MD Thesis:

The Epidemiology and Prediction of Gestational Diabetes Mellitus

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1. Abstract

Declaration of originality

I can confirm that this thesis is my own work and all else is appropriately referenced.

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1. Abstract

1. Abstract

Objectives
To examine the relationship between particular traditional risk factors and their effect on the development of gestational diabetes mellitus (GDM) and birthweight (Part 1) as well as to assess first trimester maternal biochemical predictors of development of GDM (Part 2).

Methods
Part 1. A retrospective study of prospectively collected data from fifteen maternity units in North West London between 1988-2000- the St Mary's Maternity Information System (SMMIS) dataset. The dataset was modified to include only those who were nulliparous (thus ensuring that only one pregnancy per woman was included) and excluding women with pre-existing diabetes (thus studying only women who either did or didn't develop gestational diabetes). Birthweight z-scores were calculated.

Part 2. A nested case-control study using first-trimester (11^{+0} to 13^{+6} weeks of gestation) samples. that were obtained as part of a large prospective observational on-going study aimed at identifying first-trimester predictors of adverse pregnancy outcomes. Maternal levels of lipids (cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), non-fasting triglycerides, C- reactive protein (CRP), γ-glutamyl transferase (γ - GT), adiponectin, E-selectin, tissue plasminogen activator (t-PA) and vitamin D (25(OH)D) were measured. Statistical Package for the Social Sciences (SPSS) Version 17.0 and R (version 2.11.0) was used for statistical analysis.

Results
Part 1. There was a strong association between advancing maternal age and increasing body mass index (BMI) on the development of GDM ($p<0.01$ for both). This varied within each racial group and was more pronounced in Black African and South Asian groups. Using White European women with a BMI of 18.5-24.9 as a reference group, Black African and South Asian pregnant women had higher Odds Ratios (ORs) for GDM development within all BMI categories compared to the reference group.

Maternal BMI was positively associated with birthweight z-scores within all racial groups ($p<0.001$ for all) irrespective of glycaemic status but its effect was much greater in women with GDM. The difference in birthweight z-scores between GDM and non-GDM women varied according to racial group and was much higher in non-white racial groups and at high rather than at low BMIs.

Part 2. Simple maternal demographic and clinical characteristics obtained at the first antenatal visit provide a good prediction of GDM. Low levels of HDL and high levels of t-PA are independent predictors of GDM. ($p=0.001$ and $p<0.001$ respectively). First trimester maternal serum 25(OH)D levels are not associated with the development of GDM.

Conclusions
Maternal age and BMI interact with racial group in relation to the development of GDM. Both factors are important in the development of GDM, particularly so in Black African and South Asian women. GDM strongly accentuates the effect of BMI on birthweight, especially within non-white populations. First trimester prediction of GDM can be enhanced by the measurement of specific maternal biomarkers.

2. Background

2.1 Diabetes Mellitus

Diabetes Mellitus (DM) was first described as long ago as1550 BC, in ancient Egypt, as a polyuric state. But it was only in the 2nd century AD that the term 'diabetes' was used by Aretaeus of Cappadocia, coming from the Greek meaning 'siphon' or 'pass through'.[1] Today, DM is defined as a disorder of carbohydrate metabolism, characterised by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action or both. Diabetes is usually irreversible and although most patients can have a reasonably normal lifestyle, it is considered to be a major public health problem due to its associated long-term micro- and macro-vascular complications, premature mortality, increasing prevalence and considerable use of health resources.[2]

The prevalence of DM in the UK was estimated in the year 2000 among adults ≥ 20 years old as approximately 1.7 million, and the prevalence of DM is continuing to increase worldwide due to population growth, aging, urbanisation, increasing prevalence of obesity and physical inactivity. Although the prevalence varies within regions worldwide, the World Health Organisation (WHO) has provided estimates worldwide and predicted that if obesity levels remain constant, the prevalence of DM will rise from 170 million in 2000 to 366 million in 2030, producing a 'Diabetes Epidemic'.[3] (Fig 2.1) If obesity levels rise, then the numbers affected will be even greater.

The prevalence of DM worldwide has also been shown to vary in different populations. Race and ethnicity, which are not the same, are two useful concepts when attempting to understand differential health risks and disparities,[4] particularly within the context of diabetes. Race is defined as a division of humankind that has distinct physiological characteristics and suggests a biological component.[5] Ethnicity is defined as belonging to a social group that has a common national or cultural tradition.[5]

2. Background

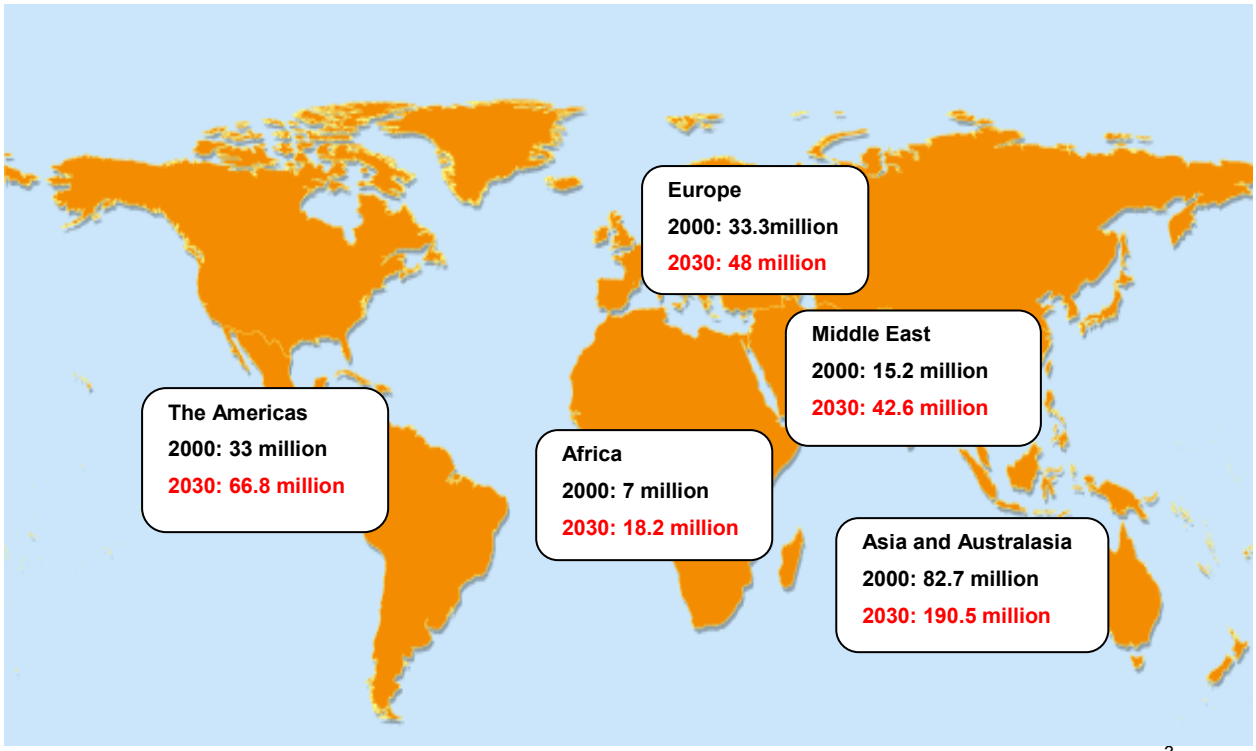


Fig 2.1 Prevalence of Diabetes Worldwide in 2000 and the estimated prevalence in 2030.[3]

2.2 Physiology of Diabetes Mellitus

2.2.1 Insulin and glucose metabolism

Insulin is a 51 amino acid peptide hormone comprising two polypeptide chains, A and B, which are linked by disulphide bridges. Insulin is the principle anabolic hormone of the body and exerts several metabolic actions, such as the regulation of the storage and release of glucose, fat and amino acids. Insulin is synthesised and secreted by the β-cells of the pancreatic islets principally in response to glucose, though other hormonal, neuronal and macronutrients may alter this response.

A large function of insulin is to promote storage of ingested nutrients. The three major tissues specialised for energy storage are the liver, muscles and adipose tissue.

2.2.2 Insulin and the Liver

Insulin enters into the portal circulation, which carries it to the liver, its primary target organ. The liver is the principle organ of glucose metabolism. Insulin exerts its action in two ways:

1. Promotes anabolism

   Insulin promotes glycogen synthesis and storage at the same time and inhibits glycogen breakdown. It also increases both protein and triglyceride formation by the liver and promotes glycolysis.
2. Inhibits catabolism

2. Background

Insulin acts to reverse the catabolic events of the post-absorptive state by inhibiting hepatic glycogenolysis, ketogenesis and gluconeogenesis.[6]

Other tissues such as muscle and fat are facultative glucose consumers (Fig 2.2).[2]

2.2.3 Insulin and other major tissues

1. Muscle

   Insulin promotes protein synthesis in muscle by increasing amino acid transport. In addition, insulin promotes glycogen synthesis to replace glycogen stores that are expended by muscle activity.

2. Adipose tissue

   Adipose tissue in the form of triglyceride is the most efficient means of storing energy. Insulin acts to promote triglyceride storage in adipocytes by a number of mechanisms.

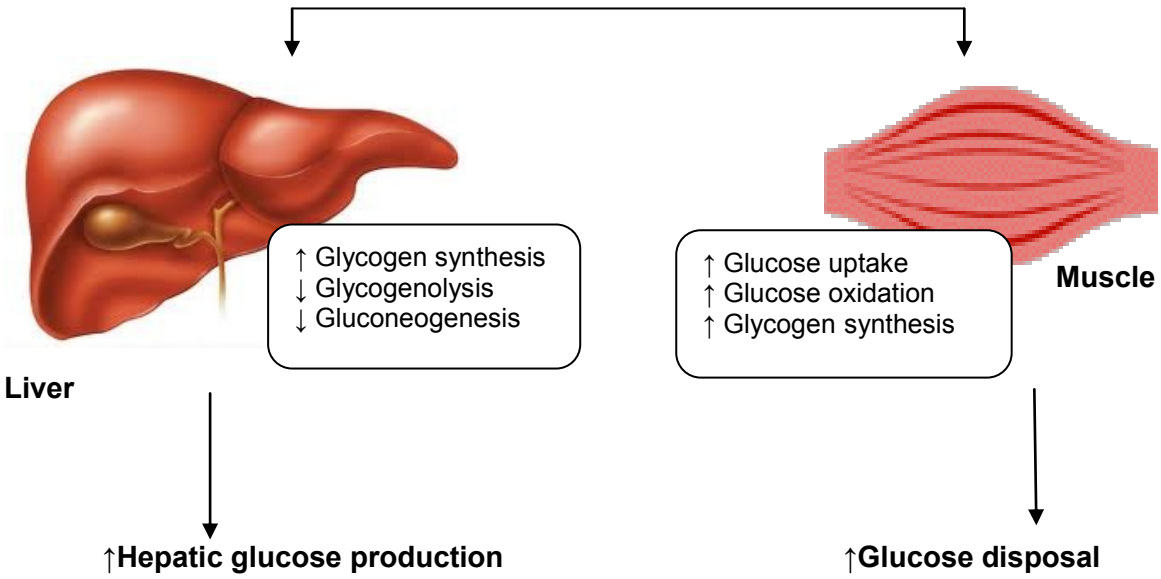


Fig 2.2 Regulation of glucose metabolism by insulin.

Secretion of insulin is tightly matched to circulating glucose concentrations. Between meals, insulin is secreted at a low basal level, accounting for approximately 50% of insulin secretion. Concentrations rise rapidly following meals. In healthy individuals, venous plasma glucose concentrations are maintained in the range of 5-7mmol/L.

2. Background

Insulin has major anabolic actions on intermediate metabolism affecting glucose, lipid and protein metabolism. In the majority of people, a balance is achieved between the rate of the appearance of glucose in the circulation and the rate of disappearance from the circulation.[2]

In diabetic women, disturbances not only in glucose metabolism but also lipid metabolism have an influence on fetal growth and may influence the metabolic profile of their offspring. Diabetic mothers tend to exhibit abnormal lipid profiles, and it is the resulting increase in fatty acids and triglycerides as well as alterations in the delivery of amino acids, which contribute to the intrauterine environment and fetal growth.

2.3 Classifications of Diabetes

There are two main types of diabetes, Type 1 and Type 2. This classification reflects differences in the underlying cause/defect.

- Type 1 DM is caused by an absolute deficiency of insulin, usually as the result of a T-cell mediated destruction of the β-cells of the pancreatic islets.
- Type 2 DM is a heterogeneous disorder that results from an interaction between a genetic predisposition and environmental factors and leads to a combination of deficiency in insulin production and decreased insulin effectiveness due to insulin resistance.

Other specific types of diabetes include[7]:

- Genetic defects of β-cell function
    - Associated with monogenic defects in β-cell function and frequently characterised by onset of mild hyperglycaemia at an early age and usually autosomal dominant pattern. Referred to as maturity-onset of the young (MODY) and characterised by impaired insulin secretion with minimal or no defects in insulin action.
- Genetic defects in insulin action
    - Genetically determined abnormalities of insulin action associated with mutations of the insulin receptor.
- Diseases of the exocrine pancreas
    - Any process that diffusely injures the pancreas eg pancreatitis, trauma, infection, cystic fibrosis
- Drug or chemical induced
    - May impair insulin secretion or may precipitate diabetes in individuals with insulin resistance.

2. Background

2.4 Epidemiology of DM

The epidemiology and clinical features of Type 1 and 2 DM are different (Table 1). The UK currently has an overall prevalence of approximately 4%.[8]

Table 1 Incidence, epidemiology and clinical features of pre-existing DM.[1]

	Type 1 DM	**Type 2 DM**
Prevalence (Adults and Children)	Accounts for 15% of DM	Accounts for 85% of DM
Epidemiology	Patients are: • Younger-usually present in children or young adults • Most commonly affects Europeans who are not usually overweight	Patients are: • Older • Often overweight • Affects all racial groups but in the UK is more common in women of Asian, Afro-Caribbean and Middle Eastern origin
Clinical features	Absolute insulin deficiency and if untreated can cause: - thirst - polyuria - blurred vision - weight loss - ketoacidosis	May have hyperglycaemia for a long period of time without clinical symptoms - Do not tend to become ketotic

The rapidly rising global prevalence of DM, as well as changing trends in the development of Type 2 DM as more adolescents and young children receive the diagnosis, has resulted in the need to identify those individuals at risk. This would allow preventative measures to be targeted towards these groups.

Risk factors for Type 2 DM can be split into two groups, those that are modifiable and those that are not, which reflects the combination of predisposing and environmental factors that result in the disease (Table 2).[9]

Table 2 Modifiable and non-modifiable risk factors for Type 2 DM.[9]

Modifiable risk factors • Overweight/Obesity • Physical inactivity • Dietary factors • Smoking • Abnormal lipids • Hypertension • Inflammation • Intrauterine environment
Non-Modifiable risk factors • Age • Gender • Ethnicity/Race • Family history of Type 2 DM • History of GDM • Polycystic ovarian syndrome

2.5 Physiology of pregnancy

2.5.1 Glucose metabolism and insulin resistance

Pregnancy is associated with profound changes in maternal metabolism to ensure a continuous supply of nutrients to the developing fetus, despite intermittent maternal food intake.[9] Glucose handling is significantly altered in pregnancy as in normal pregnancy fasting levels of glucose are decreased with advancing gestation[10] and serum levels following a meal are increased compared to the non-pregnant state.[10,11]

The following explanations for the above have been suggested:

- haemodilutional effects of early pregnancy (increasing plasma volume)
- increased utilisation of glucose- either by the feto-placental unit or increased maternal uptake of glucose secondary to increased β-cell function
- inadequate production of glucose[12-13]

2. Background

The response to the decrease in fasting glucose is an increase in hepatic glucose production. During pregnancy, there is also a change in insulin sensitivity. In early pregnancy, insulin secretion increases, while insulin sensitivity is unchanged, decreases, or may even increase[14,15] but with advancing gestation there is a marked fall in insulin sensitivity.[12] Parallel patterns of increasing concentrations of placental hormones such as human placental lactogen, prolactin, cortisol and progesterone, which have been suggested to antagonise the actions of insulin, lead to a state of relative insulin resistance and reduced insulin sensitivity as pregnancy progresses, which has the effect of facilitating the provision of fuel substrate to the fetus.[14]

In women with normal β- cell function, insulin resistance associated with pregnancy is compensated for by an increase in insulin secretion.[16] Therefore, in the majority of pregnancies the balance between progressive insulin resistance and the supply of insulin is readily met.[17] However, in some women insulin resistance becomes dominant, the women become hyperglycaemic during pregnancy and GDM ensues. This is not usually present until the second or third trimester of pregnancy as the levels of diabetogenic placental hormones increase with placental size. In women with GDM, hyperglycaemia is progressive until delivery. However, in most cases, insulin resistance resolves in the postpartum period[18] and glucose control returns to a more normal pattern.

2.5.2 Intrauterine environment

The intrauterine environment is critical for fetal development. Several factors influence transfer across the placenta such as uteroplacental and umbilical blood flows, area available for exchange, placental metabolism, and activity/expression of specific transporter proteins in the placental barrier. Fetal growth is dependent on the availability of a constantly adequate supply of glucose, lipids and amino acids from the mother for energy, production of new tissues and protein synthesis.[19]

Glucose is the main energy substrate for the fetus. Fetal production of glucose is minimal and the fetus is therefore almost entirely dependent on the placental supply of glucose from the maternal circulation. Net glucose transfer is highly dependent on maternal-fetal concentration gradients. Glucose crosses the placenta by carrier mediated facilitated diffusion via specific glucose transport proteins (GLUTs).[6]

It has been shown that the fetus can produce insulin as early as 11 weeks gestation. The production of insulin enhances glucose utilisation among the insulin sensitive tissues such as skeletal muscle, liver, heart and adipose tissue. GLUT transporters increase or decrease

glucose transport in response to changes in fetal glucose. Insulin also increases amino acid uptake and protein synthesis in the fetus.[20] Alterations in maternal metabolic processes that reduce or increase the availability of these substrates put the mother and fetus at risk.

2.6 Gestational Diabetes Mellitus

Gestational diabetes is defined as carbohydrate intolerance of varying severity, with onset or first recognition during pregnancy, irrespective of glycaemic status post pregnancy.[21] Gestational diabetes was originally defined due its association with an increased risk of Type DM in this group of women after pregnancy. Its prevalence in England and Wales is thought to be approximately 3-5%. [22]

2.6.1 Pathophysiology

Gestational diabetes is accompanied by alterations in glucose metabolism that deviate from the normal metabolic changes that occur in pregnancy. Most cases of GDM have been suggested to result from inadequate insulin secretion as a result of β- cell dysfunction. [23-24] This has been supported by the majority of studies investigating maternal β- cell function in pregnancy, which consistently find decreased β-cell function in GDM in comparison to women who maintain normal glucose tolerance throughout pregnancy.[13,24-28] The underlying mechanisms of β- cell dysfunction has been suggested to result from circulating immune markers as well as from genetic variants (particularly those found within individuals who are obese and have a metabolic state of chronic insulin resistance).[2,29]

It has also been suggested that GDM may develop not only as a result of imbalance between insulin secretion and demand but also due to the fact that insulin secretion begins at a lower starting point in these women compared to those that remain normoglycaemic during pregnancy. This would suggest that β-cell dysfunction in women with GDM is chronic rather than just acquired during pregnancy but it only becomes apparent in pregnancy, when glucose metabolism is physiologically challenged, and continues to deteriorate over time, resulting in the long-term maternal complications of Type 2 DM.[30, 31] Consequently, GDM appears to represent the presence of chronic metabolic abnormalities that predate pregnancy but are first detected during pregnancy.

2.6.2 Risk factors for GDM

Gestational diabetes mellitus results from inadequate insulin secretion for the degree of insulin resistance. Therefore, risk factors must either relate to impaired insulin secretion or contribute directly to the insulin resistant state, or both. Established risk factors for GDM development are given in Table 3.

Table 3 Summary of reported risk factors for GDM.[32]

Maternal factors
*Older age
*High parity
*Increased pre-pregnancy weight
*Increased pregnancy weight gain
*Raised BMI
*Short stature
*Low birth weight
*α-Thalassaemia trait
*Polycystic ovarian syndrome
*High intake of saturated fat
Family history
*Family history of DM
*Family history of GDM
Previous obstetric history
*Congenital malformation
*Stillbirth
*Macrosomia
*Caesarean section
*Previous GDM
Pregnancy factors
*High blood pressure in pregnancy
*Multiple pregnancy
*Increased iron stores
Protective factors
*Young age
*Alcohol use

GDM and Type 2 DM share common aetiologies and pathogenesis. The two are thought to be aetiologically indistinct from each other, with GDM preceding the onset of Type 2 DM.[32]

2.6.3 Complications of GDM

2.6.3.1 Fetal outcomes – short term

In 1952, Pedersen hypothesised that maternal hyperglycaemia was transmitted to the fetus, causing the fetus to produce and release large amounts of insulin, resulting in fetal hyperinsulinaemia.[33]

The effects of fetal hyperinsulinaemia include:

Macrosomia

An overgrowth of insulin-sensitive tissues such as adipose tissues, especially around the chest, shoulders and abdomen,increasing the risk of shoulder dystocia, perinatal death, birth trauma and the need for caesarean section. Fetal complications in GDM are further highlighted in the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study, which examined the frequency of adverse outcomes in women with carbohydrate intolerance that was less severe than overt diabetes. The study noted strong associations (although less than those in overt diabetes) between maternal glucose levels and adverse pregnancy outcomes such as increased birthweight (birthweight >90th percentile), increased rate of caesarean section, neonatal hypoglycaemia and increased cord serum c-peptide levels (>90th percentile), suggestive of fetal hyperinsulinaemia.[34]

Congenital abnormalities

The incidence of congenital abnormalities is increased in GDM, but not as much as in pregnancies complicated by pre-existing DM. This is because major hyperglycaemia occurs later in pregnancy and therefore does not usually reach teratogenic levels in the first trimester.[35]

2.6.3.2 Fetal outcomes – long term

In the long term, intrauterine exposure to diabetes can place the offspring at risk of obesity, metabolic syndrome and in later life, DM.[34] Various studies have been conducted investigating the long term effects of GDM on offspring, some with conflicting results.

Vohr et al[36] investigated the long term effects of GDM on the adiposity of offspring at 4-7 years of age. Comparisons were made between the offspring of mothers with GDM who were large for gestational age (birthweight >90th centile), and appropriate for gestational age (birthweight > 10th centile but < 90th centile) with GDM, large for gestational age in women without GDM and appropriate for gestational age in women without GDM. Offspring who were large for gestational age in pregnancies complicated by GDM were likely to be heavier, have a higher BMI and other anthropometric measurements, and a higher blood pressure in comparison to other groups. By the age of 7, 35.2% of offspring large for gestational age at delivery in pregnancies complicated by GDM had a BMI>90th percentile. However, they reported no difference in blood pressure or glucose values between the babies whose mother had GDM and control babies.

These findings were supported by the data of Wright et al[37], who found that at the age of 3, children of mothers with GDM had greater adiposity when assessed by the sum of skin folds in comparison to children of mothers who did not develop diabetes during pregnancy. Although in contrast to Vohr et al[36], they found in addition higher systolic blood pressures. These findings contrast to those of Whitaker et al[38], who found that at the age of 8-10 years there was no change in childhood adiposity, BMI or glucose tolerance test values between offspring of mothers with and those without GDM. However, in this study, the main comparison of interest was between the offspring of mothers with GDM that were treated with diet alone and the offspring of mothers with no diabetes. No comparisons were made between women with more severe forms of GDM requiring pharmacological treatment, such as insulin, which may result in an increased risk of childhood obesity.

Plagemann et al[39], noted an increase in the frequency of impaired glucose tolerance in the offspring of pregnancies complicated by diabetes. There were higher frequencies of impaired glucose tolerance noted in the GDM group compared to the pre-existing DM group. (11.1% vs 9.4% between ages 1-4 and 20% vs 17.4% between the ages of 5-9). This is markedly elevated in comparison to the usually quoted 5% frequency of impaired glucose tolerance in a normal population.

Clausen et al[40], found that the risk of the offspring being overweight doubled in pregnancies complicated by GDM (diet controlled) or Type 1 DM in comparison to offspring from the background population with pregnancies not complicated with DM. The risk of metabolic syndrome increased 4-fold in offspring of mothers with GDM and 2.5-fold in those with Type 1 DM.

2.6.3.3 Maternal Outcomes

There are increased rates of induction of labour, operative deliveries and shoulder dystocia associated with GDM.[41] Increased risks of pre-eclampsia and gestational hypertension have also been documented.[34]

In the long-term, women with GDM have an increased risk of developing diabetes later on in life, the rate of which varies between studies. Women with GDM have an18-50% risk of developing diabetes within 5 years following the index pregnancy and an increased risk of other cardiovascular risk factors such as obesity, hypertension, dyslipidaemia and subclinical atherosclerosis.[42-44]

Lee at al[45] performed a large cohort study examining the long term risk of developing Type 2 DM and potential predictors of this risk. The risk of DM was found to increase in both GDM and control groups over time. The risk of women with previous GDM developing diabetes was 1.7% at first year postpartum, 17% at 10 years and 25% at 15 years postpartum. This is in comparison to the controls where none developed DM within the first 5 years postpartum. The risk of DM within the control group was 2% at 10 years and 3.9% at 15 years postpartum. The use of insulin during pregnancy was suggested to be the biggest predictor in the long term of developing Type 2 DM in the GDM group.

2.7 Traditional risk factors and GDM

Gestational diabetes mellitus is an important disease and early recognition and treatment during pregnancy may reduce the incidence of complications. Screening for GDM remains controversial. Despite extensive research into GDM, there remains no general consensus regarding screening and different screening policies have been employed worldwide. In the UK, the National Institute for Clinical Excellence (NICE)[22] recommended selective screening for GDM, using risk factors associated with a higher rate of GDM.

2.7.1 Maternal age and GDM

In the past few decades, changes in social attitudes have resulted in women delaying their first pregnancy. The mean age of mothers at childbirth in the UK has been increasing since the mid-1970s, from 26.5 years in 1975 to 29.6 in 2010. This reflects the worldwide tendency of women to delay their childbearing to older ages.[46] Advanced maternal age has long been considered to be an obstetric risk factor with an increased risk of chromosomal abnormalities, spontaneous miscarriages, stillbirths, hypertensive disorders and GDM.[47-62] However, it is unclear whether it is maternal age itself or maternal age related co-morbidity that contributes to poor pregnancy outcomes, in older women.

2.7.2 Race and GDM

The prevalence of GDM varies in direct proportion to the prevalence of Type 2 DM in any particular population or racial/ethnic group. The prevalence of GDM is highly variable and different racial/ethnic groups in the same environment experience widely variable risk.[31] The average prevalence of GDM in England and Wales is thought to be approximately 3-5% but with some racial groups such South Asian and Black Caribbean women being at increased risk.[22]

Racial origin is thought to be important because it reflects a particular grouping of genetic factors. Genetic factors are known to be important in the development of many diseases; for

example sickle cell disease in West Africans, thalassaemia in East Europeans, cystic fibrosis in white Europeans and haemochromatosis in people of Celtic origin. Many diseases of genetic origin are not exclusive to one racial group, but there are often major differences in prevalence between racial groups, reflecting their geographical origin and differing evolutionary pressures during the progress of human colonisation around the globe.

'Ethnicity' is often used instead of racial origin, but in fact the term describes a cultural rather than genetic grouping. A good example, related to the aetiology of GDM, is diet. It is thought that some racial genetic differences have evolved in parallel with local diets, which are often environmentally determined. "Syndrome X" describes a cluster of various interrelated cardiometabolic factors that promote the development of atherosclerotic cardiovascular disease and Type 2 DM. It is thought that the common occurrence of "syndrome X" in South Asians has evolved to suit the intermittent nature of the food supply in the Indian subcontinent, linked to the Monsoon weather patterns. The incidence is noted to be increasing rapidly in India and other South Asian countries. Relative fasting is often obligatory when food supplies fail and the need to rationalise this may have resulted in fasting being incorporated into some religious practices. Intermittent fasting can normalise carbohydrate metabolism in people with "syndrome X" and in polycystic ovarian syndrome (which is common in the subcontinent), and provide a reservoir of women who become fertile during food shortage whereas their "non-syndrome X" cousins become underweight and infertile.[63]

Race is commonly contiguous with ethnicity for evolutionary reasons but when it becomes disconnected, a change in ethnicity can cause particular problems. Thus translating people from the rural subcontinent to urban centres both in that area and as immigrants to developed countries, exposes them to an ad-lib continuous diet high in carbohydrates and unsaturated fats. This probably accounts for the very high rates of diabetes, hypertension, renal disease and vascular disorders, including coronary atherosclerosis, in people of South Asian origin living in urban centres and developed countries.[64-7]

India has a higher number of diabetic patients than any other country.[3] This increase has been attributed to the so-called epidemiologic, nutritional and economic transition. The increase in food production and consumption, rural to urban immigration with the associated increase in 'junk food' consumption and decline in physical activity, result in a rise in obesity, and are contributor factors to the rise in the prevalence of DM. This does not exclude an underlying genetic susceptibility in the Asian group compared with other racial groups. Scholfield et al[68] conducted a prospective cohort study comparing indices of glucose

homeostasis between non-diabetic vegetarians living in Washington DC, 29 of whom were of South Asian (Indian) origin and 33 'Americans' (presumably of white European origin, although this was not explicitly stated in the paper). There were 14 males and 15 females in the South Asian group, and 16 males and 17 females in the 'American' group. During a 3hr glucose tolerance test, the South Asians had significantly higher insulin levels at all times except for the 3hr sample. Asian Indian men also had significantly higher plasma glucose at 1hr and 2 hrs of the glucose tolerance test. Insulinogenic scores, calculated from insulin responses to the glucose challenge measured over a 3hr period, showed significantly higher values within the Asian Indian group in comparison to the American one. The results suggest an underlying racial predisposition to glucose intolerance in the Asian population, although the differences were mainly observed within males, rather than females.

Several cohort studies have investigated the relationship between race and rates or risk of GDM. High rates of GDM are consistently reported in non-white populations, with the highest rates in Asian, Black and Hispanic/ Latino American populations.[69-76]

Thorpe et al[73] conducted a retrospective cohort study that investigated birth trends and maternal characteristics in women diagnosed with GDM who delivered in New York City between 1990 and 2001. The study showed a continuing increase in GDM over the time period from 2.6% in 1990 to 3.8% in 2001, despite a 12% decline in the number of births. The prevalence of GDM increased significantly within all racial groups except non-Hispanic white women. The highest increase was noted in South Asians, with an increase of 90% over the study period.

Berkowitz et al[74] examined the epidemiological characteristics of GDM in an ethnically diverse cohort of 10,187 women who had undergone standardised screening for glucose intolerance. The rates of GDM were lowest in white Europeans, followed by black people, Hispanics and Orientals and those who were classified as belonging to an 'other' racial/ ethnic group. Non-white racial groups were found to have an independent association with the development of GDM.

Similar ethnic variation was shown more recently by Savitz et al[75] who aimed to characterise the pattern of the occurrence of GDM among a wide range of ethnic groups in New York City. They looked at 951,920 singleton live births. This study found that, compared to the non-Hispanic white population, the adjusted relative risk (aRR) for African Americans (aRR =1.2, 95% CI (confidence intervals) 1.2- 1.3) and Sub Saharan Africans (aRR = 1.2, 95% CI 1.2-1.4) were slightly elevated, and even more elevated for non-Hispanic Caribbeans (aRR=

1.6, 95%CI 1.6-1.7), Central Americans (aRR= 1.5, 95%CI 1.4-1.5) and South Americans (aRR=2.0, 95%CI 1.9-2.1). The aRR was highest for women from South Central Asia (4.7, 95%CI 4.6-4.9) suggesting an almost 5 fold increase in the prevalence of GDM within this group.

The increased prevalence of GDM in Asian women was further highlighted by Gunton et al[76], who investigated the relationship between BMI, insulin resistance and beta-cell function in pregnant women. A comparison in outcomes was made between white and Asian women. Compared to white women, Asians were more likely to have GDM (31.7% vs 14%) and lower BMI, resulting in the suggestion that Asians develop GDM at lower BMI than their white counterparts.

The influence of race on maternal and fetal outcomes is also highlighted by Nystrom et al[77] who conducted a retrospective study of Asian, White and Asian-White couples and compared the differences in perinatal outcomes (adjusted odd ratios: aOR) between the groups. In comparison to White couples, Asian–White couples had an increased incidence of GDM (aOR 2.4 for White mother/Asian father and aOR=2.6 for Asian mother/White father) though not as high as Asian couples (aOR=4.7).

2.7.3 BMI and GDM

Obesity and being overweight are defined as abnormal or excessive fat accumulation that may impair health (Table 4). The prevalence of maternal obesity has slowly increased over the last few decades and is a growing concern due to the associated maternal morbidity and mortality.

Table 4 WHO BMI classification and categories.[78]

Classification	**BMI (kg/m^2)**
Underweight	<18.5
Normal	18.5- 24.9
Overweight	≥25.0
Obese	≥30.0
• Class I • Class II • Class III	≥30.0- 34.9 ≥35.0- 39.9 ≥40.0

2. Background

There is an increasing number of women of reproductive age who are overweight and obese; In England in 2010 32% of women between the ages of 35-64 were overweight and 21% were obese.[79] Therefore obese women are becoming increasingly more common in antenatal clinics. In the UK, the prevalence of women with a BMI ≥ 35 at any point in pregnancy who reach 24+0 weeks gestation and above is approximately 5% with 8% developing GDM. [80]

It has long been acknowledged that increased maternal weight and obesity are also linked to other adverse pregnancy outcomes, such as infection, morbidity, postpartum haemorrhage, macrosomia and stillbirth. Obesity is as an independent risk factor for developing GDM.

Several studies have looked at the relationship between pre-pregnancy BMI, weight gain during pregnancy and GDM.[71,81-89] The relationship between BMI and GDM is also unique in that BMI is the only modifiable risk factor that, if acted upon by successful dieting, could reduce the risk and rates of GDM.

Kabiru et al [88]conducted a prospective cohort study investigating the effect of increased BMI category between the first and last visits on pregnancy outcomes. 5131 women were included in the study. Women with an initially normal BMI, who increased in BMI category during the pregnancy, had an increased rate of complications such as GDM compared to those that remained in their pre-pregnancy BMI category (GDM rate: 3.7% vs 0.5 %; p =0.005, lacerations at delivery 31.7% vs 24.0%; p<0.001, failed induction of labour: 15.9% vs 4.7%: p<0.001, and caesarean section delivery: 21.0% vs 8.2%: p<0.001). Women who were overweight at the initial visit and then increased by one BMI category during the pregnancy also displayed increased complications in comparison to those whose BMI did not change category during their pregnancy. Complications included GDM (3.3% vs 1.0%, p=0.005), pre-eclampsia (3.7% vs 2.8%, p=0.002), failed induction of labour (10.3% vs 7.9%, p<0.001), operative vaginal delivery (11.4% vs 8.4%, p<0.001) and caesarean section for failure to progress (6.9% vs 3.5%, p=0.002).

A systematic review examined the effect of pre-pregnancy BMI on the risk of GDM, also aiming to quantify the change in the risk of GDM as the BMI increased.[86] For each study that was pooled into the systematic review, the unadjusted odds ratio (OR) was calculated. The review indicated that pre-pregnancy BMI is directly associated with the incidence of developing GDM. The unadjusted ORs for overweight, moderately and morbidly obese women were 1.97 (95% CI 1.77 to 2.19), 3.01 (95% CI 2.34 to 3.87) and 5.55 (95% CI 4.27 to 7.21) respectively compared to normal weight women. Torloni et al[86] calculated that with

every 1kg/m² increase in BMI, the prevalence of GDM would increase by 0.97%. The findings of the above are supported by a meta-analysis of 20 studies that examined the risk of GDM with increasing BMI.[87] The unadjusted ORs of developing GDM were 2.14 (95% CI 1.82–2.53), 3.56 (95%CI 3.05–4.21), and 8.56 (95% CI 5.07–16.04) among overweight, obese and severely obese women respectively compared with normal weight pregnant women. This illustrates the marked increase in the risk of GDM with increasing BMI.

Although the above studies highlight the association between weight gain, raised BMI and an increased rate of GDM, Glazer et al[89] explored the effect of modifying weight pre-pregnancy and risk of GDM. Their study included 4102 women, all of whom weighed over 200lbs; 32% lost weight but 61% gained weight. Women who lost at least 10lbs between pregnancies had a decreased risk of GDM in comparison to those who lost less than 10lbs (RR=0.63). Women who gained at least 10lbs had an increased risk of GDM (RR=1.47). This emphasises the importance of reducing obesity in women of reproductive age. Obesity is a modifiable risk factor associated with GDM and indeed Type 2 DM.

2.8 Screening for GDM

The goal for any screening test is to detect a disease reliably to allow cure or amelioration of the disease or minimise morbidity/ mortality either in the short or long term. To assess the value of screening, the prevalence of the disease in the general population, incidence in the study population and the sensitivity, positive and negative predictive values of the screening tests should be known.[90] Screening is also dependent on the efficiency of the local health care system and the economic condition of the country. In order that the cost effectiveness of the screening programmes can be determined, short and long term complications of the detected disease should be well established. Early treatment or intervention for the disease must be known to decrease mortality and morbidity. Quality of the life for the individual should be improved.[91]

Screening for GDM was originally established to identify a group of pregnant women who are at risk of developing Type 2 DM in the future and GDM is therefore a surrogate outcome.[31] The issue of screening is important due to the increasing prevalence of GDM and Type 2 DM. The potential benefits of treating GDM were demonstrated by the ACHOIS randomised control trial where [92] intervention and treatment of GDM led to a significant reduction in serious perinatal complications (1% vs 4%; p=0.01), defined as death, shoulder dystocia, bone fracture and nerve palsy, in comparison to routine antenatal led care.

2.8.1. Methods of screening (Universal vs Selective)

Several different methods are used for screening for GDM and which is the best screening method remains controversial. Some advocate universal biochemical screening, others advocate selective screening. Many women who are screen positive may not meet the diagnostic criteria for GDM and therefore represent a group of false positive women.

Traditionally, women have been screened on the basis of clinical risk factors. Risk based selective screening has the potential to be hampered by inadequate patient history, failure of appropriate referral and the possibility that a significant proportion of women with GDM may be missed. Although in theory universal screening should result in earlier diagnosis of GDM, the value of universal screening programmes is made less certain by the lack of data on the benefit of intervention in women that would have been screened negative based on clinical risk factors.

In the UK in 2002, The Health Technology Assessment group conducted a systematic review of screening for GDM and found that screening by risk factors such as BMI, advanced maternal age, family history of diabetes, minority ethnic background, increased weight gain in early adulthood and smoking resulted in low sensitivities (50-69%) and specificities (58-68%).[93]

Griffin et al[94], conducted a prospective randomised study where patients were randomised to universal or selective screening. The universal screening group underwent a 50-g glucose challenge test performed and if their plasma glucose at 1 h was above or equal to 7.8 mmol/l they had a formal 3-h 100-g oral glucose tolerance test (GTT). The selective screening group had a 3-h 100-g OGTT at 32 weeks if any risk factor for GDM was present. Universal screening was associated with a higher prevalence of diagnosed GDM (2.7% vs 1.45%: $p<0.03$), as expected. Universal screening facilitated earlier diagnosis, intervention and lower rates of obstetric complications such as macrosomia, caesarean section rates, pre-eclampsia and admissions to neonatal units.

A large observational multi ethnic cohort study by Cosson et al[91] compared the effectiveness of screening for GDM with universal or selective screening methods. The cohorts consisted of 159 women with GDM with risk-based screening, 265 women with GDM who underwent universal screening and 1255 women with no GDM who were the controls. This study also looked at whether either screening method could improve prognosis. Universal screening increased the prevalence of detected GDM from 8.3% to 12.6% and consequently selective screening potentially missed about 30% of all women with GDM. There was evidence of

health benefits for children when using universal rather than selective screening for GDM, especially for outcomes such as large for gestational age (7.5 % vs 17.6%; p<0.05), preterm delivery (6.0% vs 10.1%; p<0.05), neonatal jaundice (3% vs 7.5%; p<0.05) and the need for hospitalisation (12.5 % vs 23.3%; p<0.05).

However, there are potential harms of screening on women, which can be both physical and psychological.[95] A false positive screening result can result in women being inconvenienced, and/or experiencing a significant decline in the perception of their own health. There is the additional time, cost, physical discomfort and psychological burden of screening and diagnostic testing if a woman screens positive.[96] Rumbold et al[96] looked at women's experiences of being screened for GDM. This involved 158 women who were reviewed prior to screening and again after screening. More women who screened negative (i.e normal) related their experience as positive compared to women who screened positive (77% vs 57%, p<0.01). Women, who screened positive for GDM, had lower perceptions of their health.

2.8.2 Current screening guidelines in the UK

During the time of this study (1988-2000), there was no national policy on screening for GDM. An HTA[93] set up on behalf of NICE revealed that there was still considerable variation in practice across the UK. A survey of UK obstetric units in 1999[97] revealed that the majority screened for GDM but there was little consensus on the most appropriate method. The inconsistencies of screening practices provided evidence of the need for guidelines.

NICE guidelines in 2003 stated that there was not enough evidence for routine screening of GDM and it was therefore not recommended.[98] However, since then new NICE guidelines addressed the issue of screening for GDM, with the aim of improving quality of care based on the best available evidence of clinical and cost effectiveness at the time.

Since 2008, the NICE in the UK has put in place recommendations for screening for GDM.[22]

In a mainly healthy population, the current recommendation is screening based on risk factors. At booking, women should be investigated for the following risk factors:

- BMI above 30 kg/m^2
- Previous macrosomic baby (weighing 4.5kg or above)

- Previous GDM
- Family history of DM (first degree relative with DM)
- Racial origin with a high prevalence of DM (e.g. South Asian)

Women with any one of these risk factors should be offered diagnostic testing for GDM between 24-28 weeks gestation or earlier (between 16-18 weeks) if they have a previous history of GDM. Women with previous GDM should also have a repeat diagnostic test at 28 weeks if the early one at 16-18 weeks was normal. A 2-hour 75 g OGTT should be used to test for GDM and the diagnosis made using the criteria defined by the WHO.
Screening for GDM using fasting plasma glucose, random blood glucose, glucose challenge test and urinalysis for glucose should be considered with caution, as screening by these methods has produced varied results.[22]

The later in pregnancy biochemical screening such as glucose tolerance tests are performed, the higher the detection rate of GDM. This is because as pregnancy advances insulin resistance progressively increases. However, the earlier in pregnancy GDM is diagnosed, the greater is the potential to reduce adverse outcomes.

Although currently risk factor screening methods are widely used to identify women at risk of GDM, none of the variables such as BMI, ethnicity or family history have a high enough predictive value to allow truly efficient targeting and earlier intervention. Screening on the basis of risk factors alone has been shown to result in low sensitivities and specificities with sensitivities ranging from 50-68%, specificities from 58-68% and positive predictive values between 3-5%. Current UK guidelines recognise the benefits of treating GDM, as shown in the ACHOIS trial, but despite this, screening by the use of risk factors is considered to be more practical, more cost effective and less disruptive to women than universal screening.

Risk factor based screening is the current practice in most of Europe and outside of the US. However, in the US, the American Diabetes Association (ADA), recommend biochemical screening for undiagnosed Type 2 DM at the time of initial antenatal visit, in those with risk factors.[99] (Table 5). Women with an abnormal GTT in early pregnancy will receive a diagnosis of overt diabetes and not gestational diabetes.

Table 5: ADA recommended risk factors for Type 2 DM requiring OGTT

Overweight (BMI ≥ 25 kg/m^2 and have additional risk factors:
Physical inactivity
First-degree relative with diabetes
High-risk race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
Women who delivered a baby weighing >=9 lb or were diagnosed with GDM
Hypertension (≥140/90 mmHg or on therapy for hypertension)
HDL cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)
Women with polycystic ovarian syndrome
Hb A1C ≥5.7%, IGT, or IFG on previous testing
Other clinical conditions associated with insulin resistance (e.g., severe obesity, acanthosis nigricans)
History of cardiovascular disease

All women not known to have pre-existing diabetes should be screened for GDM between 24-28 weeks.

The American College of Obstetricians and Gynaecologists (ACOG)[100] also recommend universal screening for GDM, where all pregnant women between 24-28 weeks gestation undergo a 50g, 1hour glucose tolerance test.

There are substantial differences between UK and US guidelines for screening for GDM. Other than the use of universal screening recommended in the US in comparison to the recommended selective screening in the UK, there is also the difference in the attempt at an early identification of those women with undiagnosed type 2 DM. NICE guidelines take into consideration the cost-effectiveness of the screening programme to direct the guideline recommendations.

2.9 Prediction of GDM

The identification of individuals at high risk of developing GDM who would benefit from targeted preventative measures, has resulted in the investigation of novel biomarkers and potential use of them as predictors, due to their associations in the pathogenesis of Type 2 DM. Biomarkers are defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention.

Previous studies investigating the value of biomarkers in the prediction of Type 2 DM have reported conflicting and inconsistent results. This may be due to many of the factors being interlinked and sharing similar metabolic pathways and the lack of consistency in the diagnostic criteria of DM between studies.[101]

However, the close relationship between the epidemiology, risk factors and pathogenesis between Type 2 DM and GDM has stimulated the examination of screening biomarkers for the development of GDM. The association between these markers and the development of GDM has predominantly been studied within the second and third trimesters.

Most of the biomarkers that have been identified and suggested to play a role in the patho-physiology of DM can be separated into four groups: adipose derived, liver derived, inflammatory derived and endothelial derived factors. The roles of these markers as potential predictors of GDM will be discussed individually.

2.10 Adipose derived factors

2.10.1 Adiponectin

Adiponectin is a protein that is secreted exclusively by adipocytes with insulin sensitising, anti-inflammatory and anti-atherogenic properties.[102-3] It is abundantly present in the peripheral circulation and considered to be an important mediator of insulin resistance, where hypoadipenctinaemia has been found to be characteristic of obesity, Type 2 DM and atherosclerotic disease.[104-107] This is further highlighted by studies that have involved the Pima Indians, a population located in Arizona who have the highest reported prevalence of obesity, DM and insulin resistance in the world, where low levels of adiponectin were associated with DM and obesity, and the degree of hypoadiponectinaemia increased this association.[108]

2. Background

Low adiponectin levels have been shown to be significantly correlated with endothelial dysfunction, increased intima media thickness and coronary artery calcification progression, independent of other cardiovascular risk factors.[109-15] Low adiponectin levels have also been found in diseases associated with obesity such as Type 2 DM, hypertension, metabolic syndrome and coronary artery disease.[112] This is further highlighted by the association of smoking, a risk factor for cardiovascular disease and low adiponectin levels. Adiponectin levels have been reported to be lower in current smokers in comparison to never smokers and ex-smokers.[113] Adiponectin levels have also been found to vary among racial groups with lower levels found in non-white racial groups, even after adjusting for BMI.[114]

Adiponectin levels have been measured during pregnancy and postpartum.[115-117] Ritterah et al[117] measured maternal adiponectin levels at 16, 22, 30 and 36 week of gestation. They found a general trend towards declining adiponectin levels as pregnancy advanced with a sharper decline from 30 weeks gestation onwards. However, the cause of this sharp decline remained unidentified as there was no significant correlation between adiponectin and other factors measured during pregnancy such as changes in BMI, weight or carbohydrate metabolism.

A summary of studies investigating the role of adiponectin as a predictor of GDM is given in Table 6. Only a few studies have explored the association between first trimester levels of maternal adiponectin and the development of GDM. These studies have found significantly lower levels of adiponectin in women who subsequently developed GDM in comparison to those who remained normoglycaemic during pregnancy.

Williams et al[115] compared maternal plasma adiponectin concentrations in 41 cases and 70 controls in early pregnancy and found that adiponectin concentrations were significantly lower in women who subsequently developed GDM than in controls. Furthermore, they found that women with adiponectin concentrations less than 6.4 mg/ml (lower tertile of distribution of control values) experienced a 4.6-fold increased risk of GDM, as compared with those with higher concentrations. The risk of developing GDM increased by 20% for each 1 μg/ml decrease in plasma adiponectin and this was independent of confounding factors such as maternal age, family history of diabetes and adiposity in early pregnancy. This significant difference in adiponectin prior to the development of the disease encourages its potential use as a predictive biomarker of GDM.

Lain et al[116] also investigated adiponectin levels in the first trimester of pregnancy (mean gestational age 9.3 +/-2.6 weeks). Mean adiponectin concentrations were lower in women who developed GDM in comparison to those who did not (p<0.001). Categorising women in quartiles of adiponectin levels revealed that women in the lowest quartile (≤2.5μg/ml) were 11 times more likely to develop GDM in comparison to controls (OR: 11·0, 95% CI 2·0, 60·6). This finding persisted after adjustment for confounding factors (e.g. maternal age and BMI).

Georgiou et al[118] also confirmed hypoadiponectinaemia in the first trimester in women who subsequently went on to develop GDM and were able to evaluate its predictive value for GDM. Although the predictive model had a high sensitivity and specificity, it did not assess adiponectin in isolation but also took into account insulin levels, which are predictive in themselves.

A recent longitudinal study had findings, which contradict previous conclusions. Paradisi et al[119] investigated 50 women at high risk of developing GDM; 12 of whom went on to develop GDM. Although the study revealed that adiponectin levels decreased as pregnancy continued (consistent with other studies), Paradisi et al did not find a significant association between first trimester adiponectin levels and subsequent development of GDM.

Inconsistency in results may be influenced by small numbers (<50) of subjects studied as well as a consistent lack in information regarding diabetic status pre-pregnancy. Recent conflicting results encourage doubt about the validity of adiponectin as a predictor of GDM, and therefore justify further investigations.

Table 6 Summary of studies assessing the maternal adiponectin levels in GDM.

Study	Cohort	Gestation	Results	Comments
Ranheim 2004[120]	Controls=29 GDM=22	3rd trimester	8.1 ± 1.2 μg/ml vs. 12.2 ± 1.1 μg/ml; p = 0.04	Lower in GDM with BMI<25
Retnakaran 2004[121]	Asians=28 White=116 South Asians=31	2nd and 3rd trimesters	Median adiponectin levels in South Asian group (9.7μg/ml), White (15.8 μg/ml); p < 0.01	Lower in South Asians
Williams 2004[115]	Controls =111 GDM=41	1st trimester	<6.4mcg/ml OR 4.6 (96%CI 1.8-11.6)	Lower in GDM

Tsai 2005[128]	Controls=219 GDM=34	3rd trimester	8.3 µg +/- 3.8 vs 9. µg7+/- 3.9, p<0.05	Lower in GDM Correlated negatively with BMI
Fuglsang 2006[129]	N=11	1st, 2nd, 3rd trimesters and 5-8 wks post partum	From 18/40, significant inverse correlation between BMI and adiponectin (p<0.03). No correlation at week 8.	Fall in levels from mid-late pregnancy. Negative correlation with BMI.
Weerakiet 2006[124]	Controls =299 GDM=60	3rd trimester	For cut-off value of 10 mg/ml, the sensitivity, specificity, PPV and NPV were 91.7%, 30.8%, 21.0% and 94.9%, respectively.	Lower in GDM. Independent predictor for GDM.
Cortelazzi 2007[130]	Controls =13 GDM=18	3rd trimester	5·2±0·5mg/l *vs.* 8·2 ±0·8 mg/l; p< 0·01	Lower in GDM.
Retnakaran 2008[125]	Controls =80 GDM=41	3rd trimester	Adiponectin (3.6 vs. 5.3µg/ml, p<0.01	Lower in GDM. Adiponectin is positively associated with insulin sensitivity
Ritterath 2008[117]	N=32	1st, 2nd, 3rd trimesters and post partum (6 wks)	Relationship with triglycerides: 16th wk r = -0.41; p = 0.05, 22nd wk r = -0.41; p = 0.02, 30th wk r = -0.53; p < 0.01, 36th wk r= -0.37; p < 0.05, 6wks postnatal r= -0.37; p <0.05	Negative correlation with BMI. No difference between NGT and GDM/IGT groups. Fall in levels during pregnancy.
Gao 2008[126]	GDM=22, GIGT= 10 Controls = 22	1st, 2nd, 3rd trimesters	14-20wks GDM 5.06 ±1.25 ng/ml, GIGT 6.88 ±1.15 ng/ml, Controls 9.18 ±1.95 ng/ml, 24-30wks 3.00 ±1.03 ng/ml, 4.50 ±1.42 ng/ml, 5.50 ± 1.60 ng/ml, All above p<0.01	BMI negatively correlated with adiponectin levels. Fall in adiponectin during pregnancy. Lower in GDM.
Vitoratos 2008[127]	Controls = 22, GDM =22	3rd trimester and postpartum	GDM: 5381.2 ±552.09 ng/ml Controls: 8449.9 ±829.3 ng/ml Postnatal GDM=3278.5 ±841.61 ng/ml Controls 6958.1 ±694.0 ng/ml ROC curve (cut off 5253) sensitivity 86.4% and specificity 59.1%	Lower in GDM during pregnancy and postpartum

Lain 2008[116]	Controls =59 GDM =30	1st, trimester	OR 0.69 (0.52-0.92) < 25th% OR 10.2 (1.3-78.7)	Lower in GDM
Georgiou 2008[118]	Controls =14 GDM=14	1st trimester	Threshold <35µ/ml, model for prediction: sensitivity 87.5%, NPV + PPV 85.7%	Lower in GDM Potential for prediction
Paradisi 2010[119]	Controls=38 GDM=12	1st trimester	2.32±0.16 vs 1.98±0.22ng/ml: p>0.05	No difference in every trimester but decline in levels with advancing gestation.
Mrizak 2013[122]	Controls =24 GDM=28	3rd trimester	10.02 ± 1.1 µg/ml vs 8.58 ± 1.7 µg/ml, p=0.01	Lower in GDM
Lacroix 2013[123]	Controls=407 GDM=38	1st and 2nd trimesters	OR, 1.12 per 1 µg/mL decrease of adiponectin levels; p = 0.02, adjusted for BMI and HbA1c at 1st trimester	Lower in GDM

N: Number overall in study, GIGT: Gestational Impaired Glucose Intolerance, NGT: Normal Glucose Tolerance, NPV: Negative Predictive value, PPV:Positive Predictive Value, OR: Odds Ratio, r= correlation coefficient.

2.10.2 Leptin

The role of leptin is very different to that of adiponectin. Leptin is a polypeptide produced in many tissues such as the stomach, intestines and placenta. Although mainly derived from adipocytes, leptin plays a key role in the regulation of food intake, energy balance and metabolism. Leptin has been suggested to also play a role in cardiovascular disease by stimulating vascular inflammation, oxidative stress and vascular smooth muscle hypertrophy.[131]

Leptin levels are altered by periods of fasting and eating. They also display a circadian rhythm with levels being highest between midnight and early morning.[132] Leptin levels have been positively correlated with the percentage of body fat and BMI. Levels are increased with overfeeding and decreased in periods of starvation with excessive levels noted in obesity and Type 2 DM.[133]

In an uncomplicated pregnancy, leptin concentrations have been found to increase during pregnancy, peaking at approximately 28 weeks gestation, plateauing thereafter, declining slightly just before delivery and falling post-partum. This was previously thought to be due to the increase in maternal weight and metabolic and hormonal changes that occur during pregnancy. However, the majority of the increase in circulating levels of leptin has been shown to occur in early pregnancy prior to any significant metabolic or weight changes and is thought to be predominantly hormonally influenced.[134]

The use of leptin as a predictor of GDM has been investigated. In the few studies that are reported, conflicting results have been found. Qui et al[130] found an association between maternal leptin levels taken at 13 weeks of gestation and risk of GDM, independent of adiposity. Women with leptin concentrations of 31.0 ng/mL or higher experienced a 4.7-fold increased risk of GDM (95% CI 1.2- 18.0) as compared to women with concentrations of 14.3 ng/mL or lower. Gao et al[126] found a significant difference between leptin levels taken between 14-20 weeks of gestation in women who developed GDM, compared with those who did not. The levels of leptin were lower in GDM groups in comparison to non-GDM ($p<0.01$). Kirwan et al[136] reported similar results. In contrast, Georgiou et al,[118] did not find a significant difference in first trimester maternal leptin levels between women who later on developed GDM and those that remained normoglycaemic.

The studies that assessed maternal leptin levels at later gestations also give conflicting results.[137-40] Lopez-Tinoco et al[139] found that leptin levels taken during the third trimester were significantly higher in the GDM group compared to women who remained normoglycaemic during pregnancy. There was also a positive correlation between leptin and insulin resistance. However, Saucedo et al[140] found that leptin levels during the third trimester were not significantly different between GDM and non-GDM groups.

Table 7 Summary of studies assessing the maternal leptin levels in GDM

Study	Cohort	Gestation	Results	Comments
Festa 1999[143]	Controls =166 GDM=55	22-29 wks	26.9 ng/ml vs. 19.4 ng/ml, p<0.01	Leptin levels lower in GDM (adjusted for BMI) Leptin higher during pregnancy
Vitoratos 2001[144]	Controls =17 GDM=17	3rd trimester	16.52 ± 5.07 ng/ml vs 10.61 ± 1.47 ng/ml, p<0.01	Higher in GDM
Qiu 2004[135]	Controls =776 GDM=47	<16 wks	Levels ≥31.0 mg/ml lead to RR= 4.7 (95% CI 1.2-18.0) vs levels <14.3mg/ml	Higher in GDM
Ranheim 2004[120]	Controls =29 GDM=22	3rd trimester	23.3 ±1.7 ng/ml vs 22.7±1.3ng/ml, p= 0.6	No significant difference
Gao 2008[126]	Controls= 22 GDM=22, GIGT= 10	1st, 2nd, 3rd trimesters	14-20 wks: GDM 12.79±2.31 ng/ml, NGT 4.98±1.41 ng/ml GIGT 9.61±0.96 ng/ml p<0.01	Higher in GIGT and GDM

			24-32 wks: GDM 22.64 ±5.8 ng/ml, NGT 8.88± 2.41 ng/ml, IGT 15.11±2.75 ng/ml:p<0.01	
Saucedo 2010[140]	Controls=60 GDM=60	3rd trimester	22.7±4.9ng/ml vs 23.3±11.7ng/ml, p>0.05	No difference
Soheilykhah 2010[138]	Controls =27 GDM=29 GIGT=26	3rd trimester	GDM 30.60 (33.40), GIGT 24.10 (17.07) NGT 21.60 (12.60), p= 0.03	Higher in GDM, correlates with insulin resistance
Horosz E 2011[141]	Controls =86 GDM=48	3rd trimester	24.14 ± 12.77 vs 21.34 ±11.67ng/ml	No difference
Roca-Rodriguez 2012[142]	Controls=21 Prev GDM=41	Post partum	32 025.5 ±19 917.3 ng/mL vs 20 258.9±16 359.9 ng/mL, p=0.02 (GDM vs controls)	Leptin higher post partum in women with prev GDM

N: Number overall in study, GIGT: Gestational Impaired Glucose Tolerance, NGT: Normal Glucose Tolerance, RR= Relative risk

2.10.3 Resistin

Resistin is a cysteine rich protein which forms part of the group of adipocyte derived proteins previously mentioned. Resistin has been suggested to cause insulin resistance and play a role in glucose homeostasis and glucose tolerance in mice.[145] The role of resistin in humans remains controversial. It has been suggested that although it may affect glucose metabolism and its levels are elevated in obesity and Type 2 DM, resistin may also have an inflammatory role and influence the development of cardiovascular disease.[146-7] Increased placental expression suggests a possible role in the insulin resistant state that typically characterises pregnancy.[148] The role of resistin in GDM remains unclear (Table 8).

First trimester maternal serum resistin levels have been found to be unchanged from pre-pregnancy levels.[149] Serum resistin samples taken pre-delivery and within the first five days post-delivery were found to be significantly higher in women with GDM in comparison to women with an uncomplicated pregnancy. There was a general downward trend of maternal serum levels post-delivery in both groups.[149] These findings were consistent with those of Vitoratos et al[150], and both highlight the significant contribution of resistin from the placenta. However, there have been conflicting findings in other studies. Cortelazzi et al[130] found no difference in maternal plasma resistin levels between women with GDM and those with an uncomplicated pregnancy at 37-41 weeks of gestation.

2. Background

The small number of studies assessing levels of resistin during pregnancy[152] and particularly those studies assessing first trimester maternal levels of resistin and future development of GDM implies that further studies will be required but based on the above studies it is unlikely that resistin will be an important predictor of GDM development.

Table 8 Summary of studies assessing the maternal resistin levels in GDM.

Study	Cohort	Gestation	Results	Comments
Cortelazzi 2007[130]	Controls=13 GDM=18	3rd trimester	8·5 ± 2·3ng/ml vs 6·9 ± 0·7ng/ml, p>0.05	No difference
Nien 2007[153]	Normal BMI and pregnant= 261 Overweight and pregnant =140 Non pregnant and normal BMI=40	1st, 2nd and 3rd trimesters	10.2 (5–16) ng/ml, range: vs 12.5 (5-40) ng/ml, p=0.04 (non pregnant, normal BMI vs pregnant normal BMI)	Resistin higher in pregnancy (1st trim)
Chen 2007[151]	Controls=20 GDM=20	3rd trimester	62·38 ± 13·6 ng/ml vs 22·21 ± 8·14 ng/ml, p<0·01	Higher in GDM
Megia 2008[154]	Non pregnant (normal BMI) =40 Pregnant (normal BMI)=261 Pregnant with BMI>25=140	3rd trimester and post-partum (1-6mths)	3rd trim: 4.32 ± 1.56 ng/ml vs 9.30 ±1.32 ng/ml, p<0.01 Post-partum: 4.24±1.56 ng/ml vs 3.11±1.63 ng/ml, p<0.01	Lower in GDM Lower post-partum
Lain_2008[116]	Controls=59 GDM=30	1st trimester	17.2 ng/ml vs 15.8 ng/ml, p=0.45 (GDM vs controls)	No difference
Kuzmicki 2009[155]	Controls=81 GDM=82	3rd trimester	21.9 (17.55–25.40) ng/ml vs 19.03(15.92–23.91) ng/ml, p<0.05	Higher in GDM
Vitoratos 2011[150]	Controls=30 GDM=30	3rd trimester	0.28 ng/ml vs 0.21 ng/ml, p = 0.02	Higher in GDM
Nanda 2012[149]	Controls =240 GDM=60	1st trimester	8.32ng/ml vs 8.28ng/ml p = .549	No difference

2.10.4 Visfastin

Secreted by visceral adipose tissue, the role of visfastin in DM has remained controversial because of contradictory results from studies investigating the association with pre-existing

DM. Visfatin has been proposed as an insulin mimicking adipocytokine. Increased levels have been found in insulin resistant states including obesity and Type 2 DM[156-8]

In relation to GDM, maternal visfastin levels have been found to be both lower [159-63] and higher in women with established GDM.[163-67] However these studies involved maternal samples taken at time of diagnosis of GDM i.e. during the third trimester (Table 9). Limited data exist investigating the role of visfastin in early pregnancy. Ferreira et al[166] measured first trimester maternal serum visfastin and found significantly higher levels in the GDM group in comparison to the non GDM group (Table 9). This finding supports the role of visfastin in insulin resistant states.

Table 9 Summary of studies assessing the maternal visfatin levels in GDM.

Study	Cohort	Gestation	Results	Comments
Krzyzanowska 2006[163]	Controls =30 GDM=24	3rd trimester and post partum	64.0 (50.9–74.8) ng/ml vs 46.0 (36.9–54.6) ng/ml, p<0.01	Higher in GDM
Chan, 2006[161]	Controls =20 GDM=20	3rd trimester	9.4 ±3.8 ng/ml vs 12.6 ± 4.5 ng/ml; p=0.02	Lower in GDM
Haider 2007[160]	Controls =10 GDM=10	3rd trimester	1.9±0.8 ng/ml vs 5.2±4.4 ng/ml, p=0.05	Lower in GDM
Lewandowski 2007[164]	Controls =20 GDM=16	3rd trimester	76.8 ng/ml (95% CI 70.2–83.4) vs 93.1 ng/ml(95% CI 86.2–99.9); p<0.01	Higher in GDM
Szamatowicz 2009[165]	Controls =63 GDM=61	3rd trimester	14.8 (10.8-17.3) mg/l 15.3 (11.8-19.4) mg/l, GDM vs NGT, p>0.05	No difference
Ferreira 2011[166]	Controls= 300 GDM=100	1st trimester	1.34 (0.70-2.87) MoM, vs 1.00 (0.53-1.92) MoM; p < 0.01	Higher in GDM
Rezvan 2012[162]	Controls =35 GDM=35	3rd trimester	5.29 ± 0.47 ng/ml vs 7.76 ± 0.53 ng/ml, p < 0.01	Lower in GDM

NGT: Normal Glucose Tolerance, MoM=multiple of the median

2.10.5 Lipids

Insulin resistance and Type 2 DM are associated with a dyslipidaemic profile, characterised by reduced high density lipoproteins, a predominance of low density lipoproteins and elevated triglycerides.[168-71] This dyslipidaemic profile has also been found in pregnancies complicated by GDM. However, results have been inconsistent. There are very few studies

that have investigated lipid levels during the first trimester in order to examine any associated risk of GDM development.

Enquobahrie et al[172] in a cohort study compared lipid levels at approximately 13 weeks gestation between 47 women who developed GDM and 804 who did not. A positive association between triglyceride levels and risk of GDM was established. Women with a triglyceride level >137mg/dL experienced a 6-fold increase in risk of GDM compared to women with triglyceride levels in the lower tertile. (RR = 6.1; 95% CI: 2.1–17.4). There was no association found between risk of GDM and other lipids such as total cholesterol, high-density lipoprotein, and low-density lipoprotein.

Table 10 Summary of studies assessing the maternal lipids levels in GDM.

Study	Cohort	Gestation	Results	Comments
Nolan 1995[174]	N=388	1st trimester	TGs: p<0.01, r=0.60	Predictive of GDM
Enquobahrie 2005[172]	Controls= 851 GDM=47	1st trimester	TGs ≥137 mg/dL RR 3.45 (1.14-10.50), p<0.05 vs lowest tertile	Elevated TGs associated with increased risk GDM No difference for total cholesterol, HDL and LDL
Sánchez-Vera 2007[173]	Controls =107 GDM=62	2nd trimester	TGs: 5.2 mmol/L vs 4.2 mmol/L, Cholesterol: 1.14 mmol/L vs 0.8 mmol/L Glucose: 5.2mmol/L vs 4.4mmol/L p<0.05 for all factors (GDM vs controls)	GDM: increased triglycerides, cholesterol and glucose
Paradisi 2010[119]	Controls =38 GDM=12	2nd trimester	TGs> 158mg/dl (RR=5.87)	Increased risk of GDM with elevated TGs

N: Number in study overall, HDL= High Density Lipoproteins, LDL= Low Density Lipoproteins, TGs= triglycerides, RR= relative risk.

2.11 Liver Derived factors

2.11.1 Gamma- glutamyltransferase

Gamma- glutamyltransferase (γ-GT) is an enzyme found in hepatocytes and biliary epithelial cells. Outside pregnancy it is widely used as a marker of liver disease and alcohol consumption and is strongly associated with excess fat deposition in the liver, termed non-alcoholic fatty liver disease.[175] It is also thought to contribute to anti-oxidant systems, where an increase in γ-GT may be a marker of oxidative stress.[176] Oxidative stress may also play a role in the pathophysiology of DM.

A large population study that investigated the distribution and population determinants of γ-GT found an association between increasing BMI and γ-GT. This was found particularly in the upper percentiles for BMI in women.[177]

Few studies have examined the role of γ-GT as a predictor of GDM (Table 10). Tan et al[178], measured levels of γ-GT in the late second trimester at the time of diagnosis. A cut off ≥ 24 IU/L was associated with an increased risk of developing GDM (aOR 2.1 (95% CI 1.2–3.8)).

2.11.2 Alanine transaminase

The liver plays an important role in normal glucose homeostasis. Alanine transaminase (ALT) is primarily found in the liver and its levels are usually associated with non-alcoholic fatty liver disease. However, some studies have suggested an association between high ALT levels and later development of DM; elevated ALT is associated with a decline in hepatic insulin sensitivity which promotes the development of DM.[180-2]This association is independent of adiposity and ALT, along with other liver enzymes, has been found to be an independent predictor of Type 2 DM.[182]

Table 11 Summary of studies assessing the maternal γ-GT and ALT levels in GDM.

Study	Cohort	Biomarker	Gestation	Results	Comments
Tan 2008[178]	Controls =29 GDM=27	γ-GT	2nd trimester	aOR 2.3 (95% CI 1.2–4.2), p < 0.01	Higher in GDM. Independent risk factor for GDM
Alanbay 2012[179]	Controls =42 GDM=37	γ-GT	2nd and 3rd trimesters	ROC analysis (γ-GT cut off 16IU/L): sensitivity: 86%, specificity:	γ-GT independent predictor of

				37%	GDM
Tan 2012[180]	N =2610 GDM=319	γ-GT and ALT	2nd and 3rd trimesters	γ-GT: 18 ± 12 IU/L vs. 16 ± 11 IU/L, p = 0.03 RR 1.35 (95%CI 1.0–1.8); p = 0.04 (unadjusted), p>0.05 (adjusted)	γ-GT Higher in GDM ALT - no difference between groups

N: Number in study overall, aOR= Adjusted Odds Ratio, ROC: Receiver Operator Curve, RR: relative risk.

The extent of the association between ALT and insulin resistance has not been extended to the context of pregnancy. Only one study has examined the association between second and third trimester maternal ALT levels and GDM development and found no correlation.[180]

2.11.3 Sex hormone binding globulin

Sex hormone binding globulin (SHBG) is a glycoprotein that is synthesised and secreted in the liver. Its principal function is to act as a transport protein and regulate circulating concentrations of free sex hormones. However, the concentration of SHBG is affected by various clinical conditions. Elevated concentrations have been found in conditions such as thyrotoxicosis and low SHBG levels have been found consistently within insulin resistant states such as obesity and DM.[183] Ding et al conducted a large nested case control study of post-menopausal women and found that higher levels of SHBG were associated with a lower risk of DM.[184] Other studies have supported the association of reduced SHBG concentrations as a potent marker of hyperinsulinaemia[185] and insulin resistance.[186]

However, the literature surrounding SHBG and prediction of GDM is limited (Table 12). Caglar et al[187] found significantly lower levels of SHBG at 13-16 weeks gestation in women who later developed GDM in comparison to women who remained normoglycaemic. This has been confirmed in further studies[149] and highlights the association of SHBG with insulin resistance. A further study by Spencer et al[188] compared women with pre-existing DM and uncomplicated pregnancies. They found that during the first trimester of pregnancy, there were significant reduced levels of SHBG in women with pre-existing DM in comparison to women with uncomplicated pregnancies.

Table 12 Summary of studies assessing maternal SHBG levels in GDM.

Study	Cohort	Gestation	Results	Comments
Bartha 2000[190]	Controls =32 GDM=34	3rd trimester	309.54±112.22 nmol/l *vs* 460.54±144.54 nmol/l, p<0.01	Lower in GDM

Thadhani 2003[189]	Controls =94 GDM=44	1st trimester	OR 0.69 (0.48-0.99)	Lower in GDM
Smirnakis 2005[191]	Controls =108 GDM=35	<20 weeks	OR 4.5 (1.4-14.1) Lowest tertile as reference	Lower in GDM
Spencer 2005[188]	Controls=400 GDM/PED=64	1st trimester	Median MoM=0.81, p<0.01	Lower in GDM
McElduff 2007[184]	Controls =158 GDM=62	2nd and 3rd trimester	335±87nmol/l vs 339±101nmol/l; p=0.78	No difference
Caglar 2012[187]	Controls=63 GDM=30	13-16 weeks	Threshold 97.47nmol/L, sensitivity 80%,specificity 84.6%, PPV 50%, NPV 95.7% OR 12.3 (95%CI 1.7-83)	Lower in GDM

NPV= Negative Predictive value, PPV=Positive Predictive Value, MoM= Multiples of the Median, OR=Odds ratio.

2.12 Endothelial derived factors

2.12.1 Tissue plasminogen activator

Tissue plasminogen activator (t-PA) is a haemostatic factor that converts plasmin to plasminogen and is therefore involved in fibrinolysis, tissue remodelling and cell migration.[192]Tissue plasminogen activator is a marker of endothelial dysfunction and has been shown to be a strong independent predictor of DM independent of insulin resistance.[193]

Tissue plasminogen activator has been found to be positively correlated with waist circumference, visceral fat and glycosylated haemoglobin.[194-6] Weight reduction has been shown to decrease the risk of cardiovascular morbidity resulting in a significant reduction in markers of endothelial dysfunction[195-6], encouraging intervention and lifestyle modification towards these patients deemed to be at risk.

The Framingham offspring study examined the association between insulin levels and haemostatic factors in a mixed population (men and women) with normal and impaired glucose tolerance. Tissue plasminogen activator levels were significantly higher in those with impaired compared to those with normal glucose tolerance, suggesting a mechanism for subsequent increased cardiovascular risk in this group.[197]

Although elevated t-PA has been reported in insulin resistance, Type 2 DM and obesity, there are no previous studies that have investigated its role in GDM.

2.12.2 E-selectin

E-selectin is a cell adhesion molecule that is exclusively expressed in the endothelium. Outside pregnancy, E-selectin is associated with BMI, race and smoking.[198-200] E- selectin is

thought to be positively correlated with BMI and weight reduction in non-diabetic obese women resulted in a significant decrease in E-selectin level compared to those who did not lose weight.[198] Racial variation also exists between endothelial markers and significant differences between Hispanic vs white and black populations have been reported, with elevated levels in non-white racial groups.[199] Measured levels of E -selectin in a healthy population were significantly higher in smokers vs non-smokers and displayed a significant positive trend with increasing levels of smoking.[200]

Very few studies have examined the association of E-selectin with GDM (Table 13). Studies that have been performed have found variable results.[201-3] One study found no difference in the third trimester maternal E-selectin levels between women with GDM, normoglycaemic controls and the non-pregnant.[202] This contrasted with another study that found significantly higher E-selectin levels between women with GDM and those with normal glucose tolerance in comparison to non-pregnant controls.[203]

Furthermore, a study assessing postnatal levels of E-selectin found that its levels remained elevated in women that had GDM compared to those that remained normoglycaemic in whom a significant drop in E-selectin levels was noticed.[201]

In the postnatal period- between 2 and 24 months- another study found that women with a previous history of GDM and an abnormal oral glucose tolerance test postnatally had significantly elevated E-selectin levels compared to women with no previous history of GDM.[204]

There are no previous studies that have examined first trimester maternal E-selectin levels and the subsequent development of GDM.

Table 13 Summary of studies assessing maternal E-selectin levels in GDM.

Study	Cohort	Gestation	Results	Comments
Kautzky-Willer 1997[203]	Controls =8 GDM=9	3rd trimester and 3 weeks post-partum	GDM vs controls, p<0.05	Higher in GDM and remain raised post delivery
Telejko 2009[201]	Controls =68 GDM=56	3rd trimester	35.8 (25.0–46.4) ng/ml vs 34.2 (25.8–46.2)	No difference in GDM

			ng/ml, p>0.05	

2.13 Inflammatory factors

2.13.1 C-reactive protein

Chronic subclinical inflammation has been proposed to play a key role in the pathophysiology of DM development and cardiovascular disease. C-reactive protein (CRP) is produced in the liver and is a sensitive marker of inflammation produced in the liver and has emerged as an independent predictor of cardiovascular disease in within previously healthy populations.[205-16]

Circulating levels of CRP have been shown to correlate with measures of body fat, including BMI.[209] Several studies have confirmed the strong association between obesity and raised CRP, suggesting that obesity is state of chronic inflammation and contributes to the pathology of subsequent cardiovascular disease in these groups.[208-9]

C-reactive protein has been studied extensively during pregnancy but predominantly within the context of subclinical infection in preterm prelabour rupture of membranes. A rise in CRP levels has been noted as early as 4 weeks gestation. However, the study population was undergoing in vitro fertilisation treatment and therefore may have had underlying confounding factors accounting for subfertility that may also influence the results.[211] An increase in CRP has been noted in other studies as well as in other stages of pregnancy.[211]

Early pregnancy CRP levels have been shown to be significantly higher in women who went onto develop GDM in comparison to normoglycaemic pregnancies (Table 14)[191, 211-2]

Table 14 Summary of studies assessing maternal CRP levels in GDM.

Study	Cohort	Gestation	Results	Comments
Wolf 2003[211]	Controls =94 GDM=43	1st trimester	aOR 3.6 (1.2-11.4) vs the reference group*	Higher in GDM
Retnakaran 2003[213]	Controls =93 GDM=48	3rd trimester	Correlation between BMI and CRP (r = 0.38, p<0.01)	BMI important determinant of CRP and not glycaemic status during pregnancy
Qiu 2004[212]	N=851 GDM=38	<16 weeks	OR 3.5 (1.2-9.8) vs the reference group*	Higher in GDM
Chen 2006[214]	N=172 GDM=35	15-16 wks	≥10.76 mg/L aOR 2.67 (1.16-6.17)	CRP higher but not after adjustment for

				BMI
Smirnakis 2007[189]	Controls =73 (GDM=35)	1st trimester	OR 2.2 (0.7-2.5) vs the reference group*	Higher in GDM

N: Number in study overall, aOR: Adjusted Odds Ratio, OR: odds ratio

* Studies have made comparison with control groups using CRP tertiles. The lowest tertile served as the reference group.

2.13.2 Tumour necrosis factor alpha

Tumour necrosis factor alpha (TNF-α) is a cytokine involved in systemic inflammation and has been found to play a role in insulin resistance. The placenta is a major source of TNF-α and levels have been found to vary throughout pregnancy, depending on gestation, showing a downward trend from pre-pregnancy to early pregnancy and an increase in late pregnancy.[126, 214-5]

The majority of studies conducted measured TNF-α levels during the third trimester of pregnancy. Studies on TNF-α during the first trimester of pregnancy have found varying results. Kirwan et al[136] found that although there is a downward trend of TNF-α levels from pre-pregnancy to early pregnancy, there is then an increase in levels as pregnancy advances to the third trimester. These changes were consistent, irrespective of glycaemic status during pregnancy. During the third trimester, TNF-α levels were also found to be significantly higher in those women who developed GDM in comparison to those who remained normoglyacemic. TNF-α was shown to be a significant predictor of insulin sensitivity (Table 15).

Table 15 Summary of studies assessing maternal TNF-α levels in GDM.

Study	Cohort	Gestation	Results	Comments
Winkler 2002[218]	Controls =35 GDM=30	2nd and 3rd trimesters	6.3 ± 0.6 ng/l vs 4.1±0.4 ng/l, p<0.05	Higher in GDM
Kirwan 2002[136]	Controls =15 GDM=5	Pre-preg,1st - 3rd trimesters	2.84pg/ml vs 1.15pg/ml, p<0.01 (late pregnancy vs pre-pregnancy)	TNF-α significantly higher in late pregnancy vs pre pregnancy
Kinalski 2005[217]	Controls =30 GDM=30	3rd trimester	1.71 ± 0.92 pg/ml vs 1.27 ± 0.42 pg/ml, p = 0.02	Higher in GDM

Altinova 2007[216]	Controls =31 GDM=34	3rd tirmester	20.5 ± 2.4 pg/ml vs 14.0 ± 1.5 pg/ml, p = 0.04	Higher in GDM
Gao 2008[126]	Controls =10 GDM=22	2nd and 3rd trimesters	14-20 wks: 165.37±33.60 pg/ml vs 30.60±11.83 pg/ml, p<0.01 24-32 wks: 290.61±60.05 pg/ml vs 8.37±2.41pg/ml, p<0.01	Higher in GDM

Other biomarkers associated with GDM

2.14. Vitamin D

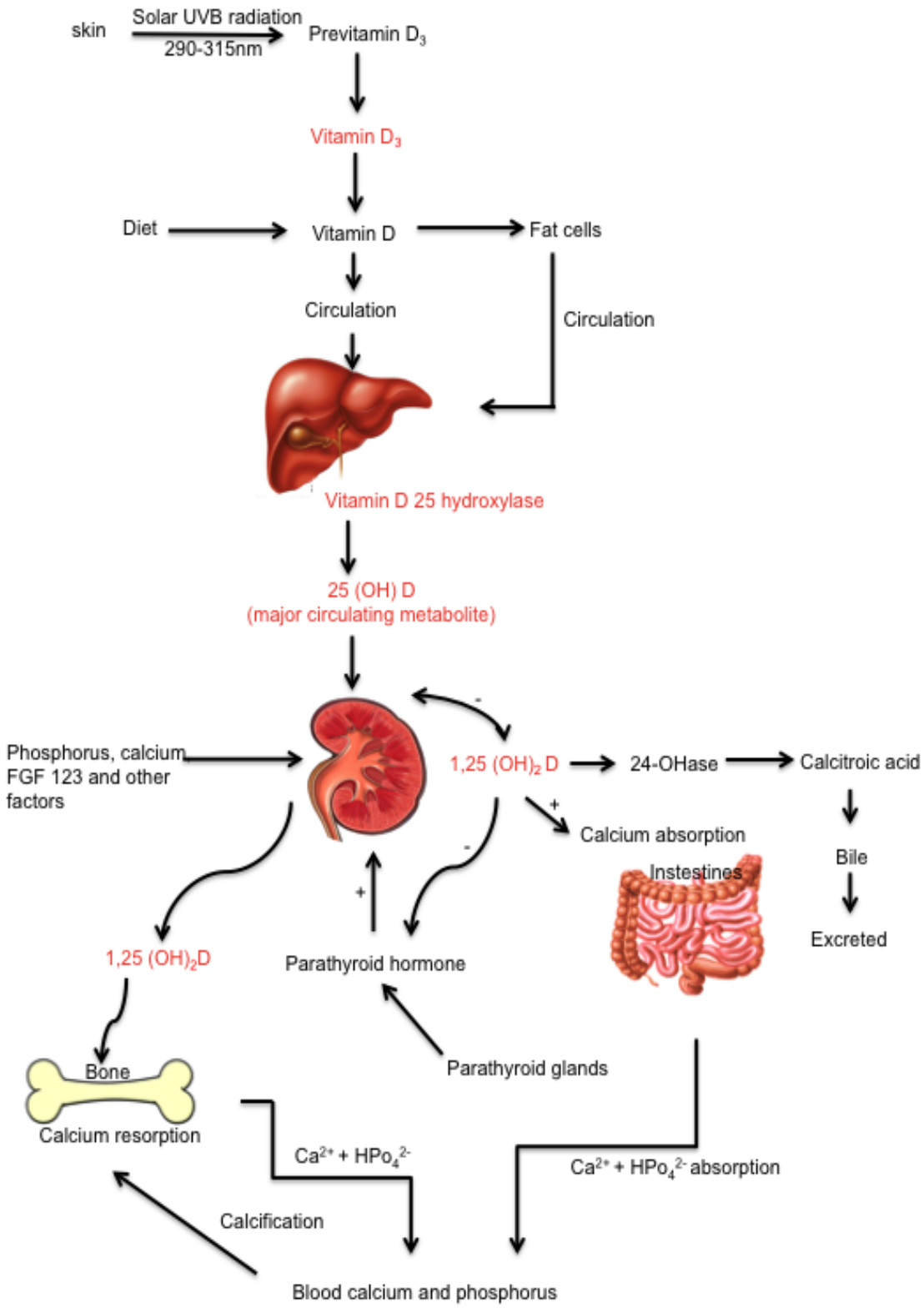
2.14.1. Physiology of Vitamin D

Vitamin D is a fat-soluble vitamin which is predominately made in skin exposed to sunlight. The two important forms of vitamin D are vitamin D2 (ergocalciferol), which is the dominant form in plants and invertebrates and vitamin D3 (cholecalciferol), which is the dominant form in vertebrates including humans. Vitamin D3 is present naturally in a small range of foods. The majority is then made endogenously in the skin and circulates bound to vitamin D3 binding protein.

In the liver, Vitamin D3 is metabolised to 25 hydroxyvitamin D (25 (OH) Vit D), which is the major circulating metabolite. In the kidneys 25-OH Vit D is further oxidised to its active form 1, 25 dihydroxy-vitamin D (1, 25 $(OH)_2$ D). The production of 1, 25 $(OH)_2$ D is tightly regulated by parathyroid hormones and serum calcium and phosphorus levels. Fibroblast factor 23 also plays a role in vitamin D synthesis where it acts to suppress 1, 25 $(OH)_2$ D synthesis (Fig 2.13). Vitamin D is critical for skeletal mineralisation, as 1, 25 $(OH)_2$ D increases the efficiency of absorption of renal calcium and intestinal calcium and phosphorus.[219]

Vitamin D plays a large role in calcium and phosphorus homeostasis and is responsible for bone mineralization, where a deficiency in vitamin D levels can predispose an individual to an increased risk of fractures. Vitamin D also has several non-skeletal functions. Vitamin D receptors have been found in organs such as the brain, breast, colon, immune cells and pancreas. Low levels of vitamin D have been associated with cardiovascular disease, DM, cancer and immune dysfunction. [220, 223]

Fig 2.3 Vitamin D synthesis and functions



2.14.2 Vitamin D deficiency

Measurement of blood 25 (OH) vitamin D is used to determine a patient's vitamin D status as it reflects the patient's total vitamin D stores from sunlight exposure, dietary intake and adipose stores in the liver.[219] Factors that influence vitamin D levels include dietary intake, variations in skin synthesis secondary to differences in skin pigmentation and clothing use, age, and factors that cause malabsorption from the bowel such as inflammatory bowel disease and gastric surgery and drugs such as long term use of glucocorticoids and anti-epileptic drugs.[224] Racial differences and the influence of skin pigmentation on vitamin D levels indicate its evolutionary importance and may account for the increased rates of low vitamin D levels in non-white racial groups. Serum vitamin D and blood glucose levels vary between seasons and are lower in winter due to decreased sunlight exposure.[225]

It is estimated that over a billion of the global population have Vitamin D insufficiency and this is a growing concern due to the association between low levels of 25 (OH) vitamin D and bone fractures and other conditions associated with the other non-skeletal functions of vitamin D.[225-30]

There is controversy regarding the blood level of 25 (OH) Vitamin D needed for good bone health and also the use of vitamin D supplementation in an attempt to reduce the disease risks associated with vitamin D deficiency. There is a lack of randomised control trials and therefore insufficient evidence to support routine supplementation, particularly in population groups with a low incidence of deficiency.[229]

2.14.3 Vitamin D and Type 2 DM

The prevalence of both Vitamin D deficiency and Type 2 DM is continuing to increase and has potential public health implications. A link between vitamin D deficiency and Type 2 DM is supported by the two sharing similar risk factors such as obesity, increasing age and racial origin.[230-6] There are also biological factors which support the link between Type 2 DM and vitamin D deficiency, such as the expression of the vitamin D receptor (VDR) in pancreatic beta cells that secrete insulin and peripheral target tissues that respond to insulin such as adipose tissue.

An association between insulin resistance and vitamin D has been proposed due to the effects of vitamin D on pancreatic β-cells and insulin sensitivity and studies have shown an inverse relationship between insulin resistance and vitamin D[230-41, 237-8] Although studies have largely shown a significant association between low levels of vitamin D and an increased risk of Type 2 DM some of the results are inconsistent.[225-236, 241-53]

2. Background

Seasonal variation has been noted in the incidence of Type 1 and Type 2 DM. Peak incidence has been recorded during March and April with a trough during September and October. Glycosylated Haemoglobin (Hba1c) levels and plasma glucose have been measured to explore the relationship with the seasons. One hypothesis for the increase in incidence and diagnosis of DM during the winter months is the potential increase in body weight secondary to reduced exercise and increased food intake during the winter months. However, changes in the incidences have been noted even without any appreciable difference in body weight.[245] A similar seasonal pattern of cardiovascular events has also been noted.[244-249]

Vitamin D production is closely linked to exposure to sunlight and seasonal and geographical variation in 25 (OH) D levels have been noted, with vitamin D displaying a cyclical pattern of peaks in late summer and troughs during winter months.[250] This seasonal change in Vitamin D may play a role in the glucose homeostasis and the subsequent development of DM.

Janghorbani et al conducted a large prospective cohort study of 4,942 white women and examined the prevalence and seasonal variation of GDM. Although the prevalence of GDM was higher in summer months (2.9% in June) and lower in winter months (1.1% in November), the difference was not statistically significant for the change in months or seasons.[251] The association between vitamin D levels, seasons and GDM requires further exploration.

2.14.4 Vitamin D and pregnancy

The metabolism of vitamin D is uniquely different during pregnancy than any other time in human physiology. Although the conversion of vitamin D to 25(OH) VitD remains the same in pregnant and non-pregnant individuals, the similarities end there. During pregnancy the levels of 1, 25 $(OH)_2$ D, the active form of vitamin D, increase approximately 2 fold by the first trimester and reach a maximum during the third trimester.[252-3] Pregnancy itself is thought to be the main driver for the large increase in 1, 25 $(OH)_2$ D but the exact mechanism of this the changes in metabolism remains unclear and is thought to be independent of calcium and parathyroid hormones.[254-66]

1, 25 $(OH)_2$ D does not readily cross the placenta and this is supported by low levels of 1, 25 $(OH)_2$ D in the fetus in comparison to mothers. However, 25(OH) VitD readily crosses the placenta, resulting in similar 25(OH) VitD levels in both mother and fetus.[257]

2. Background

In pregnancy, the calcium demand increases as more calcium is needed to support not just maternal calcium requirements but also those for fetal growth and development. The transport of calcium to the fetus is facilitated by the concentrations of 1, 25 $(OH)_2$ D being higher in the maternal circulation than in the fetal circulation. Vitamin D has an important role during pregnancy and influences both maternal and fetal outcomes.

Maternal vitamin D levels are associated with maternal health and obstetric outcomes. In the mother, in some studies, vitamin D deficiency is associated with shorter gestations[258-262], pre-eclampsia and hypertensive disorders of pregnancy.[258] In the fetus, vitamin D deficiency influences fetal development and therefore is associated with neonatal hypocalcaemia and rickets.[259] In the long term, children deficient in vitamin D have an increased risk of low bone mineral density, diabetes and eczema.[260-1]

Yu et al[262] examined the distribution of vitamin D levels within a multi-ethnic population and found that maternal serum concentration of 25(OH)D measured in the first trimester increased with maternal age and decreased with BMI. Maternal serum levels were also lower in non-white racial groups in comparison to white racial groups and levels were higher if sampled during the summer months. These variations make it difficult to describe normal levels of vitamin D during pregnancy, which can be applied to all pregnant women, without determining if there is a link between the various levels and maternal/fetal outcomes. This in turn makes it problematic to make recommendations regarding supplementation. In addition, some studies have failed to find an association between vitamin D levels and poor obstetric outcome. As a result of the variations in 25(OH)D levels described, and concerns to avoid overdosage, which may be harmful, some authorities have recommended a cut-off for the diagnosis of vitamin D deficiency in pregnant women as low as 10ng/ml in blood.[262]

Current guidelines from NICE [263] recommend maternal daily vitamin D supplementation (10mcg) in order to maintain adequate vitamin D stores during pregnancy. The importance of vitamin D supplementation should be stressed to those women who are considered at an increased risk of vitamin D deficiency and associated poorer obstetric outcomes. These include the following:

- women of South Asian, African Caribbean or Middle Eastern family origin
- women who have limited exposure to sunlight, such as women who are predominantly housebound or usually remain covered when outdoors

- women who eat a diet particularly low in vitamin D, such as women who do not consume oily fish, eggs, meat, vitamin D-fortified margarine or breakfast cereal
- women with a pre-pregnancy BMI above 30 kg/m^2

The appropriate supplementation dosage of Vitamin D during pregnancy remains uncertain and requires further investigation.

2.14.5 Vitamin D and GDM

The association between maternal 25(OH)D levels and development of GDM has been controversial (Table 16). The data regarding the potential use of 25(OH)D as a biomarker in the first trimester in the prediction of GDM are both sparse and conflicting.[259-64] Baker et al,[264] conducted a nested case control study exploring the relationship between first trimester maternal vitamin D levels and prediction of GDM. The prevalence of vitamin D deficiency, classified as serum 25(OH)D <50nmol/l was similar between GDM and controls (5/60 vs 8/120, p=0.90). These findings contrast with Zhang et al[265] who measured plasma 25(OH)D levels at 16 weeks gestation and found that plasma 25(OH)D levels were significantly lower in those who later developed GDM in comparison to those who remained normoglycaemic (24.2 vs. 30.1 ng/ml, p<0.01). This difference remained even after adjusting for confounders and vitamin D deficiency was associated with a 2.66-fold (OR: 2.66 (95%CI 1.01–7.02)) increased GDM risk. However, the small number of incident cases of GDM (<60) may account for the inconsistent results between studies.

Table 16 Summary of studies assessing maternal Vitamin D levels in GDM

Study	Cohort	Gestation	Results	Comments
Clifton-Bligh 2008[269]	N=307 GDM=81	3rd trimester	48.6 ± 24.9 nmol/l vs 55.3 ± 23.3 nmol/l, p = 0.04	Lower Vit D levels in GDM but ORs not significant
Zhang 2008[265]	Controls =114 GDM=57	16 wks	24.2ng/ml vs 30.1 ng/ml, p<0.01	Lower Vit D levels in GDM
Wang 2012[267]	Controls =200 GDM=200	26-28 wks	25OHD levels <25 nmol/L had a 1.8-fold higher risk of GDM , aOR= 1.588 (1.03-2.44)	Vit D deficiency was associated with increased risk of GDM
Burris 2012[266]	N=1087 GDM=68	2nd and 3rd trimesters	25(OH)D levels of <25 nmol/L in 13.2% with GDM vs 4% NGT OR=2.2 (95%CI 0.8–5.5)	Vit D deficiency was associated with increased risk

				of GDM
Baker 2012[264]	Controls =120 GDM=60	1st trimester	Deficiency defined as 25(OH)D < 50 nmol/L, p<0.05 between groups	Vit D deficiency not associated with GDM

aOR= Adjusted Odds Ratio, N: Number overall in study, NGT: Normal Glucose Tolerance, OR= Odds Ratio

3. Hypotheses and Aims

3.1 Hypotheses

I **hypothesised** that:

1. There is an interaction between maternal characteristics, in particular maternal age, BMI and racial origin on the prevalence of GDM and birth weight.

> '*there is a differential effect of maternal racial origin on the association between advancing maternal age and BMI on the prevalence of GDM and there is an influence of advancing maternal age, BMI, racial group and diabetic status (GDM or not) on birth weight'*

and

2. First trimester maternal biomarkers can be used to improve early prediction of GDM.

> *'maternal biomarkers measured in the first trimester can improve the early prediction of gestational diabetes'*

3.2 Aims

1. To examine the relationships between maternal age, BMI and race separately and together, and the development of GDM.

2. To examine the relationships between maternal age, BMI, race, GDM, and birth weight

3. To investigate the role of traditional and novel first trimester maternal biomarkers on the prediction of GDM.

4. Research Methods

Contributions

Hyothesis 1:

I was involved with formulating the hypotheses based on the wide literature search, the description of the SMMIS dataset and creating the study populations for the analysis. I carried out the analysis of the study population and the interpretation of the results under the guidance of my supervisors. Confirmation of the use of the appropriate statistical methods was obtained during consultation with Dr Ioanna Tzoulaki of the Department of Epidemiology and Public Health at St.Mary's Hospital.

Hypothesis 2:

I was responsible for organising the collection of blood samples for the creation of the dataset, from October 2008- April 2009. I also organised the transportation of the samples for analysis of the biomarkers, which was carried out at the research centre in an accredited laboratory at the University of Glasgow. Statistical analysis and interpretation of the data was carried out under the guidance of my supervisors.

4.1 The Dataset: St. Mary's Maternity Information System (SMMIS)

4.1.1 Background

The St. Mary's Maternity Information System (SMMIS) was first designed and piloted at St Mary's Hospital in Paddington in 1978, trialled in other maternity units from 1984, and then used in the majority of maternity units located in the former North West Thames Health region from 1988-2000. The region has a population of 3.5 million, a wide variety of racial groups, and included rural areas, suburbs and metropolitan areas. The boundaries of this region were defined by the Regional Health Authorities that existed in England until 1994 and covered northwest London, Hertfordshire and Bedfordshire.

Participating hospitals used SMMIS to collect data on every pregnancy booked at their unit. From 1988 to 2000, data was collected prospectively from all pregnancies (585, 291) booked at 15 maternity units in North West London (Table 17).

Table 17: Hospitals in the area formerly covered by the North West Thames region and the number of births within each unit between1988-2000.

Hospital Name	Births from 1988-2000
Edgware Hospital	47170
Ealing Hospital	18909
Northwick Park Hospital	50894
QE2 Hospital (Welwyn Garden City)	36775
Lister Hospital (Stevenage)	38565
Hemel Hempstead Hospital	40745
Watford Hospital	46185
Hillingdon Hospital	48675
Ashford Hospital	16207
West Middlesex Hospital	34604
Central Middlesex Hospital	18569
St Mary's Hospital	46681
Chelsea and Westminster Hospital	44814
Luton and Dunstable Hospital	62854
Bedford Hospital	33644

The SMMIS database with attributable data was stored on a private network in the Department of Epidemiology and Public Health located at St. Mary's Hospital. The database contained over 200 variables, which can be grouped into different sections: demographic, medical information about the pregnancy, labour, delivery and pregnancy outcome. The data were cleaned and validated (by running cross-checks and plausibility assessments) and then copied to a non-attributable dataset with the identifiers such as the mother's name and address removed. All subsequent analyses have been done on this non-attributable dataset. Because it is possible to identify women and their babies by cross-checking items such as date of birth of the baby, and maternal characteristics such as height and weight, against the original hospital files, the non-attributable dataset is correctly called 'pseudo-anonymised' rather than anonymised. However, a harmonised edition of the Governance Arrangements for Research Ethics Committees (GAfREC) was issued by the UK Health Departments in May 2011 and came into effect from 1 September 2011.

4. Research Methods

The harmonised GAfREC set out the requirements for review of research by Research Ethics Committees within the UK Health Departments' Research Ethics Service. These include both policy requirements and requirements under legislation applicable to the whole of the UK or to certain countries of the UK, and research involving previously collected, non-identifiable information. Under the 2001 edition, REC review was required for any research involving the data of NHS patients. Under the 2011 edition, REC review continues to be required for research involving collection of information from patients or service users for research (rather than for routine clinical care).

"REC review is also required for research involving use of previously collected information from which patients or service users could be identified by researchers outside the usual care team (either directly from that information or in combination with other information in, or likely to come into their possession).

However, REC review is not required under the harmonised GAfREC for research limited to use of previously collected, non-identifiable information. This exception also applies to research undertaken by staff within a care team using information previously collected in the course of care for their own patients or clients, provided that data is anonymised or pseudonymised in conducting the research. Such research would involve no breach of the duty of confidentiality owed by care professionals." [270]

Accordingly, no REC permission was required for the analysis of the non-attributable dataset used in this study. This was confirmed in an email from Mr Atul Patel, NRES (REC) Coordinator for Proportionate Review Service London' Health Research Authority, NRES Committee London – Dulwich, East, Fulham, Surrey Borders and Wandsworth, Research Ethics Committee (REC) Centre Charing Cross, Room 12, 4th Floor West, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, dated 18th January 2012.

Demographic variables

Mother's date of birth, age at booking, racial group, smoking, marital status and Carstairs Index were recorded in the database. Carstairs index (CI) is a measure of social deprivation allocated on the basis of postcode (calculated from four previous census indicators: low social class, lack of car ownership, overcrowding and male unemployment). The higher the score, the higher the measure of deprivation.

4. Research Methods

Medical variables about the mother

Height and weight at booking, as well as a history of DM or epilepsy, pre-pregnancy BMI and blood pressure at booking.

Information about the pregnancy

Weeks of gestation at booking, DM in pregnancy and highest diastolic pressure in pregnancy were recorded.

Information about the labour, delivery and outcome

Labour onset, augmentation, analgesia administered, meconium liquor, mode of delivery, perineal trauma, birthweight, admission to Neonatal Intensive Care Unit (NICU) and perinatal morbidity and mortality were recorded.

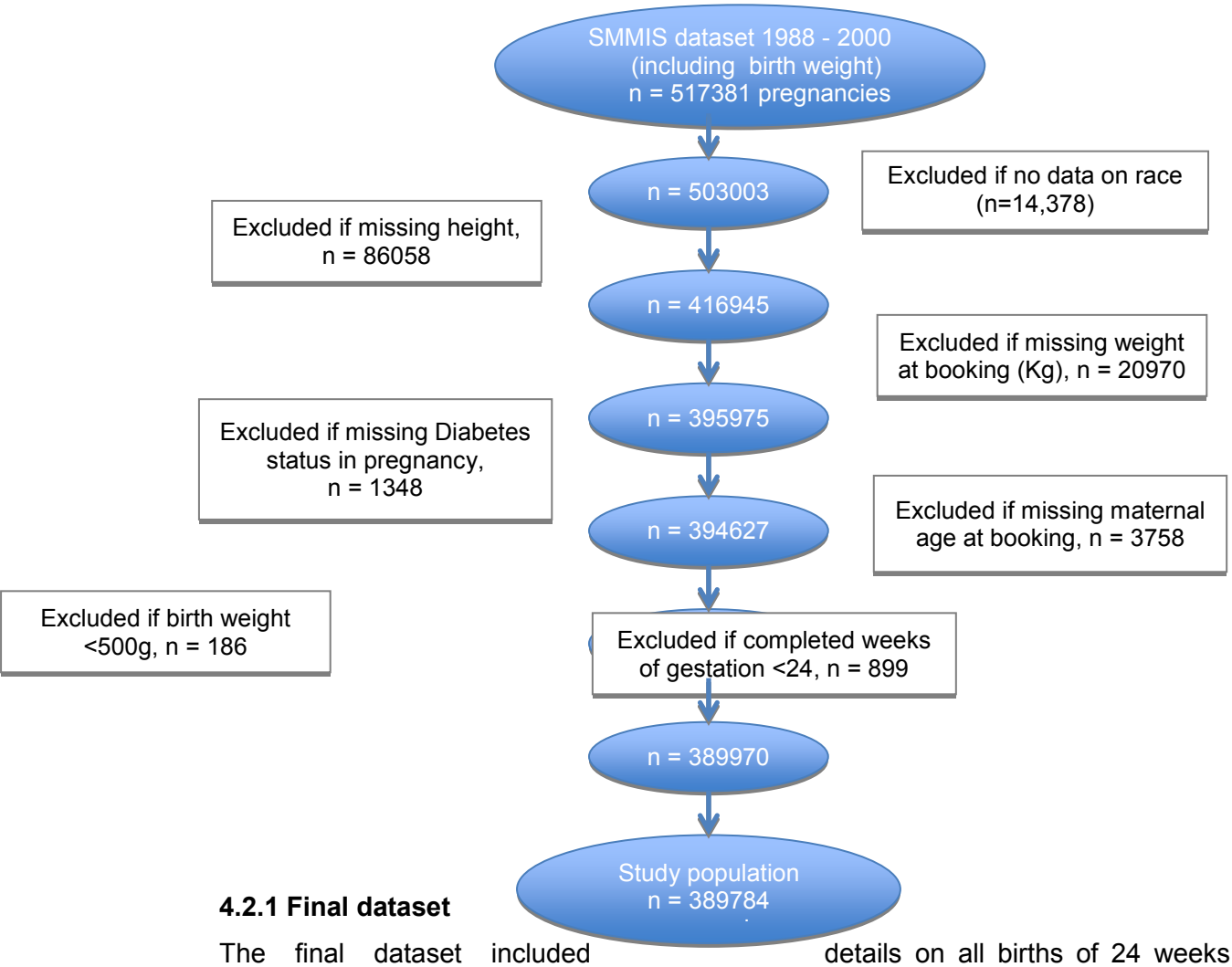
The data have been extensively validated[271] and the analysis of subsets reported in many papers to date.[272- 293]

At the first antenatal visit, BMI was calculated as weight (kg) (self-reported or measured by weighing if the woman did not know her weight), divided by height (m) squared. Racial groups were classified by self-reporting and are therefore self perceived classifications. Initially the categories available were White European, Black African, Black Caribbean, South Asian, Oriental, Mediterranean and as 'other racial group'. As the decade advanced, more categories were available, but they have subsequently been mapped to the same seven main groups to facilitate analysis. We chose to focus on White European, Black African, Black Caribbean and South Asian racial groups as these comprise the majority of the subjects in the dataset. Information regarding diabetes in pregnancy was reported by the midwives at delivery and classified as no diabetes in pregnancy, pre-existing diabetes and diabetes that was diagnosed for the first time during the index pregnancy (GDM). At delivery, mean birthweight percentile calculation was based on percentiles derived from the Aberdeen survey.[296] The percentiles take into consideration the neonatal gender and gestational age at delivery.

4.2 Description of the study population

There were 517,381 births in the SMMIS dataset from 1988 to 2000 that included information about birthweight. All births of 24 weeks gestation and over with a birthweight of at least 500g that included information regarding development of diabetes during pregnancy, maternal age, height, weight, BMI at booking and maternal ethnic group were included in the study. Births before 24 weeks gestation and weighing less than 500g were not considered to be viable. 389,784 births fitted the inclusion criteria (Fig 4.1). This was irrespective of parity.

Fig 4.1 Flow diagram of study subset derived from the original dataset



4.2.1 Final dataset

The final dataset included details on all births of 24 weeks gestation and over with a birthweight of 500g and over that included information regarding development of diabetes during pregnancy, maternal age, height, weight, BMI at booking and maternal race.

I excluded subjects with missing information regarding: racial group (n=14,378), height (n=86,058), weight (n=20,970), diabetes status during pregnancy (n=1,348), maternal age at booking (n=3,758), women giving birth before 24 weeks gestation (n=899) or with babies weighing less than 500g (n=186) as their babies were not considered to be viable. 389,784 births fitted the inclusion criteria (Fig 4.1).

4.2.2 Special cases

The outcomes of the 389784 pregnancies were checked by looking at the variables labelled as perinatal death by timing (i.e. less than 24 weeks gestation, antepartum, intrapartum or neonatal death) and recompleted weeks at delivery. Three pregnancies were identified as being coded as either a miscarriage or termination of pregnancy (TOP). Two occurred at 24 completed weeks of gestation and the other at 26 completed weeks of gestation. Miscarriage is defined as the spontaneous loss of a pregnancy before 24 weeks gestation and therefore these pregnancies were investigated as follows:

24 weeks gestation

Both pregnancies had birthweights over 500g. They also both had a record of amniocentesis, chorionic villous sampling, induced abortion and congenital abnormalities. Therefore they remained as being coded as TOP, possibly for fetal abnormalities.

26 weeks gestation

One pregnancy was identified and had a birthweight of 800g recorded. This pregnancy had no congenital abnormalities, no Apgar scores were recorded, no fetal monitoring was performed during labour and labour was induced. This was recoded as an antepartum stillbirth.

The above study population was split into three datasets:

- Group 1 (diabetespregnullips) – This dataset includes all women in their first ongoing viable pregnancy who meet the inclusion criteria.
- Group 2 (diabetespregpara 1) - This dataset includes all the women during their second ongoing viable pregnancy, who meet the inclusion criteria.

- Group 3 (diabetespreg) - This dataset includes all the women regardless of parity who meet the inclusion criteria.

The analyses reported in this Thesis were performed on the dataset of Group 1 (diabetespreg nullips).

The reason for creating the separate parity groups and focusing the analyses on Group 1 was to exclude any possible bias due to including the same women more than once in the analysis. The combined data set does not allow us reliably to link women's first, second etc pregnancies. Thus, some women are represented in the dataset more than others, and if for example highly parous women are more likely to be diabetic, this could introduce bias. However, a woman can have her first baby only once, ditto her second baby. Thus the datasets of nullips and second baby by definition include women only once in each dataset, making this a true cross-sectional study.

It should also be noted that by examining only women in their first pregnancy, this group represents a small sub group of the women who will develop GDM. By not including parous women it excludes a group of women who are at increased risk of GDM.

The analysis of Group 1 (diabetespreg nullips) will be reported in this Thesis.

4.3 Description of the variables

The following table lists all the variables that will be used in the study:

Table 18 Outcome and explanatory variables within dataset

Variable	Description
Explanatory variables Maternal age Maternal weight at booking Maternal height Maternal BMI Mother's racial group	 years kg cm kg/m² White European, Black African, Black Caribbean, South Asian, other (including Mediterranean, Oriental)
Outcomes Diabetes in pregnancy Birthweight GA at delivery Sex of the neonate	 0=non-diabetic, 1=gestational diabetes, 2=pre-existing diabetes grams weeks female or male

4.3.1 Diabetes in pregnancy

The following table shows the distribution of women with diabetes in pregnancy (gestational and established diabetes) and no diabetes within the study population (dataset diabetespreg). Gestational diabetes had a prevalence of 1.2% in this study population.

Table 19 Frequency of diabetes in pregnancy in study population

Diabetes in pregnancy	Frequency, N	Percentage (%)	Cumulative Percentage (%)
None	383883	98.5	98.5
Gestational	4756	1.2	99.7
Pre-existing	1145	0.3	100.0
Total	389784	100.0	

The demographic and clinical characteristics of the study population (diabetes preg) according to the development of diabetes are given in Table 19.

Table 20 Demographic and clinical characteristics of the study population according to the development of diabetes prior or during pregnancy. Numerical data are expressed as mean (standard deviation).

Variables	Non-Diabetic	Gestational	Pre-existing

	N=383883	Diabetes, N=4756	Diabetes, N=1145
Maternal Age (yrs)	28.5 (5.3)	31.6 (5.3)*	29.5 (5.2)*
Maternal Height (cm)	162.6 (6.8)	160.1 (7.1)*	162.2 (7.4)*
Maternal Weight at booking (kg)	64.3 (12.37)	71.2 (16.4)*	69.4 (13.8)*
BMI at booking (kg/m^2)	24.3 (4.4)	27.7 (5.8)*	26.4 (5.0)*
Birthweight (kg)	3319.8 (569.4)	3420.1 (656.9)*	3444.6 (735.1)*

*p<0.001 in comparison with the non-diabetic group

As explained previously, for this Thesis, only first pregnancies were studied to avoid bias.

This resulted in one third of the SMMIS dataset being appropriate to study (Fig 4.2)

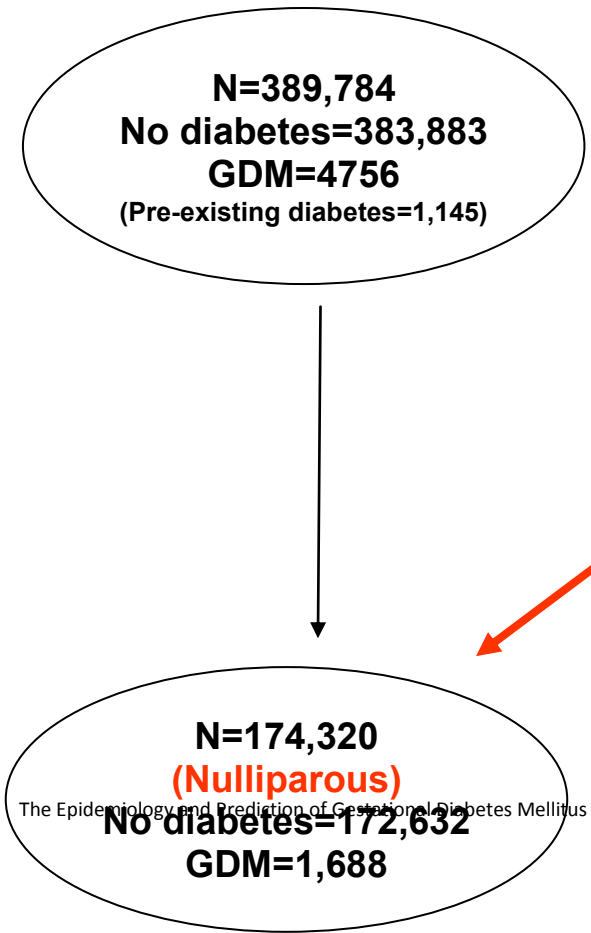


Fig 4.2 Final dataset used for subsequent analysis

Initial numbers in the dataset

Study population in this thesis
(diabetespreg nullips)

The final dataset (diabetespreg nullips) consisted of the following distribution of women with diabetes during pregnancy within the study population (Table 20). Gestational diabetes had a prevalence of 1.0% in this study population.

Table 21 Frequency of diabetes in pregnancy within the study population (nulliparous women only)

Diabetes in pregnancy	Frequency	Percentage (%)
None	172,632	99.0 %
Gestational diabetes	1688	1.0 %
Total	174,320	100%

4.3.2 Maternal Characteristics

Maternal Age

The following table shows the range of maternal age within the dataset irrespective of diabetic status (Table 22).

Table 22 Description of maternal age within the study population irrespective of diabetic status

	Number	Min	Max	Mean (SD)
Maternal age (at booking)	174320	12	50	26.8 (5.2)

SD= Standard deviation

Maternal BMI

The following table shows the range of BMI within the dataset irrespective of diabetic status (Table 23).

Table 23 Description of body mass index within the study population irrespective of diabetic status

	Number	Min	Max	Mean (SD)
Body Mass Index	174320	11	70	23.6 (4.1)

SD= Standard deviation

Maternal race

The following table (Table 24) shows the numbers in each racial group within the dataset.

Table 24 The distribution of racial groups within the dataset irrespective of diabetic status

Racial group	Frequency, (N)	Percentage (%)
White European	131201	75.3
Black African	4927	2.8
Black Caribbean	4698	2.7
South Asian	20086	11.5
Any other ethnic group	13408	7.7
Total	174320	100.0

White European women formed the majority of the dataset. South Asians were the largest non-white racial group.

4.3.3 Birthweight

At delivery, mean birthweight percentile calculation was based on percentiles derived from the Aberdeen survey.[294] These birthweight percentiles take into consideration the baby's gender and gestational age at delivery. Z-scores (birthweight in relation to the mean and standard deviation for each gestational week) were produced as an alternative to the actual birthweight.

4.4 Hypotheses

4.4.1 Hypotheses 1(a):

- There is a differential effect of maternal racial origin on the association between advancing maternal age and BMI on the prevalence of GDM.

Statistical analysis

The statistical analysis involved descriptive statistics and exploratory analysis to study the data and look at patterns. This was done using the statistical package SPSS Version 17.0 for Windows.

Exploratory analysis

Maternal characteristics and pregnancy outcomes in the two groups studied (GDM and non-GDM) were compared using the Student's t-test and Chi-square x^2 test for numerical and categorical data respectively. Maternal age was divided into the following groups: below 20, 20-24, 25-29, 30-34, 35-39 and 40 and above years of age. Maternal BMI was also divided according to the WHO international classification of BMI as follows: less than 18.5 (underweight), 18.50-24.99 (normal weight), 25.00-29.99 (overweight) and more or equal to 30.00 (obese).[78]

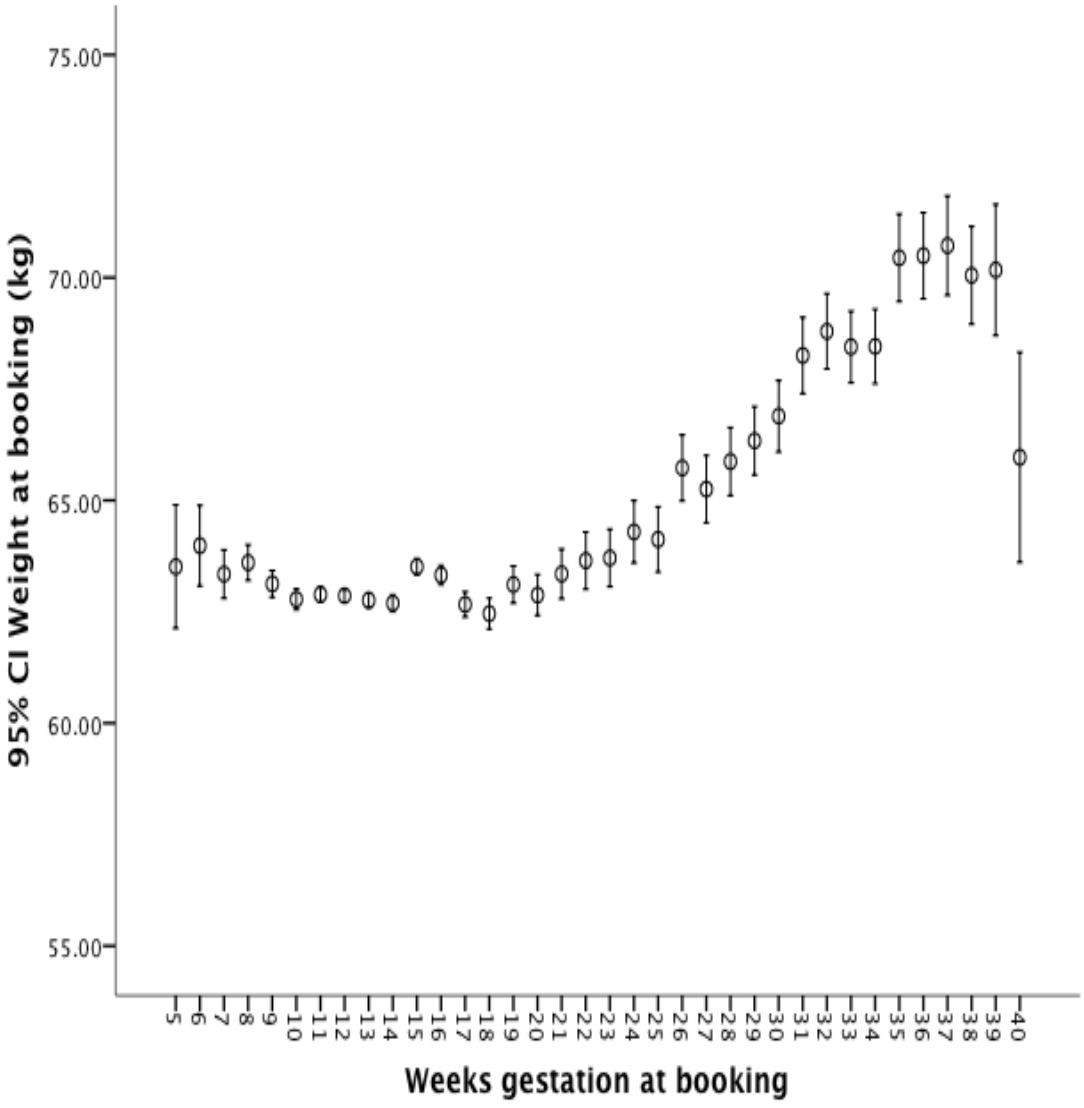
Univariate analysis was initially used to examine the influence of maternal age and BMI on GDM development in each racial group. Binary logistic regression was used to develop statistical models for development of GDM including the risk variables of maternal age, BMI and race as well as the interactions between these variables. Models were constructed to examine the magnitude and significance of predictor variables on the prevalence of GDM. The results are presented as Odds Ratios (ORs), 95% Confidence Interval (CI) and p-values.

Hypothesis 1(b):

- There is an association of advancing maternal age, BMI, racial group and diabetic status (GDM or not) with birth weight.

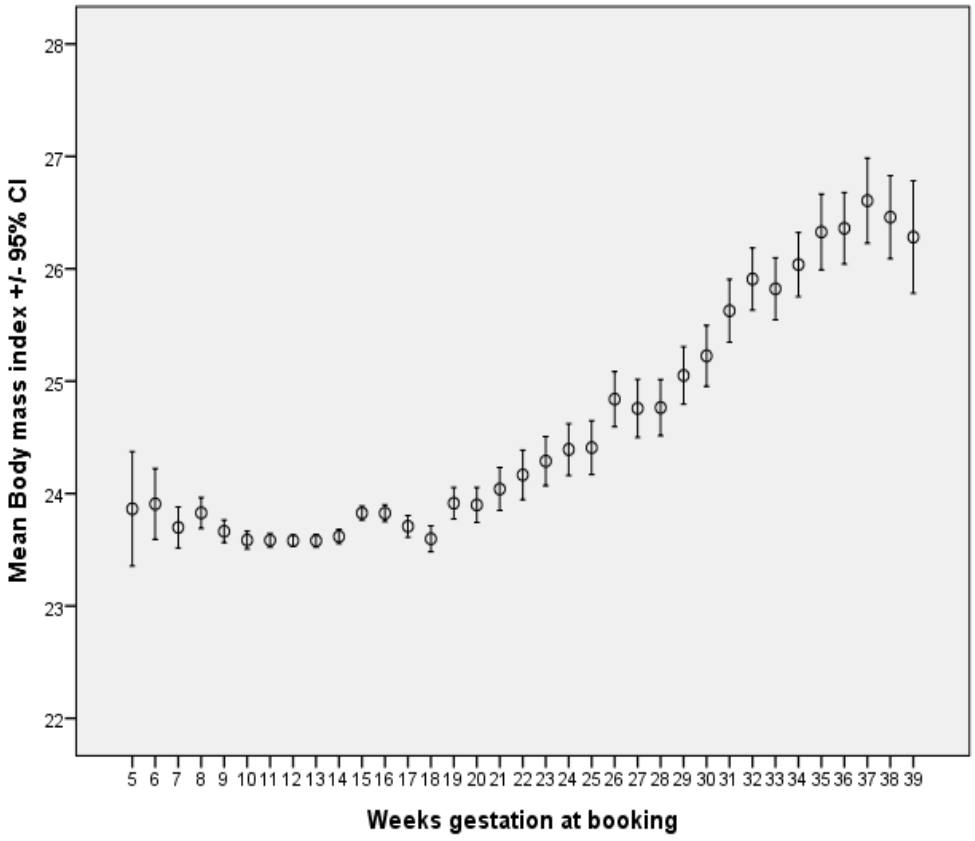
The previous dataset was modified further. Cases where the baby's gender was classified as 'indeterminate' (n = 20) were excluded. As maternal booking weight and BMI change very little up to 20 weeks of gestation, but do increase thereafter (see fig 4.3.1 and 4.3.2), all women booking after 20 weeks of gestation were also excluded from the analysis (n = 43751). This created a further dataset for the investigation of maternal characteristics and the influence on birthweight.

Fig 4.3.1 Maternal weight variation with advancing gestation



Nulliparous women. N=177,590

Fig 4.3.2 Maternal BMI variation with advancing gestation



Nulliparous women. N = 162,047

The gender specific percentile birthweight for gestational age was calculated, based on the Aberdeen survey norms.[294] Gender specific birthweight z-score, a measure of how many standard deviations the birthweight was from the expected mean was also calculated in relation to the total population.

A Z-score is a measure of how far a measurement is from the mean, measured in standard deviations and was calculated by the following equation:

$$Z = \frac{\text{Observed birthweight- expected birthweight}}{\text{Standard deviation (SD)}}$$

4. Research Methods

The outcomes studied were birthweight, percentile and z-scores, in GDM and non-GDM groups, within the three main racial groups: White European, Black and South Asian, who comprised the majority of the subjects in the dataset.

Simple and multiple linear regressions were used to explore the influence of variables on birthweight z-scores.

4.4.2 Hypothesis 2:

- First trimester maternal biomarkers can improve early prediction of GDM

Material and Methods

We performed a nested case-control study using first-trimester samples that were taken and stored as part of a large prospective observational ongoing study aimed at identifying first-trimester predictors of adverse pregnancy outcomes. The study involved women attending for their routine hospital visit at King's College Hospital. In this visit, which is held at 11+0 to 13+6 weeks' gestation, all women have an ultrasound scan to confirm gestational age, diagnose any major fetal abnormalities, and measure fetal nuchal translucency thickness, which together with maternal free β-chorionic gonadotrophin and pregnancy-associated plasma protein A, is used for screening for chromosomal abnormalities. Patients were asked to complete a questionnaire on maternal age, racial group, cigarette smoking during pregnancy, method of conception, parity and obstetric and family history of diabetes. The maternal weight and height were measured and BMI was calculated. Peripheral maternal blood pressure was measured in a seated position using an ambulatory blood pressure monitor (3BTO-A2;Microlife Medical, Taipei, Taiwan), which has been validated in pregnancy.[295] Maternal blood was taken and stored at -80°C for future biochemical analyses. All women had phenotypically normal neonates. Women had given written consent and the project had been approved by the local Research Ethics Committee.

All women underwent routine screening for GDM between 24-28 weeks. This involved a random blood sugar, which if abnormal i.e. ≥ 6.7 mmol/l was followed by a 2 hour 75g OGTT. If women had a prior history of GDM then they would have a OGTT between 16-20 weeks and at 28 weeks if the previous result was normal. If after 34 weeks following a negative screen at 28 weeks GDM was suspected due to the developed of apparent risk factors such as polyhydramnios or a large-for-gestational-age fetus a one-hour post meal glucose would be performed as well as an HbA1c. A plasma glucose ≥ 6.7 mmol/l or an HbA1c over 5.5% would require referral to the Diabetes Centre. Gestational diabetes mellitus was defined according to the WHO criteria. For each woman who developed GDM, two control subjects were selected who were matched according to the date of the ultrasound scan and consequently storage time of the samples.

4. Research Methods

We measured novel and traditional maternal serum biomarkers including adiponectin, lipids (HDL-cholesterol, LDL-cholesterol, total cholesterol and triglycerides), γ-GT, E-selectin, t-PA and CRP. In a subset of the study population, maternal serum vitamin D ($25OHD_3$ and D_2) was also measured.

Biochemical analysis

Lipids, CRP and γ-GT were measured by routine automated methods in an accredited laboratory (University of Glasgow, UK). adiponectin, e-selectin, and t-PA were measured by enzyme-linked immunosorbent assay (R&D Systems, Abingdon, UK) on previously unthawed serum samples stored at -80°C. Samples were processed by technicians who were blinded to the identity of the samples.

Maternal first trimester serum $25OHD_3$ and D_2 were measured using an automated solid-phase extraction (SPE) procedure with liquid chromatography-tandem mass spectrometry. Results are reported as total 25OHD ($25OHD_2$ + $25OHD_3$). Vitamin D deficiency was defined as 25(OH)D<25nmol/l as this a common definition used in clinical practice.

Statistical analysis

Descriptive statistics were used to summarise maternal characteristics, comparing those with GDM to controls. Categorical variables were compared using Fisher's exact test and continuous variables were compared using Student's t-test or Wilcoxon rank sum tests as appropriate.

Regression analysis was used to examine the role of each variable in the prediction of GDM. Logistic regression models were fitted and adjusted for confounding factors. HDL cholesterol, triglycerides, and CRP were log transformed as this enhanced model fit. Stepwise variable selection procedures were used to identify the most important predictors and effect estimates were reported along with 95% CIs and p values. The predictive abilities of the different models were illustrated using ROC curves. The same analyses were repeated in the subgroup of mothers who had not had previous GDM.

5. Results

5.1 Hypothesis 1 (a):

- There is a differential effect of maternal racial origin on the association between advancing maternal age and BMI on the prevalence of GDM

5.1.1 Hospital

A concern in the current study was that 15 Hospitals with different screening policies for GDM were included which could result in a different proportion of the population being designated as 'gestational diabetic'. This could be important, because not all the hospital services used the same screening techniques to assess carbohydrate tolerance. Moreover, this was further complicated by the fact that not all the hospitals had the same racial mix. We found that the addition of centre did add to the models by explaining variation over and above the effects of race, maternal age and BMI.

In order to investigate this variable more fully we created a figure showing the rates of GDM within each hospital (Fig 5.1) and then the racial mix within each hospital (Fig 5.2).

The highest rates of GDM were in West Middlesex hospital (1.58%), St Mary's hospital (1.43%) and Hemel Hempstead Hospital (1.11%). However, the racial mix within these hospitals was very different. It would have been expected that Hemel Hempstead Hospital would have had the lowest rate of GDM due to its population, which consisted mainly of White Europeans. The most likely explanation is different screening programmes within each hospital as there wasn't at the time of the study (and isn't) a general consensus regarding screening for GDM. Most units adopted their own screening guidelines for GDM. The rates of GDM diagnosis in White Europeans by centre was highly correlated with that in both Blacks (Pearson correlation coefficient 0.864) and South Asians (Pearson correlation coefficient 0.863) in the same centre. Multiple regression analysis including each individual centre as a variable confirmed that although five units contributed significantly to the birthweight z-scores, this contribution was much smaller than the effects of racial group, BMI, GDM or smoking (maximum t value for centre 7.8 compared with -67.1 for being South Asian, -47.9 for smoking, 46.4 for BMI, -23.1 for being Black, and

14.5 for the presence or absence of GDM). This means that although the incidence of GDM might have been underestimated in centres without a routine screening policy, this effect applied similarly across racial groups, and the differences between racial groups was consistent across the centres.

Fig 5.1 The frequency distribution of diagnosed gestational GDM within the 15 Maternity units in the SMMIS database.

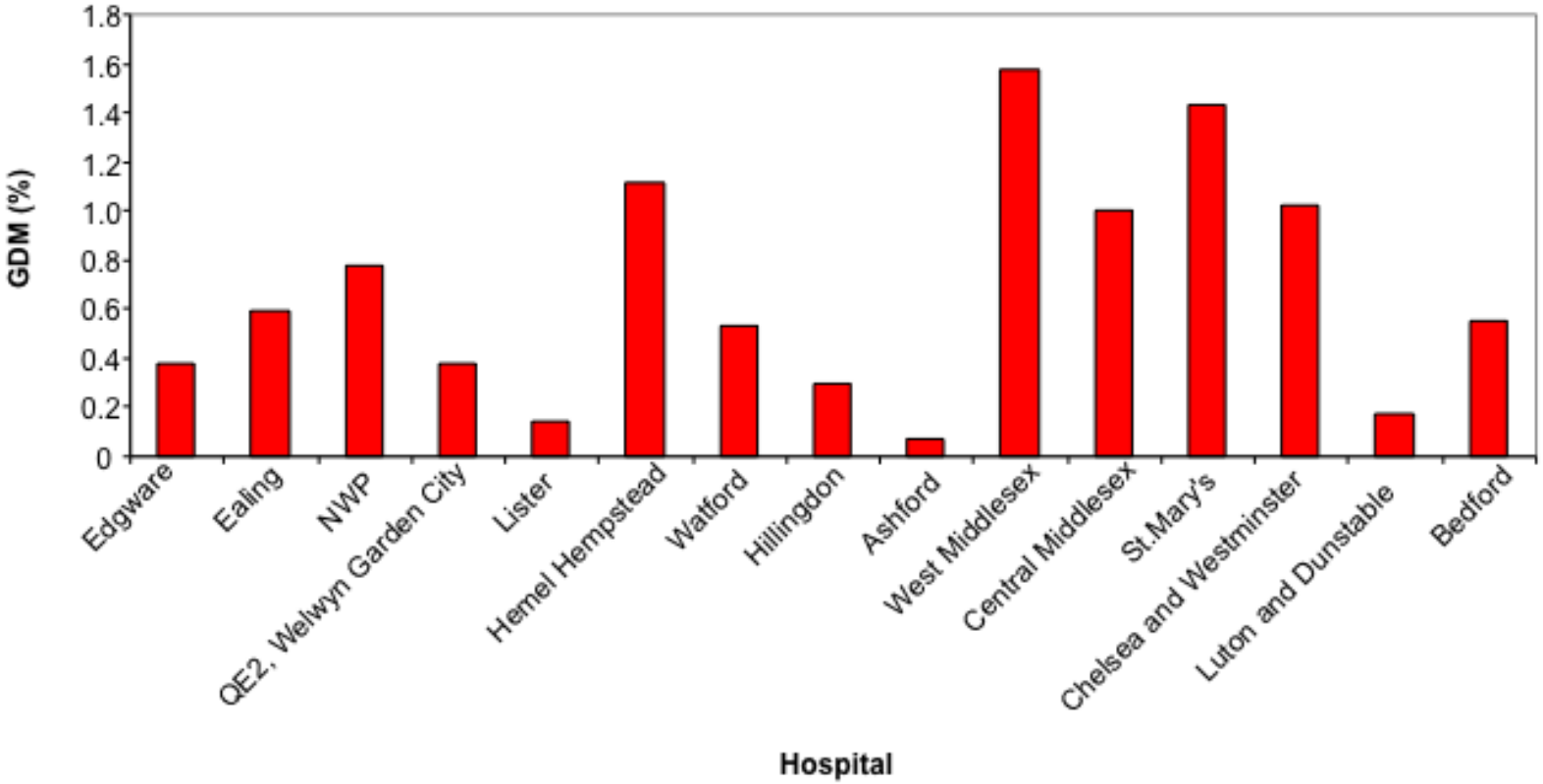
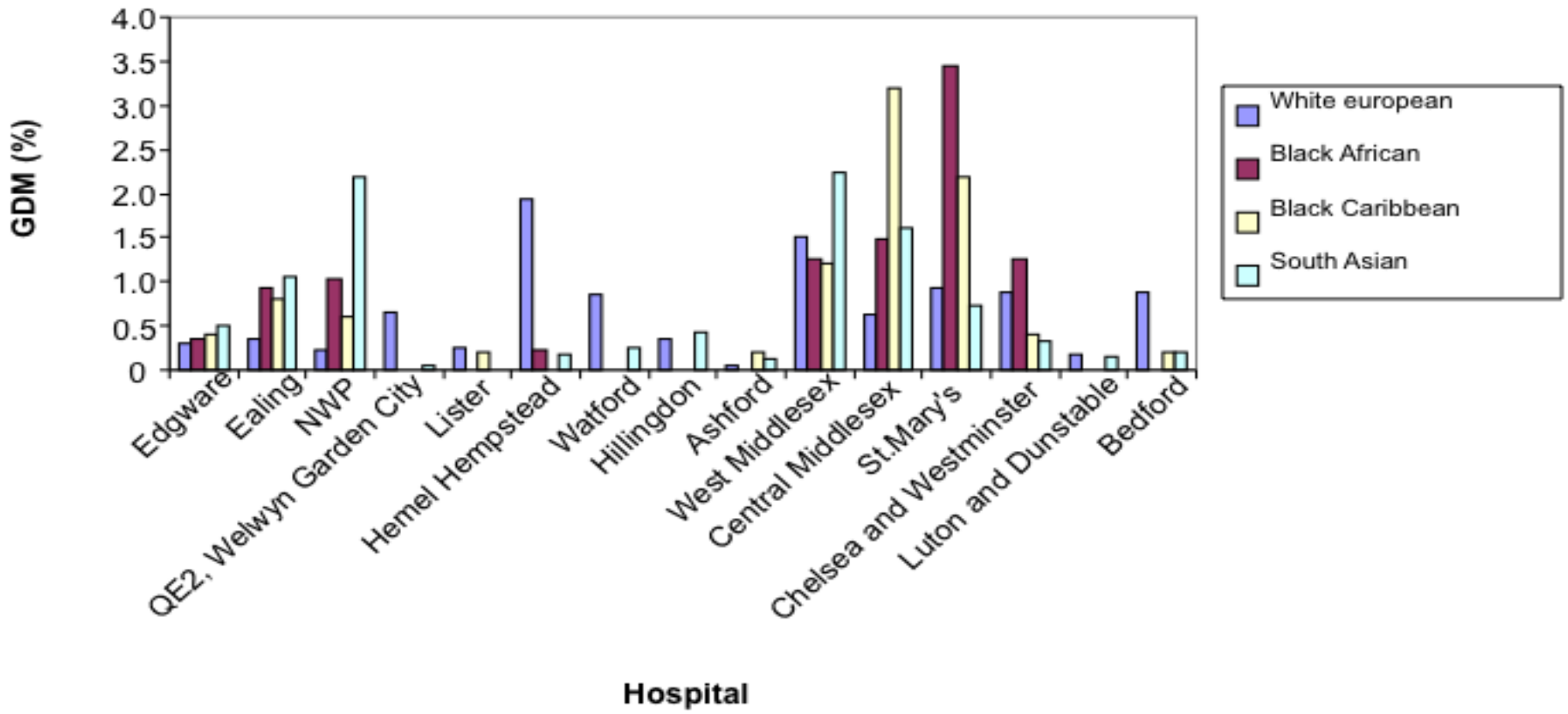


Fig 5.2 The racial variation of women with pregnancies complicated with GDM within each hospital (NWP: Northwick Park Hospital)



5.1.2 Missing Information

Women who had missing information on maternal age, BMI (weight or height), racial group, diabetes in pregnancy or birthweight were excluded from further analysis. The study population was compared with the non-studied population to exclude any bias (Table 25).

Table 25 Comparison of demographic details, clinical characteristics and pregnancy outcomes in missing dataset vs study dataset.

Maternal characteristic	**Non-studied dataset (N= 1594)**	**Study dataset (N= 174,320)**	**P value**
Maternal age (yrs)	27.7 ± 5.5	26.8 ± 5.2	p<0.001
BMI (kg/m^2)	25.7 ± 4.6	23.8 ± 4.0	p<0.001
Racial Group White European, n (%) Black African, n (%) Black Caribbean, n (%) South Asian, n (%) Oriental, n (%) Mediterranean, n (%) Other, n (%)	 1038 (67.2) 87 (5.6) 83 (5.4) 176 (11.4) 26 (1.7) 50 (3.2) 85 (5.5)	 131,201 (75.3) 4,927 (2.8) 4,698 (2.7) 20,086 (11.5) 2,949 (1.7) 4,469 (2.6) 5,990 (3.4)	p<0.001
Diabetes in pregnancy No diabetes, n(%) Gestational diabetes,n(%)	 1787 (52.3) 3 (0.2)	 172,632 (99.0) 1,688 (1.0)	p<0.001
Birthweight (grams)	1711.92 ±1557.92	3254.3 ± 567.6	p<0.001
Mean birthweight centile	44.42 ± 39.39	57.9 ± 28.2	p<0.001
Mode of delivery Vaginal, n (%) Caesarean section, n (%)	 731 (60.5) 478 (39.5)	 142,865 (82.0) 31,450 (18.0)	p<0.001

Values are expressed as mean ± standard deviation or as median (interquartile range)

Women in the missing dataset were significantly older, had a higher BMI and were more likely to be in the non-white racial group than the study dataset. Women in the missing dataset were also more likely to deliver smaller babies and more likely to deliver by Caesarean section.

The study population had a different proportion of women with pregnancies complicated by gestational diabetes (1% in the study population vs 0.2% in the non studied population). However, the non-studied population only contained 3 women with GDM and therefore the non-studied population was not considered further as it was felt that the study population adequately represented the whole population in the database in terms of the outcome “diabetes” in pregnancy.

The study included 1,688 women that developed GDM and 172,632 that did not. The prevalence of GDM in our study population was 1%.

5.1.3 Variables

Initially, it was necessary to determine whether the continuous variables such as maternal age and BMI, were normally distributed. This would influence the types of analyses that could be used to compare the variables. However, we were unable to confirm the normality of the data with N-Par tests due to the size of the database. Histograms displaying normal curves were therefore created. From these histograms, it can be seen that variables such as maternal age and BMI within our study population are sufficiently close to a Gaussian distribution for parametric statistics to be applied. (Fig 5.3 and 5.4; histograms with normal curve superimposed).

Fig 5.3 The distribution of maternal age within study population

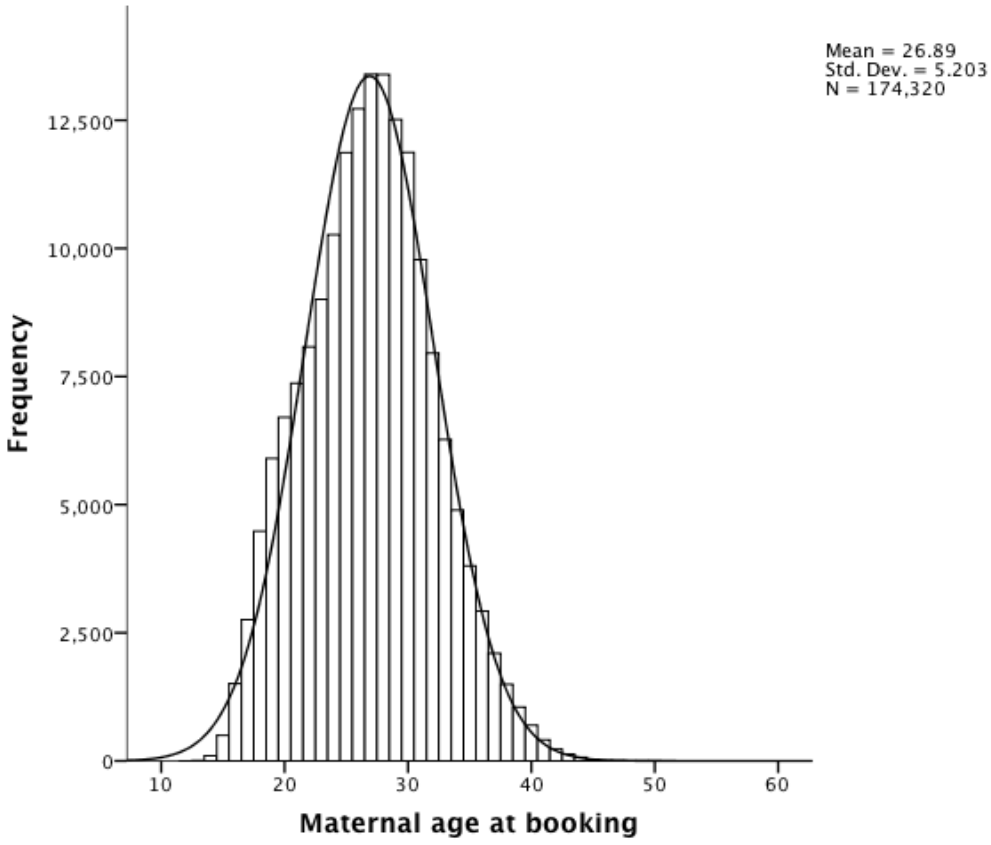
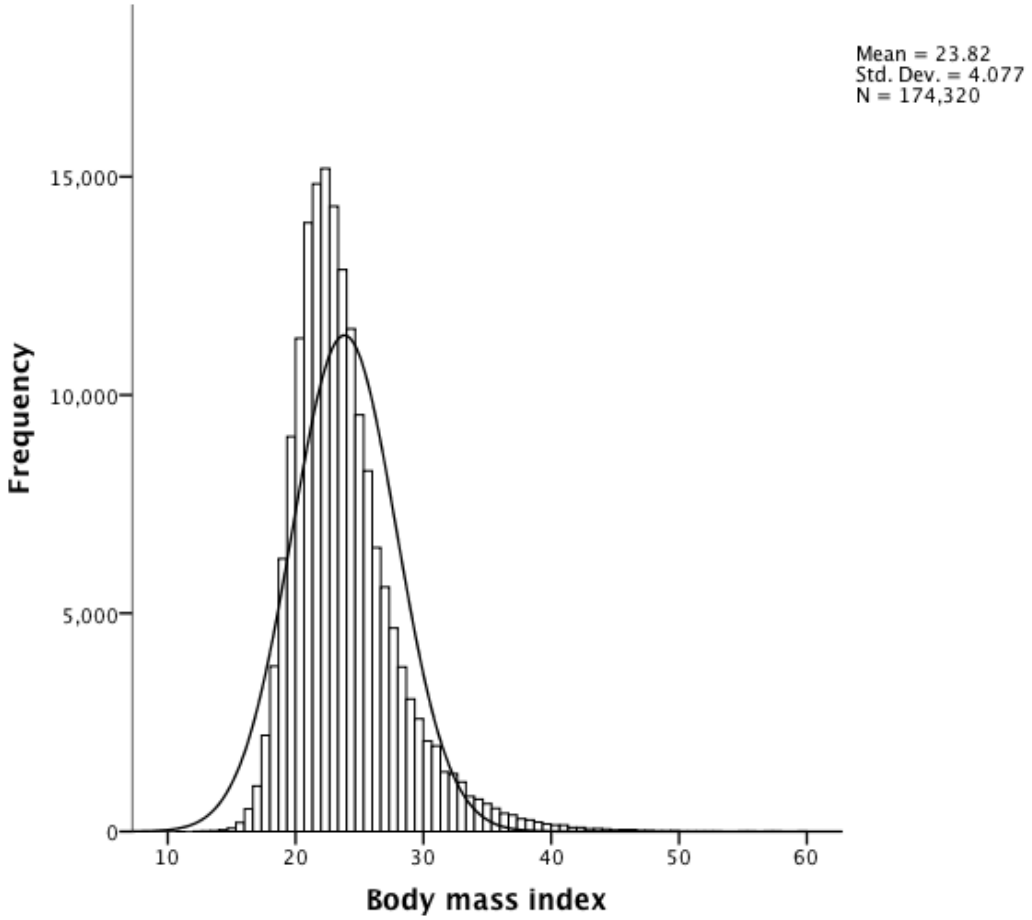


Fig 5.4 The distribution of body mass index within study (nulliparous) population

Maternal BMI



5. Results

The maternal demographic, clinical characteristics and pregnancy outcomes in the GDM and control group are given in Table 26.

Table 26 Maternal demographic, clinical characteristics and pregnancy outcomes of the non-GDM and GDM groups.

Characteristic	**Non -GDM (N=172,632)**	**GDM (N=1,688)**
Age (years)	26.8 ± 5.1	29.6 ± 5.2*
Gestational age at first antenatal visit (wks)	13.0 (12.0-16.0)	13.0 (12.0-16.0)
Body mass index (kg/m²)	23.7 ± 4.0	26.6 ± 5.5*
Racial group		
White European, n (%)	130325 (75.5)	876 (51.9)*
Black African, n (%)	4840 (2.8)	87 (5.2)
Black Caribbean, n (%)	4648 (2.7)	50 (3.0)
South Asian, n (%)	19685 (11.4)	401 (23.8)
Oriental, n (%)	2874 (1.7)	75 (4.4)
Mediterranean, n (%)	4390 (2.5)	79 (4.7)
Other, n (%)	5870 (3.4)	120 (7.1)
Gestational age at delivery (wks)	40.0 (39.0-41.0)	39.0 (38.0-40.0)*
Birthweight (grams)	3305.9 ± 639.3	3523.8 ± 566.9*
Birthweight percentile	57.9 ± 28.2	63.9± 29.6*
Birthweight >90th percentile, n (%)	16,598 (9.6)	248 (14.6)*
Mode of delivery		
Vaginal delivery, n (%)	141,764 (82.1)	1,101 (65.3)
Caesarean Section, n (%)	30,868 (17.9)	587 (34.7)

Values are expressed as mean ± standard deviation or as median (interquartile range), * p<0.001

Compared to the non-diabetic group, women who developed GDM were more likely to be older, heavier, of non-white racial origin and deliver large for gestational age babies earlier.

5.1.3.1 Maternal age

Although most cases of GDM were in the age groups 20-35 because that is when most women were pregnant (fig 5.5), there were increasing proportions of GDM with increasing age groups (fig 5.6). This was more apparent over the age of 25.

Fig 5.5 The proportions of women falling within the different maternal age groups.

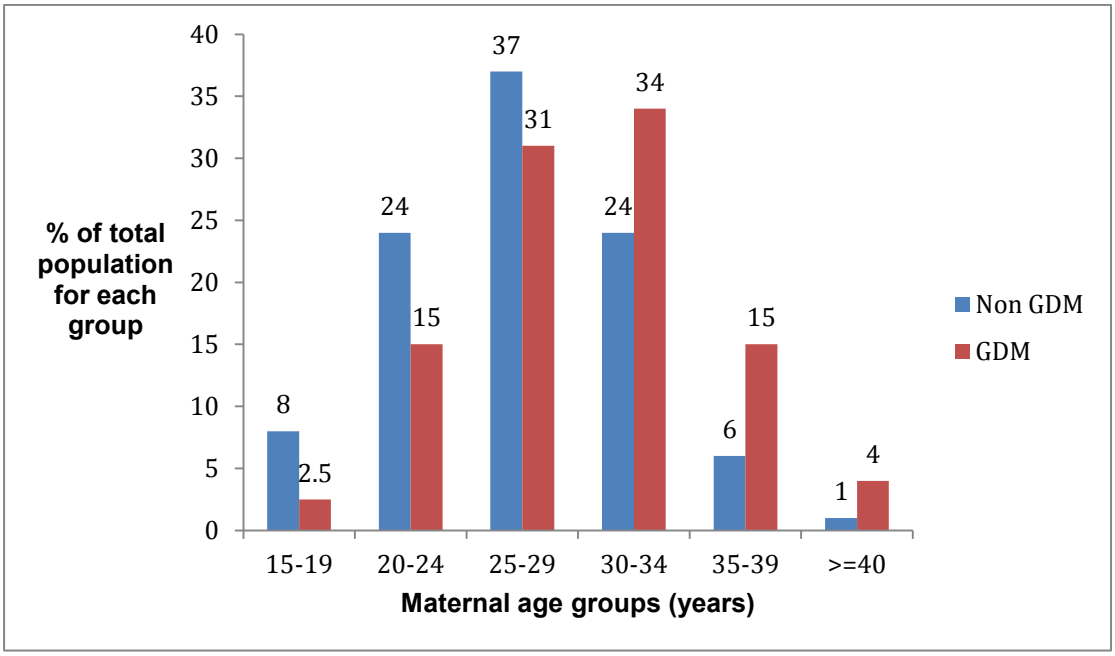
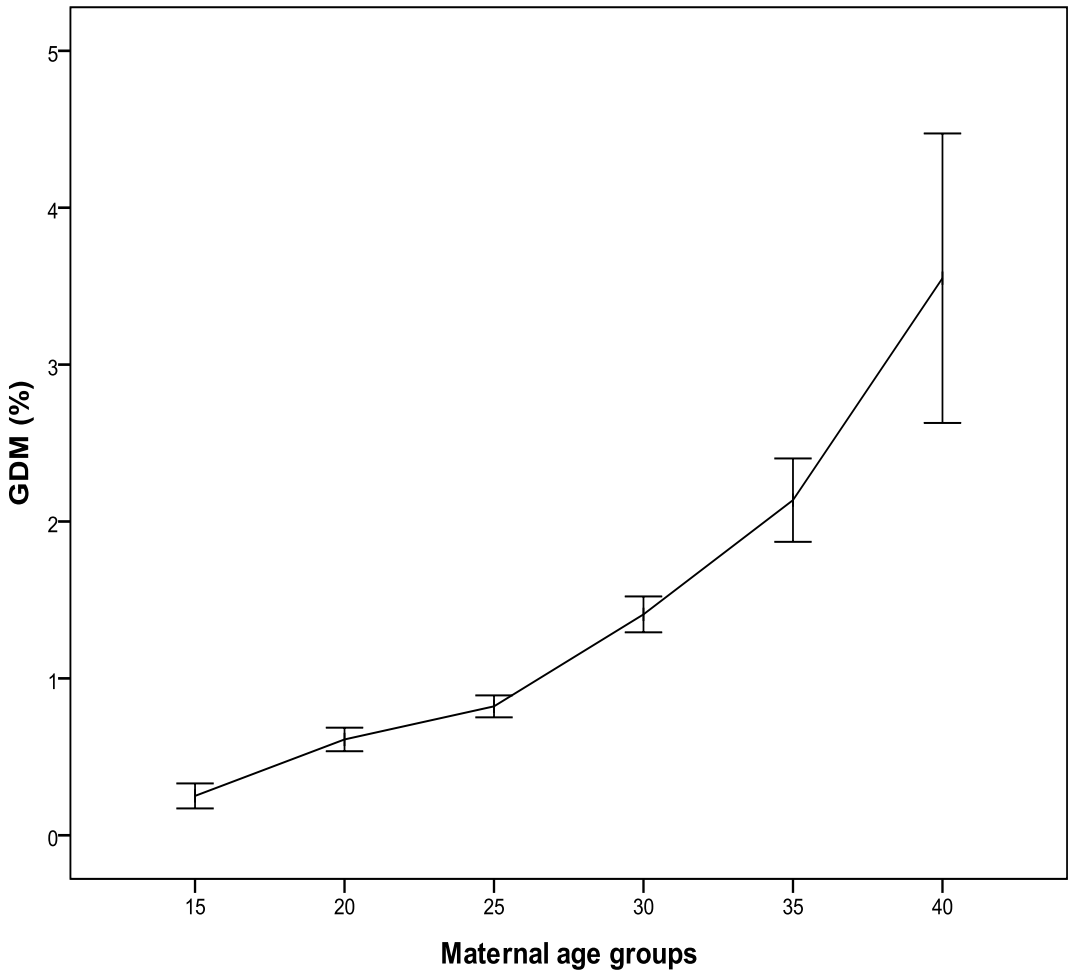


Fig 5.6 The prevalence of GDM (95% Confidence Intervals shown as error bars) in the different maternal age groups, for the total population.



The rates of GDM were generally higher within non-white racial groups. In Black Caribbean groups, rates of GDM were higher from age 25, except for age group 35-39 compared to White Europeans. Black African and South Asian groups had higher rates of GDM within all age groups compared to White Europeans (Table 27).

Table 27 The rates of GDM and non-GDM in the study population according to maternal age, within each different racial group.

Variable	White European		Black African		Black Caribbean		South Asian	
	GDM, n (%)	Non GDM, n (%)	GDM, n (%)	Non GDM, n (%)	GDM, n (%)	Non GDM, n (%)	GDM, n (%)	Non GDM, n (%)
Age groups								
<20	26 (0.2)	11478 (99.8)	1 (0.3)	351 (99.7)	1 (0.1)	825 (99.9)	7 (0.5)	1541 (99.5)
20-24	134 (0.5)	28559 (99.5)	10 (0.7)	1335 (99.3)	4 (0.3)	1320 (99.7)	77 (1.1)	7115 (98.9)
25-29	263 (0.5)	48194 (99.5)	33 (1.6)	2064 (98.4)	22 (1.5)	1441 (98.5)	129 (1.8)	7133 (98.2)
30-34	308 (0.9)	32167 (99.1)	26 (2.9)	882 (97.1)	20 (2.4)	814 (97.6)	125 (4.0)	3037 (96.0)
35-39	122 (1.4)	8128 (98.6)	12 (6.0)	187 (94.0)	2 (0.9)	217 (99.1)	50 (6.2)	750 (93.8)
≥ 40	23 (1.9)	1199 (98.1)	5 (21.7)	18 (78.3)	1 (3.1)	31 (96.9)	13 (11.4)	101 (88.6)

As expected, there was a strong positive association between advancing maternal age and the proportion of women with GDM (p<0.001) (Table 27). This positive association was present in all maternal racial groups but was more pronounced in Black Africans and South Asians in comparison to White Europeans (p<0.001 for all groups). (Table 28)

Table 28 Univariate analysis of the influence maternal age, BMI and racial group on development of GDM.

Maternal characteristic	Odds Ratio (OR)	p value
Maternal age (yrs)	1.09 (1.08-1.10)	<0.001
BMI (kg/m²)	1.11 (1.10-1.12)	<0.001
Racial origin		
Black African *	2.67 (2.14-3.34)	<0.001
Black Caribbean*	1.60 (1.20-2.13)	<0.001
South Asian*	3.03 (2.69-3.41)	<0.001

* Comparisons made between White European women age 29-24 as a reference group

Using White European women age 20-24 as a reference group, White European women over the age of 30 had higher ORs for GDM development. Non-white racial groups had significantly higher ORs at younger ages: over age 25 for Black African and Caribbean women and over 20 for South Asian women. White European women under the age of 20 had a significantly reduced OR for GDM development in comparison to the reference group. This was not the same for non-white racial groups below the age of 20 (Table 29 and Fig 5.7)

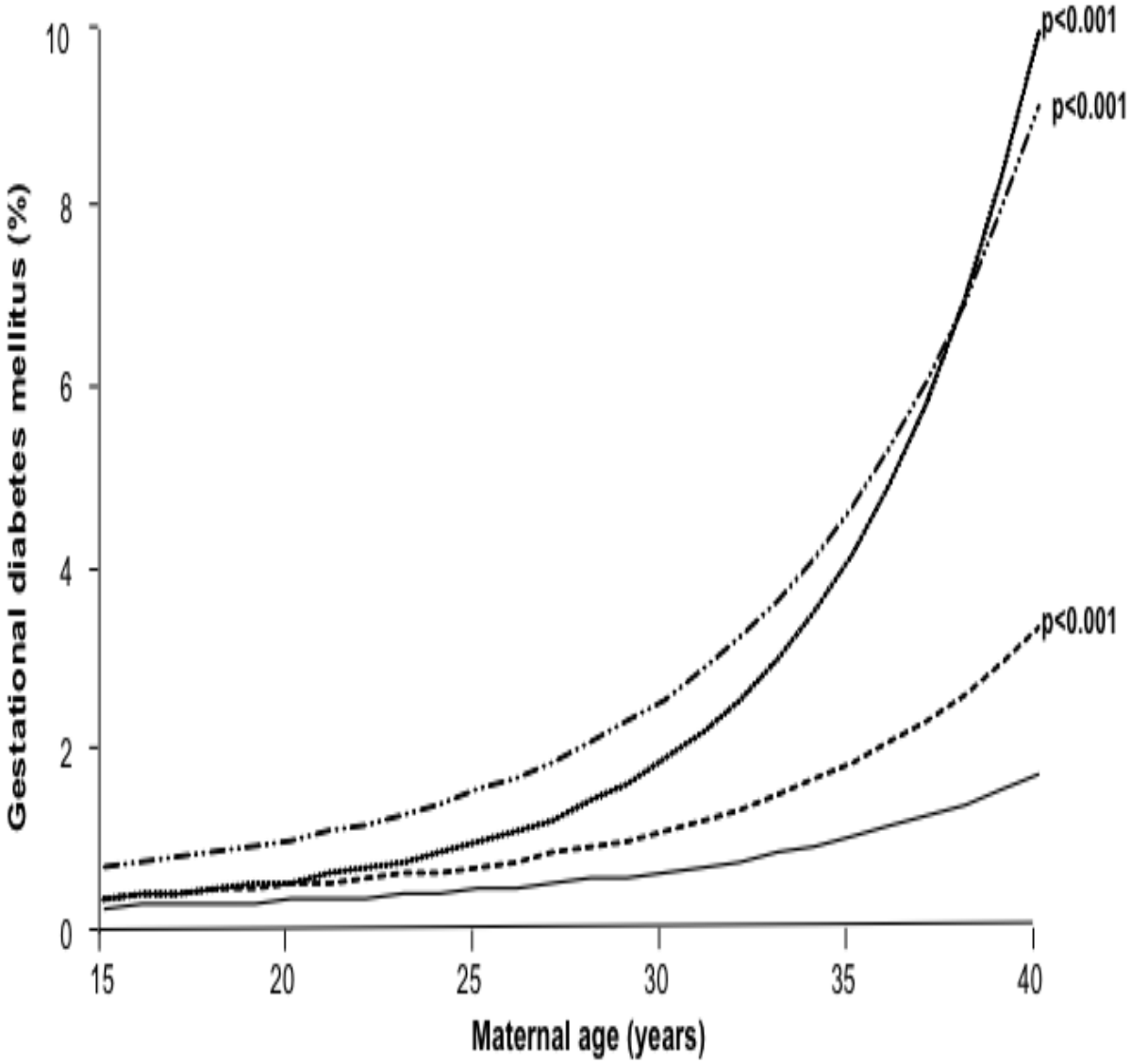
Table 29 Odds ratios (95%CI) for development of GDM according to advancing maternal age within each racial group. The reference group was White European women of 20-24 years old.

Age groups	White European	Black African	Black Caribbean	South Asian
<20	0.48 (0.31-0.73)*	0.60 (0.08-4.35)	0.25 (0.03-1.85)	0.96 (0.45-2.07)
20-24	**1.00**	1.59 (0.33-3.03)	0.64 (0.23-1.74)	2.30 (1.74-3.05)*
25-29	1.16 (0.94-1.43)	3.40 (2.32-5.00)*	3.25 (2.06-5.12)*	3.85 (3.02-4.91)*
30-34	2.04 (1.66-2.50)*	6.28 (4.10-9.61)*	5.23 (3.25-8.42)*	8.77 (6.85-11.22)*
35-39	2.97 (2.32-3.81)*	13.67 (7.44-25.11)*	1.96 (0.48-7.98)	14.05 (10.08-19.60)*
≥40	4.08 (2.61-6.38)*	59.20 (21.66-161.37)*	6.87 (0.93-50.72)	27.43 (15.02-50.08)*

*p<0.001

Fig 5.7 The prevalence of GDM in the different maternal age groups according to race. The maternal age is given as continuous variable. Regression lines for non-white racial groups are compared to White Europeans.

White European —— Black Caribbean - - - Black African ..... South Asian —·· -



5.1.3.2 Body Mass index

Although most cases of GDM were in the BMI groups 20-29.9 because that is when most women were pregnant (Fig 5.8), the prevalence of GDM was higher in higher BMI groups (Fig 5.9).

Fig 5.8 The proportions of women falling within the BMI groups.

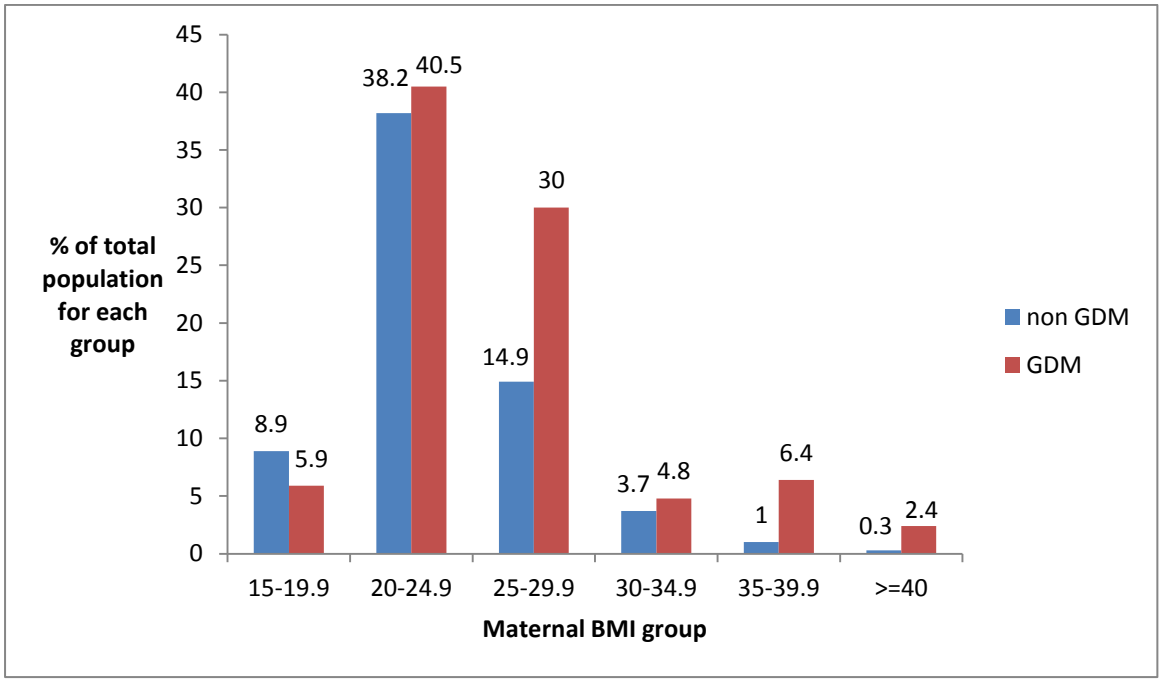
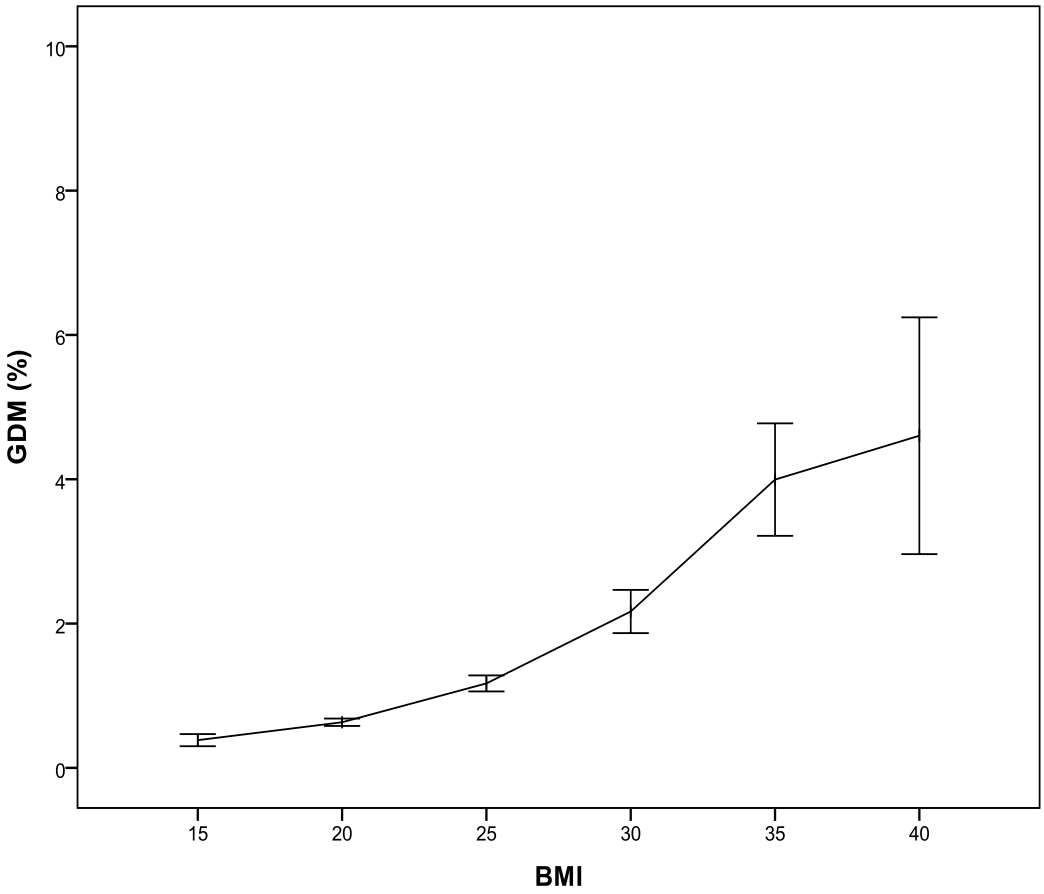


Fig 5.9 The prevalence of GDM (95%CI) in the different maternal BMI groups.



Similarly, there was a strong positive association between increasing BMI and the proportion of women with GDM ($p<0.001$) (Table 29), which was more pronounced in Black Africans and South Asians in comparison to White Europeans ($p<0.0001$ for all groups) (Table 29).

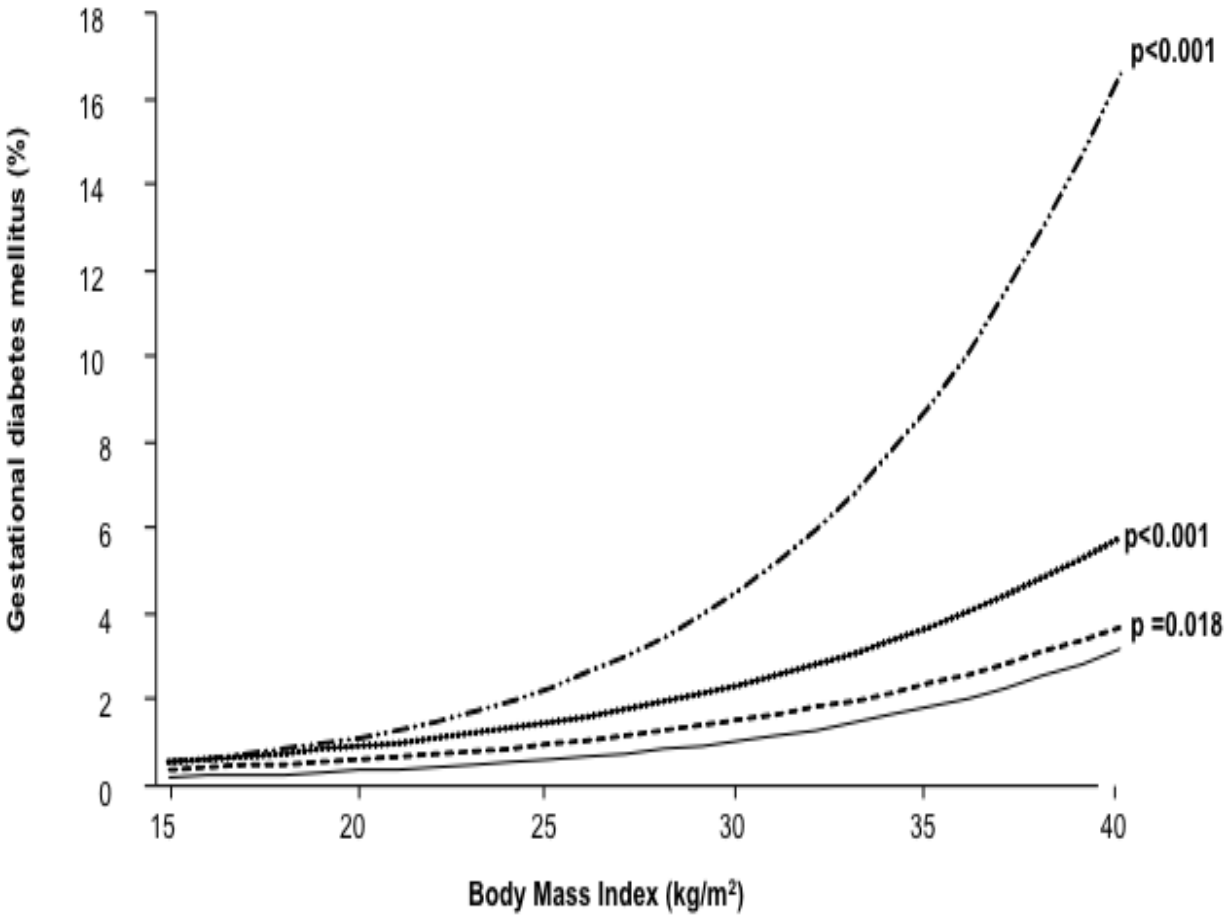
Using White European women with a BMI of 18.5-24.9 as a reference group, increasing BMI category was associated with higher ORs for GDM development within White European group. Compared to the reference group, non-white racial groups (with the exception of Black Caribbean) had higher ORs for GDM development within all BMI categories including the normal BMI group (Table 30 and Fig 5.10).

Table 30 The rates of gestational diabetes mellitus (GDM) and non-GDM in the study population according to the BMI within each different racial group.

Variable	**White European**		**Black African**		**Black Caribbean**		**South Asian**	
Body Mass Index groups	GDM, n (%)	Non GDM, n (%)	GDM, n (%)	Non GDM, n (%)	GDM, n (%)	Non GDM, n (%)	GDM, n (%)	Non GDM, n (%)
Normal (18.5-24.9)	396 (0.5)	85823 (99.5)	33 (1.2)	2729 (98.8)	15 (0.6)	2687 (99.4)	184 (1.4)	13283 (98.6)
Overweight (25.0-29.9)	217 (0.8)	26556 (99.2)	20 (1.6)	1234 (98.4)	17 (1.5)	1097 (98.5)	111(3.4)	3122 (96.6)
Obese (≥ 30)	221 (2.1)	10175 (97.9)	27 (5.6)	456 (94.4)	15 (2.6)	555 (97.4)	68 (7.4)	847 (92.6)

Fig 5.10 The prevalence of GDM (95%CI) in the different maternal BMI groups according to race. The maternal BMI is shown as continuous variable. Regression lines for non-white racial groups are compared to White Europeans

White European —— Black Caribbean - - - Black African ····· South Asian – · · –



5.1.3.3 Interactions between variables

A stepwise approach was used to create models looking at racial group, BMI and age and the interactions between the variables.

The following models were created:

- age, BMI, race and the interaction between BMI and race
- age, BMI, race and the interaction between age and race

The interaction term BMI x race was statistically significant only in the South Asians and the interaction term age x race was significant in Black Africans and South Asians, as shown in Tables 31 and 32, respectively.

Table 31 Logistic regression model: the influence of maternal age, BMI, racial group and BMI X race (interaction term) on development of GDM. Odds ratios with 95% confidence interval (95%CI) are given.

Maternal characteristic	**Odds Ratio (95%CI)**	**P value**
Age	1.103 (1.092-1.115)	<0.001
Body mass index (kg/m2)	1.120 (1.122-1.149)	<0.001
Race * -overall		0.013
Black African	3.721 (1.060-11.077)	0.018
Black Caribbean	3.027 (0.724-11.759)	0.086
South Asian	1.881 (1.291-4.732)	0.036
BMI x race interaction*-overall		0.018
Black African	0.989 (0.950-1.033)	0.565
Black Caribbean	0.977 (0.931-1.027)	0.305
South Asian	1.032 (0.998-1.046)	0.006

* in comparison to White Europeans

5. Results

Table 32 Logistic regression model: the influence of maternal age, BMI, racial group and age x race (interaction term) on development of GDM. Odds ratios with 95% confidence interval (95%CI) are given.

Maternal characteristic	Odds Ratio (95% CI)	P value
Age	1.089 (1.075-1.103)	<0.001
BMI (kg/m2)	1.124 (1.127-1.149)	<0.001
Race * -overall		0.258
Black African	0.427 (0.093-1.526)	0.230
Black Caribbean	1.093 (0.255-5.373)	0.908
South Asian	1.616 (0.626-3.221)	0.103
Age x race interaction*- overall		0.005
Black African	1.065 (1.020-1.119)	0.007
Black Caribbean	1.012 (0.959-1.063)	0.647
South Asian	1.033 (1.009-1.057)	0.005

* in comparison to White Europeans

A similar model with the interaction age X BMI was constructed. However, when the variable maternal age x BMI was included into the model for development of GDM, the interaction was not significant for any racial group (p=0.974) (i.e. the influence of BMI does not vary with age) (Table 33)

5. Results

Table 33 Multiple Regression Model: the influence of maternal age, BMI, racial group and age x BMI (interaction term) on development of GDM. Odds ratios with 95% confidence interval (95%CI) are given.

Risk Factor	Odds Ratios (95% CI)	p value
Age	1.10 (1.05-1.15)	<0.001
BMI	1.12 (1.07-1.18)	<0.001
Race* Black African Black Caribbean South Asian	 2.71 (2.16-3.39) 1.55 (1.16-2.08) 4.17 (3.69-4.72)	<0.001 <0.001 0.003 <0.001
Age x BMI interaction	1.00	0.974

* in comparison to White Europeans

Summary:**

- There is strong association between advancing maternal age and increasing BMI on the development of GDM, which varies within each racial group.
- The association between risk factors and GDM development is different within Black sub-populations- Black African and Black Caribbean.
- The influence of advancing age is on GDM development is more pronounced in Black African and South Asian groups.
- The influence of increasing BMI is more pronounced in South Asian groups.
- There is no significant interaction between maternal age and body mass index on the development of GDM.

** Summary findings relate to women in their first pregnancy

5. Results

5.2 Hypothesis 1(b): There is a relationship between advancing maternal age, BMI, racial group and diabetic status (GDM or not) and birth weight.

The dataset was modified in order to examine the influence of variables maternal age, BMI, racial origin and GDM on neonatal birthweight, represented as z-scores. A further 20 women were excluded from this analysis, because the sex of the baby was reported as indeterminate. The dataset therefore included 1,688 women with GDM and 172,612 women without GDM. As shown previously in figures 4.3.1 and 4.3.2, maternal weight and BMI increase with gestation. However, there is little variation in maternal BMI between 8 and 20 weeks gestation. The dataset was therefore modified further to exclude all women who booked after 20 weeks gestation (n=43,751).

Maternal demographic, clinical characteristics and pregnancy outcomes are given in Table 34.

Table 34 Maternal demographic, clinical characteristics and pregnancy outcomes of the non-GDM and GDM groups.

Maternal Characteristic	**Non-GDM (N=139,267)**	**GDM (N=1,307)**	**P value**
Maternal age (years)	27.14 ± 5.1	29.6 ± 5.1	<0.001
Gestational age at first antenatal visit (wks)	13.0 (9.0-17.0)	13.0 (9.0-76.0)	0.457
BMI (kg/m2)	23.6 ± 4.0	26.6 ± 5.5	<0.001
Racial group White European, n (%) Black African, n (%) Black Caribbean, n (%) South Asian, n (%) Other, n (%)	 107,199 (77.0) 3269 (2.3) 3455 (2.5) 15513 (11.1) 9831 (7.0)	 707 (54.1) 63 (4.8) 39 (3.0) 304 (23.3) 194(14.8)	<0.001

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Gestational age at delivery (wks)	40.0 (38.0-42.0)	39.0 (37.0-41.0)	<0.001
Birthweight (grams)	3273.2 ± 547.1	3337.4 ± 628.2	<0.001
Birthweight percentile	58.4 ± 28.1	64.8 ± 29.6	<0.001
Birthweight z-score	0.000± 1.0	0.421 ± 1.3	<0.001
Birthweight<10th centile, n (%)	16870 (9.7)	192 (11.3)	<0.001
Birthweight >90th centile, n (%)	16598 (9.6)	248 (14.7)	<0.001
Mode of delivery Vaginal, n (%) Caesarean section, n(%)	 114,470 (82.2) 24, 793 (17.8)	 873 (66.8) 434 (33.2)	<0.001

Values are expressed as mean ± standard deviation or as median (interquartile range).

Compared to the non-GDM group, women in the GDM group were more likely to be older, heavier, of a non-white racial group and more likely to deliver both large for gestational age babies (birthweight >90th percentile) and small for gestational age babies (<10th percentile) earlier. ($p<0.01$ for both).

The final dataset included 130,549 women of whom 82.7% were White European, 5.2% were Black and 12.1% were South Asian. The prevalence of GDM varied within the racial groups and was higher in non-White women (1.9% and 1.5% for South Asians and Blacks respectively) compared to White Europeans (0.7%; $p<0.001$ for both comparisons) (Table 35).

In pregnancies complicated by GDM, South Asian women had significantly lower BMI and smaller babies in comparison to White European women. Maternal characteristics were not significantly different between White European and Black groups. Compared to White Europeans, Black women had larger babies, although this was not statistically significant, and a higher rate of caesarean section (Table 36).

5. Results

The Carstairs Index was higher in non-white racial groups. This was irrespective of glycaemic status, which suggests increased measures of social deprivation within these groups.

Rates of macrosomia, defined as birthweight >90th percentile, were significantly higher in non-white racial groups for those pregnancies not complicated by GDM (Table 34). In those pregnancies complicated by GDM, rates of macrosomia were not different between White European and Black women but significantly lower rates of macrosomia were found for South Asian women (Table 36).

There were increased rates of babies delivered with birthweight <10th percentile in non-white racial groups in women whose pregnancy was not complicated by GDM. In those pregnancies complicated by GDM, South Asian women had higher rates of babies delivered with birthweight<10 percentile (Table 36).

Table 35 Maternal demographic, clinical characteristics and pregnancy outcomes within the racial groups in pregnancies not complicated by GDM (non-GDM). Values are expressed as mean ± standard deviation or median (interquartile range).

Maternal characteristic	**White European** ***n*= 107,199**	**Black** ***n*= 6,724**	**South Asian** ***n*= 15,513**
Maternal age (yrs)	27.3 ± 5.0	26.1 ± 5.0*	25.8 ± 4.6*
BMI (kg/m2)	23.8 ± 4.0	24.3 ± 4.4*	22.2 ± 3.6*
Gestational age at booking (wks)	13.0 ± 2.8	13.6 ± 2.9 *	13.4 ± 2.8*
Gestational age at delivery (wks)	39.4 ± 1.8	39.0 ± 2.2*	39.0 ± 1.9 *
Birthweight (g)	3,326.8 ±536.8	3,134 ±571.8*	2,993.8 ± 507.7*
Birthweight percentile	61.2 ± 27.3	52.2 ± 28.5*	42.02±27.4*
Birthweight >90th percentile, *n* (%)	12,175 (11.4)	417(6.2) *	391(2.5) *
Birthweight <10th percentile, *n* (%)	8,961 (8.4)	928 (13.8) *	2,954 (19.0) *
Birthweight (z-score)	0.091 ± 0.981	-0.173 ± 0.966*	-0.526 ± 0.932*

5. Results

Maternal characteristic	White European n= 107,199	Black n= 6,724	South Asian n= 15,513
Carstairs Index, score	-1.0 (-4.0-2.0)	1.0 (-4.0-6.0)*	0.0 (-4.0-4.0)*
Diastolic Blood Pressure at booking (mmHg)	67.5 ± 8.5	66.9 ± 8.4*	65.3 ± 8.3*
Smoking,*n* (%)	22,062 (20.6)	780 (11.6) *	266 (1.7) *
Mode of delivery			
Caesarean section,*n* (%)	18,007 (16.8)	1,594(23.7)*	3,003 (19.4)*
Vaginal,*n* (%)	89,190 (83.2)	5,129(76.3)	12,509 (80.6)

All comparisons were done with White Europeans. Variables compared using t-test/ Mann–Whitney U-testand chi-square tests for continuous and categorical data, respectively, with post hoc Bonferroni correction, where appropriate. *P<0.001

Table 36 Maternal demographic, clinical characteristics and pregnancy outcomes within the racial groups in pregnancies complicated by GDM (GDM). Values are expressed as mean ± standard deviation or median (interquartile range).

Maternal characteristic	**White European** ***n*= 707**	**Black** ***n*= 102**	**South Asian** ***n*= 304**
Maternal age (yrs)	29.4 ± 5.0	30.0 ± 4.8	28.4 ± 5.1
BMI (kg/m^2)	26.7 ± 5.8	27.3 ± 5.5	25.3 ± 4.9*
Gestational age at booking (wks)	13.1 ± 2.6	13.6 ± 3.4	13.4 ± 2.8
Gestational age at delivery (wks)	38.8 ± 1.8	38.5 ±1.8	38.4 ± 1.8
Birthweight (g)	3,408.8 ± 615.7	3,429.9± 619.4	3,172.7 ± 582.8*
Birthweight centile	67.65 ± 28.7	68.6 ± 29.0	56.4 ± 30.8*
Birthweight >90th percentile, *n* (%)	133 (18.8)	19(18.6)	28 (9.2) *
Maternal characteristic	**White European** **n= 707**	**Black** **n= 102**	**South Asian** **n= 304**

Birthweight (z-score)	0.528 ± 1.266	0.632 ± 1.341	0.092 ± 1.224*
Carstairs Index, score	-2.0 (-5.0-1.0)	2.0 (-2.0-6.0)*	1.0 (-3.0-5.0)*
Diastolic Blood Pressure at booking	70.2 ± 9.1	68.9 ± 8.6	69.4 ± 9.4
Smoking *n* (%)	121 (17.1)	6 (5.9)	5 (1.6)*
Mode of delivery **Caesarean section *n* (%)** **Vaginal *n* (%)**	 198 (28.0) 509 (72.0)	 46 (45.1) 56 (54.9)	 121(39.8) 183 (60.2)

All comparisons were done with White Europeans. Variables compared using t-test/ Mann–Whitney U-test and chi-square tests for continuous and categorical data, respectively, with post hoc Bonferroni correction, where appropriate. *P<0.001

5. Results

Babies born to women with pregnancies complicated by GDM were generally larger in comparison to those not complicated by GDM (Table 37). This was more prominent in Black women.

Table 37 Mean birthweight z -scores and differences between z-scores for Non-GDM and GDM women within each racial group.

Racial group	Non GDM	GDM	Difference
White European	0.091	0.528	0.437
Black	-0.173	0.632	0.805
South Asian	-0.526	0.092	0.618

5.2.1 Exploratory analysis

Univariate analysis revealed a significant association between advancing maternal age, BMI, racial group and diabetic status on birthweight ($p<0.001$ for all variables).

Univariate analysis also revealed that the influence of maternal age and BMI on the outcome variable birthweight z-score was very similar between Black African and Black Caribbean groups and therefore Black African and Black Caribbean racial groups were grouped together in this part of the study.

Multivariate analysis revealed that only BMI, racial group and diabetic status were significantly associated with birthweight ($p<0.001$ for all variables).

Multiple regression analysis was used to identify important determinants of birthweight and stratified according to racial groups and diabetic status (non-GDM and GDM). The final models for White Europeans are shown in Tables 38 and 39.

Table 38 Multivariate analysis investigating possible determinants of birthweight z-score for non GDM within White European women.

Dependent variable z score					95%Confidence Interval	
Parameter	Correlation Coefficient	Std Error	t	P value	Lower bound	Upper bound
Intercept	-0.464	0.025	-18.455	<0.001	-0.513	-0.414
Age	-0.004	0.001	-5.885	<0.001	-0.005	-0.003
BMI	0.300	0.001	39.268	<0.001	0.029	0.032
Smoking	-0.351	0.008	-43.799	<0.001	-0.0367	-0.336
Carstairs Index	-0.009	0.001	-6.606	<0.001	-0.012	-0.007

Table 39 Multivariate analysis investigating possible determinants of birthweight z-score for GDM within white European women.

Dependent variable z score					95%Confidence Interval	
Parameter	Correlation Coefficient	Std Error	t	P value	Lower bound	Upper bound
Intercept	-0.745	0.217	-3.432	0.001	-1.174	-0.319
BMI	0.05	0.008	6.259	0.001	0.034	0.065
Smoking	-0.329	0.123	-2.671	0.008	-0.571	-0.087

5. Results

Summary:

- Within White European women, BMI and smoking were important determinants of birthweight, irrespective of the development of GDM.
- In the non-GDM group maternal age and Carstair's index appear to be additional independent variables for birthweight.

The final models for Black women are given in Tables 40 and 41.

Table 40 Multivariate analysis investigating possible determinants of birthweight z-score for Non GDM group within Black women.

Dependent variable z score					95%Confidence Interval	
Parameter	Correlation Coefficient	Std Error	t	P value	Lower bound	Upper bound
Intercept	-0.831	0.066	-12.612	0.001	-0.96	-0.702
BMI	0.028	0.003	10.56	0.001	0.023	0.033
Smoking	-0.213	0.036	-5.852	0.001	-0.285	-0.142

Table 41 Multivariate analysis investigating possible determinants of birthweight z-score for GDM group within Black women.

Dependent variable z score					95%Confidence Interval	
Parameter	Correlation Coefficient	Std Error	t	P value	Lower bound	Upper bound
Intercept	-1.092	0.659	-1.657	0.101	-2.399	0.216
BMI	0.063	0.024	2.669	0.009	0.016	0.11

5. Results

Summary:

- Within Black women, BMI was an independent determinant of birthweight, irrespective of the development of GDM.
- In the non-GDM group smoking appears to be an additional independent variable determining birthweight.

The final models for South Asian women are given in Tables 42 and 43.

Table 42 Multivariate analysis investigating possible determinants of birthweight z-score for Non GDM group within South Asian women.

Dependent variable z score					95%Confidence Interval	
Parameter	Correlation Coefficient	Std Error	t	P value	Lower bound	Upper bound
Intercept	-1.63	0.076	-1.657	0.001	-1.779	-1.481
BMI	0.034	0.002	15.68	0.001	0.03	0.038
DBP	0.003	0.001	3.106	0.002	0.001	0.005
Age	0.006	0.002	3.65	0.001	0.003	0.009

Table 43 Multivariate analysis investigating possible determinants of birthweight z-score for GDM group within South Asian women.

Dependent variable z score					95%Confidence Interval	
Parameter	Correlation Coefficient	Std Error	t	P value	Lower bound	Upper bound
Intercept	-1.766	0.355	-4.975	0.001	-2.464	-1.067
BMI	0.073	0.014	5.325	0.001	0.046	0.1

Summary:

- Within South Asian women BMI was an independent determinant of birthweight, irrespective of the development of GDM

- In the non-GDM group, maternal age and diastolic blood pressure appear to be additional independent variables to birthweight.

The positive association between maternal BMI and birthweight, regardless of diabetic status, continued and was significant within all racial groups. As BMI was the most consistently independent predictor of birthweight z-scores within all three racial groups, we chose to focus on this variable.

Graphs with regression lines were created to illustrate the association between increasing maternal BMI and birthweight, represented as z-scores, within non-GDM and GDM groups for each racial group (Figure 5.11 a, b and c).

Two methods were used to calculate the difference in the slopes. In the first method, it was calculated at $t=(b1-b2)/S_{b1,\ =b2}$, where *b1* and *b2* are the two slope coefficients and $S_{b1,b2}$ is the polled standard error of the slope. The results according to the maternal racial group

were: for White European women, t=0.020/0.001=20, df=107 905 and two-tailed P<0.0001; for black women, t=0.036/0.003= 12.3, df=6826 and two-tailed P<0.0001; and for South Asian women, t=0.039/0.002=19, df =15 817 and two-tailed P<0.0001.

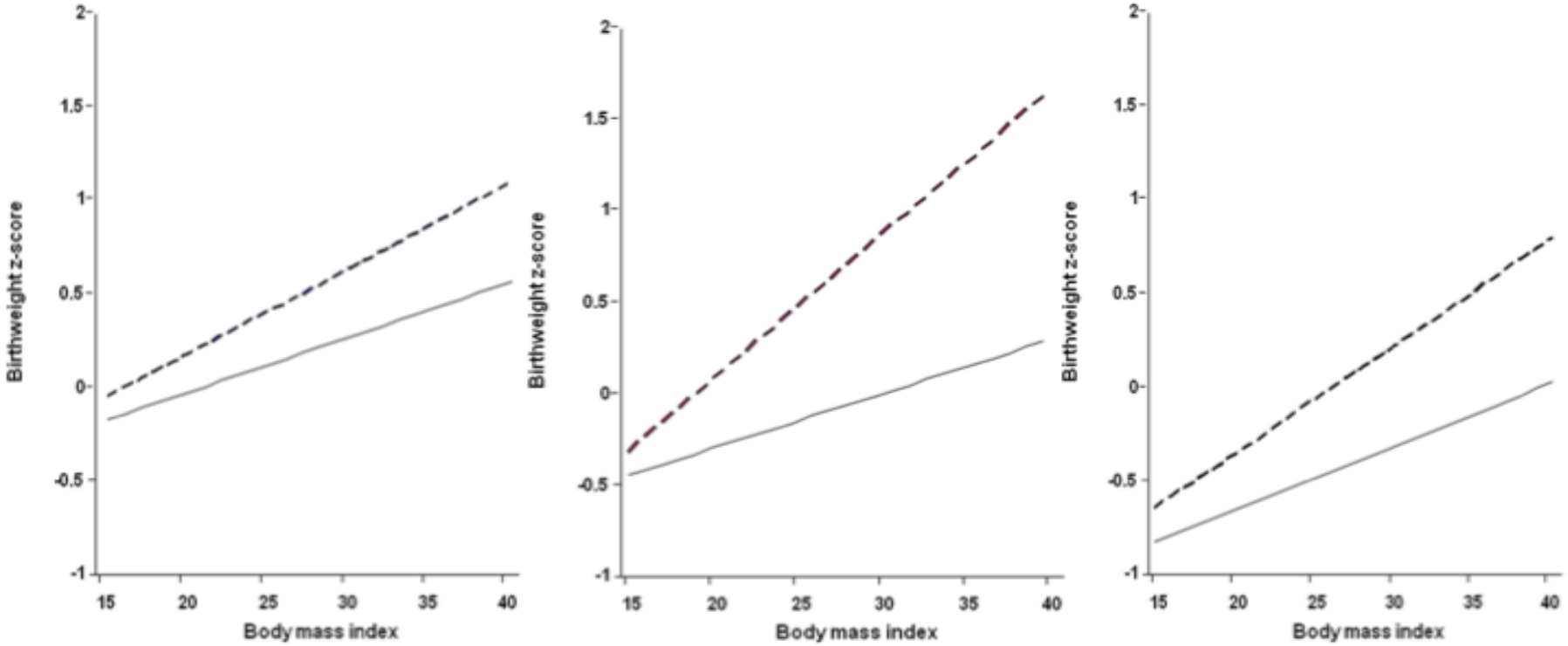
In the second method, analysis of variance was used: F=138.9 and two-tailed P<0.0001. The difference in birthweight z-scores between women who developed GDM or not was much greater at high BMIs than at low BMIs: there was a statistically significant difference between the slopes in GDM versus non-GDM pregnancies in all racial groups (P<0.001 for all). Thus, as far as birthweight is concerned, maternal BMI has a much greater effect in women with GDM than in those without, an effect that is especially marked in back women and South Asian women.

This was performed using the following equations calculated from the above multivariate analysis (Table 44).

Table 44 Multivariate analysis equations of independent predictors of birthweight for GDM and non-GDM within all racial groups.

	Non-GDM	**GDM**
White European	y = 0.030BMI - 0.004Age - 0.351Smoking - 0.009CI - 0.464 (r2=0.036)	y=0.050BMI - 0.329Smoking - 0.745 (r2=0.059)
Black	y = 0.028BMI - 0.213Smoking + 0.831 (r2=0.021)	y = 0.063BMI - 1.092 (r2=0.066)
South Asian	y = 0.034BMI + 0.006Age + 0.003DBP - 1.630 (r2=0.022)	y = 0.073BMI - 1.766 (r2=0.086)

Fig 5.11 Regression lines for the association between increasing maternal BMI and birthweight z-scores in non-GDM and GDM groups for white European, black and South Asian groups, adjusted for all significant confounding factors. There was a statistically significant difference between the slopes in GDM vs non-GDM pregnancies in all racial groups (P < 0.001 for all). Non-GDM ____ GDM _ _



5.11 a) White European b) Black c) South Asian

Only one pregnancy per woman studied

5. Results

Summary:

- The presence of maternal GDM is an independent predictor of neonatal birthweight within all racial groups, irrespective of BMI.

- There is a strong positive association between maternal BMI and birthweight z-scores within all racial groups. Development of GDM accentuates this association, which is more pronounced within the black group.

5. Results

5.3 Hypothesis 2: First trimester maternal biomarkers can improve early prediction of GDM

The dataset for hypothesis 2 consisted of 124 women who subsequently developed GDM and 248 that remained normoglycaemic. Characteristics of the populations studied are given in Table 45. Women who developed GDM were older, with greater BMI and more had prior GDM and family history of Type 2 DM. They also had higher systolic blood pressure, but there were no differences in parity, smoking history or method of conception.

Table 45 Maternal demographic, clinical characteristics and pregnancy outcomes of the non-GDM and GDM groups.

Maternal Characteristics	**Non-GDM (N=248)**	**GDM (N=124)**	**P value**
Maternal age (yrs)	32.6 ± 5.2	34.3± 5.0	0.002‡
BMI (kg/m2)	25.4 ± 5.2	29.2± 7.9	<0.001§
Parity, n(%) Nulliparous Parous	 89 (35.9) 159 (64.1)	 45 (36.3) 79 (63.7)	1.000 †
Racial group, n(%) White Black Asian Other	 148 (59.7) 62 (25.0) 29 (11.7) 9 (3.6)	 73 (58.9) 34 (27.4) 11 (8.9) 6 (4.8)	0.769 †
Previous GDM, n(%) Yes No Nulliparous	 158 (63.7) 1 (0.4) 89 (35.9)	 44 (35.5) 35 (28.2) 45 (36.3)	<0.001 †
Family history of diabetes n(%) Yes No	 218 (87.9) 30 (12.1)	 76 (61.3) 48 (38.7)	<0.001†
Smoker n(%) Yes No	 230 (92.7) 18 (7.3)	 115 (92.7) 9 (7.3)	1.000 †

5. Results

Maternal Characteristics	Non-GDM (N=248)	GDM (N=124)	P value
Gestational age at booking (days)	87.4± 3.2	87.3 ± 3.6	0.737‡
Systolic Blood Pressure (mmHg)	111.7 ± 16.6	118.4 ± 14.1	0.001§
Diastolic Blood Pressure (mmHg)	71.4 ± 8.9	72.9 ± 8.4	0.103‡
Mode of delivery, n(%) Vaginal Emergency Caesarean Elective Caesarean Ventouse	 190 (77.2) 36 (14.6) 19 (7.7) 1 (0.4)	 80 (65.0) 25 (20.3) 18(14.6) 0 (0.0)	0.037 †
Fetal sex, n(%) Male Female	 118 (47.8) 129 (52.2)	 62 (50.0) 62 (50.0)	0.741 †
Birthweight (g)	3382.8 ± 540.3	3343.2 ± 495.0	0.378‡

‡students t-test, § Wilcoxon test, † Fisher exact test. Data are means ± SD, n (%)

The levels of different biomarkers in the two study groups are given in Table 46.

Table 46 Maternal serum first trimester biomarkers in the non-GDM and GDM groups.

Biomarker	Non-GDM (N=248)	GDM (N=124)	P value	P value (adjusted)*	P value (adjusted)†
Total cholesterol (mmol/l)	4.59 ± 0.85	4.88 ± 0.90	0.003‡	0.040	0.038
HDL cholesterol (mmol/l)*	1.68 ± 0.36	1.55 ± 0.38	0.002‡	0.003	0.001
LDL cholesterol (mmol/l)	2.29 ± 0.71	2.59± 0.78	<0.001	0.003	0.003
Triglycerides (mmol/l)*	1.23 (0.95–1.62)	1.49 (1.14–2.05)	<0.001§	0.017	0.012
Adiponectin (g/mL)	9.88 ± 5.57	7.38 ± 4.19	<0.001§	0.027	0.040
E-selectin (ng/ml)	31.3 (23.2–40.4)	31.3 (24.7–44.2)	0.308§	0.867	0.827
γ-GT (U/l)	11.0 (9.0–16.0)	12.0 (9.8–17.0)	0.063§	0.632	0.566
CRP (mg/l)*	3.21 (1.62–6.97)	5.77 (2.7–11.6)	<0.001§	0.030	0.024
t-PA	5.32 (4.23–6.58)	6.41 (5.19-8.27)	0.001§	0.001	<0.001

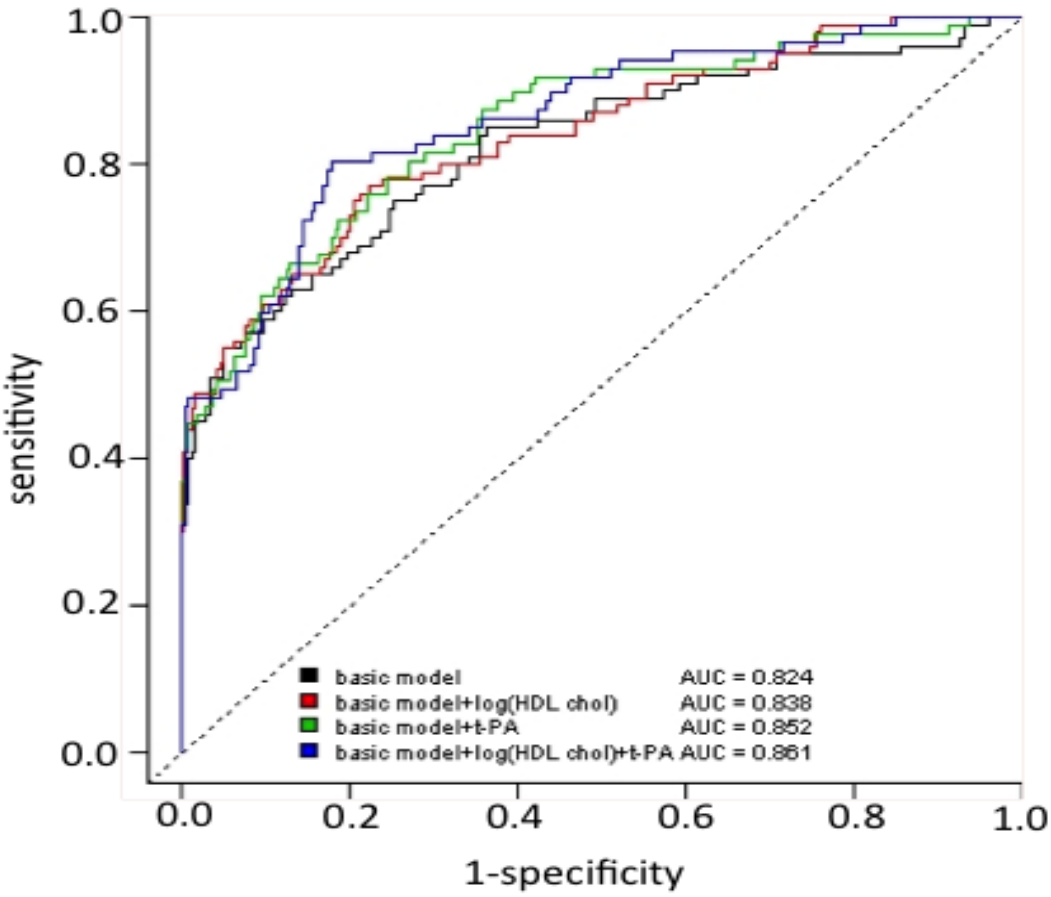
Data are means ± SD, medians (interquartile range). Log transformed for regression analysis. *Adjusted for maternal age, BMI, gestational age at sampling, smoking, ethnicity, parity, conception status and previous GDM. †Women without previous GDM (nulliparous or previous pregnancies without GDM) adjusted for maternal age, BMI, gestational age at sampling, smoking, ethnicity, parity, and conception status. ‡ student's t-test, § Wilcoxon test.

5. Results

After adjustment for confounding factors, women who subsequently developed GDM had significantly higher total cholesterol, LDL-cholesterol, triglycerides, CRP and t-PA in comparison to controls. HDL- cholesterol and adiponectin were significantly lower in women who subsequently developed GDM than controls. There was no significant difference between E-selectin and γ-GT levels between controls and women who developed GDM later on in pregnancy.

Stepwise regression analysis was used to determine the predictive value of biomarkers and revealed only an elevated t-PA and low HDL-cholesterol as significant independent predictors of GDM. The ability to predict the development of GDM using demographic and clinical information obtained at booking, demonstrated an AUC-ROC=0.824. This increased to 0.861 with the inclusion of t-PA and HDL cholesterol (Fig 5.12), which is clinically significant. Upon repeating the same analyses but removing all women with prior GDM, the AUC-ROCs were 0.751 and 0.806, respectively.

Fig 5.12 ROC curve and summary for all mothers using a basic model (including age, gestational age at sampling, BMI, race, family history of diabetes and prior GDM) and with addition of independent predictors (HDL cholesterol and t-PA).



5. Results

A subset of the dataset for Hypothesis 3 was used to investigate the association of first trimester maternal 25(OH)D and GDM development. This dataset consisted of 158 women who remained normoglycaemic during pregnancy and 90 women who developed GDM. The maternal characteristics of the population are given in Table 47.

Table 47 Maternal demographic, clinical characteristics and pregnancy outcomes of the non-GDM and GDM groups.

Maternal Characteristics	**Non-GDM (N=158)**	**GDM (N=90)**	**P value**
Maternal age (yrs)	33.1 ± 4.7	34.2 ± 4.9	0.104‡
BMI (kg/m2)	25.2 ± 4.0	30.0 ± 7.9	<0.001§
Parity, n (%) Nulliparous Parous	 67 (42.4) 91 (57.6)	 38 (42.2) 52 (57.8)	1.000 †
Racial group, n (%) White Black Asian Other	 108 (68.4) 31 (19.6) 12 (7.6) 97 (4.4)	 58 (64.4) 23 (25.6) 6 (6.7) 3 (3.3)	0.735 †
Previous GDM, n (%) No Yes	 158 (100) 0 (0)	 69 (76.6) 21 (23.3)	<0.001 †
Family history of diabetes, n (%) No Yes	 141 (89.2) 17 (10.8)	 55 (61.1) 35 (38.9)	<0.001†
Smoker, n (%) No Yes	 148 (93.7) 10 (6.3)	 83 (92.2) 7 (7.8)	0.795 †
Gestational age at booking (days)	87.4 ± 3.0	87.4 ± 3.6	0.916‡
Systolic Blood Pressure (mmHg)	112.5 ± 15.8	118.5 ± 11.6	0.003§
Diastolic Blood Pressure (mmHg)	70.2 ± 8.5	71.7 ± 7.2	0.218‡

5. Results

Maternal Characteristics	Non-GDM (N=158)	GDM (N=90)	P value
Fetal sex, n (%) Male Female	 80 (51.0) 77 (49.0)	 40 (44.4) 50 (55.6)	0.356†
Birthweight (g)	3403.1 ± 571.4	3283.6 ± 418.9	0.081‡
OGTT Fasting 2hr glucose		 5.3 ± 1.8 9.1 ± 1.8	
Hba1c		5.8 ± 0.9	
25 (OH) D (ng/ml) 25 (OH) D (nmol/l)	19.0 ± 10.7 47.6 ± 26.7	18.9 ± 10.7 47.2 ± 26.7	0.874† 0.863†
Deficient 25(OH)D (<25nmol/l) No Yes	 122 (77.2) 36 (22.8)	 72 (80.0) 18 (20.0)	0.635‡
Insufficient 25 (OH)D (< 50nmol/l) No Yes	 68 (43.0) 90 (57.0)	 34 (37.8) 56 (62.2)	0.502‡
Total Cholesterol (nmol/l)	4.62 ± 0.76	4.86 ± 0.90	0.033*
HDL Cholesterol (nmol/l)	1.74 (0.35)	1.58 (0.38)	0.001*
Truglcerides (nmol/l)	1.20 (0.95-1.56)	1.38 (1.08- 2.01)	0.002*

‡students t-test, § Wilcoxon test, † Fisher exact test. Data are means ± SD, n (%), median (interquartile range)

Women with pregnancies complicated by GDM had significantly higher BMI and were more likely to have a previous history of GDM and family history of DM. Systolic blood pressure was significantly higher in GDM group compared to non-GDM group.

Vitamin D (25OHD) levels were similar in the non-GDM and GDM groups (18.9 ng/ml vs 19.0 ng/ml respectively). Cut-offs for 25(OH)D insufficiency and deficiency revealed similar rates within GDM and non-GDM groups.

Univariate analysis revealed that 25OHD levels at booking after adjustment for confounders (maternal age, BMI, gestational age at sampling, smoking, race, parity, conception status and previous GDM) were negatively correlated with 2-hour glucose levels and positively associated with HDL cholesterol (Table 48).

5. Results

Table 48 Effect size refers to increase in variables per 1 ng/ml increase in 25OHD, $p<0.05$ after adjustment for confounding factors.

	Correlation Coefficient	95% CI	
OGTT 2hr glucose (mmol/l)	-0.738*	-1.467	-0.008
OGTT Fasting glucose (mmol/l)	-0.413	-1.089	0.264
Hba1c	-0.209	-0.559	0.142
Total Cholesterol (mmol/l)	0.164	-0.024	0.352
Triglycerides (mmol/l)	0.018	-0.107	0.142
HDL Cholesterol (mmol/l)	0.090*	0.005	0.175
Systolic blood pressure (mmHg)	1.239	-2.607	5.086
Diastolic blood pressure (mmHg)	1.652	-0.491	3.795

Summary:

- Simple maternal demographic and clinical characteristics obtained at the first antenatal visit provide a good prediction of GDM.
- Low HDL and high t-PA are independent predictors of GDM but also enhance prediction beyond the simple model of maternal demographic and clinical characteristics.
- First trimester maternal serum 25(OH)D levels are not associated with the development of GDM.
- First trimester maternal serum 25 (OH)D levels are negatively correlated with 2-hour glucose levels and positively associated with HDL cholesterol.

6. Discussion

6. 1 There is a differential effect of maternal racial origin on the association between advancing maternal age and BMI on the prevalence of GDM

This retrospective study of prospectively collected data has demonstrated strong interactions between traditional risk factors such as advancing maternal age, increasing BMI and racial group on the development of GDM. As the average maternal age at first pregnancy in the UK population has been increasing markedly over the last three decades [296] there is an increasing need to study the link between advancing maternal age and pregnancy outcomes. Traditionally, women having their first pregnancy over the age of 30 were categorized as 'elderly primigravidae'. However, most of the risks associated with advancing age rise only slowly from age 30 to 35, but accelerate after the age of 35 (e.g. the risk of fetal chromosomal abnormalities, hypertension etc). Therefore, currently, advanced maternal age is usually considered as those aged 35 or more. However, sociological developments and advances in reproductive techniques have encouraged women to have their first babies even at the age of 40 years or more – '40 is the new 30'.

Many studies have found an association between advancing maternal age and rates of GDM.[58-60, 297-9] Some of these studies suggest a threshold for maternal age associated with greater risk, however these thresholds vary between studies from >25 to >35 years of age. Hoseini et al[297] conducted a retrospective case control study examining the impact of pre-gravid maternal risk factors and risk of GDM. They found an increased risk of GDM beyond 26 years of age, noting a 2-fold increase in GDM (OR=2.1, p=0.028). This was supported by Nilofer et al,[298] who explored the prediction of GDM and found that by using a threshold for maternal age of over 25, sensitivities and specificities of 98% and 69% respectively were produced.

Carolan et al[299] conducted a population-based cross-sectional study that investigated the effect of maternal age and country/region of birth on GDM incidence demonstrated that the overall incidence of GDM within their study population was 4.8%, which is considerably higher than that of the UK. There was a strong trend of rates of GDM to increase with advancing maternal age, and higher rates of GDM were found particularly in women over the age of 35. Women over the age of 35 had six times the rates of GDM in comparison to women less than 20 years of age. This particular study also found a strong influence of maternal origin of birth, with higher rates of GDM in those women whose origin of birth was Asian. In comparison to Australian born women, Asian women had rates of GDM that were

almost three times higher, particularly in the older age group (>30 years of age).

Our study found an increased rate of GDM with maternal age over 25 years of age, increasing steadily as age increased. The association between advancing maternal age and risk of GDM was maintained even after adjusting for confounding factors such as race and BMI. This is thought to be subsequent to declining β-cell function and insulin sensitivity with advancing age, where the development of GDM is influenced by an individuals' reduced ability to secrete insulin. Importantly, advancing maternal age was much more important in women of South Asian and Black African origin than in White Europeans. A non-white pregnant woman of 25-29 years of age has a similar risk of developing GDM as a White European woman of 40 years of age; at least 3-4 times higher than a young (20-24 years old) White European. Advancing maternal age is also important in Black Caribbeans but we can only be confident about our results between 24-34 years old, because the number of subjects in the older age groups was small.

Large population studies have observed a variation in the prevalence of GDM between racial groups, with a higher prevalence of GDM in non-white racial groups.[67-75] However, the effect of obesity within racial groups does not seem to parallel the distribution of rates of GDM. Non-white racial groups, especially Asian women, develop GDM at a lower BMI. Racial differences may be due to a combination of factors.

- Differences in body composition between Asian and White populations
- Thrifty gene hypothesis vs thrifty phenotype hypothesis
- Migration, urbanization and changes in lifestyle and diet within Asian populations

6.1.1 Body composition in Asians

The differential association between increasing BMI and development of GDM within racial groups, particularly within the South Asian group, was not surprising. There is a growing body of evidence showing differences in percentage body fat and body fat distribution between racial groups, particularly with South Asian groups in comparison to their non-white counterparts.[300- 312]The Asian population is unique in that they present with a 'thin-fat' body composition.

Asians have a higher proportion of body fat at lower BMIs in comparison to European and

Black African groups. Higher levels of central obesity (waist circumference, waist hip ratio, visceral fat and skin fold thickness) [305-12] have been reported in Asian groups in comparison to other racial groups. A higher proportion of Asians display a dyslipidaemic profile with high levels of triglycerides and cholesterol and low levels of HDL cholesterol.[307-12] Increased body fat correlates positively with insulin resistance, resulting in a greater risk of diabetes and other associated metabolic disorders within this group, and the likelihood of type 2 DM is commonly underestimated in the South Asian population. Body composition and an increased propensity for insulin resistance may account for how a modest increase in weight gain within Asian populations increases the risk of diabetes substantially more than comparable increases in White populations.

Increased insulin resistance in Asian women has further been conveyed by the suggestion that Asian women are more likely to have an abnormal post pregnancy glucose challenge in relation to diagnosing Type 2 DM later on in life.[313-16] McClean et al conducted a retrospective study over a 10 year period. They examined glucose tolerance test 6 weeks postpartum in a multiethnic population who had developed GDM during pregnancy. Non-European women, particularly South Asian Women, were found to have high higher rates of persistent abnormalities than European women (31.9% vs 15%, $p<0.001$).[317]

These differences have led to controversy surrounding the current WHO classification of BMI. [78] The WHO has a global classification for BMI with overweight being classified as a BMI ≥ 25. BMI classifications are supposed to be a reflection of risk for type 2 DM and cardiovascular disease. However, in view of larger proportions of Asians at greater risk of Type 2 DM and cardiovascular disease occurring at lower BMIs different cut-offs have been proposed for Asian groups. Nevertheless, the WHO recommends continuing the current global classification of BMI, as absolute prevalence and incidence of type 2 DM varies greatly within Asian sub-groups[318]- a higher prevalence being noted in Indian Asian and Fillipino subgroups.[319]

6.1.2 Asian population and the thrifty hypotheses

A suggested explanation for Asian populations displaying higher rates of GDM is the thrifty gene hypothesis. This hypothesis suggests that fetal programming plays an important role in the origin of diabetes, metabolic syndrome and cardiovascular disease. The exposure to periods of famine has resulted in selection in favour of the thrifty genotype which enhanced survival by minimising energy loss and facilitating energy storage in form of fat in ‘feast’ so as to improve survival in ‘famine’.[320] Therefore, it suggests that these individuals have a genetic tendency to rapidly deposit fat in times of plenty as this would be advantageous to

the survival of some populations and considers the likelihood to develop diabetes an adaptive trait that evolved into a pathological trait due to changes in diet and lifestyle.

This compares to the thrifty phenotype hypothesis, which suggests that rather than factors placing an individual at increased risk of DM and associated morbidities being genetic, they are programmed within the intra-uterine environment. The intra-uterine environment plays a major role in the structure and development of the fetus and is heavily influenced by maternal metabolic and nutritional status.[321-32] Therefore an adverse intra-uterine environment potentially determines the origin of diabetes, metabolic syndrome and cardiovascular disease. It has also been suggested that maybe the two hypotheses are not exclusive of each other but perhaps interlinked and the explanation involves a combination of factors.[322]

The hypotheses have not been rigorously tested and others suggest an underlying genetic predisposition to obesity resulting in a propensity to increased insulin resistance and associated disorders.[323]

6.1.3 Asians, migration and urbanisation

Although initially individuals who migrate tend to be healthier than those that do not (migration requiring the fitness, energy and enthusiasm to take on an often demanding challenge), it is also known that subsequent generations from migrant groups generally tend to suffer from worse health and display disadvantageous risk factor profiles.[323] The migration of ethnic minority groups to higher income countries and urban areas may account for the increased risk of metabolic syndrome in these groups. [324]

Urbanisation caused by the geographical growth of cities, and population migration into cities to seek work, is associated with an increased supply of foods rich in calories, sugar, fat and refined carbohydrates as well as a change in lifestyle to one that is less physically active.[325] Also dietary acculturation, the process by which a minority group adapts to the food and eating patterns of the host country, leads a the change in traditional diets to include more energy-rich components.[326] This change in diet compounds an unhealthier lifestyle and increases the risk of obesity, diabetes and cardiovascular disease. The adverse effects of the change in diet are more likely to be revealed in those individuals prone to insulin resistance.

Bodansky et al showed that the incidence of Type 1 DM increased in immigrants to the UK from 3.1/100000 per year to 11.7/100000 per year over a period of 10 years.[327] This

highlights the influence of environmental factors such as diet and acculturation within a population with an already increased genetic background risk of diabetes.

6.1.4 Black populations and diabetes

The largest migration of individuals of Black African descent from West Africa and the Caribbean into the UK was post-second world war. The increased risk of diabetes within these populations is well documented.[328-31] However, the suggested explanations for this racial disparity are less clear than those accounting for the risk of diabetes in South Asian populations. Factors involved in the risk may involve both genetic and environmental components.

Black Afro-Caribbean populations tend to have an unusual risk factor for diabetes. They are known to be more insulin resistant than their white counterparts, displaying raised levels of glucose, glycosylated haemoglobin and insulin.[332-339] However, the lipid profile is not consistent with the insulin resistant state, where typically Afro-Caribbean populations have lower triglyceride and higher HDL- cholesterol levels.[335] This more favourable lipid profile may account for the low levels of coronary heart disease mortality that Black populations demonstrate despite the increased risk of diabetes.[340]

A difference in anthropometric measurements has also been noted, with Black populations generally having higher BMIs, but unlike South Asian populations, measurement of visceral fat, which is consistent with insulin resistance, is lower in comparison to white populations. It has been suggested that it is the increased subcutaneous fat that may contribute to the diabetes risk.[341]

Population studies that have explored trends in diabetes and pregnancy outcomes within racial groups have tended to investigate Afro-Caribbean or African American groups. However, this dismisses the possible genetic admixture within these racial groups and assumes these women behave similarly metabolically. In our study, we have separated the two groups and have shown that not all subgroups within the black population are the same. One study that has explored health outcomes within Black African and Black Caribbean populations has found that Black Africans tend to have worse self-reported health outcomes than Black Caribbeans. The two groups also differ in terms of educational, economic and social dimensions, which may further influence this disparity.[342]

If studies involving Asian subgroups have highlighted that not all Asians are alike, then perhaps the same should be considered for Black populations, where in this Thesis, Black

Caribbeans tend to behave similarly to White Europeans with regards to the interaction of maternal age and BMI on rates on GDM. Modern humans originated from Africa and black Africans are known to have the highest levels of genetic variation of any geographical human population.[343] As Black Africans in Western countries have genetic origins in different parts of Africa, this genetic variation may account for the differences within Black populations. This is reflected in the variation in prevalence of type 2 DM within African regions, with the highest prevalence within sub-saharan African regions.[344]

Most studies assess the interaction between traditional risk factors as part of a mathematical model created to predict the development of GDM. Our study explicitly investigated the interrelationships of the aforementioned risk factors. There are few previous studies that explored the interrelationship between risk factors for GDM. Ramos et al[345] conducted a large retrospective cohort study evaluating the interrelationship between ethnicity and obesity on obstetric outcomes such as caesarean section and pre-eclampsia rates as well as GDM. They found that the prevalence of GDM and other pregnancy outcomes increased with increasing BMI category. The prevalence of GDM was highest in obese (BMI>29) Latino and Asian groups. The prevalence of GDM associated with obesity was lowest in White and African American groups, with the lowest prevalence in the latter group. Rodrigues et al[346], found pre-gravid weight is an independent predictor for GDM in Cree Women and non-Native women (mostly whites). However, an increase in weight increased the risk of GDM in Cree women, demonstrating an interaction between race and BMI on GDM development.

Studies, published after our study, have further examined the interrelationship between race, BMI and GDM. Similarly to our findings, Heddersen et al[347], conducted a large population study (n=123,040) exploring the relationship between risk factors for GDM and found a significant variation in prevalence of GDM, within BMI categories for different racial groups. Asian women showed an increased risk of GDM at lower BMI cut-offs points (BMI 19.0-24.9) in comparison to Non-Hispanic Whites and African Americans. However, African Americans had a generally lower prevalence of GDM at higher BMI, which contrasts with our study, which showed a high prevalence of GDM, within the Black African group with increasing BMI. Inconsistencies between studies may be due to differing diagnostic criteria for GDM, differences in geographical origin within Africa or the small numbers of Black subgroups.

There are different opinions regarding BMI cut offs for Asian racial groups when screening for GDM should be considered. In the UK, NICE[22] recommends screening for GDM on the basis of risk factors related to maternal race, BMI >30 and previous obstetric and family history. These risk factors are assumed to be independent from one another and therefore

do not accurately reflect the interaction between race, maternal age, and BMI.

The importance of BMI and the effect on pregnancy outcome within different racial groups is also highlighted. Racial disparities have been suggested to continue post pregnancy surveillance regarding the subsequent development of diabetes following a pregnancy affected by GDM, particularly in relation to non-white women. [348]

Special attention and additional health resources should be directed towards non-white racial groups, where education regarding changes in lifestyle and diet to prevent increasing obesity and subsequent adverse health and pregnancy outcomes within these groups would be particularly relevant.

6.2 There is an influence of advancing maternal age, BMI, racial group and diabetic status (GDM or not) on birth weight

Birthweight is an important predictor of infant morbidity and mortality as well as a factor that influences the future risk of obesity, diabetes and cardiovascular disease. Birthweight is influenced by both constitutional and environmental factors but the magnitude of the impact of these factors remains unclear.

This retrospective study of prospectively collected data has demonstrated racial variation in birthweight in non-GDM and GDM populations even after adjusting for maternal BMI and age. Previous studies have shown racial differences in birthweight at term with a shift towards lower birthweight in non-white racial groups.[349-354] The causes of the racial variation in birthweight remain unclear.

Previous studies have attempted to determine whether non-modifiable factors contribute more to determining birthweight than environmental factors or modifiable factors such as BMI.[355- 367] Maternal constitutional factors such as height have been shown in several studies to be important contributing factors to the racial differences and this suggests an underlying genetic influence.[358-63] Studies have predominantly focused on the relationship between race and low birthweight. However, macrosomia is also associated with adverse pregnancy and long term outcomes[364-371] and is a well established complication of pregnancies affected by GDM. Macrosomia predisposes babies to electrolyte and metabolic disturbances such as hypoglycaemia, hyperbilirubinaemia, hypomagnesia[369], as well as increased risk of birth trauma and operative delivery. In the long term there is an increased risk of obesity in childhood/ early adulthood[370] and an increased risk of cardiovascular and metabolic

disturbances.[373-86] This occurs in the absence of a maternal history of pre-existing diabetes or GDM.[376]

Large cohort studies have documented variable rates of macrosomia, ranging between 5-20%. This may be due to different definitions of macrosomia such as birthweight over 4000g, over 4500g or birthweight over the 90th percentile. [376-381] In this study we chose to use the definition of birthweight over the 90th percentile, taking into consideration the gestational age and gender of the baby to calculate birthweight z-scores. The rates of macrosomia in this study are consistent with other studies.

Predisposing factors such as advancing maternal age, parity, BMI, gestational age and weight gain during pregnancy are recognised as being positively associated with fetal macrosomia.[382-393] However, there is substantial variation in the literature regarding the magnitude of the effect of these factors on macrosomia. The influence of maternal body composition on birthweight in non-diabetic pregnancies has previously been investigated.[382-386] and a significant positive association between pre-pregnancy BMI and birthweight has been observed. [386]Jensen et al[387], found that independent of maternal glucose levels, there was an association of between obesity in women (WHO classification of BMI) and adverse pregnancy outcomes such as large-for-gestational age infants and neonates weighing over 4000g.

There is a wealth of literature confirming a variation in birthweight by racial origin [388-402] in pregnancies not complicated by GDM. The majority of studies have recognised racial disparities predominantly between White and Black populations.[403-15] Studies in the US have persistently found lower birthweight in Black in comparison to white populations as well as an increased prevalence of adverse perinatal outcomes such as pre-term delivery [396] and fetal growth restriction. Similar findings of racial variation persist in studies conducted in the UK with South Asian offspring being consistently lighter in comparison to White populations' offspring even when correcting for maternal height and weight.[397] The underlying mechanisms for these effects probably involve maternal and fetal dysregulation of glucose, insulin, lipid and amino acid metabolism. Further studies have explored racial disparities in rates of macrosomia. Stotland et al[377], examined the epidemiology of macrosomia, where rates of macrosomia remained lower within non-white groups in comparison to white groups, regardless of the definition of macrosomia (>4kg or>4.5kg). Rates of macrosomia were greater in White than Black populations. However, this study did not take into consideration other influencing maternal characteristics such as maternal BMI. Similarly, Ramos et al,[345] showed that rates of macrosomia rise with increasing maternal BMI. They did not quantify

the racial differences in rates of macrosomia within increasing BMI categories but have shown a reduced risk of macrosomia in Black obese populations compared to obese White populations.

Our study confirmed the above findings. Furthermore, in women who developed GDM, there was an accelerated growth pattern within non-white groups, with a higher rate of macrosomia in Black populations.

Studies that have also examined the influence of GDM on birthweight have recognised higher rates of macrosomia [396-400] in pregnancies complicated by GDM compared to those that remained normoglycaemic. However, there have been fewer studies that have examined the influence of GDM on birthweight within different racial groups. Those that have been published have yielded inconsistent results. Saldana et al[400] found that there was no significant effect of GDM on birthweight within either white or black racial groups. Only in black women with pregnancies complicated by impaired glucose tolerance, a degree of glucose intolerance less severe than GDM, were there significantly increased rates of macrosomia and higher birthweights in comparison to Black women who had normal glucose tolerance. This difference was not seen in the White racial group. This would suggest genetic background as an important predictor of birthweight as well as environmental factors, such as pre-existing diabetes or GDM.

Esakoff et al[401] also examined the effect of racial variation on perinatal outcomes in pregnancies in women with GDM where Latina women with GDM had the highest ORs of macrosomia (delivering neonates over 4kg and 4.5kg) in comparison to African American and Asian racial groups. African American women had a lower prevalence of delivering a macrosomic neonate in comparison to the white group, suggesting that the effect of GDM within this group was less dramatic.

Keiffer et al[402] demonstrated a racial disparity in the impact of diabetes on birthweight where the differences in the birthweights of infants of diabetic compared with non-diabetic mothers were much more dramatic for black infants. They found a larger difference in mean birthweight in infants of black mothers (211.67g) in comparison to infants of white mothers (115.75g). This study also found that pre-pregnancy BMI and diabetic status were both independently associated with birthweight. In our study, the influence of increasing BMI on birthweight is more marked in pregnancies complicated by GDM, particularly within Black populations. Although the findings are similar to those in our study, Keiffer et al did not make

a distinction between pregnancies complicated with GDM and those who had pre-existing DM.

Recent studies have recognized the importance of insulin like growth factor as an important growth factor and its role in modifying placental transfer of nutrients such as glucose and amino acids.[403] In non diabetic pregnancies it is positively associated with birthweight.[400] Racial variation may still persist and influence fetal growth. Lower levels of insulin like growth factor have been suggested as explanations for a lower birthweight in non-white racial groups, particularly Black/African racial groups, in comparison to white racial groups.[403-8]

Differences in the baby's body composition have also been suggested as an explanation for lower birthweight in Black populations in comparison to white populations with lean body mass being significantly less in Black vs white babies.[408] However, there are no studies exploring racial variation in neonatal body composition in pregnancies complicated by GDM.

In multiple regression, maternal age was not significantly associated with birthweight in pregnancies complicated with GDM within all racial groups, suggesting that BMI, racial origin and diabetic status mediate/ modulate the influence of maternal age on birthweight.

The limitations of both of the above studies (hypothesis 1 & 2) are that they were retrospective (although the data were collected prospectively) including 15 different maternity units in North West Thames and consequently there would have been some inconsistency in the screening methods and diagnosis of GDM. This may have led to an underreporting of GDM cases in some units and consequently the inclusion of some women with occult diabetes in the non-GDM group. However, such misclassification would have expected to reduce the differences between groups, and so the associations reported in the current manuscript are likely, if anything, to be an underestimate of the true differences. The use of self-reporting of racial group is also a limitation as this may not match with the individual's genes and predisposition to genetic conditions.

Other insulin resistant states and the influence of risk factors such as maternal age, Body mass index and race on outcomes.

Polycystic ovary syndrome (PCOS) is a common, heritable endocrine disorder that affects women throughout their lives. PCOS is characterized by oligo- an- ovulation, clinical and/or

biochemical signs of hyperadrogenism and polycystic ovaries. PCOS represents another insulin resistant state where the presence of insulin resistance and hyperinsulinaemia is common and ranges from 50-70%.[409] Insulin resistance occurs independent of obesity, however individuals with PCOS are believed to have an underlying genetic predisposition, where insulin resistance is further exacerbated by factors such as obesity. Women with PCOS also display an increased risk of type 2 DM and cardiovascular disease.[409]

Infertility and delayed first pregnancy affects approximately 40%[410] of women with PCOS resulting from an-ovulatory infertility. Irrespective of PCOS, pregnancy achievement and maintenance is adversely affected by obesity. Obese women have a lower chance of pregnant resulting in delayed first pregnancy. The first line management in women with PCOS and increased BMI is weight loss. A loss of 5-10% has been shown to increase ovulation and pregnancy rates.[411] The influence of BMI on fertility in PCOS is further emphasised by response to fertility treatments. There is the suggestion that women who are obese and have PCOS are less likely to respond to assisted reproductive techniques. Weight reduction in these women can increase their chance of conception spontaneously and with ovulation induction..[412]

Advancing maternal age is known to be associated with a decline in fertility. In PCOS there is a decline in hyperadrongenism and menstrual cycles become more regular with advancing age.[413] Younger age has been found to be predictive of successful pregnancy and live birth; more advanced maternal age was predictive only of ovulation. Although, older women are more likely to ovulate, the reduction in quality of the oocyte influences the ability to maintain a pregnancy and further affects a delay in first pregnancy.[414]

Less is known regarding the influence of race on PCOS, particularly the influence of race on metabolic consequences and fertility rates. Ladson et al, performed a case control study aiming to explore the metabolic and reproductive differences between white and Black women with PCOS. There was no difference between reproductive hormone levels between racial groups, determined through measurement of luteinising hormone (LH), follicle-stimulating hormone (FSH), dehydroepiandrosterone (DHEAS) and sex hormone binding globulin (SHBG). [415]

6.3 First trimester maternal biomarkers can improve early prediction of GDM

This study was a multi ethnic population study and supports the importance of maternal characteristics in the prediction of GDM in early pregnancy but also encourages the potential use of novel biomarkers in conjunction with traditional risk factors as a means of enhancing prediction.

Despite the vast documentation and number of studies surrounding GDM, including the pathology, complications, risk factors, and benefits of diagnosing and treating it, there remains no general consensus regarding screening. There is a continuing debate regarding the relative value of universal vs selective screening, where currently in the UK universal screening on the basis of risk factors for GDM is recommended. However, changing the way in which women are screened could improve detection rate and be more cost effective.

Previous studies have attempted to further improve the prediction of GDM in early pregnancy by creating 'prediction tools' based on clinical risk factors for GDM, with variable results. Naylor et al[416], pre HAPO, looked into the possible benefits of screening women for GDM with the use of a clinical scoring system. This was a model based on risk factors that were judged to be independent predictors of GDM such as maternal age, BMI and race Data was collected from approximately 3000 women. They then randomly selected data from half of the women and categorized them into three groups (low, intermediate and high risk) based on a clinical scoring system using weighted risk factors such as age, pre-pregnancy BMI and race. The remainder of women were screened with a 50-g oral glucose challenge test, with the threshold for a positive result based on their risk score. Naylor et al, found that this selective screening approach resulted in a 34.6% reduction in the number of screening tests performed, without a decrease in the detection rate of GDM.

Caliskan et al[417] also found high detection rates on the basis of selective screening, however, the number of incident cases of GDM in the study was low (n=14). The study also ignored racial origin as a risk factor for GDM, and all clinical risk factors were weighted equally.

The development of Type 2 DM risk models that could be used in pregnancy has yielded AUC-ROC values ranging between 0.74-0.85.[101] Van Leeuwen et al[418] aimed to create models to estimate the probability of GDM from maternal characteristics and achieved AUC-ROC 0.77 (95% CI 0.69-0.85) which is a similar value to the current study. However, the

number of incident GDM cases in the study by Van Leeuwen et al was considerably lower (n=22) than in the current study. Teede et el[419], generated a similar AUC-ROC value with a larger number of GDM cases (n=2880) and also took into consideration, when establishing clinical risk scores, different weighting within particular Asian subgroups.

Several studies have examined the association between novel biomarkers and the risk of Type 2 DM and transferred this association into the context of GDM, in an attempt to predict it and reduce the prevalence of its associated complications. Many of the studies have also examined the use of biomarkers in combination and not in isolation. These markers are known to share similar metabolic pathways, which may influence the findings.

Maternal hypo-adiponectinaemia during the first trimester has been previously found to be associated with increased risk of developing GDM. This association persisted even after adjustment for the confounding factor of BMI and is consistent with the current study. A lipid profile characterized by low HDL and raised triglycerides, LDL and total cholesterol levels are also consistent with previous studies examining lipid profiles in those individuals who later develop GDM and Type 2 DM. The current study highlighted the dyslipidaemic profile (raised triglycerides and LDL with low HDL) in women who subsequently developed GDM. This compares to the studies by Enqubahrie et al[172] and Montelongo et al[420] who found no association between the risk of GDM and other lipids such as total cholesterol, HDL and LDL. However, differences may be due to numbers of cases, study populations, where racial groups have been shown to exhibit different lipid profiles in pregnancies with and without GDM.

Evidence of increased inflammation in women who subsequently develop GDM was demonstrated with significantly raised CRP levels in comparison to controls and is consistent with previous studies.[211, 193] However, although the association between these traditional and novel factors is noted, many of these biomarkers were not independent predictors of GDM. Only t-PA and HDL were found to be first trimester independent predictors of GDM. This may be due to the fact that a lot of biomarkers examined have overlapping metabolic functions and therefore are not completely independent.[101]

Hyperglycaemia is a key prognostic factor in the development of vascular diseases in those patients with DM and good glycaemic control has been shown to reduce the associated vascular complications.[421]Tissue plasminogen activator (t-PA) is not only a marker of endothelial dysfunction and an independent predictor of cardiovascular disease but is also associated with inflammation and insulin resistance, which is higher in diabetics. [421-5]

Endothelial dysfunction during pregnancy is not well understood. The current study found that t-PA is significantly increased in early pregnancy in those who develop GDM in comparison to those who remain normoglycaemic during pregnancy. Other studies have found that this difference remains post partum, where women with a previous history of GDM have found to have raised t-PA levels and other markers of endothelial dysfunction, suggesting an underlying endothelial dysfunction in these women that predisposes them to GDM and diabetes in later life.[204]

The predictive ability of biomarkers in the first trimester is limited. Previous studies have either focused on one or two biomarkers in isolation and the association with GDM but have not further examined the predictive ability of these biomarkers. This is further hindered by previous studies conducted during the first trimester have yielded inconsistent results regarding the association with development of GDM, which encourages the need for larger studies.

At present, there is little clinical utility for the use of biomarkers in the prediction of GDM. However, this thesis conveys the potential of particular biomarkers and possibly provokes a need for further larger studies to explore their clinical use.

Vitamin D deficiency during pregnancy and its associated complications is an important public health problem. However, previous studies examining the association between 25(OH)D concentrations and pregnancy complications, particularly GDM have given inconsistent results.[264-8] The majority of studies that have been conducted concentrated on levels of 25(OH)D in the second and third trimesters of pregnancy. Some studies found that although levels of 25(OH)D were inversely correlated with fasting glucose and insulin but there was no significant increased risk of GDM with lower levels of 25(OH)D.[269]

Our study conducted within a multiethnic population found no difference in 25(OH)D levels measured in the first trimester in women who either developed GDM or remained normoglycaemic during pregnancy. Baker et al[264], have since conducted a nested case control study and measured 25(OH) D levels at 13 weeks gestation. There was no difference in 25(OH)D levels in those pregnancies that developed GDM and those who did not. Low vitamin D levels were not associated with an increased risk of GDM. Zhang et al[265], measured levels of 25(OH) D at 16 weeks gestation within a Chinese population and found significantly lower 25(OH)D levels in those who developed GDM in comparison to those who

remained normoglycaemic . This significance remained after adjustment for BMI. Further larger population studies are needed to explore this further.

Differences in results may be attributed to procedures used to measure 25(OH) D levels. Methods used include immunoassays, high performance liquid chromatography (HPLC) and liquid chromatography tandem-mass spectrometry (LC-MS/MS). Immunoassays are the most popular method predominantly due to their convenience and speed of results. Immunoassays have been found to generate results that vary strongly, due to different standards and artificial calibrators being used by the suppliers.[426] This then makes it difficult to compare the results generated with other studies. HPLC and LC-MS/MS has a higher sensitivity and selectivity and LC-MS/MS is currently regarded as the gold standard, as it can separate and accurately quantify both 25(OH)D_2 and 25(OH)D_3. [427-32]

Currently in the UK, it is advised that women receive information regarding their health and nutrition during pregnancy.[263] All pregnant women are advised to take daily supplementation of vitamin D (10mcg/day). It is advised that high-risk groups receive particular attention as to whether they are taking this supplement. However, the clinical significance and improvement in pregnancy outcomes with Vitamin D supplementation is yet to be determined.

The reference ranges for 25 (OH)D in pregnancy are variable and a consensus about normal ranges has not yet been reached. Yu et al [262] aimed to examine the normal range of values of 25 (OH)D within a large population with uncomplicated pregnancies. They found that first trimester maternal levels of vitamin D were influenced by maternal characteristics and the season during which the vitamin D measurements were taken; vitamin D levels were higher with advancing maternal age and during the summer months but lower with increasing BMI. The 5th percentile for 25(OH)D was recorded as 10.8 ng/ml in this study. This is much lower than the suggested cut-off of 30ng/ml to suggest a diagnosis of vitamin D deficiency.

Although vitamin D has been found to be associated with adverse pregnancy outcomes as mentioned previously, there is a lack of evidence to support supplementation during pregnancy. Lawlor et al[433] found no association between maternal vitamin D during pregnancy and bone mineral content in children, on average 10 years of age. The optimum concentration of vitamin D in pregnancy and the dose required to achieve it have not been determined. Single oral doses of vitamin D or daily oral doses during pregnancy have previously been explored. Although oral daily supplementation was thought to be a better

method of achieving this, only a third of women achieved vitamin D sufficiency.[434] Further research is needed here.

7. Conclusions

There is a significant interaction between maternal race, age and BMI on the development of GDM. Advancing maternal age and BMI are more important risk factors for GDM in South Asian and Black African women than in White European or Black Caribbean women. This is more pronounced in South Asians who develop GDM at younger ages and at lower BMI.

Maternal BMI and diabetic status are important determinants of birthweight. The influence of BMI on birthweight is more pronounced at higher BMI's and in pregnancies complicated by GDM. This is particularly so for Black women.

Several studies have examined the association between novel biomarkers and the risk of Type 2 DM and transferred this association within the context of GDM in an attempt to predict it before the onset of the disease and its associated complications. Many of the studies have also examined the use of biomarkers within groups and not in isolation. These markers are known to share similar metabolic pathways, which may influence the findings. Further research is needed to understand the role of these biomarkers in isolation to allow us to further explore their potential role in prediction of GDM as early as the first trimester. However, based on our study, it appears that HDL and t-PA can improve first trimester prediction of GDM above the traditional risk factors.

The prevalence of diabetes is continuing to increase worldwide. It is already one of the biggest health challenges in the UK and indeed worldwide at the present. This is due to the huge health and socio-economic consequences associated with the disease. The development of diabetes in pregnancy highlights a group of women at increased risk of diabetes and associated complications, in the future. It also highlights a group of children at increased risk of obesity and diabetes. Early detection will enable these groups to be targeted and also increase these individuals' chances of adopting sustainable lifestyle choices.

This study highlights the strong influence of race and genetic predisposition on an individual during pregnancy and how this affects pregnancy outcome. Further research into the interaction between risk factors is needed as this may enable us to develop more specific screening criteria in the future.

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Abbreviation	Meaning
DM	Diabetes Mellitus
GDM	Gestational Diabetes Mellitus
GIGT	Gestational Impaired Glucose tolerance
NGT	Normal glucose tolerance
SPSS	Statistical Package for Social Sciences
SMMIS	St Mary's Maternity Information System
LDL	Low Density Lipoprotein
CRP	C-reactive protein
HDL	High Density Lipoprotein
γ-GT	Gamma-glutamyl transferase
TG	Triglyceride
ALT	Alanine transaminase
25(OH)D	25 hydroxylase vitamin D
t-PA	Tissue plasminogen activator
BMI	Body Mass Index
OR	Odds Ratio

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aOR	Adjusted odds ratio
RR	Relative risk
aRR	Adjusted relative risk
CI	Confidence interval
aOR	Adjusted odds ratio
GLUT	Glucose transport proteins
HAPO	Hyperglycaemia and Adverse Pregnancy Outcome
ACHOIS	Australian Carbohydrate Intolerance Study in Pregnant Women
vs	versus
NICE	National Institute of Clinical Excellence
GTT	Glucose tolerance test
OGTT	Oral glucose tolerance test
N	Number in over study/ population
NPV	Negative predictive value
PPV	Positive predictive value
r	Correlation coefficient
Prev	Previous

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wks	Weeks
MoM	Multiples of the median
trim	Trimester
ROC	Receiver operator curve
SHBG	Sex hormone binding globulin
TNF-α	Tumour necrosis factor alpha
VDR	Vitamin D receptor
Hba1c	Glycosylated haemoglobin
GAfREC	Governance arrangements for Research and ethics committee
REC	Research and ethics committee
NICU	Neonatal intensive care unit
TOP	Termination of pregnancy
Kg	Kilograms
m	Metres
g	Grams
gm	Centimetres
GA	Gestational age

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yrs	Years
Min	Minimum
Max	Maximum
SD	Standard deviation
SPE	Solid phase extraction
Std error	Standard error
r2	Coefficient of determination
AUC-ROC	Area under the curve – receiver operator curve

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Publications

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