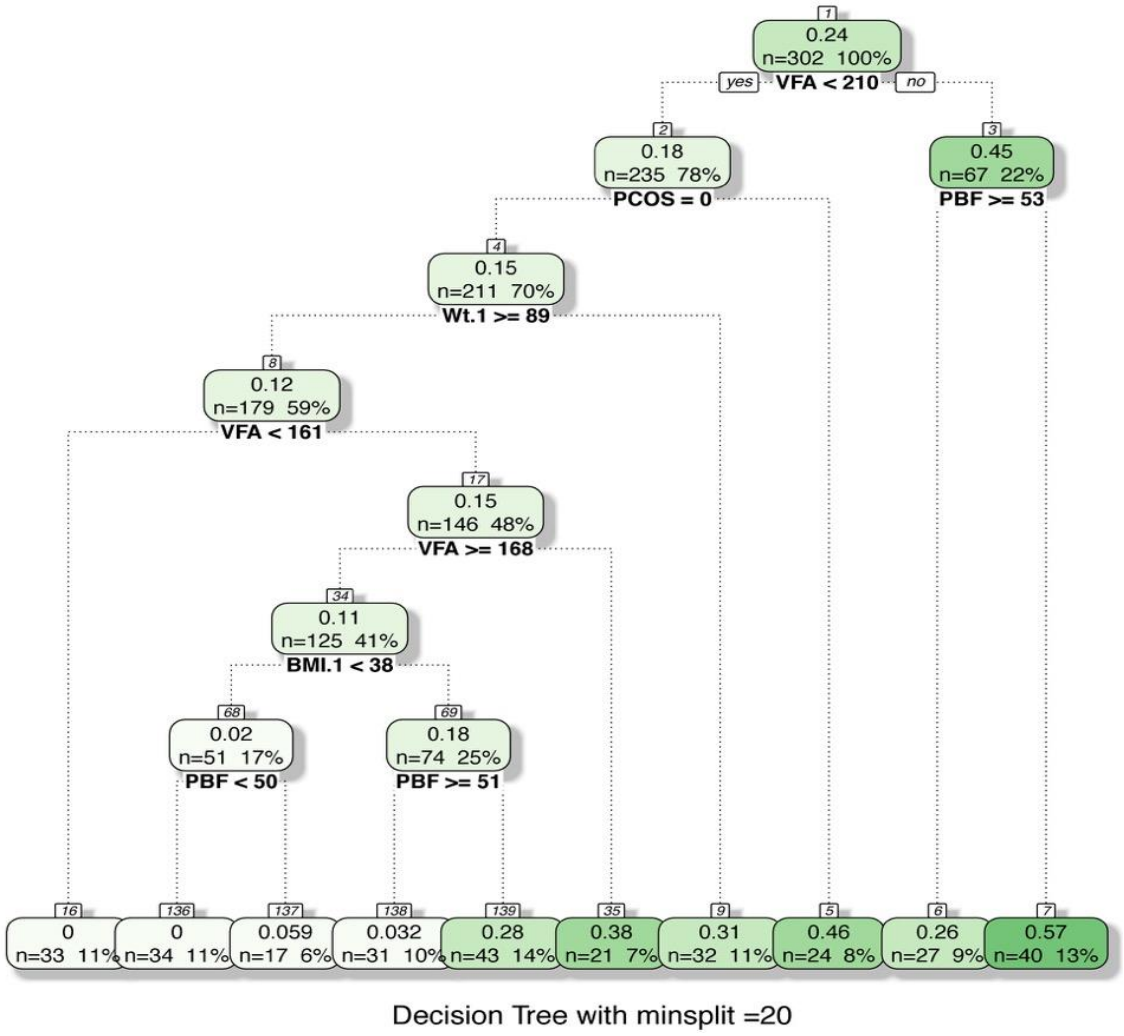


Figure 2-12: Decision tree for predicting GDM

The decision tree for predicting GDM used VFA < 210 as the first split

The birth centile is classified into 3 groups in routine clinical practice: birth centile <10 as low birth centile (small for gestational age), birth centile between 10 and 90 as normal birth centile and birth centile > 90 as high birth centile (LGA). PCA was carried out as before by reducing the variables into two principal components and plotting the variance. There was no distinct clustering of samples seen between the three birth centile classes and hence no evident separation. 98.9% of the variance was captured in PC1, 0.7% of the variance was captured in PC2 and 0.2% of the variance in PC3. However, there was no evident separation in both PC1 versus PC2 and PC1 versus PC3 (Figure 2-13 & Figure 2-14).

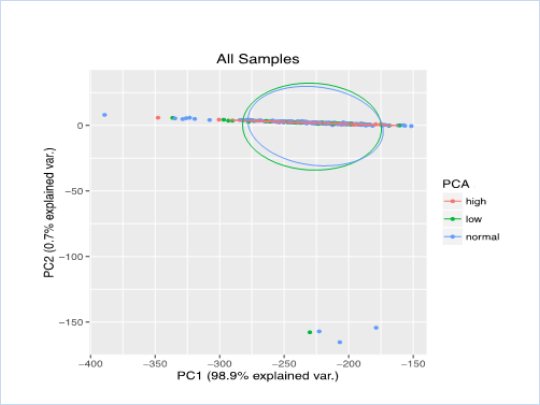


Figure 2-13: Principal Component Analysis (PC1 versus PC2) according to birth centile classes

Figure 2-14: Principal Component Analysis (PC1 versus PC3) according to birth centile classes.

The blue dots represent normal birth centile, the red dots represent high birth centile and the green dots represent low birth centiles. 98.9% of the variance was captured in PC1, 0.7% of the variance was captured in PC2 and 0.2% of the variance in PC3.

The analysis is repeated with important variables only, excluding age and height. Again, there was no evident separation seen (Figure 2-15 & Figure 2-16).

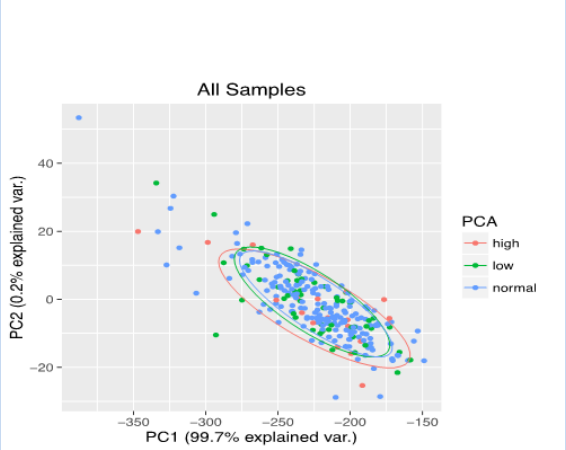


Figure 2-15: Principal Component Analysis (PC1 versus PC2) according to birth centile class (without age and height)

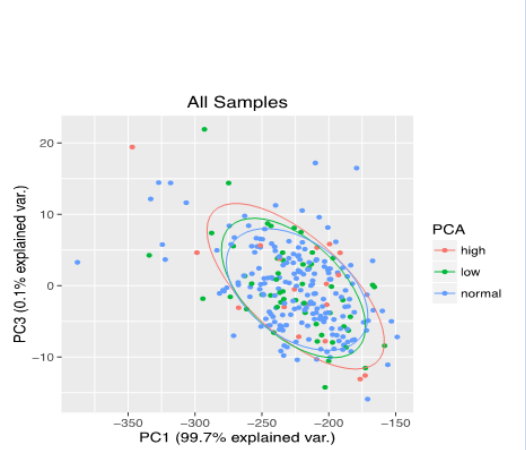


Figure 2-16: Principal Component Analysis (PC1 versus PC3) according to birth centile class (without age and height)

The blue dots represent normal birth centile, the red dots represent high birth centile and the green dots represent low birth centiles. There were no distinct clusters of samples seen between the classes. 99.7% of the variance was captured in PC1, 0.2% of the variance was captured in PC2 and 0.1% of the variance was captured in PC3.

The PCA failed to classify correctly and hence the Random Forest algorithm was used.

Random Forest to predict birth centile classes

A birth centile predictive model was developed using the RF algorithm on similar lines as the development of the GDM predictive model. The input data managed to predict birth centile class correctly with an average of 68%.174 out of 191 (91.1%) from the normal birth centile class were predicted correctly. In the low birth centile class, only 4 were predicted right out of 52 and none of the ones from the high birth centile class were predicted right (Figure 2-17). The model was well trained in predicting normal birth centile but not so in extremes.

	real.test		
predicted	high	low	normal
high	0	2	4
low	2	4	13
normal	16	46	174

Figure 2-17: Confusion matrix for birth centile classes

The matrix showed that the model predicted birth centile classes with an average of 68% accuracy.174/191 normal birth centiles were predicted correctly.

2.7 Discussion

The literature search did not reveal any published studies to date that examined the possible role of directly measured VFM and its relation to pregnancy outcomes in obese women. Therefore, to my knowledge, this was the first study examining the role of VFM in obese pregnant women.

The results showed that VFM in early pregnancy is a novel risk factor for GDM [142]. There was a moderately positive correlation of VFM with fasting glucose and HbA_{1c} in all patients who developed GDM. Similar correlations of VFM with fasting hyperglycaemia and HbA_{1c} have been shown in patients with T2DM [110]. There was a weak correlation between total PBF and fasting glucose but no correlation between total PBF and HbA_{1c}. This suggests that non-visceral

subcutaneous fat may not have the same metabolic effect as visceral fat. This fits in with the proposal that GDM should be considered as a part of metabolic syndrome [143][144]. The study results confirmed a significant association between the higher baseline VFM (≥ 75th percentile) and risk of subsequent gestational diabetes. Obese women with a VFM ≥ 75 percentile measured in early pregnancy have a 3-fold risk of subsequent GDM (Table 2-11).

In the general population, it is already well-established that excess visceral fat and insulin resistance, but not general adiposity, are independently associated with pre-diabetes and T2DM in obese adults [107][108]. The results of my study suggested a similar association between VFM and risk of GDM in obese pregnant women.

In my study, Asian women who developed GDM have a lower BMI and a significantly lower VFM compared with Caucasians. This is in conformity with a recently published study by Hedderson *et al* [145]. This study concluded that clinicians should be aware that the BMI thresholds for increased risk of GDM varies by racial/ethnic group and that risk is high even at relatively low BMI cut offs in Asian and Filipina women. Hence, Asian women may benefit from different prevention strategies for GDM in addition to weight management [145]. In the Asian cohort of my study, those developing GDM had a higher VFM and markers of central obesity. Despite small numbers in this study, these observations are in keeping with the suggestion that Asians are particularly susceptible to diabetes even at lower BMIs. The pathogenic link between visceral fat and onset of diabetes is likely to be through the development of insulin resistance. Studies in T2DM have shown that visceral fat accumulation decreases insulin sensitivity and has a negative impact on glycaemic control [110]. Visceral adipocytes are known to release a variety of inflammatory cytokines such as interleukin 1 and 6, tumour necrosis factor and resistin which have been suggested to induce insulin resistance. I did not measure cytokines or any of the inflammatory markers in this study, which is one of the limitations of the study.

There is an increased risk of fetal macrosomia in pregnancies complicated by GDM and obesity. I found no significant difference between the neonatal birth weights and other neonatal outcomes in the two groups, which possibly again was a reflection of the intensive management of GDM in these women (Table 2-7).

According to the decision tree developed, VFM emerged as the most important variable in determining the risk of GDM, followed by BMI, SMM, weight, and PBF and waist hip ratio. Factors like previous GDM, history of polycystic ovarian syndrome, family history of diabetes and previous macrosomia were less important variables (Figure 2-11).These results add to the growing evidence of the importance of central obesity and in particular, VFM in the development of GDM. The decision tree used a value of VFM < 210 as the first spilt which was the most important split in the decision tree.

The model managed to predict GDM with an average prediction accuracy of 77.5%, which is an overall good performance. However, even though it gave a good performance in predicting no-GDM, two-thirds of the true GDMs were wrongly classified. This is largely due to the unbalanced distribution of both classes of patients, as only 24% of patients in the original dataset developed GDM. Hence, the model shows a bias in prediction towards the no GDM class. [146]. Similarly, with the birth centile model, it correctly predicted birth centile classes with an average of 68%. The model was well trained in predicting normal birth centile but not as accurate in predicting low or high birth centile. Hence the prediction of the birth centile model showed a slight bias towards the normal birth centile class. Studies have shown us that GDM alone predicts macrosomia poorly and hence the birth centile model would be very useful [74] [76] [92].

This was probably the first attempt to create a mathematical model to predict GDM and LGA baby using VFM in addition to classical risk factors. Measuring the VFM with the InBody 720 is a simple and non-expensive test which can easily be done in a clinical setting. The clinical significance of this study is the potential for early and personalised risk stratification for GDM allowing low risk

obese women to avoid unnecessary diagnostic testing, additional clinic visits and growth scans. Conversely those at high risk can start dietary and lifestyle interventions early to reduce the risk of complications.

The strengths of this study include direct measurement of fat distribution *in vivo* in early pregnancy in ambulant women attending a single centre with standardised dietary and exercise advice. A range of clinically relevant and novel predictors of GDM were simultaneously measured rather than one novel factor measured in isolation. As such, the model created had greater validity. A limitation of the study is that there was no comparison with a cohort of non-obese women. Also, ethnicity was not included in the model as our dataset contained predominantly Caucasian women. Another limitation was that insulin sensitivity was not measured in this study. The recommendation is to design a clinical study with a larger number of pregnant women, across the BMI spectrum in order to confirm the findings, train the model better and improve its accuracy. One study showed that obesity in a multi-ethnic population cannot be defined by a single set of new cut points for BMI, but varied cut offs depending on the outcome assessed [47].

2.8 Conclusions

This study showed that visceral fat mass is a novel risk factor for the development of GDM in obese pregnant women. Obese pregnant women with VFM ≥ 75th percentile have a 3-fold higher risk of developing GDM. A mathematical model was developed with good overall performance in predicting gestational diabetes and LGA babies in obese pregnant women early in pregnancy. The model will require further training with data from a larger cohort of obese pregnant women to confirm the findings and improve its performance so that it can be adopted as a clinical tool in the management of obese pregnant women.

3 METFORMIN IN OBESE NON-DIABETIC PREGNANT WOMEN (MOP) TRIAL

This chapter begins with an outline of the complications of obesity in pregnancy and a description of the aims and objectives of the "Metformin in obese non-diabetic pregnant women trial (MOP Trial)". Lifestyle intervention programmes in pregnancy have shown some benefits on maternal outcomes such as GWG but no evidence of benefits for the neonate [113][147][148][149]. Therefore, the MOP trial, which investigated the possible beneficial effects of metformin in obese pregnant women, fulfilled an unmet need in a timely manner. This chapter goes on to describe the methodology and results of the MOP trial.

3.1 Introduction

The proportion of women with obesity in the UK is a matter of concern. The Health Survey of England 2013 reported that approximately 1 in 4 women were obese and 2 out of 3 women had a high or very high waist circumference [150]. The CMACE [69] reported that nearly 5% of pregnant women in UK had a BMI of > 35 kg/m^2. The literature review showed that obesity is associated with a number of serious adverse outcomes including gestational diabetes and preeclampsia [68][151]. Being overweight or obese contributes to over half of maternal mortality [69]. Obesity during pregnancy is associated with an increased risk of adverse short-term and long-term consequences for both mother and baby [131]. Excessive weight gain in pregnancy is also associated with an increased risk of complications [71].

As lifestyle intervention programmes in pregnancy have shown no evidence of benefits for the neonate, investigators have turned to pharmacological interventions and, in particular the insulin-sensitising agent metformin [113] [147][148]. Since insulin resistance is increased in obesity and is strongly associated with birth weight and fetal adiposity [131], metformin is a rational choice to improve outcomes in this population. Metformin primarily acts on the liver but also affects skeletal muscle, adipose tissue, endothelium and ovaries.

It reduces fasting serum insulin by 40% and leads to weight reduction by 5.8% [133]. It improves insulin sensitivity, reduces hepatic gluconeogenesis and increases peripheral glucose uptake [41]. Metformin is eliminated by the kidney and has increased clearance in pregnancy [130].

Because it crosses the placenta, there have been concerns about its safety in pregnancy. Data from its use in pregnancy in obese women with polycystic ovarian syndrome has shown it to be safe with no evidence of teratogenicity [42]. Metformin is currently classified as a Category B drug for use during pregnancy. The NICE has recommended metformin as first-line therapy for gestational diabetes when dietary intervention fails to control the blood glucose [125].

The Metformin in GDM (MiG) study [40] concluded that in women with GDM, metformin (alone or with supplemental insulin) is not associated with increased perinatal complications as compared with insulin. GWG was significantly lower in the metformin treated women although there were no differences in birth weight between the groups. In my previous work, done as part of the St Helier diabetes team, I showed that women with GDM treated with metformin showed a 20% birth weight centile reduction and lesser number of LGA babies versus insulin treated women. Also, women in the metformin group gained significantly less weight in pregnancy [152]. A small study in women with polycystic ovaries showed that metformin during pregnancy reduces insulin, insulin resistance and development of gestational diabetes [133]. Outside of pregnancy, the Diabetes Prevention Program research group showed that metformin reduced the incidence of T2DM in patients with impaired glucose tolerance by 31% [153].

3.2 Rationale

Based on the literature review, there is substantial evidence that obesity in pregnancy contributes to increased morbidity and mortality for both mother and baby [67].The important complications associated with obesity in pregnancy are an increased risk of GDM, pregnancy induced hypertension, preeclampsia, thromboembolic complications and LGA babies [68]. Lifestyle intervention

programmes have shown no benefit for the neonate [112][148][149]. Research studies and new interventions which could decrease the incidence and severity of complications for both mother and baby are therefore very important and make this study very timely.

Pregnancy is an insulin resistant state and more so, in obese women. Metformin inhibits gluconeogenesis and reduces free fatty acid levels and thereby improves insulin sensitivity [40]. It also stimulates Glucagon-like-peptide 1 (GLP-1) release and insulin secretion. Weight loss has been observed during metformin treatment. Metformin has been classified as a Class B1 drug in pregnancy. It crosses the placenta but there is no evidence of adverse fetal effects [40]. Limited or no weight gain during pregnancy in obese pregnant women is associated with a significantly lower risk of PET, CS and LGA and a more favourable pregnancy outcome [88].

Several small studies suggest that metformin could be beneficial in pregnancy due to its insulin sensitizing action, thereby reducing the risk of GDM and incidence of LGA babies [133][154]. It may also decrease the risk of pregnancy induced hypertension and preeclampsia [155]. Thus, treatment with metformin from the beginning of the 2nd trimester may improve the overall pregnancy and neonatal outcomes in obese non-diabetic pregnant women. It is further hypothesised that metformin may be more effective in a subgroup of obese pregnant women having high baseline insulin resistance and treatment of this selective subgroup of women with metformin may reduce the risk of GDM.

3.3 Aims of the study

1. To investigate whether metformin improves pregnancy outcomes (incidence of LGA (≥90% birth weight centile) babies, onset of maternal GDM, hypertension, preeclampsia, macrosomia, shoulder dystocia, admission to SCBU in obese non-diabetic women.

We aim to compare perinatal outcomes in women randomised to the two groups

Group 1: Standarised life style intervention and placebo

Group 2: Standarised life style intervention and metformin

2. To determine whether there is an association between baseline insulin resistance and adverse pregnancy outcomes such as gestational diabetes, pregnancy-induced hypertension and preeclampsia.

3. To investigate whether metformin will improve body fat distribution with particular emphasis on VFM during pregnancy

4. To examine the hypothesis that metformin is most effective in those patients with the highest baseline insulin resistance and treatment with metformin throughout pregnancy will reduce the risk of gestational diabetes in this group of women.

3.4 Objectives of the study

1. To set up a randomised controlled trial in obese non-diabetic pregnant women with BMI ≥ 35 kg/m^2.
2. To obtain ethical approval and Clinical Trial Authorisation from Medicines and Health Regulatory Agency (MHRA).
3. To organise manufacture and packaging of placebo to match the metformin.
4. To obtain informed written consent of each participant.
5. To randomise recruited women to metformin or placebo.
6. To organise Oral glucose tolerance test (OGTT), fasting insulin and other blood tests at recruitment.
7. To measure the body composition of women at recruitment and repeat at 22 weeks, 28 weeks, 36 weeks and postnatal.
8. To record the results of the OGTT conducted at 28 weeks of gestation.
9. To record all adverse events in the participants.

10. To perform statistical analysis and compare the baseline characteristics, body composition, pregnancy and neonatal outcomes between the women randomised to metformin or placebo.
11. To calculate insulin resistance at booking and 28 weeks of gestation using the Homeostatic model assessment (HOMA) model.
12. To perform statistical analysis to compare the change of fasting insulin, insulin resistance, visceral fat and CRP at 28 weeks of gestation from baseline in the metformin and placebo groups.

3.5 Material and Methods

3.5.1 Ethical approval

I obtained the ethical approval from the London-Surrey Borders Research Ethics committee (REC no 08/H0806/80) (EudraCT no. 2008-005892-83) on 19th November 2008 (Appendix B.1). I obtained the Clinical Trial Authorisation (CTA) from the MHRA on 14th January 2009 (Appendix C.1). I registered the trial at clinicaltrials.gov (NCT01273584). I also obtained the Cranfield University Health Research Ethics Committee (CUHREC) approval on 05-07-2013 (Project Reference No. 24/13) (Appendix D.1).

I informed the subject's general practitioner of the intention to enrol a subject into the study. No study specific procedures was undertaken on any subject until that subject gave written informed consent. The study was conducted in accordance with the principles of Good Clinical Practice.

3.5.2 Inclusion Criteria

1. Obese pregnant women with BMI ≥ 35 kg/m^2
2. Gestation between 12 and 16 weeks
3. Age greater than 18 years.
4. Informed written consent

3.5.3 Exclusion Criteria

1. Pre-existing known diabetes or previous gestational diabetes
2. Presence of contra-indication to metformin (renal, liver, heart failure)
3. Moving out of study area for pregnancy management
4. Participants who suffer with hyperemesis
5. Multiple Pregnancy
6. Known sensitivity to metformin or its excipients

3.5.4 Study design

It was a randomised, prospective, double-masked, placebo controlled trial conducted at St Helier Hospital and 2 other NHS hospitals in the UK. All women attending the antenatal obesity clinic and fulfilling the eligibility criteria were invited to take part in the MOP trial. I discussed the trial with the prospective participants in detail, and gave a participant information sheet to patients who expressed an interest. 378 obese pregnant women were screened for eligibility. The women were given at least 24 hours to confirm participation. All women who agreed to participate in the trial provided written informed consent. 93 women were excluded due to various reasons like established diabetes or other systemic illness. All women received standardised personal advice on healthy eating and carbohydrate content of food, emphasizing low glycaemic index foods.

133 women attending the antenatal obesity clinic at the St Helier Hospital and fulfilling the eligibility criteria consented to take part in the study. Of these, 118 women completed the study. These 118 women recruited at St Helier Hospital formed the cohort for my study (Figure 3-1).

Before randomisation, I recorded the demographics, medical and obstetric history for each participant. Participants had baseline blood tests including OGTT, HbA_{1c}, fasting insulin and CRP measurements. I performed the body composition analysis by bioelectrical impedance using InBody^{TM}720 at baseline on all participants and repeated it at each visit.

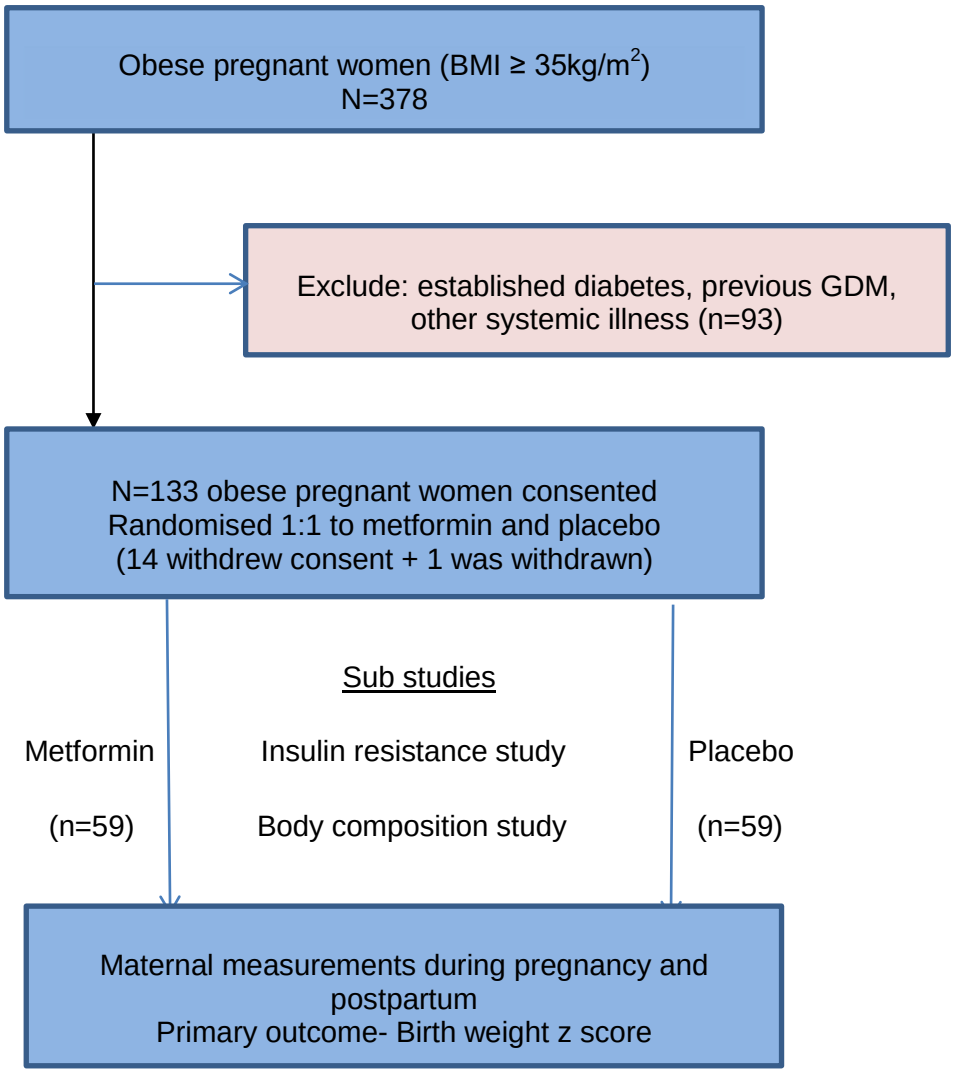


Figure 3-1: Flowchart for women participating in the MOP Trial

The flowchart shows that 133 obese pregnant women consented to the MOP trial and randomised 1:1 to metformin or placebo.

Randomisation and Trial medications

Eligible women were randomised in a 1:1 ratio to either active treatment with metformin or to placebo. The randomisation was computer generated by a statistician. The implementation of the random allocation sequence was carried out by the local pharmacist at the site, using sequentially numbered containers.

Both the participants and the clinical research team were blinded to the type of intervention. .

Composition of the placebo

The placebo tablets were manufactured to look identical to the metformin tablets by University College of London (UCL) Hospitals NHS Foundation Trust (Manufacturers License Number MIA (IMP) 17022).

The composition of the placebo tablets was

Lactose Ph Eur	84.25% w/w
Microcrystalline cellulose (Avicel ® PH 102)	15.00 w/w
Magnesium Stearate BP	0.75%

The UCL Hospitals pharmacy was also the central randomisation facility. Women in both groups were prescribed metformin/placebo on their first visit after randomisation. All women received standardised personal advice on healthy eating, emphasizing low glycaemic index foods, and were encouraged to undertake 30 minutes of physical activity daily.

Clinical Assessments

The schedule of clinical assessments is shown in Table 3-1.

Clinicians performing these assessments were blinded to treatment allocation. I saw all participants at 4-6 week intervals and assessed them clinically including weight, blood pressure, maternal assessment. Fetal assessment was done by a midwife. All women had their urine tested & recorded for proteins and ketones at every visit. I recorded the clinical data of the participants at each antenatal visit, delivery and neonatal outcomes for each participant. I monitored and recorded subject compliance with trial medications with history and tablet counts at each antenatal visit. I recorded the details of adverse events, if any, for each participant throughout the pregnancy. I also recorded overnight admissions to hospitals, if any in the case record forms.

Table 3-1 Schedule of visits for participants of the MOP Trial

Visit No	1 Screening BMI>35	2 Recruit	3	4	5	6	7	8
Week of gestation	10-12	12-16	20-22	26-28	32-34	36-38	Delivery/ Neonatal	Postnatal
Inclusion/ Exclusion	X							
Informed Consent		X						
Randomisation to Metformin/ Placebo		X						
Medical and obstetric History	X	X						
Blood Pressure, Urine, fetal check	X X	X X	X X	X X	X X	X X	X X	X
Body Composition	X	X	X	X	X	X		X
OGTT		X		X				X GDM+
Fasting Insulin		X		X				
CRP, HbA1c		X X	X	X		X		
Adverse Event			X	X	X	X	X	
Dispensing of Placebo/ Metformin		X	X	X	X	X		
Delivery and baby details							X	

All obese pregnant women with BMI ≥ 35kg/m^2 were screened for eligibility to participate in the trial. Inclusion and exclusion criteria were applied.

The dosing schedule of the trial medications is 500 mg twice a day in week 1 and thrice a day in week 2. In week 3, this was gradually increased to 1000 mg with breakfast and 500 mg with lunch and dinner. In week 4, the dose was further increased to 1000 mg with breakfast and dinner and 500 mg with lunch. Finally, in week 5, the women reached the maximum dose of 1000 mg of the trial medications with each meal and this dose was continued till the delivery of the baby.

I advised women to take metformin during meals to reduce the risk of gastrointestinal side-effects. In women unable to tolerate the full dose, the maximum tolerated dose was continued till the birth of the baby. I recorded this reduced dose in the case record form and created an adverse event form. Unblinding of treatment allocation was only permitted on the recommendation of the Data Safety Monitoring Committee where knowledge of the treatment was necessary for clinical reasons or for management of an adverse event.

I advised participants not to take metformin within 48 hours of having a general anaesthetic. If participants were to undergo an emergency operation, I would ensure that the anaesthetist was informed that they have been taking metformin.

I performed body composition analysis on all participants using the InBody^{TM}720 body composition analyser at booking and at each visit including the postnatal visit. The InBody^{TM}720 performed body composition analysis using Direct Segmental Multi-frequency Bioelectrical Impedance Analysis Method (DSM- BIA Method). I saw all women at 6-8 weeks postpartum and recorded their weight, blood pressure and general well-being. In patients who developed GDM, the OGTT was repeated postnatal to screen for persistent glucose intolerance or type 2 diabetes.

Blood Tests

All recruited women had blood tests at recruitment, 22 weeks, 28 weeks and 36 weeks of gestation. An OGTT and a baseline HbA_{1c} was performed in all women soon after randomisation. The results of this test were masked to

patients and clinicians. All women had a second OGTT at 28 weeks of gestation in line with standard practice for screening for GDM at St Helier Hospital. Trial medications were stopped for 1 week prior to the date of the test so as to exclude any influence on the OGTT results. The WHO 1999 criteria for diagnosis of GDM were used.

All women had fasting glucose and insulin measurements at baseline in order to assess insulin sensitivity. The serum was separated and frozen at -20^{o} C and stored for analysis of fasting insulin. Samples were batched and analysed together to avoid inter-assay error. In a subset of 43 patients, fasting insulin levels was repeated at 28 weeks to assess changes in insulin resistance. Insulin was measured by radioimmunoassay at the SAS Peptides Hormone Section, Royal Surrey County Hospital, UK with the Mercodia Iso-Insulin ELISA kit and I participated in these measurements. This is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation, insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to the micro-titration well. A simple washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with tetra methyl benzidine. The reaction is stopped by adding Sulphuric acid to give a colorimetric endpoint that is read spectrophotometrically. The lower limit of detection in this kit is 1mU/L. The intra-assay and inter-assay coefficient of variation at a concentration of 15.9 mU/L were 3% and 3.9% respectively.

I referred the women with abnormal results on the OGTT to a joint antenatal specialist diabetes clinic. They were given specialist advice by a dietitian and were taught home glucose monitoring. They were advised to continue the study medication and to continue home glucose monitoring till birth of the baby. If target blood glucose values are not achieved on dietary modifications, insulin was added to the study medications. Women with normal OGTT results continued with the trial medications as before. I reviewed the home glucose readings at periodic intervals throughout the pregnancy (Figure **3-2**)**.**

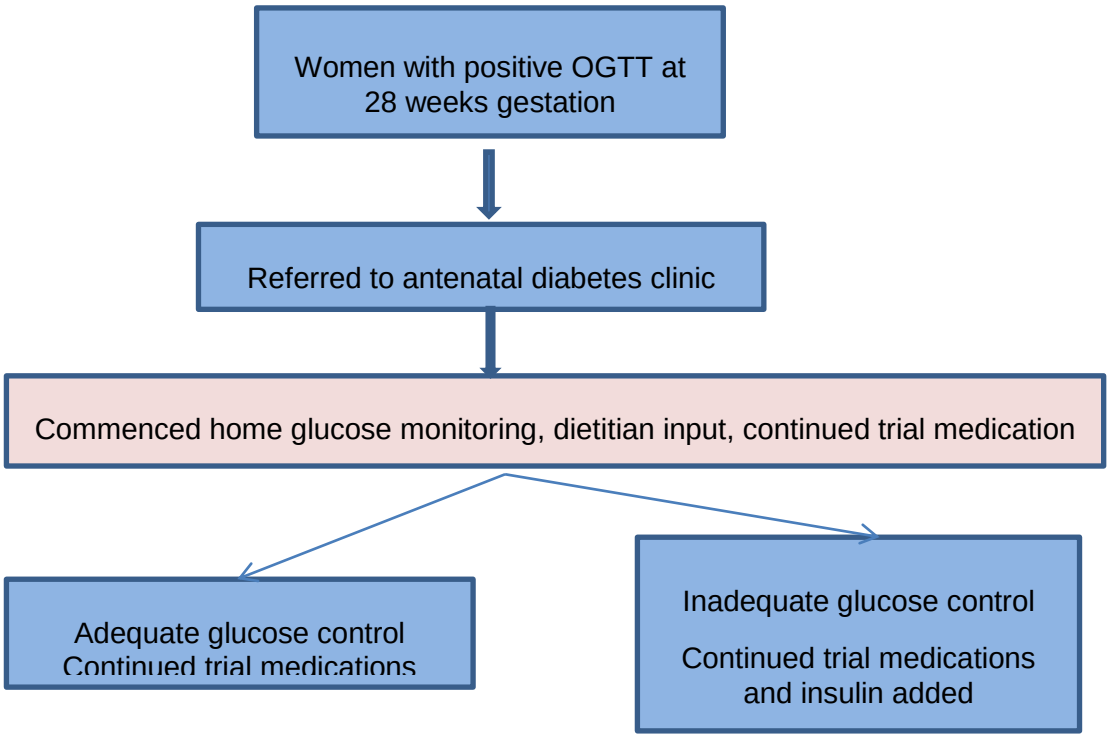


Figure 3-2: Flow chart for women who developed GDM

All women who developed GDM were referred to the antenatal diabetes clinic. They were seen by the dietitian and asked to start home glucose monitoring. If adequate glucose control was achieved, women continued trial medications as before. If glucose control was inadequate, insulin was added in addition to the trial medications.

I used the Homeostatic model assessment (HOMA) as a surrogate marker of insulin resistance. The HOMA model is derived from a mathematical assessment of the interaction between β cell function and insulin resistance in an idealised model that is then used to compute steady state insulin and glucose concentrations. The output of the model is calibrated to give normal beta cell function of 100% and normal insulin resistance of 1. The relationship between glucose and insulin in the basal state reflects the balance between hepatic glucose output and insulin secretion, which is maintained by a feedback loop between the liver and the β cells [156]. The hyperinsulinaemic-euglycaemic clamp method is the gold standard to measure insulin sensitivity but it is an expensive and time consuming. Hence I used HOMA-IR as a surrogate estimation of insulin sensitivity, based on fasting glucose and insulin. It correlates well with the clamp method [156]. However, HOMA is based on

fasting glucose and insulin concentrations and GDM, at least to begin with, is a postprandial disease and this needs to be considered. I calculated HOMA-IR using the following equation (Equation 3-1).

Equation 3-1: HOMA-IR

$$\text{HOMA-IR} = \frac{\text{Fasting Insulin (microU/L)} \times \text{Fasting glucose (mmol/L)}}{22.5}$$

Ultrasound Scans

All women participating in the study were scanned at 30 and 34 weeks of gestation to assess fetal growth. Additional growth scans were performed in those who develop GDM in line with the standard clinic protocol for GDM at St Helier Hospital.

Adverse events

Patients were advised to contact me or the midwife in the event of any new symptoms. I recorded any adverse event in the patient's notes and in the case record. Details recorded include the nature of the event, time of onset, severity, treatment needed and any relation to the trial medication. I reported all serious adverse events (SAE) to the sponsor immediately. Hospitalisation for the following reasons was not considered as Serious Adverse Events in this study.

1. Irregular uterine contractions requiring observation for < 24 hours.
2. Vaginal bleeding requiring observation for < 24 hours.
3. Show or Spontaneous Rupture of membranes

Any admission to the hospital during the pregnancy for greater than 24 hours was considered as a SAE.

Withdrawal of subjects

An obese pregnant women participating in the study was withdrawn from the study, if her fetal growth scan showed:
Estimated fetal weight (efw) < 5th centile

and

Either reduced end diastolic flow in the umbilical artery or

Oligohydramnios defined as amniotic fluid index < 2cm

Outcome measures

Primary outcome

The primary outcome measure was the median neonatal birth-weight z score (difference between observed and expected birth weight, with adjustment for gestational age, divided by the fitted standard deviation). The expected birth weight, corrected for gestational age, was derived from the local population of phenotypically normal neonates born alive at 24 weeks of gestation or later. [157].

Secondary outcomes

Maternal secondary outcome measures included maternal gestational weight gain (defined as the difference in maternal weight between the last antenatal visit and the day of randomisation), GDM, preeclampsia, pregnancy induced hypertension, delivery by Caesarean section, and postpartum haemorrhage, defined as blood loss of 1 litre or more.

Secondary neonatal outcomes included miscarriage at less than 24 weeks of gestation, stillbirth at 24 weeks or more, preterm birth at less than 37 weeks, a LGA neonate (birth weight > 90^{th} percentile adjusted for gestational age), birth trauma (shoulder dystocia, brachial plexus injury or fracture), Apgar score < 7 at 5 minutes, admission to level 2 or 3 neonatal unit, hypoglycaemia (plasma glucose levels < 2.6 mmol/l on two occasions at greater than 30 minutes apart), hyperbilirubinemia requiring phototherapy and respiratory distress defined as the need for more than four hours of respiratory support or oxygen.

3.5.5 Statistical Analysis

I performed the statistical analysis using R. Each continuous variable was tested individually for normality using the skewness and kurtosis test. The normally distributed data have been expressed as mean ± standard deviation.

Welch's t-test or Independent two samples t-test which is a parametric test was used to compare the means of two groups. Fisher's test was used to compare categorical variables and the level of significance was $P<0.05$. The association between variables in a normally distributed data was investigated with the Pearson's correlation coefficient.

Data which was not normally distributed has been expressed as median and interquartile range. The Mann-Whitney U test, which is a non-parametric test was used to explore the difference between the variables in the two groups with and without GDM and the level of significance was $P<0.05$. The association between variables in a data not normally distributed was investigated with the Spearman's rank correlation. Chi-square test or Fisher's test was used to compare categorical variables and the level of significance is $P<0.05$.

3.6 Results

The study period was October 2010-June 2015 at the St Helier University Hospitals NHS Trust. I assessed a total of 378 obese pregnant women without diabetes and with a BMI ≥ 35 kg/m2 and a singleton pregnancy for eligibility. 93 women were excluded for various reasons like established diabetes, previous GDM or other systemic illnesses. In the 285 eligible women, 133 women (46.6%) agreed to participate in the study. The obese women tend to attend their first antenatal visit later than normal weight women, but in spite of that, I managed to recruit women between 12 and 16 weeks of gestation. After randomisation 14 women withdrew their consent, 6 in the metformin group and 8 in the placebo group. In one of the patients, the results of the OGTT were inadvertently unblinded. This was a positive test and therefore the patient was excluded from the trial for ethical reasons. The remaining 118 women who completed the study formed the cohort of my study (Figure 3-1).

Data distribution

At the outset, I analysed the distribution of the continuous variables by performing the skewness test (Table 3-2).

Table 3-2: Tests of normality for maternal and neonatal characteristics

Variable	Minimum	Quartile 1	Median	Mean	Quartile 3	Maximum	Skew	Normality
Age (years)	21	29.2	33	32.5	36	43	-0	Yes
Weight at recruitment (kg)	75.7	96.5	104.9	107.7	114.7	170.5	1	Yes
HbA_{1c} at recruitment (mmol/l)	26	32	34	34.3	36	47	0.3	Yes
BMI at recruitment (kg/m^2)	34.1	36.7	38.9	40.4	42.7	62.1	1.6	No
Waist Hip Ratio at recruitment	0.92	0.98	1.0	1.01	1.04	1.5	3.06	No
Systolic BP at recruitment (mm of Hg)	80	110	117	117.1	123.3	158	0.3	Yes
Diastolic BP at recruitment (mm of Hg)	55	70	77	75.9	83	100	-0.3	Yes
Visceral fat mass at recruitment (units)	136.3	166.8	188	197.5	222.8	374.7	1.5	No
OGTT-1 Fasting BG (mmol/l)	3.7	4.2	4.5	4.5	4.8	6.4	0.7	Yes
OGTT-1 2-hour BG (mmol/l)	2.7	4.8	5.9	5.6	6.4	8.4	-0.2	Yes
OGTT-2 Fasting BG (mmol/l)	4.6	4.3	4.4	4.5	4.2	4.8	1.4	No
OGTT-2 2-hour BG (mmol/l)	3.4	4.8	5.6	5.8	6.9	10.0	0.6	Yes
Fasting insulin at recruitment pmol/l	24	73	94	105	133	253	0.9	Yes
HOMA-IR at recruitment (score)	0.42	1.29	1.68	1.88	2.39	4.5	0.9	Yes
CRP at recruitment (mg/l)	2.1	7.6	11.2	13.2	17.1	48.7	1.6	No
Fasting insulin at 28 weeks (pmol/l)	4.5	76.2	116	125	157	448	2.1	No
HOMA-IR at 28 weeks (score)	0.56	1.42	2.09	2.31	2.71	6.9	1.64	No
CRP at 28 weeks (mg/l)	1.3	5.9	10.4	13.0	16.7	53.9	1.6	No
VFM at term (units)	143.2	179.8	200.5	207.7	231.4	343.8	1.1	No
VFM postnatal (units)	125.6	163.6	183.3	192.6	216.7	354.2	1.4	No

Birth weight (grams)	1100	3141	3500	3494	3882	5040	-0.66	Yes

Some of the variables have a normal distribution while others have a non-normal distribution

Baseline characteristics

Among the 118 women who completed the study, 59 women were randomised to metformin and 59 to placebo at recruitment. Women allocated to metformin or placebos were similar in age, booking weight, BMI and baseline fasting and 2-hour glucose values during OGTT (Table 3-3).

Table 3-3: **Maternal baseline data: metformin and placebo groups**

	Metformin group n=59 Mean (± SD) [a] Median (IQR)[b]	**Placebo group n=59 Mean (± SD) [a] Median (IQR)[b]**	**P value**
Age (years)	33.3 (± 4.9)	32 (± 5.3)	0.2[a]
Weight (kg)	106.8 (± 15.5)	109.7 (± 18.2)	0.4[a]
BMI (kg/m^2)	39.4 (36.7-42.5)	38.1 (36.8-43.4)	0.8[b]
Baseline OGTT-fasting BG, mmo/l	4.5 (± 0.7)	4.6 (± 0.7)	0.4[a]
Baseline OGTT-2-hour BG, mmol/l	5.7 (± 1.4)	5.5 (± 1.3)	0.4[a]
HbA_{1c} ,mmol/mol	34.2 (± 10.9)	34.5 (± 7.2)	0.9[a]

[a] Independent two samples t-test, [b] Mann-Whitney U test, IQR-interquartile range. There was no significant difference between the maternal baseline characteristics between the metformin and placebo groups.

The ethnic distribution of women was not different between the groups. In the metformin group, 79.7% of the women were Caucasians, 11.9% were Asians and 8.4% Blacks. In the placebo group, 77.9% were Caucasians, 10.2% Asians and 11.9% Blacks (Table 3-4).The number of women with polycystic ovarian syndrome and hypertension were similar in both groups. There was no significant difference in the number of smokers in the metformin or the placebo group (P=0.7)

Table 3-4: **Maternal baseline characteristics: Statistical comparison between the metformin and placebo groups.**

	Metformin group (n=59)	Placebo group (n=59)	P value [a]
Ethnicity n (%)			
Caucasians	47 (79.7)	46 (77.9)	0.82
Asians	7 (11.9)	6 (10.2)	0.77
Blacks	5 (8.4)	7 (11.9)	0.8
History of Polycystic ovarian syndrome, n (%)	13 (22)	11 (18.6)	0.8
Hypertension, n (%)	7 (11.9)	5 (8.4)	0.8
Cigarette Smoking, n (%)	3 (5.1)	5 (8.5)	0.7

[a] Fisher's exact test. There was no between-groups difference in the ethnic distribution. There are no significant differences in number of women with history of polycystic ovarian syndrome and hypertension between the two groups.

The OGTT results showed, similar median fasting glucose (4.6 mmol/l (4.3-4.9) vs. 4.6 mmol/l (4.4-5.1), P=0.4) and similar mean 2-hour glucose values (5.9 ± 1.6 mmol/l vs. 5.8 ± 1.9 mmol/l, P=0.8) in women who received metformin or placebo (Table 3-5).

Table 3-5: **28-week Oral Glucose Tolerance Test results**

	Metformin n=59 Mean (± SD) [a] Median (IQR) [b]	Placebo n=59 Mean (± SD) [a] Median (IQR) [b]	P Value
OGTT-fasting glucose (mmol/l)	4.6 (4.3-4.9)	4.6 (4.4-5.1)	0.4
OGTT- 2-hour glucose (mmol/l)	5.9 (±1.6)	5.8 (±1.9)	0.8

[a] Independent two samples t-test, [b] Mann-Whitney U test, IQR- interquartile range. There is no significant difference between the fasting and 2-hour glucose values between the metformin and placebo groups.

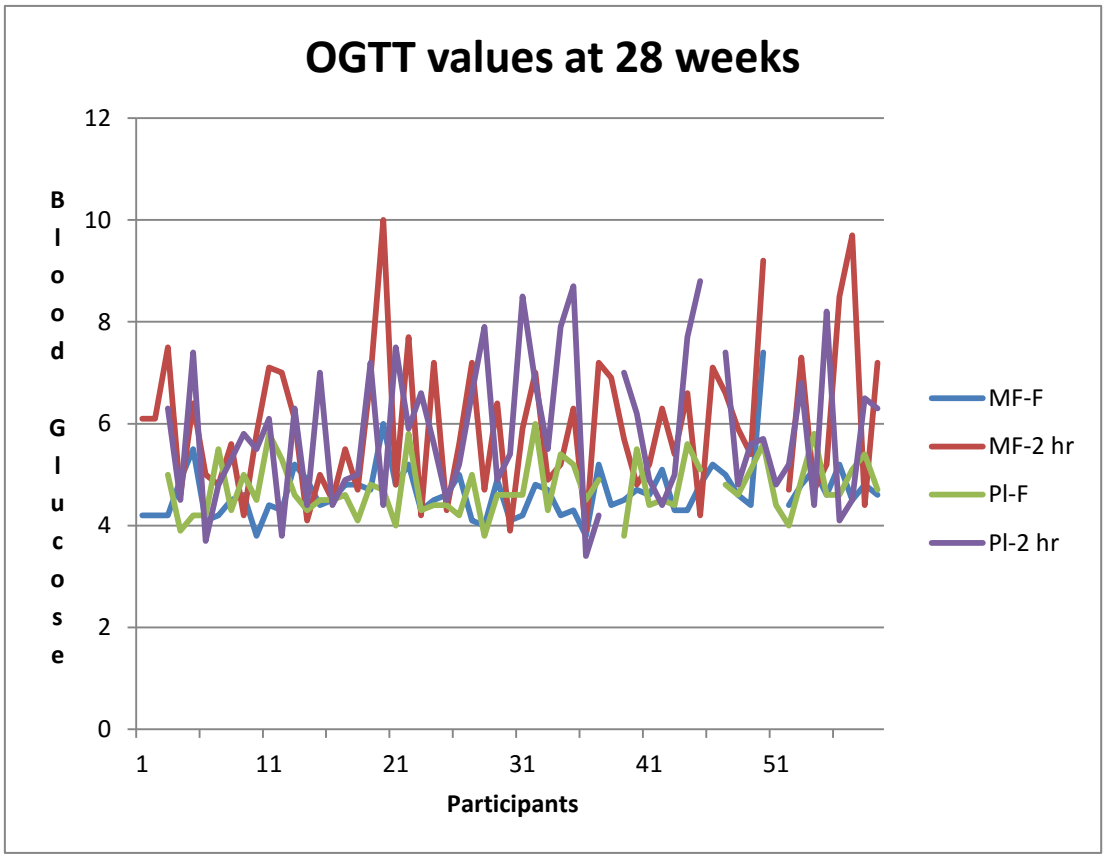


Figure 3-3: Fasting and 2-hour glucose values in the metformin and placebo groups at 28 weeks

MF-F – Fasting glucose in the metformin group, MF-2 hr – 2 hour glucose in the metformin group, Pl-F – Fasting glucose in the placebo group, Pl-2 hr – 2 hour glucose in the placebo group.

At baseline, women in the two study groups had similar BMI, WHR, PBF and visceral fat mass (Table 3-6). Those allocated to metformin had considerably less weight gain during pregnancy (3.9 ± 4.6kg vs. 7.0 ± 4.5 kg, P=0.0003). There was a trend for women in the metformin group to have a lower median visceral fat at term as compared to the placebo group (199 units [180-229 units] vs. 202 units [180-230 units], P=0.97) (Table 3-6). A similar trend was also seen at 6 weeks postnatal (181.8 units [155-219 units] vs. 185.8 units [168-213 units], P=0.4). However, this difference is not statistically significant

Table 3-6: **Changes in maternal body composition at 28 weeks- statistical comparison between the metformin and placebo groups**

	Metformin group n=59 Mean (± SD) [a] Median (IQR) [b]	Placebo group n=59 Mean (± SD) [a] Median (IQR) [b]	P value
Baseline Body Mass Index (kg/m^2)	39.4 (36.7-42.5)	38.1 (36.8-43.4)	0.8[b]
Baseline Waist-Hip ratio	1.01 (0.99-1.04)	0.99 (0.96-1.04)	0.09[b]
Baseline Visceral fat mass (units)	189.2 (171-226)	186.8 (164.8-211.9)	0.4[b]
Baseline total percentage body fat	49.9 ± 3.3	48.9 ± 4.07	0.2[a]
Visceral fat mass at term (units)	198.8 (179.8-229.1)	201.5 (180.4-229.6)	0.97[b]
Visceral fat mass Postnatal (units)	181.8 (154.5-218.8)	185.8 (167.9-213.1)	0.4[b]
Gestational Weight Gain (kg),	3.9 ± 4.6	7 ± 4.5	<0.05[a]

[a] Independent two samples t-test [b] Mann-Whitney U test, IQR-interquartile range. There was no significant difference in baseline BMI, WHR, VFM and PBF between the metformin and placebo groups. There was also no significant difference in the VFM at term and postnatal between the two groups. However, the GWG was significantly lower in the metformin group was compared to the placebo group.

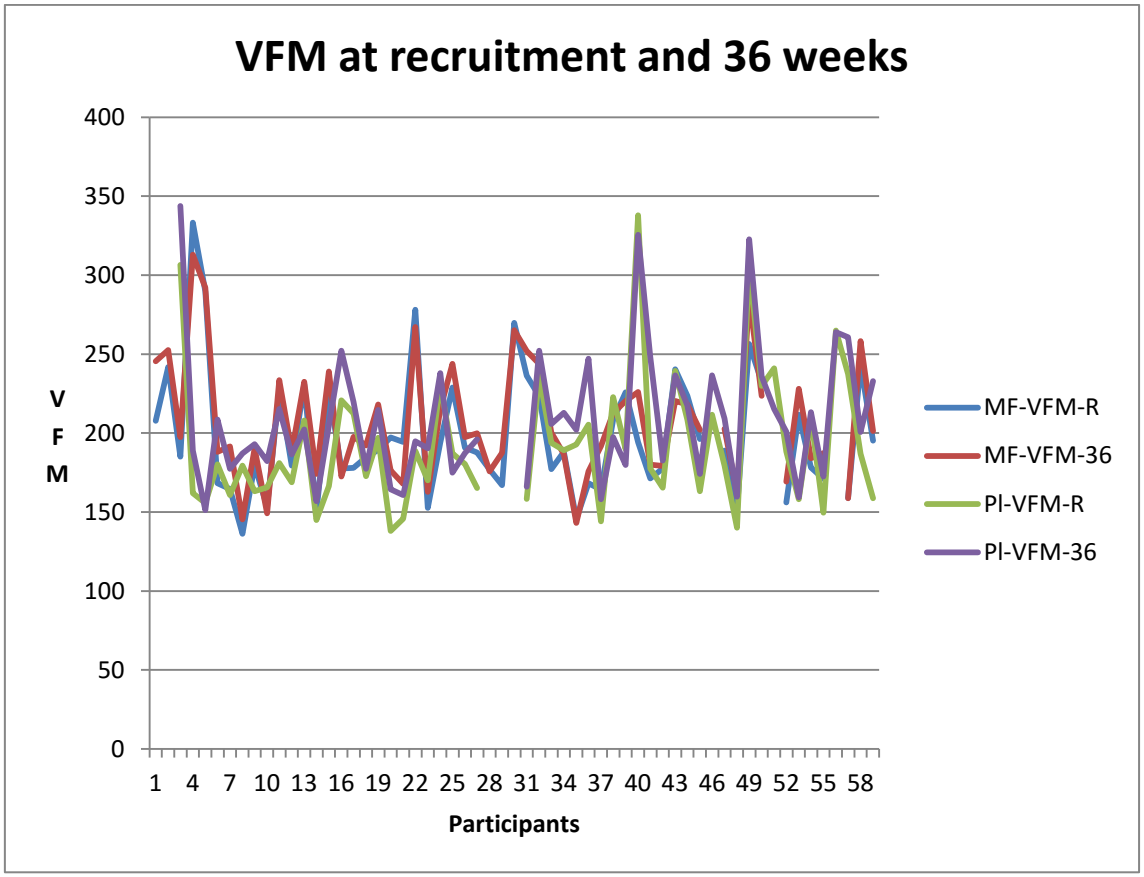


Figure 3-4: Visceral fat mass at recruitment and 36 weeks in the metformin and placebo groups

MF-VFM-R - VFM in the metformin group at recruitment, MF-VFM-36 - VFM in the metformin group at 36 weeks, Pl-VFM-R - VFM in the placebo group at recruitment, Pl-VFM-36 - VFM in the placebo group at 36 weeks. A trend is seen for women in the metformin group to have a lesser increase in visceral fat mass at term.

At baseline, women in the two study groups had similar fasting insulin levels, HOMA- IR scores and CRP levels (Table 3-7). We could only repeat the fasting insulin measurements in a small subset of patients (25 patients on metformin and 18 patients on placebo) due to financial constraints. Those allocated to metformin showed lower fasting insulin at 28 weeks compared to placebo (95 pmol/l [71-121] vs. 158.5 pmol/l [102.8-195], P=0.009). Similarly, those allocated to metformin also showed lower HOMA-IR score at 28 weeks compared to placebo (1.98 score [1.29-2.2] vs. 2.81 score [1.92- 3.72], P=0). There was a trend towards a lower CRP at 28 weeks in the metformin group as

compared to the placebo group. (9.4 mg/l [5.6-14.4] vs. 11.7 mg/l [7.4-21.9], P=0.1). However, the difference was not significant.

Table 3-7: **Changes in fasting insulin, insulin resistance and C-reactive protein at 28 weeks- statistical comparison between the metformin and placebo groups**

	Metformin group (n=59) Mean (± SD) [a] Median (IQR) [b]	Placebo group (n=59) Mean (± SD) [a] Median (IQR) [b]	P value
Baseline fasting insulin (pmol/l),	106.1(± 56.2)	105.3 (± 53.2)	0.9[a]
Baseline insulin resistance (HOMA-IR score),	1.88 (± 0.95)	1.88 (± 0.91)	1.00[a]
Baseline C-reactive protein(mg/l),	10.85 (7.1-16.6)	11.8 (7.9-18)	0.4[b]
Fasting insulin at 28 weeks (pmol/l),	(n=25) 95 (71-121)	(n=18) 158.5 (102.8-195)	<0.05[b]
Insulin resistance at 28 wks (HOMA-IR score),	1.98 (1.29-2.2)	2.81 (1.92-3.72)	0.03[b]
C-reactive protein at 28 weeks (mg/l),	9.4 (5.6-14.4)	11.7 (7.4-21.9)	0.1[b]

[a] Independent two samples t-test, [b] Mann-Whitney U test, IQR- interquartile range. The baseline fasting insulin, insulin resistance and C-reactive protein were not significantly different in the two groups. There was a significant rise in the fasting insulin and insulin resistance at 28 weeks in the placebo group as compared to the metformin group. The rise in the CRP at 28 weeks was not significantly different in the metformin and placebo group.

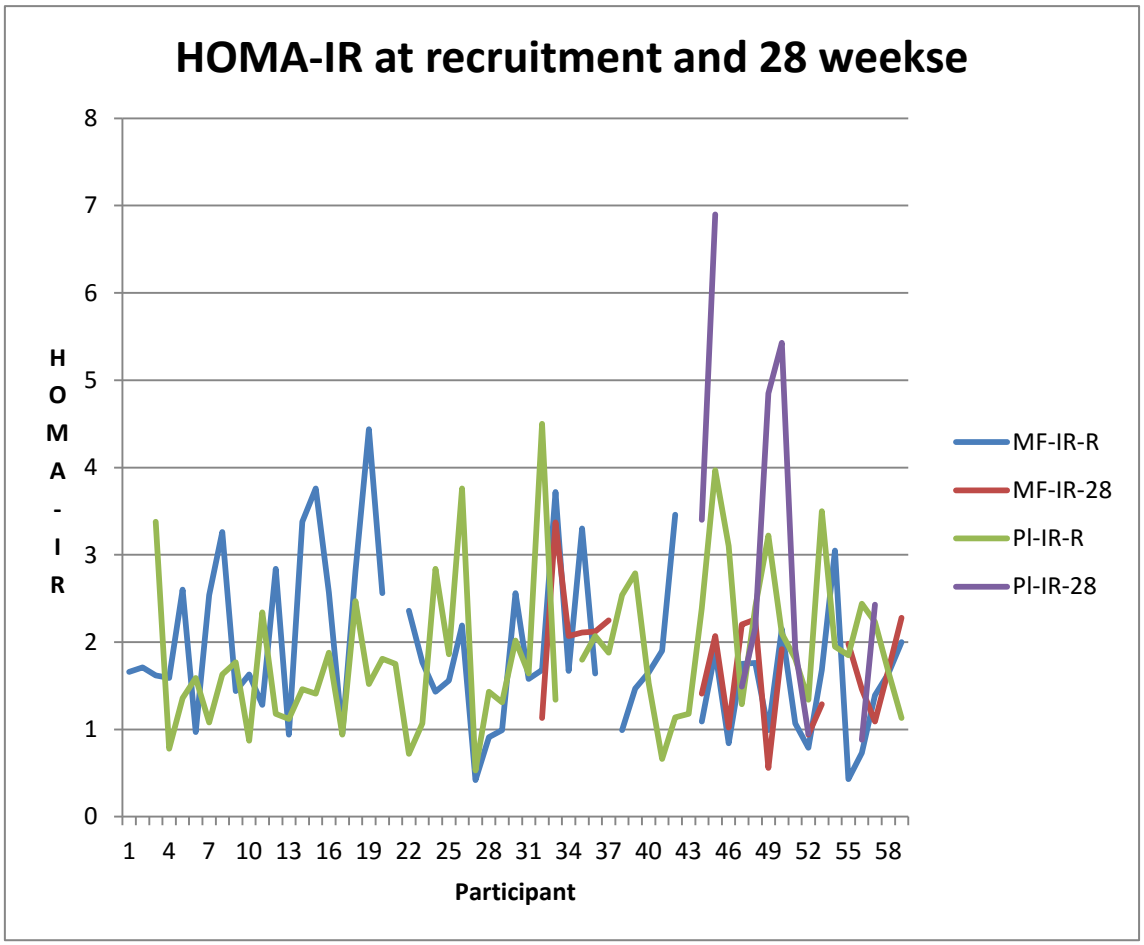


Figure 3-5: Insulin resistance (HOMA-IR) at recruit and at 28 weeks in the metformin and placebo groups

MF-IR-R – HOMA-IR in the metformin group at recruitment, MF-IR-28 – HOMA-IR in the metformin group at 28 weeks, Pl-IR-R – HOMA-IR in the placebo group at recruitment, Pl-IR-28 – HOMA-IR in the placebo group at 28 weeks. Women in the metformin group showed a lesser increase in HOMA-IR at 28 weeks from recruitment as compared to the placebo group.

Table 3-8 shows independent samples t-test for differences in means between those on metformin and those on placebo. The change in values between 28 weeks and baseline for fasting insulin, insulin resistance (HOMA-IR), CRP, VFM at term and postnatal, was calculated in both the metformin and placebo groups. The mean of the changes for all these variables was determined for each group. The difference in mean change (28 weeks – baseline) for fasting insulin between the metformin and the placebo group is - 47.8 (P=0.007) and

for insulin resistance was -.76 (P=0.04). There was a no significant difference in change in CRP at any of the measurement time points. There was a significant difference in mean change in VFM at term (-6.9, P=0.03) and postnatal (-8.2, P=0.01), compared to baseline between the two groups. There was a 50.5% increase in fasting insulin concentration and a 49.4% rise in insulin resistance from booking to 28 weeks of pregnancy in the group of women receiving placebo. However, there was only a 5.3% rise in insulin resistance at 28 weeks in the metformin group.

Table 3-8: **t-test for equality of means- Difference in fasting insulin, insulin resistance, VFM and CRP at 28 weeks to baseline compared between treatments**

	t-test for Equality of Means			
	Significance (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
			Lower	Upper
Change in fasting insulin (28wks – baseline)	<0.05	-47.8	-81.5	-14.1
Change in insulin resistance (28wks – baseline)	0.04	-.76	-1.46	-.05
Change in CRP (28 weeks-baseline)	0.1	-2.68	-5.98	.62
Change in VFM (term-baseline)	0.03	-6.9	-13.09	-.77
Change in VFM (postnatal-baseline)	0.01	-8.2	-14.63	-1.82

Mean difference – average difference between readings, Sig (2 tailed) – equivalent to P value (<0.05 denotes significant difference), 95 % Confidence interval of the difference – range within which true mean difference lies (95% confident)

Pregnancy induced hypertension and preeclampsia were not significantly different in the two groups. Similarly, the rates of CS were also not significantly different in the groups (44.1% in the metformin group vs. 40.7% in the placebo group; P=0.8). There was a trend towards a lower incidence of GDM in the metformin group as compared to the placebo group. However, the difference

did not reach statistical significance (6 [10.2%] versus 11[18.6%], P=0.3) (Table 3-9).

Table 3-9: **Maternal pregnancy outcomes - statistical comparison between metformin and placebo groups**

	Metformin (n=59)	Placebo (n=59)	P value [a]
Pregnancy induced hypertension n, (%)	5 (8.5)	4 (6.8)	0.72
Preeclampsia n, (%)	0	3 (5)	0.2
Mode of delivery n, (%) **Vaginal** **Instrumental** **Elective C/section** **Emergency C/section**	 31 2 9 17	 31 4 13 11	 1.0 0.4 0.5 0.3
Gestational Diabetes, n (%)	6 (10.2)	11(18.6)	0.3

[a] Fisher's exact Test. PIH, PET were not significantly different between groups. There was also no significant difference in the number of women delivering by Caesarean section between the two groups. Lesser women in the metformin group developed GDM as compared to the placebo group although this difference did not reach statistical significance.

There was a trend towards higher baseline HOMA-IR in the 16 women who developed GDM than in women without GDM (1.82 [1.4-2.5] vs. 1.68 [1.3-2.4], P=0.6). However, this difference did not reach statistical significance (Table 3-10).

Table 3-10: **Baseline HOMA-IR in women with GDM and no GDM**

	GDM (n=16) Median (IQR)	No GDM (n=98) Median (IQR)	P value [a]
Baseline HOMA-IR	1.82 (1.4-2.5)	1.68 (1.3-2.4)	0.6

IQR-interquartile range, [a] Mann-Whitney U test.

Five women in the metformin group and 4 women in the placebo group developed pregnancy-induced hypertension. Three women in the placebo group

developed preeclampsia compared to none in the metformin group (P=0.2). The baseline HOMA-IR was similar in those developing preeclampsia compared to those who did not (1.63 units [1.5-2.6] vs. 1.69 units [1.3-2.4], P=0.5). There was a trend towards a higher GWG in women who developed preeclampsia, compared to women who did not, but the difference was not significant (10.8 kg [7.1-12.8] vs. 6.8 kg [4-8.7], P=0.3) (Table 3-11).

Table 3-11: **HOMA-IR in women with preeclampsia**

	Preeclampsia (n=3) Median (IQR)	**No Preeclampsia (n=111) Median (IQR)**	**P value [a]**
HOMA-insulin resistance, units	1.63 (1.5-1.6)	1.69 (1.3-2.4)	0.5
Gestational weight gain, kg	10.8 (7.1-12.8)	6.8 (4-8.7)	0.3

IQR-interquartile range, [a] Mann-Whitney U test.

There was no significant difference between the metformin group and the placebo group in the median neonatal birth-weight z score. The distribution of the baby birth weight was normal and hence is presented as mean ± SD. The mean birth weight in the two groups were not significantly different (3421± 564 g vs. 3374 g ± 876; P=0.7). There was no significant difference in the mean birth centile between the two groups (52.3 ± 30.8 vs. 53.0 ± 30.4; P=0.8). Similarly, the percentage of large for gestational age babies (LGA) was similar in both groups (20.3% vs. 18.6%, P=1). There was also no significant difference in the rate of admission to neonatal care units (5.1% vs. 3.4%, P=1), neonatal jaundice (3.4% vs. 5.1%, P=1), neonatal hypoglycaemia (5.1% vs. 3.4%, P=1) and shoulder dystocia (0% vs. 0%; P=1) in both the groups (Table 3-12).

Table 3-12: **Neonatal outcomes-statistical comparison between the metformin or placebo groups**

	Metformin group (n=59) Mean (± SD) [a] Median(IQR) [b]	Placebo group (n=59) Mean (± SD) [a] Median(IQR) [b]	P value
Median birth-weight z score (IQR)	0.0 (-0.7 - 0.7)	0.1(-0.5 - 0.8)	0.9 [b]
Birth-weight (gram), mean ± SD	3421 ± 564	3374 ± 876	0.7 [a]
BW Centile, mean ± SD	52.3 ± 30.8	53.0 ± 30.4	0.8 [a]
BW centile > 90, n (%)	12 (20.3)	11(18.6)	1[c]
BW centile < 10, n (%)	6 (10.2)	10(16.9)	0.4 [c]
Preterm < 37 weeks, n (%)	4(6.8)	4(6.8)	1.0[c]
Jaundice requiring phototherapy, n (%)	2(3.4)	3(5.1)	0.65[c]
Hypoglycaemia [d], n (%)	3(5.1)	2(3.4)	0.65[c]
Neonatal unit admissions, n (%)	3(5.1)	2(3.4)	0.65[c]
Shoulder dystocia, n (%)	0	0	1.0[c]

[a] Independent two samples t-test, [b] Mann- Whitney U test, [c] Fisher's exact test, [d] Capillary glucose <2.6mmol/l . There was no significant difference in any of the neonatal outcomes.

I stratified the women into two groups according to the baseline insulin resistance, those with baseline insulin resistance ≥ 75th percentile and others with insulin resistance < 75th percentile. In the metformin group, only 1 women of the 15 with baseline insulin resistance ≥ 75th percentile developed GDM. In the placebo group, 4 of the 9 women with baseline insulin resistance ≥ 75th percentile developed GDM. In the group of women with baseline insulin

resistance < 75th percentile, there was not much difference in the incidence of GDM between the groups (Table 3-13).

When comparing the risk of GDM in the placebo group alone, stratified by HOMA IR, four out of 9 women (44.4%) with baseline HOMA-IR ≥ 75th percentile developed GDM, whereas, only six of the forty eight women (12.5%) with HOMA IR ≤ 75th percentile developed GDM.

Table 3-13: **GDM incidence according to baseline insulin resistance in the metformin and placebo groups**

	Metformin (n=59)	**Placebo (n=59)**
Baseline insulin resistance > 75th percentile, n (%)	15 (26.8)	9 (15.8)
developed GDM, n (%)	1(6.6)	4 (44.4)
Baseline insulin resistance < 75th percentile, n (%)	41 (73.2)	48 (84.2)
developed GDM, n (%)	5 (12.2)	6 (12.5)

Only 1 woman with baseline insulin resistance ≥ 75th percentile randomised to metformin developed GDM out of 15 women compared to 4 women from the placebo group developing GDM out of 9 women in the group with baseline insulin resistance < 75th percentile.

I calculated the odds ratio to estimate the risk of GDM. In the group with baseline insulin resistance ≥ 75th percentile, the OR was 0.09; this suggests that the metformin group are much less likely to develop GDM than the placebo group. However, the 95% confidence interval puts the risk somewhere between 0.008 and 1.0016, indicating that the difference in risk between the two groups is not statistically significant (Table 3-14).

Table 3-14: Estimates of relative risk of GDM between metformin and placebo groups in women with baseline insulin resistance ≥ 75th percentile

Estimates of the Relative Risk (Row1/Row2)			
Type of Study	**Value**	**95% Confidence Limits**	
Case-Control (Odds Ratio)	**0.09**	0.0080	1.0016

The odds ratio between the 2 groups was 0.09 suggesting that women randomised to metformin are less likely to develop GDM. However, this was not statistically significant.

In the group with baseline insulin resistance group < 75th percentile, the OR is 0.97. This suggests that the metformin group are more or less just as likely to develop GDM as the placebo group. The 95% confidence interval puts the risk somewhere between 0.27 and 3.45, indicating that the difference in risk between the two groups is not statistically significant (Table 3-15).

Table 3-15: **Estimates of relative risk of GDM between metformin and placebo groups in women with insulin resistance < 75th percentile**

Estimates of the Relative Risk (Row1/Row2)		
Type of Study	**Value**	**95% Confidence Limits**
Case-Control (Odds Ratio)	**0.97**	**0.2737-3.4535**

The odds ratio between the 2 groups is 0.97 suggesting that women randomised to metformin were more or less just as likely to develop GDM.

Similarly, when both groups, women with insulin resistance > 75th percentile and women with insulin resistance < 75th percentile, were considered together the OR was 0.54. This suggests that the metformin group are less likely to develop GDM than the placebo group, but the 95% confidence interval puts the risk somewhere between 0.19 and 1.55, indicating that the difference in risk between the two treatment groups was not statistically significant (Table 3-16).

Table 3-16: **Estimates of relative risk of GDM between metformin and placebo groups in women when both groups were considered together**

Estimates of the Common Relative Risk (Row1/Row2)			
Type of Study	**Method**	**Value**	**95% Confidence Limits**
Case-Control (Odds Ratio)	**Mantel-Haenszel**	**0.54**	**0.1876- 1.5515**

The odds ratio between the 2 groups was 0.5394 suggesting that women randomised to metformin were less likely to develop GDM. However, the 95% confidence interval is between 0.19 and 1.55, which was not statistically significant.

I used the Breslow–Day test to compare the risks of GDM in the two groups, but the difference was not statistically significant at 5% (P=0.07) (Table 3-17).

Table 3-17: **Breslow-Day test for homogeneity of the Odds Ratios**

Breslow-Day Test	
Chi-Square	3.2831
DF	1
Pr > ChiSq	0.07

The graph below (Figure 3-6) also indicates that there was no significant difference in the risk of developing GDM in patients taking metformin between the high and low insulin groups, as confidence intervals for the risks overlap.

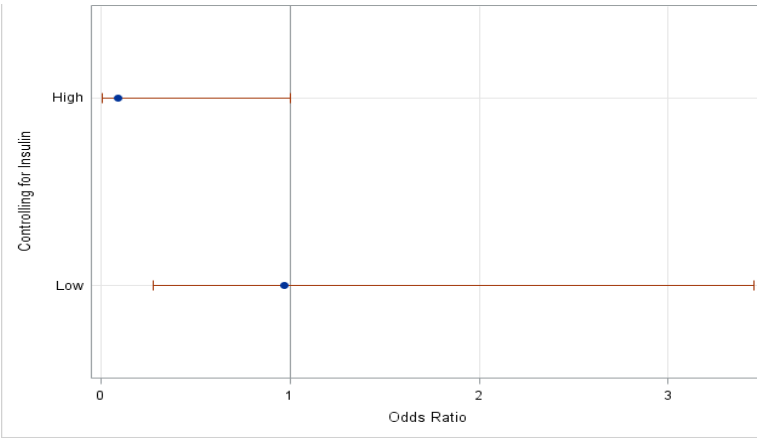


Figure 3-6 Odds ratio of developing GDM with 95% confidence limits in the high and low insulin resistance groups

The confidence intervals for the risk of developing GDM overlapped between the high insulin resistance group and the low insulin resistance group indicating there was no significant difference in the risk of developing GDM between the two groups

The GWG was significantly reduced in women receiving metformin (3.9 kg vs. 7 kg, P<0.05). When stratified by baseline insulin resistance, GWG was significantly reduced by metformin in women with HOMA-IR greater than 75th percentile (3.1 ± 0.6 kg vs. 6.6 ± 2.5 kg, P<0.05) and also in those with baseline HOMA-IR values less than 75th percentile (4.6 ± 4.5 kg vs. 6.9 ± 4.9 kg, P=0.02). No correlation was found between GWG and HOMA-IR in the metformin (r=-0.15) and placebo (r=-0.15) groups.

The result of baseline HOMA-IR ≥ 75 percentile were significantly more likely among those who subsequently developed GDM, compared with women who did not develop GDM in the placebo group. The odds ratio was 5.7 (95% confidence interval 1.2-27.5), p=0.02 (Table 3-18).

However, there was no such association seen with pregnancy-induced hypertension and preeclampsia. The number of patients with HOMA-IR ≥ 90th percentile was very small (n=5). Of these 5 women, one developed GDM and PIH and another women developed preeclampsia.

Table 3-18: Baseline HOMA-IR and risk of subsequent GDM, PIH and PET in the placebo group

			Odds ratio (95%CI)	P value
Baseline HOMA IR ≥ 75th percentile, n=9	GDM, %[a] 40	No GDM, %[b] 10.4	5.7 (1.2-27.5)	0.02
	PIH, %[c] 25	No PIH, %[d] 14.8%	1.9 (0.17-20.8)	0.5
	PET, %[e] 0	No PET, %[f] 16.3%	0.7 (0.03-14.7)	0.8

[a] n=10 women who developed GDM in the placebo group

[b] n=48 women who did not develop GDM in the placebo group

[c] n=4 women who developed PIH in the placebo group

[d] n=54 women who did not develop PIH in the placebo group

[e] n=3 women who developed PET in the placebo group

[f] n=55 women who did not develop PET in the placebo group

Adverse Events

There was no significant difference in the incidence of SAE between the groups, but the incidence of side effects like nausea, vomiting and diarrhoea were higher in the metformin group as compared to the placebo group. Eleven patients receiving metformin and four receiving placebo complained of nausea and vomiting. Similarly, more patients in the metformin group complained of diarrhoea (nine patients) as compared to the placebo group (two patients). One patient from the metformin group was admitted overnight with diarrhoea (Table 3-19). In three patients, one in the metformin group and two in the placebo group, fetal scan showed fetal growth restriction with estimated fetal weight < 5th percentile and abnormal fetal Doppler studies. The trial medications were stopped in these patients as per protocol guidelines.

Table 3-19: Comparison of side-effects in the metformin and placebo groups

Side effects	Metformin	Placebo	P value
At least one side effect, n (%)	27 (45.8)	10 (16.9)	<0.05
Nausea and vomiting, n (%)	11 (18.6)	4 (6.8)	0.09
Diarrhoea, n (%)	9 (15.3)	2 (3.4)	0.05
Abdominal pain or heartburn, n (%)	4 (6.8)	4 (6.8)	1.0
Consequences of side-effects			
Stopped tablets, n (%)	2 (7.4)	2 (20)	0.3
Reduced dose, n (%)	15 (55.6)	5 (50)	0.76
Continued dose, n (%)	10 (37)	3 (30)	0.99

[a] Fishers exact test

Adherence to the study regimen was assessed as good (> 50% of tablets taken) in 97 (82.2%) of the 118 participants and poor (< 50% of tablets taken) in 21 (17.8%). The adherence was poor in 10 women receiving metformin and 11 women receiving placebo. The maximum daily tolerated dose of metformin/placebo was 3 g. There was no significant difference in degree of compliance between subjects receiving metformin or placebo. The percentage of women taking ≥ 2500 mg of metformin per day was an overall 88.1%.

There was no significant difference in the anthropometric measurements at birth in the neonates of mothers receiving metformin or placebo (Table 3-20).

Table 3-20: Comparison of neonatal anthropometric measurements between the metformin and placebo groups.

Measurements	Metformin Median (IQR)	Placebo Median (IQR)	P value[a]
Head circumference, cm (n=104)	35 (34-35.8)	34.5 (33-35)	0.1
Chest circumference, cm, (n=104)	33.5 (32-35)	34 (32.8-35)	0.5
Abdominal circumference, cm, n=104)	32 (30-33.5)	32 (30-34)	0.9
Length, cm,(n=104)	51 (49.5-53)	52 (49.5-54)	0.3
Crown-rump length, cm, (n=56)	31 (29-33)	31.5 (30-33)	0.3
Biceps skin fold, mm, (n=56)	11 (10-11.5)	11 (10-12)	0.06
Triceps skin fold, mm, (n=56)	5.4 (4.6-6.2)	5.3 (4.9-5.9)	0.8
Subscapular skinfold, mm, (n=56)	5 (4.4-6)	5.6(4.9-6.1)	0.5

[a] Mann-Whitney U test, There is no difference in the neonatal anthropometric measurements between the two groups.

3.7 DISCUSSION

The results of the study showed that in obese, non-diabetic pregnant women with BMI ≥ 35 kg/m^2, treatment with metformin did not reduce the median neonatal birth-weight z score or the incidence of LGA neonates. However, metformin therapy reduced GWG, reduced the rise in VFM during pregnancy, and attenuated the expected physiological rise in insulin resistance seen at 28 weeks of gestation.

Only 10.2% of the women in the metformin group developed gestational diabetes compared with 18.6% in those allocated to placebo, but this difference was not significant. Therefore, even though metformin blunted the rise in insulin resistance at 28 weeks, the incidence of GDM was not significantly reduced. This is probably because some of these women with a high BMI > 35 kg/m^2 are relatively resistant to the insulin sensitising effects of metformin. Studies in women with polycystic ovaries have shown that those who develop GDM are more hyperinsulinemic and more insulin resistant [158]. There was a trend for a higher baseline insulin resistance in women who developed GDM as compared to no-GDM women but again this difference was not significant. This perhaps suggests that metformin initiation at 12 weeks of gestation in obese women may be a late start and the adverse effects of obesity have already begun. Also, the study was not adequately powered for detection of the difference in rate of gestational diabetes between groups.

Women receiving metformin had a significantly lower increase in visceral fat compared with those on placebo. Also, the effects of metformin persisted into the post-partum period as reflected by the greater reduction in visceral fat and lower VFM after delivery in this group. This effect may be potentially beneficial in reducing the risk for future T2DM and cardiovascular disorders in these women. Visceral fat is associated with insulin resistance and the metabolic abnormalities associated with obesity, known as the 'metabolic syndrome' [111]. Visceral fat is also associated with subclinical inflammation and markers of inflammation including CRP are reported to be higher in obese pregnant women compared to controls [159]. There was a trend towards a lower CRP at 28 weeks in the metformin group as compared to the placebo group, but this difference was not statistically significant.

There was no significant difference in the incidence of pregnancy-induced hypertension between the two groups. Three women developed preeclampsia in the placebo group compared to none in the metformin group. The baseline insulin resistance in the group of women developing PIH or preeclampsia was similar to the women who did not develop PIH or preeclampsia. There was a

trend towards a higher GWG in women who developed preeclampsia as compared to women who did not. The greater GWG could also contribute to the increased incidence of preeclampsia. Previous studies have shown that the prevalence of preeclampsia increased, both with increasing pre pregnancy BMI and increasing gestational weight gain [88][160]. The decrease in GWG seen in women on metformin could be multifactorial and is closely related to decreased food intake [161].

No significant differences were found comparing metformin or placebo treated patients in relation to mode of delivery, neonatal jaundice, neonatal hypoglycaemia, admission to SCBU, shoulder dystocia.

One of the aims of the study was also to determine whether there is an association between baseline insulin resistance and adverse pregnancy outcomes such as gestational diabetes, pregnancy-induced hypertension and preeclampsia. When comparing the risk of GDM stratified by HOMA IR in the placebo group alone, four out of 9 women (44.4%) with baseline HOMA-IR ≥ 75^{th} percentile developed GDM whereas only six of the forty eight women (12.5%) with HOMA IR ≤ 75^{th} percentile developed GDM (P=0.04). Also, there was a significant association with baseline HOMA-IR ≥ 75^{th} percentile and subsequent GDM [Odds Ratio (CI), 5.7 (1.2-27.5)]. This suggests that women with high baseline insulin resistance have a higher risk of developing GDM. There was no such association of high baseline insulin resistance with PIH or preeclampsia in the placebo group. However, the overall rate of preeclampsia is very low in our study and hence it is difficult to comment.

The study also aimed to examine the hypothesis that metformin is most effective in those patients with the highest baseline insulin resistance and treatment with metformin throughout pregnancy will reduce the risk of gestational diabetes in this group of women. When stratified by insulin resistance, there was a trend towards a lower risk of GDM for women in the high IR group receiving metformin as compared to placebo. One of the fifteen women with high HOMA-IR randomised to metformin developed GDM compared with four of the nine with high HOMA-IR randomised to placebo.

However, there was no statistically significant difference between the groups. Therefore, the hypothesis that metformin is most effective in women with highest baseline insulin resistance in preventing GDM, is not substantiated by the study results.

There was no significant difference in the incidence of SAE between the groups but the incidence of side effects like nausea, vomiting and diarrhoea was higher in the metformin group as compared to the placebo group. Despite this, adherence to the study regimen was assessed as good in 82.2% of the participants.

There was also no difference in the neonatal anthropometric measurements at birth in both the groups. The MiG TOFU study has shown a more favourable pattern of fat distribution in two year old children of mothers who had received metformin in pregnancy [127]. However, in that study, the comparison was between neonates of mothers randomised to metformin or insulin. There was no long term follow up of the babies born to mothers who participated in the MOP Trial.

Genetic polymorphisms in drug uptake transporter genes have been increasingly recognised as a possible mechanism accounting for variation in metformin response [162] . It has also become increasingly clear that the pharmacokinetics of metformin are primarily determined by membrane transporters, including the plasma membrane monoamine transporter (PMAT), the organic cation transporter (OCTs), the multidrug and toxin extrusion-1 transporter (MATE-1) and the critical AMPK [162]. Some genetic variants of membrane transporters have been proved to determine the pharmacokinetics of metformin and a differential response after treatment in obese subjects e.g. the glucokinase regulatory protein (GCKR), the peroxisome proliferator activated receptor gamma, coactivator 1 alpha (PPARGC1A) and the fat mass and obesity associated protein (FTO gene) [163]. The GoDARTS and UKPDS metformin pharmacogenetics study groups investigated the genetics of metformin response in a discovery cohort of 1024 Scottish individuals with type 2 diabetes and incident metformin use. A locus on chromosome 11, tagged by

rs11212617 was associated with metformin response. Although promising, this locus only explains 2.5% of the variance in metformin response [164].

Comparison with other relevant studies

There are very few studies published to date which quantitate the effect of metformin on insulin resistance in pregnancy. The mean HOMA-IR score measured at 28 weeks in the placebo group (3.07 ± 1.7) in my study was similar to the insulin resistance measurements in the standard care group of the UPBEAT study [113] (3.04 ± 2.1, P=NS) [20] and the control group of the LiP study (3.4 ± 1.8, P=NS) [112], described earlier. The MOP trial showed a 59.8% increase in the fasting insulin concentration at 28 weeks of gestation in the placebo group. This was similar to a previous study which showed 65% increase in fasting insulin concentration in the control group during pregnancy [165]. There was only a 5.3% increase in insulin resistance in the metformin group at 28 weeks of gestation, whereas in the placebo group, there was a 49.4% increase in insulin resistance. Glueck *et al.* reported a 4.4% decrease in HOMA-IR score at 28 weeks of gestation in women with polycystic ovaries treated with metformin in pregnancy [133]. This effect of metformin in attenuating the rise in HOMA-IR normally seen at 28 weeks of pregnancy was also observed in the EMPOWaR trial [114].

Women allocated to metformin gained considerably less weight during pregnancy. The gestational weight gain in the placebo group was similar to that reported in the standard care group participants of the UPBEAT study (7.76 ± 4.6 vs. 7 ± 4.5; NS) [113] and the intervention group of the LiP study (7 ± 4.5 vs. 7.4 ± 4.6, P=NS) [147]. This suggests that the lifestyle intervention used in the MOP trial in the entire cohort was effective and comparable to that reported in previous lifestyle intervention studies. The instant read-out of results from the InBody 720 bio impedance device at each antenatal clinic visit served as a very effective motivational tool helping to avoid excessive weight gain.

There was a trend towards a decrease in CRP levels in women on metformin and a similar finding was reported in the EMPOWaR trial [114] and in non-

pregnant adults treated with metformin in the Diabetes Prevention Program [159].

There are a few studies investigating the effect of metformin on pregnancy outcomes in women with polycystic ovary syndrome [154][155][166][167]. Most studies show that metformin has no significant effect on neonatal birth weight or on the incidence of preeclampsia or GDM. One study using a dose of metformin of 2000 mg per day, shows significantly less maternal weight gain compared to placebo [166]. Another trial on 40 women shows that metformin is associated with a significantly lower rate of preeclampsia than placebo[167]. Studies in women with polycystic ovaries, which is characterised by insulin resistance, also suggest that obese women are either refractory to the effects of metformin or may require increased dosage[168][169].

The EMPOWaR trial showed no significant differences in the median birth weight, maternal GWG, the rate of preeclampsia or the rate of adverse perinatal events between the metformin and the placebo groups. Only Caucasians were included in this study unlike in the MOP trial in which all races were included, so that the results can be extrapolated to the whole population. The MOP trial used the higher cut off point for BMI at 35 kg/m^2 instead of 30 kg/m^2 used in EMPOWaR, in order to have adequate power with a smaller sample size. The adherence to the study regimen was also higher in the MOP trial with nearly 80% of women having taken at least 50% of the total number of tablets prescribed. In the EMPOWaR study, women were considered to have adhered to the study regimen if they took a minimum of 1 tablet of 500 g for at least 29% of the days and only 67% fulfilled these criteria [114].

The strengths of the MOP Trial were its randomised controlled design, the racially heterogeneous nature of the participating group of women, the high percentage of eligible women who agreed to participate and high levels of compliance with study medication. A limitation of it is that it was not adequately powered for the secondary outcomes like gestational diabetes and preeclampsia. The number of women with high insulin resistance in each study group was very small making comparisons difficult to interpret. Future studies

should also examine body composition in the offspring of mothers receiving metformin as evidence of benefit in childhood fat distribution is beginning to emerge [127].

Comparison of the St Helier cohort with the entire MOP Trial

The results of the St Helier cohort of the MOP trial are essentially similar to the results seen in the entire MOP trial (n=450), reported in the New England Journal of Medicine (NEJM) in February 2016 [134]. There were no significant differences between the metformin and the placebo groups in neonatal birth weight z score, incidence of LGA neonates or adverse fetal or neonatal outcomes. In the entire MOP trial, the incidence of preeclampsia was significantly lower in the metformin group than in the placebo group (3.0% vs. 11.3%; odds ratio 0.24; 95% confidence interval, 0.10 to 0.61; p=0.001) [134]. This effect was not seen in our St Helier cohort as I had only 3 cases of preeclampsia in my study and all were in women from the placebo arm. I conducted a secondary analysis to examine whether the reduced incidence of preeclampsia in women treated with metformin in the entire MOP trial is mediated by changes in insulin resistance [170]. The results of the analysis showed that median HOMA-IR was significantly lower in the metformin group at 28 weeks of gestation. Logistic regression analysis demonstrated that there was a significant contribution in the prediction of preeclampsia from maternal history of chronic hypertension and gestational weight gain, but not HOMA-IR either at randomisation (p=0.514) or at 28 weeks (p=0.643). The study concluded that the reduced incidence of preeclampsia in non-diabetic obese pregnant women treated with metformin is unlikely to be due to changes in insulin resistance [170]. Metformin could have a potential benefit in reducing the risk of preeclampsia because of its modulatory effect on endothelial dysfunction.

In summary, my cohort showed that metformin given to non-diabetic obese pregnant women from 12-18 weeks of gestation until delivery, did not reduce the neonatal birth weight centile or the incidence of LGA babies. However, metformin therapy reduced GWG, reduced the rise in VFM during pregnancy, and attenuated the expected physiological rise in insulin resistance seen at 28

weeks of gestation. Surprisingly, this did not lead to an overall significant reduction in the incidence of gestational diabetes or have a beneficial effect on other pregnancy outcomes such as the incidence of macrosomic babies. The study showed a trend towards a reduction in gestational diabetes in obese pregnant women with high baseline insulin resistance randomised to metformin as compared to placebo. However, these differences are not statistically significant. Similarly, there appears to be a potential effect of metformin in reducing preeclampsia, though the number of women developing preeclampsia in the St Helier cohort was very small. My study was not powered for the secondary outcomes like GDM and preeclampsia and larger studies of metformin in obese pregnant women in pregnancy are warranted.

4 DISCUSSION AND CONCLUSIONS

The CEMACH in its 2003-2005 triennia report has highlighted obesity in pregnancy as a cause for increased morbidity and mortality in mother and baby [67]. The literature review showed that obesity is associated with a number of serious adverse outcomes including GDM and preeclampsia [68][151]. The prevalence of GDM is rising and this is concerning because of the risk of pregnancy complications such as macrosomia, shoulder dystocia, Caesarean section and neonatal hypoglycaemia and also because of the risk to the mother and offspring of diabetes and cardiovascular disease in later life [68][104] [131].

The literature search did not reveal any published studies that investigated VFM as a risk factor for adverse pregnancy outcomes in obese women. Therefore, to my knowledge, the VFM study was the first study examining the role of VFM in determining pregnancy and neonatal outcomes in obese women.

The results of the VFM study showed that baseline VFM is a novel risk factor for GDM. There was clearly a significant association between higher baseline VFM (≥ 75^{th} percentile) and risk of subsequent GDM. Obese women with a baseline VFM ≥ 75 percentile had a 3-fold risk of GDM. There was a correlation between VFM and fasting glucose and HbA_{1c} in all women who developed GDM. Similar correlations of VFM with fasting hyperglycaemia and HbA_{1c} have been shown in patients with T2DM [110]. This suggests that visceral rather than non-visceral subcutaneous fat has a metabolic effect [143][144]. In the general population, it is already well-established that excess visceral fat and insulin resistance, but not general adiposity, are independently associated with pre-diabetes and T2DM in obese adults [107] [108]. Body fat composition measurements by InBody are easy to perform, take less than 5 minutes per test and are non-expensive.

To the best of my knowledge, this was also the first attempt to create a mathematical model to predict GDM and LGA baby using VFM. PCA failed to classify GDM correctly. Hence, supervised learning methods were applied to design and develop a predictive model using random forest and decision tree

modelling. The mathematical model managed to predict GDM with an average prediction accuracy of 77.5%. However, the model got better trained in detection of no-GDM than GDM. The birth centile model could correctly predict birth centile classes with an average of 68%. The model was well trained in predicting normal birth centiles but not as accurate in predicting low or high birth centiles. As I discussed earlier, GDM and fetal macrosomia could be considered a metabolic complication of diabetes like macrovascular disease rather than a microvascular complication of diabetes. Hence, it would be very important to predict the risk of macrosomia with the birth centile model.

The current method of screening for GDM is based on the presence of clinical risk factors which provides a detection rate of approximately 60% with a 40% false positive rate [125]. Currently, those women identified with even a single risk factor undergo an oral glucose tolerance test at 24-28 weeks gestation. Risk stratification for GDM early in pregnancy may reduce the need for OGTT in women at low risk, resulting in savings in costs and in healthcare personnel time. It could also help to avoid extra clinic visits and extra scans in low risk obese women. In current settings, by the time GDM is diagnosed at 28 weeks of gestation, the effects of hyperglycaemia on the fetus may be already evident on the ultrasound growth scans. Conversely those at high risk can start lifestyle interventions early to reduce the risk of complications.

Lifestyle intervention programmes in pregnancy have shown no beneficial effects on the neonate [113][147][148][149]. Since insulin resistance is increased in obesity, and obesity is strongly associated with birth weight and fetal adiposity [131], metformin, an insulin sensitiser, was a rational choice for the MOP trial. The results of the St Helier cohort of the MOP trial showed that in obese, non-diabetic pregnant women, treatment with metformin did not reduce the median neonatal birth weight z score, incidence of LGA neonates or other adverse fetal and neonatal outcomes. However, metformin therapy reduced GWG, reduced the rise in visceral fat mass during pregnancy and attenuated the expected physiological rise in insulin resistance seen at 28 weeks of gestation. 10.2% of the women in the metformin group developed gestational

diabetes compared with 18.6% in those allocated to placebo, but this difference was not significant. Therefore, even though metformin blunted the rise in insulin resistance at 28 weeks, the incidence of GDM was not significantly reduced. There was a trend for a higher baseline insulin resistance in women who developed GDM as compared to no-GDM women but again this difference was not significant. This raises the question whether the metformin initiation at 12 weeks of gestation in the MOP trial was a delayed intervention, and the adverse effects of obesity on the fetus had already set in.

There was a significant reduction in the GWG in the metformin group. Women receiving metformin had a significantly lesser increase in visceral fat at term, compared with those on placebo. Also, these effects persisted into the post-partum period. This may be potentially beneficial in reducing the risk for future T2DM and cardiovascular disorders in these women.

In the placebo group alone, more women with baseline HOMA-IR ≥ 75th percentile developed GDM, compared to women with HOMA IR ≤ 75th percentile (44.4% vs. 12.5%, P=0.04). Also, there was a significant association with baseline HOMA-IR ≥ 75th percentile and subsequent GDM [Odds Ratio (CI), 5.7 (1.2-27.5)]. This suggests that women with high baseline insulin resistance have a higher risk of developing GDM. There was no such association of high baseline insulin resistance with PIH or preeclampsia in the placebo group. However, the overall rate of preeclampsia is very low in our study and hence it is difficult to comment.

The hypothesis that metformin is most effective in women with highest baseline insulin resistance in preventing GDM, was not substantiated by the study results. When stratified by insulin resistance, there was a trend towards a lower risk of GDM for women in the high IR group receiving metformin as compared to placebo. This difference again was not significant.

The strengths of the MOP trial were its randomised controlled design, the racially heterogeneous nature of the participating group of women, and high levels of compliance with study medication. A limitation was that it was not adequately powered for the secondary outcomes like gestational diabetes and

preeclampsia. Also, the number of women with high insulin resistance in each study group was very small, making comparisons difficult to interpret.

There was a MHRA inspection of the MOP trial at St Helier Hospital. They checked the intricate details of the conduct of the trial and were satisfied that the trial was conducted in accordance to the Good Clinical Practice guidelines.

The mechanisms responsible for adverse pregnancy outcomes in obesity may be mediated by several metabolic pathways besides glucose [171]. Maternal blood glucose is subtly increased among obese women. The HAPO study has shown that even modest increments can influence fetal growth and adiposity. Other important parameters include raised maternal triglycerides and fatty acids. Obese mothers have a more atherogenic lipid profile in early pregnancy compared to normal weight women and this may influence placentation and be the link to adverse pregnancy complications like preeclampsia [172]. Maternal insulin resistance contributes to plasma lipid perturbations due to an increase in adipose tissue lipolysis. Increased lipolysis provides a surplus of plasma free fatty acid substrates for hepatic triglyceride synthesis. These physiologic adaptations in pregnancy may differ between normal weight and obese women [172]. Recent studies have shown that women who developed GDM had significantly increased triglycerides, cholesterol, LDL concentrations, LDL/HDL ratios and decreased HDL concentrations in early pregnancy compared to controls [173]. One study demonstrated that circulating maternal lipids, but not glucose, correlate with fetal growth at different time points during the 3rd trimester in a population of well-controlled GDM pregnancies [174]. In this study, they found that maternal triglycerides and free fatty acids correlated with fetal abdominal circumference at 28 weeks, and at delivery they correlated with neonatal birth weight, BMI and fat mass [174].

The mechanism for the association between early pregnancy maternal dyslipidaemia and GDM risk is unknown. Triglyceride concentrations increase in pregnancy to two or three times the nonpregnant levels [175]. This is probably a result of increased adipose tissue lipolysis as a consequence of insulin resistance and enhanced non esterified fatty acid (NEFA) delivery to the liver

which is then associated with increased very low density lipoprotein concentrations [175][176]. Reduced lipoprotein lipase activity leads to a reduced capacity for triglyceride removal from the circulation [175]. Maternal hypertriglyceridemia is associated with maternal insulin resistance [177]. The maternal and cord blood leptin concentration is elevated and there is evidence of higher levels of CRP and IL-6 in the mother, reflecting a low grade inflammatory state. In non-pregnant adults, this elevation of inflammatory mediators is linked to insulin resistance [171].

Obesity is associated with fetal hyperinsulinemia even in the absence of maternal diabetes. Increased influx of amino acids could stimulate fetal hyperinsulinemia. Obese women have higher triglyceride levels which could be broken down by placental lipases to free fatty acids which could cross the placenta. The increased energy influx and fetal hyperinsulinemia together could explain macrosomia in obese women without diabetes.

Besides its anti-hyperglycaemic effect, metformin also causes suppression of fatty acid oxidation and a reduction in hypertriglyceridemia. This is associated with decreased synthesis and increased clearance of VLDL. Reduction in triglyceride levels reduces insulin resistance [14]. Studies have shown that (Buchanan et al) mild gestational diabetes is characterised by an impairment of β cell function rather than an exaggeration of the normal insulin resistance of late pregnancy [24]. Protection against the insulin resistance induced β cell failure with metformin could be important.

In conclusion, this study showed that visceral fat mass is a novel risk factor for the development of GDM in obese pregnant women. Obese pregnant women with VFM ≥ 75^{th} percentile have a 3-fold higher risk of developing GDM. A mathematical model was developed with good overall performance in predicting gestational diabetes and LGA babies in these women. To summarise, the addition of VFM to conventional risk factors in the predictive model may help discriminate between high and low risk pregnancies but this needs to be confirmed in larger studies with diverse populations including non-obese women. The clinical significance of this model lies in the potential for early and

personalised risk stratification for GDM allowing those at high risk to start dietary and lifestyle interventions early to reduce the risk of complications. The MOP trial showed that metformin given to non-diabetic obese pregnant women did not reduce the neonatal birth weight centile or the incidence of LGA babies. However, metformin therapy reduced GWG, reduced the rise in VFM during pregnancy, and attenuated the expected physiological rise in insulin resistance. The study showed a trend towards a reduction in gestational diabetes in obese pregnant women with high baseline insulin resistance randomised to metformin as compared to placebo.

Future research recommendations

- A clinical study involving body composition analysis of a larger number of obese pregnant women belonging to one ethnicity would provide a large training set for the mathematical model to improve its accuracy to predict GDM, preeclampsia and LGA babies.
- Large randomised placebo controlled trial, adequately powered to investigate the effect of metformin in reducing GDM and preeclampsia. The St Helier cohort of the MOP trial did show a trend towards beneficial effects of metformin in decreasing the incidence of GDM in women with high baseline insulin resistance, but was not adequately powered to comment on these effects of metformin. The entire MOP trial showed benefit of metformin in reducing the incidence of preeclampsia, but adequately powered studies would be needed to confirm the findings.
- Future studies should also examine body composition in the offspring of mothers receiving metformin, as evidence of benefit in childhood fat distribution is beginning to emerge.
- In the placebo group, baseline HOMA-IR ≥ 75 percentile was a more likely finding among those women who subsequently developed GDM, compared with women who did not. This suggests that obese pregnant women who develop GDM have a high insulin resistance at around 12 weeks of gestation or perhaps much earlier in pregnancy. Hence, it

would be logical that metformin therapy should be started prenatally, 3 months before conception, to see its effects on prevention of GDM and preeclampsia. Future studies should look into prenatal intervention programmes, both with lifestyle modification and metformin therapy.

- Randomised placebo controlled trial to determine if antenatal dietary supplementation with Myo-inositol from early pregnancy till delivery will reduce the risk of gestational diabetes in obese pregnant women. Myo-inositol, an isomer of inositol, is a naturally occurring sugar commonly found in cereals, corn, legumes and meat. It is one of the intracellular mediators of insulin signalling and improves insulin sensitivity. A few small studies with Myo-inositol in women with polycystic ovaries have shown beneficial effect in preventing gestational diabetes. A large number of obese women will have high insulin resistance in early pregnancy and may benefit with Myo-ionositol.

List of relevant poster presentations at Conferences

1. **Archives of Disease in Childhood- Fetal and Neonatal Edition**

   April 2012. Maternal body composition in obese women and pregnancy outcome

   J Balani, S Hyer, A Johnson, H Shehata

2. **International Symposium on Diabetes, Hypertension,**

   Metabolic Syndrome and Pregnancy, Florence, March 2013.

   Visceral Fat mass and not total body fat is an adverse prognostic factor in obese pregnant women.

   J Balani, S Hyer, A Johnson, H Shehata

3. **Diabetes in Pregnancy: National conference, London,**

   November 2016 - Poster presentation

   A mathematical model for predicting gestational diabetes in obese pregnant women using Machine Learning.
   Jyoti Balani, Steve Hyer, Hassan Shehata, Fady Mohareb

4. **Diabetes in Pregnancy conference, Barcelona, March 2017**
   Insulin resistance, gestational weight gain and incidence of gestational diabetes in obese non-diabetic women receiving metformin.
   Balani J, Hyer S, Johnson A, Syngelaki A, Akolekar R, Nicolaides K, Shehata H

Chapter in a book

"Obesity, Polycystic Ovaries and Impaired Reproductive Outcomes"
Jyoti Balani, Stephen Hyer, Marion Wagner and Hassan Shehata
Chapter 22 in a book on "Obesity", Elsevier publications

Publications related to my study

1. **The importance of Visceral fat mass in obese pregnant women and relation with pregnancy outcomes.**
   Jyoti Balani, Steve Hyer, Antoinette Johnson, Hassan Shehata
   Obstetric Medicine 2014, Vol 7 (1), 22-25

2. **Metformin versus Placebo in Obese Pregnant women without diabetes mellitus**
   A Syggelaki, K Nicolaides, **Jyoti Balani**, Steve Hyer et al
   N Engl J Med, 2016; 374:434-43

3. **Association between insulin resistance and preeclampsia in obese non-diabetic women receiving metformin**
   Jyoti Balani, M.D., Steve Hyer, M.D., Argyro Syngelaki, Ranjit Akolekar, M.D., Kypros H. Nicolaides, M.D., Antoinette Johnson, M.D., Hassan Shehata, M.D.
   Obstetric Medicine, 2017, Vol. 10 (4), 170-173

4. **Visceral fat mass as a novel risk factor for predicting GDM in obese pregnant women**
   J Balani[1], S L Hyer[1], H Shehata[2], F Mohareb[3]
   Obstetric Medicine, 2018, 0 (0), 1-5

5. **The effect of reducing maternal insulin resistance in the prevention of gestational diabetes: Results from the MOP trial**
   Jyoti Balani, Steve Hyer, Antoinette Johnson, Hassan Shehata
   Pending approval from other authors

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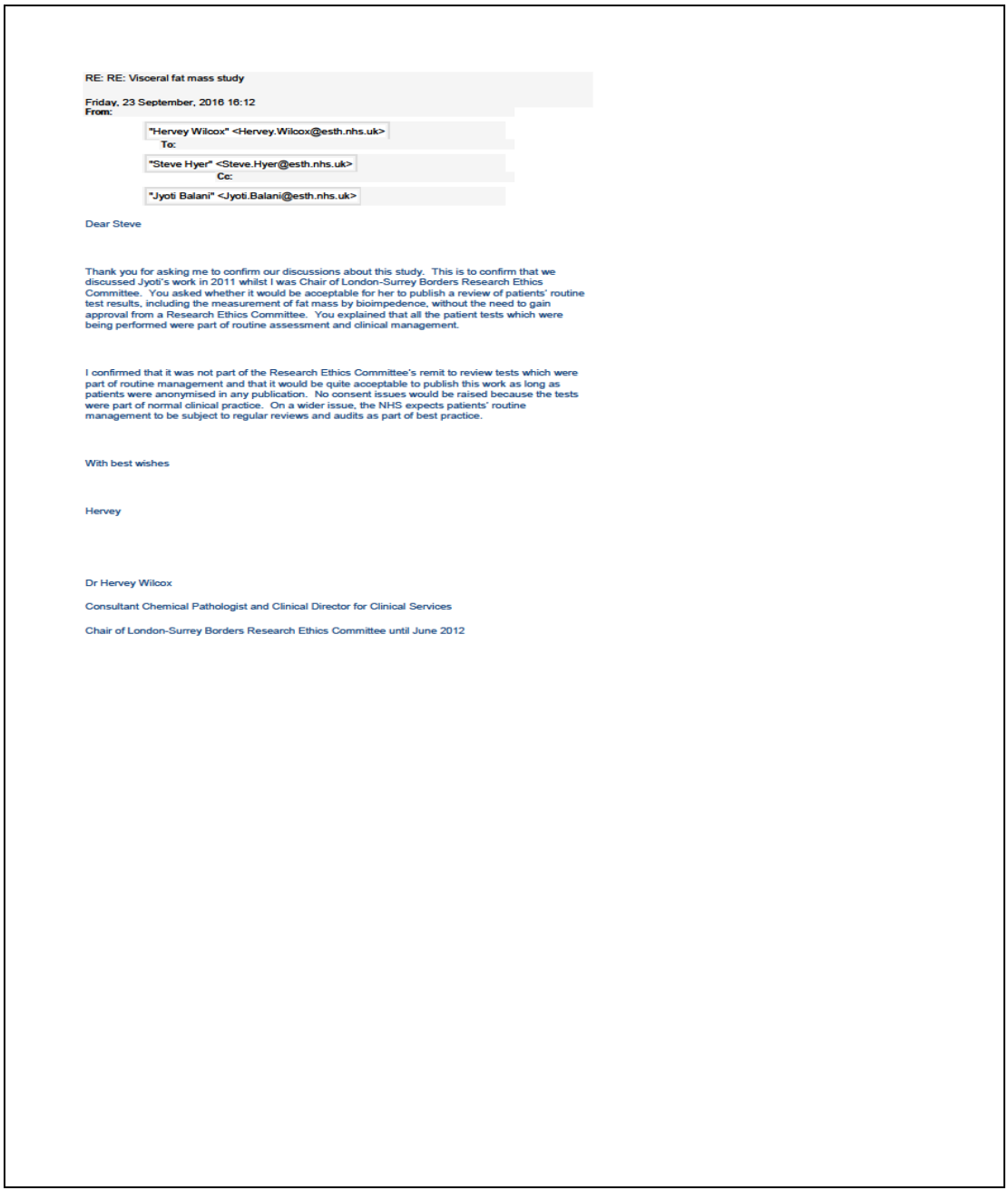
Appendix A Ethics documents for VFM study

This appendix section provides details of the documentation related to ethics for the VFM study.

A.1 Ethics letter for the VFM study



RE: RE: Visceral fat mass study

Friday, 23 September, 2016 16:12
From:

"Hervey Wilcox" <Hervey.Wilcox@esth.nhs.uk>
To:

"Steve Hyer" <Steve.Hyer@esth.nhs.uk>
Cc:

"Jyoti Balani" <Jyoti.Balani@esth.nhs.uk>

Dear Steve

Thank you for asking me to confirm our discussions about this study. This is to confirm that we discussed Jyoti's work in 2011 whilst I was Chair of London-Surrey Borders Research Ethics Committee. You asked whether it would be acceptable for her to publish a review of patients' routine test results, including the measurement of fat mass by bioimpedence, without the need to gain approval from a Research Ethics Committee. You explained that all the patient tests which were being performed were part of routine assessment and clinical management.

I confirmed that it was not part of the Research Ethics Committee's remit to review tests which were part of routine management and that it would be quite acceptable to publish this work as long as patients were anonymised in any publication. No consent issues would be raised because the tests were part of normal clinical practice. On a wider issue, the NHS expects patients' routine management to be subject to regular reviews and audits as part of best practice.

With best wishes

Hervey

Dr Hervey Wilcox

Consultant Chemical Pathologist and Clinical Director for Clinical Services

Chair of London-Surrey Borders Research Ethics Committee until June 2012

A.2 File note regarding ethics for VFM study

Epsom and St. Helier NHS
University Hospitals
NHS Trust

FILENOTE

25/01/2011

Study Title: Visceral Fat Mass study

File note regarding Ethics permission for the above study

This trial did not require ethical approval as advised by the chair of the London Surrey Borders ethics committee as all tests being performed were part of routine assessment and clinical management.

Signed by Investigator:

Dr Steve Hyer

Department of Diabetes and Endocrinology

St Helier Hospital, Wrythe Lane

Carshalton, Surrey, SM5 1AA

Department of Diabetes & Endocrinology
St Helier Hospital
Wrythe Lane
Carshalton
Surrey SM5 1AA

Appendix B Ethics Approval for the MOP Trial

This appendix section provides details of the documentation related to ethical approval for the MOP Trial

B.1 Ethical Approval of the MOP Trial

London - Surrey Borders Research Ethics Committee
St Georges University of London
South London REC Office 1
Corridor 1 - Room 1.13
1st Floor, Jenner Wing
Tooting
London
SW17 0RE

Telephone: 0208 725 0262
Facsimile: 0208 725 1897

19th November 2008

Mr Hassan Shehata
Consultant & Honorary Senior Lecturer in Maternal Medicine
Epsom & St Helier University Hospitals NHS Trust
Women's Health Department
St. Helier University Hospital
Wrythe Lane,
Carshalton,
Surrey
SM5 1AA

Dear Mr Shehata

Full title of study:	**Does metformin improve pregnancy outcomes (onset of maternal GDM, hypertension, PET, macrosomia, shoulder dystocia, admission to SCBU) in obese non-diabetic women?**
REC reference number:	**08/H0806/80**
Protocol number:	**1**
EudraCT number:	**2008-005892-83**

Thank you for your letter of , responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered at the meeting of the Sub-Committee of the REC held on 12 November 2008. A list of the members who were present at the meeting is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA). The favourable opinion for the study applies to all sites involved in the research. There is no requirement for other Local Research Ethics Committees to be informed or SSA to be carried out at each site.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission at NHS sites ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application	1.1	12 September 2008
Investigator CV		20 August 2008
Participant Consent Form	1.0	30 August 2008
Letter of invitation to participant		
Response to Request for Further Information		
Participant Information Sheet	1.1	20 October 2008
Covering Letter		28 October 2008
Protocol	1.1	20 October 2008
Signature Sheet & Delegation of Duties Log		

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

08/H0806/80	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Dr Hervey Wilcox
Chair

Email: lsbrec@stgeorges.nhs.uk

Enclosures: *List of names and professions of members who were present at the meeting.*
"After ethical review – guidance for researchers"

Copy to: Mrs Yvonne Reilly, R&D Manager
Epsom & St Helier University Trust
St Helier Hospital
Wrythe Lane
Carshalton
SM5 1AA

London - Surrey Borders Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on the 12th November 2008

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr Hervey Wilcox - Chair	Consultant Chemical Pathologist	No	
Canon Christopher Vallins – Vice Chair	Regional Chaplaincy Adviser	Yes	
Mrs Wendy Brooks - Alternate Vice-Chair	Stroke Nurse Consultant	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Ms Joan Bailey	London-Surrey Borders REC Co-ordinator

B.2 Ethics Approval for Substantial Amendment 1

NHS

National Research Ethics Service
London - Surrey Borders Research Ethics Committee

St Georges University of London
South London REC Office 1
Corridor 1 - Room 1.13
1st Floor, Jenner Wing
Tooting
London
SW17 0RE

Tel: 0208 725 0262
Fax: 0208 725 1897

Date: 5th October 2009

Mr Hassan Shehata
Consultant & Honorary Senior Lecturer in Maternal Medicine
Epsom & St Helier University Hospitals NHS Trust
Women's Health Department
St. Helier University Hospital
Wrythe Lane, Carshalton,
Surrey, SM5 1AA

Dear Mr Shehata

Study title:	**Does metformin improve pregnancy outcomes (onset of maternal GDM, hypertension, PET, macrosomia, shoulder dystocia, admission to SCBU) in obese non-diabetic women?**
REC reference:	**08/H0806/80**
Protocol number:	**1**
EudraCT number:	**2008-005892-83**
Amendment number:	**Substantial Amendment 1**
Amendment date:	**20th July 2009**

The above amendment was reviewed at the meeting of the Sub-Committee held on the 7th August 2009.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

However, the Sub-Committee raised the following points:

- The Participant Information Sheet on page 2 - the term '**placebo**' will need to be clearly explained in lay language.
- The Participant Information Sheet ~ '**What will I have to do?**' will need to be amended to 'If you need to undergo an emergency operation, you must stop taking metformin or placebo tables immediately and inform your doctors including the anaesthetist that you have been taking **in a trial** metformin or placebo'.
- Within in the 'Notification of Amendment (CTIMP's) in the section which details the brief changes to the study – Point Number 3 the Sub-Committee would like

further clarification as to why an extra serum and plasma sample would need to be obtained for future testing?

- Within in the 'Notification of Amendment (CTIMP's) in the section which details the brief changes to the study – Point Number **4** – the Sub-Committee were unclear as to whether the '**In Body 720'** which is a body composition analyser is a validated tool?

- Consent Form – The version number and date will need to be amended within point 1 which states 30th August, Version 1.0 to match the Participant Information Sheet which is Version 1.3, dated 14th July 2007.

- Consent Form – Initial Boxes will need to be enlarged.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Covering Letter		21st July 2009
Annex 2 Notification of Amendment (CTIMPs)	October 2005	20th July 2009
Reasons for Amendments		
Description of Amendments		
List of Revised documents		
Supporting Data for Amendments		
Protocol	1.2	14th July 2009
Participant Information Sheet	1.3	14th July 2009
Participant Consent Form	1.1	14th July 2009

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

The Participant Information Sheet, dated the 14th July 2009 now has the correct version number stated in the above list of approved documents. Therefore, this letter supersedes the letter of approval issued on the 20th August 2009.

08/H0806/80:	Please quote this number on all correspondence

Yours sincerely

Ms Joan Bailey
Committee Co-ordinator

E-mail: lsbrec@stgeorges.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Copy to:

Mrs Yvonne Reilly, R&D Manager
Epsom & St Helier University Trust
St Helier Hospital
Wrythe Lane
Carshalton, SM5 1AA

Dr Joyti Balani
Clinical Research Fellow
Diabetes & Maternal Medicine
Epsom & St Helier University Trust
St Helier Hospital
Wrythe Lane, Carshalton, SM5 1AA

B.3 Ethical approval for Substantial Amendment 2

National Research Ethics Service
London - Surrey Borders Research Ethics Committee
St Georges University of London
South London REC Office 1
Corridor 1 - Room 1.13
1st Floor, Jenner Wing
Tooting
London
SW17 0RE

Tel: 0208 725 0262
Fax: 0208 725 1897

Date: 16th November 2009

Mr Hassan Shehata
Consultant & Honorary Senior Lecturer in Maternal Medicine
Epsom & St Helier University Hospitals NHS Trust
Women's Health Department
St. Helier University Hospital
Wrythe Lane, Carshalton,
Surrey, SM5 1AA

Dear Mr Shehata

Study title:	**Does metformin improve pregnancy outcomes (onset of maternal GDM, hypertension, PET, macrosomia, shoulder dystocia, admission to SCBU) in obese non-diabetic women?**
REC reference:	**08/H0806/80**
Protocol number:	**1**
EudraCT number:	**2008-005892-83**
Amendment number:	**Substantial Amendment 2**
Amendment date:	**21st October 2009**

The above amendment was reviewed at the meeting of the Sub-Committee held on the 11th November 2009.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
European Commission Notification of Substantial Amendment Form	October 2005	21st October 2009
Covering Letter		21st October 2009
Participant Information Sheet	1.4	26th August 2009
Participant Consent Form	1.5	24th September 2009
Protocol	1.3	21st October 2009
Description of Amendments		26th October 2009

Reason for Proposed Amendments		21st October 2009
Supporting Information on Placebo		24th September 2009

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

08/H0806/80:	**Please quote this number on all correspondence**

Yours sincerely

Ms Joan Bailey
Committee Co-ordinator

E-mail: lsbrec@stgeorges.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Copy to: Mrs Yvonne Reilly, R&D Manager
Epsom & St Helier University Trust
St Helier Hospital
Wrythe Lane
Carshalton
SM5 1AA

Dr Joyti Balani
Clinical Research Fellow
Diabetes & Maternal Medicine
Epsom & St Helier University Trust
St Helier Hospital
Wrythe Lane, Carshalton, SM5 1AA

B.4 Ethical approval for Substantial Amendment 3

NHS
National Research Ethics Service
London - Surrey Borders Research Ethics Committee

St Georges University of London
South London REC Office 1
Corridor 1 - Room 1.13
1st Floor, Jenner Wing
Tooting
London
SW17 0RE

Tel: 0208 725 0262
Fax: 0208 725 1897

Date: 11th December 2009

Mr Hassan Shehata
Consultant & Honorary Senior Lecturer in Maternal Medicine
Epsom & St Helier University Hospitals NHS Trust
Women's Health Department
St. Helier University Hospital
Wrythe Lane, Carshalton,
Surrey, SM5 1AA

Dear Mr Shehata

Study title:	**Does metformin improve pregnancy outcomes (onset of maternal GDM, hypertension, PET, macrosomia, shoulder dystocia, admission to SCBU) in obese non-diabetic women?**
REC reference:	**08/H0806/80**
Protocol number:	**1**
EudraCT number:	**2008-005892-83**
Amendment number:	**Substantial Amendment 3**
Amendment date:	**18th November 2009**

The above amendment was reviewed at the meeting of the Sub-Committee held on the 9th December 2009.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
European Commission Notification of Substantial Amendment Form		18th November 2009
Covering Letter		16th November 2009
Description of Amendments		16th November 2009
Reasons for Proposed Amendments		16th November 2009
Protocol	1.4	16th November 2009

NHS
National Research Ethics Service

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

08/H0806/80:	**Please quote this number on all correspondence**

Yours sincerely

Ms Joan Bailey
Committee Co-ordinator

E-mail: lsbrec@stgeorges.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Copy to:

Mrs Yvonne Reilly, R&D Manager
Epsom & St Helier University Trust
St Helier Hospital
Wrythe Lane
Carshalton
SM5 1AA

Dr Joyti Balani
Clinical Research Fellow
Diabetes & Maternal Medicine
Epsom & St Helier University Trust
St Helier Hospital
Wrythe Lane, Carshalton, SM5 1AA

B.5 Ethical approval for Substantial Amendment 4

NHS
National Research Ethics Service
South West London REC 4
St Georges University of London
South London REC Office 1
Corridor 1 - Room 1.13
1st Floor, Jenner Wing
Tooting
London
SW17 0RE

Tel: 0208 725 0262
Fax: 0208 725 1897

Date: 15th June 2010

Dr Joyti Balani
Clinical Research Fellow
Diabetes & Maternal Medicine
Epsom & St Helier University Trust
St Helier Hospital
Wrythe Lane,
Carshalton, SM5 1AA

Dear Dr Balani

Study title:	**Does metformin improve pregnancy outcomes (onset of maternal GDM, hypertension, PET, macrosomia, shoulder dystocia, admission to SCBU) in obese non-diabetic women?**
REC reference:	**08/H0806/80**
Protocol number:	**1.3**
EudraCT number:	**2008-005892-83**
Amendment number:	**Substantial Amendment 4**
Amendment date:	**25th May 2010**

The above amendment was reviewed at the meeting of the Sub-Committee held on the 9th June 2010.

Ethical opinion

Favourable Opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
European Commission Notification of Substantial Amendment Form		25th May 2010
Covering Letter		25th February 2010
Description of Amendments		25th May 2010
List of Revised Documents		

NHS
National Research Ethics Service

Clinical Trial Information Card		
Participant Information Leaflet On Medication Dose Escalation for MOP-Trial		

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

08/H0806/80:	**Please quote this number on all correspondence**

Yours sincerely

Ms Joan Bailey
Committee Co-ordinator

E-mail: lsbrec@stgeorges.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

South West London REC 4

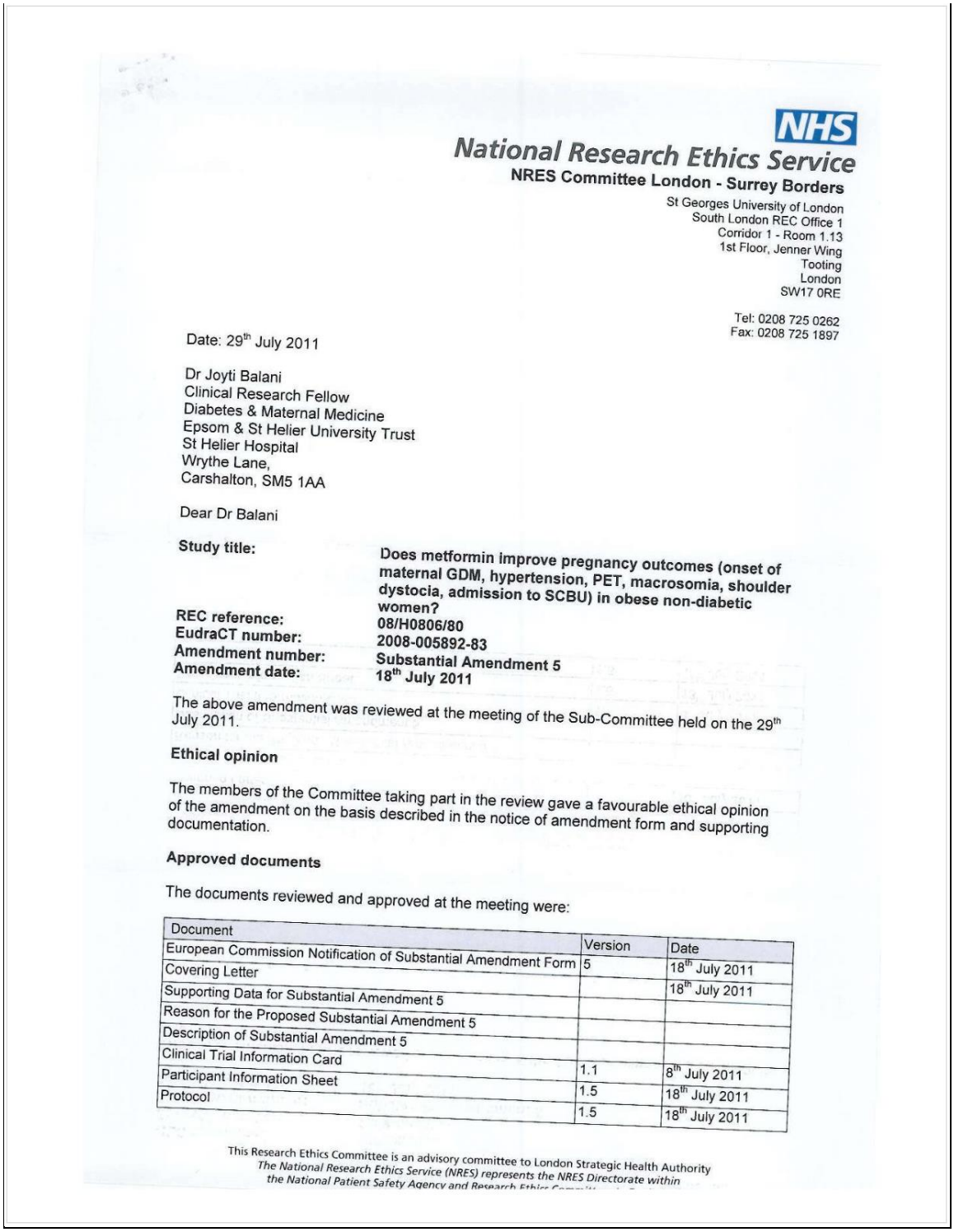
Attendance at Sub-Committee of the REC Meeting held on the 9th June 2010

Name	*Profession*	*Capacity*
Dr Hervey Wilcox ~ Chair	Consultant Chemical Pathologist	Expert
Canon Christopher Vallins ~ Vice Chair	Regional Chaplaincy Adviser	Lay
Mrs Wendy Brooks ~ Alternate Vice Chair	Stroke Nurse Consultant	Expert

Also in attendance:

Name	*Position (or reason for attending)*
Ms Joan Bailey	South West London REC (4) Co-ordinator

B.6 Ethical approval for Substantial Amendment 5



NHS
National Research Ethics Service
NRES Committee London - Surrey Borders

St Georges University of London
South London REC Office 1
Corridor 1 - Room 1.13
1st Floor, Jenner Wing
Tooting
London
SW17 0RE

Tel: 0208 725 0262
Fax: 0208 725 1897

Date: 29th July 2011

Dr Joyti Balani
Clinical Research Fellow
Diabetes & Maternal Medicine
Epsom & St Helier University Trust
St Helier Hospital
Wrythe Lane,
Carshalton, SM5 1AA

Dear Dr Balani

Study title: **Does metformin improve pregnancy outcomes (onset of maternal GDM, hypertension, PET, macrosomia, shoulder dystocia, admission to SCBU) in obese non-diabetic women?**
REC reference: **08/H0806/80**
EudraCT number: **2008-005892-83**
Amendment number: **Substantial Amendment 5**
Amendment date: **18th July 2011**

The above amendment was reviewed at the meeting of the Sub-Committee held on the 29th July 2011.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
European Commission Notification of Substantial Amendment Form	5	18th July 2011
Covering Letter		18th July 2011
Supporting Data for Substantial Amendment 5		
Reason for the Proposed Substantial Amendment 5		
Description of Substantial Amendment 5		
Clinical Trial Information Card	1.1	8th July 2011
Participant Information Sheet	1.5	18th July 2011
Protocol	1.5	18th July 2011

This Research Ethics Committee is an advisory committee to London Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within
the National Patient Safety Agency and Research Ethics

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

08/H0806/80:	Please quote this number on all correspondence

Yours sincerely

Dr Hervey Wilcox
Chair

E-mail: lsbrec@stgeorges.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Copy to:

Mr Hassan Shehata
Consultant & Honorary Senior Lecturer in Maternal Medicine
Epsom & St Helier University Hospitals NHS Trust
Women's Health Department
St. Helier University Hospital
Wrythe Lane, Carshalton,
Surrey, SM5 1AA

Mrs Yvonne Reilly
R&D Office
First Floor, Block B
St Helier Hospital
Wrythe Lane, Carshalton
Surrey, SM5 1AA

B.7 Ethical approval for Substantial Amendment 6

NHS
Health Research Authority

NRES Committee London - Surrey Borders
HRA
Research Ethics Committee (REC) London Centre
Ground Floor
Skipton House
80 London Road
London
SE1 6LH

Tel: 020 797 22580

10 May 2013

Dr Jyoti Balani
Department of Diabetes and Maternal Medicine
Epsom & St Helier University Hospitals NHS Trust
Wrythe Lane, Carshalton,
Surrey, SM5 1AA

Dear Dr Balani

Study title:	**Does metformin improve pregnancy outcomes (onset of maternal GDM, hypertension, PET, macrosomia, shoulder dystocia, admission to SCBU) in obese non-diabetic women?**
REC reference:	**08/H0806/80**
EudraCT number:	**2008-005892-83**
Amendment number:	**SA 6- Revison to Sample Size/Minor Changes to Protocol**
Amendment date:	**10 April 2013**
IRAS project ID:	**7506**

The above amendment was reviewed at the meeting of the Sub-Committee held on 08 May 2013.

Ethical opinion

No ethical issues.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Reasons for Proposed Substantial Amendment 6		10 April 2013
Description of Substantial Amendment 6		
Protocol	1.6	10 April 2013
European Commission Notification of Substantial Amendment Form	SA 6- Revision to Sample Size/Minor Changes to Protocol	10 April 2013

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

08/H0806/80:	**Please quote this number on all correspondence**

Yours sincerely

pp

Derek Cock
Committee Vice Chair

E-mail: NRESCommittee.London-SurreyBorders@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Mrs Yvonne Railey, Epsom & St. Helier Hospital NHS trust*

B.8 Ethical approval for Substantial Amendment 7

NHS
Health Research Authority

NRES Committee London - Surrey Borders

HRA
Research Ethics Committee (REC) London Centre
Ground Floor
Skipton House
80 London Road
London
SE1 6LH
Tel: 02079722580

17 October 2013

Mr Hassan Shehata
Consultant & Honorary Senior Lecturer in Maternal Medicine
Epsom & St Helier University Hospitals NHS Trust
Women's Health Department
Epsom & St. Helier University Hospi
Wrythe Lane,Carshalton, Surrey
SM5 1AA

Dear Mr Shehata

Study title:	**Does metformin improve pregnancy outcomes (onset of maternal GDM, hypertension, PET, macrosomia, shoulder dystocia, admission to SCBU) in obese non-diabetic women?**
REC reference:	**08/H0806/80**
EudraCT number:	**2008-005892-83**
Amendment number:	**SA7 (our ref: AM13)**
Amendment date:	**23 September 2013**
IRAS project ID:	**7506**

The above amendment was reviewed at the meeting of the Sub-Committee held on 09 October 2013.

Ethical opinion

In the covering letter, it says '... if any centres cannot store its own blood samples, these samples will be shipped to another participating study site for storage.' You were asked to provide the names of the sites. You responded with the following: the blood samples collected from recruited women at Medway Maritime Hospital will be stored at Kings College Hospital which is also participating in the study as there are no storage facilities at Medway Hospital. The subcommittee were satisfied with this response.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

The documents reviewed and approved at the meeting were:

Document	Version	Date
Participant Consent Form	1.7	23 September 2013
List of Revised Documents with Substantial Amendment 7		
Protocol	1.7	23 September 2013
Description of Substantial Amendment 7		23 September 2013
Covering Letter	Letter from Dr Balani	23 September 2013
Reasons for the proposed Substantial Amendment 7		23 September 2013
European Commission Notification of Substantial Amendment Form		23 September 2013
Clinical Trial Information Card	1.3	23 September 2013
Participant Information Sheet	1.6	23 September 2013
GP/Consultant Information Sheets	1.3	23 September 2013

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

Yours sincerely

Canon Christopher Vallins
Chair

E-mail: NRESCommittee.London-SurreyBorders@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Mrs Yvonne Railey, Epsom & St. Helier Hospital NHS trust*

B.9 Ethical approval for Substantial amendment 8

NHS
Health Research Authority

NRES Committee London - Surrey Borders
Research Ethics Committee (REC) London Centre
Ground Floor
Skipton House
80 London Road
London
SE1 6LH

Tel: 020 797 22561
Fax: 020 797 22592

20 October 2014

Mr Hassan Shehata
Consultant & Honorary Senior Lecturer in Maternal Medicine
Epsom & St Helier University Hospitals NHS Trust
Women's Health Department
Epsom & St. Helier University Hospital
Wrythe Lane, Carshalton, Surrey
SM5 1AA

Dear Mr Shehata

Study title:	**Does metformin improve pregnancy outcomes (onset of maternal GDM, hypertension, PET, macrosomia, shoulder dystocia, admission to SCBU) in obese non-diabetic women?**
REC reference:	**08/H0806/80**
EudraCT number:	**2008-005892-83**
Amendment number:	**SA8**
Amendment date:	**03 September 2014**
IRAS project ID:	**7506**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering letter on headed paper		03 September 2014
GP/consultant information sheets or letters	1.4	03 September 2014
Notice of Substantial Amendment (CTIMP)	SA8	03 September 2014
Other [Reasons for the proposed amendment]		03 September 2014
Other [Description of Amendment]		03 September 2014

Other [List of Revised Documents]		
Other [Email]		15 September 2014
Other [Email regarding amendment]		22 September 2014
Participant consent form	1.8	03 September 2014
Participant information sheet (PIS)	1.7	03 September 2014
Research protocol or project proposal	1.8	03 September 2014
Sample diary card/patient card	1.4	03 September 2014
Validated questionnaire [The Epworth Sleepiness Scale]		

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

08/H0806/80:	**Please quote this number on all correspondence**

Yours sincerely

pp

Sir Adrian Baillie
Chair

E-mail: nrescommittee.london-surreyborders@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Mrs Yvonne Railey, Epsom & St. Helier Hospital NHS trust*

Appendix C Medicines and Health Regulatory Agency (MHRA) approval

C.1 MHRA approval for the MOP Trial

Safeguarding public health

MHRA

Dr J S Balani
EPSOM & ST HELIER UNIVERSITY HOSPITALS NHS TRUST
ST. HELIER HOSPITAL
WRYTHE LANE
CARSHALTON
SURREY
SM5 1AA
UNITED KINGDOM

14/01/2009

Dear Dr J S Balani

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our reference:	29429/0001/001-0001
Eudract Number:	2008-005892-83
Product:	RELONCHEM METFORMIN TABLETS 500MG
Protocol number:	WCH/2008/001

NOTICE OF ACCEPTANCE

I am writing to inform you that the Licensing Authority accepts your request for a clinical trial authorisation (CTA), received on 15/12/2008.

The authorisation is effective from the date of this letter although your trial may be suspended or terminated at any time by the Licensing Authority in accordance with regulation 31. You must notify the Licensing Authority within 90 days of the trial ending.

Finally, you are reminded that a favourable opinion from the Ethics Committee is also required before this trial can proceed.

Yours sincerely,

Clinical Trials Unit
MHRA

Medicines and Healthcare products Regulatory Agency
Market Towers 1 Nine Elms Lane London SW8 5NQ
T 020 7084 2000 F 020 7084 2353 www.mhra.gov.uk

An executive agency of the Department of Health

C.2 MHRA approval for Substantial Amendment 2 (including Amendment 1) and Substantial Amendment 3

Safeguarding public health

MHRA

Dr J S Balani
EPSOM & ST HELIER UNIVERSITY HOSPITALS NHS TRUST
ST. HELIER HOSPITAL
WRYTHE LANE
CARSHALTON
SURREY
SM5 1AA
UNITED KINGDOM

20/11/2009

Dear Dr J S Balani

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference:	29429/0001/001-0003
Eudract Number:	2008-005892-83
Product:	RELONCHEM METFORMIN TABLETS 500MG
Protocol number:	WCH/2008/001
Substantial Amendment Code Number:	SA2, date 21-10-2009

NOTICE OF ACCEPTANCE OF AMENDMENT

I am writing to inform you that the Licensing Authority accepts the proposed amendment to your clinical trial authorisation (CTA), received on 26/10/2009.

This amendment may therefore be made.

You are reminded that where it is appropriate, the Ethics Committee should also be notified of amendments.

Yours sincerely,

Clinical Trials Unit
MHRA

Medicines and Healthcare products Regulatory Agency
Market Towers 1 Nine Elms Lane London SW8 5NQ
T 020 7084 2000 F 020 7084 2353 www.mhra.gov.uk

An executive agency of the Department of Health

Safeguarding public health

MHRA

Dr J S Balani
EPSOM & ST HELIER UNIVERSITY HOSPITALS NHS TRUST
ST. HELIER HOSPITAL
WRYTHE LANE
CARSHALTON
SURREY
SM5 1AA
UNITED KINGDOM

20/11/2009

Dear Dr J S Balani

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference:	29429/0001/001-0004
Eudract Number:	2008-005892-83
Product:	RELONCHEM METFORMIN TABLETS 500MG
Protocol number:	WCH/2008/001
Substantial Amendment Code Number:	SA3 , date-16-11-2009

NOTICE OF ACCEPTANCE OF AMENDMENT

I am writing to inform you that the Licensing Authority accepts the proposed amendment to your clinical trial authorisation (CTA), received on 17/11/2009.

This amendment may therefore be made.

You are reminded that where it is appropriate, the Ethics Committee should also be notified of amendments.

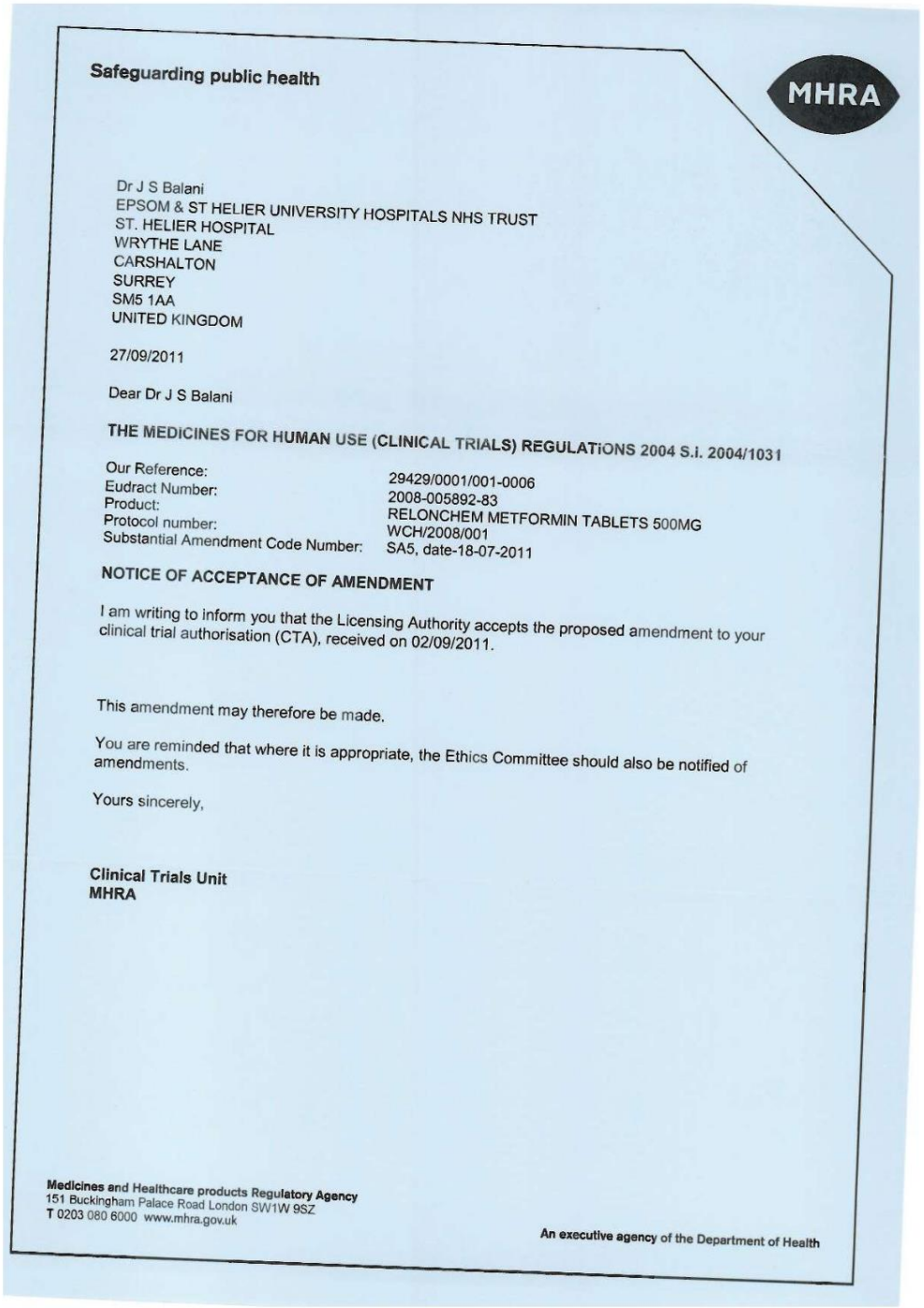
Yours sincerely,

Clinical Trials Unit
MHRA

Medicines and Healthcare products Regulatory Agency
Market Towers 1 Nine Elms Lane London SW8 5NQ
T 020 7084 2000 F 020 7084 2353 www.mhra.gov.uk

An executive agency of the Department of Health

C.3 MHRA approval for Substantial Amendment 5



Safeguarding public health

MHRA

Dr J S Balani
EPSOM & ST HELIER UNIVERSITY HOSPITALS NHS TRUST
ST. HELIER HOSPITAL
WRYTHE LANE
CARSHALTON
SURREY
SM5 1AA
UNITED KINGDOM

27/09/2011

Dear Dr J S Balani

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference:	29429/0001/001-0006
Eudract Number:	2008-005892-83
Product:	RELONCHEM METFORMIN TABLETS 500MG
Protocol number:	WCH/2008/001
Substantial Amendment Code Number:	SA5, date-18-07-2011

NOTICE OF ACCEPTANCE OF AMENDMENT

I am writing to inform you that the Licensing Authority accepts the proposed amendment to your clinical trial authorisation (CTA), received on 02/09/2011.

This amendment may therefore be made.

You are reminded that where it is appropriate, the Ethics Committee should also be notified of amendments.

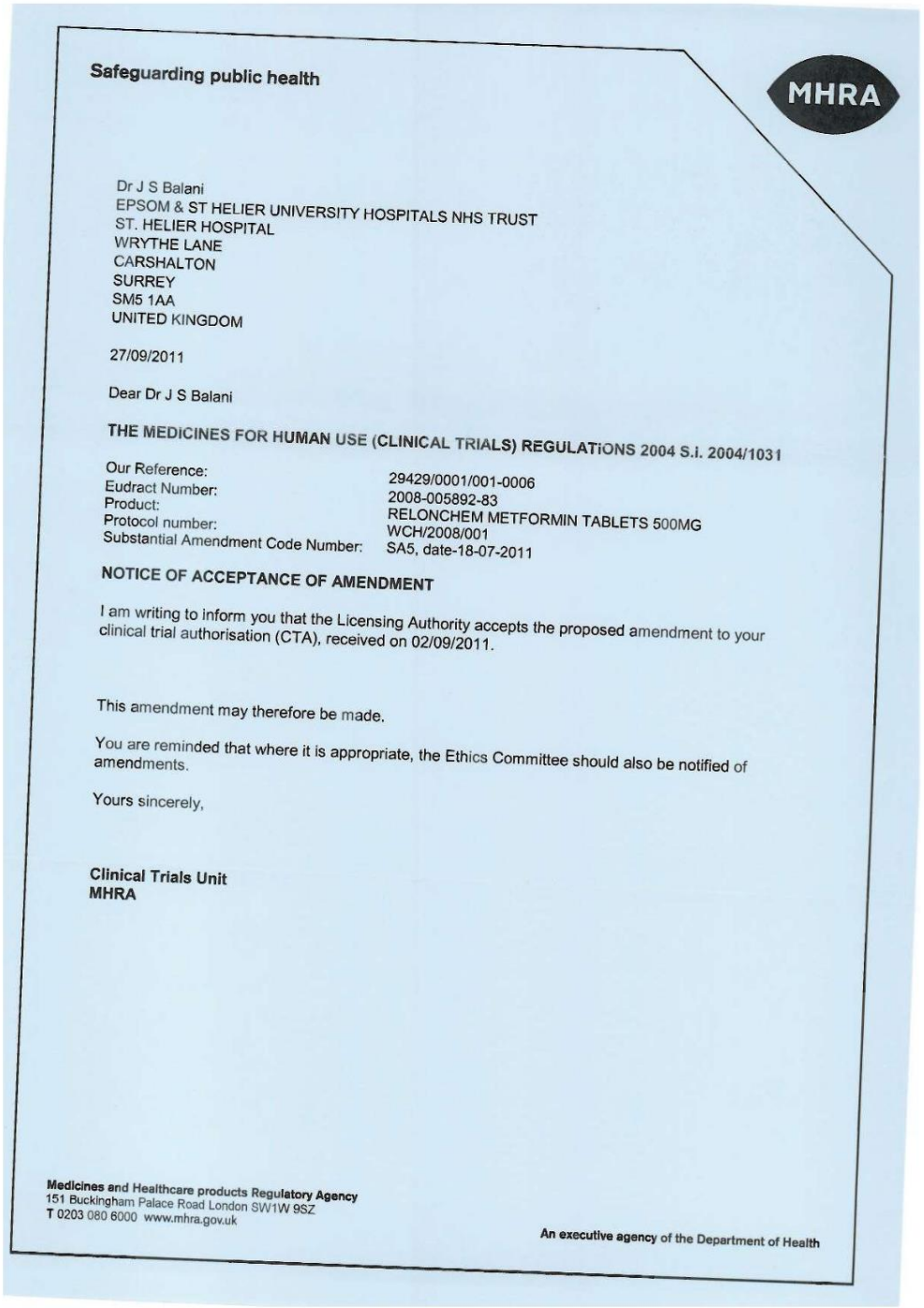
Yours sincerely,

Clinical Trials Unit
MHRA

Medicines and Healthcare products Regulatory Agency
151 Buckingham Palace Road London SW1W 9SZ
T 0203 080 6000 www.mhra.gov.uk

An executive agency of the Department of Health

C.4 MHRA approval for Substantial Amendment 6



Safeguarding public health

MHRA

Dr J S Balani
EPSOM & ST HELIER UNIVERSITY HOSPITALS NHS TRUST
ST. HELIER HOSPITAL
WRYTHE LANE
CARSHALTON
SURREY
SM5 1AA
UNITED KINGDOM

27/09/2011

Dear Dr J S Balani

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference:	29429/0001/001-0006
Eudract Number:	2008-005892-83
Product:	RELONCHEM METFORMIN TABLETS 500MG
Protocol number:	WCH/2008/001
Substantial Amendment Code Number:	SA5, date-18-07-2011

NOTICE OF ACCEPTANCE OF AMENDMENT

I am writing to inform you that the Licensing Authority accepts the proposed amendment to your clinical trial authorisation (CTA), received on 02/09/2011.

This amendment may therefore be made.

You are reminded that where it is appropriate, the Ethics Committee should also be notified of amendments.

Yours sincerely,

Clinical Trials Unit
MHRA

Medicines and Healthcare products Regulatory Agency
151 Buckingham Palace Road London SW1W 9SZ
T 0203 080 6000 www.mhra.gov.uk

An executive agency of the Department of Health

Appendix D Ethics Approval for MOP trial from Cranfield University

Cranfield Health

Cranfield UNIVERSITY

Vincent Building
Cranfield University
Cranfield
Bedfordshire
MK43 0AL
England
T: +44 (0)1234 758300
F: +44 (0)1234 758380
www.cranfield.ac.uk/health

Dr Selim Cellek
Cranfield Health
Cranfield University
Cranfield, MK43 0AL

5 July 2013

Dear Selim

Project Reference No 24/13: Metformin in obese nondiabetic pregnant women

Thank you for submitting your application to Cranfield University Health Research Ethics Committee (CUHREC). As the study has already received ethical approval by the London – Surrey Borders Research Ethics Committee and is not being sponsored by Cranfield University I can confirm that the CUHREC Committee is happy for the study to continue.

Please note that applicants will still be responsible for:

- reporting of any adverse incidents occurring during the course of the study to the committee, even if the incident is not directly related to the study (e.g. a complaint by a participant);
- notifying the committee of any major changes to the protocol and obtaining further ethical approval as appropriate;
- notifying the committee when the study has ended.

The committee may revoke approval for a submission if they become aware of any unethical or other improper practices during the execution of the research.

Yours sincerely

pp T. Groves

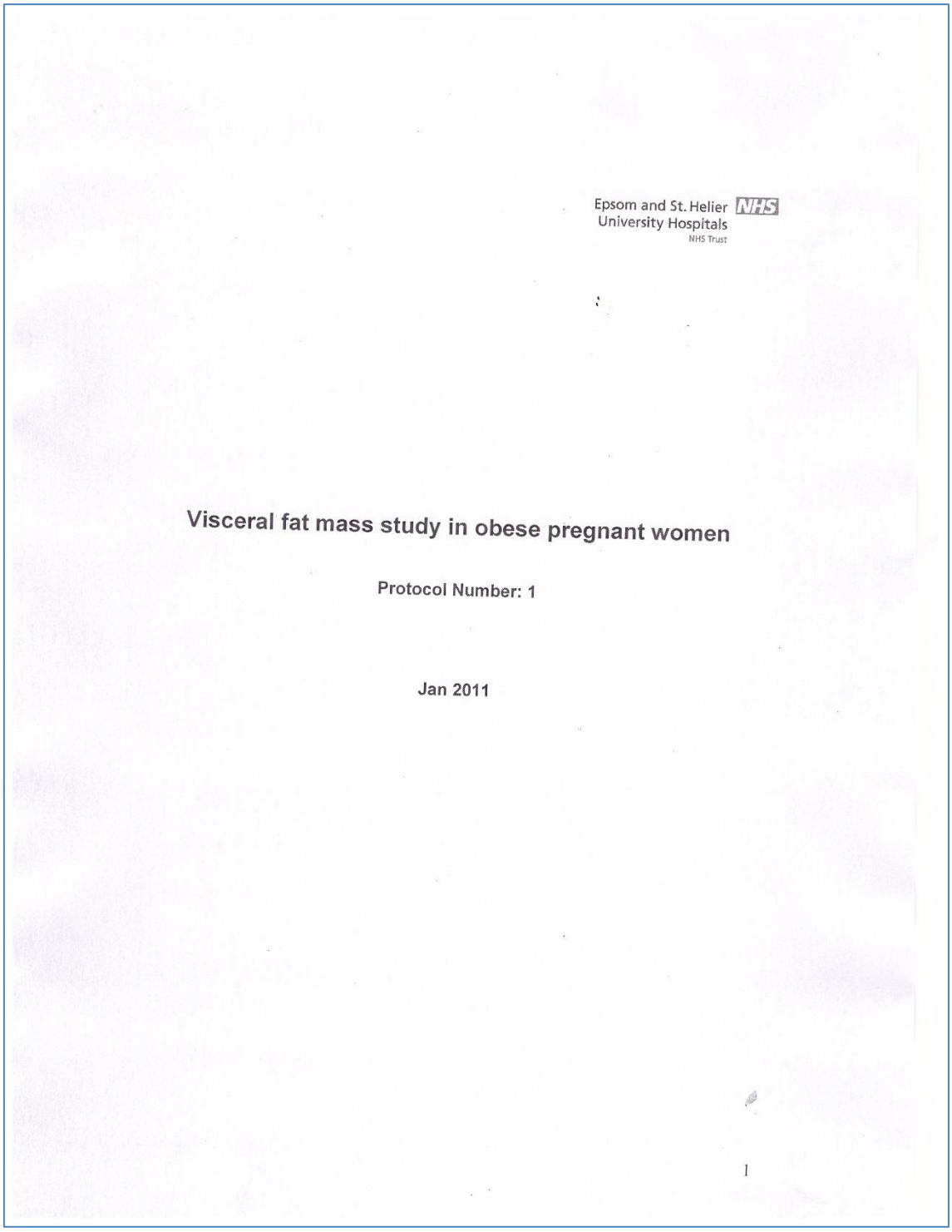
Professor Paul Harrison
Chairman
Cranfield University Health Ethics Committee

REGISTERED

The Queen's Anniversary Prizes
For Higher and Further Education
2011

Appendix E Supporting information for the VFM study

E.1 Protocol of the VFM study


Epsom and St. Helier NHS
University Hospitals
NHS Trust

Visceral fat mass study in obese pregnant women

Protocol Number: 1

Jan 2011

1

INVESTIGATORS

Dr Jyoti Balani

Honorary Consultant in Medicine & Diabetes
Epsom & St Helier University Hospitals NHS Trust
Wrythe lane, Carshalton, Surrey,
SM5 1AA
Tel: 0208296 2114

Mr Hassan Shehata

Consultant & Honorary Senior Lecturer in Maternal Medicine
Epsom & St Helier University Hospitals NHS Trust
Wrythe lane, Carshalton, Surrey,
SM5 1AA
Tel: 0208296 2986
Fax: 0208296 3866

Dr Steve Hyer

Consultant Physician & Endocrinologist
Epsom & St Helier University Hospitals NHS Trust
Wrythe lane, Carshalton, Surrey,
SM5 1AA
Tel: 0208296 2114

TABLE OF CONTENTS

Contents

INTRODUCTION

There is increasing evidence that obesity in pregnancy contributes to increased complications for both mother and baby. Obese women spend an average of 4.83 more days in hospital and the increased levels of complications in pregnancy and interventions in labour represent a 5 fold increase in cost of antenatal care [1] . The costs associated with newborns are also increased, as in babies born to obese mothers there is a 3.5 fold increase in admission to Neonatal Intensive Care Unit (NICU).

Audit data for our population showed that 12% of women have a BMI >30 and 7% with morbid obesity (BMI>35).

Obese women are at increased risk of gestational diabetes (GDM) compared with normal weight women. A prospective study of more than 16,000 patients with body mass index (BMI) between 30 kg/m2 and 40 kg/m^2, showed that the odds ratio (OR) for GDM were 2.6 [95% CI 2.4 to 6.0] compared with women with BMI < 30 kg/m^2 [2].

A meta-analysis on studies conducted in patients with diabetes outside of pregnancy, suggested there was a strong association between measures reflecting abdominal obesity and the incidence of type 2 diabetes, and the pooled odds ratio was 2.14 (95% CI 1.70 to 2.71; P<0.0001) [3]. There is no published data on the possible association of visceral fat mass in pregnancy and risk of gestational diabetes. Hence, the aim of the study is to evaluate the relation of visceral fat mass and the risk of GDM in obese pregnant women with no known diabetes. We want to investigate whether a higher visceral fat mass would increase the risk of subsequent gestational diabetes.

Body composition can be analysed by measuring body impedance using instruments such as Inbody 720^R.This instrument performs body composition analysis using Direct Segmental Multi-Frequency Bioelectrical Impedance Analysis Method (DSM- BIA Method). The InBody 720 gives a quantitative value for the various body compartments which equals the weight of each compartment, when added together they equal the person's weight. It measures

BMI, WHR and various body compartments like lean body mass, total percentage body fat (PBF) and visceral fat mass (VFM). The bioelectrical impedance analysis (BIA) method is based on the electric resistance difference between the fat and components of other organs [4]. The InBody 720 has been validated and correlates well with intraabdominal fat area assessed by DEXA [5]. InBody 720 has also been used in studies of patients with obesity [6] [7].

It will be clinically significant if GDM could be predicted early in pregnancy. Lifestyle and pharmacological interventions to improve baby outcomes could then be tried.

Aims and Objectives

To investigate whether a higher baseline visceral fat mass confers a higher risk of subsequent gestational diabetes in obese pregnant women.

Material and Methods

Ethical Approval

The London-Surrey Borders Research Ethics committee advised us that ethical approval will not be required for the study as all women would only undergo routine clinical investigations and management. No study specific procedure would be undertaken on these women.

Inclusion criteria

- Obese pregnant women
- Gestation between 12 and 18 weeks

Exclusion Criteria

- Pre-existing established diabetes
- Moving out of pregnancy area for pregnancy management

- Multiple foetus

Study methods

The study would be conducted at St Helier Hospital. Women attending the antenatal weight management clinic at St Helier Hospital would be recruited. Their demographic, medical and obstetric history will be recorded.

All women would receive standardised personal advice on healthy eating. They would be encouraged to eat low glycaemic index foods and exercise 30 minutes at least 5 days in a week.

All women underwent body composition analysis using an instrument called Inbody 720^{R} at booking. They also had a 75g oral glucose tolerance test at around 28 weeks of gestation, as per the hospital protocol for a pregnant woman with high BMI. The World Health Organisation (WHO) 1999 criteria for the diagnosis of GDM was used [8].

Statistical Analysis

The sample size is 300 obese pregnant women. The maternal baseline characteristics, pregnancy and neonatal outcomes in patients developing GDM will be compared with those with normal glucose tolerance. The normally distributed data will be expressed as mean ± SD. Welch's t-test will be used to compare means of the two groups. Fisher's test will be used to compare categorical variables and the level of significance will be $P<0.05$.
Data that is not normally distributed will be expressed as median and interquartile range. Non-parametric tests like Mann-Whitney U test will be used to compare these variables between the GDM and no GDM group.

DISSEMINATION OF THE RESULTS OF THE RESEARCH

The dissemination of research findings will include ublishing results in peer-reviewed journals.

- Multiple foetus

Study methods

The study would be conducted at St Helier Hospital. Women attending the antenatal weight management clinic at St Helier Hospital would be recruited. Their demographic, medical and obstetric history will be recorded.

All women would receive standardised personal advice on healthy eating. They would be encouraged to eat low glycaemic index foods and exercise 30 minutes at least 5 days in a week.

All women underwent body composition analysis using an instrument called Inbody 720^{R} at booking. They also had a 75g oral glucose tolerance test at around 28 weeks of gestation, as per the hospital protocol for a pregnant woman with high BMI. The World Health Organisation (WHO) 1999 criteria for the diagnosis of GDM was used [8].

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Data that is not normally distributed will be expressed as median and interquartile range. Non-parametric tests like Mann-Whitney U test will be used to compare these variables between the GDM and no GDM group.

DISSEMINATION OF THE RESULTS OF THE RESEARCH

The dissemination of research findings will include ublishing results in peer-reviewed journals.

BENEFITS OF RESEARCH

We expect benefits in improving pregnancy outcomes in obese pregnant women. The anticipated timescale for the benefits to patients, the public and the NHS will be immediate from the time the project's data are analysed and published in peer review journals. Implementation of the findings of this programme of research into NHS practice will lead to major benefits for mothers with obesity and their babies.

QUALITY CONTROL AND QUALITY ASSURANCE

The study will be conducted in accordance with ICH GCP.

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7. Sartorio A, Malavolti M, Agosti F, Marinone PG, Caiti O, Battistini N, et al. Body water distribution in severe obesity and its assessment from eight-polar bioelectrical impedance analysis. Eur J Clin Nutr. 2005 Feb;59(2):155–60.
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Appendix F Supporting Information for MOP Trial

F.1 Protocol Version 1.8

Epsom and St. Helier NHS
University Hospitals
NHS Trust

Proposed Study

Metformin in Obese non-diabetic Pregnant women

(MOP Trial)

Protocol Number: 1

Version 1.8

3rd September 2014

1

INVESTIGATORS

Epsom and St Helier University Hospitals NHS Trust

Chief Investigator

Mr Hassan Shehata, MRCOG MRCPI
Consultant & Honorary Senior Lecturer in Maternal Medicine
Epsom & St Helier University Hospitals NHS Trust
Wrythe lane, Carshalton, Surrey, SM5 1AA
Tel: 0208296 2140 Fax: 02082962866
Email: Hassan.shehata@esth.nhs.uk

Co- Investigators

Dr Steve Hyer, MD, FRCP
Consultant Physician & Endocrinologist
Epsom & St Helier University Hospitals NHS Trust
Wrythe lane, Carshalton, Surrey, SM5 1AA
Tel: 0208296 2119
Email: steve.hyer@esth.nhs.uk

Dr Jyoti Balani, MD
Consultant in Diabetes
Departments of Diabetes
Epsom & St Helier University Hospitals NHS Trust
Wrythe lane, Carshalton, Surrey, SM5 1AA
Tel: 0208296 2140
Email: jyoti.balani@esth.nhs.uk

Prof Kypros Nicolaides PhD, MRCOG
Harris Birthright Research Centre for Fetal Medicine,
Kings College Hospital Medical School,
Denmark Hill, London, SE5 8RX
Email: FMFEducation@fetalmedicine.com

Dr Ranjit Akolekar, MBBS, MRCOG
Consultant, Fetal Medicine
Medway Hospital NHS Trust
Windmill Road, Gillingham, Kent ME7 5NY
Email: ranjit.akolekar@nhs.net

Dr Antoinette Johnson MRCOG
Consultant in Obstetrics & Gynaecology
Epsom & St Helier University Hospitals NHS Trust
Wrythe lane, Carshalton, Surrey, SM5 1AA
Tel: 02082962140 Fax: 0208296 3866
Email: Antoinette.johnson@esth.nhs.uk

Mr Nick Anim PhD, FRCOG
Kingston Hospital NHS Trust
Galsworthy Rd, Kingston upon Thames, KT2 7Q
Tel: 02089342197 Fax:02089343318
Email: Nick.anim-nyame@kingstonhospital.nhs.uk

Dr Arunava Kundu MD,DCH, MRCP,FRCPCH
Consultant Paediatrician
Queen Mary's Hospital for children
Wrythe lane, Carshalton
Surrey, SM5 1AA
Email: arunava.kundu@esth.nhs.uk
Tel: 02082963021

Dr Rosie O' Neil, BSc Hons, PhD, PCTHLE
Senior Lecturer in Statistics, Faculty of CISH
Kingston University Penrhyn Road,
Kingston upon Thames, Surrey, KT2 2EE

Dr Lesley Roberts BSc (Hons.) MRCOG
Royal Surrey County Hospital NHS Trust
Egerton Road, Guildford GU2 7XX
Email: lesleyroberts@alexander-house.co.uk

Professor Andrew Hattersley
Professor of Molecular Medicine, Peninsula Medical School
Barrack Road, Exeter, EX2 5DW

Dr Andrea Petroczi, MSc, PhD
Reader in Public Health, Kingston University
Penrhyn Road, Kingston upon Thames, Surrey, KT1 2EE

TABLE OF CONTENTS

1 INTRODUCTION

Obesity in Pregnancy has been identified by CEMACH (2008-2011) as a major health risk to mother and baby. There is substantial evidence that obesity in pregnancy contributes to increased morbidity and mortality for both mother and baby. CEMACH found that approximately

- 35% of women who died (who had a recordable BMI in the 2000-2003 triennia were obese (i.e. had a BMI of 30 or greater)
- 30% of the mothers who had a stillbirth or a neonatal death were obese

Obese women spend an average of 4.83 more days in hospital and the increased levels of complications in pregnancy and interventions in labour represent a 5 fold increase in cost of antenatal care (Galtier-Dereure et al, 2000). The costs associated with newborns are also increased, as in babies born to obese mothers there is a 3.5 fold increase in admission to Neonatal Intensive Care Unit (NICU).
Audit data for our clinic population showed that 12% of women have a BMI >30 and 7% with morbid obesity (BMI>35). In an audit of pregnancy outcomes for women with Gestational Diabetes Mellitus, we found a 20% birth weight centile reduction in metformin versus insulin in women when metformin was started after 28 weeks of gestation.

The risks of obesity in pregnancy are:-

- Antepartum risks
  - pregnancy-induced hypertension/PET
  - Miscarriages
  - gestational diabetes
  - TED
  - Ultrasound difficulties
- Intrapartum risks
  - labour induction, CS, and failed VBAC, perineal trauma
  - Dystocia
  - Anaesthetic risks
  - Surgical difficulties
- Postpartum risks
  - increased rates of puerperal infection
  - decreased rates of breastfeeding initiation or continuation
  - PND
  - TED
- Offspring risks
  - higher risk for having congenital anomalies
  - Stillborn
  - Childhood and adult obesity

A population-based cohort study of 120,251 pregnant, obese women delivering full-term, liveborn, singleton infants was examined to assess the risk of four pregnancy outcomes

including pre-eclampsia (PET), Caesarean section (CS), small for gestational age (SGA), and large for gestational age (LGA) by obesity class and total gestational weight gain. The results showed that gestational weight gain incidence for overweight or obese pregnant women, less than the currently recommended 15 lb, was associated with a significantly lower risk of PET, CS, and LGA and higher risk of small for gestational age birth. The authors concluded that limited or no weight gain during pregnancy in obese pregnant women results in a more favourable pregnancy outcome. The same study showed that in obese women the LGA incidence is 15%. (Kiel et al 2007)

Metformin improves insulin sensitivity, reduces hepatic gluconeogenesis and increases peripheral glucose uptake. It also reduces fasting serum insulin by 40% and causes weight reduction by 5.8%. It is eliminated by kidney and has increased clearance in pregnancy. It does cross placenta. Retrospective data has shown no serious adverse events. The Metformin in GDM (MiG) study (Rowan et al 2008) concluded that in women with GDM, metformin (alone or with supplemental insulin) is not associated with increased perinatal complications as compared with insulin. The women preferred metformin to insulin treatment. Retrospective data from UK (n=100) showed no increase in PET/perinatal loss, neonatal morbidity (Glueck et al 2004). Other studies showed no evidence of teratogenicity (Diabetes control group 1996) and no increase in birth defects and PET (Diabetes in Pregnancy Consultation Document 2007). CEMACH (2005) showed that growth and development of offspring is normal at 18 months.
Studies in obese women with polycystic ovary syndrome using metformin in a maximum dose of 2.5 g/day has shown beneficial effects on insulin resistance and weight gain (Glueck et al, 2004). In an audit of pregnancy outcomes for women with gestational diabetes treated with metformin, we found that 34% of the women required more than 1.5g/day of metformin to achieve therapeutic efficacy (Balani et al, 2009). Also in the MiG Trial, a maximum dose of 2.5 g/ day of metformin was used in the treatment of gestational diabetes. Studies on the effect of pregnancy on the pharmacokinetics of metformin have concluded that the clearance of metformin increases in pregnancy as a result of enhanced renal elimination and hence possibly a larger dose of Metformin is required to maintain therapeutic efficacy (Hughes RC et al, 2006). This study will be investigating women who are very obese (BMI>35) and hence will need a larger dose of metformin. We intend to titrate the dose gradually to a maximum dose of 3 g/ day

Three small randomized controlled trials evaluated metformin for the treatment of obesity among non pregnant adolescents. These studies did show modest reductions in BMI (approximately 1-2 kg/m^2) and weight (3 to 4 kg).

We aim to assess the efficacy of Metformin in the management of obesity in pregnancy.

2 OBJECTIVES

Title of Research

Does metformin improve pregnancy outcomes (incidence of LGA (≥90% birth weight centile) babies, onset of maternal GDM, hypertension, PET, macrosomia, shoulder dystocia, admission to SCBU) in obese non-diabetic women?

Purpose of proposed investigation

The purpose of the study is whether management of obese non-diabetic pregnant women with standarised life style intervention (diet and physical activity) and metformin will lead to improved maternal and perinatal outcomes compared to life style intervention alone.
We aim to compare perinatal outcomes in women randomised to the two groups.

Group 1: Standarised life style intervention and placebo
Group 2: Standarised life style intervention and metformin

3 STUDY DESIGN

3.1 Design

It is a randomised, double blind, placebo controlled trial.
Women (non-diabetic at booking) with BMI>35 will be recruited at ≈ 12-18 weeks of gestation. Women will be randomised to the two groups after a written informed consent has been obtained. Women will undergo an oral glucose tolerance test (OGTT) at recruitment.

The women will have fasting insulin measurements along with the fasting glucose test at baseline during the initial OGTT. It will allow us to examine the hypothesis that metformin is most effective in those patients with the highest insulin resistance. The serum will be separated and frozen at -20° C and fasting serum insulin measurements as well as fasting glucose and the 2-hour glucose measurements would be carried out on a later date as a batch to avoid inter-assay error. Blood samples will also be collected for HbA1c, CRP, C-peptide, Leptin and Adiponectin. These blood tests will be repeated at 22 wks, 28 wks and again at 36 wks. All women will have their urine tested & recorded for ketones at every visit.

We will also be collecting blood samples from all women to test the FTO gene variant and its association with the metformin response.. 5 ml of blood would be collected in a EDTA bottle and frozen at -80° C. The blood could be collected any other time if the test is missed out at the first blood collection. Informed consent would be taken specifying that the blood would be frozen for genetic testing at a later date. Hattersley et et al have found a strong link between the FTO gene variant and body mass index while they were doing a genome wide search for susceptibility to type 2 diabetes. The strength of the genetic influence depends on whether an individual has inherited one or two copies of the FTO gene variant. (Frayling et al, 2007)

All additional blood tests mentioned above will be carried out only in centres equipped with facilities for blood collection, processing and storage. If any centre cannot store its own blood samples, these samples will be shipped to another participating study site for storage. The patient would be informed about this.

Women will have C-reactive protein (CRP) measurements once each trimester. Central obesity is a state of chronic inflammation and CRP is an important marker for inflammation (Dullaart 2008).

We wish to analyse body composition by bioimpedence using InBody 720, which is a body composition analyser after the end of 1st trimester of pregnancy. We would measure skeletal muscle mass, soft lean mass, fat free mass, Percent Body fat, Waist Hip ratio, Visceral Fat Area, obesity degree and various other parameters. These measurements would be done around 12-14 wks, 16 wks, 22 wks, 28 wks, 32 wks and 36 wks and after

birth of baby. Insulin resistance is thought to be mediated largely by visceral fat. However, there is controversy regarding the specific mechanism by which fat in the visceral compartment confers greater risk than subcutaneous fat. Many investigators have suggested that one or more moieties secreted by the visceral adipocytes might mediate insulin resistance, such as the free fatty acids themselves (portal theory) or the adipose tissue related cytokines (adipokines) such as interleukin-1, interleukin-6, and tumour necrosis factor α, resistin, or a reduction in adiponectin which has been repeatedly shown to be associated with reduced insulin resistance. (Bergman et al 2006). We would be able to examine whether treatment with Metformin reduces insulin resistance by altering the visceral fat content and its effect on the various cytokine levels.

Women will complete the "Epworth Sleepiness scale" questionnaire at recruitment and in the middle of the study.

3.2 Randomisation

Randomisation will be performed centrally using computer generated random numbers.

Obese women with BMI > 35 attending the weight maintenance clinic will be randomised to 2 groups, control and treatment group.

3.3 Treatment

Women in the two groups will receive the following treatments.

Group 1 (Control): Standardised dietary advice and exercise advice by midwife/doctor (minimum of 30 minutes brisk walking per day) plus **2** placebo tablet with each meal.

Group 2 (Treatment) Standardised dietary advice and exercise advice by midwife (minimum of 30 minutes brisk walking per day) plus oral metformin 500mg 2 tablets with each meal.

Women in Group 1 will receive 2 Tablets of placebo daily on their first visit after inclusion into trial. The dose of the placebo will be increased gradually 1 tablet every week to reach a maximum dose of 2 tablets 3 times a day with meals.
Women in Group 2 will receive 2 Tablets of Metformin 500mg daily on their first visit after inclusion into trial. The dose of Metformin will be increased gradually 1 tablet every week to reach a maximum dose of 2 tablets 3 times a day with meals. This gradual increase in doses will reduce the possible side effects of Metformin.
All women will be assessed clinically including weight, BP, urine protein, maternal and fetal assessment by midwife and fetal heart sounds.
The women in Group 1 and 2 will then be seen every 4-6 weeks till term. They would undergo a second oral glucose tolerance test at 28 weeks. The medication will be stopped for 1 week prior to the test.

Women with positive results for oral GTT done at 28 weeks in both groups will be referred to the diabetic clinic for further management. They would continue their trial medications as before. If target blood glucose values are not achieved, insulin will be added to their existing trial medications.

Table 1

Gestation		
≈ 10-12 wks Visit 1	Offer of recruitment to women attending the obesity antenatal clinic/ nuchal scan clinic with BMI > 35 (Excluding known diabetics)	
≈12-14 wks Visit 2	Informed consent Oral Glucose Tolerance Test (1) Fasting serum Insulin, HbA1c, C-peptide, CRP, Leptin, Adiponectin, FTO gene variant analysis Serum samples for all the above tests frozen for measurements to be done at a later date Clinical review, Wt, BMI, Body comp analyser (InBody 720) Randomise	
	Group 1 (control) Low GI diet, Exercise +	Group 2 (treatment) Low GI diet, Exercise +
Week 12	Start Tab Placebo 1-1	Start Tab Metformin 500 1-1
Week 13	↑ Tab Placebo 1-1-1	↑ Tab Metformin 500 1-1-1
Week 14	↑ Tab Placebo 2-1-1	↑ Tab Metformin 500 2-1-1
Week 15	↑ Tab Placebo 2-1-2	↑ Tab Metformin 500 2-1-2
Week 16	↑ Tab Placebo 2-2-2	↑ Tab Metformin 500 2-2-2

(if starting at week 14, finish at week 18)

≈ 16wks Visit 3	Clin review, wt, Body comp analy continue placebo	Clin review, wt, Body comp analy continue metformin	
≈ 22 wks Visit 4	Repeat blood tests* Clin review, wt, Body comp analy Anomaly scan	Repeat blood tests* Clin review, wt, Body comp analy Anomaly scan	
≈ 27 wks (Telephone)	stop placebo	stop metformin	
≈ 28 wks	OGTT (2) Repeat blood tests* Clin review, wt, Body comp analy ↓ Negative GTT Restart placebo	OGTT (2) Repeat blood tests* Clin review, wt, Body comp analy ↓ Negative GTT Restart metformin	*Positive GTT* *Ref to Diabetes clinic* *(Table 2, Appendix2)*

≈ 32 wks	Clin review, wt, Body comp analy Growth scan Continue Placebo	Clin review, wt, Body comp analy Growth scan Continue Metformin
≈36 wks	Repeat blood tests* Clin review, wt, Body comp analy Growth scan Continue Placebo upto term	Repeat blood tests* Clin review, wt, Body comp analy Growth scan Continue Metformin upto term
At Birth	Record baby outcome + anthropometric measurements	Record baby outcome + anthropometric measurements
6-8 weeks postnatal	Wt, Body composition analysis	Wt, Body Composition Analysis
Baby at ≈ 2 yrs of age	↓ anthropometric measurements + Schedule of Growing Skills	↓ anthropometric measurements + Schedule of Growing Skills

*HbA1c, C-peptide, CRP, Leptin, Adiponectin
All women will have their urine ketones tested and recorded in every visit.

Table 2

Gestation	Women with positive OGTT (2) done at 28 wks ↓
≈ 28-30 wks	Refer to diabetic clinic Commence Home Blood Glucose monitoring Continue the current medications Clin review, wt, Body comp analy Growth scan Add Insulin if blood glucose targets not achieved
≈ 32 wks	Continue trial medication +/- Insulin Clin review, wt, Body comp analy Growth scan
≈ 36 wks	Repeat blood tests* Clin review, wt, Body comp analy Growth scan Continue trial medication ±Insulin till term
At birth	Record baby outcomes + anthropometric measurements
6-8 wks postpartum	Repeat Oral GTT (3), Wt, Body composition analysis
Baby at ≈ 2 yrs of age	anthropometric measurements + Schedule of Growing Skills

* HbA1c, C-peptide, CRP, Leptin, Adiponectin
All women will have their urine ketones tested and recorded in every visit.

Trained personnel will perform anthropometric measurements including crown-heel length, crown rump length, head circumference, chest circumference, abdominal circumference, mid upper arm circumference, triceps skin fold thickness and subscapular skin fold thickness (to be done extra for trial), on the baby at around 48 hours of birth.

At 6-8 weeks postpartum, women are seen again. The women's weight, blood pressure and postpartum OGTT results (in patients who develop Gestational Diabetes) are recorded.

At around 2 years of age details of infants health are recorded and anthropometric measurements are repeated in addition to Schedule of Growing Skills and neurodevelopmental milestones.

A cohort of children will have more detailed neurodevelopmental assessments at 2 yrs of age in centres with suitable facilities.

Duration of treatment

Treatment will be continued till delivery of the baby. The procedures at each visit have been outlined in the study scheme in Appendix 1.

Supporting Information on Metformin and Placebo

Metformin Tablets, 500mg

Manufacturing Authorisation Holder: Relonchem Limited
Product Licence number: PL 20395/0027.
Repackaged and labelled in packs of 84 by the manufacturer of placebo

Placebo tablets (Lactose Tablets)

Manufacturer: University College London Hospitals NHS Foundation Trust
Manufacturer's Licence Number: MIA (IMP) 17022.

Packed and labelled in packs of 84

Formula for Placebo tablets:

Lactose Ph Eur	84.25%w/w
Microcrystalline cellulose (Avicel®PH 102)	15.00 w/w
Magnesium Stearate BP	0.75 %

Finished Product Specifications

TEST	SPECIFICATION	
	METFORMIN	PLACEBO
Appearance	Plain, white coloured film coated round biconvex tablets	Plain, white uncoated round biconvex tablets
Diameter	11.0 ± 0.1mm	11.0 ± 0.1mm
Thickness	6.0 ± 0.2mm	6.0 ± 0.2mm
Friability	As per marketing authorisation	Complies with B.P.
Average Weight	590.77 mg ± 5%	Approximately **625** mg
Uniformity of weight	As per marketing authorisation	Complies with B.P.
Disintegration Time	NMT 30 minutes	NMT 30 minutes
Dissolution test	NLT 70% after 45 mins	Not tested
Identification - Metformin	As per marketing authorisation	Absent
Assay	As per marketing authorisation	Not tested
Microbiological Quality	As per marketing authorisation	According to Ph Eur Category 3B

Setting: Multicentre Trial

Mr Hassan Shehata will be the Chief Investigator of the clinical trial and will coordinate the research at all centres.

Site	Investigator
Epsom and St. Helier University Hospitals NHS Trust, Wrythe Lane, Carshalton, Surrey SM5 1AA	Chief Investigator- Mr Hassan Shehata Co-Investigators- Dr Steve Hyer, Dr Jyoti Balani, Dr Antoinette Johnson Dr Arunava Kundu Research Midwife-Zeni Koutsi
Kings College, London, Denmark Hill, London, SE5 8RX	Principal Investigator :Professor Kypros Nicolaides Co-Investigator Research Midwife- Argyro Syngelaki
Medway Hospital NHS Trust Windmill Road, Gillingham, Kent ME7 5NY	Principal Investigator- Dr Ranjit Akolekar, MBBS, MRCOG Consultant Fetal medicine
University College Hospital, London 25 Grafton Way, London WC1E 6DB	Principal Investigator Prof Kypros Nicolaides
Kingston Hospital NHS Trust Galsworthy Rd, Kingston upon Thames, KT2 7QB	Principal Investigator Mr Nick Anim PhD, MRCOG
Southend Hospital NHS Foundation Trust, Prittlewell Chase, Westcliff on sea Essex SS0 0RY	Principal Investigator Prof Kypros Nicolaides
The Princess Alexandra Hospital Hamstel Road, Harlow, Essex CM20 1QX	Principal Investigator Prof Kypros Nicolaides
Royal Surrey County Hospital, Egerton Road, Guildford, GU2 7XX	Principal Investigator Dr Lesley Robert

3.4 Data recording

The Consultant and his healthcare team would record the clinical data of the participants at each antenatal visit.
Delivery notes and neonatal outcomes for each participant would be recorded by the healthcare team.

Monitoring of subject compliance

Subject compliance with Metformin treatment will be monitored with tablet counts at each antenatal visit.

4. SELECTION OR WITHDRAWL OF SUBJECTS

4.1 Inclusion Criteria

Subjects must meet all the following criteria:

i. Obese pregnant women with BMI > 35
ii. Informed written consent.

4.2 Exclusion criteria:

i. Diabetes at booking
ii. Presence of contra-indication to metformin (renal, liver, heart failure)
iii. Moving out of study area for pregnancy management
iv. Participants who suffer with hyperemesis.
v. Participants who are 18 years and below.
vi. Participants with significantly raised creatinine
vii. Participants with high alcohol intake.

Participants will be advised not to take metformin within 48 hours of having a General Anaesthetic.

If participants were to undergo an emergency operation it would be ensured that the anaesthetist is informed that they have been taking metformin.

Withdrawal of subjects

An obese pregnant women participating in the study would be withdrawn if her fetal growth scan shows

Estimated fetal weight (efw) < 5^{th} centile
and
Either reduced end diastolic flow in the umbilical artery/
reversed flow in the ductus arteriosus

Or oligohydramnios- amniotic fluid index < 2cm

5. OUTCOME MEASURES

5.1 Outcomes

Primary outcome:
Birth weight centile (z-score)

Secondary Outcomes:
Maternal outcomes
a. Maternal weight gain
b. Maternal development of GDM
c. Maternal development of hypertension/PET
d. Caesarean section
e. Postpartum haemorrhage
f. Maternal Insulin Resistance and relation to metformin efficacy
g. Changes in circulating levels of cytokines including CRP, adiponectin, leptin and metabolic markers like uric acid and lipid profile.
h. FTO gene variant and its relation to baby outcome
i. Changes in body composition as measured by Bioimpedence – comparison between the two groups

Neonatal Outcomes
a. Neonatal Hypoglycaemia – 2 capillary plasma glucose levels <2.6 mmol/l at least 30 minutes apart.
b. Prematurity < 37 weeks gestation
c. Hyperbilirubinemia requiring phototherapy
d. Polycythaemia – cord blood haematocrit > 0.6
e. Respiratory distress – 4 or more hours of respiratory support or oxygen with associated diagnosis
f. Macrosomia/LGA - Birth weight >90th centile based on appropriate growth standards
g. Birth trauma – shoulder dystocia, brachial plexus injury or fracture.
h. Apgar score < 6 at 5 minutes.
i. Admission to level 2 or greater neonatal unit including length of stay.
j. Stillbirth/ Intrauterine deaths
k. 2nd trimester miscarriages
l. Neonatal body composition including skin fold thickness

Psychological outcomes:

Satisfaction with care will be measured using the National Survey for Women's Experience with Maternity Care, which is currently being used by the National Perinatal Epidemiology Unit, Oxford, to survey 4,800 women's experience in the UK. This comprehensive measure covers a range of pregnancy, birth, and postnatal factors, as well as cross-cutting themes such as information, choice, and quality of care. This will enable us to compare women's experience of maternity care within the trial.

Economic outcomes:

A cost effectiveness evaluation will be undertaken in relation to the two randomised groups. The analysis will consider costs associated with the intervention (metformin). Incidence and costs of ante-natal care and complications during and following delivery will be derived. The principal measure of effectiveness will be the neonatal morbidity. Bootstrap estimates will be derived to determine the incremental cost effectiveness ratio (ICER).

5.2 Indication of timescale and milestones to be achieved

Our proposal is a 4.5 year project, all of which will be completed, data analysed and papers written before the 4.5 years are out.

October 2010 – December 2014 Recruitment and consenting of patients and data collection

January 2015 – June 2015: Data completion, analysis and writing up.

6. ASSESSMENT OF SAFETY

The safety measures to be measured in the study will be as follows

Physical examination

Patients will be examined at baseline, at each antenatal visit and at time of delivery. Any change in findings will be recorded.

Adverse events

Adverse events will be recorded in the patient's notes and then transferred to the case record form. Details recorded will include the nature of he event, time of onset, severity and treatment needed.

The investigators will immediately notify the sponsor of all serious adverse events.

Hospitalisation for the following in pregnancy would not be considered as Serious Adverse Events

1. Irregular uterine contractions for observation
2. Bleeding PV for observation
3. Show or Spontaneous Rupture of membranes

7. STATISTICS

(1) Sample size calculations
Assuming power 80%, significance level 5% and 2-sided testing, we will recruit 200 women per arm. This will allow the detection of a difference in mean centile (z-score) of 0.30 standard deviations and allows for a drop out rate of 20%.

This can usefully be translated into the equivalent difference in the proportion large-for-gestation (LGA) assuming that the z-score follows a Normal distribution. It equates to a reduction in LGA from 19.7% in the untreated group to 11.2% in the treated group, a relative change of 40%.

We would like to recruit 50 more women into the study in order to ensure that the study is adequately powered.

(2) Statistical analysis

The primary outcome and all continuous secondary outcomes will be compared in the two groups using a 2-sample t tests or paired t-tests as appropriate. The results will be presented as mean differences (in centiles) with 95% confidence intervals (CI). Where appropriate data may be transformed to produce approximately Normal distributions to validate parametric tests or alternatively non-parametric tests will be used if more suitable. In general, binary outcomes will be analysed and presented as relative risks with corresponding 95% Confidence Intervals. All analysis will be conducted according to the intention to treat.

8. DISSEMINATION OF THE RESULTS OF THE RESEARCH

The dissemination of research findings will include presentations at scientific meetings and publishing results in peer-reviewed journals.

9. BENEFITS OF RESEARCH

We anticipate improved pregnancy outcomes for women treated with metformin. Furthermore, we expect improved patient satisfaction. We also anticipate major incremental cost effectiveness ratio and good economic value from the planned interventions.

The anticipated timescale for the benefits to patients, the public and the NHS will be immediate from the time the project's data are analysed and published in peer review journals. Implementation of the findings of this programme of research into NHS practice will lead to major benefits for mothers with obesity and their babies.

10. CRITERIA FOR SUSPENSION OF THE TRIAL

We will be appointing a Data Monitoring Committee (DMC) comprising two experienced clinicians and a biostatistician and which will operate according to the Damocles guidelines (DAMOCLES Study Group, Lancet 2005 365) to monitor the ctrial conduct and review interim data with respect to adverse events and patient safety. The DMC will report to the trial steering committee and may recommend that the trial is suspended if there is excessive unexplained maternal morbidity or mortality and/or excessive fetal morbidity or mortality.

11. ACCESS TO SOURCE DATA

Direct access to all source data will be available to the sponsor for purposes of monitoring and audit and to relevant regulatory authorities for the purpose of inspection.

12. QUALITY CONTROL AND QUALITY ASSURANCE

The study will be conducted in accordance with ICH GCP.
Quality assurance will take the form of an audit by the sponsor.
All investigators will be GCP trained.

13. ETHICS

Ethics approval will be obtained from the London Surrey Borders Research Ethics Committee before the study commences.
The subject's general practitioner will be informed of the intention to enrol a subject into the study.
No study- specific procedures will be undertaken on any subject until that subject has given written informed consent using the documentation in Appendix 2.

14. DATA HANDLING AND RECORD KEEPING

All data will be collected on a paper case record form using only code numbers to identify individual subjects. No data from which an individual subject could be identified will be recorded on this case record form.
Source data will be stored for a minimum of 5 years in adults and to the age of 18 years for babies.

15. REFERENCES

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APPENDIX 1
Study Scheme

	Screening	Control Group								Treatment Group							
	BMI > 35																
Visit No		1 Screen	2 Recruit ,gtt	3-4	5	6	7	8-9	10	1	2	3-4	5	6	7	8-9	10
Week of Gestation	booking	≈ 10-12	≈ 12-14	≈ 16+22	≈ 28	≈ 32	≈ 36	Postnatal	≈ 2 Years	≈ 10-12	≈ 12-14	≈ 16+24	≈ 28	≈ 32	≈ 36	postnatal	≈ 2 years
Informed Consent			X								X						
Inclusion/ Exclusion Criteria		X								X							
Clinical Examination BP, Urine, Body Comp analyser	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	
Oral Glucose Tolerance Test	(freez samp)		X		X*						X		X*				
Fasting Insulin FTO gene	X (freez samp)		X								X						
CRP, HbA1c, Leptin, Adiponectin			X	X	X		X				X	X	X		X		
Adverse Event Enquiry				X	X	X	X	X				X	X	X	X	X	
Dispensing of Placebo/ Metformin tablets			X	X	X	X	X				X	X	X	X	X		
Recording baby outcome at								X								X	

30

birth																	
Schedule of Growing Skills									X								X

*Stop trial medications for 1 week prior to test

APPENDIX 2

Women with BMI > 35 with positive Oral GTT (2) results done at 28 wks

Joint antenatal diabetic clinic

Week of Gestation	≈ 28	≈ 32	≈ 36	At birth	At 6-8 weeks	≈ 2 years
Visit No	5	6	7	8	9	10
Clinical Examination BP, Urine, Body Comp analy	X	X	X		X	
Oral Glucose Tolerance Test	X				X	
CRP , HbA1c, C-peptide, Leptin, Adiponectin	X		X			
Original medications (Metformin/Placebo)	X	X	X			
± Insulin	X	X	X			
Recording baby outcome at birth				X		X
Schedule of Growing Skills						X

F.2 Patient Information sheet for the MOP Trial

PATIENT INFORMATION SHEET

Epsom and St. Helier NHS
University Hospitals
NHS Trust

Date: 03/09/2014
Version 1.7

Study Title

Metformin in Overweight Non-diabetic Pregnant Women (MOP) Trial

Invitation

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish.
Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

We aim to assess whether metformin will achieve less weight gain and avoid pregnancy complications in overweight women and improve the baby outcome.

Why have I been invited?

All pregnant women with Body Mass Index >35 attending the antenatal overweight clinics in our Hospital and who are not diabetic (high blood sugar) will be invited to participate in the study. Women with diabetes will not be included. Women who are more than 24 weeks of gestation will not be included in the study.

Do I have to take part?

It is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

What will happen to me if I take part?

We will have 2 groups: Control group and the treatment group.

Group 1(control) will receive diet and exercise advice and placebo
Group 2(treatment) will receive diet and exercise advice and metformin tablet

A placebo is a tablet, which appears similar to the actual medicine tablet but has no ingredients of the drug. To try to make sure the groups are the same to start with, each patient is put into a group by chance (randomly). You have equal chance of falling into either group.

What will I have to do?

You will need to come regularly for your antenatal visits to the weight management clinics. You will be a part of the trial until your delivery and the immediate postnatal period. Your baby data will also be recorded by the healthcare team. Your baby will have another assessment at 2 years of age.

You will receive all the standerdised treatment during your pregnancy and delivery. No normal treatment will be withheld from you at any point in the study.

You should take the Metformin tablets or placebo tablets regularly as directed and you can continue to take your regular medications or other prescribed or over-the-counter drugs.
If you need to undergo an emergency operation, you must stop taking metformin or placebo tablets immediately and inform your doctors including the anaesthetist that you have been taking **part** in a trial **of** metformin or placebo**.**

Also, you should not restart your metformin or placebo tablets within 48 hours of having a general anaesthetic.

What is Tablet Metformin?

This is a tablet given by mouth, which works by making the body more sensitive to insulin. With Metformin, we expect women to gain less weight in pregnancy and therefore avoid complications and improve outcome for both mother and baby.
Metformin has been used for several years to treat diabetics, but has only been used recently to treat pregnant women with diabetes.

Is Metformin safe for the baby?

Experience from several years from using Metformin in a condition called Polycystic Ovary Syndrome, shows that it does not cause harmful effects to the baby when used in pregnancy. A very big study in Australia (MiG Trial) confirmed its safety and

effectiveness in pregnant women with gestational diabetes. The government agency that looks at all treatments (NICE, the National Institute of Clinical Excellence) has recently approved its use in pregnant women with diabetes. Despite this, at the present time, Metformin is not licensed for use in pregnancy. The advice on the data sheet for Metformin that advises that it should not be used in pregnancy should be disregarded.

Are there any side effects?

Metformin can cause abdominal discomfort, nausea, vomiting, diarrhoea and disturbed taste. These side- effects are reduced by starting with a low dose and slowly building up. Side-effects are much less if the drug is taken with food rather than on an empty stomach. Very rarely, it can cause a skin rash, in which case, we would have to discontinue it.

Would any special blood tests be done?

Yes. Scientists have found a strong link between a particular gene and body mass index. A blood sample would be collected and frozen to allow future genetic (DNA) analysis relating to analysing genetic factors related to the outcome of pregnancy for you and your baby.

If your hospital is unable to store your blood samples, it will be securely shipped to another participating site for storage and you will be informed about it.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. Your GP will be informed about your participation in the study.

What if relevant new information becomes available?

Sometimes we get new information about the treatment being studied. If this happens, your research doctor will tell you and discuss it with you.

What will happen if I don't want to carry on with the study?

You can withdraw from treatment but keep in contact with us to let us know your progress. Information collected up to your withdrawal may still be used. However, if you do not want us to use any of your information, we could also do that and your information could be completely deleted from the study.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions.

What kind of information would you collect?

The pregnancy information required is standard clinical information regarding weight gain, blood pressure, glucose control and details of the delivery. We will also record details of the baby including baby weight and any complications.

How long will you store the information specific to the trial?

The entire information specific to the trial will be safely stored electronically as well as paper case record forms for 5 years. The electronic data will be deleted and the case record forms will be destroyed in a paper shredder at the end of 5 years.
You can write to the researcher for a copy of the information held on you pertaining to the study.

What will happen to the results of the research study?

The results of the study will be presented in scientific conferences and local groups and through these to patients.
Also results will be published in peer-reviewed journals.

Who is organising and funding the research?

The research has been organised and funded by the Research and Development Department, Epsom & St Helier NHS Trust.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by London Surrey Borders Research Ethics Committee.

A copy of this information sheet and a copy of your signed consent form will be given to you to keep.

For further information, contact
MOP Team Tel: 02082962140
Mr Hassan Shehata- Chief Investigator
Dr Jyoti Balani- Co-Investigator
Dr Antoinette Johnson Co-Investigator
Katherina Gross-Gibbs Midwife 02082962764
Epsom and St Helier University Hospitals NHS Trust

F.3 Consent Form

CONSENT FORM

Epsom and St. Helier University Hospitals NHS Trust

03-09-2014 Version 1.8

Title of Project: Metformin in Obese Non-diabetic Pregnant Women (MOP) Trial

Name of Researcher: Mr Hassan Shehata
Consultant & Honorary Senior Lecturer in Maternal Medicine
Epsom & St Helier University Hospitals NHS Trust

Please initial box

1. I confirm that I have read and understand the information sheet dated 03-09-2014 ☐
   Version 1.7, for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the research group, from regulatory authorities or from the Epsom & St Helier university Hospitals NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. I agree to my GP being informed of my participation in the study ☐

4. I agree to a sample being taken to allow future genetic (DNA) analysis relating to analysing genetic factors related to the outcome of pregnancy for mother and baby. ☐

5. I agree to take part in the above study. ☐

_____	__ _____	_____
Name of Patient	Date	Signature
_____	____ _____	_____
Name of Person taking consent	Date	Signature

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

F.4 Clinical Trial Information Card

Epsom and St. Helier NHS
University Hospitals
NHS Trust

Clinical Trial Information Card

Name of Clinical Trial: Metformin in obese non-diabetic pregnant women (MOP Trial)

Name of Chief Investigator- Mr Hassan Shehata

If you have any questions during your clinical trial, you can contact either
Zeni Koutsi - Research Midwife on 02082962764 / Pager: 07659108486 or
Dr Jyoti Balani – Clinical Research Fellow on 02082962140 between 9am and 5 pm Monday to Friday. This number has a message service if we are unable to answer the telephone when you call.

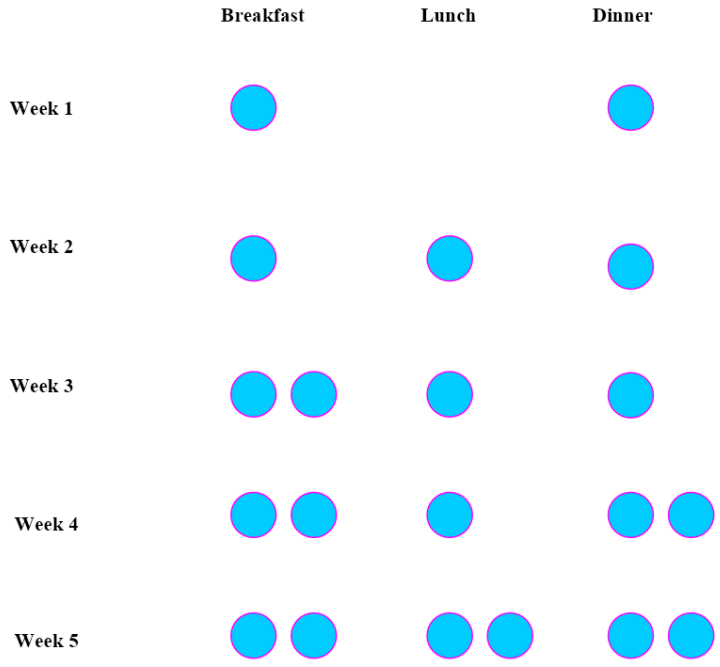
If you are unwell, please contact the labour ward at St Helier Hospital on 02082962479 or the labour ward at Epsom Hospital on 01372 735208

In case of emergency, if code break is necessary, please contact Mr Issac Manyonda on 07956212036 for code break information only.

F.5 Dose escalation sheet

PATIENT ID NUMBER **Start date:**

Participant Information Leaflet On Medication Dose escalation for MOP Trial



Dose changes would be made on Wednesday of each week.

For any queries Contact Zeni Koutsi Research Midwife on 02082962764
Pager 07659108486 or
Dr Jyoti Balani on 02082962140
between 9 am and 5pm Monday to Friday

F.6 Clinical Trial Prescription Form

Epsom and St. Helier NHS University Hospitals NHS Trust

CLINICAL TRIAL PRESCRIPTION FORM

Clinical Trial Name	**MOP**
Protocol Number	**WCH/2008/001**
EudraCT Number	**2008-005892-83**
Patient Name/ Date of Birth	
Hospital Number	
Patient Randomisation number	
Visit Date	

Items to be dispensed

Screened by ……..
(Not applicable to KCH)

Medication	Form	Directions	Dispensed by/ Checked by	Date
Metformin 500mg/ Placebo	**Tablet**	**As directed by the doctor**		

SUPPLY: 1 month per visit (2 bottles x 84 tablets)

Prescribing Doctor's Signature: Date:

Prescribing Doctor's Name (Print): Bleep/ extension no:

Collected by: Date:

Patient Advice and Liaison service (PALS) 02082962508
Main Switchboard 02082962000

F.7 Chemical pathology form

For lab use only

Stick barcode numbers here

Epsom and St. Helier NHS University Hospitals NHS Trust

Chemical Pathology MOP study only

Pathology Request form

Please apply clinical trial label

Ward/Dept **MOPZ1**

Consultant Code **MOPZ1**

Ext 2140 Mr Shehata's PA

Send report to MOP Trial Co-ordinator,.

Specimen Type Blood ☐ Urine ☐ Other ☐ Date of Sample __/__/__ Time of sample (24 hr clock) ____

GESTATION:

Clinical features (if any)

Panel 1	**Panel 2**	**Panel 3**	**Panel 4**
At Recruitment ☐ HBA1c CRP LEPTIN/ADIPONECTIN Samples required: 1 EDTA (purple top) 2 clots (yellow/gold top)	At Recruitment ☐ Fasting Insulin FTO gene Samples required: 1 EDTA (purple top) 1 clot (yellow/gold top)	At Recruitment ☐ Oral Glucose Tolerance Test Samples required: 1 fluoride (grey top) at time = 0 1 fluoride (grey top) at time =120	28 weeks ☐ Post natal ☐ Oral Glucose Tolerance Test Fasting Insulin Samples required: 1 clot (yellow/gold top) 1 fluoride (grey top) at time = 0 1 fluoride (grey top) at time =120
22 weeks ☐ 28 weeks ☐ 36 weeks ☐ CRP LEPTIN/ADIPONECTIN Samples required: 2 clots (yellow/gold top)			

Version 6 September 2013

E:\OTHERS\Backup from Sunil Computer\MOP Trial updated August 2015\Current\Current in use\Current 2013\2014\chempath.MOPstudyform.v6.doc 11/15/2016

F.8 Adverse Event reporting form

Adverse Event Worksheet

R&D No: **REC Ref:** **EudraCT No:**
Site name and number: **Center number:** **Subject number:**

EVENT e.g. Headache	Start Date and Time	Stop Date and Time	Outcome	Intensity	Effect on Study Drug	Causality
SAE YES ☐ NO☐ * If yes , complete SAE form	___/___/___ ___:___	___/___/___ ___:___	☐ recovered/resolved ☐ recovering/resolving ☐ not recovered/not resolved ☐ recovered with sequelae ☐ unknown ☐ fatal	☐ mild ☐ moderate ☐ severe	☐ withdrawn ☐ reduced dose ☐ increased dose ☐ not changed ☐ unknown ☐ NA	☐ unrelated ☐ possibly / probably related ☐ Definitely relate
PI Signature: _____				Date: _____		

The criteria for the casual relationship to the study drug are as follows:

- Unrelated to the study drug: A clinical event with a temporal relationship to drug administration which makes a casual relationship improbable and/or in which other drugs, chemicals or underlying disease provide plausible explanations
- Possible: A clinical event with a reasonable time sequence to administration of the drug but which could also be explained by con current disease or other drugs or chemicals.
- Probable: A clinical event with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on re administration or withdrawal.

Criteria for defining the severity of an adverse event:

Mild	No disruption of daily activities
Moderate	Affected normal daily activities
Severe	Inability to perform daily activities

Title: Safety Reporting: Adverse Events
Version number 4
March 2010

F.9 Study Withdrawal form

1

Study withdrawal / discontinuation form

Subject initials: Subject study number:
R&D number: Ethics number:
Study Title: Metformin in obese non-diabetic pregnant women

Last known date subject took study drug:
Last known time subject took study drug:

Please state primary reason for discontinuation or withdrawal from the study.

Tick one of the following boxes

1. Adverse Event ☐
2. Serious Adverse Event ☐
3. Abnormal laboratory result ☐
4. Abnormal test procedure result ☐
5. Unsatisfactory therapeutic effect ☐
6. The subjects condition no longer requires treatment ☐
7. Non-compliance- Early ☐
   - Late ☐
8. w consentSubject withdre ☐
9. Lost to follow up ☐
10. Death ☐

Principal cause of death:
Date of death:

Comments

Investigator name	Investigator signature	Date
……	……..	……

F.10 SUSAR reporting form

SAE / SUSAR Fax Cover Form

Metformin in **O**bese non – diabetic **P**regnant women

TO THE R&D DEPARTMENT AND THE CLINICAL TRIALS UNIT - ST HELIER HOSPITAL

PLEASE CIRCLE ONE OF THE FOLLOWING:

1. Initial report **2. Follow - up report** **3. Final report**

E- mail: Yvonne.reilly@esth.nhs.uk Sheila.jackson@esth.nhs.uk

FAX R&D : 0208 296 3165 **TELEPHONE NUMBER:** 0208 296 3330

FAX CLINICAL TRIALS UNIT: 0208 644 2831 **TELEPHONE NUMBER:** 0208 296 2519

DATE: ____/__ __ __ /____ **TOTAL PAGES:** _____
(dd / mmm / yyyy) including cover page)

FROM: _____ **PHONE:** _____

PROTOCOL: _____ **SUBJECT:** _____

R&D No: WCH/2008/002 **REC Ref: 08/H0806/80** **EudraCT No:**

INVESTIGATOR NAME: MR SHEHATA **SIGNATURE:** _____

Check all documents included with this Fax:

_____ **SAE Report Form**

_____ **Discharge Summary (when the SAE involves hospitalization)**

_____ **Other relevant information (medical record progress notes, lab/diagnostic test reports, Autopsy etc.)**

Comments:

Metformin in **O**bese non – diabetic **P**regnant women

SAE / SUSAR Reporting Form - Fax Report to: 0208 296 3165 and 0208 644 2831

R&D No: WCH/2008/002 **REC Ref: 08/H0806/80** **EudraCT No:**

Site name and number: **Centre number:** **Subject number:**

Date site aware of event _____ Initial Report Date _____ Follow-up Report Date_____

Final report Date_____

Please circle the SAE / SUSAR category from the following choices (all that apply):

1. Death **2**. Result in Congenital Anomaly **3**. Hospitalization or Prolonged Hospitalization. **4**. Immediately Life-threatening

5. Persistent/Significant Disability/Incapacity **6**.Serious as assessed by the Investigator **7**.Other, specify

1. Study Product Name *Note: If blinded, indicate as such*	**2. Dose, Route, Schedule of Study Product(s) at SAE Onset For example: BD or PO**	**3. Date Study Product First Started** DD/MMM/YYYY	**4. Date Study Product Last Taken prior to onset date of this event** DD/MMM/YYYY	**5. Relationship** to Study product: 1.Not associated 2.Possible / Probable 3.Associated 4. Unknown	**6. If Not Associated** Is event related to: 1-study procedure 2-other condition/ illness 3-other drug 4-other

nt Cause of)	**9. Onset Date** DD/MMM/YYYY	**10. Severity** (Complete only one column. Mark only one box in that column.)		**11 Outcome of Event is** (choose only o
_____ _____	____/_____/__	Mild Moderate Severe Life- threatening Death	Grade 1 Grade 2 Grade 3 Grade 4 Grade 5 (Death)	Ongoing Resolved without sequelae Date ____/_____/_____ (DD/M Resolved with sequelae Date _____/____/_____ (DD/M State sequelae: _____ Death Date of Death: ____/_____/____ (DD/MMM/YYYY Autopsy: Not Done or Done (Provide Report) Planned

List Relevant Lab/Diagnostic results below OR attach copies of the results. When Faxing results of tests please anonymise and use only the subject's initials and study number.

12. Relevant Laboratory Tests or Pending tests

Test	Collection Date DD/MMM/YYYY	Result	Site Normal Range	Collection Date of test previous to this SAE	Result of test previous to this SAE

13. Relevant Diagnostic Tests (EX: MRI, CT scan, and Ultrasound)

Test	Date Performed (DD/MMM/YYYY)	Results/Comments

14. CONCOMITANT MEDICATIONS: List relevant concomitant medications the subject was taking up to 1 month prior to SAE onset. Continue on a separate page if necessary.

Medication	Start Date DD/MMM/YYYY	Stop Date DD/MMM/YYYY	Total Daily Dose	Indication	Suspect
1.			_____ Unknown?		Yes No
2.			_____ Unknown?		Yes No
3.			_____ Unknown?		Yes No

15. EVENT SUMMARY

(Include chronological details of event, associated signs and symptoms, alternative etiologies including concomitant medications suspected, medical management and relevant past medical history below, or attach summary. Include all information. Attach additional pages if needed.

Completed by (signature): _____ Completed by (print): _____

Date: ___/___/____

Investigator(signature): _____ Investigator (print): _____

Date: ___/___/____

Appendix G Publications

G.1 Obesity, Polycystic Ovaries and Impaired Reproductive Outcome- Chapter in a textbook on Obesity, First Edition-2013

22 Obesity, Polycystic Ovaries and Impaired Reproductive Outcome

Jyoti Balani[1], *Stephen Hyer*[1], *Marion Wagner*[2] *and Hassan Shehata*[2]

[1]Department of Diabetes and Endocrinology, Epsom and St Helier University Hospitals, NHS Trust, Surrey, UK, [2]Department of Maternal Medicine, Epsom and St Helier University Hospitals, NHS Trust, Surrey, UK

People of such constitution cannot be prolific. . .fatness and flabbiness are to blame. The womb is unable to receive the semen and they menstruate infrequently and little.

Hippocrates Essay on the Scythians [1]

Introduction

Obesity is a complex and multi-factorial chronic condition with an increasing global prevalence especially in the western countries. According to the World Health Organization (WHO), obesity has more than doubled worldwide since 1980. The WHO defines obesity as abnormal or excessive fat accumulation that may impair health. Body mass index (BMI), calculated as weight (kg)/height (metres) [2], is used as an estimate of obesity – a BMI greater than 25 kg/m^2 is classified as overweight and a BMI equal to or greater than 30 kg/m^2 is categorised as obesity. Obesity is further subdivided into class I (BMI 30–34.9 kg/m^2), class II (BMI 35–39.9 kg/m^2) and class III (BMI ≥ 40 kg/m^2). Overweight and obesity rank fifth as leading risks for global deaths. In addition, 44% of the burden of diabetes, 23% of ischaemic heart disease and between 7–41% of certain cancers are attributable to overweight and obesity [2].

The fundamental problem in obesity is an energy imbalance between calories consumed and calories expended. An increased intake of energy-dense foods and a decrease in physical activity associated with increasing urbanisation are important contributory factors to the increased prevalence of obesity globally.

Obesity. DOI: http://dx.doi.org/10.1016/B978-0-12-416045-3.00022-4

Obesity and Reproductive Health

Overweight women have a higher incidence of menstrual dysfunction, anovulation and infertility compared with women of similar reproductive age. Spontaneous abortions after natural conception or after using assisted reproductive technology occur more frequently in obese women compared with women having normal BMI. Lashen et al. [3] reported a significantly higher risk for early miscarriages among obese patients compared in normal weight controls. Maheshweri et al. [4] documented similar results in obese women after assisted reproduction.

Obesity is a known risk factor for subfertility due to anovulation. A recently published study by Van der Steeg et al concluded that obesity is also associated with lower pregnancy rates in subfertile ovulatory women [5]. For every BMI unit above 29 kg/m^2, the probability of a successful pregnancy was reduced by approximately 5%, equivalent to the impact on pregnancy of being 1 year older.

Obese men have erectile dysfunction and reduced coital frequency [6] and this is thought to relate to decreased testosterone concentration and elevated pro-inflammatory cytokines which induce endothelial dysfunction through the nitric oxide pathway [7]. Obesity results in increased peripheral aromatisation of androgens resulting in low testosterone levels. Increased scrotal fat and raised testicular temperature may result in reduced spermatogenesis.

Pathogenic Mechanisms

There is new and increasing evidence that point to the importance of genetics influencing body fat mass. Approximately 20 different genes have been implicated in human monogenic obesity, but they account for only a small percentage of cases [8]. Alterations in energy balance in humans have been linked to mutations in the leptin–melanocortin pathway which helps regulate energy homeostasis acting through the satiety centre in the hypothalamus [9].

The pathogenic mechanisms responsible for obesity having a negative impact on reproductive health are uncertain. One hypothesis is that obesity affects the hypothalamic–pituitary–ovary axis. Excess free oestrogen resulting in part from increased peripheral aromatisation of androgens to oestrogen in adipose tissue, combined with decreased availability of gonodotropin-releasing hormone, could interfere with the hypothalamic–pituitary regulation of ovarian function resulting in irregular or anovulatory cycles.

Hyperinsulinaemia is another important factor implicated in fertility disorders in obesity. It may be directly responsible for the development of androgen excess through its effects in reducing sex hormone–binding globulin (SHBG) synthesis and in stimulating ovarian androgen production rates. Hyperandrogenaemia in turn leads to altered ovarian function. Preliminary results from our department show that fat is preferentially distributed centrally in obese women during pregnancy. Visceral fat area as measured by bio-impedance is elevated whilst lean mass in the

lower limbs is reduced. The significance of this for pregnancy outcomes is currently being investigated.

It is now well accepted that the adipocyte is effectively an endocrine cell capable of releasing many active substances including interleukins, tumour necrosis factor, leptin, complement factors and plasminogen activator inhibitor. Leptin is thought to inhibit ovarian follicular development through both the induction of insulin resistance and a direct impairment of ovarian function. Alterations in the secretion and action of insulin and other hormones as leptin, resistin, ghrelin and adiponectin in obese women may affect follicle growth, corpus luteum function, early embryo development, trophoblast function and endometrial receptivity [10].

There is recent in vitro data indicating that leptin may exert a direct inhibitory effect on ovarian function by inhibiting human granulosa and theca cell steroidogenesis, probably by antagonising stimulatory factors, such as insulin growth factor-1, transforming growth factor-β, insulin and luteinising hormone [11] Also, in vitro and in vivo studies have demonstrated that high leptin concentrations in the ovary may interfere with the development of a dominant follicle and oocyte maturation [12]. There is also evidence that the endometrium may also have a subtle role in the detrimental effects of obesity on reproduction [13].

Psychosocial factors have been implicated in reduced fertility in obese women. Obese people do not have sexual intercourse as frequently as slimmer people even if they have a cohabiting sexual partner. This could be explained in part by decreased sex drive resulting from decreased dopamine activity and increased serotonin activity in the brain caused by overeating [14,15].

Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is one of the most common disorders to cause infertility from anovulation and affects 4–7% of women. Nearly one-half of the women with PCOS are obese. A consensus conference held in Rotterdam established that at least two of the following criteria are sufficient for the diagnosis of PCOS: oligo and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries at ultrasound [16].

The clinical features of PCOS are heterogeneous and may change throughout life and are largely influenced by obesity and metabolic alterations. It was originally known in its severe form as the Stein–Levinthal syndrome. PCOS is characterised by multiple small cysts in the ovary and hyperandrogenism. Excess androgen production arises from the ovaries and to a lesser extent from the adrenals. It is still unclear whether the basic defect is in the ovary, adrenal, pituitary or a more generalised metabolic defect. The androgens are normally converted to oestrogens in adipose tissue, but in PCOS, androstenedione is secreted and converted to testosterone in peripheral tissues.

Obesity is strongly associated with the PCOS; obesity is present in at least 30% of cases and in some series up to 75% of cases [17]. Levels of SHBG tend to decrease with increasing body fat leading to higher circulating free androgens delivered to target sensitive tissues [18]. SHBG is regulated by a complex of factors, including oestrogen and growth hormone as stimulating factors and androgens and insulin as inhibiting factors [19]. Hyperinsulinaemia in obesity overcomes insulin resistance and inhibits SHBG synthesis in the liver.

Leptin may exert a direct inhibitory effect on ovarian function. Higher circulating levels of leptin than those expected in relation to BMI, or normal concentrations of leptin, have been reported in obese women with PCOS [20]. However, it is presently unknown whether high leptin levels in the peripheral circulation and/or in the ovarian tissues may play a role in determining anovulation in obese women with PCOS.

The possible role of a dysfunctional endocannabinoid system in the pathophysiology of obesity-related PCOS is currently being studied. One study has also shown that PCOS and maternal obesity affect oocyte size in in vitro fertilisation/intracytoplasmic sperm injection cycles. Women with PCOS and obesity have smaller oocytes compared with control subjects and both PCOS and obesity independently influence oocyte size [21].

The therapeutic efficacy of weight loss indirectly supports the pathogenic role of obesity in PCOS. Lifestyle interventions with hypocaloric diet with or without associated increased physical activity have proved their efficacy [22]. A recent study examined the effect of a 48-week period of intensive lifestyle intervention; dietary advice and a standardised physical activity programme with or without metformin treatment showed a significant positive effect on ovulatory performance which was related to the amount of weight loss, rather than the effect of metformin [23].

Obesity in Pregnancy

Obesity in pregnancy has been identified by the Confidential Enquiry into Maternal and Child Health (CEMACH) (2008–2011) as a major health risk to mother and baby (Figure 22.1). There is substantial evidence that obesity in pregnancy contributes to increased morbidity and mortality for both mother and baby. CEMACH found that approximately

- 35% of women who died were obese [24] and
- 30% of the mothers who had a stillbirth or a neonatal death were obese.

Obese women spend an average of 4.83 more days in hospital and the increased levels of complications in pregnancy and interventions in labour represent a five-fold increase in cost of antenatal care [25]. The costs associated with babies born to obese mothers are also increased as there is a 3.5-fold increase in admission to Neonatal Intensive Care Unit (Figure 22.1).

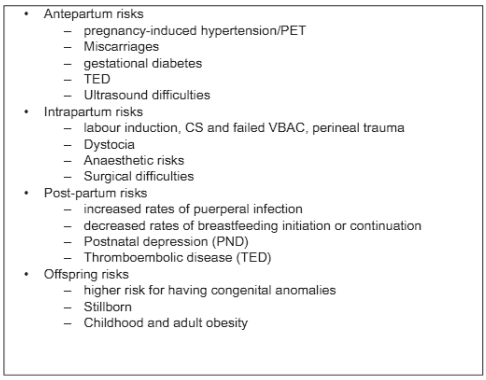


Figure 22.1 The risks of obesity in pregnancy.

Gestational Weight Gain in Obese Women

A population-based cohort study of 120,251 pregnant, obese women delivering full-term, live born, singleton infants investigated the risk of four pregnancy outcomes including pre-eclampsia (PET), Caesarean section (CS), small for gestational age (SGA) and large for gestational age (LGA) by obesity class and total gestational weight gain. The results showed that gestational weight gain of less than 15 lb for overweight or obese pregnant women was associated with a significantly lower risk of PET, CS, and LGA and higher risk of SGA birth. The authors concluded that limited or no weight gain during pregnancy in obese pregnant women results in a more favourable pregnancy outcome. The same study showed that in obese women the LGA incidence is 15% [26].

Weight gain in pregnancy is a complex biological phenomenon that supports the function of growth and development of the foetus. Gestational weight gain is influenced not only by changes in maternal physiology and metabolism but also by placental metabolism. The placenta functions as an endocrine organ, a barrier and a transporter of substances between maternal and foetal circulation. Changes in maternal homeostasis can modify placental structure and function and thus impact on foetal growth rate. Conversely, placental function may influence maternal metabolism through alterations in insulin sensitivity and systemic inflammation.

The Institute of Medicine (IOM) has revised its guidelines for weight gain in pregnancy in 2009 [27]. According to its new recommendations, normal weight women

should gain 25–35 lb (11.5–16 kg), overweight women should gain 15–25 lb (7–11.5 kg) and obese women (BMI > 30 kg/m^2) only 11–20 lb (5–9 kg). The recommended rates of weight gain in the second and third trimesters are 0.42 kg/week for normal weight women, 0.28 kg/week for overweight women and 0.22 kg/week for obese women. These calculations assume a 0.5–2 kg weight gain in the first trimester.

The IOM committee intends that its recommendations be used in concert with good clinical judgement. If the woman's gestational weight gain is not within the proposed guidelines, clinicians should consider other modifiable factors that may cause excessive or inadequate weight gain especially the presence of fluid retention/oedema. The adequacy and consistency of foetal growth should be assessed before suggesting modified target weight gain.

Guidelines for Management for Obese Women of Reproductive Age

Full implementation of the IOM Committee guidelines to improve reproductive health in obese women would mean:

Offering pre-conception services, including dietary counselling, advice on physical activity and contraception, to all overweight or obese women to help them reach a healthy weight before conceiving. This should reduce their obstetric risk as well as improve long-term health of their offspring.

Offering lifestyle advice services to all pregnant women to help them achieve the recommended weight gain targets and thereby reduce post-partum weight retention, improve their long-term health, normalise infant birthweight and offer an additional tool to help reduce childhood obesity.

Offering lifestyle advice services to all post-partum women. This may help them to conceive again at a healthy weight as well as improve their long-term health.

Currently, there are ongoing studies in the United Kingdom to determine whether the addition of insulin sensitisers like metformin to lifestyle interventions would improve the neonatal and pregnancy outcomes in obese non-diabetic pregnant women.

Management of Obesity in Pregnancy

Dietary Approaches

Intermittent fasting during pregnancy is not recommended and is associated with a higher incidence of gestational diabetes mellitus and induction of labour [28]. However, milder caloric restrictions of 1600–1800 Kcal/day are beneficial for weight gain without the risks of ketosis to the foetus [29]. Based on a Cochrane

review, severe protein and energy restriction for obese and overweight women are unlikely to be beneficial and may even be harmful [30].

The available evidence supports balanced dietary interventions in overweight women before conception. Even though the results are often conflicting, there is evidence to suggest that diets rich in protein, fat or high gastrointestinal carbohydrates as well as very low caloric diets are best avoided in pregnancy. Nevertheless, most of the studies are observational in nature, and despite some strong associations, the content of the diet cannot necessarily be implicated in the pregnancy outcomes. Additionally, methodological limitations include a large number of confounding variables, selection and recall bias.

Physical Activity

A Cochrane review on the effects of physical activity on pregnancy concluded that the available data were 'insufficient to infer important risks or benefits to the mother or infant' [31]. Guidelines from the American College of Obstetricians and Gynaecologists [32] recommended that pregnant women should exercise for 30 min or more on most days of the week and participate in moderate intensity exercise unless there were medical or obstetric complications. Whilst these recommendations have been widely adopted they are consensus rather than evidence based.

Behavioural Interventions

Claesson et al. [33] used a 'motivational' talk approach in early pregnancy followed by an invitation to an aqua-aerobic class and then followed weekly by a midwife. This programme resulted in obese women in the intervention group gaining significantly less weight compared to the control group (8.7 vs 11.3 kg) independently of socio-demographic background. The authors credited the increased frequency of contact with the health care professional for the success of their lifestyle intervention compared to previous studies.

More recently Asbee et al. [34] offered pregnant women with BMIs of less than 40 kg/m^2 a simple intervention consisting of a single contact with the dietician at the initial visit where standardised dietary counselling was provided aiming for a diet consisting of 40% carbohydrates, 30% protein, 30% fat and moderate intensity exercise. This approach reduced the weight gain in the intervention group compared to the control group (13 vs 16.1 kg). It should be noted that none of the above lifestyle interventions had any significant effect on birthweight or any other pregnancy outcomes.

Pharmacological Interventions

The only available anti-obesity medication, Orlistat, is not licensed for use in pregnancy. Metformin has been increasingly used in pregnant women with diabetes mellitus or polycystic ovarian syndrome, i.e. states of increased insulin resistance.

Limited data suggest that in combination with caloric restriction it reduces pregnancy weight gain in PCOS [35]. A trial of metformin in pregnancy is needed to clarify its potential benefits for obese pregnant women. The authors are currently undertaking such a study.

Bariatric Surgery

Two recent reviews of case control and cohort trials showed that bariatric surgery improved fertility and unlike lifestyle intervention, decreased a number of maternal and foetal/neonatal complications associated with obesity [36,37].

Patients undergoing these procedures must be managed by an experienced multidisciplinary team and monitored for nutritional deficiencies and surgical complications in any subsequent pregnancies. The safety and timing of gastric surgical procedures need to be further investigated by controlled clinical trials.

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G.2 The importance of visceral fat mass in obese pregnant women and relation with pregnancy outcomes

Published in Obstetric Medicine, 2013

Original Article

Obstetric Medicine

Obstetric Medicine
2014, Vol. 7(1) 22–25

DOI: 10.1177/1753495X13495192
obm.sagepub.com
SAGE

The importance of visceral fat mass in obese pregnant women and relation with pregnancy outcomes

Jyoti Balani[1], Steve Hyer[1], Antoinette Johnson[2] and Hassan Shehata[2]

Abstract

Background: Maternal obesity is a well established risk factor for gestational diabetes but it is not known if the pattern of maternal fat distribution predicts adverse pregnancy outcomes.

Methods: Body composition was assessed by bioimpedance using Inbody 720® in 302 consecutive obese pregnant women attending a weight management clinic. The relation of visceral fat mass and total percentage body fat with the development of gestational diabetes and perinatal outcomes was evaluated.

Results: Women developing gestational diabetes (Group 1; $n = 72$) were older, had higher body mass indices and greater central obesity (waist:hip ratio, visceral fat mass) compared with those remaining normoglycaemic. Visceral fat mass, but not percentage body fat, correlated with fasting glucose in all patients ($r = 0.2$, $p < 0.001$) and particularly those in Group 1 ($r = 0.35$, $p = 0.002$). Visceral fat mass, but not percentage body fat, also correlated strongly with glycaemia, particularly in Group 1 ($r = 0.47$, $p < 0.0001$). Visceral fat mass also showed a weak but significant correlation with baby weight ($r = 0.17$, $p = 0.01$).

Discussion: Central obesity, as assessed by early pregnancy waist:hip ratio and particularly by visceral fat mass, is a predictor of gestational diabetes in addition to classical risk factors and may help identify those obese patients at increased risk of complications.

Keywords

Gestational diabetes, obesity, visceral fat mass, total percentage body fat, bioimpedance

Introduction

There is substantial evidence that obesity in pregnancy contributes to increased complications including mortality for both mother and baby. The Confidential Enquiry into Maternal and Child Health (2007) reported that 35% of women who had died had a recorded body mass index (BMI) of 30 or more and furthermore 30% of the mothers who had experienced a stillbirth or neonatal death were obese.[1]

Obese women are also at increased risk of gestational diabetes (GDM). In a prospective study of more than 16,000 patients with BMI 30–40, the odds ratio (OR) for GDM were 2.6 (95% confidence interval (CI) 2.4–6.0) compared with women with BMI <30.[2] More recently, a meta analysis of 70 studies found the OR for GDM was 3.01 (95% CI 2.34–3.87) for moderate obesity and 5.55 (95% CI 4.27–7.21) for women with morbid obesity (BMI >40) compared with normal weight women.[3] Similar results were reported by Chu et al.[4]

Despite these consistent results, there is still uncertainty regarding the relative importance of the distribution of the fat and the risk of GDM. While central obesity and by implication, visceral fat mass (VFM), is well established as a risk factor for type 2 diabetes and the metabolic syndrome, there are no published data on the possible association of VFM in pregnancy and risk of GDM. We investigated the relation of fat distribution (total percentage fat, VFM and waist: hip ratio (WHR)) and risk of GDM in a cohort of obese women with no known diabetes attending a weight management clinic.

Methods

Subjects

We enrolled 302 consecutive obese pregnant women with no known diabetes attending the weight management clinic at St Helier Hospital, Carshalton, Surrey, UK. The median age of these women was 31 years (range, 26–34 years) and the median BMI was 38.2 kg/m^2 (range, 36.1–41.4); 74.5% of these women were Caucasians. All women received standardised dietary and exercise advice. Maternal baseline characteristics are shown in Table 1.

Gestational diabetes

All women underwent a 75 g oral glucose tolerance test at around 28 weeks gestation; GDM was defined by WHO criteria.[5] Seventy-two of the 302 enrolled patients subsequently developed GDM (23.8%) and were medically managed in the joint antenatal obstetric and diabetic clinic by a standard protocol. They performed home glucose monitoring four times daily and if three or more tests were outside target range (<5.6 mmol/l (fasting), <8 mmol/l (1 h post-prandial) and <7 mmol/l (2 h post-prandial), they were commenced on metformin. Additional insulin was prescribed if tests remained suboptimal despite maximal metformin.

Fat distribution

All women underwent body composition analysis at booking (median gestation = 15 weeks; range, 14–17 weeks) using Inbody 720® bioimpedence. The instrument performs body composition analysis using Direct Segmental Multi-frequency Bioelectrical Impedence Analysis Method (DSM-BIA Method). It measures weight, BMI, WHR, lean

[1]Department of Diabetes, Epsom and St Helier University Hospitals NHS Trust, Carshalton, Surrey, UK
[2]Department of Maternal Medicine, Epsom and St Helier University Hospitals NHS Trust, Carshalton, Surrey, UK

Corresponding author:
Steve Hyer, Doreen Kouba Diabetes Centre, St Helier Hospital, Wrythe lane, Carshalton, Surrey SM5 1AA, UK.
Email: Steve.hyer@esth.nhs.uk

Table 1. Maternal baseline characteristics.

	Group 1 (GDM) ($n=72$)	Group 2 (non-GDM) ($n=230$)	p Value
Age (mean ± SD)	32.1 ± 5.5	29.6 ± 5.8	<0.01*
Ethnicity *n* (%)			
White	60 (83.3)	165 (71.7)	NS
Asian	7 (9.7)	30 (13)	NS
Blacks	3 (4.2)	27 (11.7)	
Others	2 (2.8)	8 (3.5)	
History of PCOS, *n* (%)	15 (20.8)	16 (7)	≤0.01*
Family history of diabetes, *n* (%)	41(56.9)	79 (34.3)	≤0.001*
Previous GDM, *n* (%)	12 (16.8)	0	<0.0001*
Smokers, *n* (%)	4 (5.6)	16 (7)	NS

GDM: gestational diabetes; PCOS: polycystic ovary syndrome. *$p<0.05$.

Table 2. InBody 720® body composition data at booking.

	Group 1 (GDM) ($n=72$)	Group 2 (non-GDM) ($n=230$)	p Value
BMI (kg/m^2) (mean ± SD)	40.2 ± 4.6	38.5 ± 3.9	≤0.01*
Waist:hip ratio (mean ± SD)	1.02 ± 0.07	0.99 ± 0.05	≤0.01*
Total percentage body fat (mean ± SD)	49.8 ± 3.5	49.2 ± 3.6	NS
Visceral fat mass[a] (units) (mean ± SD)	199.2 ± 40.5	183.8 ± 31.5	≤0.01*

GDM: gestational diabetes; BMI: body mass index.
[a]Normal value <100 units. *$p<0.05$.

Table 3. Maternal outcomes.

	Group 1 (GDM) ($n=72$)	Group 2 (non-GDM) ($n=230$)	p Value
Hypertension,[a] *n* (%)	8 (11.1)	21 (9.1)	NS
Pre-eclampsia, *n* (%)	1 (1.4)	2 (0.9)	NS
Mode of delivery, *n* (%)			
Vaginal	34 (47.2)	122 (53)	NS
Instrumental	3 (4.2)	23 (10)	NS
Elective c/section	13 (18.1)	32 (13.9)	NS
Emergency c/section	22 (30.6)	53 (23)	NS
DVT/PE, *n* (%)	0	1 (0.4)	NS

GDM: gestational diabetes; NS: not significant; DVT: deep vein thrombosis; PE: pulmonary embolism; BP: blood pressure.
[a]BP>150/100 requiring treatment or incremental rise in BP from booking >30/20.

Table 4. Neonatal outcomes.

	Group 1 (GDM) ($n=72$)	Group 2 (non-GDM) ($n=230$)	p value
Birth weight (mean ± SD)	3452.8 ± 626.3	3506.7 ± 564.1	NS
Large for gestational age, *n* (%)	13 (18.3)	42 (18.1)	NS
Admissions to neonatal unit, *n* (%)	8 (11.2)	22 (9.6)	NS
Major malformations, *n* (%)	0	0	NS
Neonatal hypoglycaemia,[a] *n* (%)	3 (4.2)	1 (0.4)	<0.05*
Neonatal jaundice, *n* (%)	3 (4.2)	2 (0.8)	NS
Shoulder dystocia, *n* (%)	0	1 (0.4)	NS

GDM: gestational diabetes.
[a]Capillary glucose <2.6 mmol/l.

body mass, total percentage body fat (PBF) and VFM. It is a validated tool correlating well with intraabdominal fat area assessed by computed tomography (CT)[6] and dual-energy X-ray absorptiometry (DEXA).[7] It has also been used in patients with obesity.[8,9] It has been shown to be safe in the second and third trimesters of pregnancy and validated against deuterium and hydrodensitometry techniques for body composition analysis.[10,11] DSM-BIA is also an accurate technique for assessing body water distribution, which changes during pregnancy.[12] There is no evidence that the physiological alterations in body water are different in pregnancies complicated by GDM. In this study, Inbody® assessment was repeated at 36 weeks gestation in Group 1 patients to assess changes during pregnancy on treatment.

Statistical analysis

The maternal baseline characteristics, pregnancy and neonatal outcome in patients developing GDM (Group 1, $n=72$) were compared with those with normal glucose tolerance (Group 2, $n=230$). Group data were compared by χ^2 2×2 contingency tables and by unpaired *t*-tests; significance was taken as $p<0.05$. Results are expressed as mean (SD) or median (range) if non-parametric distribution. Pearson's correlation coefficient was used to compare continuous variables.

Results

Women developing GDM (Group 1) were significantly older, more likely to have a history of previous GDM, or a family history of diabetes, or a past history of polycystic ovaries when compared with those remaining normoglycaemic (Group 2) (see Table 1). Group 1 women had significantly higher mean fasting glucose (5.04 ± 2.01) compared with Group 2 (4.57 ± 0.83) and higher mean 2-h glucose values (6.32 ± 2.91 versus 5.44 ± 1.29); $p<0.01$. Group 1 women also had higher mean BMI, greater WHR and significantly greater VFM compared with those in Group 2 (see Table 2). However, total PBF was very similar in both groups.

The maternal and neonatal outcomes were not significantly different in the two groups except for neonatal hypoglycaemia, which was significantly higher in the diabetic group (see Tables 3 and 4).

VFM but not total PBF correlated with fasting glucose values in the whole cohort ($r=0.21$, $p<0.001$) and particularly in Group 1 ($r=0.35$; $p<0.002$). There was no significant correlation between VFM and 2 h glucose values in the whole cohort or in the two groups.

There was a significant correlation between glycaemia (HBA1c) and maternal BMI ($r=0.39$, $p<0.001$), and between HbA1c and VFM ($r=0.47$, $p<0.0001$) (see Figure 1). No significant correlation was found between HbA1c and total PBF ($r=0.16$, $p=$NS).

We also studied possible associations with baby birth weight (BW). Maternal BMI ($r=0.14$; $p=0.02$), VFM ($r=0.17$; $p=0.002$) but not PBF for the whole cohort and particularly in Group 2 ($r=0.21$; $p=0.001$) had weak but significant correlations with baby weight.

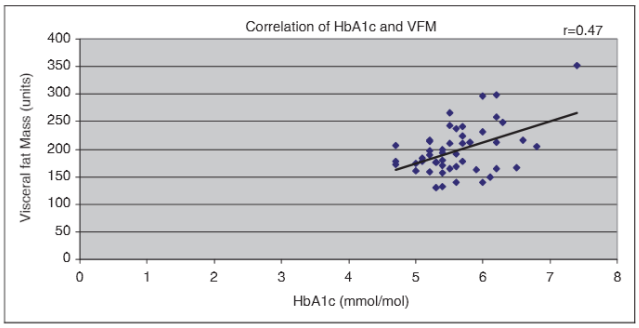

Figure 1. Visceral fat mass versus HbA1c in Group 1 patients. HbA1c: glycaemia

Although the expected increase in mean VFM at 36 weeks was reduced by metformin compared with those in Group 1 on dietary measures alone, the difference was not statistically significant (3.76 ± 3.1(SEM) versus 8.24 ± 2.6 (SEM); NS).

Discussion

To our knowledge, this is the first article to examine the possible role of directly measured VFM in relation to pregnancy outcomes. The novel finding in our study is that in addition to well-established predictors of GDM (maternal age, BMI, family history of GDM, history of polycystic ovary syndrome), visceral fat (and not total fat) assessed at antenatal booking is another risk factor for GDM.[13] VFM correlated with fasting glucose in all patients, particularly those developing GDM, as well as long-term measures of HbA1c. Proxy measures of visceral fat, such as waist circumference and WHR showed the expected correlation.

Although obese patients will be expected to have higher VFM, we found no correlation between total PBF and indices of HbA1c (fasting or 2 h glucose), suggesting that non-visceral fat (e.g. subcutaneous fat) does not have the same metabolic implications. This is in keeping with concept of the metabolic syndrome and the proposal as long ago as 1997 that GDM should be considered a component of the metabolic syndrome.[14,15]

A strong association between measures of abdominal obesity (waist circumference, WHR and CT-assessed intra-abdominal fat area) and the development of type 2 diabetes is well established: a meta-analysis of 15 cohorts from 10 longitudinal studies gave a pooled OR for the incidence of diabetes of 2.14 (95% CI: 1.70–2.71)[16] compared with controls. Visceral fat assessed by CT remained a significant predictor of incident diabetes even after adjustment for BMI, total body fat and subcutaneous fat. In a large prospective study of obese non-diabetic subjects, baseline VFM measured by DEXA and magnetic resonance imaging but not general adiposity was independently associated with risk of development of prediabetes and diabetes.[17] These studies point to an important role for visceral fat accumulation in the development of glucose intolerance.

In agreement with a recently published study, Asians developing GDM had lower BMI, lower WHR and lower VFM compared with Caucasians.[18] However, among the Asian cohort, those developing GDM had higher VFM and markers of central obesity. Despite only small numbers in this study, these observations are in keeping with the suggestion that Asians are particularly susceptible to diabetes even at lower BMIs.

The pathogenic mechanism linking visceral fat and the onset of diabetes is likely to be through the development of insulin resistance although we cannot completely exclude the possibility of an effect on insulin secretion. In patients with established type 2 diabetes, visceral fat accumulation has a significant negative impact on glycaemic control through decreased insulin sensitivity.[19]

Visceral adipocytes release a variety of inflammatory cytokines that are able to induce insulin resistance such as interleukin-1, interleukin-6, tumour necrosis factor and resistin as well as others such as adiponectin, which improves insulin sensitivity.[19,20] Adiponectin is down-regulated in obesity and plasma levels are lower in obese subjects compared with controls.[21] Future studies need to examine the concentration of these substances in normal pregnancies and those complicated by obesity and GDM.

A major concern in pregnancies complicated by GDM or obesity is the increased risk of fetal macrosomia. The risk associated with obesity is increased two- to three fold and appears to correlate with the degree of obesity.[22] We noted a positive correlation between maternal BMI and baby BW and between VFM (but not PBF) and BW. On further analysis, these correlations were not found in Group 1 women, reflecting the influence of treatment (metformin and insulin) on perinatal outcomes. We have previously shown a favourable effect of metformin on the incidence of macrosomia in GDM women.[23] Furthermore, we found no significant difference between BWs in the two groups in this study, which is likely to reflect the beneficial effect of metformin in GDM babies. In addition, a reduction in the expected increase in VFM was demonstrated in metformin-treated women, although this observation needs confirmation with larger numbers of patients.

Strengths of this study are direct measurement of fat distribution in vivo in early pregnancy in ambulant women attending a single centre with standardised dietary and exercise advice. There was complete data on all 302 women.

Limitations are the lack of a normal weight cohort of women to act as controls to allow calculation of OR for VFM. We were not able to measure cytokines such as adiponectin or inflammatory markers such as C-reactive protein, which might have added useful information regarding the metabolic syndrome. Also insulin sensitivity and secretion were not measured in this study. Future research will address this issue.

Conclusions

The results of this study add to the growing evidence of the importance of central obesity and in particular VFM in the development of GDM.

While BMI is a convenient measure of obesity, routine measurement of waist circumference or WHR in early pregnancy ideally complemented by VFM assessments may help identify those patients at increased risk.

Acknowledgements

We acknowledge the help of the midwives and diabetes specialist nurses, who greatly assisted this project.

Conflicts of interest

None declared.

Funding

This research was funded by the Diabetes and Maternal Medicine Charity Fund.

Ethical approval

The study was considered by the ethics committee and considered an audit rather than research, and therefore not requiring ethical approval. Patients receiving metformin were given an information sheet detailing its use in pregnancy and the fact that although approved for use in pregnancy, it wasn't licensed for use in pregnancy.

Guarantor

N/A.

Contributorship

JB: Collecting data, analysis of results, initial draft manuscript; SH: Supervision of project, revising manuscript; AJ: Help with data collection, discussion; HS: Conceived idea for project, final version of manuscript.

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G.3 Metformin versus placebo in obese pregnant women without Diabetes Mellitus – Publication in the New England Journal of Medicine, February 2016

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Metformin versus Placebo in Obese Pregnant Women without Diabetes Mellitus

Argyro Syngelaki, Ph.D., Kypros H. Nicolaides, M.D., Jyoti Balani, M.D., Steve Hyer, M.D., Ranjit Akolekar, M.D., Reena Kotecha, M.D., Alice Pastides, M.D., and Hassan Shehata, M.D.

ABSTRACT

BACKGROUND

Obesity is associated with an increased risk of adverse pregnancy outcomes. Lifestyle-intervention studies have not shown improved outcomes. Metformin improves insulin sensitivity and in pregnant patients with gestational diabetes it leads to less weight gain than occurs in those who do not take metformin.

METHODS

In this double-blind, placebo-controlled trial, we randomly assigned pregnant women without diabetes who had a body-mass index (BMI; the weight in kilograms divided by the square of the height in meters) of more than 35 to receive metformin, at a dose of 3.0 g per day, or placebo (225 women in each group) from 12 to 18 weeks of gestation until delivery. The BMI was calculated at the time of study entry (12 to 18 weeks of gestation). The primary outcome was a reduction in the median neonatal birth-weight z score by 0.3 SD (equivalent to a 50% reduction, from 20% to 10%, in the incidence of large-for-gestational-age neonates). Secondary outcomes included maternal gestational weight gain and the incidence of gestational diabetes and of preeclampsia, as well as the incidence of adverse neonatal outcomes. Randomization was performed with the use of computer-generated random numbers. The analysis was performed according to the intention-to-treat principle.

RESULTS

A total of 50 women withdrew consent during the trial, which left 202 women in the metformin group and 198 in the placebo group. There was no significant between-group difference in the median neonatal birth-weight z score (0.05 in the metformin group [interquartile range, −0.71 to 0.92] and 0.17 in the placebo group [interquartile range, −0.62 to 0.89], P=0.66). The median maternal gestational weight gain was lower in the metformin group than in the placebo group (4.6 kg [interquartile range, 1.3 to 7.2] vs. 6.3 kg [interquartile range, 2.9 to 9.2], P<0.001), as was the incidence of preeclampsia (3.0% vs. 11.3%; odds ratio, 0.24; 95% confidence interval, 0.10 to 0.61; P=0.001). The incidence of side effects was higher in the metformin group than in the placebo group. There were no significant between-group differences in the incidence of gestational diabetes, large-for-gestational-age neonates, or adverse neonatal outcomes.

CONCLUSIONS

Among women without diabetes who had a BMI of more than 35, the antenatal administration of metformin reduced maternal weight gain but not neonatal birth weight. (Funded by the Fetal Medicine Foundation; ClinicalTrials.gov number, NCT01273584; EudraCT number, 2008-005892-83.)

From the Harris Birthright Research Centre for Fetal Medicine, King's College Hospital (A.S., K.H.N., R.A., R.K., A.P.), and the Departments of Endocrinology (J.B., S.H.) and Maternal Medicine (H.S.), Epsom and St. Helier University Hospitals NHS Trust, London, and the Department of Fetal Medicine, Medway Maritime Hospital, Gillingham, Kent (R.A.) — all in the United Kingdom. Address reprint requests to Dr. Shehata at the Department of Maternal Medicine, Epsom and St. Helier University Hospitals NHS Trust, London SM5 1AA, United Kingdom, or at hassan.shehata@nhs.net.

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The prevalence of obesity is increasing both in developed countries and in developing countries, and obesity is considered to be a global pandemic.[1] An estimated one fifth of pregnant women in the United Kingdom and one third of those in the United States are obese.[2,3] Obesity during pregnancy is associated with an increased risk of adverse short-term and long-term consequences for both mother and baby.[4-11] Attempts at reducing the incidence of pregnancy complications associated with obesity have focused on dietary and lifestyle interventions, but these have generally been unsuccessful.[12-17]

An alternative strategy is the use of metformin, which reduces insulin resistance. Metformin has been used extensively in the treatment of gestational diabetes mellitus,[18] and there has been no evidence of an increase in the incidence of birth defects associated with its use.[19] Hyperglycemia and increased insulin resistance occur with obesity[20] and may explain the association between obesity and fetal macrosomia, as well as other pregnancy complications.[21] Studies involving women with gestational diabetes mellitus have shown that metformin reduces gestational weight gain.[18,22] The Metformin in Obese Nondiabetic Pregnant Women (MOP) trial was designed to test the hypothesis that metformin, as compared with placebo, would be associated with a lower median neonatal birth-weight z score when administered to pregnant women without diabetes who had a body-mass index (BMI; the weight in kilograms divided by the square of the height in meters) of more than 35.

METHODS

TRIAL DESIGN AND PARTICIPANTS

In this study, we randomly assigned women without diabetes who had a BMI of more than 35 and were at 12 to 18 weeks of gestation with a singleton fetus to receive metformin or placebo. Participants were from three National Health Service (NHS) maternity hospitals in the United Kingdom (King's College Hospital, London; Medway Maritime Hospital, Kent; and Epsom and St. Helier University Hospitals NHS Trust, London). In these hospitals, all women receiving pregnancy care are offered an ultrasonographic examination at 11 to 13 weeks of gestation as part of combined screening for trisomy 21. Pregnancy dating was based on the measurement of the fetal crown–rump length at that scan. The BMI was calculated at the time of study entry (12 to 18 weeks of gestation). The demographic characteristics of the mothers and the medical history were recorded in a database.

Exclusion criteria were a maternal age of less than 18 years; a major fetal defect observed on the scan performed at 11 to 13 weeks of gestation; a history of gestational diabetes mellitus; kidney, liver, or heart failure; a serious medical condition; hyperemesis gravidarum; treatment with metformin at the time of screening; known sensitivity to metformin; and miscarriage before randomization. Potential trial participants were given written information about the trial; they then had at least 24 hours to consider participation. All the women who agreed to participate in the trial provided written informed consent.

Ethical approval for the study was obtained from the London–Surrey Borders Research Ethics committee, and clinical trial authorization was obtained from the Medicines and Healthcare Products Regulatory Agency. The study protocol, including the statistical analysis plan, is available with the full text of this article at NEJM.org.

Two of the authors wrote the first draft of the manuscript, and all the authors contributed to its revision and made the decision to submit the manuscript for publication. Study funding was provided by the Fetal Medicine Foundation, which had no role in the study design, the collection, analysis, or interpretation of the data, or the writing of the report. Quality control of screening and verification of adherence to protocols at the various centers were performed on a regular basis by the trial coordinators.

RANDOMIZATION AND STUDY-GROUP ASSIGNMENTS

Eligible women were randomly assigned, in a 1:1 ratio, with the use of computer-generated random numbers, to receive either metformin or placebo. In the random-sequence generation there were no restrictions, such as block size or stratification according to study site. The appearance, size, weight, and taste of the placebo tablets were identical to those of the metformin tablets; both were purchased at full cost from University College London Hospitals NHS Foundation Trust.

The women in each group were prescribed metformin or placebo on their first visit after randomization. All the women received standard-

ized personal advice on healthy eating, with an emphasis on low-glycemic-index foods, and were encouraged to exercise for 30 minutes each day.

The metformin or placebo was given with meals; metformin was initiated at a daily dose of 1.0 g in week 1, and the dose was increased by 0.5 g per week to a maximum dose of 3.0 g in week 5. Women with serious side effects while taking the full dose were asked to continue taking the maximum tolerated dose. The study regimen was stopped if fetal growth restriction — defined by an estimated fetal weight lower than the fifth percentile and abnormal results of fetal Doppler studies — was detected.

FOLLOW-UP VISITS

Follow-up visits were scheduled at intervals of 4 to 6 weeks for prescription of metformin or placebo and for maternal assessment, including measurement of weight and blood pressure and urinalysis for proteins and ketones. We assessed adherence to taking metformin or placebo by counting the tablets returned by the patients at each visit; if during a given visit a patient forgot to return the tablets, we relied on verbal report and on the results of previous and subsequent visits. Adherence was considered to be good if the total number of tablets consumed was at least 50% of the total number prescribed and poor if it was less than 50%.

All the women underwent an 75-g oral glucose-tolerance test (OGTT) at 28 weeks of gestation; metformin or placebo was stopped for 1 week before the date of the test. Women with abnormal results on the OGTT (i.e., results that met the World Health Organization 1999[23] criteria for gestational diabetes mellitus) were advised to continue the assigned study regimen as before and to commence home glucose monitoring. If target blood-glucose values were not achieved, insulin was added to their existing regimen. Women with normal OGTT results continued with the study regimen as before.

The clinical data of the participants were recorded in the study database at each visit. Details regarding delivery and neonatal outcomes were added as soon as they became available.

OUTCOME MEASURES

The primary outcome measure was the median neonatal birth-weight z score (difference between observed and expected birth weight, with adjustment for gestational age, divided by the fitted standard deviation). The expected birth weight, corrected for gestational age, was derived from our population of phenotypically normal neonates born alive at 24 weeks of gestation or later.[24]

Maternal secondary outcome measures included gestational weight gain, which was defined as the difference in maternal weight between the day of randomization and the last antenatal visit, gestational diabetes mellitus, preeclampsia,[25] pregnancy-induced hypertension,[25] delivery by cesarean section, and postpartum hemorrhage, which was defined as blood loss of 1 liter or more. Key secondary outcomes for the fetus or neonate included death before 24 weeks of gestation, stillbirth at 24 weeks of gestation or later, preterm birth before 37 weeks of gestation, status of being large for gestational age (birth weight >90th percentile, with adjustment for gestational age),[24] birth trauma (shoulder dystocia, or brachial plexus injury or fracture), an Apgar score of less than 7 at 5 minutes, admission to a level 2 or 3 neonatal unit, hypoglycemia (plasma glucose levels <46.8 mg per deciliter [2.6 mmol per liter] on two occasions ≥30 minutes apart), hyperbilirubinemia requiring phototherapy, and respiratory distress, which was defined by the need for more than 4 hours of respiratory support or supplemental oxygen.

ADVERSE EVENTS

Patients were advised to contact their local investigator if any adverse events occurred. The nature, time of onset, and severity of the event, the treatment needed, and any relation to the assigned study regimen were recorded. All serious adverse events were reported to the sponsor.

STATISTICAL ANALYSIS

The sample-size estimation was based on our data from 72,013 singleton pregnancies for which routine screening was performed for trisomies at 11 to 13 weeks of gestation. At that screening visit, the maternal weight and height were measured and the BMI was calculated. In that large population, the neonatal birth weight was normally distributed, with a median (±SD) of 3381±563 g.

In the subgroup of pregnancies in which the mother's BMI was 35 or less (67,354 women), the median neonatal birth weight was 3351 g, and the prevalence of large-for-gestational-age neonates was 10%. In the subgroup of pregnan-

cies in which the mother's BMI was more than 35 (4659 women), the median neonatal birth weight was 3516 g, and the prevalence of large-for-gestational-age neonates was 20%. Therefore, the median birth weight in neonates whose mothers had a BMI of more than 35 was 0.3 SD (165÷563) higher than in those whose mothers had a BMI of 35 or less.

Since metformin is associated with less gestational weight gain[18,22] and since birth weight is related to both maternal BMI and gestational weight gain,[10,26] we hypothesized that the use of metformin in women with a BMI of more than 35 might result in a reduction in the mean neonatal birth weight by 0.3 SD — down to the value observed in neonates born to women with a BMI of 35 or less. We estimated that 400 patients would need to undergo randomization to give the study 80% power to detect such a reduction at a 5% significance level; after allowing for an expected withdrawal of 20%, we calculated that we would need to recruit 450 patients. The analysis was performed according to the intention-to-treat principle.

Baseline data for the mothers in the two study groups were summarized with the use of medians and interquartile ranges. Comparisons between groups were performed with the use of the Mann–Whitney U test. Univariate comparisons of dichotomous data were performed with the use of the chi-square test or Fisher's exact test.

RESULTS

STUDY POPULATION

The study period was from October 2010 through June 2015 at Epsom and St. Helier University Hospitals, from June 2013 through June 2015 at King's College Hospital, and from September 2013 through June 2015 at Medway Maritime Hospital. In all the hospitals, there was a 5-month gap in recruitment because of problems with the manufacture of the drugs. At Epsom and St. Helier University Hospitals, several periods of interruption occurred because of problems with personnel.

A total of 1071 women without diabetes who had a BMI of more than 35 and a singleton pregnancy were assessed for eligibility, but 227 were excluded (Fig. 1). Of the 844 eligible women, 450 (53.3%) agreed to participate in the study. After randomization, 50 women (23 women in the metformin group and 27 in the placebo group) withdrew consent. Withdrawal of consent occurred within 10 days after enrollment in 42 of the 50 cases (84%) and at 14 to 49 days in the remaining 8 cases.

The maternal characteristics and obstetrical history of the 202 participants in the metformin group and the 198 participants in the placebo group are shown in Table 1. There were no significant between-group differences in the characteristics at baseline apart from maternal age, which was higher in the metformin group than in the placebo group.

OUTCOME MEASURES

There were no significant differences between the metformin group and the placebo group in the median neonatal birth-weight z score, the incidence of large-for-gestational-age neonates, or the incidence of adverse fetal or neonatal outcomes (Table 2). The median gestational weight gain in the mother and the incidence of preeclampsia were lower in the metformin group than in the placebo group, but there were no significant between-group differences in the other secondary outcomes (Table 2; and Tables S1 through S4 and Fig. S1 in the Supplementary Appendix, available at NEJM.org). In the total cohort of participants, there was a significant association between maternal gestational weight gain and the incidence of preeclampsia ($r=0.17$, $P=0.001$).

ADVERSE EVENTS

There was no significant between-group difference in the incidence of serious adverse events, but the incidence of side effects was higher in the metformin group than in the placebo group (Table 3, and Table S5 in the Supplementary Appendix). In response to side effects, 17.6% of the women stopped taking their tablets, 41.8% reduced the dose, and 40.6% continued with the full dose; there were no significant between-group differences with regard to these decisions. In seven patients (two patients in the metformin group and five in the placebo group), the study regimen was stopped because of fetal growth restriction, as evidenced by an estimated fetal weight below the 5th percentile and abnormal fetal Doppler studies.

ADHERENCE

The maximum tolerated daily dose of metformin or placebo was 3.0 g in 254 of the 400

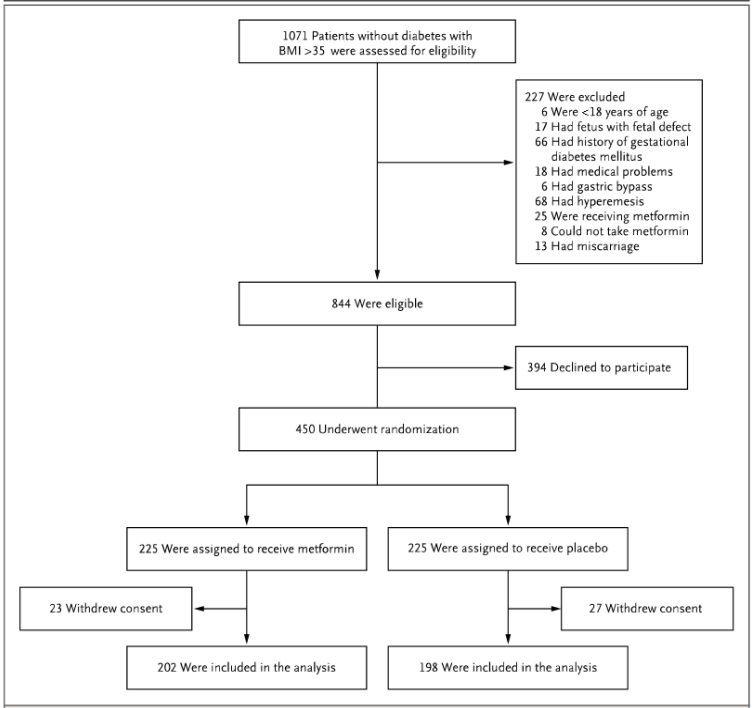

Figure 1. Screening, Randomization, and Analysis Populations.
The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.

women (63.5%), 2.0 or 2.5 g in 57 women (14.2%), and less than 2 g in 89 women (22.2%); the number of women taking each dose was used as the denominator in calculating the rate of adherence (Table S6 in the Supplementary Appendix). Adherence was good in 318 women (79.5%) and poor in 82 (20.5%). The prevalence of good adherence was directly related to the final maximum tolerated dose of medication; the prevalence was 93.5% among women taking the full dose of 3.0 g and only 53.3% among those who were taking less than 2.0 g of the drug. There were no significant between-group differences in the degree of adherence.

DISCUSSION

Our trial showed that in pregnant women without diabetes who had a BMI of more than 35, the daily administration of metformin from 12 to 18 weeks of gestation until delivery did not reduce the median neonatal birth-weight z score or the incidence of large-for-gestational-age neonates. However, metformin was associated with less maternal gestational weight gain and a lower incidence of preeclampsia than were seen with placebo. There was no significant difference between the groups in the incidence of other pregnancy complications or of adverse fetal or neonatal outcomes.

Side effects, including nausea and vomiting, diarrhea, and headache, were as expected during gestation, but the incidence of side effects was significantly higher in the metformin group than in the placebo group. However, among the women with side effects, there were no significant between-group differences with regard to the decision of whether to continue with the full dose, reduce the dose, or stop the study regimen. Regardless of side effects, adherence to the study regimen was good (≥50% of tablets taken) in nearly 80% of the women and did not differ significantly between the two groups. The rate of adherence was considerably higher among women taking the full dose of 3.0 g per day than among those taking less than 2.0 g per day, which suggests that adherence was not driven by the presence or absence of side effects but by the motivation of the patients to adhere to the demands of the study.

The major strengths of our trial include the racially heterogeneous nature of the participating women, who had moderate-to-severe obesity and were selected from a screened population of women receiving routine pregnancy care. In addition, a high percentage of eligible women agreed to participate and they also had a high rate of adherence to the study regimen.

The study has certain limitations. It was not adequately powered for the secondary outcomes. In a screening study involving 120,492 women with singleton pregnancies in our population, the incidence of preeclampsia was 2.2%,[9] and in the subgroup of 7152 women (5.9%) with a BMI of more than 35, the incidence was 5.5%. For a randomized trial to have 80% power to detect a reduction in the incidence of preeclampsia from 5.5% to the observed 3.0% in the metformin group, at a 5% significance level, 2050 patients would need to be recruited.

We found that, among obese women, less gestational weight gain was associated with a lower prevalence of preeclampsia. This finding is compatible with the results of several previous studies that showed that the prevalence of preeclampsia increased with both increasing prepregnancy BMI and increasing gestational weight gain.[9,10,26-28]

Most previous studies that have investigated the effect of metformin on pregnancy outcome have involved women with the polycystic ovary syndrome.[29-32] In four randomized, controlled trials, metformin or placebo was given from 5 to 6 weeks of gestation until delivery. One trial involving 40 women who received metformin at

Table 1. Maternal Characteristics and Obstetrical History, According to Study Group.*

Characteristic	Metformin (N=202)	Placebo (N=198)
Median maternal age (IQR) — yr	32.9 (27.3–36.2)	30.8 (26.6–34.4)
Median maternal weight (IQR) — kg†	104.7 (95.7–116.2)	105.4 (97.0–115.5)
Median maternal height (IQR) — cm	165 (160–168)	165 (160–169)
Median body-mass index (IQR)‡	38.6 (36.5–41.5)	38.4 (36.3–41.9)
Median gestational age at randomization (IQR) — wk	15.1 (13.7–17.0)	14.9 (13.6–17.3)
Race or ethnic group — no. (%)§		
White	142 (70.3)	128 (64.6)
Black	50 (24.8)	55 (27.8)
South Asian	7 (3.5)	12 (6.1)
East Asian	1 (0.5)	0
Mixed	2 (1.0)	3 (1.5)
Medical history — no. (%)		
Chronic hypertension	13 (6.4)	17 (8.6)
Polycystic ovary syndrome	26 (12.9)	18 (9.1)
Cigarette smoking	15 (7.4)	21 (10.6)
Conception — no. (%)		
Spontaneous	197 (97.5)	194 (98.0)
Ovulation induction	2 (1.0)	3 (1.5)
In vitro fertilization	3 (1.5)	1 (0.5)
Parity — no. (%)		
Nulliparous	55 (27.2)	68 (34.3)
Parous with previous preeclampsia	14 (6.9)	13 (6.6)
Parous with previous large-for-gestational-age neonate	39 (19.3)	31 (15.7)

* Comparison between groups was performed with the use of the Mann–Whitney U test for continuous variables and the chi-square test for categorical variables. There were no significant ($P<0.05$) between-group differences in any of the characteristics listed here except for maternal age, which was higher in the metformin group than in the placebo group ($P=0.02$). IQR denotes interquartile range.
† The pregnancy weight was measured at the time of study entry (12 to 18 weeks of gestation).
‡ The body-mass index (the weight in kilograms divided by the square of the height in meters) was calculated at the time of study entry (12 to 18 weeks of gestation).
§ Race or ethnic group was self-reported.

Table 2. Pregnancy Outcomes, According to Study Group.*

Outcome	Metformin (N=202)	Placebo (N=198)	Odds Ratio (95% CI)	P Value
Primary outcome				
Median birth-weight z score (IQR)	0.05 (−0.71 to 0.92)	0.17 (−0.62 to 0.89)	—	0.66
Fetal or neonatal outcomes				
Miscarriage — no. (%)	0	3 (1.5)	—	0.12
Stillbirth — no. (%)	1 (0.5)	2 (1.0)	0.49 (0.04 to 5.42)	0.62
Neonatal death — no. (%)	0	1 (0.5)	—	0.49
Live birth — no. (%)	201 (99.5)	192 (97.0)	6.28 (0.78 to 52.66)	0.12
Delivery at <37 weeks of gestation — no./total no. (%)	13/202 (6.4)	21/195 (10.8)	0.57 (0.28 to 1.17)	0.12
Median birth-weight percentile (IQR)	51.8 (23.9 to 82.1)	56.6 (26.8 to 81.4)	—	0.66
Large for gestational age — no./total no. (%)†	34/202 (16.8)	30/195 (15.4)	1.11 (0.65 to 1.90)	0.79
Birth trauma — no. (%)	3/202 (1.5)	3/195 (1.5)	0.96 (0.19 to 4.84)	1.00
Apgar score at 5 min <7 — no. (%)	1/202 (0.5)	3/195 (1.5)	0.32 (0.03 to 3.09)	0.36
Admission to NICU — no./total no. (%)	11/202 (5.4)	14/195 (7.2)	0.74 (0.33 to 1.68)	0.47
Hypoglycemia — no./total no. (%)	9/202 (4.5)	11/195 (5.7)	0.78 (0.32 to 1.93)	0.58
Hyperbilirubinemia — no./total no. (%)	11/202 (5.4)	15/195 (7.7)	0.69 (0.31 to 1.54)	0.36
Respiratory distress syndrome — no./total no. (%)	9/202 (4.5)	13/195 (6.7)	0.65 (0.27 to 1.56)	0.33
Maternal outcomes				
Median weight gain (IQR) — kg	4.6 (1.3 to 7.2)	6.3 (2.9 to 9.2)	—	<0.001
Gestational diabetes mellitus — no./total no. (%)	25/202 (12.4)	22/195 (11.3)	1.11 (0.60 to 2.04)	0.74
Preeclampsia — no./total no. (%)	6/202 (3.0)	22/195 (11.3)	0.24 (0.10 to 0.61)	0.001
Pregnancy-induced hypertension — no./total no. (%)	13/202 (6.4)	13/195 (6.7)	0.96 (0.43 to 2.13)	0.93
Delivery by cesarean section — no./total no. (%)	80/202 (39.6)	82/195 (42.1)	0.93 (0.62 to 1.38)	0.79
Postpartum hemorrhage — no./total no. (%)	19/202 (9.4)	16/195 (8.2)	1.16 (0.58 to 2.33)	0.67

* The percentages for delivery before 37 weeks of gestation, birth trauma, Apgar score less than 7 at 5 minutes, admission to the neonatal intensive care unit (NICU), hypoglycemia, hyperbilirubinemia, and the respiratory distress syndrome and all secondary maternal outcomes were calculated after the exclusion of three patients with miscarriage in the placebo group. Data on median birth-weight z score and percentile were missing for three neonates in the placebo group. The comparison between groups was performed with the use of the Mann–Whitney U test for continuous variables and the chi-square test for categorical variables. In view of multiple comparisons, a P value of less than 0.0025, rather than less than 0.05, was considered to indicate statistical significance. CI denotes confidence interval.
† Large-for-gestational-age status was defined by a neonatal weight that was higher than the 90th percentile.

a dose of 1.7 g per day or placebo showed that metformin had no significant effect on neonatal birth weight or on the incidence of preeclampsia or maternal gestational diabetes mellitus.[29] These results were confirmed by a larger study involving 273 women and a higher dose of metformin of 2.0 g per day; that study showed that women who received the drug had significantly less maternal gestational weight gain than did those who received placebo but that metformin was not associated with a significantly lower median neonatal birth weight or incidence of preeclampsia, gestational diabetes mellitus, or preterm birth.[30] In contrast, a trial involving 40 women showed that metformin at a dose of 1.7 g per day was associated with a significantly lower rate of preeclampsia than the rate among women who received placebo.[31] Another study involving 40 women showed that metformin at a dose of 850 mg per day, as compared with placebo, had no significant effect on the incidence of gestational diabetes mellitus.[32]

One recent randomized, controlled trial, the Effect of Metformin on Maternal and Fetal Out-

Table 3. Serious Adverse Events among Trial Participants.*

Category and Event	Metformin (N = 202)	Placebo (N = 198)
Fetal death		
Miscarriage	0	3
Stillbirth	1	2
Fetal defect		
Arachnoid cyst, diagnosed prenatally at 32 wk	1	0
Moderate unilateral hydronephrosis, diagnosed prenatally at 34 wk	0	1
Transposition of the great arteries, diagnosed postnatally	1	0
Trisomy 21, diagnosed postnatally	1	0
Fetal disease		
Congenital hyperinsulinism	1	0
Fetal anemia due to Rh hemolytic disease, with delivery at 33 wk	1	0
Maternal disease		
Admission for acute fatty liver at 32 wk	1	0
Admission for chest pain postnatally	0	1
Admission for dehydration at 27 wk	1	0
Admission for fibula and tibia fracture at 33 wk	1	0
Admission for gestational asthma at 30 wk	1	0
Admission for headache and neurologic symptoms at 16 wk	0	1
Admission for numbness in both legs at 32 wk	0	1
Admission for pancreatitis at 33 wk	1	0
Admission for psychosis at 37 wk	0	1
Admission for pyelonephritis at 34 wk	0	1
Admission for tachycardia at 33 wk	1	0
Scar dehiscence in woman with four previous cesarean sections, at 28 wk	0	1
Preeclampsia or fetal growth restriction		
Admission for preeclampsia and subsequent preterm delivery	1	6
Admission for preeclampsia and subsequent full-term delivery	0	2
Admission for gestational hypertension and subsequent full-term delivery	0	1
Admission for fetal growth restriction and subsequent preterm delivery	1	2
Preterm birth		
Admission for preterm prelabor amniorrhexis	4	6
Admission for cervical cerclage for short cervix	1	1
Admission for preterm labor, with subsequent full-term delivery	1	1
Spontaneous early preterm birth	1	2
Vaginal bleeding		
Admission for vaginal bleeding prepartum	0	5

* None of these serious adverse events was considered by the investigators to be associated with metformin or placebo.

comes in Obese Pregnant Women (EMPOWaR) trial, examined the effect of metformin at a dose of 2.5 g per day, administered from 16 to 18 weeks of gestation to delivery, in 449 white women without diabetes who had a BMI of more than 30.[33] That study showed no significant differences between the metformin group and the placebo group in the median birth weight, ma-

ternal gestational weight gain, the rate of preeclampsia, or the rate of adverse perinatal events. In our study, all racial groups were included so that the results would potentially be applicable to the general population. We also used a BMI cutoff point of 35 rather than 30 because the incidence of adverse pregnancy outcomes is much higher when the mother's BMI is more than 35 than when it is more than 30; the cutoff point of 35 enabled the study to have adequate power with a smaller sample size.[10] Finally, we used a 3.0-g dose of metformin, as compared with the 2.5-g dose used in the EMPOWaR trial, to avoid potential criticisms, in the event of no effect, that the dose was inadequate, particularly in women with a very high BMI.

The EMPOWaR trial had 15 participating centers; our study had only 3 participating centers, which allowed closer supervision of the study and direct contact with most patients by a small group of researchers. This difference may have contributed to the higher rate of eligible women who agreed to participate and remain in the trial (47% [400 of 844 women] in our study vs. 13% [443 of 3329 women] in the EMPOWaR trial). Similarly, adherence to the study regimen was higher in our study, in which it was estimated that nearly 80% of women consumed at least 50% of the total number of tablets prescribed. In the EMPOWaR trial, women were considered to have adhered to the study regimen if they took a minimum of one tablet of 500 g for at least 29% of the days between randomization and delivery; only 67% of women fulfilled these criteria.

The failure of the EMPOWaR trial to show that the use of metformin was associated with less gestational weight gain and a lower incidence of preeclampsia than were seen with placebo — findings that were observed in our study — may be the consequence of lower adherence to an adequate dose of medication. In our study, nearly 66% of the women in the metformin group took a minimum dose of 2.5 g for at least 50% of the days between randomization and delivery. In the EMPOWaR trial, 2.5 g of metformin was taken for only 38% of the days between randomization and delivery in the group of patients receiving this dose, but the proportion of patients who were in this dose subgroup was not specified.

In conclusion, in pregnant obese women without diabetes mellitus, prophylactic therapy with a daily dose of 3.0 g of metformin from 12 to 18 weeks of gestation until delivery was associated with less maternal gestational weight gain than that observed with placebo but not with a lower median neonatal birth weight.

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No potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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G.4 Association between insulin resistance and preeclampsia in obese non-diabetic women receiving metformin

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Association between insulin resistance and preeclampsia in obese non-diabetic women receiving metformin

Jyoti Balani[1], Steve Hyer[1], Argyro Syngelaki[2], Ranjit Akolekar[3], Kypros H Nicolaides[2], Antoinette Johnson[4] and Hassan Shehata[4]

Abstract

Objectives: To examine whether the reduced incidence of preeclampsia in non-diabetic obese pregnant women treated with metformin is mediated by changes in insulin resistance.

Methods: This was a secondary analysis of obese pregnant women in a randomised trial (MOP trial). Fasting plasma glucose and insulin were measured in 384 of the 400 women who participated in the MOP trial. Homeostasis model assessment of insulin resistance (HOMA-IR) was compared in the metformin and placebo groups and in those that developed preeclampsia versus those that did not develop preeclampsia.

Results: At 28 weeks, median HOMA-IR was significantly lower in the metformin group. Logistic regression analysis demonstrated that there was a significant contribution in the prediction of preeclampsia from maternal history of chronic hypertension and gestational weight gain, but not HOMA-IR either at randomisation ($p = 0.514$) or at 28 weeks ($p = 0.643$).

Conclusions: Reduced incidence of preeclampsia in non-diabetic obese pregnant women treated with metformin is unlikely to be due to changes in insulin resistance.

Keywords

Metformin, preeclampsia, insulin resistance, obesity, pregnancy

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Background

Obesity in pregnancy is associated with a number of serious adverse outcomes including gestational diabetes and preeclampsia.[1–3] A recent randomised double-blind placebo-controlled trial of metformin from 12–18 weeks' gestation until delivery in pregnant non-diabetic women with body mass index >35 kg/m^2 (MOP trial), reported that metformin had no significant effect on birth weight centile, which was the primary outcome, nor did it reduce the risk of GDM.[4] However, in the metformin group, compared to placebo, there was a lower median gestational weight gain (4.6 kg, interquartile range (IQR) 1.3–7.2 vs. 6.3 kg, IQR 2.9–9.2, $p < 0.0001$) and incidence of preeclampsia (3.0% vs. 11.3%; odds ratio (OR) 0.24, 95% confidence interval (CI) 0.10–0.61; $p = 0.001$).[4] This is a secondary analysis of the MOP trial[4] to investigate whether the reduced incidence of preeclampsia in non-diabetic obese pregnant women treated with metformin is mediated by changes in insulin resistance.

Materials and methods

Individuals included in this study were participants of the MOP trial that has been previously reported.[4] In this trial, 400 women were randomised in a 1:1 ratio to either active treatment with metformin or to placebo. In 384 participants, written informed consent was obtained for fasting blood samples at randomisation at 15 (12–18) weeks' gestation and again at 28 (25–33) weeks. Ethical Approval was obtained from the London-Surrey Borders Research Ethics committee (REC no 08/H0806/80) (EudraCT no. 2008-005892-83).

The plasma was separated and frozen at −20°C. Samples were batched and analysed together to avoid inter-assay variation. Plasma glucose reagents were provided by Siemens Healthcare Diagnostics Ltd, Surrey, UK; the lower limit of detection of the assay was 0.4 mmol/L and the intra-assay and inter-assay coefficients of variation at a concentration of 4.4 mmol/L were 0.6% and 1.6%, respectively. Plasma insulin was measured by a two-site sandwich immunoassay (reagent supplied by Siemens Healthcare Diagnostics Ltd, Surrey, UK); the lower limit of detection of the assay was 0.5 mIU/L and the intra-assay and inter-assay coefficients of variation at a concentration at a concentration of 45.72 mIU/L were 3.2% and 2.6%, respectively. All samples were analysed in duplicates and those with a coefficient of variation exceeding 10% were re-analysed. None of the samples in this study were previously thawed and refrozen. Insulin resistance was assessed by the homeostatic model (HOMA-IR) score, which correlates well with direct evaluation using a glucose clamp[5] and has been validated in pregnancy.[6] HOMA-IR was calculated by multiplying fasting insulin in mIU/L with fasting glucose in mmol/L divided by 22.5.

Statistical analysis

The distribution of glucose, insulin and HOMA-IR were assessed for normality using histograms and probability plots. Logarithmic transformation was necessary to achieve Gaussian normality. Mann–Whitney *U* test was used to compare the median $\log_{10}$ values of glucose, insulin and

[1]Department of Endocrinology, Epsom and St Helier University Hospitals NHS Trust, Surrey, UK
[2]Harris Birthright Research Centre for Fetal Medicine, Kings College Hospital, London, UK
[3]Department of Fetal Medicine, Medway Maritime Hospital, Kent, UK
[4]Department of Maternal Medicine, Epsom and St Helier University Hospitals NHS Trust, Surrey, UK

Corresponding author:
Jyoti Balani, Department of Endocrinology, Doreen Kouba Diabetes Centre Epsom and St Helier Hospital University Hospitals NHS Trust, Wrythe Lane, Carshalton, Surrey, SM5 1AA, UK.
Email: jyoti.balani@nhs.net

Table 1. Baseline maternal characteristics in the preeclampsia and non-preeclampsia groups.

Characteristics	Metformin $n=196$	Placebo $n=188$	p Value	Preeclampsia $n=22$	No preeclampsia $n=362$	p Value
Maternal age in years, median (IQR)	32.8 (27.3–36.1)	30.8 (26.6–34.5)	0.036	32.5 (26.0–36.5)	31.6 (27.2–35.4)	0.993
Maternal weight in kg, median (IQR)	104.8 (95.7–116.3)	105.3 (97.2–114.4)	0.719	109.7 (103.9–113.3)	104.8 (96.0–116.0)	0.134
Maternal height in cm, median (IQR)	165 (159.8–168)	165 (160–169)	0.453	165 (159–172)	165 (160–168)	0.355
Body mass index, median (IQR)	38.7 (36.5–41.5)	38.4 (36.3–41.9)	0.653	39.7 (37.2–43.4)	38.4 (36.3–42.0)	0.314
Gestational age at randomisation in weeks, median (IQR)	15.1 (13.7–16.9)	14.7 (13.6–17.1)	0.429	14.5 (13.4–18.3)	15.3 (13.9–17.5)	0.410
Gestational age at second sampling in weeks, median (IQR)	28.1 (27.7–28.6)	28.1 (27.7–28.7)	0.156	28.1 (27.9–28.8)	28.1 (27.7–28.6)	0.467
Racial origin			0.621			0.476
Caucasian, *n* (%)	139 (70.9)	123 (65.4)		13 (59.1)	248 (68.5)	
Afro-Caribbean, *n* (%)	47 (24.0)	51 (27.1)		7 (31.8)	91 (25.3)	
Asian, *n* (%)	8 (4.1)	12 (6.4)		2 (9.1)	18 (5.0)	
Mixed, *n* (%)	2 (1.0)	2 (1.1)		0	5 (1.4)	
Medical history						
Chronic hypertension, *n* (%)	11 (5.6)	17 (9.0)	0.196	7 (31.8)	21 (5.8)	<0.001
Cigarette smokers, *n* (%)	14 (7.1)	20 (10.6)	0.228	2 (9.1)	32 (8.8)	1.000
Conception			0.784			0.415
Spontaneous, *n* (%)	191 (97.5)	184 (97.9)		21 (95.5)	354 (97.8)	
Assisted reproduction, *n* (%)	5 (2.6)	4 (2.1)		1 (4.5)	8 (2.2)	
Parity						
Nulliparous, *n* (%)	55 (28.1)	65 (34.6)	0.169	12 (54.5)	108 (29.8)	0.030
Parous with previous preeclampsia, *n* (%)	14 (7.1)	11 (5.9)	0.608	3 (13.6)	22 (6.1)	0.165

IQR = interquartile range. Comparisons between outcome groups were performed by Chi-square test for categorical variables and Mann–Whitney test for continues variables.

HOMA-IR between the metformin and placebo groups and between the preeclampsia and non-preeclampsia groups. Multivariable logistic regression analysis was used to determine whether nulliparity, chronic hypertension, weight at randomisation, gestational weight gain and glucose, insulin and HOMA-IR at 15 and 28 weeks provided significant independent contribution in prediction of preeclampsia. The statistical software IBM SPSS Statistics version 22.0 with adjustment for unequal allocated groups was used for data analyses.

Results

Of the 384 patients who participated in this study, 196 women received metformin and 188 women received placebo. The maternal characteristics and history of the preeclampsia and non-preeclampsia groups are presented in Table 1. In the women who developed preeclampsia, compared to the non-preeclampsia group, there was a higher incidence of nulliparity and chronic hypertension.

Comparison between the metformin and placebo groups demonstrated that at randomisation there were no significant differences in fasting plasma glucose, fasting plasma insulin and HOMA-IR, but at 28 weeks' gestation, in the metformin group fasting plasma insulin and HOMA-IR were significantly reduced (Table 2). Comparison between the preeclampsia and non-preeclampsia groups demonstrated no significant differences in fasting plasma glucose, fasting plasma insulin or HOMA-IR, either at randomisation or at 28 weeks' gestation (Table 2). The median 2-hour postprandial glucose values were similar at randomisation (5.2 vs. 5.3 mmol/L; p:NS) and at 28 weeks gestation (5.3 vs. 5.5; p:NS).

Logistic regression analysis demonstrated that in the prediction of preeclampsia there was a significant contribution from chronic hypertension (OR 2.7; 95% CI 4.6–45.0, $p<0.001$) and gestational weight gain (OR 1.14; 95% CI 1.06–1.23, $p=0.001$), but not from weight at randomisation ($p=0.517$), parity ($p=0.061$), glucose, insulin or HOMA either at randomisation ($p=0.504$, $p=0.492$, $p=0.514$, respectively) or at 28 weeks' gestation ($p=0.397$, $p=0.924$, $p=0.643$, respectively).

Discussion

The findings of this study suggest that the reduced incidence of preeclampsia in non-diabetic obese pregnant women treated with metformin is not due to changes in insulin resistance. There were no significant differences in fasting plasma glucose, fasting plasma insulin or HOMA-IR between the preeclampsia and non-preeclampsia groups either at randomisation or at 28 weeks' gestation. Furthermore, neither plasma glucose nor plasma insulin or HOMA-IR either at randomisation or at 28 weeks provided a significant contribution in the prediction of preeclampsia.

Some studies reported that in women who develop preeclampsia maternal HOMA-IR is increased and this increase precedes the clinical onset of the disease and it may be apparent from the first trimester of pregnancy.[7–12] However, several other studies found no significant differences in HOMA-IR between preeclamptic and non-preeclamptic groups in the first, second or third trimesters of pregnancy or at the time of delivery.[13–19]

Preeclampsia has a complex pathophysiology and the primary cause is thought to be impaired placentation leading to placental hypoxia and

Table 2. Comparison between outcome groups for biomarkers of insulin resistance at 15 and 28 weeks' gestation.

Marker	Metformin $n = 196$	Placebo $n = 188$	p Value	Preeclampsia $n = 22$	No-preeclampsia $n = 362$	p Value
At 15 weeks' gestation (enrolment)						
Fasting glucose mmol/L, Median (IQR)	4.4 (4.2–4.6)	4.5 (4.2–4.8)	0.052	4.5 (4.2–4.8)	4.4 (4.2–4.7)	0.331
Fasting insulin mIU/L Median (IQR)	18.4 (12.9–27.7)	18.1 (12.3–26.8)	0.584	21.0 (15.9–29.7)	18.1 (12.3–27.1)	0.170
HOMA-IR Median (IQR)	3.8 (2.4–5.4)	3.6 (2.4–5.5)	0.908	4.2 (3.2–6.0)	3.6 (2.3–5.4)	0.110
At 28 weeks' gestation						
Fasting glucose mmol/L Median (IQR)	4.4 (4.1–4.8)	4.4 (4.1–4.8)	0.804	4.5 (4.0–4.9)	4.4 (4.1–4.8)	0.990
Fasting insulin mIU/L Median (IQR)	19.7 (14.0–31.0)	22.9 (15.2–33.6)*	0.046	24.3 (15.3–39.7)	21.5 (14.6–32.0)	0.220
HOMA-IR Median (IQR)	3.9 (2.6–5.7)	4.6 (3.0–6.8)**	0.005	4.8 (3.4–8.5)	4.2 (2.7–6.0)	0.115

IQR = interquartile range. Comparison between groups was performed by Mann–Whitney U test.
*$p < 0.05$; **$p < 0.01$.

oxidative stress with consequent release of anti-angiogenic factors, such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sENG), into the maternal circulation, which in turn lead to endothelial dysfunction and multisystem organ injury.[20–25] A recent *in vivo* study demonstrated that metformin reduces the release of sFlt-1 and sENG from placental and endothelial cells and the release of sFlt-1 from placental villous explants from women with severe preeclampsia.[26]

Our study demonstrated as expected, that metformin treatment compared to placebo, was associated with a reduction in plasma insulin and HOMA-IR at 28 weeks' gestation. There is extensive evidence to support this effect of metformin in pregnant and non-pregnant individuals.[27,28] A large RCT on 2155 non-diabetic adults with elevated fasting and post-load plasma glucose concentrations reported that use of metformin, compared to placebo, was associated with a 31% lower incidence of type 2 diabetes mellitus after an average follow up of 2.5 years.[29] During pregnancy, metformin has been widely used in women with polycystic ovarian syndrome and several studies have demonstrated its association with improved insulin sensitivity.[28,30–32] Normal pregnancy is associated with an incremental rise of fasting insulin at 28 weeks of gestation and this increase is attenuated by metformin. One study showed that metformin decreases HOMA-IR score by 4.4% at 28 weeks of gestation.[28] A recent randomised controlled trial (EMPOWaR) in non-diabetic women with BMI >30 kg/m^2 found no significant differences between the metformin and placebo groups in either the primary outcome, which was median birth weight, or in any of the secondary outcomes, including gestational weight gain and rate of preeclampsia.[33] The authors reported significantly lower HOMA-IR scores and fasting glucose in the metformin group at 28 weeks gestation but not at 36 weeks. Nevertheless, we and the authors of the EMPOWaR trial failed to demonstrate a reduction in the incidence of GDM in the metformin group.[32] Metformin was discontinued for a week prior to OGTT in our study but not in the EMPOWaR study. There was no significant difference in the rate of preeclampsia in women receiving placebo (22 (10%)) versus those treated with metformin (19 (8%)) in the EMPOWaR trial. However, failure of the EMPOWaR trial to demonstrate, as in our study, that the use of metformin reduces the rate of preeclampsia may be the consequence of poor adherence to the study regimen, wherein, women took 2.5 g of metformin for only 38% of the duration of the trial.[33] The attenuated rise in HOMA-IR induced by metformin at 28 weeks of gestation seen in the MOP trial was also observed in the EMPOWaR trial, although by 36 weeks, differences in HOMA-IR in that trial had become non-significant. The strength of this study is its randomised controlled design. A major limitation of the study is that the primary outcome of the MOP trial was birthweight and the study was not adequately powered for the secondary outcomes such as preeclampsia.

Conclusions

Whilst metformin significantly reduced fasting insulin and HOMA-IR at 28 weeks, we observed no statistical differences in plasma glucose, plasma Insulin or HOMA-IR comparing women in the preeclampsia and non-preeclampsia groups. We conclude that the finding of a reduced incidence of preeclampsia in obese non-diabetic pregnant women treated with metformin is unlikely to be mediated via changes in insulin resistance. Further studies will be necessary to investigate the potential beneficial effects of metformin in obese non-diabetic pregnant women.

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Ethical approval

Ethical approval was obtained from the London-Surrey Borders Research Ethics committee (REC no 08/H0806/80) (EudraCT no. 2008-005892-83). Informed written consent was obtained from all the participants.

Guarantor

JB.

Contributorship

JB: collecting data, analysis of results, initial manuscript; SH: revising manuscript; AS: collecting data, analysis of results, initial manuscript; RA: analysis of results, initial manuscript; KN: supervision of the project; AJ: helped with data collection; HS: supervision of the project. All authors contributed to the revision and made the decision to submit the manuscript for publication.

Note

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G.5 Visceral fat mass as a novel risk factor for predicting gestational diabetes in obese pregnant women

Original Article

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Visceral fat mass as a novel risk factor for predicting gestational diabetes in obese pregnant women

J Balani[1], SL Hyer[1], H Shehata[2] and F Mohareb[3]

Abstract

Objective: To develop a model to predict gestational diabetes mellitus incorporating classical and a novel risk factor, visceral fat mass.

Methods: Three hundred two obese non-diabetic pregnant women underwent body composition analysis at booking by bioimpedance analysis. Of this cohort, 72 (24%) developed gestational diabetes mellitus. Principal component analysis was initially performed to identify possible clustering of the gestational diabetes mellitus and non-GDM groups. A machine learning algorithm was then applied to develop a GDM predictive model utilising random forest and decision tree modelling.

Results: The predictive model was trained on 227 samples and validated using an independent testing subset of 75 samples where the model achieved a validation prediction accuracy of 77.53%. According to the decision tree developed, visceral fat mass emerged as the most important variable in determining the risk of gestational diabetes mellitus.

Conclusions: We present a model incorporating visceral fat mass, which is a novel risk factor in predicting gestational diabetes mellitus in obese pregnant women.

Keywords

Gestational diabetes, obesity, visceral fat mass, predictive model, principal component analysis, machine learning

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Introduction

The rising prevalence of gestational diabetes is concerning because of the risk of pregnancy complications such as macrosomia, shoulder dystocia, caesarean section and neonatal hypoglycaemia and also because of the risk to the mother and offspring of diabetes and cardiovascular disease in later life.[1–3] Changes in the diagnostic criteria for gestational diabetes mellitus (GDM), the obesity epidemic, increasing maternal age and unhealthy lifestyles have all been implicated in the increasing prevalence of GDM.[4,5]

Identifying women at greatest risk of GDM early in their pregnancy would allow lifestyle modification interventions and possibly drug treatments to be implemented in order to reduce the risk of complications.[6] Metformin, for example, can be used to reduce the risk of GDM in women with polycystic ovaries.[7]

Various strategies are adopted to detect overt or gestational diabetes in pregnancy depending on the local prevalence of diabetes. Some centres in the UK have adopted the IADPSG strategy, which recommends universal testing though our local policy was to continue using WHO criteria.[4] Our current policy of GDM screening is based on selective screening of women at high risk of GDM based on (i) maternal age, (ii) body mass index (BMI), (iii) history of polycystic ovarian syndrome as defined by the Rotterdam criteria,[8] (iv) family history of diabetes, (v) previous GDM, (vi) ethnicity and (vii) previous macrosomia. Selective screening using risk factors above has low sensitivity (50–69%) and specificity (58–68%) and in one study, 39% of women with GDM would have been missed if only selective risk factor testing had been used.[9] Better selection processes for selective screening may reduce the need for oral glucose tolerance testing in women at low risk with resulting savings in costs and in burdensome diagnostic testing.

Obesity is a strong predictor for GDM with odds ratios compared with normal weight women of about 3 for women with Class I obesity[10] and 5–8 for Class II and III obesity.[11] Nevertheless, only 24% of Class I obese[12] or Class II and III obese[13] women developed GDM in the control arms of two recent prospective trials investigating the possible beneficial effects of metformin in these women. Abdominal obesity may be a better predictor both for GDM and future development of diabetes outside pregnancy.[10,14]

In a prospective study of 302 obese pregnant women, we found that central obesity as assessed by early pregnancy waist-hip ratio (WHR) and visceral fat mass (VFM) measured by bioimpedance

[1]Department of Endocrinology, Epsom and St Helier University Hospitals NHS Trust, Surrey, UK
[2]Department of Maternal Medicine, Epsom and St Helier University Hospitals NHS Trust, Surrey, UK
[3]Department of Bioinformatics, Cranfield University, Cranfield, UK

Corresponding author:
J Balani, Department of Endocrinology, Doreen Kouba Diabetes Centre, Epsom & St Helier University Hospitals NHS Trust, Wrythe Lane, Carshalton, Surrey SM5 1AA, UK.
Email: jyoti.balani@nhs.net

was an independent predictor of GDM in addition to classical risk factors.[15]

The aim of this study was to develop a mathematical model to accurately predict GDM in obese pregnant women in early pregnancy. We used principal component analysis (PCA) initially but since the PCA showed no clear clustering of the GDM and non-GDM groups, machine learning using decision tree and random forests were used.

Patients and methods

The London–Surrey Borders Research Ethics committee advised us that ethical approval is not required for the study as all women would only undergo routine clinical investigations and management. No study specific procedure is undertaken on any of the participants.

Details of the study methods have been previously published.[15] In brief, we enrolled 302 obese pregnant women with no established diabetes attending the weight management clinic at St Helier Hospital, Carshalton, Surrey, UK in 2010–2011. The median age of these women was 31 years (range 26–34 years), the median BMI was 38.2 kg/m^2 (range 36.1–41.4 kg/m^2) and the median VFM was 182.8 units (range 164.3–207.7 units). About 74.5% of the women were Caucasian. All women underwent 75 g oral glucose tolerance test between 24 and 28 weeks of gestation. GDM was defined by the, 1999 WHO criteria.[16] Seventy-two of the 302 enrolled women (23.8%) subsequently developed GDM and were medically managed in the joint antenatal obstetric and diabetic clinic by a standard protocol. All women underwent body composition analysis at booking (median gestation (weeks): 15[14–17]) by Direct Segmental Multi-Frequency Bioelectrical Impedance Analysis Method (DSM-BIA Method) using an Inbody 720$^{R.}$ machine. This method is based on the electric resistance difference between the fat and other components.[17] The device measures body mass index, WHR, lean body mass, total percentage body fat (PBF) and visceral fat area. The InBody 720 has been validated and correlates well with intraabdominal fat area assessed by CT scan[18] and DEXA.[19] It has been also been shown to be safe in the second and third trimesters of pregnancy and has also been validated against deuterium and hydro-densitometry techniques for body composition analysis.[20,21]

Data mining and analysis

The dataset consisted of the following variables; maternal age, weight, body mass index, percentage body fat, visceral fat mass, lean body mass, history of polycystic ovarian syndrome, family history of diabetes, history of hypertension and previous macrosomia. PCA was performed on this dataset. PCA is a multivariate analysis for clustering input data according to their variance. PCA showed no clear clustering of the GDM and non-GDM groups. We then applied decision tree and random forests algorithms to the data after feeding the computer programme with the training dataset to recognise the presence or absence of gestational diabetes. This process is termed supervised machine learning.[22–24]

A decision tree algorithm classifies data items by asking a series of questions about the features associated with the items. Each question is contained in a node, and every internal node points to one child node for each positive answer to its question. There is a hierarchy in the questioning, encoded as a tree. In its simplest form, yes-or-no questions are asked, and each internal node has a 'yes' child and a 'no' child. An item is sorted into a class as it passes down from the topmost node, the root, to a node without children, a leaf, depending on the answers. The item is then assigned to the class that has been associated with the leaf it reaches. If trained on high-quality data, decision trees can make very accurate predictions.[23]

Random forest (RF) is an ensemble algorithm of decision trees aggregated together. This method constructs multiple versions of the training data by sampling with replacement (bootstrapping), and combining the machine learning algorithms to make predictions.[21]

RF was implemented with 200 trees using the 'randomForest' function from the 'randomForest' package in R.[25] The performance of the developed model was validated using the Monte Carlo cross-validation method.[26] For $K = 100$, the samples from each dataset were randomly distributed into training and testing datasets in 100 different splits. Then, the performance was calculated as an average of the performance of the 100 models. Firstly, the input dataset ($n = 302$) was randomly split over 100 iterations into a training dataset, which contained 70% of the samples ($n = 227$), and a testing dataset ($n = 75$) composed by the remaining samples. The training dataset was then used to build the model while the testing dataset was used to calculate the performance of such model. As the performance is calculated as a mean of 100 individually trained and optimised models, the outcome is less likely to suffer from optimistic prediction accuracy and/or over-fitting.

Results

Mathematical modelling

The optimisation confusion matrix (Figure 1) indicates that the model achieved 100% classification accuracy where all 227 training samples were correctly classified. The model validation achieved an initial prediction accuracy of 81.13%; where 61 out of 75 samples were correctly predicted (Figure 1). Upon running a series of 200 iterations, while randomly reshuffling samples within the training and testing subsets, the model stabilised after 20 iterations as shown from the performance accumulative mean, achieving a mean performance of 77.53%. However, 14 patients were wrongly classified.

Visceral fat mass emerged as the most important variable for predicting GDM by the RF method as shown in Figures 2 and 3. This was followed by BMI, weight, PBF and waist hip ratio. The less important variables were family history of diabetes, hypertension, previous big baby and history of polycystic ovarian syndrome. The

	real.test	
predicted	0	1
0	172	0
1	0	55

	real.test	
predicted	0	1
0	55	12
1	2	6

Figure 1. 0 represents no GDM and 1 represents GDM. The figure on the left hand side is the optimisation confusion matrix for prediction of GDM. The model achieved 100% classification accuracy where all 227 training samples were correctly classified as GDM or no GDM. The figure on the right hand side is the validation confusion matrix. The model predicted 61 out of 75 samples correctly achieving an initial prediction accuracy of 81%.

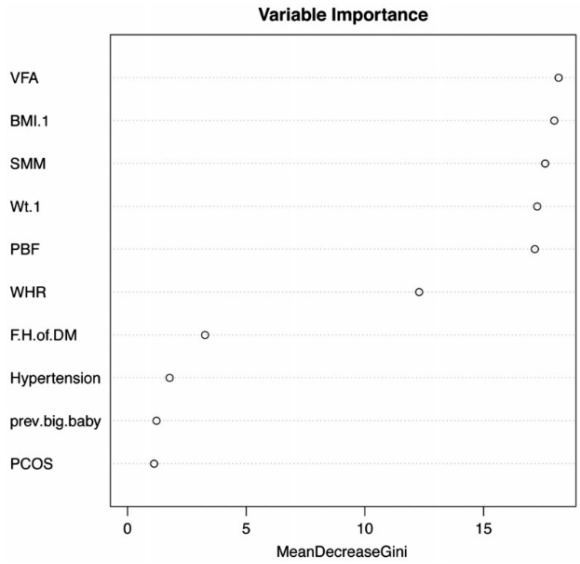

Figure 2. Ranking of variables in predicting GDM. Visceral fat area emerged as the most important input variable followed by BMI, weight, percentage body fat (PBF) and waist hip ratio (WHR). Less important variables included family history of diabetes, hypertension, previous big baby and history of polycystic ovarian syndrome.

decision tree used a value of VFM < 210 as the first split in the decision tree.

Discussion

In this analysis, VFM emerged as the most important variable in determining the risk of GDM, followed by BMI, weight, PBF and WHR. Traditional predictors like previous GDM, history of polycystic ovarian syndrome, family history of diabetes and previous big baby were less important. These results add to the growing evidence of the importance of central obesity and in particular, visceral fat mass in the development of GDM.

The model correctly classified all 227 training samples and achieved a mean validation performance of 77.53% thereby providing good prediction accuracy. However, even though 97% of the no GDM were classified correctly, only one third of the GDM were correctly classified. Since only 24% of patients developed GDM in the original training dataset, there was an unbalanced distribution of samples among both classes, resulting in a slight bias in the model prediction towards the no GDM class. A larger training database with consequently more positive GDM would be required for training the model better thereby improving the predictive performance of the model.

To our knowledge, this is the first attempt to create a mathematical model to predict GDM incorporating VFM. Traditional predictors based on maternal history are easy to measure and widely applicable. The importance of central obesity and features of the metabolic syndrome in the development of GDM has long been recognised.[24]

A strong association between measures of abdominal obesity (waist circumference, WHR and CT-assessed intra-abdominal fat area) and the development of type 2 diabetes is also well established.[14] Measuring VFM by bioimpedance is simple and can easily be done in the clinical setting. In our experience, midwives very quickly learn how to perform this measurement and the test takes less than 5 min. We have previously reported that VFM but not PBF correlates with fasting glucose and HbA1c particularly in women developing GDM.[15] This finding emphasises the importance of metabolically active visceral fat.

The clinical significance of this study is the potential for early and personalised risk stratification for GDM allowing low-risk women to avoid unnecessary diagnostic testing, repeated clinic visits and additional growth scans. Conversely, those at high risk can start lifestyle interventions early to reduce the risk of complications.

The strength of this study is that we measured a range of clinically relevant and novel predictors of GDM simultaneously rather than one novel measure measured in isolation. As such, the model created has greater validity. We also acknowledge limitations. The sample size was relatively small and a larger dataset will be needed to further train the model and improve its accuracy. In addition, our dataset was predominantly Caucasian and hence we were unable to include ethnicity in the model.

In summary, existing prognostic models for GDM lack a strong predictive value and are not commonly used in routine clinical care

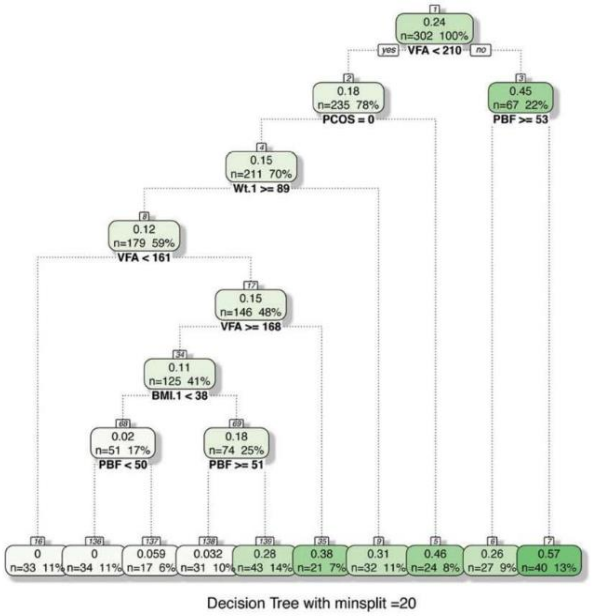

Figure 3. Decision tree in predicting GDM. Visceral fat area emerged as the most important input variable. The decision tree used a value of VFM < 210 as the first split in the decision tree.

nor are they recommended by current clinical guidelines. The addition of VFM in early pregnancy in the predictive model helps discriminate between high- and low-risk pregnancies but this need to be confirmed in larger studies with diverse populations.

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Ethical approval

The London–Surrey Borders Research Ethics committee advised us that ethical approval will not be required for the study as all women would only undergo routine clinical investigations and management.

Guarantor

JB

Contributorship

JB: collecting and analysing data, development of predictive model, initial and final draft manuscript. SH: supervision, final manuscript. HS: final manuscript. FM: predictive model, final manuscript.

ORCID iD

J Balani http://orcid.org/0000-0001-9634-2010

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