

Hyperglycaemia, Ethnicity and Neonatal Outcome Study

A study conducted to review the influence of ethnicity
on neonatal outcomes in pregnancies complicated with
diabetes.

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by

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1 Summary of the project

In England and Wales, about 2-5% of pregnancies are complicated with diabetes each year. Diabetes is a particular problem in the South Asian (SA) ethnic group with the prevalence of Type 2 Diabetes and GDM being about 6 times and 11 times higher respectively as compared to White British (WB) women. My PhD project was undertaken to study the influence of ethnicity and maternal hyperglycaemia during pregnancy on neonatal outcomes. This project consists of two retrospective studies and one prospective pilot study.

The first retrospective study was undertaken to compare the neonatal outcomes in WB and SA infants born to mothers with gestational or pre-gestational diabetes (Type 1 and Type 2 diabetes).

The second retrospective study was undertaken to compare the risk of morbidity and mortality between large for gestational age infants with a birthweight $\geq$ 97th centile and appropriate for gestational age infants with birthweight between 10th – 90th centile, both born to mothers without diabetes.

Maternal hyperglycaemia during pregnancy leads fetal exposure to high blood glucose levels, which in turn leads to fetal hyperinsulinism. The neonatal complications seen in infants of diabetic mothers are due to persistent fetal hyperinsulinism after birth. Currently there is no clinical or biochemical test to identify, at birth, the infants who are at risk of neonatal complications. A prospective pilot study was undertaken to evaluate the feasibility of using cord blood C-peptide (surrogate marker of insulin) to identify infants born to mothers with diabetes and LGA infants of non-diabetic mothers at risk of postnatal complications. Such a test would enable early implementation of interventions to avoid complications and at the same time free the vast majority of infants from unnecessary medicalisation of their postnatal care.

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Definitions and Glossary of terms

Body Mass Index (BMI): BMI is a simple index of weight-for-height that is commonly used to classify underweight, overweight and obesity in adults. It is defined as the weight in kilograms divided by the square of the height in metres.

Dr. Priscilla White's classification of diabetes mellitus according to age of onset, duration, vascular disease, and need for insulin

Gestational Diabetes:

Class A1 - any onset, any duration, no insulin

Class A2 - any onset, any duration, insulin

Pre-gestational Diabetes (all require insulin):

Class B - onset >20 year, duration <10 year

Class C - onset 10-19 years or duration 10-19 years

Class D - onset <10 years or duration >20 years, presence of vascular disease

Class F - any onset/duration, nephropathy

Class R - any onset/duration, retinopathy

Disposition index: This is a gold standard measure of B-cell function, which is a product of insulin secretion and insulin sensitivity(1, 2)

Euglycaemic clamp technique: The plasma insulin concentration is raised and maintained by a continuous insulin infusion while the plasma glucose concentration is held constant at basal levels by a variable glucose infusion. When a steady state is achieved the rate of glucose infusion equals glucose uptake by all the tissues in the body and is a measure of tissue insulin sensitivity.

Hyperglycaemic clamp technique: The plasma glucose concentration is acutely raised above the basal level and maintained by a continuous, variable glucose infusion to maintain hyperglycaemic plateau based on the rate of insulin secretion and glucose metabolism. As the plasma glucose concentration is maintained at a constant level, the rate of glucose infusion equals insulin secretion and glucose metabolism.

Insulin sensitivity index: Measures the ability of endogenous insulin to decrease glucose in extracellular fluids by inhibiting glucose release from the liver and stimulating the peripheral consumption of glucose.

Miscarriage: Spontaneous loss of pregnancy before 20 weeks of gestation. Most miscarriages occur before 14 weeks and a more than 50% of the fetuses have major congenital or genetic disorder.

Neonatal death: Death of a live born infant before the age of 28 days. Early neonatal death is up to 7 days. Late neonatal death is from 7 and up to 28 days.

Pre-gestational diabetes: Type 1 diabetes mellitus or Type 2 diabetes mellitus with onset at least 1 year before the woman's estimated delivery date. This excludes women with Type 2 diabetes mellitus who present for the first time in pregnancy as in these women, the diagnosis cannot be confirmed until after delivery.

Perinatal: Describes the period surrounding birth and includes the time from fetal viability from about 24 weeks of gestation up to either 7 or 28 days of life.

Perinatal Mortality: Combination of fetal deaths after 24 completed weeks of gestation and neonatal death before 7 completed days.

Pre-eclampsia: Defined as Pregnancy Induced Hypertension and proteinuria (either 1+ on urine dipstick or $\geq 0.3\text{g/L}$).

Pregnancy Induced Hypertension (PIH): Defined as a diastolic blood pressure of more than 90mmHg at rest. In case of pre-existing hypertension a rise of $\geq 15\text{mmHg}$ in diastolic blood pressure is regarded as PIH.

Stillbirth: A child that has issued forth from its mother after the 24th week of pregnancy and which did not at any time after being completely expelled from its mother breathe or show any other signs of life (Section 41 of the Births and Deaths Registration Act 1953 as amended by the Stillbirth Definition Act 1992).

Abbreviations

ACOG	American College of Obstetricians and Gynaecologists
ADD	American Association of Diabetes
AGA	Appropriate for gestational age
BMI	Body Mass Index
CASE	Clinical Audit Standards and Effectiveness
CEMACH	Confidential Enquiries into Maternal and Child Health
CI	Confidence Interval
CS	Caesarean section
DESMOND	Diabetes Education and Self Management for Ongoing and Newly Diagnosed
DIAMOND	Diabetes Mondiale Study
EURODIAB	Europe and Diabetes study
GDM	Gestational Diabetes Mellitus
HAPO	Hyperglycaemia and Adverse Pregnancy Outcome
HIE	Hypoxic Ischaemic Encephalopathy
HTA	Health Technology Assessment
IDF	International Diabetes Federation
IADPSG	International Association of Diabetes and Pregnancy Study Group
IOL	Induction of labour
LGA	Large for Gestational Age
LGH	Leicester General Hospital
LMS	Lambda-Mu-Sigma
LRI	Leicester Royal Infirmary
MODY	Maturity Onset Diabetes of the Young
NICE	National Institute of Health and Care Excellence
NICU	Neonatal Intensive Care Unit
NNU	Neonatal Unit
OGCT	Oral Glucose Challenge Test
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio

PP	Post prandial
PIH	Pregnancy Induced Hypertension
PNW	Post Natal Ward
RCOG	Royal College of Obstetricians and Gynaecologists
SA	South Asian
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TOBY	TOtal BodY hypothermia for treatment of perinatal asphyxia
TTN	Transient Tachypnoea of Newborn
UHL	University Hospitals of Leicester
WB	White British
WHO	World Health Organisation

2 CHAPTER

2.1 Definition of Diabetes Mellitus

“Diabetes mellitus” is a metabolic disorder of multiple aetiology, characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs (WHO 1999) (3).

2.2 Types of Diabetes

There are three main types of diabetes:

1. Type 1 Diabetes Mellitus (T1DM) usually develops in childhood or adolescence and patients require lifelong insulin injections for survival. This primarily occurs due to a decrease in or lack of production of insulin by the pancreas. It was previously also called insulin-dependent diabetes mellitus (IDDM) or juvenile diabetes (3-5).
2. Type 2 Diabetes Mellitus (T2DM) usually develops in adulthood and results from insulin resistance, a condition in which cells fail to use insulin appropriately in addition to varying degree of insulin deficiency. 90% of diabetic cases worldwide have T2DM and treatment may involve lifestyle changes and weight loss alone, oral medications or insulin injections. It has also been called non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes (3-5).
3. Gestational Diabetes Mellitus (GDM) is a state of hyperglycaemia, which develops during pregnancy, usually in the second trimester. It resolves after delivery and may precede development of T2DM. This is the commonest form of diabetes in pregnancy, constituting 90% of diabetic pregnancies (3-5).

Other more rare causes of diabetes include:

1. Diabetes associated with genetic conditions such as Down's Syndrome, Turner Syndrome, Prader - Willi Syndrome, Friedrich's ataxia (6).
2. Diabetes due to diseases such as cystic fibrosis and acquired processes such as pancreatitis (3).
3. Steroid diabetes induced by high doses of glucocorticoids (7) and
4. Several forms of monogenic diabetes. Monogenic diabetes occurs when there is an inherited or de novo mutation in a single gene of an individual. So far, more than 20 genes have been linked to monogenic diabetes. Some examples include:
 - a) Maturity Onset Diabetes of the Young (MODY), most commonly caused by mutations in the HNF1A gene or the GCK gene (8).
 - b) Neonatal diabetes, most commonly caused by mutations in the KCNJ11, ABCC8 or INS genes (8).

In addition to these well-defined diabetic states, intermediate states of hyperglycaemia (impaired fasting glucose or impaired glucose tolerance) have also been defined. These states are significant in that they can progress to diabetes, but with weight loss and lifestyle changes, this progression can be prevented or delayed (3).

2.3 Normal physiological and metabolic changes in pregnancy

Pregnancy is a complex metabolic state that involves dramatic alterations in the maternal hormonal milieu as well as adaptation to the increasing burden of fuel utilisation by the fetus as the pregnancy advances. Pregnancy is a physiological, diabetogenic state, which is associated with progressive carbohydrate intolerance, impaired insulin sensitivity and insulin resistance to allow transfer of nutrients to the fetus. Fasting plasma glucose begins to fall by the 8th week of pregnancy reaching a nadir by 12 weeks and this is due to increase in renal clearance of glucose in early pregnancy (9). This is followed by a rise in fasting and glucose mediated insulin secretion and an increase in insulin resistance, which has been noted as early as 10 weeks of gestation (10, 11). Insulin sensitivity progressively declines during the last 20 weeks of gestation and is reported to be 50% in pregnant women compared to non-pregnant women becoming comparable to levels seen in T2DM (12, 13). In healthy women, this pregnancy-related insulin resistance is met with a corresponding increase in insulin secretion so that plasma glucose values remain normal. This is achieved by pancreatic beta (β) cell hypertrophy and hyperplasia (14). There is a 2-3 fold increase in the fraction of the pancreas occupied by beta cells and insulin secretion increases to 2-3 times that of the non-pregnant state during late pregnancy (particularly during the 2nd trimester) (14-21). This carbohydrate intolerance and maternal insulin resistance developing in pregnancy is a beneficial physiological mechanism found in all mammalian species. Maternal hyperglycaemia promotes glucose transfer to the fetus by passive transport across the placenta and is an important component of mammalian evolution. Moderately increased fetal glucose supply leads to a small increase in lean body mass but a greater increase in fetal fat mass (22). Presence of some adiposity at birth has adaptive advantage as these metabolic reserves are essential both in the immediate postnatal period for thermogenesis and in the long term to allow survival and preservation of energy supply to the brain in case of gastrointestinal diseases or inadequate maternal care (23, 24). Thus, the placenta has evolved processes biased towards the prevention of fetal under nutrition, which was more common in the past compared to fetal over nutrition, which is rather more common now (25). The exact cause of pregnancy induced insulin resistance is

unknown. Some literature suggests that the major contributors for increased insulin resistance seem to be placental hormones such as human placental lactogen, progesterone, cortisol, growth hormone and prolactin. These hormones cause decreased phosphorylation of insulin receptor substrate-1 and thus profound insulin resistance. In addition to this many other defects such as alteration in insulin signalling pathways and reduced insulin mediated glucose transport in skeletal and fat cells have been attributed to insulin resistance (26-28). Gestational Diabetes Mellitus can therefore be envisaged as a more extreme outcome of an evolved physiological process that is normally seen in pregnancy. This increase in demand differs only slightly between normal and gestational diabetic women. However diabetic women fail to increase insulin secretion to match the demand. This diabetogenic state of pregnancy is transient and returns back to normal following delivery and this change is seen as early as 2-3 days after delivery (29).

2.4 Gestational Diabetes Mellitus

2.4.1 Definition

Gestational Diabetes Mellitus (GDM) is defined by the World Health Organisation as carbohydrate intolerance of varying severity with onset or first recognition during pregnancy (30). This definition applies whether or not there is a need for insulin and whether or not it disappears after pregnancy. It does not apply to gravid women with the pre-gestational diagnosis of T1DM or T2DM.

2.4.2 Aetiology and pathogenesis of Gestational Diabetes Mellitus

In pregnancies complicated by GDM, women undergo the above-mentioned physiological changes in carbohydrate tolerance; but maternal adaptation is insufficient to maintain normoglycaemia. Both impaired pancreatic beta cell function and higher than normal insulin resistance contributes to impaired glucose disposal leading to glucose intolerance and maternal hyperglycaemia. When insulin levels and responses are expressed relative to each individual's degree of insulin resistance, women with GDM consistently show significantly impaired beta cell

function. Both lean and obese women who are at high risk for developing GDM show a distinct decrease in their ability to secrete appropriate amounts of insulin to stimulate adequate glucose disposal and to suppress gluconeogenesis (12, 31). In addition to this, the majority of women with GDM have β -cell dysfunction that occurs on the background of chronic insulin resistance that would have been present from the preconception period.

2.4.2.1 Decrease secretion of insulin from pancreatic β -cells

Women with GDM have an inability to increase insulin secretion to compensate for and to match for the degree of increased insulin resistance that occurs during pregnancy. The extent of the defect may be influenced by maternal factors such as pre-gestational BMI, age, parity, ethnicity, gestational weight gain and a family history of diabetes, which are all potentially related to the degree of insulin resistance in the pre-pregnancy period.

The exact mechanism for the impaired pancreatic endocrine function is not known. Solomon et al in their recent randomized, crossover study in healthy controls and T2DM patients showed that insulin secretion, insulin sensitivity and disposition index was lower in diabetic patients compared to normal controls. They also showed that healthy individuals with normal glucose tolerance, when subjected to a 24-hour experimental diabetic-like hyperglycaemia, showed reduced disposition index (product of insulin sensitivity and insulin secretion) and impaired insulin secretory responses. Hence a possible explanation could be that the hyperglycaemia in women with GDM may be responsible for the impaired pancreatic endocrine function and reduced insulin secretion (32).

The first manifestation of β -cell dysfunction is the relative decrease in first-phase insulin response as observed in GDM women compared to women with normal glucose tolerance (12, 13).

Persson et al. using the frequently sampled intravenous glucose tolerance test, studied the early insulin response to glucose (EIR) and insulin sensitivity (S_i) in a

heterogeneous group of 14 pregnant women with GDM of varied severity treated either with diet alone or diet and insulin and 10 normal control pregnant women. They reported a significant reduction in EIR ($p < 0.001$) and S_i ($p < 0.01$) in women with GDM compared to controls. Fasting hyperglycaemia and the reduction in early insulin response were less marked in diet-controlled women with GDM as compared to those who were managed with diet and insulin. They showed that the severity of hyperglycaemia increased in direct relation to the impairment of insulin secretion and increased insulin resistance during pregnancy (33).

Homko et al determined pre-hepatic insulin secretion rates (ISRs) in seven pregnant women with GDM and in eight age- and weight-matched non-diabetic pregnant women during the third trimester and again postpartum. Women with GDM had significantly lower ISRs (689 vs. 849 pmol/min, $p < 0.05$) and were more insulin resistant than non-diabetic controls. Postpartum, women with GDM had similar ISR as controls however they continued to have a higher insulin resistance (34).

Akbay et al in their study using continuous glucose infusion in 21 women with diet controlled GDM i.e. women with only mild hyperglycaemia that required diet modification showed that β -cell function was reduced in women with GDM as compared to 21 normal pregnant controls without GDM. However, the difference did not reach significance due to the small number of women in the study (35).

Saisho et al in a more recent retrospective study of 277 Japanese women, have similarly shown that women with GDM have impaired insulin secretion and disposition index compared to normal glucose tolerant women, irrespective of obesity, which is the commonest reason for increased insulin resistance. The level of β -cell dysfunction in GDM was directly proportionate to the severity of glucose intolerance and the total insulin dosage required (36). Xie et al also had similar results in Chinese women (37).

Although overweight and obese women are at the greatest risk of developing GDM, this risk also exists in lean women although it might be operational through different factors. Buchanan et al and Kautzky-Willer et al showed that increase in

insulin resistance was similar between normal women and women with GDM. However GDM women (n=21) had a significant decrease in the first-phase and the second-phase of insulin response and this decrease in insulin secretion was present in both obese and lean women with GDM, although to varying degrees (12, 31).

In summary, although pregnant women with GDM secrete more insulin than in the non-pregnant state they have a decreased ability to increase insulin secretion to match the degree of increased insulin resistance that occurs during pregnancy. This leads to hyperglycaemia that requires either dietary modification or insulin treatment to normalise glycaemic control.

2.4.2.2 Insulin resistance in women with GDM

As noted above, pregnancy induces marked insulin resistance, which is most severe during the third trimester approaching the degree of resistance seen in non-pregnant individuals with T2DM (20). In women with GDM, this physiological insulin resistance may further add to undiagnosed chronic pre-gestational insulin resistance that may be present in some women. Hence pregnant women with GDM tend to have even greater insulin resistance than normal pregnant women.

Ryan et al were the first to demonstrate the mechanisms responsible for insulin resistance. They studied three groups of women, non-pregnant women with normal glucose tolerance (N= 7, mean age 32.9 ± 2.1 years), pregnant women without GDM (N = 5, mean age 24.8 ± 3.5 years) and pregnant women with GDM (N = 5, mean age 34.6 ± 2.6 years).

Figure 2.1: Comparison of glucose tolerance test in women with and without GDM (38)

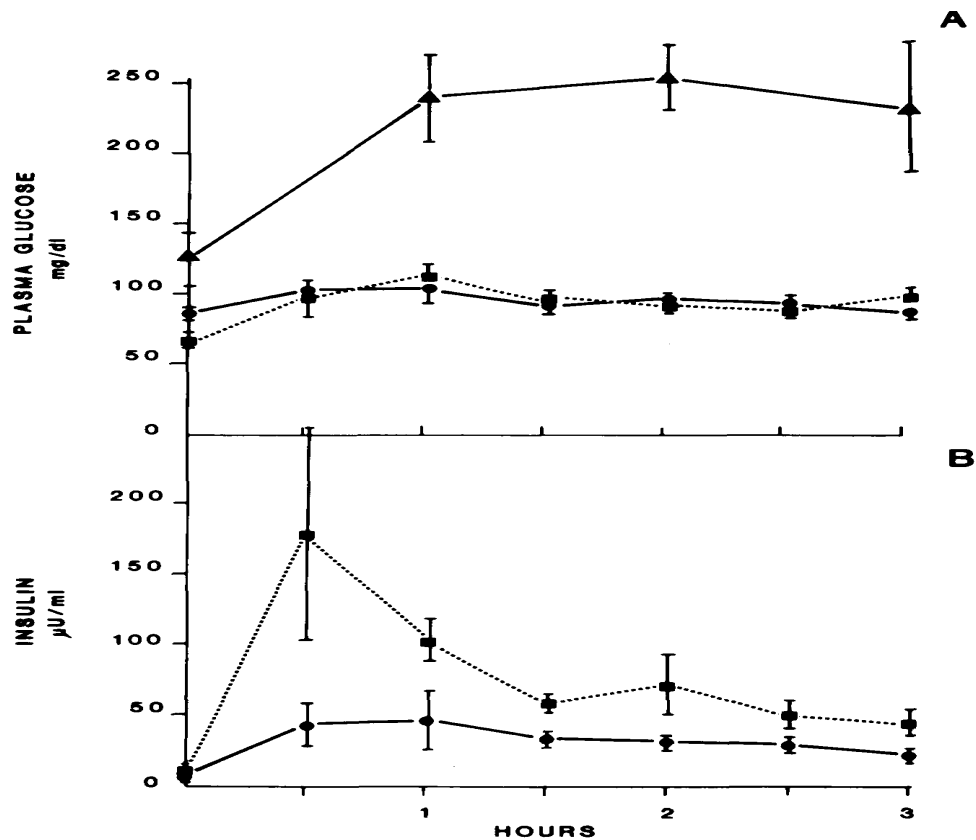


FIGURE 1. Results of oral glucose tolerance tests. (A) Glucose values are shown for groups 1 (NDNP, ●), 2 (NDP, ■), and 3 (GD, ▲). Group 3 shows clear glucose intolerance. (B) Insulin values available only for groups 1 and 2. Note that despite comparable glycemia, group 2 demonstrates higher insulin levels than group 1.

Graph A shows that non-diabetic, non-pregnant women and non-diabetic pregnant women had similar blood sugar levels while the blood sugar levels in women with GDM were markedly increased. Graph B shows that in spite of similar blood sugar levels, insulin levels were increased in non-diabetic pregnant women compared to non-diabetic, non-pregnant women suggesting a physiological increase in insulin resistance during pregnancy.

Further, by using the euglycaemic glucose clamp technique with low dose insulin infusion to attain physiological insulin concentration and high dose insulin infusion to attain pharmacological insulin concentration (insulin was infused at a constant rate of 40 mU/m² per min and 240 mU/m² per min respectively along with a simultaneous glucose infusion to maintain blood sugar levels at 4.2 mmol/L. This

gives a measure of insulin resistance which is inversely proportional to the glucose infusion rate), they showed that non-diabetic pregnant women had a 33% reduction in the glucose infusion rate ($p < 0.02$) and women with GDM had a 73% reduction in the glucose infusion rate ($p < 0.00005$) compared to non-diabetic, non-pregnant women, reflecting an increase in insulin resistance in these groups. A similar reduction in glucose infusion rates was also noted with high insulin infusion. (38).

Xiang et al compared 150 Hispanic pregnant women with GDM to 50 well-matched pregnant controls using a hyperinsulinaemic, euglycaemic clamp and a frequently sampled iv glucose tolerance test method. They showed a small but significant reduction in glucose clearance and insulin sensitivity in women with GDM. Women with GDM also had a 67% reduction in pancreatic β -cell compensation for insulin resistance and blunted suppression of glucose and free fatty acid production compared to normal controls (31, 39).

2.4.2.3 Rare causes for GDM

In GDM women, defects in β -cell function can also be due to autoimmune destruction of pancreatic β - cells, as in T1DM. This is characterized by circulating immune markers directed against pancreatic islets (anti-islet cell antibodies) or β -cell antigens (such as glutamic acid decarboxylase, GAD, or insulin autoantibodies). These patients appear to have evolving T1DM. A second cause for defective β -cell function in GDM is due to inheritance of an autosomal dominant mutation, known as maturity-onset diabetes of the young (MODY), with genetic subtypes denoted as MODY-1, MODY-2, etc.). These women will continue to be diabetic even after pregnancy, but may have slightly better diabetic control.

2.4.3 Predisposing factors for developing GDM

2.4.3.1 Maternal obesity

Maternal pre-pregnancy weight and BMI are one of the most important modifiable risk factors for the development of T2DM and GDM in pregnancy. The incidence of GDM is increasing in parallel with the global epidemic of obesity and T2DM (25). In Europe the prevalence of obesity in the adult population has increased from 10% to 40% in the last two decades (40). A greater proportion of women each year are entering pregnancy at higher weights than in the past (41-44). Several mechanisms have been postulated to explain the link between obesity and GDM. Obesity increases insulin resistance, causes insulin receptor and post receptor defects and these changes are further exacerbated by pregnancy (45). Obese patients show evidence of systemic inflammation as they have higher circulating levels of serum C-reactive protein, interleukin-6 (46, 47) and ferritin (48). These proinflammatory cytokines have been reported to be responsible for altered glucose metabolism (49). Torloni et al. conducted a systematic review including 70 studies to assess the impact of all categories of BMI on the risk of developing GDM. They reported that compared to normal weight women, the odds ratio (OR) for developing GDM was 0.75 in underweight women ($\text{BMI} < 20 \text{ kg/m}^2$), 1.97 in overweight women ($\text{BMI} 25\text{-}29.9 \text{ kg/m}^2$), 3.01 in moderately obese women ($\text{BMI} 30\text{-}34.9 \text{ kg/m}^2$) and 5.5 in morbidly obese women ($\text{BMI} > 35 \text{ kg/m}^2$). For each 1 kg/m^2 increase in BMI the prevalence of GDM increased by 0.92% (50). Maternal overweight and obesity in women with GDM is associated with adverse pregnancy (51, 52) perinatal and neonatal outcomes (53-58). Kim et al, retrospectively analysed data for 656,925 live, singleton pregnancies in Florida from 2003 to 2007. They showed that there was a continuous, dose-dependent, linear effect between maternal weight and GDM across all ethnicities (59). Maternal obesity is one of the few modifiable risk factors for GDM and hence is an important factor that would benefit from public health intervention to help decrease maternal obesity and GDM.

2.4.3.2 Maternal age

Advanced maternal age has been historically associated with increased risk for developing GDM and T2DM(60, 61). It has been shown to have an independent influence on the development of GDM after controlling for other risk factors such as maternal weight, BMI, parity and ethnicity. The risk of developing GDM with increasing age is a continuum, without a threshold age beyond which the risk significantly increases, so there is no consensus on the age cut-off for screening of women at risk of GDM. A cross sectional survey of 14,613 women with singleton pregnancies by Solomen et al in 1997, showed that the crude relative risk for developing GDM increased by 4% (95% CI 2-6%) with each year over 25(62). Lao et al conducted a retrospective review of 15,827 singleton pregnancies, to determine the age threshold for increased risk of GDM. After controlling for various confounding factors, the risk for the older women was as follows OR (95% CI): 25-29 years, 2.59 (1.84-3.67); 30-34 years, 4.38 (3.13-6.13); 35-39 years, 10.85 (7.72-15.25); and > 40 years, 15.90 (10.62-23.80) as compared to women less than 25years old(63). This supports the recommendation by the American Association of Diabetes (ADA) to class pregnant women above 25 years as high risk for developing GDM(64). Maternal age has not been included as one of the risk factors in the risk factor based selective screening for GDM as currently recommended by NICE (65). This is because over recent years, worldwide there has been an increase in maternal age at the time of first and subsequent pregnancies. Including age as a risk factor would mean offering a diagnostic test to a high proportion of pregnant women resulting in a low yield of cases. This would result in utilisation of a significant proportion of economic and health resource without proven long-term clinical benefit and would also result in a significant psychological stain on these women who are otherwise at a low risk of adverse pregnancy outcomes (66).

2.4.3.3 Maternal ethnicity

Certain ethnic groups are at significantly higher risk of developing GDM and T2DM. A systematic review by Health Technology Assessment, which included 135 studies, reported that the South Asian (Indian, Pakistani, Bangladeshi) and black Caribbean ethnic groups were at highest risk of developing GDM (66). A UK based study, which was included in the systematic review, reported that women from ethnic minority groups were more likely to develop GDM as compared to Caucasian women (55.4% versus 15.3% $p < 0.0001$). In the U.S., Native Americans, Asians, Hispanics, and African-American women are at higher risk for GDM than non-Hispanic white women (67-70). In Australia, GDM prevalence was found to be higher in women whose country of birth was China or India than in women whose country of birth was Europe or Northern Africa. In Europe, GDM has been found to be more common among Asian women than among European women. A prospective study conducted in Southern India with a universal screening programme in urban, semi-urban and rural areas detected a prevalence rate of 17.8%, 13.8% and 9.9% respectively. This reported prevalence in urban areas is similar to the reported prevalence rate of GDM in-migrant South Asian population in developed nations (71, 72). The WHO Ad Hoc Diabetes Reporting Group, in a study on the prevalence of diabetes in a diverse population of women aged of 20-39 years, found that the prevalence of diabetes was lowest amongst rural indigenous groups and highest amongst urban migrant groups(73). It is beyond doubt that certain ethnic groups are at a higher risk of developing GDM and hence the current NICE guideline includes ethnicity as a risk factor for GDM screening.

2.4.3.4 Family history

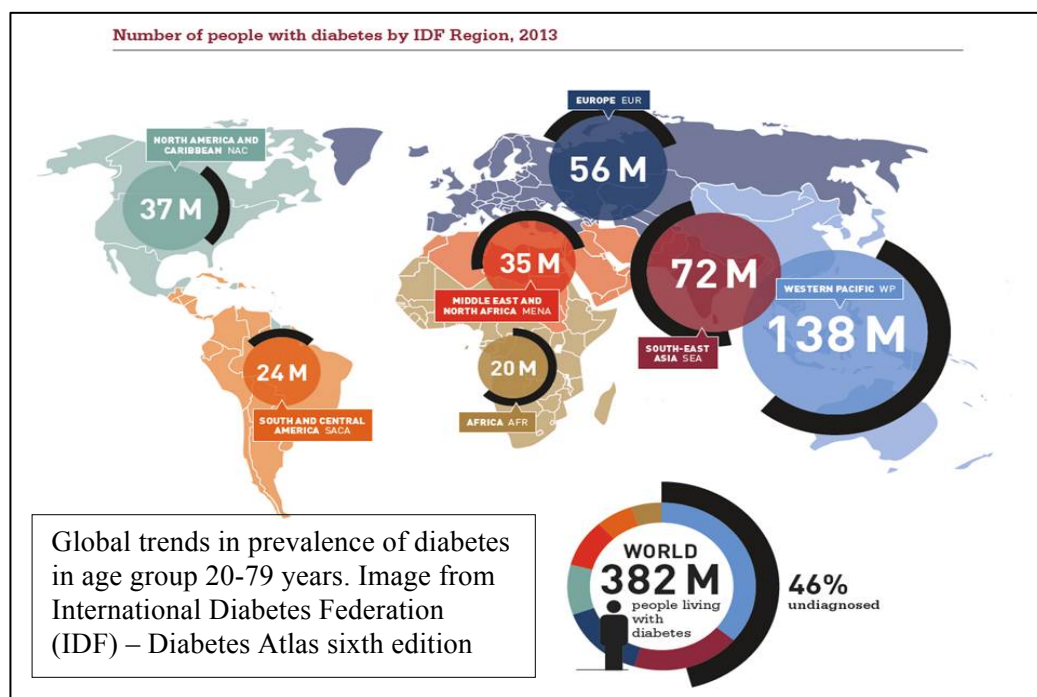
A positive family history of T2DM in a first or second degree relative has long been known as a risk factor for GDM. Both genetic and epigenetic (environmental) factors are likely to play a role. In 1926, in his report of six patients treated with insulin, Lambie was the first to draw attention to the close association between family history of diabetes and GDM (74). Since those early days in the history of

diabetes in pregnancy, a positive family history of diabetes was considered as a significant risk factor. The fourth International Workshop on GDM concluded a positive family history of diabetes would classify a pregnant woman into a high-risk group for GDM. The HTA systematic review of 135 studies also found family history as a risk factor for GDM(66). Positive family history of diabetes is included in the risk factor based selective screening recommended by NICE(65).

2.5 Global prevalence of diabetes

The overall prevalence of diabetes is increasing globally. The Diabetes Population Prevalence Model gave prevalence estimates of 0.3% in people aged below 30 years and 3.4% in people aged between 30 and 60 years old(75). The Diabetes Mondiale Study (DIAMOND)(76)the Europe and Diabetes study (EURODIAB)(77)and the SEARCH for Diabetes in the Youth Study(78)have been instrumental in monitoring trends in the incidence of T1DM. T1DM is increasing in children, mainly in those under the age of five with an estimated annual increase of 3% and there is a strong indication of geographical variation in trends of T1DM. T2DM is increasing in all age groups, including children and young people, but predominantly among the Black, Asian and other ethnic minority groups(79). The International Diabetes Federation (IDF), which is an umbrella organization of over 230 national diabetes associations in 170 countries and territories, in their sixth diabetes atlas, reported the prevalence of diabetes in adults (20 - 79 years) in the various IDF regions.

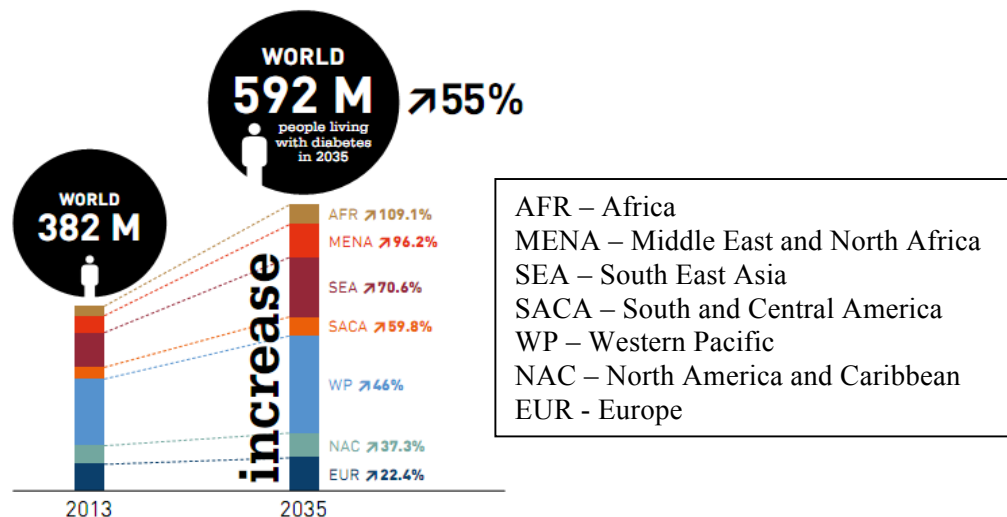
Figure 2.2: Prevalence of diabetes in various regions across the globe (80).



The overwhelming burden of the disease is disproportionately distributed amongst low- and middle-income countries from where four out of five cases of diabetes originate(80). This disproportionate increase in the prevalence of diabetes in low and middle income countries is attributed to environmental factors due to the nutritional transition from traditional dietary practices to more industrial and urban diets and more sedentary lifestyle, both of which lead to an increase in obesity(80). Hence indigenous populations to these nations and migrant populations belonging to ethnic groups representing these nations have the highest risk of diabetes(81). For example indigenous South Asians from the Southern Asian continent contribute about 20% to the global burden of diabetes and similarly South Asians in other nations also have an increased prevalence of diabetes (80, 82, 83).

If current demographic patterns continue, more than 592 million people will be affected with diabetes within a generation. This figure takes into account changes in the population and patterns of urbanisation and is almost certainly an underestimate (80).

Figure 2.3: Projection of number of people with diabetes by 2035 (80).



2.6 Global prevalence of diabetes in pregnancy

With the increase in the background prevalence of T1DM and T2DM and advancing maternal age at the time of first pregnancy, more and more women are entering pregnancy with a diagnosis of pre-gestational diabetes. In addition to this more and more women are developing GDM in pregnancy. In 2013 more than 21million livebirths (17%) worldwide were affected by diabetes in pregnancy (80).

Table 2:1: Pregnancies complicated by diabetes in 2013 (80).

Total live births in women aged 20 – 49 years (millions)	127.1
Diabetes in pregnancy in women (20 – 49 years)	
Global prevalence (% of total live births)	16.9
Number of live births following hyperglycaemia in pregnancy, millions	21.4

Table 2:2: Hyperglycaemia in pregnancy (20-49 years) by IDF region 2013 (80).

International Diabetes Federation Region	Live births affected by hyperglycaemia during pregnancy (millions)	Prevalence (% of total live births)
South East Asia	6.3	25.0
Middle East and North Africa	3.4	17.5
Africa	4.6	14.47
Europe	1.7	12.6
Western Pacific	3.7	11.9
South And Central America	0.9	11.4
North America and Caribbean	0.9	10.4

2.6.1 Prevalence of T1DM and T2DM in pregnancy

The Confidential Enquiry into Maternal and Child Health (CEMACH) in 2002 provided information about the prevalence of T1DM and T2DM in pregnant women in England, Wales and Northern Ireland. The prevalence of diagnosed T1DM and T2DM in women of childbearing age group was estimated at 0.68% and 0.36% respectively(84, 85). Another study, based on the Northern Diabetic Pregnancy Survey reported an increase in T1DM from 0.29% in 1996 - 1998 to 0.35% in 2002 - 2004 ($p=0.024$) and an increase in T2DM from 0.02% in 1996 - 1998 to 0.12% in 2002 - 2004 ($p<0.0001$) (86) . There was a 50% rise in T1DM and T2DM (pre-gestational diabetes) in less than a decade and the increase was predominantly due to a six-fold increase in T2DM. Data from Northern, North-western and East Anglian audits showed a 58% increase in T2DM with the absolute prevalence rising from 26 % in 2002–2003 to 40 % in 2007–2008 of the total pregnancies complicated with pre-gestational diabetes(87). A report from New South Wales, Australia similarly showed an increase in the prevalence rate of pre-gestational diabetes from 0.3% in 1998 to 0.4% in 2002 (88). A more recent study from Saudi Arabia in 2012 reported a significantly high prevalence of 3.7% for pre-existing diabetes in pregnant women and this represents a fivefold increase in last 14 years. This rise might be partly explained by improved screening and detection however the main rise was attributed by the authors to the increase in the background prevalence of T2DM in the population which ranges from 21 - 23%(89). Lawrence et al in a retrospective review of 209,287 singleton deliveries at Kaiser Permanente hospitals in southern California identified an increase in the race/ethnicity adjusted prevalence of pre-existing diabetes from 0.81% in 1999 to 1.82% in 2005 ($p<0.001$) amounting to 112% increase in the prevalence of pre-existing diabetes (90). The above-mentioned studies are from developed nations, which have established data collecting and reporting systems. Developing nations will have experienced a similar if not larger increase in the prevalence of pre-gestational diabetes.

2.6.2 Prevalence of GDM in pregnancy

There is a wide variation in the reported prevalence of GDM between countries due to the heterogeneity in the definition of GDM, screening practices (universal vs. selective), diagnostic methods and blood sugar thresholds used for the diagnosis of GDM. These limitations make comparison of the prevalence of GDM between countries very difficult and somewhat meaningless. In spite of the above-mentioned difficulties in assessing the prevalence of GDM, a few studies are reviewed providing information on the trends for GDM prevalence across the globe.

The current prevalence of GDM in the UK is 2-5%. However prevalence as high as 11% has been reported in the Indian ethnic group (65, 72).

Dabelea et al undertook a retrospective review of 36,403 singleton pregnancies at Kaiser Permanente of Colorado, to compare the prevalence of GDM diagnosed by universal screening over three time periods from 1994 to 2002 in four different ethnic categories (non-Hispanic Whites, Hispanics, African Americans and Asians) (91). The overall prevalence of GDM increased from 2.1% in 1994 to 4.1% in 2002. There was a 12%/year rise in GDM ($p < 0.0001$). This rise in GDM paralleled the reported rise in T2DM, which increased from 3.4% to 5.1%, and reported rise in obesity, which doubled in the same time period (92). Studies from other regions in the US have reported a similar rise in GDM prevalence (82, 93, 94). The current reported prevalence of GDM in the US ranges from 1-14% with the commonest rate being 2-5% (62, 67, 68, 70, 82, 91, 93, 95, 96).

Chamberlain et al similarly in their retrospective study in eight Australian jurisdictions reported an increase in the crude GDM prevalence among indigenous women from 4.74% (95% CI, 4.47–5.01) in 1990–1999 to 5.10% (4.96–5.24) in 2000–2009 compared to an increase from 3.06% (3.03–3.10) to 4.54% (4.51–4.56), among non-indigenous women (81). GDM prevalence increased significantly in indigenous women by an average of 2.6% annually and in non-indigenous women by an average of 3.2% annually.

2.7 Complications of diabetes in pregnancy

T1DM, T2DM and GDM during pregnancy are associated with adverse maternal and neonatal outcomes. In T1DM and T2DM there is a risk of fetal exposure to hyperglycaemic intrauterine environment right from the onset of conception and hence fetuses of mothers with pre-gestational diabetes mellitus are at higher risk of adverse outcomes especially major congenital anomalies and increased perinatal mortality as compared to GDM where hyperglycaemia has its onset in the mid or towards the end of second trimester when fetal organogenesis would have been completed long before. With increasing prevalence of T2DM, more and more women in the reproductive age group have a pre-conceptional diagnosis of T2DM. Although women with T2DM have more obstetric risk factors in that they are older, more overweight and obese, of higher parity and more likely to take potentially harmful medications at conception (for treatment of hypertension and/or hyperlipidemia), they have a less severe glycemic disturbance and a shorter duration of diabetes as compared to women with T1DM. They are less likely to seek advice for safe planning of pregnancy or receive pre-conceptional counseling (97). Although at first, T2DM was perceived as a more benign condition than T1DM, potentially modifiable by diet and lifestyle changes, there is now increasing evidence that serious adverse perinatal outcomes in T2DM are similar to T1DM (98, 99). The maternal and fetal complications that occur beyond the second trimester are similar in all types of diabetes and depend on the severity of hyperglycaemia and its control.

2.7.1 Short term maternal complications

2.7.1.1 Miscarriage

Pre-gestational diabetes increases the risk of spontaneous miscarriage secondary to congenital anomalies as hyperglycaemia in the first trimester is teratogenic. Jovanovic et al reported that the risk of spontaneous abortion was 12.4% with first-trimester HbA1C < 9.3% and 37.5% with HbA1C > 14.4% (risk ratio 3.0; 95% confidence interval 1.3–7.0). There was a similar rise in congenital anomalies with increasing HbA1C (100).

2.7.1.2 Congenital malformation

Women with T1DM diabetes have 2 – 8 fold increase in the incidence of congenital malformation(101-105). This association is secondary to the teratogenic effect of abnormally raised blood glucose, which impacts most organ systems and has its effect before the 7th week of gestation(106, 107). It is suggested that maternal ketosis secondary to poor glycaemic control also plays a role in the occurrence of congenital anomalies(108). Almost any organ can be affected due to maternal diabetes but the commonest are cardiac defects followed by nervous and genitourinary system abnormalities, all of which occur 3 – 4 times more frequently than in the general population(109). Limb and spinal defects are less common but are more specifically associated with diabetes(110). In women with T2DM the pattern and prevalence of congenital anomalies is similar to women with T1DM indicating the same underlying metabolic pathogenesis(111, 112). Appropriate pre-conceptional care, guidance to achieve better glycaemic control and commencement of folic acid well before pregnancy has shown to decrease pregnancy loss due to major congenital anomalies in women with T1DM and T2DM(113-117).

2.7.1.3 Pregnancy Induced Hypertension (PIH) and Pre-eclampsia

PIH and pre-eclampsia are common complications in women with pre-gestational and gestational diabetes with rates 2 - 4 times higher than the general population (118-121). The exact mechanism of action for the development of hypertension in women with diabetes is not known. It is postulated that insulin modulates blood pressure through several pathways, including stimulation of sympathetic neural activity, direct vasculopathic actions, changes in cellular ion flux and by promotion of sodium retention (122). Hyperinsulinism even with mild glucose intolerance has been linked to hypertensive disorders in pregnancy (123). Duration and severity of diabetes, presence of hypertension and end organ damage especially nephropathy and retinopathy increases pre-eclampsia risk(124-129). A large Swedish population based study covering >98% of pregnancies similarly showed that T1DM women were at significant higher risk of preeclampsia OR 4.47 (95% CI, 3.77 – 5.31) (130) as compared to women without diabetes. ATLANTIC DIP, an Irish prospective study also reported a three times higher risk of preeclampsia in women with T1DM as compared to general population(131, 132). Cundy et al in their study of 100 women with T1DM and T2DM each, showed that the overall incidence of PIH was similar in T1DM and T2DM (41% vs. 45%), but women with T2DM had more chronic hypertension while women with T1DM had higher pre-eclampsia and a higher associated risk of adverse outcomes (133). Better glycaemic control may prevent or at least decrease the severity of hypertensive disorders in pregnancy(119, 126).

2.7.1.4 Perinatal mortality

Maternal hyperglycaemia and subsequent fetal hyperglycaemia is responsible for the generation of oxygen radicals, which are linked to mechanisms of cellular damage(107, 134-137). A high incidence of congenital malformations in fetuses of diabetic mothers contributes to high perinatal mortality in women with diabetes and this relationship is directly proportionate to glycaemic control in the peri-conceptional period and during pregnancy(111). A series of studies have also demonstrated that fetal hyperinsulinaemia is also associated with cord blood

acidaemia and hypoxemia, which is related to increased rates of stillbirth and neonatal deaths seen in diabetic mothers along with other complications like birth trauma and neonatal hypoglycaemia(138-140). In a review study of perinatal mortality in diabetic women in the last decade, Cundy reported that women with T1DM and T2DM have 2 – 6 fold higher perinatal mortality rates than the general population (141). In T1DM increased perinatal mortality was secondary to major congenital anomalies or prematurity while in T2DM it was due to stillbirths, chorioamnionitis and hypoxic birth injury(142). A similar high prevalence of perinatal mortality was also noted by CEMACH(104, 105). There was an improvement in perinatal mortality from 1960 to 1980, following the discovery of insulin. However since then even though there has been an improvement in perinatal mortality in the general population the relative risk for perinatal mortality associated with diabetes has remained unchanged(143).

2.7.1.5 Caesarean section

Women with both pre-gestational and gestational diabetes have an increased risk of preterm and term caesarean section (CS) and preterm induction of labour. A Canadian retrospective case-control study involving 776500 women reported women with pre-gestational diabetes had a significantly higher rate of caesarean section and induction of labour 1.78 (95% CI 1.60 – 1.98) as compared to women without diabetes(144). Another retrospective study from the US, reported that the risk of being delivered by caesarean section increased by OR (95% CI) 1.78 (1.21 – 2.59) in diet-controlled GDM, 3.17 (1.80 – 5.55) in insulin controlled GDM to 4.51 (2.81 – 7.22) in women with pre-gestational diabetes as compared to the control women(145). Another study showed that women with treated GDM had higher caesarean section rates even though they had lower rates of macrosomia of 10.5% vs. 28.7% in the untreated GDM group and 13.7% in the women without diabetes. This suggests higher caesarean section in women with GDM may be iatrogenic due to lower clinician threshold to ensure safe delivery in these women (146). The CEMACH enquiry in the UK also reported higher rates of obstetric intervention in women with pre-existing diabetes 39% vs. 21% in the general population(147).

2.7.1.6 Diabetic nephropathy

The presence of nephropathy has a significant impact on pregnancy outcome for three reasons (i) Increased risk of maternal hypertension and its complications(148), (ii) Increased risk of preterm delivery due to worsening pre-eclampsia and growth restriction(149)and (iii) Increased risk of fetal growth restriction and associated fetal distress(150, 151). The rate of pre-eclampsia in women with diabetic nephropathy is 53 – 64%(149, 152-154) especially in the presence of reduced renal function, presence of hypertension at the onset of pregnancy(148)or in the presence nephrotic proteinuria(153, 154). With better control of hypertensive complications in women with diabetic nephropathy, the rate of perinatal mortality is similar in women with T1DM with and without nephropathy(122).

2.7.1.7 Diabetic retinopathy

About 20 – 30% of women with diabetes in the reproductive age have some evidence of retinopathy. In some women, pregnancy can accelerate the progression of retinopathy and this depends on the severity of diabetic retinopathy before conception(155-158), duration of diabetes(156-160), glycaemic control (155-158, 161-163)and co-existent hypertension(156, 158, 164). The CEMACH enquiry reported that women with T1DM were more likely to have retinopathy as compared to T2DM (36% vs. 9%, $p < 0.001$) and they were more like to have progression of retinopathy during pregnancy (18% vs. 11%). However women with T1DM were more likely to receive retinopathy screening as compared to T2DM and hence this report might have underestimated retinopathy in T2DM (147). Women with GDM usually do not develop retinopathy as their hyperglycaemia occurs mainly in the pregnancy and is of shorter duration.

2.7.2 Short term neonatal complications

2.7.2.1 Macrosomia

This is the commonest complication seen in the infants of diabetic mothers and is due to excessive fetal growth. The definition of macrosomia varies with some defining it as a birthweight more than 4000grams or 4500grams. This term is used interchangeably with large for gestational age (LGA), which is defined as birthweight more than 90th centile, or 97th centile for gestation and sex. The Pedersen's hypothesis, which was suggested more than sixty years ago, links fetal overgrowth/macrosomia to the transplacental passage of excessive maternal glucose, which leads to fetal hyperglycaemia and excessive fetal insulin release (165). Several studies have since supported this hypothesis (166, 167). The recent HAPO study has shown a positive linear association between maternal hyperglycaemia, fetal hyperinsulinism and birthweight (168). They also showed a linear relationship between maternal hyperglycaemia, fetal insulin and neonatal body fat (see section 4.2.7.2). Treatment of maternal diabetes and strict glycaemic control during pregnancy significantly reduces the risk of macrosomia. More recently, other mechanisms of fetal overgrowth have also been reported, including excessive transplacental passage of lipids and amino acids.

2.7.2.2 Preterm birth

Infants of mothers with diabetes in pregnancy are at increased risk of spontaneous or iatrogenic preterm birth due to fetal macrosomia or maternal complications such as poor glycaemic control, hypertension and fetal growth restriction(120, 124, 169-171). Sibai et al reported in a prospective observational study that the risk of both spontaneous and indicated preterm (<37 weeks gestation) delivery in diabetic mothers was 38% vs. 13.9% in women without diabetes(172). Similarly, a recent retrospective study from Austria reported that diabetes in pregnancy independently increased the risk of spontaneous preterm delivery after controlling for various confounding covariates, $p = 0.002$ (173). CEMACH reported a neonatal death rate of 4.1% in the infants of diabetic mothers as compared to 1.2% in the general

population, which was attributed to prematurity following spontaneous preterm birth(147). However, some other studies have reported no relationship between maternal diabetes and preterm delivery(174, 175). Hence professional opinion regarding the risk of spontaneous preterm delivery in mothers with diabetes remains divided.

2.7.2.3 Birth trauma

Infants of diabetic mothers are at an increased risk of birth trauma such as shoulder dystocia, clavicular fracture, brachial plexus injury (Erb's palsy) and hypoxic ischaemic encephalopathy mainly due to pathological overgrowth, which results in cephalo-pelvic disproportion thereby increasing the risk of obstructed labour. Infants of diabetic mothers have a 2 – 4 fold increase in the risk of shoulder dystocia compared to the same birthweight infants born to non-diabetic mothers (176, 177). Brachial plexus injury is the most important complications of shoulder dystocia and is seen in 0.2 - 3% of newborns of diabetic mothers(178). However most of them resolve with fewer than 10% resulting in permanent neurological damage(179, 180). A decision-analysis model estimated that in diabetic women with an estimated fetal weight of more than 4500grams, 443 caesarean sections would be needed to prevent one permanent brachial plexus injury compared to 3695 caesarean sections in non-diabetic population. Hence, NICE recommends that during antenatal fetal well-being assessment, estimation of fetal weight (although not very accurate) should be obtained and plans for either early induction of labour or elective caesarean section should be made(181). Shoulder dystocia in the most severe form can result in hypoxic ischaemic encephalopathy and is associated with an increased risk of perinatal mortality or long-term adverse neurodevelopmental outcome.

2.7.2.4 Neonatal hypoglycaemia

The complication of neonatal hypoglycaemia seen in the infants of diabetic mothers is due to fetal hyperinsulinism (Pedersen's hypothesis)(137). Maternal hyperglycaemia leads to fetal hyperglycaemia, which in turn results in fetal hyperinsulinism due to pancreatic β -cell hypertrophy and hyperplasia. Fetal hyperinsulinism during the antenatal period results in fetal macrosomia and its persistence after birth results in neonatal hypoglycaemia. The pathophysiology of neonatal hypoglycaemia is explained in detail in section 4.2.7.

2.7.2.5 Neonatal respiratory distress

Delayed lung maturity is a known complication of pregnancies complicated with diabetes. The exact pathophysiology of this complication is not known but it is postulated that both hyperglycaemia and hyperinsulinism play a role in decreasing pulmonary surfactant secretion by either lowering the substrate availability for surfactant production(182)or by hampering the glucocorticoid-induced lung maturity(183). Becquet et al in a large retrospective review of 18,095 singleton infants born beyond 33 weeks of gestation without congenital/chromosomal anomaly reported that the treatment of maternal diabetes with insulin during pregnancy (indicating more severe diabetes) independently increased the risk of respiratory distress in neonates after controlling for confounding factors like gestational age and mode of delivery, RR 1.44 (1.00 – 2.08)(184). Similarly, a study by Vignoles et al reported that gestational diabetes was an independent risk factor for respiratory distress in infants born beyond 34 weeks of gestation OR (95% CI) 11.5 (3.9 – 33.9), $p < 0.001$ after controlling for variables like gestational age, caesarean section and fetal growth retardation(185). The commonest reasons for respiratory distress in infants of diabetic mothers are transient tachypnoea of newborn respiratory distress syndrome, pneumonia and persistent pulmonary hypertension.

2.7.2.6 Neonatal polycythaemia and hyperbilirubinaemia

Studies in animal models have shown that polycythaemia in the offspring of diabetic mothers is associated with a significant reduction in arterial oxygen content resulting in chronic fetal hypoxia and a reciprocal increase in plasma erythropoietin (186, 187). High levels of cord blood erythropoietin were found in infants of diabetic mothers after intrauterine fetal demise(188). Shannon et al reported similar findings in infants of diabetic mothers(189). Green and Nelson demonstrated that neonatal haematocrit co-related well with maternal glycaemic control and HbA1c at the time of delivery(190, 191). Polycythaemia results in hyperbilirubinaemia due to the break down of excess haemoglobin. In addition to this, bruising, haematomas and birth trauma seen more commonly in macrosomic infants of diabetic mothers contributes to the increased incidence of hyperbilirubinaemia. Peevy et al demonstrated that LGA infants of diabetic mothers had a significantly elevated serum bilirubin (12.3mg/dl) compared to appropriate for gestational age infants of diabetic mothers (7.6mg/dl) and infants of non-diabetic mothers (7.8mg/dl) (192) . Hyperbilirubinaemia is one the most common reasons for prolonged hospital stay after birth and for re-admission in infants of diabetic mothers.

2.7.2.7 Neonatal hypocalcaemia and hypomagnesaemia

Maternal glycosuria-induced renal loss of magnesium results in maternal hypomagnesaemia and consequent fetal hypomagnesaemia(193). Fetal hypomagnesaemia is thought to be responsible for fetal functional hypoparathyroidism, which results in significantly lower levels of parathyroid hormones in infants of diabetic mothers as compared to control infants (born to mothers without diabetes) in the first four days of life leading to hypocalcaemia(194). Hypocalcaemia and hypomagnesaemia is reported to occur in about 25 – 30% of infants of diabetic mothers and it is directly related to maternal glycaemic control during pregnancy(195). The incidence of neonatal hypocalcaemia is decreasing with better glycaemic control in mothers during pregnancy(196).

2.7.3 Long term neonatal complications of diabetes in pregnancy

Many studies have provided evidence for the hypothesis that the developmental origins of many adult diseases lie in the fetal period. These relationships are due to fetal programming from altered nutritional stimuli, which results in epigenetic modification of gene expression. This phenomenon of fetal programming was first explained by Barker's hypothesis(197). A large number of epidemiological studies have demonstrated that infants of diabetic mothers are susceptible to complex adult diseases like obesity(198), T2DM(199), metabolic and cardiovascular complications(200, 201)and even cancer(202). Even macrosomic infants born to obese mothers without the diagnosis of GDM are at risk of chronic adult diseases like obesity, T2DM, and cardiovascular diseases(203, 204). Studies of siblings born to mothers before and after diabetes confirm that these later life phenotypic changes are related to intrauterine exposure to hyperglycaemia(205).

2.8 Conclusion

It is already well established that infants born to mothers with T1DM, T2DM and GDM have increased risks of adverse neonatal outcomes. Hence the currently recommended clinical care pathway for infants of diabetic mothers in the UK was widely implemented following the Confidential Enquiry into Maternal and Child Health's report on pregnancy in women with T1DM and T2DM. What has not been previously established is if this risk of adverse neonatal outcomes varies in the different ethnic groups to such an extent to justify a different care pathway, which would be more appropriate to their needs. The SA ethnicity is of particular interest as they have an eleven times higher risk of developing GDM and a six times higher risk of developing T2DM as compared to the WB ethnic group. The NICE guideline on diabetes in pregnancy has identified the need for further research focusing on the impact of diabetes during pregnancy on the management and outcome of the newborn infants as a priority. Similarly the 5th international conference on GDM highlighted that there was a lack of information about neonatal outcomes from the various ethnic groups, which is needed to guide clinical care. Diabetes UK and the South Asian Health Foundation have also identified that

research related to maternal hyperglycaemia and neonatal outcomes in the SA women is a priority. Hence a cohort study to compare the neonatal outcomes between the infants of SA and WB mothers with diabetes during pregnancy was undertaken to establish if the SA infants of mothers with diabetes had a significantly lower rate of adverse outcome to justify a different care pathway. As the SA ethnic group is the second largest ethnic group (30%) in Leicester where the study was conducted, comparisons were made between the SA and the WB infants. At the time of study design, it was decided that the neonatal outcomes in the other ethnic minority groups would not be compared as the numbers in these would be small and at the time that the study was designed there was no evidence of any ethnic differences in neonatal outcome to justify a more extensive comparison. It was decided that if the current study showed statistically and clinically significant ethnic differences in the neonatal outcomes, then future studies including other ethnic groups would be important.

2.9 Study design and methodology

This was a retrospective study to review the neonatal morbidity and mortality of South Asian (SA) neonates born to mothers with known diabetes in pregnancy (T1DM, T2DM and GDM) vs. White British (WB) neonates born to mothers with known diabetes (T1DM, T2D and GDM). It was a population based, retrospective, cohort study.

2.9.1 Research question

Is the rate of neonatal morbidity and mortality in infants born to SA mothers with diabetes in pregnancy significantly lower than that seen in infants of WB mothers with diabetes in pregnancy and of sufficient clinical difference to merit a different care pathway?

2.9.2 Study population

University Hospitals of Leicester NHS Trust (UHL) has deliveries on two sites: Leicester General Hospital and Leicester Royal Infirmary. Annually there are about 11,000 deliveries in total at both sites. They share a common maternity database and the diabetes specialist midwife at each site maintains a database of pregnant women with diabetes in pregnancy (T1DM, T2DM and GDM). Ethnicity recorded in the maternity and the diabetes database was based on self-report as registered in the maternal notes. From these data sources 160 infants born from 1st January 2009 to 31st December 2010 to South Asian mothers with diabetes in pregnancy (T1DM, T2DM and GDM) were identified and recruited to the study. For every selected SA infant of a diabetic mother, the first available WB infant in the diabetes database born to mother with the same type of diabetes as the SA mother was selected. There were no exclusion criteria.

2.9.2.1 Identification of the diabetic women

2.9.2.2 Diagnosis of women with pre-gestational diabetes

Most women with established T1DM and T2DM were diagnosed before pregnancy and were already known at the start of pregnancy.

2.9.2.3 Diagnosis of women with GDM

At UHL, risk factor based selective screening as recommended by NICE guidelines is used for the diagnosis of GDM.

Risk factors:

1. Body mass index above 30 kg/m.
2. Previous macrosomic baby weighing 4.5 kg or above
3. Previous gestational diabetes
4. Family history of diabetes (first-degree relative with diabetes)
5. Ethnic origin with a high prevalence of diabetes:
 - South Asian (India, Pakistan or Bangladesh)
 - Black Caribbean
 - Middle Eastern (Saudi Arabia, UAE, Iraq, Jordan, Syria, Oman, Qatar, Kuwait, Lebanon or Egypt).

Pregnant women with a history of GDM in a previous pregnancy are offered a 75gram Oral Glucose Tolerance Test (OGTT) in the first trimester. If negative then they undergo a further OGTT in second trimester between 24-28 weeks of gestation. Pregnant women with other risk factors for developing GDM are offered a 75gram Oral Glucose Tolerance Test between 24-28 weeks of gestation. Blood glucose threshold levels are based on those recommended by the International Diabetes Federation 2009: fasting blood sugar level ≥ 5.5 mmol/litre and 2hr post prandial blood sugar level ≥ 7.8 mmol/litre indicate the diagnosis of GDM (65).

2.9.3 Sample size

Using CEMACH data, the anticipated rate of morbidity in infants born to diabetic mothers (all types) is approximately 30% (104, 206). A 50% lower rate of morbidity was chosen (i.e. 15%) in order to justify a different approach to the postnatal care of SA infants of diabetic mothers. In order to have 90% power, at a 5% significance level, to show such a difference, it was necessary to review approximately 160 babies in each arm of the study. Figures obtained from the diabetes database, in Leicester indicated that there are about 300 women each year with diabetes in pregnancy (all types) and of these, about 45% women are SA, 45% women WB and the remaining women belong to other ethnic groups. Therefore it was estimated that the required number of participants could be obtained within two years, therefore the appropriate local records from 1st January 2009 to 31st December 2010 were used.

Diabetes during pregnancy can be due to T1DM, T2DM or GDM and each of the three types has different aetiologies and manifestations in the mother. However the neonatal outcome largely depends on the degree of maternal glycaemic control during pregnancy. Therefore, they were considered as a single group of infants, born following exposure to maternal hyperglycaemia during their intrauterine life. The power calculation for the study included this assumption. During the selection process for the participants of the study care was taken to ensure equal numbers of SA and WB infants were recruited for each type of maternal diabetes. The first 150 consecutive SA women with gestational diabetes mellitus and the first 10 SA women with pre-gestational diabetes (T1DM and T2DM) were selected from the diabetes database and the same process was continued for the WB women.

2.9.4 Study Outcomes

2.9.4.1 Primary outcome

The primary outcome was intended to capture babies where the maternal diabetes contributed to significant neonatal morbidity comprising any of the following:

1. The need for admission for neonatal care (e.g. for neonatal hypoglycaemia, neonatal hyperbilirubinaemia, respiratory distress syndrome, congenital malformation, sepsis and hypoxic ischaemic encephalopathy)
2. Neonatal hypoglycaemia
3. Neonatal birth trauma (shoulder dystocia, nerve palsy, bone fracture or hypoxic ischaemic encephalopathy)
4. Readmission in the first week after initial discharge home and
5. Neonatal death.

2.9.4.2 Secondary outcome

The secondary outcomes comprised:

1. Mode of delivery
2. Birthweight $\geq$ 97th centile for gestation

As per the local policy, all infants born to mothers with diabetes in pregnancy received pre-feed blood sugar monitoring after birth. The infants received an early first feed followed by 3 - 4 hourly regular feeds as per mothers feeding preference. The pre-feed blood sugar was tested from second feed onwards and continued until two consecutive blood sugar levels were more than 2.6mmol/L. The capillary blood sample was collected following a heel prick with a lancet and analysed immediately at the bedside by use of HemoCue Glucose System. Biochemical hypoglycaemia was classed as blood sugar less than 2.0mmol/L. Clinical hypoglycaemia was defined as the presence of one of the following symptoms: jitteriness, apnoea, hypothermia, respiratory distress, poor feeding, bradycardia, lethargy, irritability, hypotonia or seizure along with biochemical hypoglycaemia.

The NICE recommended, gestation specific, jaundice threshold charts were used to diagnose and treat neonatal hyperbilirubinaemia.

Respiratory distress syndrome was diagnosed by one of the following 3 criteria:

1. Symptoms of respiratory distress (tachypnoea - respiratory rate $>60/\text{min}$, subcostal and intercostal recessions, tracheal tug, head bobbing,) and oxygen requirement beyond 24 hours,
2. Surfactant administration,
3. A ground glass appearance on chest X-ray in an infant with symptoms of respiratory distress. If the infant had respiratory symptoms but the chest x-ray was clear with/without fluid in the fissure then the diagnosis was considered to be transient tachypnoea of newborn (TTN - diagnosis of exclusion).

Diagnosis of hypoxic ischaemic encephalopathy was diagnosed based on the TOBY trial criteria(207).

Serum calcium, magnesium and haematocrit were not always measured. Hypocalcaemia was defined as serum calcium levels less than 2.0mmol/L and hypomagnesaemia was defined as serum magnesium levels less than 0.7mmol/L . Polycythaemia was define as a serum haematocrit more than 70% estimated from a venous blood sample.

2.9.5 Data

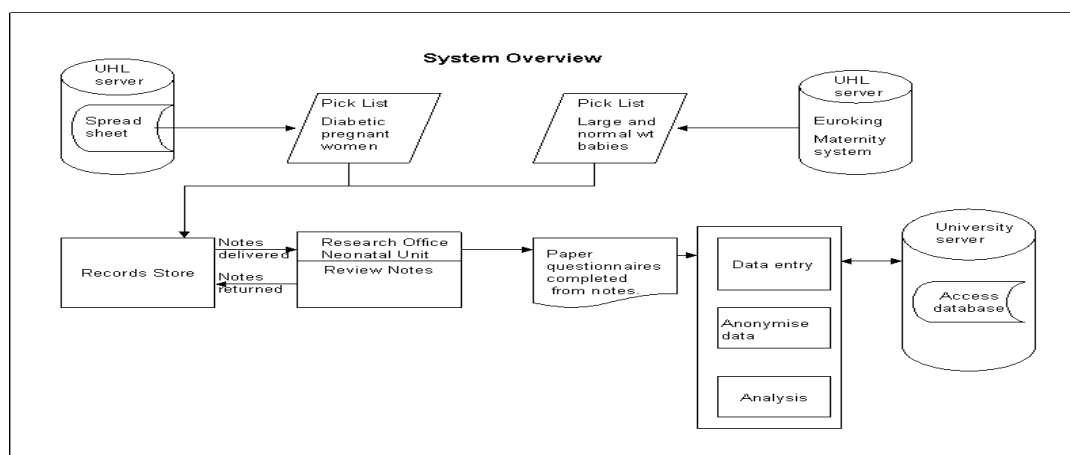
The information provided in this section applies to both the retrospective and the prospective studies (discussed in subsequent chapters). For all the studies, the ethical approval was granted by the LNR Research Ethics Committee 2, Research and Development approval was granted by the University Hospitals of Leicester and the University of Leicester provided the sponsorship.

2.9.5.1 Data collection

The maternal and neonatal data variables collected were included with the aim of identifying antenatal and perinatal factors that could influence adverse neonatal outcomes. Data collection forms used for the retrospective and the prospective studies are included in Appendix 1 and 2 in section 6.1 and 6.2. Following the design of the data collection form, comments and feedback were sought from neonatologists at the University Hospitals of Leicester, which led to a few minor changes. The research fellow recorded relevant data from maternal and neonatal medical notes directly onto the data collection forms. The medical notes were reviewed up to 28 days after birth to record any readmissions to hospital or A&E following initial discharge home.

2.9.5.2 Data handling

Figure 2.4: Data flow.



Maternal and neonatal medical notes of the recruited study participants for all the three studies were requested with the help of Clinical Audit Standards and Effectiveness (CASE) team and the antenatal clinic co-ordinators. The research fellow also received training to request the required notes through the 'track it' system at University Hospitals of Leicester. The maternal medical notes for the study were obtained from the antenatal medical records at Leicester Royal Infirmary and Leicester General Hospital with the help of antenatal clinic co-ordinators. Maternal notes were stored in locked rooms in the antenatal records department at each hospital. The neonatal medical notes for the study were requested from medical records and were delivered to the neonatal unit at Leicester General Hospital where they were stored in a locked office. The initial data collection for the studies included collection of identifiable personal data.

This was to ensure the following:

1. Data was collected only once for each participant and inadvertent duplication avoided.
2. Maternal data could be linked to the correct neonatal data
3. To permit the participant to be tracked back if incorrect or implausible data was collected but only identified during data entry onto the computer.

The following maternal identifiable characteristics were collected:

1. Maternal name and surname
2. Maternal date of birth
3. Maternal hospital number
4. Maternal address

The following neonatal identifiable characters were collected:

1. Neonatal name and surname.
2. Neonatal date of birth.
3. Neonatal hospital number.

The paper copies of the data collection forms were stored securely in the locked filing cabinet in a locked office in the Department of Health Sciences at University of Leicester.

2.9.5.3 Data entry

An Access database was developed to allow for both data entry and validation. The data collected was double entered on to the computer database. Double entering of the data helped to decrease errors in data collection and ensured the highest level of accuracy. The data was double entered by an undergraduate student trained for the task during their summer holiday placement. Any inconsistencies identified during the second entry were corrected. This process also helped to identify missing data, which was then collected and entered. Following the completion of data entry, further data checking and data cleaning was carried out. Any inconsistencies in the data and outliers for specific variables were identified, rechecked and rectified.

2.9.5.4 Data encryption

The computer used for data entry was also stored in a locked office in the Department of Health Sciences at University of Leicester. The database was encrypted and accessible only through a secure password. The data encryption service provided by IT services at University of Leicester provided advice, product recommendations and support to encrypt secure data. The data encryption implemented met NHS standards i.e. a strong algorithm, AES 256 bit was used. The encrypted computer database was held on CFS X: drives and the data storage were on central SANs in secure alarmed computer rooms in accordance with the University's corporate security policies. X: drive backups were also made and stored securely by IT Services. The X: drive was a shared departmental file store for staff. It is a "Network drive" and it was managed centrally by IT Services and regularly backed up. The University network had filtering firewall functionality at the Internet gateway, managed by IT Services.

After completion of double data entry onto the computer database, the database was anonymised and it was not possible to trace back individual study participants. This anonymised database was used for data analysis.

2.10 Results of retrospective study 1

In this retrospective, cohort study, 160 consecutive South Asian (SA) women were compared with 160 consecutive White British (WB) women with diabetes in pregnancy (150 women with GDM and 10 women with T1DM and T2DM) to compare the neonatal outcomes in the infants born to mothers in these two ethnic groups.

2.10.1 Characteristics of the mothers with diabetes in pregnancy

The characteristic of SA and WB mothers with diabetes have been summarised in table 2.3. There was a statistically significant difference in maternal age, weight, height, BMI, systolic BP, smoking and alcohol history between the SA and the WB women. There was no difference seen in diastolic BP, gravidity and previous history of diabetes. SA mothers were significantly younger, had a lower weight and BMI at the time of antenatal booking of the current pregnancy and were more likely to be within the normal BMI range. They also had a significantly lower history of smoking 6.4% vs. 43.4% ($p < 0.001$) and alcohol consumption 3.8% vs. 25.8% ($p < 0.001$) at the time of antenatal booking. Information about the history of smoking and alcohol was collected from the details recorded in the maternal notes during the first antenatal visit (antenatal booking of the pregnancy). There is a possibility that this data might reflect their smoking and alcohol habits before pregnancy, which might have decreased once the pregnancy was confirmed.

Table 2:3: Characteristics of the SA and WB diabetic women.

		South Asian (N = 160)	White British (N = 160)	P value
Maternal age (years)**		31.00 (27.00- 35.00)	33.00 (28.00 – 38.00)	0.012
Maternal weight (kg)**		66.00 (58.25 – 79.00)	84.00 (72.00 – 98.00)	< 0.001
Maternal height (cm)**		158.00 (154.50 – 162.00)	164.00 (158.00 – 164.00)	< 0.001
Maternal BMI**		26.95 (23.16 – 31.82)	31.64 (26.37 – 36.89)	< 0.001
Maternal BMI	Normal BMI*	56/156 (35.9)	30/160 (18.8)	0.001
	Overweight*	47/156 (30.1)	33/160 (20.6)	0.054
	Obese*	50/156 (32.1)	95/160 (59.4)	<0.001
Blood pressure (mmHg)	Systolic BP**	113.00 (103.00 – 122.00)	120.00 (110.00 – 130.00)	< 0.001
	Diastolic BP**	71.00 (62.25 – 78.00)	74.00 (65.00 – 80.00)	0.164
Smoking*		10 (6.4)	69 (43.4)	< 0.001
Alcohol*		6 (3.8)	40 (25.8)	< 0.001
Recreational drugs*		0	0	-
Gravida	Primigravida*	37 (23.1)	41 (25.6)	0.130
	Multigravida*	100 (62.5)	84 (52.5)	
	Grand multigravida*	23 (14.4)	35 (21.9)	
Previous history of diabetes*		32 (20.4)	34 (21.3)	0.890

Values for continuous variables presented as median (interquartile range);

Values for categorical variables presented as n (%);

**Chi-square test for categorical variables;*

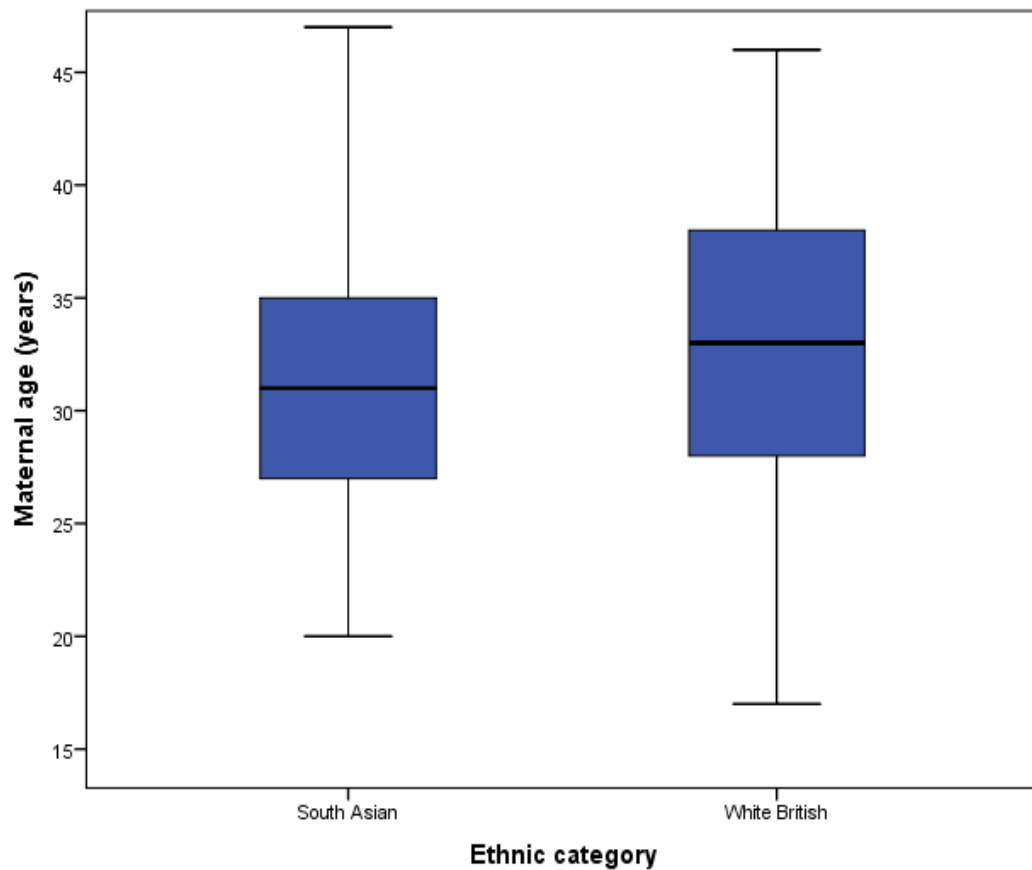
Fisher's exact test, 2-sided exact significance;

***Mann-Whitney U test for continuous variables.*

2.10.1.1 Comparison of maternal age in the two ethnic groups

In this study, SA mothers with diabetes were significantly younger as compared to WB mothers as seen in the box plot in Fig 2.5. Mann-Whitney U (non-parametric) testing revealed a significant difference between the age of SA women (median = 31.00 years, $n = 160$, interquartile range = 27.00 – 35.00 years) and WB women (median = 33.00 years, $n = 160$, interquartile range = 28.00 – 38.00 years), $p = 0.012$, $Z = -1.23$, $r = 0.07$.

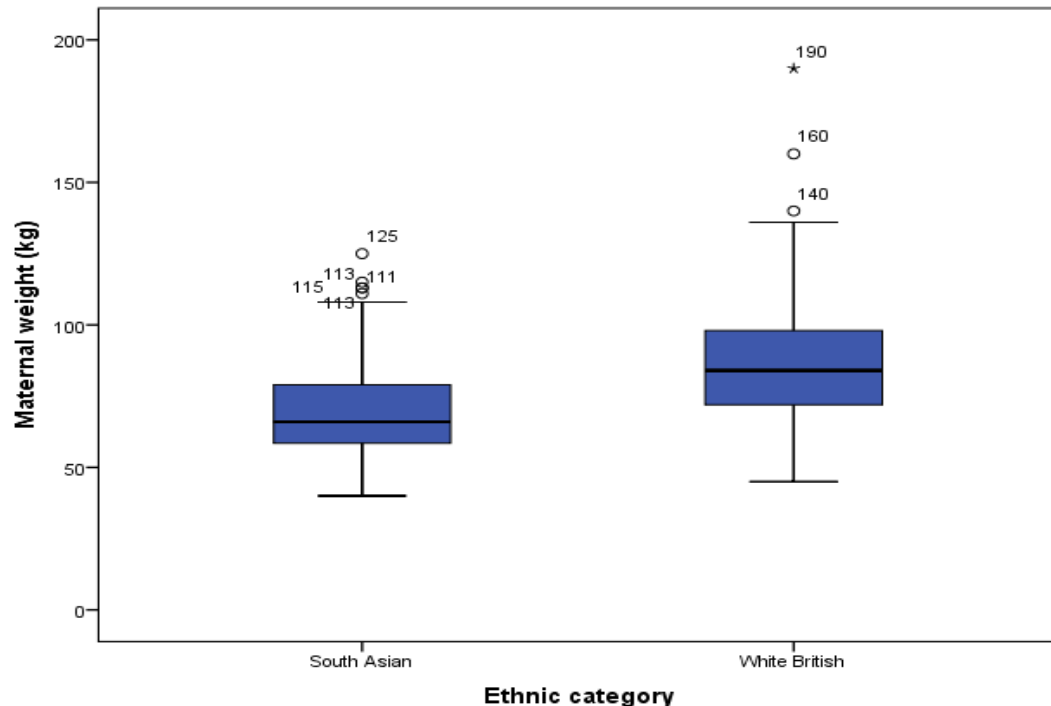
Figure 2.5: Box plot to compare maternal age between the SA and WB women.



Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values.

2.10.1.2 Comparison of maternal weight and BMI

Figure 2.6: Box plot to show the comparison of maternal weight between SA and WB women.



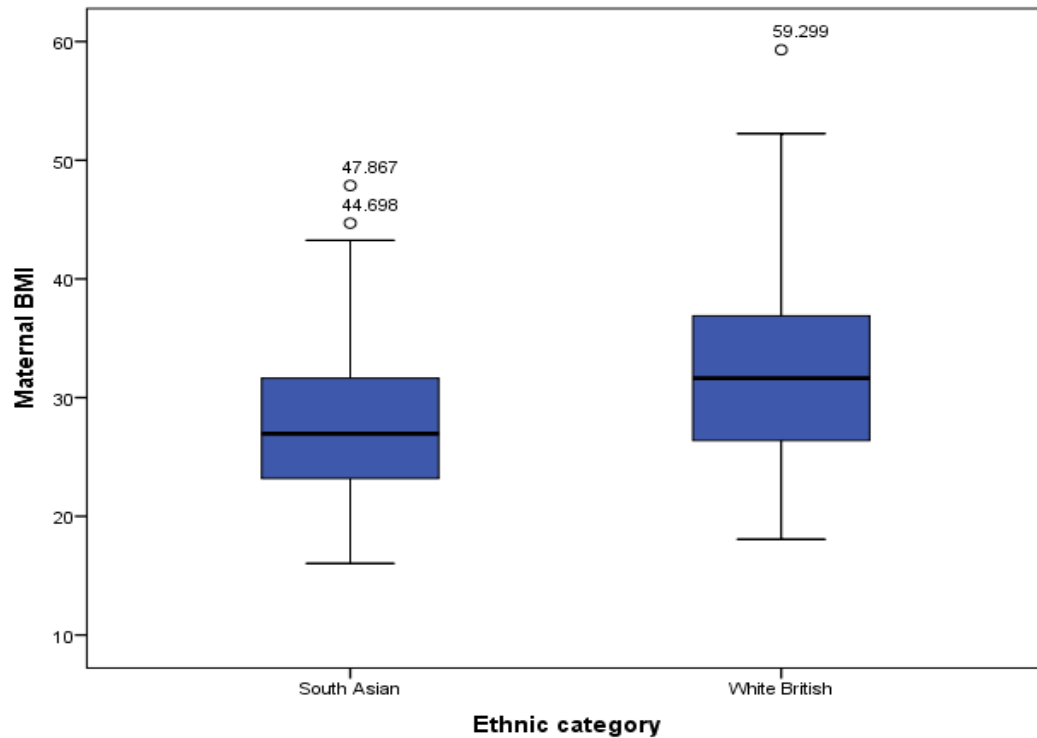
*Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values.*

SA women had a significantly lower weight at the time of antenatal booking for the index pregnancy as compared to WB women as shown in the Fig 2.6. Amongst the SA women, there were five outlier women with their weight between 113 – 125 kg and amongst the WB women there were two outlier women with their weight of 140 and 160 kg and one woman with an extreme weight of 190 kg. These values were rechecked and confirmed to be the true maternal weight.

A Mann-Whitney U test revealed a significant difference in the weight of SA mothers (median = 66.00 kg, interquartile range = 58.25 – 79.00, n = 160) as compared to WB mother (median = 84.00 kg, interquartile range = 72.00 – 98.00, n = 159), $p < 0.001$, $z = -7.322$, $r = 0.41$ (medium effect).

SA women also had a lower BMI as compared to WB women as shown in Fig 2.7. BMI was calculated by using the formula $\text{weight (kg)} / \text{height (cm)}^2$.

Figure 2.7: Box plot to compare maternal BMI between SA and WB women.

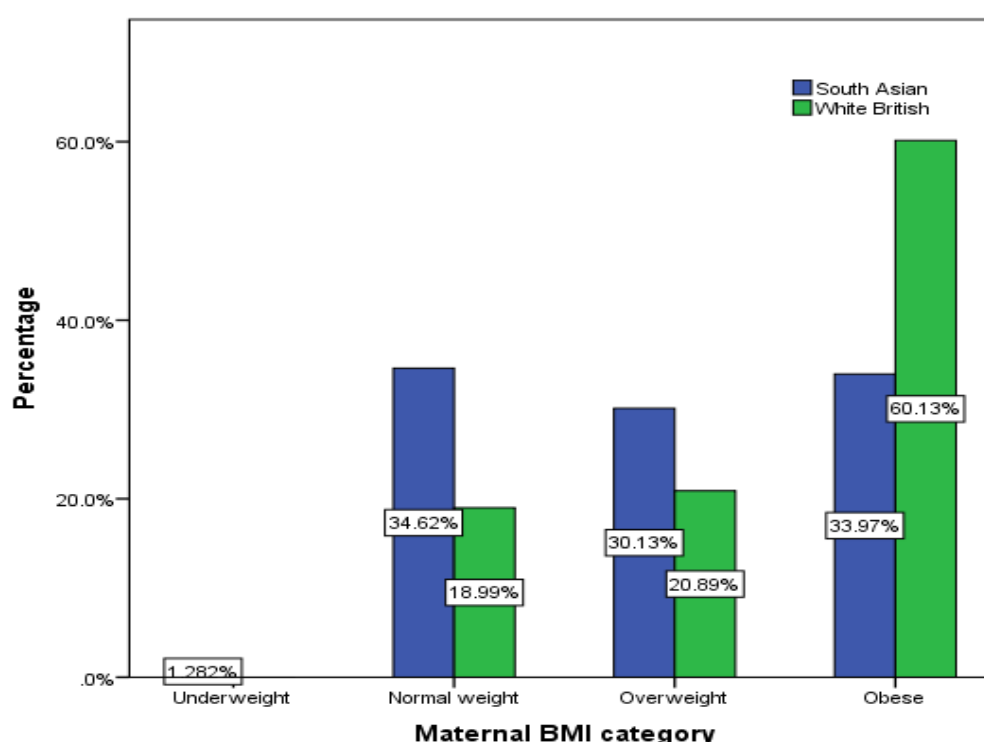


Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers.

A Mann-Whitney U testing revealed a significant difference between the maternal BMI of SA women (median = 26.95, interquartile range = 23.16 – 31.82, n = 157) and WB women (median = 31.64, interquartile range = 26.37 – 36.89, n = 158), $p < 0.001$, $z = -5.14$, $r = 0.29$ (medium effect).

As shown in the bar chart in fig 2.8, 64% of SA women and 81% WB women were overweight or obese at booking. Only 34.62% of SA women and 18.99% of WB women with diabetes in pregnancy had a normal BMI. BMI categories were defined as follow: underweight <18.5 , normal BMI $18.5 - 24.9$, overweight BMI $25.0 - 29$ and obese BMI > 30.0 .

Figure 2.8: Bar chart comparing maternal BMI of SA and WB women.



In relation to further weight gain in pregnancy, there was a significant difference with SA women gaining more weight as compared to WB women (weight gain during pregnancy was calculated as the difference between the maternal weight at the time of antenatal booking and at 36 weeks of gestation) Fig 2.9. Data regarding weight gain was available for 113 SA women and 96 WB women. As shown in table 2.5, 32.7% of the SA women and 37.5% of the WB women had a weight gain during pregnancy, which was higher than the recommendations for the weight gain during pregnancy by Royal college of Obstetrics and Gynaecology as summarised in table 2.4.

Table 2:4: RCOG recommendation for weight gain during pregnancy.

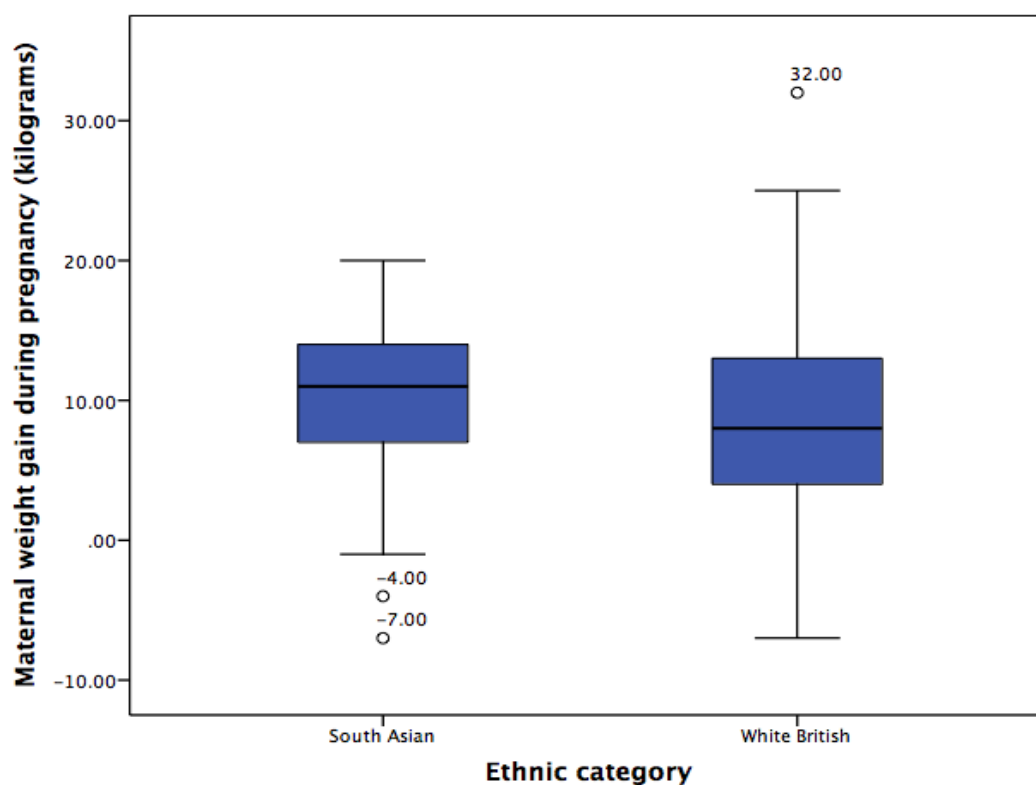
Pre-pregnancy BMI (kg/m ²)	Recommended
Underweight (< 18.5)	12.5–18
Healthy weight (18.5–24.9)	11.5–16
Overweight (25.0–29.9)	7–11.5
Obese (≥ 30.0)	5–9

Table 2:5: SA and WB women with weight gain above the recommended range.

Pre-pregnancy BMI (kg/m ²)	Weight gain range (kg)	
	SA women (N=113)	WB women (N = 96)
Underweight (< 18.5)	1/3 (33.3)	None
Healthy weight (18.5–24.9)	5/35 (14.3)	5/16 (6.25)
Overweight (25.0–29.9)	17/34 (50.0)	4/14 (28.6)
Obese (≥ 30.0)	14/41 (34.1)	22/66 (33.3)

Values for categorical variables presented as n (%)

Figure 2.9: Box plot to show maternal weight gain during pregnancy in the SA and WB women.



*Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values.*

A Mann-Whitney U test showed a significant difference in weight gain in pregnancy between SA women (median = 11.0 kg, n = 113) and WB women (median = 8.0 kg, n = 96), $p = 0.033$, $z = -2.131$, $r = 0.15$ (small effect).

2.10.1.3 Comparison of family history between the two ethnic groups

SA women had a significantly higher family history of diabetes 112 (70%) vs. 73 (45.6%), $p < 0.001$ as compared to WB women. SA women had a significantly lower family history of pregnancy induced hypertension 15 (9.4%) vs. 28 (17.5%), $p = 0.048$ and congenital anomalies 21 (13.1%) vs. 38 (23.8%), $p = 0.02$ as compared to WB women. There was no difference in the family history of previous neonatal deaths in the two ethnic groups (table 2.6).

Table 2:6: Comparison of family history between the SA and the WB women.

	South Asian (N = 160)	White British (N = 160)	P – value
FH of diabetes	112 (70)	73 (45.6)	< 0.001
FH of hypertension	4 (2.5)	3 (1.9)	1.000
FH of pregnancy induced hypertension	15 (9.4)	28 (17.5)	0.048
FH congenital anomaly	21 (13.1)	38 (23.8)	0.021
FH of neonatal death	3 (1.9)	1 (0.6)	0.623

FH – Family History

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

2.10.1.4 Comparison of previous obstetric history between the two ethnic groups

Of the total 320 women included in the analysis, 37 SA women and 41 WB women were primigravida and were excluded from the analysis of previous obstetric history leaving 123 SA women and 119 WB women. Table 2.7 shows the comparison of previous obstetric history between the two groups.

Table 2:7: Comparison of previous obstetric history between the two groups.

	South Asian N = 123	White British N = 119	P – value
Previous miscarriage	49 (39.8)	56 (47.1)	0.300
Previous stillbirth	10 (8.1)	4 (3.4)	0.168
Previous preterm birth	14 (11.4)	22 (18.5)	0.149
Previous neonatal death	4 (3.3)	0	0.122

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

There was a very high rate of reported previous miscarriage (spontaneous pregnancy loss within the first 23 weeks, as reported in the maternal antenatal notes) in both SA (39.8%) and WB (47.1%) women ($p = 0.300$). However the difference between the two groups was statistically insignificant.

Similarly there was no statistically significant difference in the reported rate of previous stillbirth (defined as spontaneous death of the fetus before birth beyond 24 weeks gestation, data was collected as reported in the maternal antenatal notes) between the SA and the WB women 8.1% vs. 3.4% ($p = 0.168$). 11.4% SA women and 18.5% WB women reported a history of one or more (maximum four) previous preterm birth ($p = 0.149$). There was no statistically significant difference between the two groups. In this study cohort, four South Asian women with diabetes reported a history of previous neonatal death. There were no deaths in the White British group.

2.10.2 Diagnosis of GDM

Of the total 160 women with diabetes in pregnancy in each group, 10 women had pre-gestational diabetes (T1DM, T2DM) and they were already known to be diabetic at the start of the pregnancy. The remaining 150 women were tested for GDM. Table 2.8 below summarises the gestational age at the time of diagnosis of GDM and the median fasting and the postprandial blood sugar levels in the two study groups.

Table 2:8: Comparison of results of OGTT in the SA and WB women.

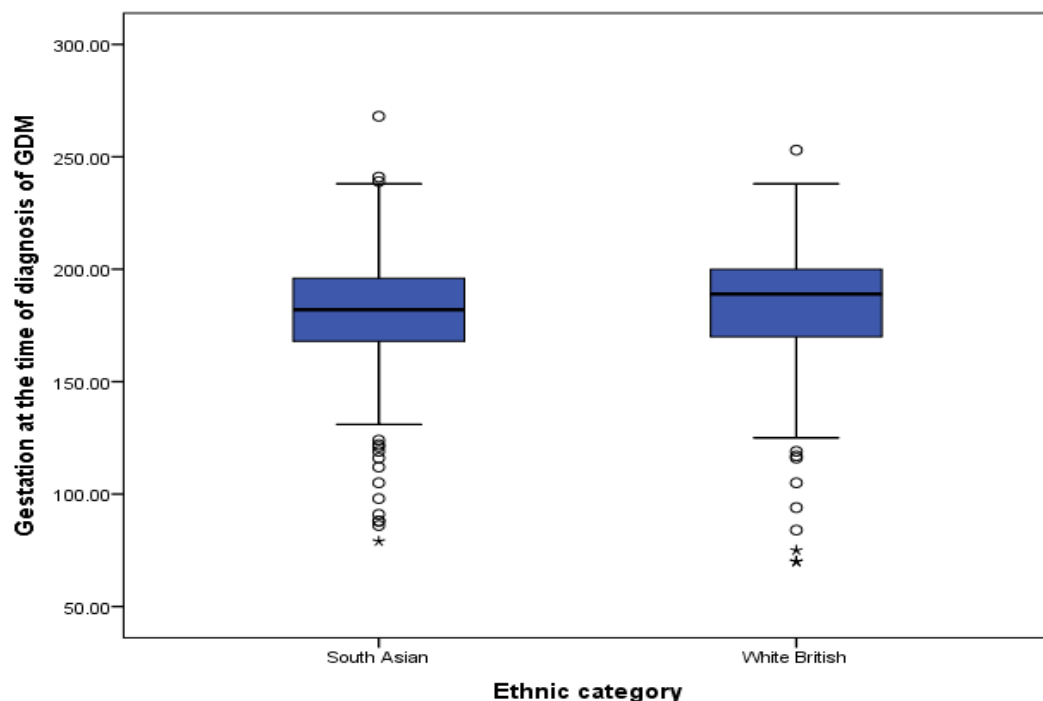
	South Asian N = 150	White British N = 150	P – value
Gestation at diagnosis (weeks)	25+4 (24+0 – 28+0)	27+0 (24+2 – 28+4)	0.069
Fasting blood sugar (mmol/L)	5.10 (4.6 – 5.7)	5.20 (4.6 – 5.85)	0.846
2-hour PP blood sugar (mmol/L)	8.8 (8.10 – 10.0)	8.45 (8.0 – 9.3)	0.014

*Values for continuous variables presented as medians (interquartile range);
Mann-Whitney U test for continuous variables.*

2.10.2.1 Comparison of gestation at the time of diagnosis of GDM

There was no statistically significant difference in the gestational age at the time of the diagnosis of GDM between SA and WB women 25+4 vs. 27+0 weeks respectively, $p = 0.069$ (table 2.8).

Figure 2.10: Box plot to compare the gestation at the time of diagnosis of GDM amongst SA and WB mothers.



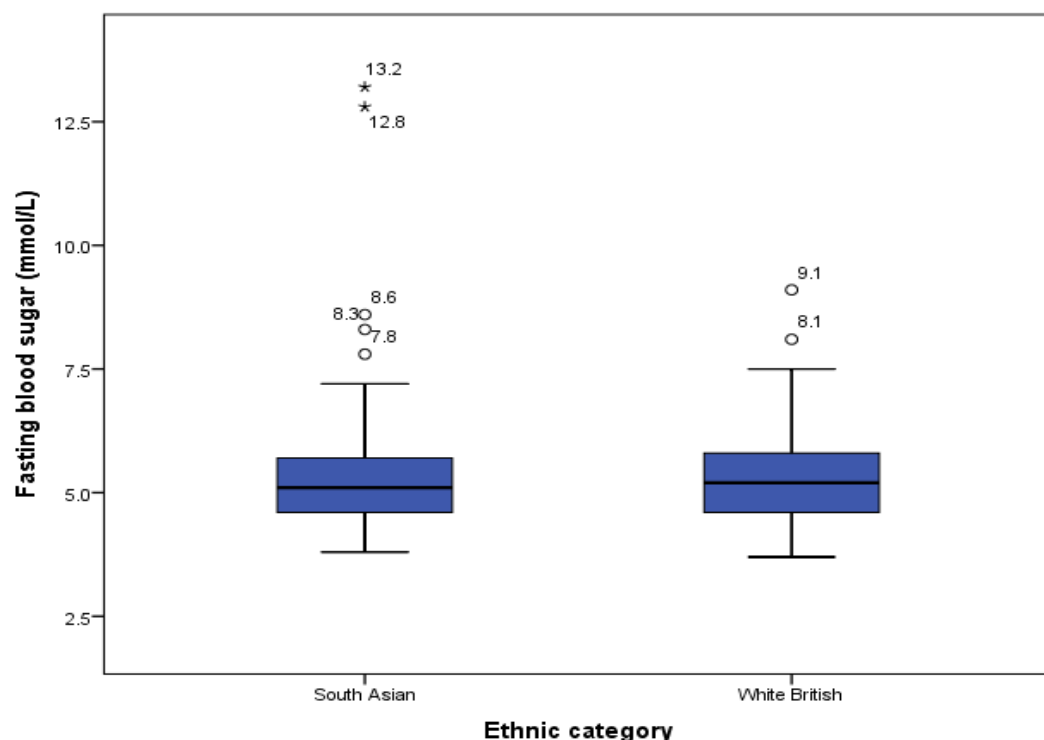
*Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ^o represents outliers and * represents extreme values.*

The box plot in Fig 2.10 shows outliers at either extremes of gestational age in both study groups. The outlier and extreme values at the lower end represents 32 SA women and 34 WB women who received screening for GDM in first trimester as they had GDM in their previous pregnancy. These women who had a positive OGTT in the first trimester could have had T2DM detected for the first time in the pregnancy. This diagnosis could only be confirmed if glycaemic derangement persisted in the postnatal period.

2.10.2.2 Comparison of maternal blood sugar levels at the time of diagnosis of GDM

Mann-Whitney U test revealed that there was not a statistically significant difference in the fasting blood sugar level between SA women (median = 5.10 mmol/L, n = 150) and WB women (median = 5.20 mmol/L, n = 150), $p = 0.846$, $z = -0.274$, $r = 0.02$ (fig 2.11).

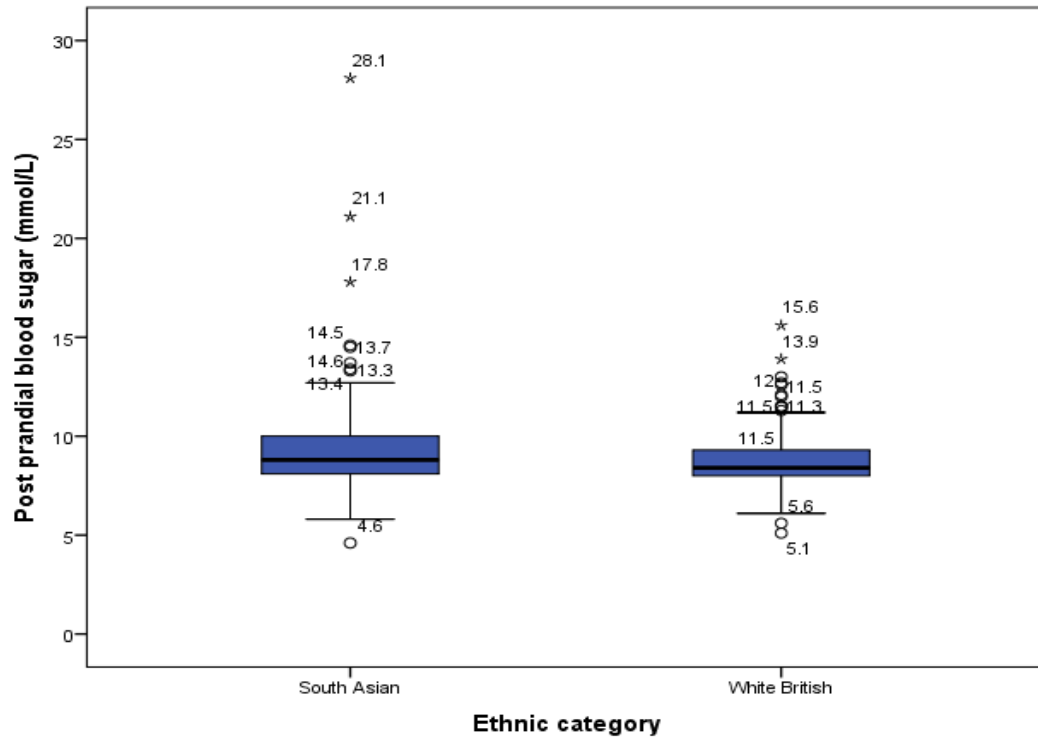
Figure 2.11: Box plot to show fasting blood sugar levels between SA and WB women.



*Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values.*

However the postprandial blood sugar levels were different in the two groups. Mann-Whitney U test showed that there was a statistically significant difference in the 2-hour post prandial blood sugar levels between the SA (median = 8.8mmol/L, n = 150) and WB women (median=8.45 mmol/L, n = 150), $p = 0.014$, $z = -2.421$, $r = 0.20$ (fig 2.12).

Figure 2.12: Box plot to show maternal 2-hour post prandial blood sugar level in SA and WB mothers.



Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values.

The two extreme values of 12.8 and 13.2 mmol/L for fasting blood sugar seen in fig 2.11 were checked and represent true maternal fasting blood sugar level. These mothers had corresponding postprandial levels of 28.1 and 21.1 mmol/L respectively (fig 2.12).

2.10.3 Glycaemic control in pregnancy

2.10.3.1 Maternal glycaemic control between 28 – 32 weeks gestation

Of the entire cohort, 142 SA women and 141 WB women were assessed for their diabetes control and treatment between 28 – 32 weeks gestation (table 2.9).

Table 2:9: Comparison of maternal blood sugar control between 28 – 32 weeks gestation.

		South Asian N = 142	White British N = 141	P – value
Diabetes Control*	Good control	72 (50.7)	69 (49.3)	0.095
	Moderate control	55 (38.7)	44 (31.4)	
	Poor control	15 (10.6)	27 (19.3)	
Diabetes Treatment *	Diet control	100 (70.4)	90 (63.8)	0.256
	Insulin treatment	42 (29.6)	49 (34.8)	
	Oral hypoglycaemics	0	2 (1.4%)	

Values for continuous variables presented as median (interquartile range);

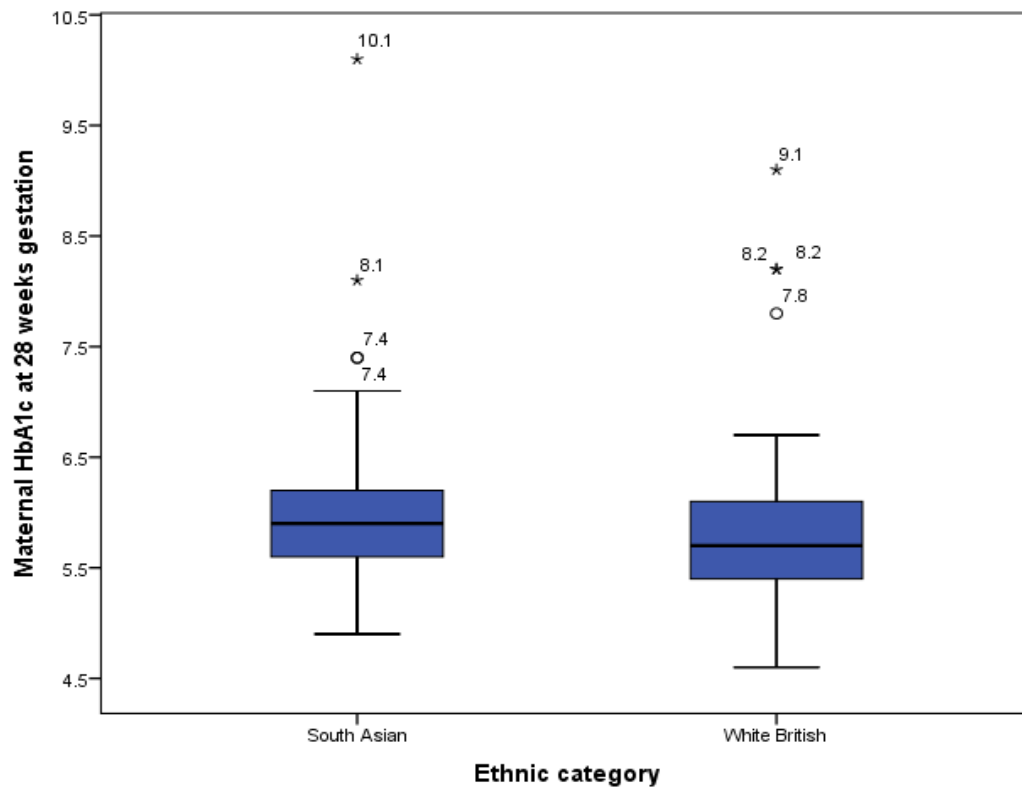
Values for categorical variables presented as n (%);

**Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance; **Mann-Whitney U test for continuous variables.*

Good control of diabetes in pregnancy was defined as both average fasting and average 2-hour postprandial blood sugar levels being within the normal range, moderate control was defined as either of these values being outside the normal range and poor control defined as both values being outside the normal range. Daily record maintained by women over a period of 3-5 weeks using capillary blood sample was used to calculate average fasting and average 2-hour postprandial blood sugar levels. These same readings were used to guide clinical treatment.

There was no significant difference with regards to blood sugar control between the women in the two groups as shown in table 2.9. SA women were more likely to be diet controlled 100 (70.4%) vs. 90 (63.8%) and were less likely to require insulin 42 (29.6%) vs. 49 (34.8%) to control their diabetes at this stage as compared to WB women. However the difference in their treatment requirement was not statistically significant ($p = 0.256$).

Figure 2.13: Box plot comparing HbA1c in the SA and WB women between 28 – 32 weeks gestation.



*Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values.*

Mann-Whitney U testing revealed a significant difference in the mean HbA1c between SA women (median = 5.9, $n = 111$) and WB women (median = 5.7, $n = 96$), $p = 0.027$, $z = -2.21$, $r = 0.15$ (small effect). Fig 2.13 shows comparison of maternal HbA1c levels between SA and WB women between 28 – 32 weeks gestation.

Table 2:10: Comparison of ultrasound findings in the SA and WB women between 28 – 32 weeks gestation.

		South Asian N = 99	White British N = 100	P – value
Antenatal ultrasound for fetal wellbeing	Normal	79 (79.8)	68 (58.6)	0.001
	LGA	12 (8.5)	42 (29.8)	0.019
	Polyhydramnios	9 (6.3)	17 (12.1)	0.585
	Growth restriction	0	1 (0.7%)	1.000

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance;

Table 2.10 is a comparison of ultrasound scanning conducted in the subset of 99 SA women and 100 WB women from the entire cohort who had a scan for fetal wellbeing between 28 – 32 weeks gestation. About one third women in both the groups did not receive antenatal ultrasound during this period of gestation and this would have been influenced by the timing of diagnosis, clinician's discretion and clinic appointments. It showed that SA women had a statistically significantly higher chance of a normal fetal ultrasound scan as compared to WB women, 79 (79.8%) vs. 68 (58.6%), $p = 0.001$. WB women had an odds ratio (95% CI) of 2.79 (1.51 – 5.15) for having an abnormal antenatal ultrasound finding between 28 – 32 weeks gestation. SA women also had a statistically significantly lower risk of carrying a LGA fetus 12 (8.5%) vs. 42 (29.8%), $p = 0.019$. WB women had an odds ratio of 4.67 (1.354 – 16.09) for carrying a LGA fetus between 28 – 32 weeks gestation. There was no difference in the risk of developing complications of polyhydramnios ($p = 0.585$) and growth restriction ($p = 1.000$) on antenatal ultrasound scans between the two groups.

2.10.3.2 Maternal glycaemic control between 32 – 36 weeks gestation

147 SA women and 150 WB women from the entire cohort were assessed for their diabetes control and treatment between 32 – 36 weeks gestation. There was no statistically significant difference in their diabetes control or treatment requirements, $p = 0.079$ and $p = 0.313$ respectively. Summary of this comparison is shown in table 2.11.

Table 2:11: Comparison of maternal blood sugar control and treatment between 32– 36 weeks gestation.

		South Asian N = 147	White British N = 150	P - value
Diabetes Control*	Good control	80 (54.4)	71 (47.3)	0.079
	Moderate control	55 (37.4)	54 (36.0)	
	Poor control	12 (8.2)	25 (16.7)	
Diabetes Treatment*	Diet control	86 (58.5)	75 (50.0)	0.313
	Insulin treatment	60 (40.8)	73 (48.7)	
	Oral hypoglycaemic	1 (0.7)	2 (1.3)	

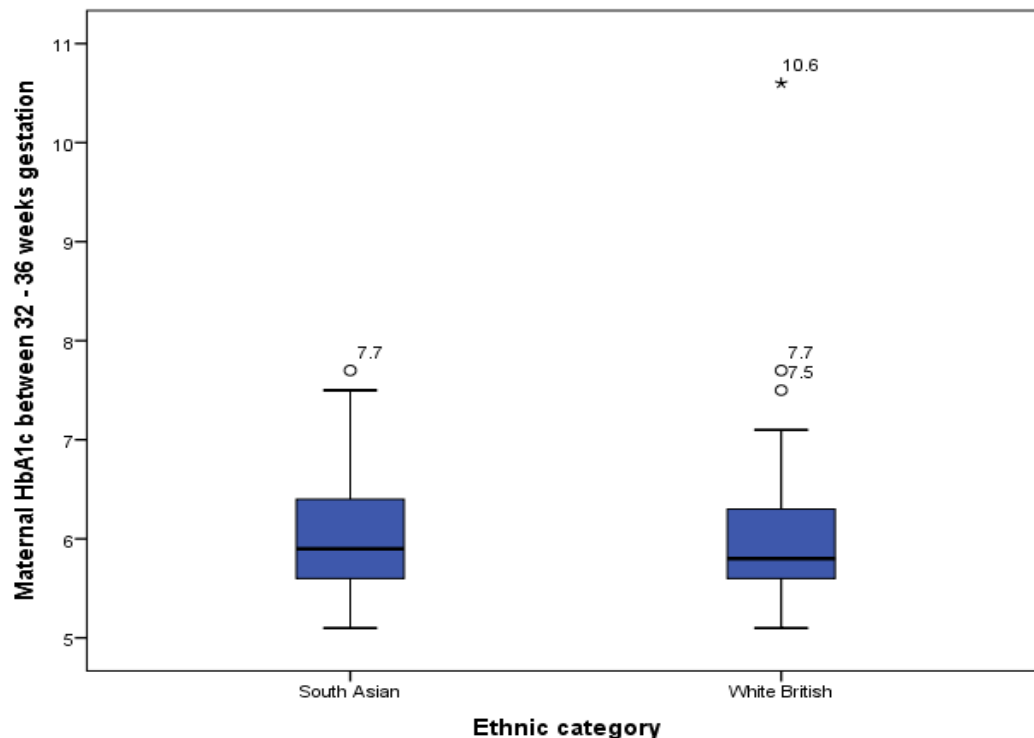
Values for continuous variables presented as median (interquartile range);

Values for categorical variables presented as N (%);

**Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance;*

***Mann-Whitney U test for continuous variables.*

Figure 2.14: Box plot showing comparison of maternal HbA1c level between SA and WB mothers at 32 – 36 weeks gestation.



*Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values.*

Fig 2.14 shows the comparison of HbA1c amongst SA and WB women who were tested between 32 – 36 weeks of gestation. Mann-Whitney U testing revealed that there was no statistically significant difference in the HbA1c levels between SA women at this gestation (median = 5.9, n = 46) and WB women (median = 5.8, n = 44), $p = 0.579$, $z = -0.554$, $r = 0.06$ (small effect). Even though there was a statistically significant difference between the HbA1c levels in SA and WB women between 28 – 32 weeks gestation, this difference was not noted between 32 – 36 weeks gestation. One of the reasons for this lack of difference may be that a very small proportion of women in both the groups (28.5% of SA women and 27.5% of WB women) had their HbA1c levels tested at this point.

Table 2:12: Comparison of ultrasound findings in SA and WB women between 32 – 36 weeks gestation.

		South Asian N = 129	White British N = 134	P – value
Antenatal ultrasound for fetal wellbeing	Normal	100 (77.5)	64 (47.8)	< 001
	Macrosomia	18 (13.9)	60 (44.8)	0.012
	Polyhydramnios	12 (9.3)	22 (16.4)	0.361
	Growth restriction	2 (1.5)	1 (0.7)	0.204

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

Table 2.12 compares the findings of ultrasound scanning performed in 129 SA women and 134 WB women for fetal wellbeing between 32 – 36 weeks gestation. This comparison using Chi-square testing revealed that SA women had a significantly higher chance of a normal fetal ultrasound 100 (77.5%) as compared to 64 (47.8%) in the WB women $p < 0.001$. WB women had an odds ratio (95% CI) of 3.77 (2.21 – 6.45) for having an abnormal finding on fetal ultrasound between 32 – 36 weeks gestation. SA women also had a significantly lower risk of carrying a macrosomic infant 18 (13.9%) as compared to 60 (44.8%) in the WB women, $p = 0.012$ giving an odds ratio (95% CI) of 0.25 (0.09 – 0.68). There was no significant difference in the rates of polyhydramnios and growth restriction at this point.

2.10.3.3 Maternal glycaemic control between 36 weeks – delivery

130 SA women and 126 WB women were assessed for their blood sugar levels and diabetes control and treatment between 36 weeks gestation and delivery. A summary of this comparison is shown in table 2.13. There was no significant difference in diabetes control and treatment between SA and WB mothers, $p = 0.84$ and $p = 0.300$ respectively.

Table 2:13: Comparison of maternal blood sugar control between 36 weeks to delivery.

		South Asian N = 130	White British N = 126	P – value
Diabetes Control**	Good control	76 (58.5)	73 (58.4)	0.840
	Moderate control	45 (34.6)	41 (32.8)	
	Poor control	9 (6.9)	11 (8.8)	
Diabetes Treatment**	Diet control	75 (57.7)	61(48.4)	0.300
	Insulin treatment	54 (41.5)	63(50.0)	
	Oral hypoglycaemic	1 (0.8)	2 (1.6)	

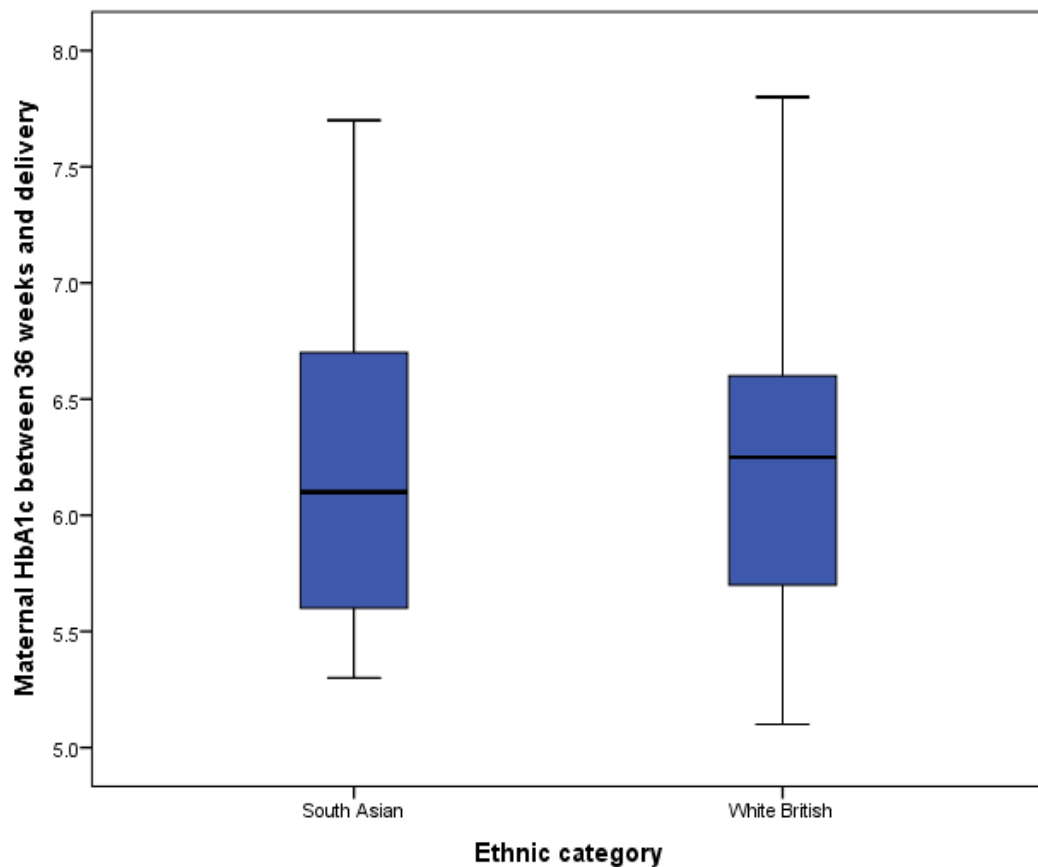
Values for continuous variables presented as median (interquartile range);

Values for categorical variables presented as n (%);

▪ *Only 13 SA women and 14 WB women had their HbA1c tested;*

***Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance; *Mann-Whitney U test for continuous variables.*

Figure 2.15: Box plot to show the difference in HbA1c level between the SA and the WB women between 36 weeks to delivery.



*Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ^o represents outliers and * represents extreme values.*

Mann-Whitney U testing revealed no significant difference between the HbA1c levels between SA (median = 6.10, n = 13) and WB mothers (median = 6.25, n = 14), $p = 1.000$. It is difficult to draw any conclusion from the results as only a very small proportion of study population were tested.

Table 2:14: Comparison of ultrasound findings in SA and WB women between 36 weeks to delivery.

		South Asian N = 78	White British N = 66	P – value
Antenatal ultrasound for fetal wellbeing	Normal	45 (57.7)	34 (51.5)	0.504
	Macrosomia	17 (21.8)	26 (39.39)	0.015
	Polyhydramnios	8	12	0.290
	Growth restriction	6	1	0.105

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance;

Ultrasound scanning performed for fetal wellbeing beyond 36 weeks gestation revealed SA women had a statistically significant lower risk of carrying a LGA in fetus as compared to WB women, 17 (21.8%) vs. 26 (39.39%), $p = 0.015$ (table 2.14). WB women had a significantly higher odds ratio of 4.078 (95% CI, 1.331 – 12.498) of carrying a LGA infant in the last four week of pregnancy as compared to SA women. There was statistically no significant difference in the complication rates of polyhydramnios ($p = 0.290$) and growth restriction ($p = 0.105$) between the two groups.

2.10.4 Mode of delivery

Table 2:15: Comparing the mode of delivery amongst SA and WB mothers.

	South Asian N=160)	White British (N = 160)	P – value
Elective C/S before due date	28 (17.5)	38 (23.8)	0.214
IOL before due date	75 (46.9)	67 (41.9)	0.431
Normal vaginal delivery	75 (46.9)	65 (40.6)	0.310
Ventouse delivery	13 (8.1)	6 (3.8)	0.056
Forceps delivery	9 (5.6)	10 (6.3)	1.000
Emergency caesarean section	35 (21.9)	41(25.6)	0.880

Values for categorical variables presented as n (%);

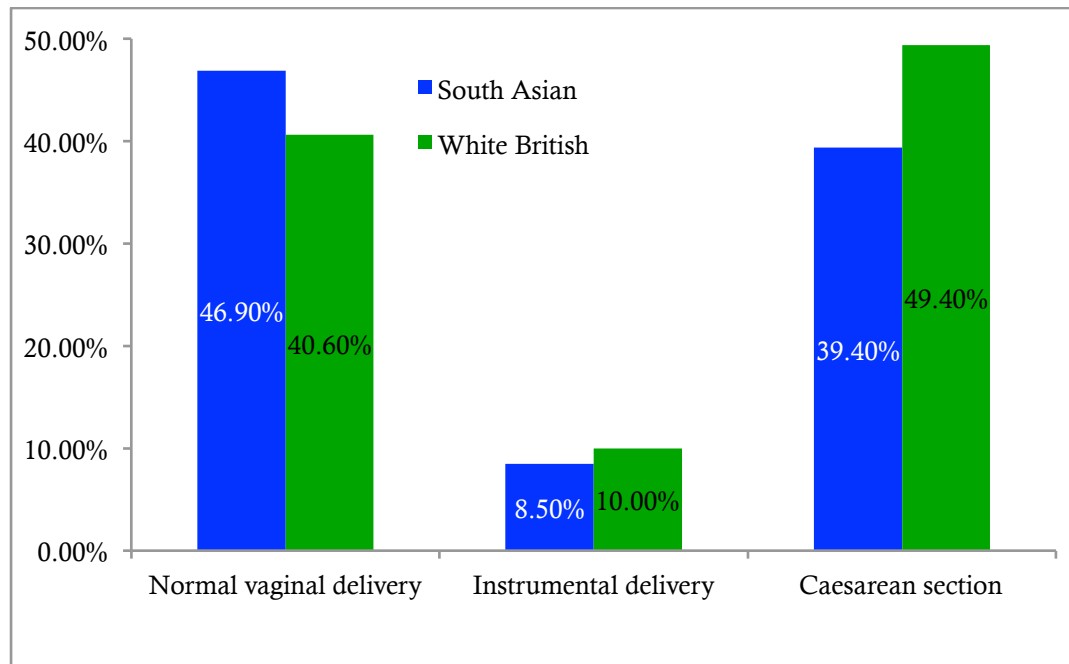
Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

Table 2.15 compares the mode of delivery (as recorded in maternity records) for the SA and WB women in the study. Due to the increased risk of sudden intrauterine fetal death in diabetic pregnancies, clinicians usually plan to deliver diabetic mothers electively after 38 weeks of gestation.

In this cohort, 103 (64.4%) SA women and 105 (65.6%) WB women were delivered electively before their due date (37 – 39 weeks) either by elective caesarean section or elective induction of labour.

There was no difference in the different modes of delivery between the two groups. 28 (17.5%) of the SA women were electively delivered by caesarean section before their due date and a further 75 (46.9%) SA women underwent elective induction of labour before their due date as compared to 38 (23.8%) and 67 (41.9%) respectively in the WB women, $p = 0.214$ and $p = 0.431$ respectively. Fig 2.16 shows a comparison of the different modes of delivery between SA and WB women.

Figure 2.16: Bar chart to show comparison of different mode of delivery between SA and WB mothers.



Of the 132 SA women (who were either electively induced, $n = 75$ or the rest who went into spontaneous labour), 75 (46.9%) delivered by spontaneous vaginal delivery, 13 (8.1%) by ventouse delivery, 9 (5.6%) by forceps delivery and 35 (21.9%) were delivered by emergency caesarean section. Of the 122 WB women (who were either electively induced, $n = 67$ or the rest who went into spontaneous labour), 65 (40.6%) delivered by spontaneous vaginal delivery, 6 (3.8%) by ventouse delivery, 10 (6.3%) by forceps delivery and 41 (25.6%) were delivered by emergency caesarean section. There was no statistically significant difference in the rates of ventouse delivery, forceps delivery and emergency caesarean section between the two groups, $p = 0.056$, $p = 1.000$, $p = 0.880$ respectively.

2.10.4.1 Maternal birth trauma

Table 2.16 compares the incidence of perineal tear amongst the SA and WB mothers with diabetes in pregnancy.

Table 2:16: Comparison of perineal tear amongst SA and WB women.

		South Asian N = 160	White British N = 160	P – value
Perineal trauma	1 st degree tear	21 (13.1)	12 (7.5)	0.007
	2 nd degree tear	29 (18.1)	16 (10.0)	
	3 rd degree tear	7 (4.4)	2 (1.3)	
2 nd and 3 rd degree tear		36 (22.5)	18 (11.3)	0.011
Episiotomy		22 (13.8)	17 (10.6)	0.495

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

Figure 2.17: Bar chart comparing different grades of perineal trauma between SA and WB women.

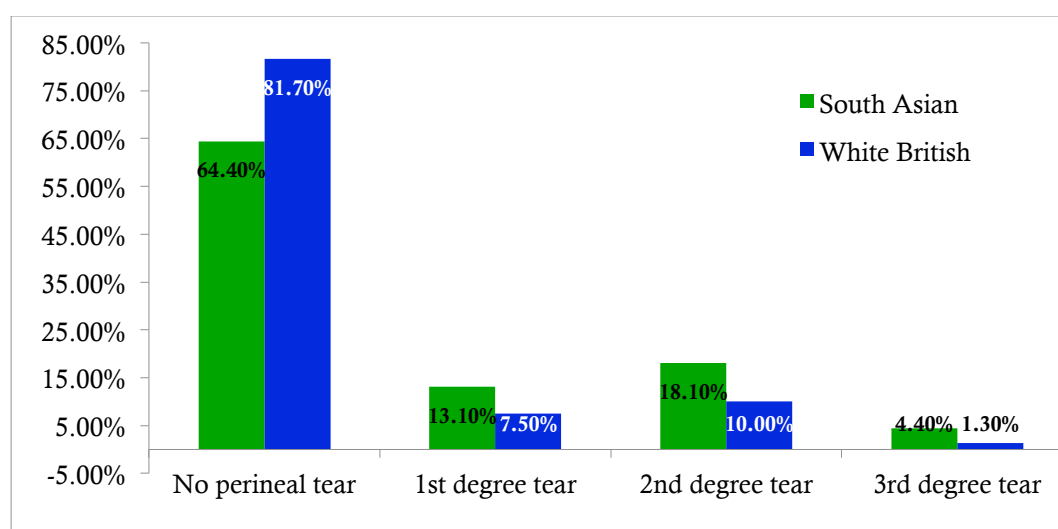


Fig 2.17 shows a bar chart to compare the different degrees of perineal tear in the SA and WB women. Chi-square testing showed that SA women had a significantly higher risk of all grades of perineal trauma as compared to WB women, $p = 0.007$. There was no difference in the rate of elective episiotomy between the two groups.

2.10.5 Characteristic of South Asian and White British infants

Characteristics of infants born to the SA and WB mothers with diabetes in pregnancy have been summarised in table 2.17. There were four stillbirths in the SA ethnic group (three women with GDM and one with T2DM) and they were excluded from further analysis, hence N = 156 in the SA ethnic group and N = 160 in the WB ethnic group. The four stillbirths had a median gestation at birth (IOR) of 31+1 (25+6 – 39+1) weeks and a median birthweight (range) of 1520 (590 – 2820) grams.

Table 2:17: Comparison of neonatal demographic characteristics.

		South Asian N = 156	White British N = 160	P – value
Birthweight (grams)**		3260 (2825– 3670)	3535 (3055 – 3975)	< 0.001
LGA (>97 th centile)		45 (28.8)	63 (39.4)	0.032
Head circumference (cm)**		34.40 (33.30 – 35.30)	34.50 (33.35 – 36.00)	0.086
Gestation**		38+6 (38+1 – 39+4)	38+3 (37+2 – 39+2)	0.112
Preterm (<37weeks gestation)		14 (9.6)	28 (17.5)	0.049
Sex	Male*	84 (53.8)	83 (51.9)	0.737
	Female*	72 (46.2)	77 (48.1)	
Stillborn		4	0	-
Congenital anomaly		8 (5.1)	11 (6.9)	0.264

Values for continuous variables presented as median (interquartile range);

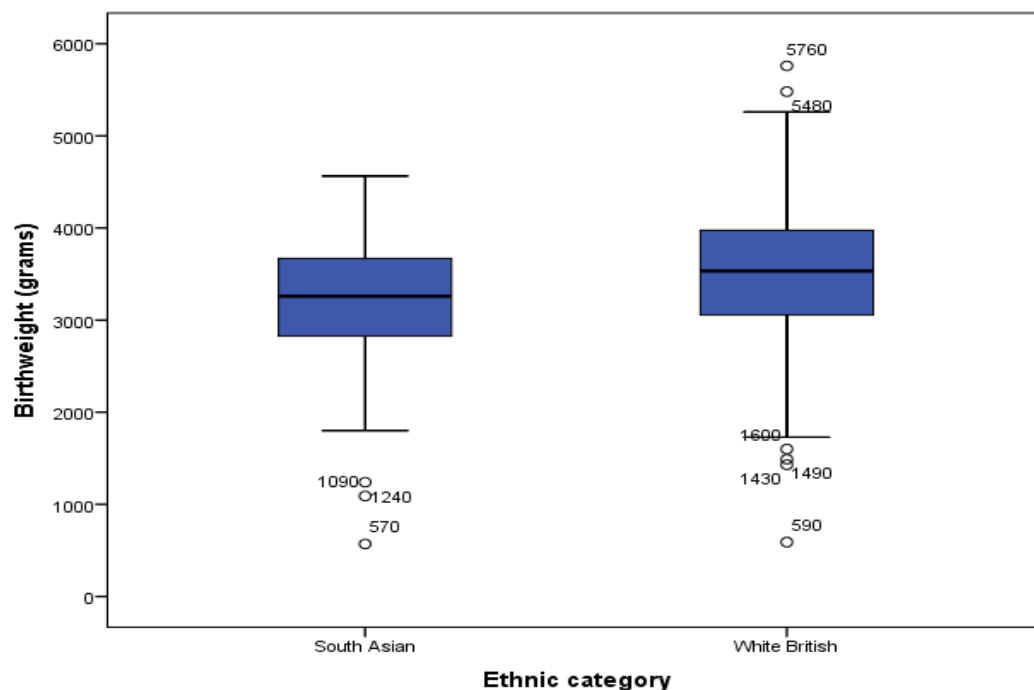
Values for categorical variables presented as n (%);

**Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance; **Mann-Whitney U test for continuous variables.*

2.10.5.1 Comparison of birthweight

Infants of SA mothers were significantly lighter with a median birthweight (interquartile range) of 3260 (2825 – 3670) grams as compared to 3535 (3055 – 3975) grams in the infants of WB mothers (fig 2.18).

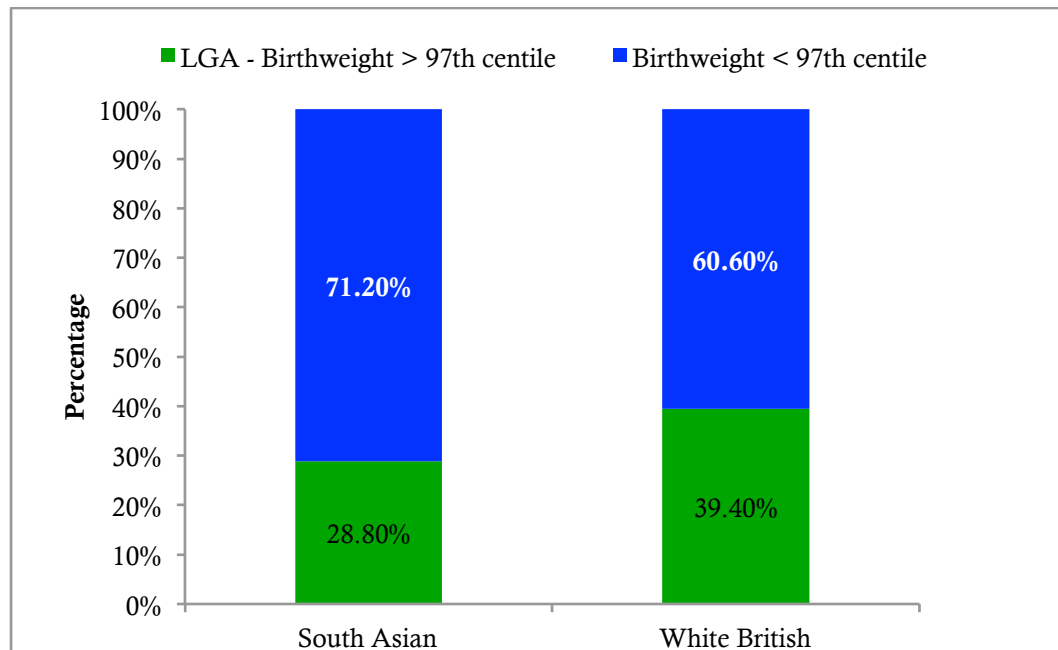
Figure 2.18: Box plot to show the distribution of birthweight between SA and WB infants.



Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers.

Mann-Whitney's U testing revealed that there was a significant difference in the birthweight between the SA infants and the WB infants, $p < 0.001$, $z = -3.560$, $r = 0.2$ (medium effect). A SA infant with an outlier birthweight of 570 grams (gestation 25+6) died within 22hrs after birth and was the only neonatal death in this study. A WB infant with an outlier birthweight of 590 grams was born at 27+0 weeks gestation and survived to discharge. Other low and high outlier birthweight values have been checked and they represent true birthweights. WB infants had a significantly higher rate of being born LGA (39.4% as compared to 28.8% in SA infants) odds ratio 1.602 (95% CI, 1.002 – 2.562), $p = 0.032$ (fig 2.19).

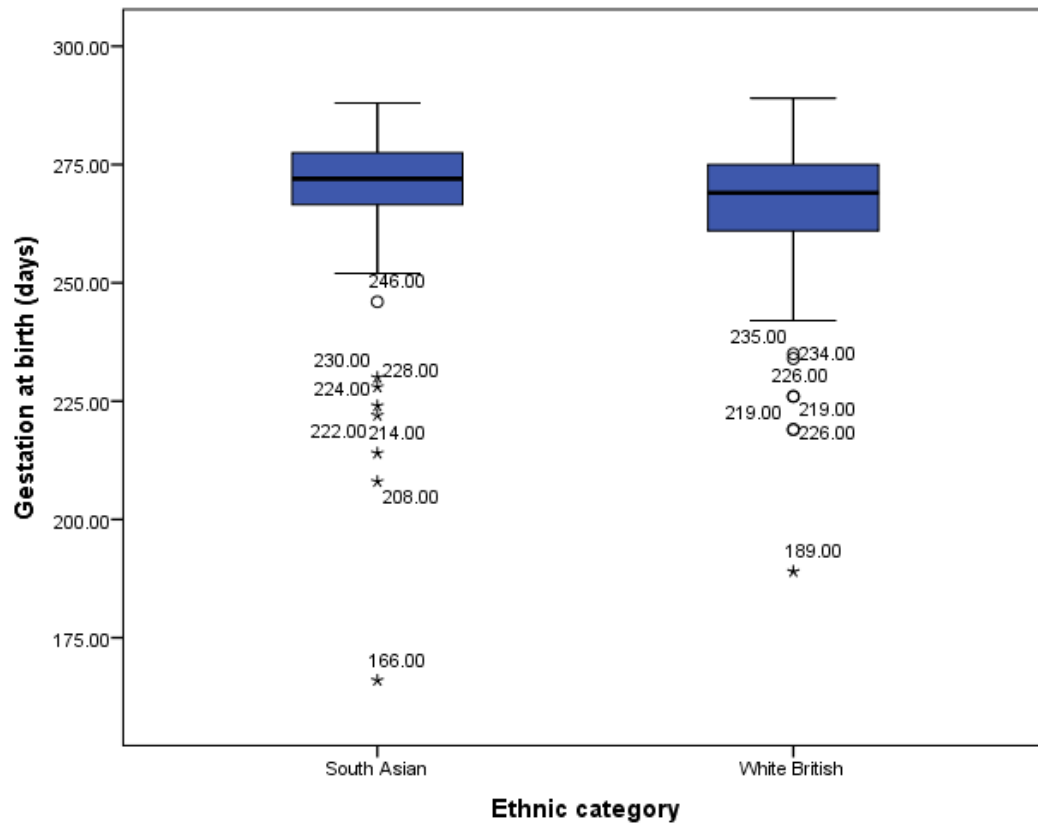
Figure 2.19: Stacked bar chart to show the comparison of LGA infants born to SA and WB mothers.



Measurements for head circumference were available in 117 SA infants and 128 WB infants. Mann-Whitney U testing revealed that there was no statistically significant difference in the head circumference between the SA infants (median = 34.4cm, n = 117) and WB infants (median = 34.50cm, n = 128), $p = 0.086$, $z = -1.716$, $r = 0.11$. Measurements for the head circumference were not available in the remaining infants due to early discharge before their routine newborn check usually at around 24 hours of age.

2.10.5.2 Comparison of gestation at birth and sex distribution

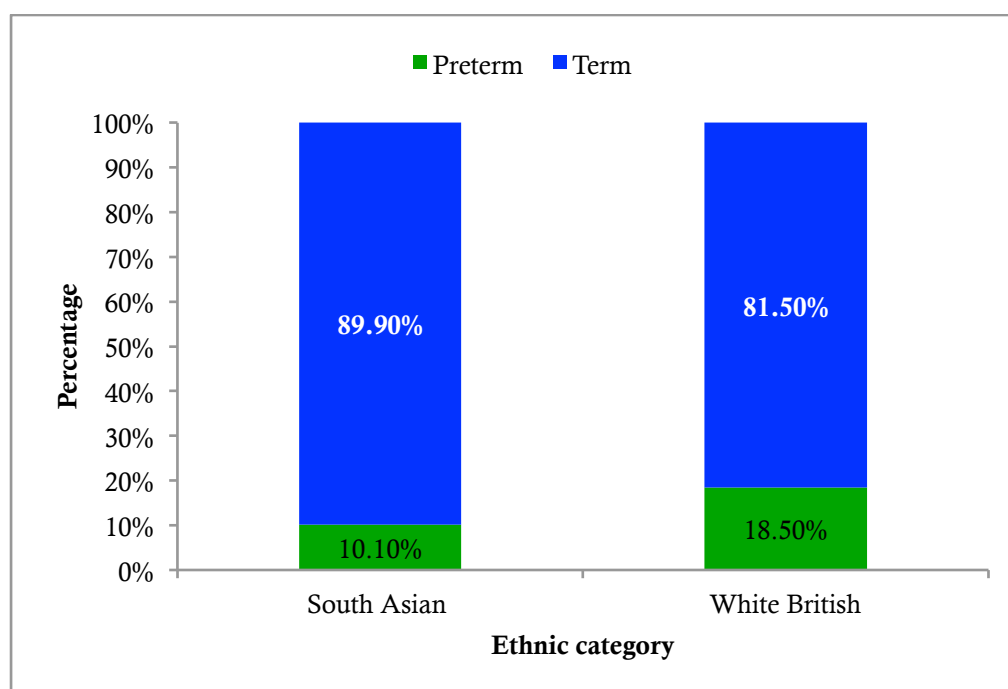
Figure 2.20: Box plot to show distribution of gestation at birth between SA and WB infants.



*Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values.*

There was a significant difference in the gestation at birth between the SA and the WB infants as revealed by Mann-Whitney U testing. SA infants were born at an older gestation (median = 38+6, n = 156) as compared to WB infants (median = 38+3, n = 160), $p = 0.005$, $z = -2.834$, $r = 0.16$ (small effect) (fig. 2.20).

Figure 2.21: Stacked bar chart to compare preterm births between the SA and the WB mothers.



WB infants were more likely to be born preterm 18.5% as compared to 10.1% in SA infants, however this difference did not reach a statistical significance, $p = 0.094$ (Fig 2.21). There was no difference in the sex distribution between the groups, $p = 0.737$.

There was no difference in the rate of congenital anomaly between the SA and WB infants 8 (5.1%) vs. 11 (6.9%), $p = 0.264$. One SA infants had major congenital anomaly (pulmonary stenosis) and two WB infants each had a major congenital anomaly (transposition of great arteries and truncus arteriosus).

2.10.6 Comparison of neonatal outcomes

The neonatal outcomes were compared in 156 SA infants and 160 WB infants (4 stillborn infants were excluded). The primary neonatal outcomes and the composite outcome in the SA and WB infants born to mothers with diabetes have been summarised in table 2.18. There was a significant difference in the composite outcome between the SA and the WB infants 29 (18.6%) vs. 51 (31.9%), $p = 0.009$. SA infants had nearly half the risk of adverse composite neonatal outcome, odds ratio (95% CI) 0.488 (0.289 – 0.823) as compared to WB infants.

Table 2:18: Comparison of primary neonatal outcome between SA and WB infants.

	South Asian (N = 156)	White British (N = 160)	P – value
Neonatal hypoglycaemia	13 (9.4)	24 (17.4)	0.076
NICU admission	18 (11.6)	34 (21.3)	0.023
Birth trauma	4 (2.6)	6 (3.8)	1.000
Neonatal death	1	0	-
Stillbirth	4	0	-
Composite outcome [▪]	29 (18.6)	51 (31.9)	0.009

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

[▪]*Composite outcome include NICU admission, neonatal hypoglycaemia, birth trauma, neonatal death and readmission*

There was one neonatal death amongst the SA infants and none in the WB group. This infant was born at 25+6 weeks of gestation by spontaneous vaginal delivery with a birthweight of 570 grams to a SA mother with insulin treated GDM. Other outcomes are discussed in detail in the subsequent sections.

2.10.6.1 Condition at birth and birth trauma

Table 2:19: Comparison of condition at birth and birth trauma between SA and WB infants.

	South Asian N = 156	White British N = 160	P – value
Apgar score at 1min**	9.00 (9.00 – 9.00)	9.00 (9.00 – 9.00)	0.723
Apgar score at 5min***	10.00 (9.00 – 10.00)	9.00 (9.00 – 10.00)	0.034
Arterial pH***	7.27 (7.21 – 7.30)	7.29 (7.23 – 7.32)	0.076
Venous pH***	7.31 (7.26 – 7.35)	7.32 (7.27 – 7.36)	0.103
Shoulder dystocia	3 (1.9)	5 (3.1)	0.723
Clavicular fracture	0	0	-
Erb's palsy	0	1 (0.6)	-
HIE	1 (0.6)	1 (0.6)	-
Birth trauma	4 (2.6)	5 (3.1)	1.000

Values for continuous variables presented as median (interquartile range);

Values for categorical variables presented as n (%);

**Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance; **Mann-Whitney U test for continuous variables.*

Mann-Whitney U testing revealed that there was no significant difference in Apgar scores at 1min between SA infants (median = 9.0, n = 156) and WB infants (median = 9.00, n = 160), p = 0.723. However it did show a significant difference in Apgar scores at 5 minutes between SA infants (median = 10.00, n = 155) and WB infants (median = 9.00, n = 157), p = 0.034, z = -2.123, r = 0.12. This difference of just 1 point in Apgar score at 5 minutes of age is of doubtful clinical significance. There was no significant difference in the rates of birth trauma between the SA and WB infants 2.6% vs. 3.1%, p = 1.000.

2.10.6.2 Neonatal hypoglycaemia

Table 2:20: Comparison of SA and WB infants with neonatal hypoglycaemia.

		South Asian (N = 13)	White British (n = 24)	P – value
Gestational age (<i>weeks</i>) **		38+1 (30.4 – 39+3)	38+1 (33+3 – 41+2)	0.672
Birthweight (<i>grams</i>) **		2780 (1240 – 3756)	3830 (2180 – 5480)	0.003
Maternal diabetes	GDM*	11/145 (7.6)	19/150 (12.7)	0.125
	Pre- gestational*	2/9 (22.2)	5/10 (50.0)	0.350
Blood sugar levels (mmol/L) **		1.75 (1.15 – 1.90)	1.8 (1.60 – 1.90)	0.441
Time to 1 st feed (hours)		1.16 (0.51 – 1.52)	1.09 (0.50 – 1.40)	0.139
Time to hypoglycaemia (hours)		5.45 (3.40 – 14.01)	4.16 (3.33 – 5.39)	0.062
Managed on PNW		8 (61.5)	14 (58.3)	1.000
NICU admission		5 (38.5)	10 (41.7)	1.000
Duration of NICU stay (<i>days</i>)**		9 (1 – 25)	2 (0 – 21)	0.165

Values for continuous variables presented as median (interquartile range);

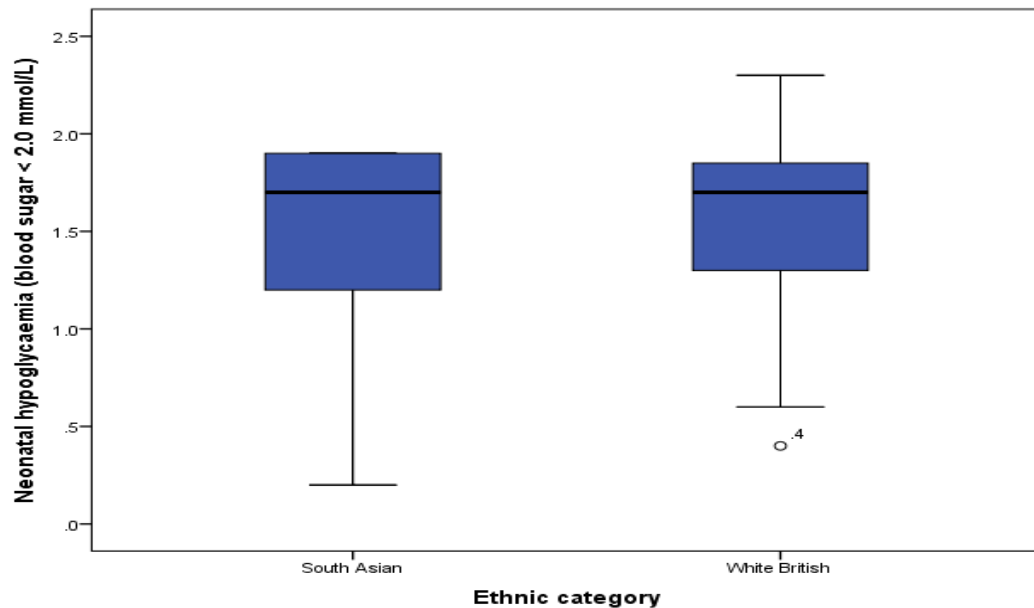
Values for categorical variables presented as n (%);

**Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance; **Mann-Whitney U test for continuous variables*

Neonatal hypoglycaemia was diagnosed in 13 (9.4%) SA infants as compared to 24 (17.4%) WB infants, $p = 0.076$. Although there was no statistically significant difference in the number of infants developing neonatal hypoglycaemia between the two groups, this complication occurred in nearly double the WB infants as compared to SA infants. WB infants had an odds ratio (95% CI) of 1.360 (1.046 – 17.85) for developing neonatal hypoglycaemia as compared to SA infants. Neonatal hypoglycaemia occurred more commonly in infants born to mothers with pre-gestational diabetes (T1DM and T2DM) 20% and 50% of SA and WB infants respectively as compared to infants born to mothers with GDM 7.6% and 12.7% of SA and WB infants respectively.

Mann-Whitney U test revealed that in the infants who developed hypoglycaemia, there was no difference in the blood sugar level between SA infants (median = 1.75 mmol/L, IQR = 1.15 – 1.9 mmol/L, n = 13) and WB infants (median = 1.80, IQR = 1.60 – 1.90, n = 24), $p = 0.441$, $z = -0.802$, $r = 0.17$.

Figure 2.22: Box plot to show the blood sugar levels in SA and WB infants who developed hypoglycaemia.



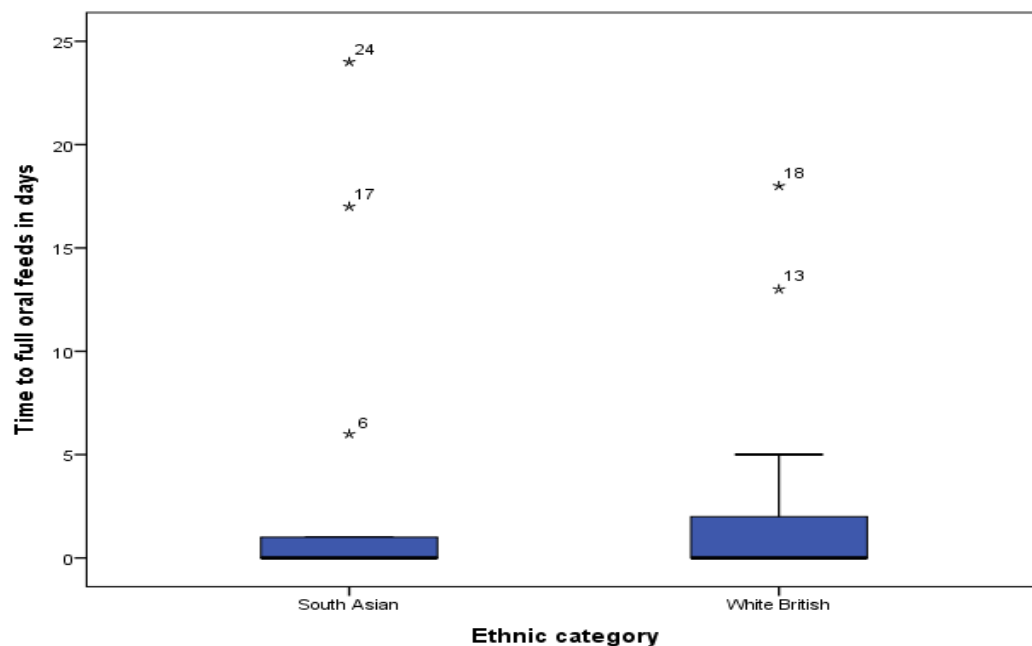
*Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ^o represents outliers and * represents extreme values.*

There was also no difference in gestational age between affected SA infants (median = 38.1 weeks, n = 13) and WB infants (median = 38+1, n = 24), $p = 0.672$, $z = -0.430$, $r = 0.07$. Mann-Whitney U testing did show that the SA infants who developed hypoglycaemia had a significantly lower birthweight (median = 2780 grams, n = 13) as compared to WB infants (median = 3830 grams, n = 24), $p = 0.003$, $z = -2.864$, $r = 0.47$. The box plot in Fig 2.22 shows the distribution of blood sugar levels in the hypoglycaemic SA and WB infants. There was no difference in the time taken establish full oral feeds in both the groups after the initial hypoglycaemia.

Of the 13 SA infants who developed hypoglycaemia, two infants were symptomatic with clinical hypoglycaemia and 11 infants had biochemical hypoglycaemia. Eight

SA infants were cared for on the postnatal ward. Their hypoglycaemia resolved with additional breast and bottle-feeding. Five SA infants needed admission to NICU (four born to mothers with GDM and one to mother with pre-gestational diabetes). Gestational age of SA infants admitted to NICU with hypoglycaemia ranged from 30+4 – 39+3 weeks with a median gestation of 38+1 weeks. In two infants hypoglycaemia resolved with additional bottle feeds, one SA infant with gestational age of 37+6 weeks, and birthweight of 1890 grams needed a 10% dextrose bolus followed by 10% and then a 12.5% dextrose infusion. Two SA infants needing admission to NICU were started directly on 10% dextrose infusion. The median duration of admission (range) was nine days (1 – 25 days). At the time of discharge, of the 13 SA infants in total, two infants were exclusively breast fed, 2 infants were exclusively bottle fed and nine infants went home with a mixed feeding plan.

Figure 2.23: Comparison of time taken to reach full oral feeds between SA and WB infants who developed hypoglycaemia.



Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values

Of the 24 WB infants who developed neonatal hypoglycaemia, six infants were symptomatic with clinical hypoglycaemia while 18 infants had biochemical hypoglycaemia detected on routine pre-feed blood sugar monitoring. 14 infants were managed on the postnatal ward and their hypoglycaemia resolved with additional breast and bottle feeding and they did not need NICU admission. 10 WB infants needed NICU admission (seven born to mothers with GDM and 3 born to mothers with pre-gestational diabetes). Six infants needed a bolus of 10% dextrose followed by maintenance 10% dextrose infusion. One infant born at a gestation of 38+5 weeks and birthweight of 5480 grams went on to require a 12.5% dextrose infusion to maintain blood sugars within the normal range. One infant was started on maintenance 10% dextrose infusion without a bolus and in three infants, hypoglycaemia resolved with additional bottle feeds on NICU. Median duration of admission (range) was 2 days (0– 18 days). At the time of discharge, of the total 24 infants, 5 infants were exclusively breast fed, 8 infants were exclusively bottle fed and 11 infants went home with a mixed feeding plan. None of the infants who developed hypoglycaemia had any difficulty with establishing oral feeds (no history of feed intolerance) (Fig 2.23).

2.10.6.3 Comparison of NICU admission

SA infants were more likely to receive their early neonatal care on the postnatal ward (PNW) 138 (89%) as compared to 126 (78.8 %) in the WB infants, $p = 0.023$. SA infants were significantly less likely to require NICU admission as already stated in section 2.4.5. The reasons for NICU admissions have been summarised in table 2.21 below.

Table 2:21: Comparison of the clinical reasons for NICU admission in SA and WB infants.

	South Asian N = 156	White British N = 160	P – value
PNW	138 (89.0)	126 (78.8)	0.023
NICU admission	18 (11.6)	34 (21.3)	0.023
Prematurity	6 (3.8)	17 (10.6)	0.031
Respiratory distress	9 (5.8)	20 (12.5)	0.038
Neonatal hypoglycaemia	5 (3.2)	10 (6.3)	0.076
Sepsis	15 (9.7)	26 (16.3)	0.095
Hyperbilirubinaemia	8 (5.2)	12 (7.5)	0.490
HIE	1 (0.6)	1 (0.6)	1.000
Hypocalcaemia	0	2 (1.3)	-
Hypomagnesaemia	0	0	-
Polycythaemia	0	0	-
Duration of hospital stay (days)	2 (1 – 3)	2 (1 – 3)	0.282

*Values for categorical variables presented as n (%);
Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.*

2.10.6.4 Prematurity

The SA infants admitted to NICU were less likely to be preterm (define as gestation < 37+0 weeks at birth) 6/18 (33.3%) as compared to 17/34 (50%) in the WB infants. Chi-square testing revealed a statistically significant difference between the SA and the WB infants, $p = 0.031$. WB infants needing admission to NICU had nearly double the risk of being preterm with an odds ratio of 1.994 (1.020 – 3.899) as compared to SA infants.

2.10.6.5 Presumed sepsis

The most common reason for NICU admission in both the ethnic groups was presumed sepsis and table 2.22 summarises the comparison between the SA and WB infants admitted to NICU with sepsis. It was noted in 15/18 (83.3%) of SA infants and 26 (76.5%) of WB infants, $p = 0.095$. The median (IQR) C-reactive protein (CRP) for SA infants was < 5 (<5 – 21.0) and for WB infants was < 5 (<5 – 19.5), $p = 0.664$. None of the SA infants had a positive blood or CSF culture. Two WB infants had positive blood cultures but none had a positive CSF culture. The median duration (IQR) for antibiotic treatment for SA infants was 4 (3 – 5 days) as compared to 3.5 (2 – 5 days) in the WB infants, $p = 0.904$.

Table 2:22: Comparison of SA and WB infants admitted to NICU with sepsis.

	South Asian N = 18	White British N = 34	P – value
Sepsis*	15 (83.33)	26 (76.5)	0.095
CRP (mg/L)**	<5 (<5 – 21.0)	< 5 (<5 - 19.5)	0.664
Positive blood culture*	0	2 (7.8)	0.524
Duration of antibiotics (days)**	4 (3 – 5)	3.5 (2 – 5)	0.904

Values for continuous variables presented as median (interquartile range);

Values for categorical variables presented as n (%);

**Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance; **Mann-Whitney U test for continuous variables.*

2.10.6.6 Respiratory distress

Respiratory distress was the second most common reason for NICU admission. 9/18 (50%) of the SA infants and 20/34 (58.8%) of the WB infants admitted to NICU had respiratory distress and Chi-square testing revealed a significant difference between the two ethnic groups, $p = 0.038$. The reasons for respiratory distress in SA infants as compared to WB infants were: respiratory distress syndrome in 44.4% vs. 40.0%, transient tachypnoea of newborn in 55.6% vs. 40.0%, congenital pneumonia and other diagnoses in 10% of WB infants respectively. The respiratory support required in the SA and WB infants admitted for respiratory distress is summarised in table 2.23.

Table 2:23: Comparison of respiratory support required in SA and WB infants admitted to NICU with respiratory distress.

	South Asian (N = 9)	White British (N = 20)	P- value
Respiratory support	4 (44.4)	12 (60.0)	0.688
FiO ₂	4 (44.4)	8 40.0)	0.516
FiO ₂ days	1.5 (1.00 - 22.25)	3.5 (2.25 – 5.75)	0.283
NCPAP	2 (22.2)	7 (35.0)	1.000
NCPAP days	6.5 (2.00 – 8.00)	2.0 (1.00 – 4.00)	0.500
Ventilation	1 (11.1)	7 (35.0)	0.569
Ventilation days	1.0	2.0 (1.00 – 6.00)	0.500
Surfactant treatment	1 (11.1)	7 (35.0)	0.371

Values for categorical variables presented as n (%);

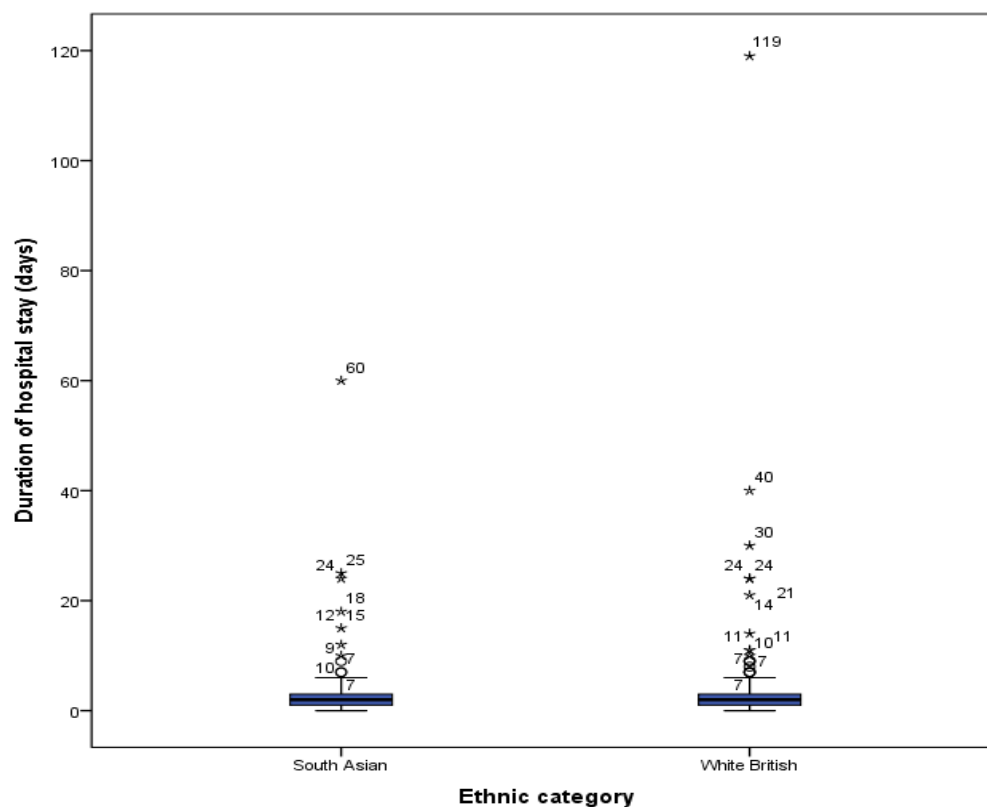
**Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.*

2.10.6.7 Neonatal hyperbilirubinaemia and other morbidities

There was no difference in the risk of hyperbilirubinaemia between SA and WB infants 8/18 (44.4%) vs. 12/34 (35.3%), $p = 0.490$. Hypocalcaemia was reported in 2/34 (5.8%) of WB infants and none in SA infants. The study infants were not routinely screened for hypocalcaemia, hypomagnesaemia and polycythaemia. There is a possibility that subclinical biochemical abnormalities were not identified in this retrospective study.

2.10.6.8 Duration of hospital stay

Figure 2.24: Box plot to compare the duration of hospital stay between the SA and the WB infants.



Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values.

Mann-Whitney U testing revealed that there was no statistically significant difference in the duration of hospital stay between the SA infants (median = 2 days,

n = 155, IQR = 1 – 3 days) and the WB infants (median = 2.0 days, n = 160, IQR = 1 – 3 days), $p = 0.282$, $z = -1.075$, $r = 0.06$. Fig 2.24 shows a box plot to compare the duration of hospital stay between the SA and WB infants. The outlier and the extreme values represent the longer duration of hospital stay in the infants born preterm in both the groups.

2.10.7 Comparison of readmission

There was no significant difference in the readmission rates between the SA infants 23 (14.83%) vs. 16 (10%) in the WB infants, $p = 0.232$ based on all the infants surviving to discharge. Mann Whitney U testing revealed that there was no statistical difference in the age at readmission between the SA infants (median = 12 days, n = 155) and WB infants (median = 5.5 days, n = 160 days), $p = 0.239$, $z = -1.202$, $r = 0.07$. The most common reason for readmission was related to physiological jaundice, none required treatment and the second most common reason was poor feeding and excessive weight loss after birth that was treated with additional top up formula feed in addition to maternal breast feeding. There were no neonatal complications related to maternal diabetes in pregnancy that were responsible for readmission.

2.10.8 Regression Analysis

Univariate analysis showed that there was a significant difference in the adverse composite outcome between SA and WB infants born to mothers with diabetes in pregnancy. The WB infants had almost double the risk of adverse composite outcome as compared to the SA infants. Various maternal, pregnancy-related and intrapartum confounding factors could have also contributed to this difference in the neonatal outcome seen in the two groups. The various maternal factors that could have influenced adverse neonatal outcome were maternal age, pre-pregnancy weight, height, pre-pregnancy BMI, ethnicity, smoking, alcohol consumption and parity. The various pregnancy-related and intrapartum factors that could have influenced adverse neonatal outcome were need for insulin during pregnancy to control diabetes, caesarean section and preterm birth. A binary logistic regression model for multilevel analysis was used to study the influence of various confounding factors stated above on composite adverse outcome in SA and WB infants born to mothers with diabetes in pregnancy. A binary logistic regression was used as it predicts the probability of an observation to fall into one of two categories of a dichotomous dependent variable based on one or more independent variables that can be either continuous or categorical.

2.10.8.1 Assumptions for binary logistic regression

Assumption 1: The dependent variable (adverse composite outcome) was measured on a dichotomous scale.

Assumption 2: The independent variables were either continuous or categorical.

Assumption 3: There was independence of observations and the dependent variable had mutually exclusive and exhaustive categories.

Assumption 4: There were a bare minimum of 15 cases per independent variable.

Assumption 5: The continuous independent variables were linearly related to the logit of the dependent variable. Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell (1962) procedure. This assessment confirmed that as the interaction term for the four continuous independent variables (maternal age, maternal weight, maternal height and maternal BMI) was not statistically significant, the original continuous independent variable was linearly related to the logit of the dependent variable as shown in table 2.24.

Table 2:24 Box-Tidwell (1962) procedure for the assessment of the linearity of the continuous variables with respect to the logit of the dependent variable

Variables in the Equation						
Step 1 ^a	B	S.E.	Wald	df	Sig.	Exp(B)
Maternal age	.469	1.333	.124	1	.725	1.598
Maternal weight	-4.328	3.200	1.829	1	.176	.013
Maternal height	3.604	5.994	.361	1	.548	36.745
Maternal BMI	11.301	7.563	2.233	1	.135	80933.754
Smoking (1)	-.346	.414	.699	1	.403	.707
Alcohol (1)	.073	.473	.024	1	.878	1.075
Ethnic group (1)	.967	.409	5.600	1	.018	2.629
Parity (1)	.837	.360	5.407	1	.020	2.309
In_Maternal_Age by Maternal_Age	-.107	.298	.129	1	.719	.898
In_Maternal_Weight by Maternal_Weight	.686	.492	1.939	1	.164	1.985
In_Maternal_Height by Maternal_height	-.493	.925	.284	1	.594	.611
In_MAT_BMI by MAT_BMI	-2.178	1.393	2.444	1	.118	.113
Constant	-194.025	219.106	.784	1	.376	.000

Assumption 6: Two or more independent variables were not highly correlated with each other and the data did not show multicollinearity.

Assumption 7: During regression analysis, the casewise diagnostic identified that there were 11 infants with studentized residuals greater than ± 2 standard deviations. These infants were reviewed in further detail and decision was made to include them in the analysis.

2.10.8.2 Results of regression analysis

2.10.8.2.1 Data coding

Case processing summary (table 2.25) showed that 311 cases (97.2%) were included in the analysis and 9 cases (2.8%) were missing.

Table 2:25: Summary of the cases included in the analysis

Case Processing Summary			
Unweighted Cases ^a		N	Percent
Selected Cases	Included in Analysis	311	97.2
	Missing Cases	9	2.8
	Total	320	100.0
Unselected Cases		0	.0
Total		320	100.0
a. If weight is in effect, see classification table for the total number of cases.			

2.10.8.2.2 Baseline analysis (Block 0, beginning block)

The beginning block was the first step of binary regression analysis. This step of the model just included the constant without any independent variables. It provided the best guess for the outcome without the influence of the independent variables. This information was later used as a comparison to the model with all the independent variables added. The model at this stage correctly identified 75.2% of the cases. (table 2.26). Table 2.27 shows that only constant was included in the model at this stage and table 2.28 shows the list of independent variables not included in the model.

Table 2:26: Classification table to show the prediction of the outcome without any independent variables.

Classification Table ^{a,b}					
	Observed		Predicted		
			Composite Outcome		Percentage Correct
			.00	1.00	
Step 0	Composite_Outcome	.00	234	0	100.0
		1.00	77	0	.0
	Overall Percentage				75.2
a. Constant is included in the model. b. The cut value is .500					

Table 2:27: Inclusion of constant in the model (without any independent variable)s.

Variables in the Equation							
		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-1.112	.131	71.578	1	.000	.329

Table 2:28: Independent variables not included in the model.

Variables not in the Equation					
			Score	df	Sig.
Step 0	Variables	Maternal_Age	1.434	1	.231
		Maternal_Weight	2.315	1	.128
		Maternal_Height	.830	1	.362
		Maternal_BMI	1.680	1	.195
		Ethnic Group (1)	8.161	1	.004
		Smoking (1)	.193	1	.660
		Alcohol (1)	.934	1	.334
		Parity	7.090	2	.029
		Parity (1)	5.480	1	.019
		Parity (2)	5.470	1	.019
		Insulin treatment (1)	4.593	1	.032
		Caesarean section (1)	15.021	1	.000
		Preterm birth (1)	6.934	1	.008
	Overall Statistics		34.126	12	.001

2.10.8.2.3 Model fit

Omnibus Tests of Model Coefficients provided the overall statistical significance of the model. Table 2.29 shows that the model was statistically significant with p value of 0.001. Another way of assessing the adequacy of the model was to analyse how poor the model was at predicting the categorical outcomes. This was done using Hosmer and Lemeshow test. Table 2.30 shows that the Hosmer and Lemeshow test was not statistically significant, $p = 0.129$, indicating that the model was not a poor fit.

Table 2:29: Omnibus tests of model coefficients.

Omnibus Tests of Model Coefficients				
		Chi-square	df	Sig.
Step 1	Step	34.324	12	.001
	Block	34.324	12	.001
	Model	34.324	12	.001

Table 2:30: Hosmer and Lemeshow test.

Hosmer and Lemeshow Test			
Step	Chi-square	df	Sig.
1	12.539	8	.129

2.10.8.2.4 Variance in the model

The explained variation in the dependent variable based on the model ranged from 10.4% to 15.5%, depending on whether the Cox & Snell R² or Nagelkerke R² methods were used, respectively.

Table 2:31 Model summary

Model Summary			
Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	313.791 ^a	.104	.155
a. Estimation terminated at iteration number 5 because parameter estimates changed by less than .001.			

2.10.8.2.5 Category prediction

The earlier classification table in section 2.10.8.2.2, which did not include any independent variables showed that 75.2% of cases overall could be correctly classified. However, with the independent variables added, the model now correctly classified 76.8% of cases (table 2.32). That is, the addition of the independent variables improved the overall prediction of cases into their observed categories of the dependent variable.

Table 2:32: Classification table to show prediction of the outcome with the independent variables.

Classification Table ^a					
	Observed		Predicted		
			Composite Outcome		Percentage Correct
			.00	1.00	
Step 1	Composite_Outcome	.00	227	7	97.0
		1.00	65	12	15.6
	Overall Percentage				76.8
a. The cut value is .500					

The sensitivity of the model was 15.6% and specificity was 97.0%. The positive predictive value was 63.2% and the negative predictive value was 22.3%.

2.10.8.2.6 Variables in the equation

A binary logistic regression was performed to ascertain the effects of maternal age, pre-pregnancy weight, height, BMI, ethnicity, smoking, alcohol consumption, parity, need for insulin treatment for diabetes in pregnancy, caesarean section and preterm birth on the likelihood of adverse composite outcome in their newborn infants. The logistic regression model was statistically significant, $\chi^2(4) = 34.3\%$, $p = 0.001$. The model explained 10.4% (Nagelkerke R²) of the variance in heart disease and correctly classified 76.8% of cases. Sensitivity was 15.6%, specificity was 97.0%, positive predictive value was 63.2% and negative predictive value was 22.3%. Of the abovementioned independent confounding factors only ethnicity was statistically significant. SA infants born to mothers with diabetes had a significantly lower risk of adverse composite outcome, adjusted OR (95% CI) 0.435 (0.215 – 0.883), $p = 0.021$ as compared to WB infants born to mothers with diabetes.

Table 2:33 Results of binary logistic regression.

Variables in the Equation								
Step 1 ^a	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP (B)	
							Lower	Upper
Maternal age	.016	.026	.408	1	.523	1.017	.967	1.069
Maternal weight	.082	.071	1.309	1	.253	1.085	.943	1.248
Maternal height	-.085	.076	1.245	1	.265	.919	.791	1.066
Maternal BMI	-.229	.194	1.394	1	.238	.795	.543	1.163
Ethnic group (1)	.830	.360	5.322	1	.021	2.294	1.133	4.644
Smoking (1)	-.335	.358	.872	1	.350	.715	.354	1.445
Alcohol (1)	-.047	.399	.014	1	.907	.954	.437	2.087
Parity			3.097	2	.213			
Parity (1)	-.470	.352	1.787	1	.181	.625	.313	1.245
Parity (2)	.056	.455	.015	1	.901	1.058	.434	2.582
Insulin treatment (1)	.453	.292	2.403	1	.121	1.573	.887	2.789
Caesarean Section (1)	.625	.347	3.238	1	.072	1.868	0.946	3.688
Preterm Birth (1)	.741	.430	2.967	1	.085	2.097	.903	4.871
Constant	11.65	12.361	.889	1	.346	115060.123		

2.11 Summary of the results

1. SA mothers were significantly younger, had a lower weight and BMI at the time of antenatal booking of the current pregnancy and were more likely to be within the normal BMI range as compared to WB mothers. They also had a significantly lower history of smoking and alcohol consumption at the time of antenatal booking as compared to WB mothers.
2. The SA mothers were diagnosed to have GDM 10 days earlier as compared to WB mothers. The SA mothers were less likely to require insulin for GDM and their fetuses were significantly less likely to be large for gestational age (LGA).
3. The SA women were more likely to have a vaginal delivery but they had a significant high risk of perineal trauma as compared to WB mothers.
4. SA infants had nearly half the risk of adverse composite neonatal outcome as compared to WB infants. They were significantly less likely to be preterm, LGA and need NICU admission as compared to WB infants.

3 CHAPTER

3.1 Introduction

A global increase in the prevalence of large for gestational age (LGA) infants has been reported in many developed and even developing nations. This increase in prevalence of LGA infants has been attributed to an increase in maternal age, pre-pregnancy weight, BMI, decreased smoking and improved standards of living. Pre-gestational or gestational diabetes increases the risk of delivering LGA infants and this has been extensively studied and hence infants of mothers with diabetes in pregnancy have a different care pathway. However a significant proportion of LGA infants are born to mothers without known diabetes in pregnancy. There is no consensus regarding their postnatal management with considerable intra and inter-hospital variation. In this chapter, I aim to provide details regarding changing trends in the prevalence and postnatal outcomes of LGA infants born to mothers without diabetes in pregnancy and to highlight the areas of controversy that have led to my choice of the subject as an area for research.

3.2 Background and Literature review

3.2.1 Definition of Large for Gestational Age

The term Large for Gestational Age (LGA) implies excessive fetal growth resulting in a birthweight, which is above the average or expected birthweight for gestation, sex and ethnicity. There is wide variation in the definitions used for the diagnosis of LGA infants. The most commonly used definition is birthweight more than 90th centile for gestation and sex. However some authors suggest restricting the diagnosis of LGA to infants with a birthweight more than 97th centile for gestation and sex. The 97th birthweight centile cut off has better risk prediction and helps to identify infants who are at the greatest risk of perinatal morbidity and mortality (208, 209).

The term 'LGA' is used interchangeably with the term macrosomia to describe the characteristic appearances in term and post term infants, which are similar to those seen in infants of diabetic mothers. Fee et al in 1963 described macrosomic infants of diabetic mothers as 'infants that are physically large in a manner that is inconsistent with gestational age, appear at first glance to be oedematous, plethoric and have a peculiar facial appearance akin to that seen in patients with Cushing Syndrome' (210). There is no consensus regarding the definition of macrosomia and so it is defined arbitrarily by using birthweight, resulting in wide variation in the interpretation and clinical application of the term macrosomia. Some clinicians define it as a birthweight more than 4000 grams irrespective of gestation (coincides approximately with birthweight around the 90th centile in a male infant born at 40 weeks of gestation) or as birthweight more than 4500 grams irrespective of gestation (coincides approximately with birthweight around the 97th centile in a male infant born at 40 weeks of gestation) (211). In a population with normal birthweight distribution the prevalence of LGA (birthweight >4000grams) should account for about 7-10% and LGA (birthweight >4500grams) should account for about 3-5%. In areas with a high prevalence of GDM, an incidence of LGA as high as 10-33% has been reported (212-214). Some authors also suggest grading macrosomia according to birthweight suggesting it is more predictive of neonatal complications and outcomes as outlined in the table 3.1 (209).

Table 3.1: Classification of LGA infants into different grades.

Grade I	Birthweight > 4000grams	Higher than usual risk of labour and neonatal complications
Grade II	Birthweight > 4500grams	More predictive of neonatal morbidity
Grade III	Birthweight > 5000grams	More predictive of neonatal mortality

The American College of Obstetricians and Gynaecologists (ACOG) recommends the use of a birthweight of 4500grams irrespective of gestation and sex as a cut-off to diagnose macrosomic infants, since neonatal morbidity and mortality increases sharply beyond this birthweight (215). Although the terms LGA and macrosomia are used interchangeably, not all LGA infants are macrosomic (infants who have a higher birthweight due to constitutional and genetic reasons) and not all macrosomic infants are LGA (infants who may have not crossed the 90th or the 97th birthweight centile but have achieved a much higher growth velocity due to excessive fat deposition as compared to lean mass).

3.2.2 Types of LGA/macrosomic infants

1. Physiological LGA/macrosomia

A subset of LGA neonates are physiologically big due to constitutional factors and have a higher birthweight due to proportionate increase in both fat and lean mass. These are symmetrical LGA infants with both birthweight and head circumference on similar centiles. In a normal healthy population this accounts for 70% of cases of LGA and is due to genetic factors and not excessive supply of nutrients in utero (212).

2. Pathological LGA/macrosomia

This subset of LGA infants are pathologically big and have an increased birthweight due to a disproportionate increase in fat mass as compared to lean mass due to fetal over nutrition which occurs throughout pregnancy and significantly increases in the third trimester. They have an increase in thoracic and abdominal circumference, which is relatively greater than head circumference (214). These are asymmetrical LGA infants and constitute about 30% of LGA infants. Metabolic derangements due to maternal high pre-pregnancy weight, BMI, increased weight gain in pregnancy above the recommended range and maternal pre-gestational and gestational diabetes are considered the biggest risk factors for pathological LGA (65, 215, 216).

3.3 Prevalence of macrosomia

Globally there has been a gradual increase in the average birthweight of newborn infants in both developed and developing nations resulting in a population-level shift to the right of the whole birthweight distribution at. In parallel to this, there has been a 15 – 25% increase in the prevalence of LGA or macrosomic infants in the last two to three decades with a corresponding decrease in small for gestational age infants (216, 217). During the last decade many countries across Europe, the United States, Australia and Asia have reported an increase in the prevalence of macrosomia and LGA infants.

3.3.1 Prevalence of macrosomia in Europe:

Power et al, in an epidemiological study, reviewed trends in birthweight of infants born in England, Wales and Scotland. During this period, in Scotland, the proportion of macrosomic infants (birthweight > 4000grams) increased from 7.7% in 1975 to 11.69% in 1992 (52% increase) accounting for a 0.40% increase each year (95% CI 0.37% to 0.44%) and in England and Wales from 8.63% in 1983 to 9.03% in 1986 (4.6% increase) accounting for a 0.35% increase each year (95% CI 0.11% to 0.60%). The major contributors for increasing birthweight were thought to be secondary to the intergenerational influence of improved socio-economic conditions, higher maternal height which is a reflection of better nutrition and decreased rates of smoking (218).

Gyselaers et al, in their epidemiological study of singleton pregnancies as registered by the Study Centre for Perinatal Epidemiology in Brussels reported an 18% increase in prevalence of macrosomia in Flanders from 7.3% in 1991 to 8.63% in 2010 ($p < 0.0001$). The increase in mean birthweight of all term singletons was 2.1grams/year (0.06%); however, amongst infants delivered by caesarean section for obstructed labour the increase was significantly higher at 17.4grams/year (0.52%) (219).

Schack-Nielsen et al undertook a study of national birthweight data from The Danish Medical Birth Registry, which included all single live births in Denmark from 1973 to 2003 ($n = 1,863,456$). Mean birthweight increased by approximately 160 grams during the study period, which represented an increase in birthweight of 5 grams/year for both boys and girls. The prevalence of macrosomia had nearly doubled over three decades (220).

3.3.2 Prevalence of macrosomia in Canada

Kramer et al analysed a hospital-based cohort of 61,437 singleton infants born between 22 and 43 weeks of gestation to describe temporal trends in birthweight, LGA (birthweight $>90^{\text{th}}$ centile) and SGA (birthweight $< 10^{\text{th}}$ centile) infants. From 1978 – 79 to 1994 – 96 there was an increase in birthweight from 3419 to 3476 grams ($p<0.001$), LGA from 8% to 11.5% ($p<0.001$) and a decrease in SGA from 11.1% to 7.2% ($p<0.001$) (216).

Wen et al reported a substantial increase in the birthweight of singleton infants in Canada from 1981 to 1997 using data from the Canadian Birth Database of Statistics. The percentage of LGA infants (birthweight $\geq 90^{\text{th}}$ centile) increased from 8.71% in 1981-83 to 10.03% in 1995-97 resulting in a 15.2% increase ($p<0.001$). The percentage of very LGA infants (birthweight $\geq 97^{\text{th}}$ centile) increased from 2.43% in 1981-83 to 2.95% in 1995-97 resulting in a 21.4% increase ($p<0.001$) (221).

3.3.3 Prevalence of macrosomia in Australia

Lahmann et al, in their study explored temporal trends in birthweight in Australia using Queensland Perinatal Data for singleton livebirths from 1988 to 2005 ($n = 830,231$). Mean birthweight increased by 1.9 grams/year. Similarly, the proportion of macrosomic infants also increased significantly from 12.4% in 1988-89 to 13.85% in 2004-05 (12% increase, $p < 0.01$) in non-indigenous infants. In indigenous infants there was a non-significant increase in macrosomic infants from 8.85% in 1988-89 to 9.35 in 2004-05 (6% increase) (222).

Hadfield RM et al undertook a population-based study of 1273,924 live-born singletons using data obtained from the New South Wales Midwives data collection system. From 1990 to 2005 there was an 18% increase from 9.2% to 10.8% in male LGA infants and a 21% increase from 9.1% to 11.0% in female LGA infants. This increase in LGA infants could only be partly explained by a decrease in maternal smoking, increasing maternal age and gestational diabetes (223).

3.3.4 Prevalence of macrosomia in Asia

Lu et al in their population based survey using data from the Perinatal Health Care Surveillance System in 12 cities in southeast China which included 594,472 term singleton births, reported an increase in LGA from 6.0% in 1994 to 8.49% in 2000 which then plateaued at 7.83% in 2005 (224).

The Chinese National Health Services in their combined report with the office of the WHO in China and the Social Development of China State Council Development Research Centre reported an increase in mean birthweight in three 5-year time periods from 3186grams in 1993 to 3284grams in 1998 and to 3307grams in 2003 (225).

Hence globally, in both developed and developing nations, there has been an increasing trend in the prevalence of LGA infants. This can partly be explained by an intergenerational improvement in general health, nutrition and environment resulting in improved maternal weight and height and a decrease in smoking rates. However the rise cannot be entirely explained by these factors and additional factors such as a global increase in the prevalence of obesity, maternal pre-pregnancy weight and BMI, maternal weight gain during pregnancy and an increase in pre-gestational and gestational diabetes are thought to play a role in this change.

3.4 Predisposing factors for fetal overgrowth in infants born to non-diabetic mothers

3.4.1 Non-modifiable factors

3.4.1.1 Race and ethnicity

Ethnicity has been shown to have an independent influence on birthweight across the entire range of birthweight centiles due to genetic or constitutional factors. In the UK, Caucasian infants are on an average 250 – 350 grams heavier than SA infants and hence a higher proportion of Caucasian infants will be classified as LGA as compared to South Asian infants (226). In a retrospective review Xiang et al compared the rates of LGA infants in various ethnic groups all born to diabetic mothers in the US. They reported that the non-Hispanic blacks had the highest risk of delivering LGA infants (17.2%) followed by pacific islanders (16.2%), Hispanics (14.5%), non-Hispanic Whites (13.1%), Asian Indians (12.8) and was lowest amongst ‘other Asians’ (9.6 – 11.1%) after controlling for various maternal confounding factors which can influence birthweight(227). Similarly, Bowers K et al in a retrospective review of 105,985 pregnancies in the Consortium on Safe Labour in the US from 2002-2008 showed that non-Hispanic white, non-Hispanic black, Hispanic and Asian women had a three factor (pre-pregnancy weight, gestational weight gain and GDM) joint effect risk of delivering a LGA infant of 11.27 (8.4 – 15.11), 7.09 (4.81 – 10.45) 10.19 (6.84 – 15.19) and 5.14 (2.11 – 12.50) respectively(228). Hence after controlling for various maternal confounding factors infants belonging to certain ethnic groups have a higher risk of being born LGA.

3.4.1.2 Maternal age

Worldwide there has been a gradual increase in maternal age at the time of first pregnancy. There is also a direct relationship between maternal age and birthweight (229). The presence of other risk factors such as higher pre-pregnancy weight, BMI and parity further amplify the age-related effects on fetal growth. A five-year

cohort study conducted by Najafian et al in Iran showed that about 60% of macrosomic infants were born to mothers above the age of 35 years(230). Li Yi et al in their 18-month retrospective study from China reported that mothers of macrosomic infants were older by a mean of 2 years as compared to mothers of normal neonates ($p<0.001$) (231). Kramer et al in their analysis of temporal trends in birthweight in Canada reported that over an 18 year period the increase in birthweight and prevalence of LGA infants was associated with a decrease in teenage pregnancy from 4.4% in 1978 – 79 to 1.0% in 1994 – 96 whereas births to women above 35 years of age nearly tripled from 7.8% to 20.1%(216).

3.4.1.3 Parity

There is a linear relationship between parity and LGA/macrosomic infants. Macrosomic infants with a birthweight ≥ 5000 grams have twice the odds of being born to a multiparous women as compared to an infant with birthweight appropriate for gestational age(209). Similarly, Alsammani et al in their study from Saudi Arabia, also showed that multiparity was significantly associated with adverse pregnancy outcomes and had an OR of 1.67 (1.00 – 2.80) for delivering a macrosomic infant(232). This relation to maternal parity persists even after controlling for various factors including maternal weight, BMI, pregnancy weight gain and GDM.

3.4.1.4 Maternal height

Increase in maternal height is an intergenerational indicator of improving maternal nutrition and quality of life. These improved environmental factors result in better fetal nutrition and growth and attainment of higher birthweight in the offspring. Many studies related to birthweight have shown that mothers of LGA/macrosomic infants are taller as compared to mothers of appropriate for gestational age infants. Kramer et al reported that an increase in birthweight and the prevalence of LGA over an 18-year period was associated with a significant decrease in maternal short stature ($<157.5\text{cm}$) from 33.5% to 24.4% and an increase in tall stature ($>135\text{cm}$) from 24.4% to 32.7%(216).

3.4.2 Modifiable factors

3.4.2.1 Maternal pre-pregnancy weight

Fetal LGA/macrosomia, which is one of the main complications seen in overweight women, increases by 2-3 fold in obese women as compared to normal weight women(233). Spellacy et al, in their two year retrospective review of 33,545 births, concluded that the risk of macrosomia increased by 3.7 fold in women with a pre-pregnancy weight of $\geq 90\text{kg}$ and 5.8 fold in women with a pre-pregnancy weight of $\geq 112.5\text{kg}$ (234). Okun et al, in their retrospective review of a birth cohort from the United States from 1995 – 97 also showed a 1.5 fold increase in macrosomia per 15 kg increase in maternal pre-pregnancy weight(235). A more recent meta-analysis showed a prevalence of macrosomia of 13.3% and 14.6% in obese and morbidly obese women respectively as compared to 8.3% in normal weight women(236). Although several factors influence excessive fetal weight gain, the prevailing evidence suggests that maternal obesity is a major factor resulting in fetal overgrowth(233, 237, 238).

3.4.2.2 Maternal weight gain in pregnancy

Another important factor that influences fetal growth is maternal weight gain during pregnancy. DeVader SR et al conducted a two-year population-based cohort study of term singleton infants in Missouri using birth data from 1999 – 2001. They showed that women gaining more than recommended weight of 25 – 35lbs during pregnancy had a 2.5 times higher risk of delivering an LGA infant compared to women with normal weight gain(239). Okun et al, in their retrospective review of a birth cohort from 1995 – 97 in the United States showed a similar result with 1.7 fold increase in macrosomia per 7kg increase in maternal weight during pregnancy (235). A systematic review of outcomes according to maternal weight gain by Siega-Riz et al similarly showed a 2 – 2.5 fold increase in the rate of LGA births in mothers with gestational weight gain above that recommended by the Institute of Medicine in a dose-dependent manner(240).

3.4.2.3 Gestational diabetes

Maternal diabetes in pregnancy is the most extensively studied factor in relation to fetal growth. Maternal hyperglycaemia due to diabetes leads to fetal hyperglycaemia and fetal hyperinsulinism, which in turn results in fetal visceral and soft tissue overgrowth resulting in fetal macrosomia due to the anabolic action of the hormone insulin. This has been extensively discussed in chapter 1.

3.4.2.4 Socio-economic status

Improvement in maternal weight and height are thought to be due to an improvement in maternal nutrition secondary to an improvement in living standards, access to healthcare, maternal education and individual and national economic prosperity. These changes have been noted in both developed and developing nations and have been attributed to the global increase in the average birthweight and LGA infants and a decline in small for gestational age infants. (216).

3.4.3 Universal vs. selective risk factor based screening

The current NICE guideline on diabetes in pregnancy recommends risk factor based selective screening for the diagnosis of GDM as outlined in chapter 1 (65). The American College of Obstetrics and Gynaecologist also recommends risk factor based selective screening (241). On the contrary, International Association of Diabetes in Pregnancy Study Group (IADPSG, endorsed by the American Diabetes Association) recommends universal screening for GDM in all women in pregnancy (242). The current screening recommendations by the various associations are based on observational studies which do not provide evidence that any particular method is optimal in preventing short term and long term adverse outcomes and is cost-effective(66). Marquette et al prospectively provided universal GDM screening to 434 pregnant women. A prevalence of 3.3% for GDM was noted in 178 women with risk factors and 2.4% in 256 women without risk factors. They reported a sensitivity of only 50% and specificity of 58% with risk factor based selective screening (243). Helton et al retrospectively reviewed their practice of universal screening for GDM in 595 pregnant women from 1988 – 1993. They reported that risk factor based selective screening would have sensitivity of 69% and specificity of 68% (244). Weeks JW et al conducted a prospective review of their practice for universal screening for GDM from 1990 – 1992. Risk factor based selective screening would have failed to identify 43% gestational diabetic women. 28% of the women, who would have been missed, required insulin treatment for their GDM (245). Moses R et al in a large Australian study where 1185 consecutive women were given 75gram OGTT reported that historically used risk factors would have identified only 60.8% women with GDM. 39.2% women would have been missed and low risk women had a GDM prevalence of 4.8% (246). A further two studies by O’Sullivan JB et al and Coustan DR et al similarly showed that risk factor based selective screening would have identified only 50% of women with GDM(247, 248). In the retrospective review of the data from the ATLANTIC DIP study, Avalos et al examined risk factor prediction using different combinations of risk factors in a mainly European population who were offered universal screening. They found that only 54 – 76% of women had at least one risk factor but the remaining women with GDM did not have any risk factors. The prevalence of

GDM amongst women with no risk factors ranged from 2.7% - 5.4% if the NICE recommended risk factor based screening criteria had been applied, 20% of women with GDM would remain undetected (249).

While risk factor based selective screening continues to be used, there is a potential risk that as many as 50% of women at risk of GDM will remain undetected and therefore untreated. These women are at risk of delivering an LGA or macrosomic infant with its associated pregnancy, perinatal and neonatal complications. There is some evidence that undiagnosed women with GDM who remain untreated are at higher risk of complications as compared to treated mothers with GDM and control mothers. A study by Langer Oded et al compared 555 women with a late diagnosis of GDM at 37 weeks and hence were untreated to 1110 treated women with GDM and 1110 non-diabetic control subjects (universal screening with 50 gram oral glucose challenge test was provided to all pregnant women). The composite adverse outcome in the infants was 59% in untreated GDM, 18% in treated GDM and 11% in control infants. Risk of LGA and hypoglycaemia was 29% and 18%, OR 3.28 (2.53 – 4.6) and 10.38 (6.51 – 16.56) in untreated GDM as compared to 11% and 6% OR 1.06 (0.81 – 1.38) and 2.98 (1.84 – 4.84) in treated GDM and 11% and 2% in control subjects respectively(250). A previous study by the same author compared 42 women with normal OGTT – controls to 42 women with abnormal OGTT - treated for GDM and 42 women with only one abnormal value on OGTT and hence not treated for GDM. The rate of LGA infants was significantly higher in the untreated group with a single abnormal value on OGTT, 34% as compared to 9% in the controls and 12% in the treated GDM group ($p<0.01$). Similarly, the infants in the untreated group had a significantly higher risk of neonatal hypoglycaemia 15% as compared to 3% in the control and the GDM treated group (251). In a similar study by Lindsay et al, 139 women with one abnormal value on OGTT who were not treated were compared to 725 control women with normal OGTT. The incidence of macrosomia was 18% in the study group, which was significantly greater than 6.6% in the control group, adjusted OR 2.55 (1.44 – 4.52) (252). In a retrospective review of 5,500 women who were mainly European and were universally screened and treated for GDM, Avalos et al reported that low risk women with GDM had a higher risk of composite adverse maternal and neonatal

outcomes 42% vs. 31% and 13% vs. 9% respectively when compared to normal control women without GDM(249).

Universal screening as recommended by IADPSG, although now being increasingly adopted, is not applied in clinical practice in all countries and the UK continues to provide risk factor based selective screening for the diagnosis of GDM as recommended by NICE guidelines(253). There is no clear evidence from well-conducted prospective studies to prove that identifying these extra cases of GDM in low risk women would help to improve short-term perinatal outcomes and long-term maternal and neonatal outcomes. There is no evidence that this would be cost-effective and on the contrary it is believed that universal screening would increase anxiety in a large number of low risk women and the test uptake rates in low risk women would be poor(254, 255). Hence until further evidence is available, risk factor based selective screening as recommended by the updated NICE guidelines continues to be the gold standard in the diagnosis and management of women with GDM in pregnancy in the UK.

In conclusion, the above mentioned studies show that risk factor based selective screening can miss up to 40% – 50 % of pregnant women with GDM and these women are at increased risk of adverse maternal and neonatal outcomes. These women, remain undiagnosed and untreated and are therefore at risk of delivering a LGA/macrosomic infant. These LGA infants born to apparently non-diabetic mothers are at an increased risk of adverse neonatal outcomes compared to infants born to mothers following a normal, low risk pregnancy and have a similar risk of neonatal complications as infants of known diabetic mothers.

3.5 Neonatal complications in LGA/macrosomic infants:

The literature in relation to neonatal outcomes in LGA/macrosomic infants provides evidence for increased risk of perinatal and neonatal morbidity and mortality in this group of infants as compared to normal weight infants born to mothers following a low risk pregnancy. However the overall incidence of complications varies from study to study. Some studies report complication rates similar to normal weight infants born to non-diabetic mothers (control infants) (256, 257) and some report complications similar to macrosomic infants of diabetic mothers (258). Most of the other studies report neonatal complication rates in LGA infants, which fall between the two ends of this spectrum. There is convincing evidence that infants born with a birthweight of 4000 grams or more irrespective of their gestational age are at a higher risk of neonatal complications. This risk continues to increase linearly as birthweight increases with an exponential increase in complications beyond a birthweight of 5000grams(259). These infants are at increased risk of neonatal mortality and morbidity: birth trauma such as shoulder dystocia, brachial plexus injury, clavicular fracture, humeral fracture and hypoxic ischaemic encephalopathy, neonatal hypoglycaemia, neonatal hyperbilirubinaemia, increased risk of NICU admission and readmission following initial discharge home due to poor feeding, higher than expected weight loss and hyperbilirubinaemia.

In the absence of universal screening for GDM, there is a high likelihood that some of these infants are born to mothers with undetected GDM or mothers with glycaemic derangements below the diagnostic threshold for GDM. Hence the cohort of LGA infants would be comprised of both physiological LGA and pathological LGA infants, which are difficult to distinguish from each other at birth.

It has been well established that macrosomic infants born to mothers with diabetes in pregnancy are at a significantly higher risk of neonatal morbidity and mortality and hence they should be monitored closely after birth in the hospital setting to enable early identification and treatment of complications. However, the majority of LGA/macrosomic infants are born to non-diabetic mothers and there is

conflicting evidence regarding management of these infants. The following discussion will focus on neonatal outcomes in LGA infants born to non-diabetic mothers.

3.5.1 Neonatal complications in LGA/macrosomic infants of non-diabetic mothers

Linder N et al in an 11 year retrospective review compared 2766 singleton term macrosomic infants to 2766 matched control infants, both born to non-diabetic mothers. A significantly higher proportion of macrosomic infants were admitted to NICU 3.6% vs. 2.1% ($p<0.001$) OR 1.78 (1.27 – 2.53) with a composite of adverse outcome of 11.7% vs 8.0% ($p<0.001$) OR 1.53 (1.27 – 1.83) as compared to controls. The main complications were neonatal hypoglycaemia OR 2.37 (1.23 – 4.81), transient tachypnoea of newborn OR 2.83 (1.53 – 5.50) and birth trauma OR 3.0 (1.78 – 5.62). The macrosomic infants who developed complications had a higher mean birthweight of 4232 grams vs. 4192 ($p<0.002$) as compared to the macrosomic infants who did not develop complications. There was a direct relationship between hypoglycaemia and birthweight. One of the major limitations of this study was that it is a retrospective review and did not clarify how pregnant women were screened for GDM and what diagnostic thresholds for blood sugar were used(259).

Onal E et al, retrospectively compared 613 term LGA Turkish infants born to non-diabetic mothers to 87 term infants born to diabetic mothers. LGA infants of non-diabetic mothers had significantly lower incidence of hypoglycaemia at 1 hour of age 5.3% vs. 12.8% ($p=0.014$) and polycythaemia 3% vs. 9.3% ($p=0.01$). They were less likely to have NICU admission 7.2% vs. 12.6% ($p=0.087$) and non-significant differences in hypoglycaemia at 4 hours 8.8% vs. 9.3% ($p=0.84$) as compared to LGA infants of diabetic mothers. This study suggested that LGA infants of non-diabetic mothers have fewer neonatal complications and may not need intensive monitoring after birth(260).

Esakoff et al performed a retrospective review of 36,241 singleton pregnancies at the University of California, San Francisco from 1982 – 2006 to determine the influence of birthweight of ≥ 4000 grams on neonatal outcomes. They compared three groups of infants:

1. Infants born to non-diabetic mothers,
2. Infants born to diabetic mothers (universal screening),
3. Infant with birthweight > 4000 grams born to diabetic and non-diabetic mothers.

In non-diabetic mothers, infants with a birthweight of more than 4000 grams had significantly higher rates of hypoglycaemia 2.4% vs. 1.2% ($p<0.01$), respiratory distress 1.7% vs. 1.2% ($p=0.02$), shoulder dystocia 6% vs. 0.9% ($p<0.001$) and brachial nerve palsy 0.7% vs. 0.1% ($p<0.001$) as compared to infants with birthweight less than 4000 grams. Similarly infants of diabetic mothers with a birthweight of more than 4000 grams had significantly higher rates of complications as compared to infants of diabetic mothers with birthweight less than 4000 grams.

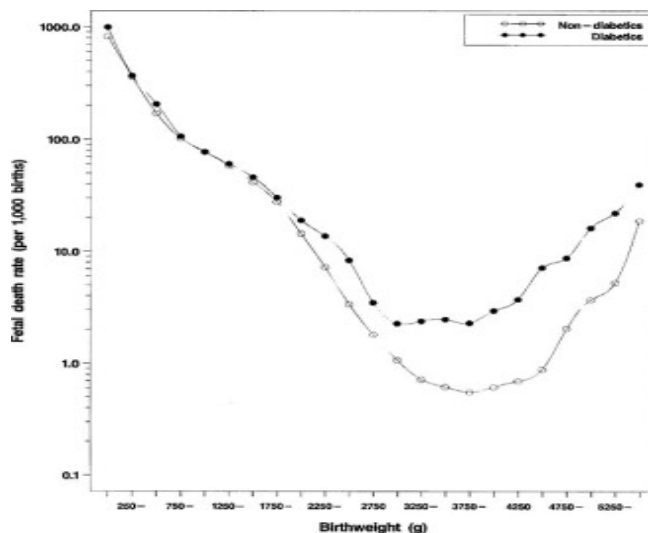
In their final comparison, they reported that LGA infants of non-diabetic mothers had significantly lower odds for developing neonatal hypoglycaemia 2.04 (1.42-2.92) vs. 2.06 (1.05-6.45), RDS 1.54 (1.02-2.33) vs. 3.10 (1.11-8.65), shoulder dystocia 9.62 (7.38-12.54) vs. 16.45 (6.71-40.33) and brachial plexus injury 6.65 (2.90-15.27) vs. 41.89 (4.05-433.64) as compared to LGA infants of diabetic mothers. However the rate of neonatal morbidity for these groups was significantly higher than that in normal weight control infants.

In summary Esakoff and his team have shown that birthweight more than 4000 grams is a predictor of adverse neonatal outcomes and that the presence of maternal diabetes in pregnancy increases this risk further. The authors suggested that as the LGA infants born to non-diabetic mothers have a significantly higher rate of complications as compared to control infants, they should be monitored in the early postnatal period(261, 262).

Das et al, in a three-year retrospective review (2003 – 2005) in Philadelphia, compared 262 singleton LGA infants (birthweight ≥ 4000 grams) born to non-diabetic mothers to 41 singleton LGA infants born to diabetic mothers (diagnosed by universal screening). LGA infants born to non-diabetic mothers had a lower risk of hypoglycaemia (blood sugar $<2.8\text{mmol/L}$) (28.6% vs. 56.1%; $p<0.01$) and respiratory distress syndrome (9.2% vs. 29.2%; $p=0.001$). They had slightly higher although statistically insignificant, risk of birth injury (8.0% vs. 2.4%; $p=0.13$). So, although LGA infants of non-diabetic mothers had less neonatal morbidity as compared to infants of diabetic mothers, more than a quarter developed hypoglycaemia and that almost 10% developed respiratory distress syndrome. The authors point out that this might be due to undetected maternal glycaemic derangements in this group(263).

Mondestin et al conducted a retrospective population-based study using the US births data from 1995 – 97. The study included 10,733,983 singleton births > 20 weeks gestation of which 271,691 (2.5%) infants were born to mothers with diabetes in pregnancy.

Figure 3.1: Relationship between fetal death rate and birthweight (264) .



The overall death rate amongst infants of non-diabetic infants was 4.0/1000 births as compared to 5.9/1000 births amongst infants of diabetic mothers. At birthweight of 4000 grams, 4500 grams, 5000 grams and ≥ 5500 grams the death rate per 1000

births in macrosomic infants of non-diabetic mothers as compared to infants of diabetic mothers was 0.6 vs. 2.9, RR 3.6 (2.7-4.8), 0.9 vs. 7.1, RR 6.4 (4.4-9.3), 3.7 vs. 15.9, RR 3.4 (1.9-6.1) and 18.3 vs. 38.9, RR 1.8 (1.7-1.9) respectively. In infants of non-diabetic and diabetic mothers, there was a decreasing rate of fetal death with increasing birth weight up to 4249 grams and 3999 grams respectively, and then an increasing risk up to ≥ 5500 g. It can be concluded that neonatal mortality in severely macrosomic infants of non-diabetic mothers increased proportionately to birthweight beyond 4250 grams however it remains lower than macrosomic infants of diabetic mothers across all weight categories (265) .

Ute M. Schaefer-Graf et al, in a 5-year retrospective review in Germany, analysed 887 LGA infants (birthweight $>90^{\text{th}}$ centile) born to mothers without diabetes in pregnancy. Of these infants 16.0% developed hypoglycaemia within the first 24 hours. The hypoglycaemia rate was 5.9% in infants of mothers with a normal OGTT (control infants), 12.2% in infants of mothers with one elevated blood glucose value on OGTT and 17.7% in infants of mothers without antenatal glucose testing. Higher rates of hypoglycaemia in LGA infants of mothers who were not screened/tested for GDM may suggest undiagnosed GDM in these women(258).

Hoegsberg et al tested the hypothesis that macrosomic infants of non-diabetic mothers have higher birthweight due to fetal hyperinsulinism and increased subcutaneous fat as compared to normal weight infants. They compared 50 macrosomic infants to 32 normal weight controls, all born to mothers with a normal oral glucose challenge test in pregnancy. They measured cord blood insulin and triceps and subscapular skin fold thickness. Macrosomic infants with a mean birthweight of 4541 ± 227 vs. 3320 ± 187 grams had significantly higher mean cord blood insulin levels of 18.75 ± 19.08 vs. 8.67 ± 6.64 $\mu\text{U/ml}$ ($p < 0.001$) as compared to control infants. There was no significant difference in the blood glucose levels during the OGCT in mothers of macrosomic and control infants 5.8 ± 1.0 vs. 5.7 ± 0.9 . However the mothers of hyperinsulinaemic infants had slightly higher blood glucose during the OGCT 6.1 ± 0.8 vs. 5.6 ± 1.0 as compared to those infants who did not have elevated insulin levels suggesting that even mild derangements in maternal blood sugar levels can result in fetal macrosomia. Mothers of macrosomic

infants also had a higher pre-pregnancy weight and higher net weight gain in pregnancy. Although this study established that macrosomic infants of non-diabetic mothers had a more than two fold increase in fetal insulin levels, it did not explore any correlation of this to adverse neonatal outcomes (266) .

3.6 Conclusion

In conclusion the above studies show a wide variation in the outcomes of LGA infants born to non-diabetic mothers. When compared to normal weight infants born to mothers following a low risk pregnancy, LGA infants of non-diabetic mothers have poorer outcomes. However, they have better outcomes than infants of diabetic mothers. This is mainly because the cohort of LGA infants comprises infants who are constitutionally LGA (physiological LGA infants) due to genetic factors and those who are pathologically macrosomic (pathological LGA infants) due to abnormal maternal metabolic factors. Physiological LGA infants are at risk of birth trauma due to adverse cephalo-pelvic ratios and obstructed labour. Hence it is important to be vigilant in pregnancy and labour to identify LGA infants and decide the best mode of delivery. However physiological LGA infants, unlike pathological LGA infants, are not at an increased risk of metabolic complications. However due to the current inability to identify such infants at birth and the lack of clear evidence regarding the optimal postnatal management of LGA infants there is a wide variation in practice depending upon clinicians' interpretation of the available literature. As part of my preliminary work, I informally contacted the medical teams at neonatal units in the Central Newborn Network and the Trent Perinatal Network (based in East Midlands) to get an overview of the range of practice and availability of guidelines for the management of LGA or macrosomic infants born to non-diabetic mothers. This survey included four Neonatal Intensive Care units, five Local Neonatal Units and five Special Care Units. Of these neonatal units only five had a separate guideline for the management of LGA/macrosomic infants (macrosomia was defined as birthweight either >4000grams or >4500grams) which involved pre-feed blood sugar monitoring. A further four units reported that the management of macrosomia was included in their guideline for the management of infants of diabetic mothers. However they

were not certain whether the guideline was followed in all LGA/macrosomic infants particularly those who were born to non-diabetic mothers. An additional five units did not have any guideline for these infants and treated LGA infants as normal infants born to low risk mothers. Hence it can be seen that the management of LGA or macrosomic infants varied widely between various NHS Trusts. Some hospitals treated infants with birth weight more than 4000grams or 4500grams (depending on local policy) similar to infants of mothers with diabetes i.e. aimed to deliver them in hospitals with provision for advanced neonatal resuscitation, monitored their pre-feed blood sugar levels to identify neonatal hypoglycaemia and kept them as inpatients for about 24 hours until breast feeding or oral bottle feeding was established. Conversely other hospitals treated them as normal infants born to low risk mothers without any medicalisation of their care.

This raises two questions:

1. Do LGA/macrosomic infants born to non-diabetic mothers have a sufficient increase in neonatal morbidity and mortality as compared to normal birthweight infants born to low risk mothers to warrant a separate care pathway?
2. Can pathological LGA infants be identified and distinguished from physiological LGA infants at birth to allow targeted postnatal monitoring, early identification and treatment of postnatal complications in those at highest risk, whilst avoiding unnecessary medicalisation of postnatal care?

3.7 Study design and methodology for retrospective study 2

3.7.1 Research question

Do LGA infants defined as those with a birthweight $\geq$ the 97th centile for gestation, sex and ethnicity, born to mothers who have not been previously identified as diabetic, have a significantly higher rate of morbidity than AGA (appropriate for gestational age) infants with birthweight between the 10th and 90th centiles for gestation, sex and ethnicity born to non-diabetic mothers?

In order to answer the above question a population based, retrospective, case control study was undertaken to review the risk of morbidity and mortality in LGA infants with a birthweight $\geq$ the 97th centile for gestation, sex and ethnicity as compared to AGA infants born with normal birthweight both born to mothers without diabetes.

3.7.2 Sample size

The estimated rate of morbidity for normal birthweight infants is 5% (267) . A rate of morbidity of 15% for LGA infants (i.e. 3 times the rate of morbidity for normal birthweight infants) was chosen to be of sufficient significance clinically to merit a specific care pathway to be developed for these infants. In order to have 90% power, at the 5% significance level, to detect a rate of morbidity of at least 15% in LGA infants, it was estimated that 200 infants would be needed in each arm. At University Hospitals of Leicester (UHL), about 11000 infants deliver each year. In 2007 and 2008, about 330 term infants each year had birthweight greater than the 97th centile. This was based on the figures obtained from a maternity database at UHL for 2007 and 2008. We determined that it would be possible to achieve the required number of participants to the study and the control arm of the study (i.e. 200 infants in each arm) using appropriate local records from 2009.

3.7.3 Study population

The study and the control infants were retrospectively recruited from a cohort of infants born in 2009 at the University Hospitals of Leicester (UHL) NHS trust. In 2009, a total of 10683 women delivered at UHL, which has deliveries across two sites Leicester Royal Infirmary and Leicester General Hospital.

3.7.3.1 Selection of the study cohort

Of the total infants born in 2009, 430 singleton infants were identified as being born LGA based on:

- Gestation between 35+0 and 41+6 weeks
- Birthweight $\geq$ 97th centile for gestation, sex and ethnicity
- Born to mothers without diabetes in pregnancy
- No antenatally detected congenital anomaly

From these, a total of 200 SA and WB infants alive at the onset of pregnancy were randomly selected and included in the study. This study included only the WB (N = 154, 77%) and the SA (N = 46, 23%) ethnic groups, as ethnic specific birthweight charts were only available for these two ethnic groups (explained in more detail in section 3.7.3.2). Since the majority of the neonates born at or below 34 weeks of gestation are routinely admitted for neonatal care (because of prematurity) they were excluded from the study.

3.7.3.2 Selection of the control cohort

For every LGA infant, the first available appropriate for gestational age (AGA) infant of the same gestation, sex and ethnicity as the LGA infant was selected from the maternity database. AGA infants had birthweight between the 10th and 90th centiles for gestation, sex and ethnicity (see below). These infants were born to mothers without any medical problems in pregnancy i.e. following a low risk pregnancy with normal antenatal scans and were planned to deliver by vaginal delivery or by elective caesarean section. Again infants with antenatally detected congenital anomalies and those from multiple births were excluded.

Ethnicity was based on self-report as recorded in the maternal notes. Ethnic specific birthweight centile charts were used for SA infants. As part of preliminary work, the research fellow and the team of statisticians from TIMMS group at Health Sciences department at University of Leicester designed ethnicity specific birthweight centile charts for infants of SA origin using the birthweight data of SA infants born in Leicester between 1st January 2003 and 31st December 2006. 24,274 WB and 7,190 SA infants were included in the analysis. The LMS (lambda-mu-sigma) statistical method was used to construct centile charts for the SA infants(226). The use of ethnic specific birthweight centile charts was important as on an average SA infants are 200 – 350 grams lighter than their WB counterparts and using a single birthweight centile chart would underestimate LGA infants and overestimate small for gestational age infants in the SA ethnic group. The currently used WHO-UK birthweight centile charts designed in 1990 using the birthweight data of Caucasian infants were used for WB infants(226).

3.7.4 Study outcomes

3.7.4.1 Primary outcome

The primary outcome was intended to capture babies who suffered significant neonatal morbidity related to their large size, comprising any of the following:

1. Need for admission to neonatal care (for neonatal hypoglycaemia, hyperbilirubinaemia, respiratory distress, congenital malformation, feeding difficulty, presumed sepsis, HIE, hypocalcaemia and hypomagnesaemia),
2. Readmission in the first week after initial discharge home,
3. Neonatal death.

3.7.4.2 Secondary outcome

The secondary outcomes comprised:

1. Mode of delivery,
2. Evidence of neonatal birth trauma not leading to admission e.g. shoulder dystocia, nerve palsy, bone fracture or hypoxic ischaemic encephalopathy,
3. Duration of hospital stay.

3.8 Results of retrospective study 2

In this study, 200 LGA infants (defined as birthweight > 97th centile for sex, gestation and ethnicity) born between 35+0 and 41+6 weeks of gestation to non-diabetic White British and South Asian mothers were compared to 200 AGA infants (defined as birthweight between 10th and 90th centile for sex, gestation and ethnicity) born to mothers following low risk pregnancy.

3.8.1 Characteristics of mothers of LGA and AGA infants

The characteristics of the mothers of LGA and AGA infants have been summarised in tables 3.2 and 3.3. There was a significant difference between the mothers of LGA and AGA infants in terms of age, booking weight, height, booking BMI, parity, booking systolic and diastolic BP and hypertension during pregnancy. There was no significant difference between history of smoking, alcohol or recreational drug use at the time of antenatal booking of the pregnancy between the mothers of the two study groups. There was an equal ethnic distribution and the White British infants constituted about 75% and South Asian about 25% of the entire cohort. The only significant difference in the previous obstetric history and family history between the mothers of LGA and AGA groups was a significantly higher rate of previous preterm birth and family history of pregnancy induced hypertension in mothers of LGA infants.

Table 3:2: Comparison of maternal demographic characteristics of LGA and AGA infants.

		LGA infants (N = 200)	AGA infants (N = 200)	P – value
Maternal age (years)**		30.00 (27.00 – 35.00)	28.00 (24.00 – 32.75)	< 0.001
Maternal weight (kg)**		76.00 (67.00 – 91.00)	64.00 (56.00 – 72.75)	< 0.001
Maternal height (cm)**		168.00 (163.0–170.0)	163.00 (158.0–168.0)	< 0.001
Maternal BMI**		27.70 (24.09 – 32.37)	23.90 (21.45 – 26.77)	< 0.001
Maternal BP (mmHg)	Systolic BP**	114.00 (105.8 – 123.0)	111.00 (102.0 – 120.0)	0.018
	Diastolic BP**	70.00 (63.00 – 77.00)	69.00 (60.00 – 75.00)	0.027
Parity	Primigravida*	40 (20.0%)	74 (37.0%)	< 0.001
	Multigravida*	132 (58.0%)	116 (66.0%)	< 0.001
	Grand- multigravida*	28 (14.0%)	10(5.0%)	< 0.001
Smoking*		67 (33.5%)	75 (37.5%)	0.403
Alcohol*		47 (23.5%)	45 (22.5%)	0.877
Recreational Drugs*		3 (1.5%)	8 (4.0%)	0.175
Ethnicity	White British	154 (77.0%)	150 (75.0%)	-
	South Asian	46 (23.0%)	50 (25.0%)	-
Pregnancy Induced hypertension*		19 (9.5%)	3 (1.5%)	0.001

Values for continuous variables presented as median (interquartile range);

Values for categorical variables presented as n (%);

**Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance. **Mann Whitney U test used for continuous variables.*

Table 3:3: Comparison of previous maternal obstetric history and family history between LGA and AGA infants.

	LGA Infants N = 200	AGA Infants N = 200	P – value
Previous miscarriage	48 (38.1)	56 (35.0)	0.622
Previous stillbirth	2 (1.6)	3 (1.9)	1.000
Previous preterm birth	11 (8.7)	4 (2.5)	0.030
Previous neonatal death	1 (0.5)	1 (0.8)	1.000
GDM in previous pregnancy	5 (2.5)	0 (0.0)	0.061
FH diabetes	63 (31.5)	58 (29.0)	0.663
FH PIH	31 (15.5)	17 (8.5)	0.045
FH of hypertension	6 (3.0)	11 (5.5)	0.322
FH congenital anomalies	45 (22.5)	34 (17.0)	0.209
FH neonatal death	3 (1.5)	1 (0.5)	0.623

Values for categorical variables presented as n (%);

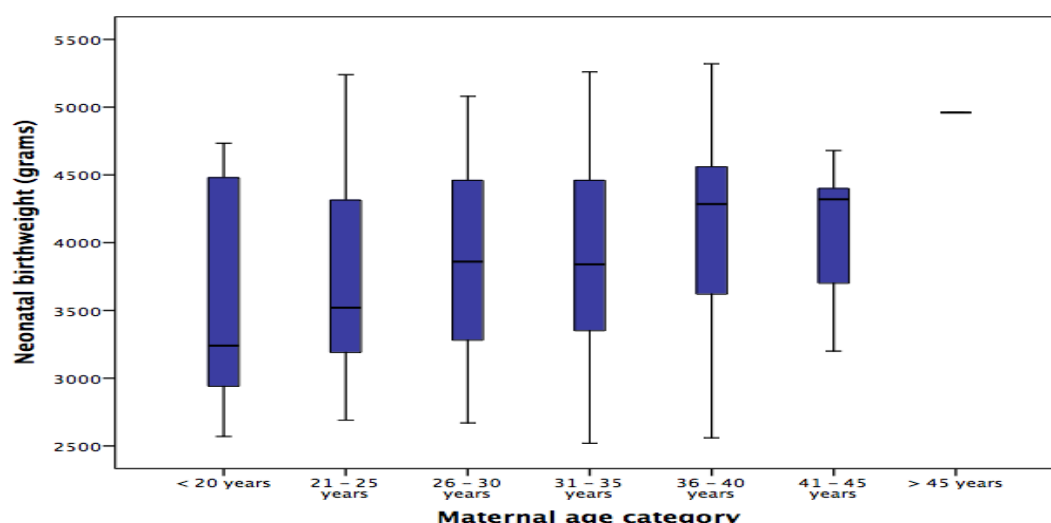
Chi-square test for categorical variables. Fisher's exact test, 2-sided exact significance.

There was no significant difference in the rates of previous miscarriages and stillbirth between the mothers of LGA and AGA infants although the number of infants in each category was small. The rate of miscarriage in this cohort was significantly higher than the rate of 20% reported in the general population. Similarly the rate of previous stillbirth in this cohort was significantly higher than the rate of 0.5% reported in the general population. Mothers of LGA infants had a significantly higher rate of previous preterm birth (defined as < 37+0 weeks of gestation) 8.7% as compared to 2.5% in the AGA infants (p=0.030). 2.5% of mothers of LGA infants had GDM in a previous pregnancy but had not been detected to have diabetes in the current pregnancy as compared to none in the AGA group. Mothers of LGA infants had a significantly greater family history of pregnancy induced hypertension 15.5% vs. 8.5% than the mothers of AGA infants (p = 0.045). There was no significant difference in any other family history between the two study groups.

3.8.1.1 Comparison of maternal age between the two study groups

For the entire cohort, there was a direct linear relationship between the birthweight of the infants and maternal age and this was observed in both LGA and AGA infants (fig. 3.2). For the analysis, maternal age was divided into seven age categories (< 20 years, 21 – 25 years, 26 – 30 years, 31 – 35 years, 36 – 40 years, 41 – 45 years, >45 years) as shown in the fig 3.2 below. There was a gradual increase in the median birthweight of the infants with each increasing maternal age category (table 3.4).

Figure 3.2: Box plot to show median birthweight in each category of maternal age for the entire cohort.



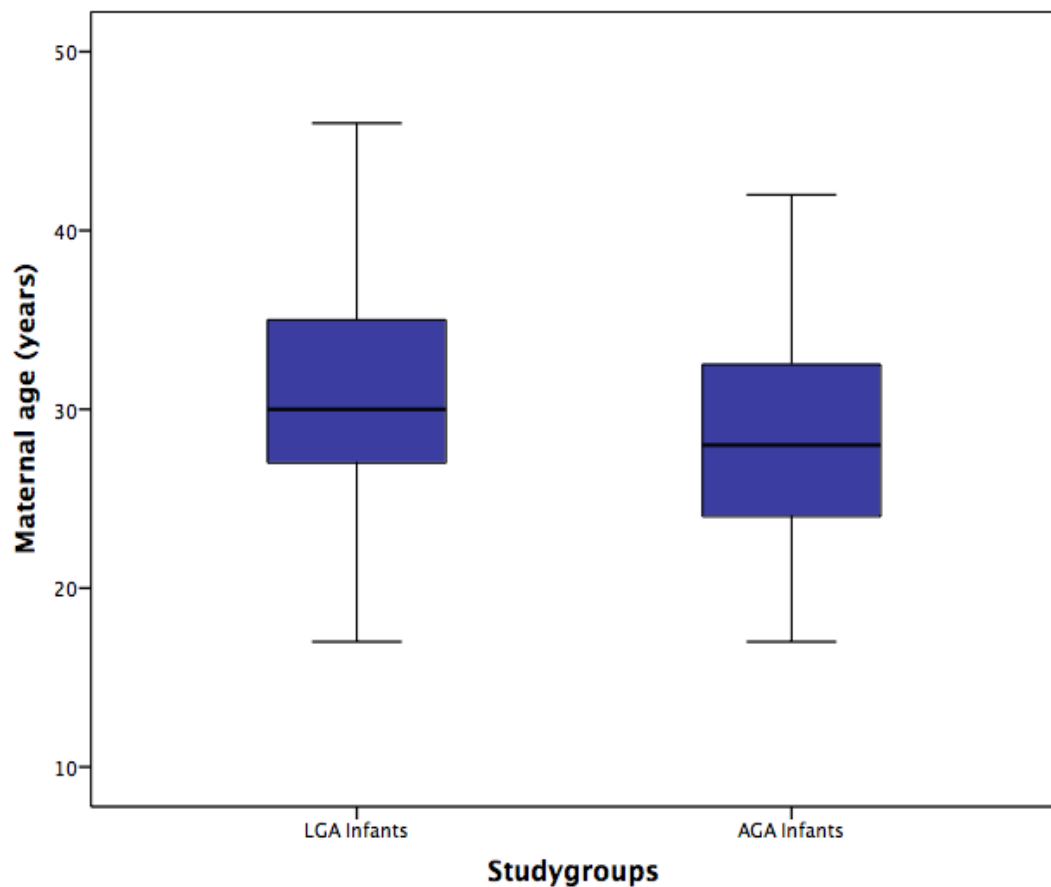
Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values.

Table 3:4: Median (IQR) birthweight of infants in each maternal age category.

	Median birthweight (grams)	IQR
< 20 years	3240.00	2915.50 – 4520.00
21 – 25 years	3520.00	3180.00 – 4320.00
26 – 30 years	3860.00	3260.00 – 4470.00
31 – 35 years	3840.00	3345.00 – 4470.00
36–40 years	4285.00	3610.00 – 4560.00
41 – 45 years	4320.00	3575.00 – 4470.00

The mothers of LGA infants were significantly older as compared to the AGA infants ($p < 0.001$) as shown in Fig 3.3. Mann-Whitney U test revealed a significant difference between maternal age of mothers of LGA infants (median = 30 years, IQR = 27 – 35 years, $N = 200$) and mother of the AGA infants (median = 28 years, IQR = 24 – 32 years, $N = 200$), $p < 0.001$, $Z = -3.712$, $r = 0.19$.

Figure 3.3: Box plot to show the comparison of maternal age of LGA and AGA infants.

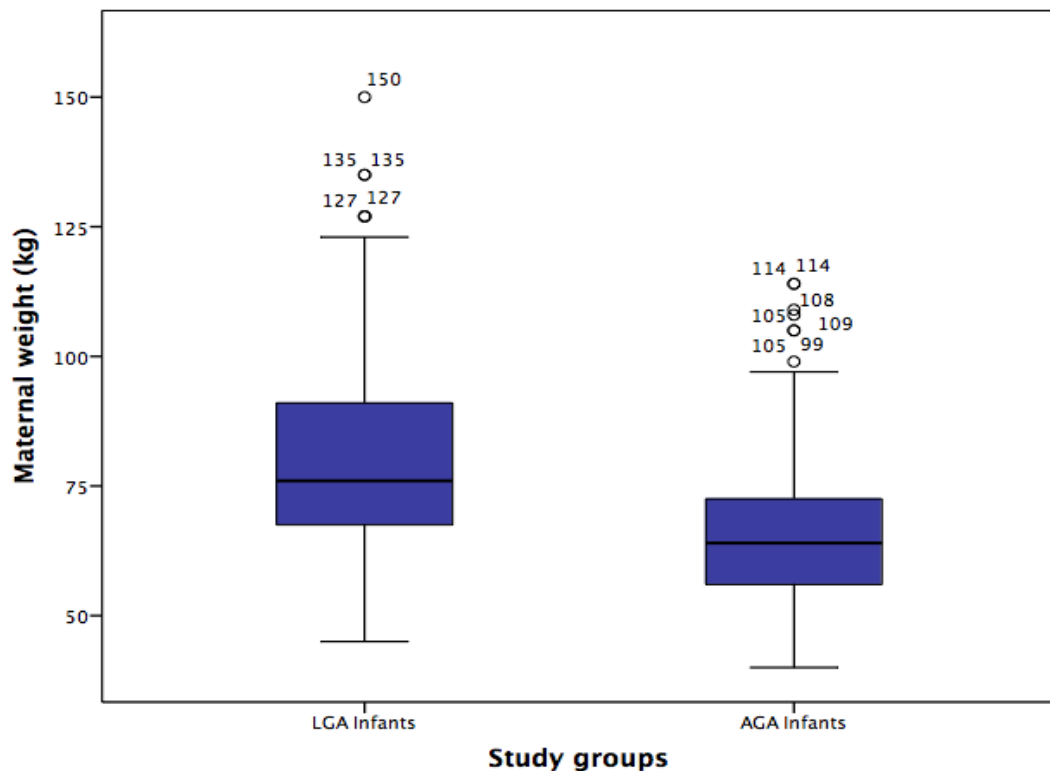


Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values.

3.8.1.2 Comparison of maternal booking weight between the two study groups

The mothers of LGA infants were significantly heavier than the mothers of AGA infants at the time of the antenatal booking of their pregnancy as shown in Fig 3.4. Amongst the mothers of LGA infants, there were five outlier women with a booking weight that ranged from 127 – 150 kg. Amongst mother of the AGA infants there were seven outlier women with booking weight that ranged from 99 – 114 kg. These values were rechecked and confirmed to be the true booking weight in these mothers.

Figure 3.4: Box plot to show the comparison of maternal booking weight of LGA and AGA infants.



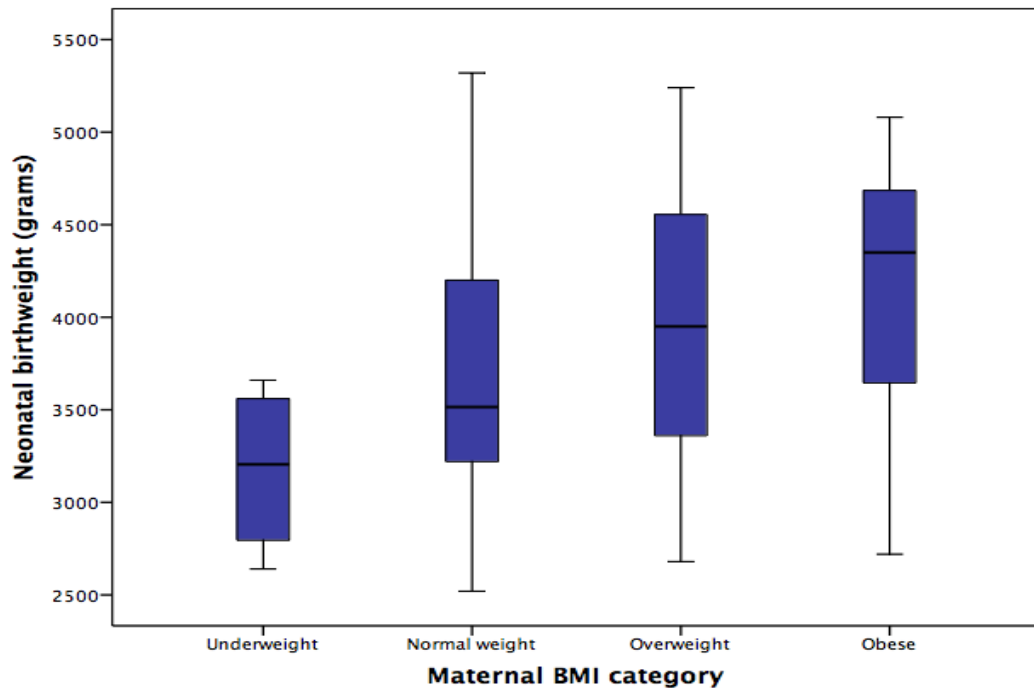
Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values and ^o represents outlier values.

Mann - Whitney U test revealed that there was a significant difference between the booking weight of the mothers of the LGA infants (median = 76.00 kg, IQR = 67.00 – 91.00, N = 199) and mothers of the AGA infants (median = 64.00 kg, IQR = 56.00 – 72.75, N = 200), $p < 0.001$, $Z = -8.47$, $r = 0.42$ (medium effect).

3.8.1.3 Comparison of maternal booking BMI between the two study groups

For the entire cohort, there was a direct linear relationship between maternal booking BMI and median birthweight of the infants as shown in fig. 3.5. Amongst LGA infants, the risk of delivering an LGA infant increased by 20% with each increase in the maternal BMI category as shown in table 3.5.

Figure 3.5:Relation of infant birthweight to maternal booking BMI.



Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values.

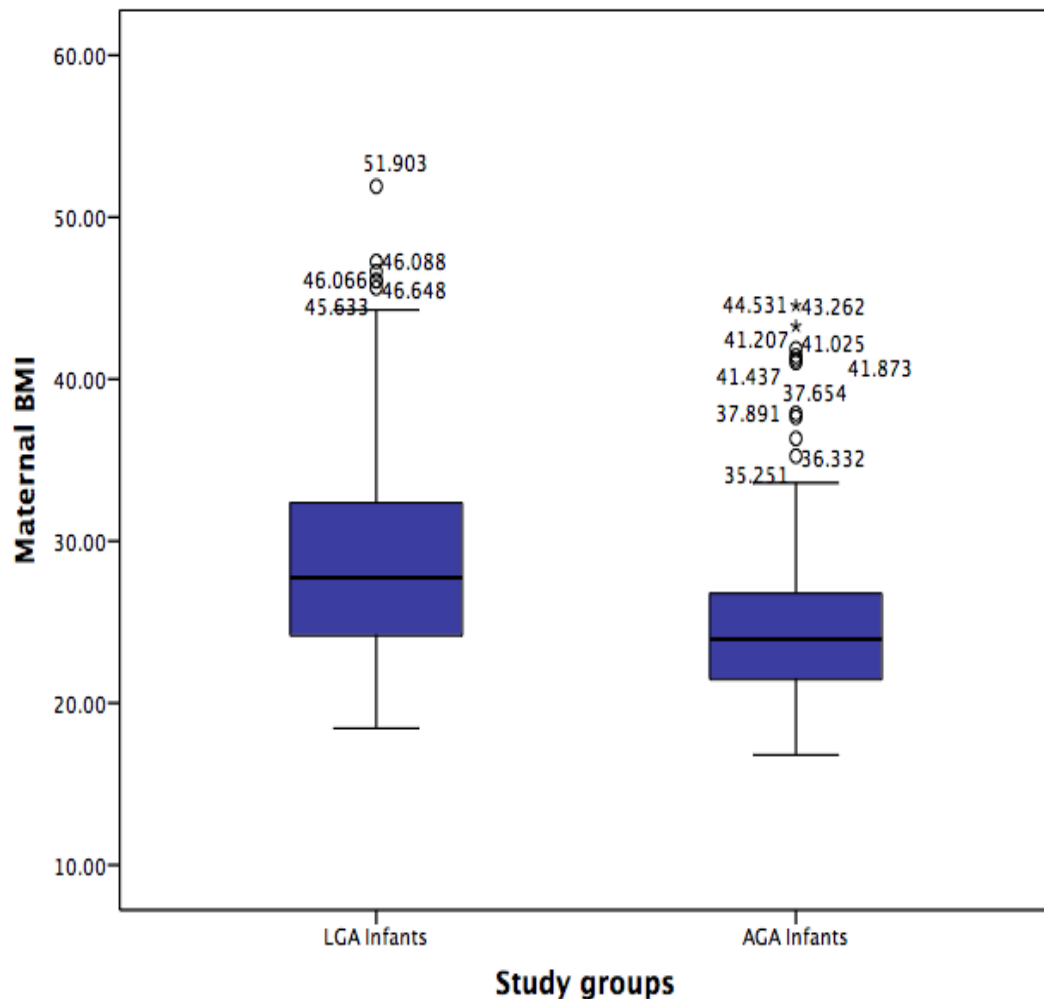
Table 3:5: LGA and AGA infants born for each maternal BMI category.

	LGA Infants N = 200	AGA Infants N = 200
Underweight (BMI < 18)	None	4 (100.0)
Normal weight (BMI 18 - 25)	63 (34.6)	119 (65.4)
Overweight (BMI 25 – 30)	66 (57.4)	49 (42.6)
Obese (BMI > 30)	70 (72.9)	26 (27.1)

Values for categorical variables presented as n (%)

The mothers of LGA infants had a significantly higher booking BMI as compared to mothers of AGA infants as shown in figure 3.6. The outlier and the extreme values for maternal BMI in both the study groups were checked and they represent true maternal BMI values.

Figure 3.6: Box plot to show comparison of maternal BMI of LGA and AGA infants.



*Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values.*

A Mann – Whitney U test revealed a significant difference in the booking maternal BMI of LGA infants (median = 27.7, IQR = 24.09 – 32.37, N = 199) and AGA infants (median = 23.9, IQR = 21.45 – 26.77, N = 200), $p < 0.001$, $Z = -7.34$, $r = 0.37$.

3.8.1.4 Relation between parity and LGA at birth

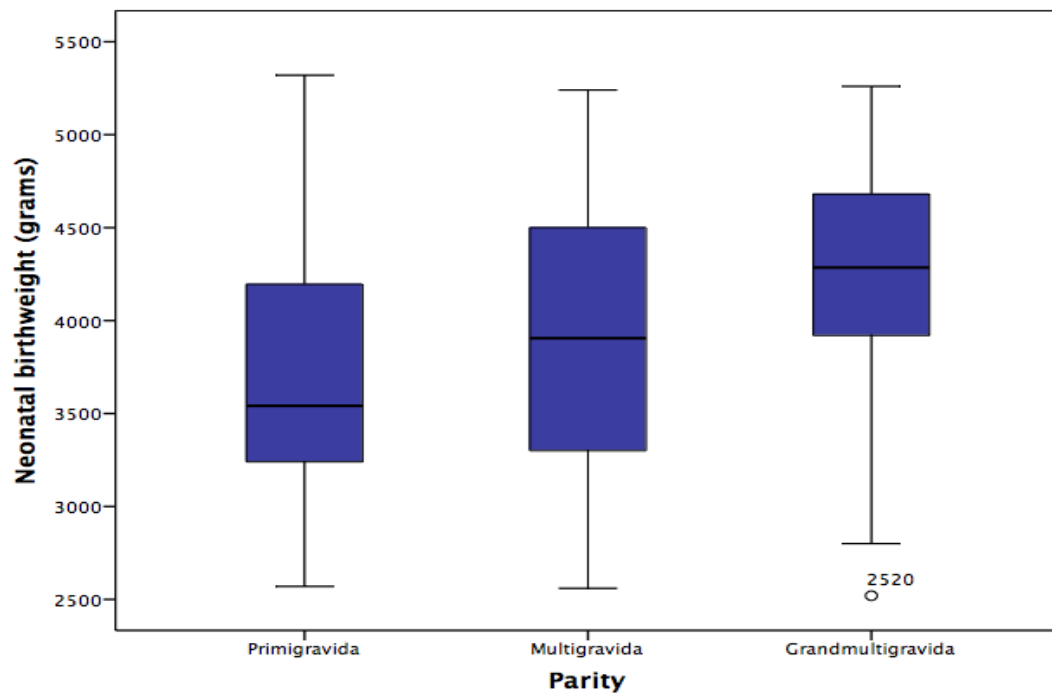
The risk of delivering an LGA infant increased with increasing parity as shown in table 3.6. In primigravida women, one-third of infants were LGA. This proportion increased to more than 50% in multiparous (gravida 1 – 4) women and amongst grand-multiparous (gravida ≥ 5) the risk of delivering a LGA infant further increased to 70%. The proportion of AGA infants decreased accordingly with increasing parity.

Table 3:6: LGA and AGA infants born in each gravida category.

	LGA Infants N= 200	AGA Infants N = 200
Primigravida	40/114 (35.1)	74/114 (64.9)
Multigravida (gravida 1 – 4)	132/248 (53.2)	116/248 (46.8)
Grand-multigravida (gravida ≥ 5)	28/38 (73.7)	10/38 (26.3)

Values for categorical variables presented as n (%)

Figure 3.7: Influence of parity on birthweight.



Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values and ^o represents outliers values.

Kruskal-Wallis test revealed a statistically significant difference in the birthweight of the infants across the three different parity groups (Group 1, N = 114, Primigravida, Group 2, N = 248, gravida 2 – 4, Group 3, N = 38, gravida ≥ 5), chi-square (2, N = 400) = 16.83, $p < 0.001$. There was a significant increase in the risk of delivering LGA infants with increasing parity ($p < 0.001$). Grand-multiparous women delivered infants with a higher median birthweight of 4285 grams as compared to multiparous women who delivered infants with a median birthweight of 3905 grams. Infants born to primiparous women had the lowest median birthweight of 3542 grams as shown in fig 3.7.

3.8.2 Comparison of mode of delivery and maternal perineal trauma

Table 3:7: Comparison of the mode of delivery between LGA and AGA infants.

	LGA Infants N = 200	AGA Infants N = 200	P – value
Vaginal delivery	111 (55.5)	150 (75.0)	< 0.001
Emergency caesarean section	37 (18.5)	16 (8.0)	0.003
Instrumental delivery	6 (3.0)	16 (8.0)	< 0.001
Caesarean section before term	46 (23.0)	18 (9.0)	< 0.001
Induction of labour before term	31 (15.5)	12 (6.0)	0.003

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

Figure 3.8: Bar chart to compare the modes of delivery between LGA and AGA infants.

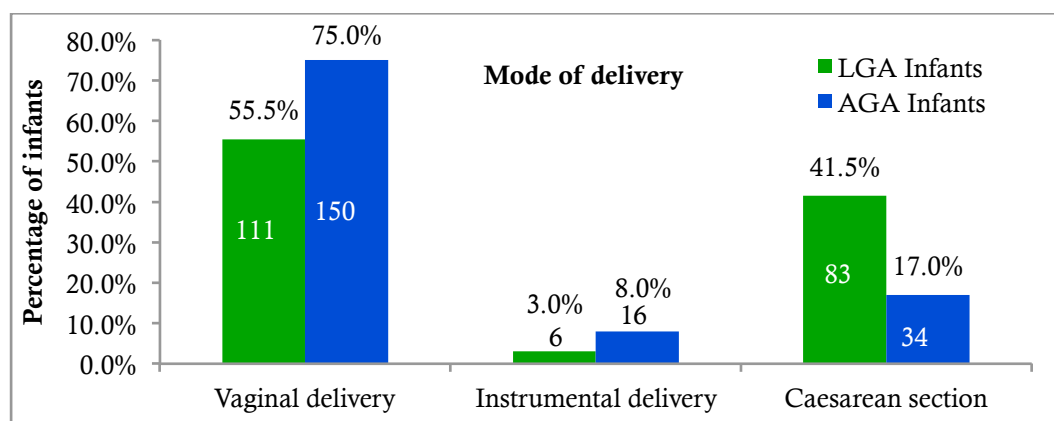


Table 3.7 shows that LGA infants had a significantly higher rate of elective caesarean section before term 46 (23.0%) vs. 18 (9.0%) ($p < 0.001$) and induction of labour before term 31 (15.5%) vs. 12 (6.0%) ($p = 0.03$) as compared to AGA infants. Fig 3.8 and table 3.8 show that LGA infants had an overall statistically higher risk of being delivered by caesarean section 41.5% as compared to 17.0% in the AGA infants ($p < 0.001$). Maternal age and LGA were two factors that significantly increased the risk of delivering by caesarean section after controlling for various confounding factors such as pre-pregnancy weight, height, BMI, parity, smoking and neonatal sex, OR (95% CI) 1.09 (1.04 – 1.14) and 2.95 (1.77 – 4.90) respectively.

Table 3:8: Comparison between LGA and AGA infants undergoing emergency and elective caesarean section.

	LGA infants N = 200	AGA infants N = 200	P – value
Elective caesarean section	46 (23)	18 (9)	< 0.001
Emergency caesarean section	37 (18.5)	16 (8)	0.003

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

Amongst LGA infants, 23.0% were born by elective caesarean section and 18.5% by emergency caesarean section. The reasons for elective caesarean section were previous caesarean section (15.5%), maternal request (2.5%), maternal complications (2.0%), breech presentation (1.5%) and LGA infant (1.5%). The reasons for emergency caesarean section were failure to progress (9.0%) and fetal distress (9.5%).

Amongst AGA infants, 9% were born by elective caesarean section and 8% by emergency caesarean section. The reasons for elective caesarean section were previous caesarean section (5.5%), breech presentation (2.5%) and maternal complications (1%). The reasons for emergency caesarean section were failure to progress (1.0%) and fetal distress (7.0%).

Mothers of AGA infants had a statistically significant higher risk of instrumental delivery 8% vs. 3% ($p < 0.001$) and perineal trauma 45.7% vs. 31.7% as compared to mothers of LGA infants ($p = 0.005$). Both first-degree tear 10.6% vs. 6.0% and second-degree tear 30.2% vs. 19.1% were higher in mothers of AGA infants as compared to LGA infants. However, third-degree tears, although did not reach a statistically significant difference were higher in mothers of LGA infants 6.5% vs. 5.0% as compared to mothers of AGA infants (table 3.9 and fig 3.9).

Table 3:9: Comparison of perineal trauma in mothers of the LGA and AGA infants.

Perineal tear	LGA Infants N = 199	AGA infants N = 199	P – value
Perineal tear	63 (31.7)	91 (45.7)	0.005
1 st degree	12 (6.0)	21 (10.6)	
2 nd degree	38 (19.1)	60 (30.2)	
3 rd degree	13 (6.5)	10 (5.0)	

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

Figure 3.9: Bar graph showing the distribution of different grades of perineal trauma amongst mothers of LGA and AGA infants.

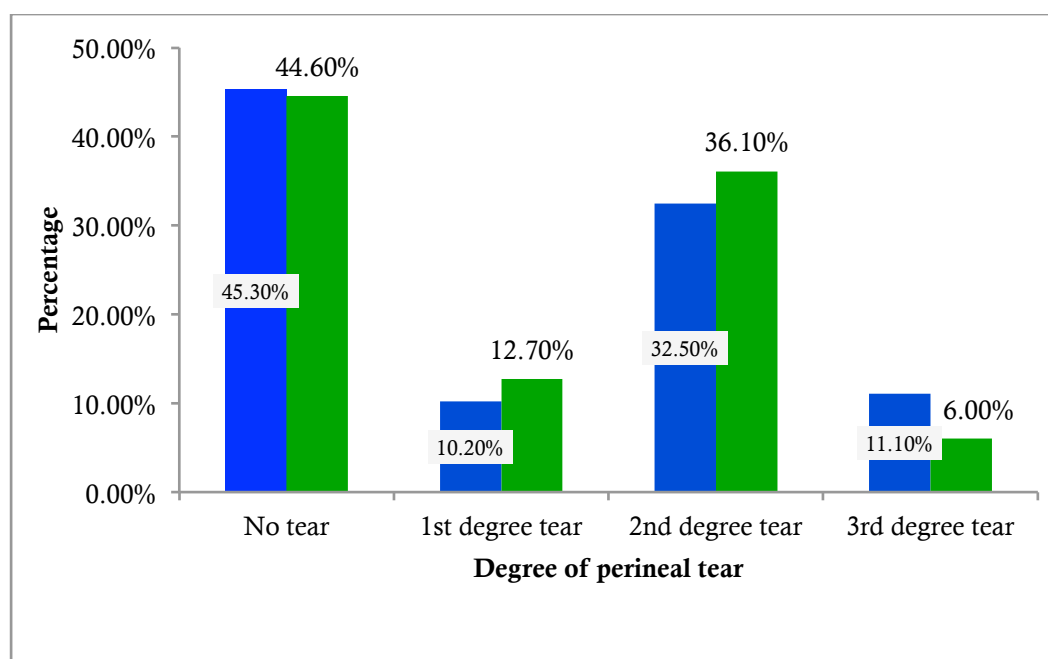


Table 3:10: Comparison of condition at birth of the LGA and AGA infants.

	LGA Infants N = 200	AGA Infants N = 200	P – value
Stillbirth	1	None	
Live birth	199	200	0.679
Arterial cord pH	7.30 (7.2 – 7.3)	7.32 (7.25 – 7.30)	0.783
Venous cord pH	7.36 (7.3 – 7.4)	7.32 (7.3 – 7.4)	0.357
Apgar score at 1min	8 (1 – 10)	9 (5 – 10)	0.006
Apgar score at 5 min	9 (4 – 10)	9 (7 – 10)	0.697

Values for continuous variables presented as median (interquartile range); Mann-Whitney U test used for continuous variables; Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

Table 3.10 shows that there was no statistical difference in arterial and venous cord blood pH at birth between LGA and AGA infants (information about arterial and venous cord pH was available in 86 LGA infants and 61 AGA infants). Information about Apgar scores was available in 189 LGA infants and 194 AGA infants. LGA infants had a statistically lower Apgar score of 8 vs. 9 ($p=0.006$) at 1min of age as compared to AGA infants. However there was no difference in the Apgar score between the two groups at 5mins of age. A difference of 1 in the Apgar score between the two groups at 1min of age would not be clinically significant especially as the Apgar score between the two groups was normal without any difference at 5mins of age. There was one stillbirth in the LGA group and none in the AGA group.

3.8.3 Characteristics of the infants in the LGA and AGA groups

Table 3:11: Comparison of neonatal demographics between LGA and AGA infants.

		LGA Infants N = 199	AGA Infants N = 200	P – value
Birthweight (grams)		4450.0 (4202.5–4700.0)	3295.0 (3042.0-3507.5)	< 0.001
Head circumference (cm)		36.0 (35.4 – 36.9)	34.5 (33.8 – 35.0)	< 0.001
Gestation (Weeks + days)		39+5 (39+0 – 40+4)	39+4 (39+0 – 40+4)	0.697
Sex	Male	111 (55.5%)	111 (55.5%)	
	Female	89 (44.5%)	89 (44.5%)	

Values for continuous variables presented as median (interquartile range);

Values for categorical variables presented as n (%);

Mann Whitney U test for continuous variables; Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

Characteristics of the LGA and AGA infants have been summarised in table 3.11. LGA infants were significantly heavier with a median (IQR) birthweight of 4450.0 (4202.50–4700.00) grams as compared to 3295.0 (3042-3507.50) grams in the AGA group of infants ($p < 0.001$). The groups showed the expected difference in birthweight, determined by the inclusion criteria for each group.

LGA also had a significantly higher head circumference of 36.0 (35.4 – 36.85) cm vs. 34.5 (33.78 – 35.03) cm in the control infants ($p < 0.001$). Measurements for the head circumference were available in only 133 LGA infants and 90 AGA infants due to early discharge (around 6 hours of age) in the remaining infants before their routine newborn check was performed (which is usually done at around 24 hours of age). As AGA infants had a higher chance of being delivered by vaginal delivery, more mothers and their infants were ready to be discharged before the newborn check as compared to LGA infants who had a higher risk of caesarean section which resulted in at least two days post-operative stay in the hospital. There was no difference in the gestation at birth between the LGA and the AGA group infants.

3.8.4 Comparison of neonatal birth trauma

Table 3:12: Comparison of birth trauma between LGA and AGA infants.

	LGA Infants N = 199	AGA Infants N = 200	P-value
Shoulder dystocia	28 (14.0%)	4 (2.0%)	< 0.001
Clavicular fracture	1 (0.5%)	0 (0.0%)	0.500
Erb's Palsy	4 (2.0%)	1 (0.5)	0.186
HIE	None	None	

Values for categorical variables presented as n (%);

Chi-square test for categorical variable; Fisher's exact test, 2-sided exact significance.

The information about various types of birth trauma that are reported to be higher in the LGA infants was collected and the results are summarised in the table 3.12. LGA infants were at a significantly higher risk of shoulder dystocia 28 (14%) as compared to 4 (2.0%) in the AGA infants ($p < 0.001$). However in this cohort there was no difference in the rates of clavicular fracture, Erb's palsy and hypoxic ischaemic encephalopathy (HIE) between the two groups. There was one stillbirth in LGA group born at 40+2 weeks of gestation with a birthweight of 4620 grams with a history of shoulder dystocia during delivery.

3.8.5 Comparison of neonatal outcomes between LGA and AGA infants

Table 3.13 shows comparison of the primary outcomes between the LGA and AGA infants. There was a significant difference in the composite primary outcome (admission to NICU, neonatal death and readmission) between the LGA and the AGA group of infants 44 (22%) vs. 25 (12.5%) ($p = 0.008$). LGA infants had a significantly higher risk of admission to the neonatal unit 21 (10.6%) as compared to 10 (5%) in the AGA infants ($p = 0.029$). They had a higher tendency for readmission after initial discharge however this did not reach a statistically significant level ($p = 0.196$). There was one stillbirth in the LGA group born at 40+2 weeks of gestation with birthweight of 4620 grams with a history of shoulder dystocia during delivery. There were no neonatal deaths in either group.

Table 3:13: Comparison of Primary Outcomes between LGA and AGA infants.

	LGA Infants N = 199	AGA Infants N = 200	P – value
Admission to NNU	21 (10.6)	10 (5.0)	0.029
Readmission	25 (12.6)	17 (8.5)	0.196
Neonatal death	0	0	-
Composite outcome	44 (22)	25 (12.5)	0.032

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

3.8.5.1 Comparison of LGA and AGA infants admitted to NICU

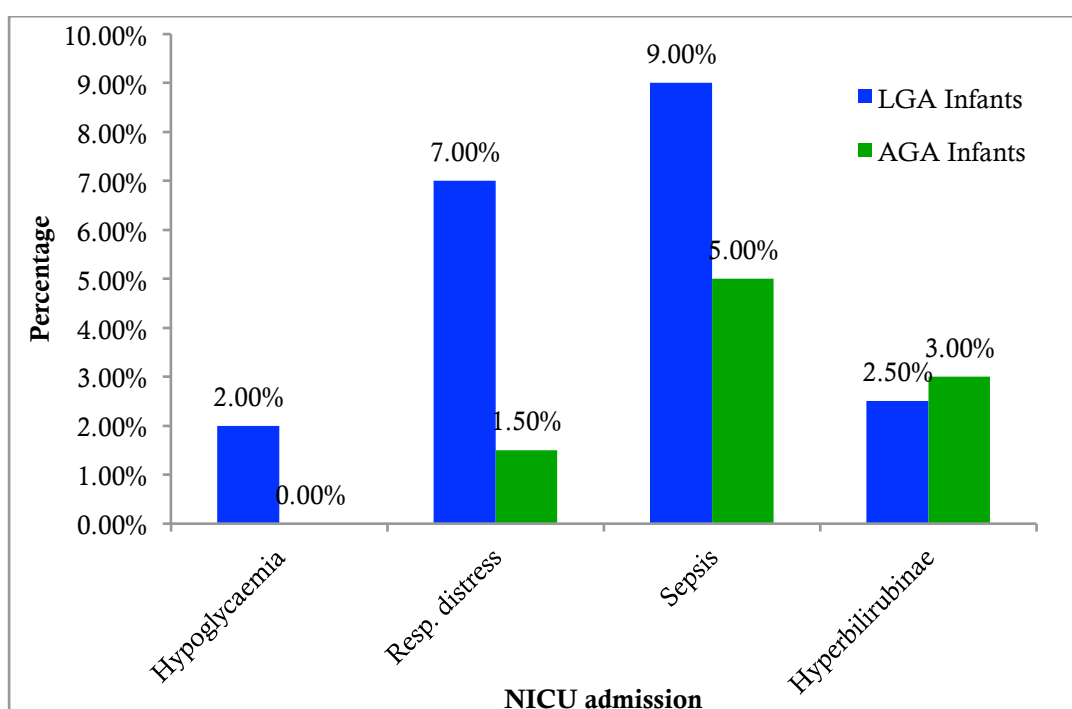
The LGA infants had a significantly higher risk of requiring NICU admission in the early postnatal period as compared to the AGA infants (table 3.14). The AGA infants were significantly more likely to receive their early neonatal care on the postnatal ward 190 (95.0%) vs. 178 (89.0%) of the LGA infants ($p=0.029$). The reasons for NICU admission are shown in table 3.14 and fig 3.10.

Table 3:14: Comparison LGA and AGA infants needing NICU admission.

		LGA Infants N = 199	AGA Infants N = 200	P – value
Admission to NNU*		21 (10.6)	10 (5.0)	0.029
Gestation (weeks)**		39+6 (39+0 – 40+6)	40+3 (39+3 – 41+2)	0.217
Birthweight (grams)**		4640.0 (4300.0-4840.0)	3335.0 (3152.5-3412.5)	<0.001
Hypoglycaemia*		4 (2.0)	None	-
Respiratory distress*		14 (7.0)	3 (1.5)	0.011
Respiratory distress	TTN*	12 (6)	2 (1)	
	Pneumonia*	2 (1)	1(0.5)	
	RDS*	0	0	
Presumed sepsis*		18 (9.0)	10 (5.0)	0.122
Lethal congenital anomaly		0	1	-
Poor feeding		0	0	-
Jaundice*		5 (2.5)	6 (3.0)	1.000
HIE*		0	0	-
Hypocalcaemia*		0	0	-
Hypomagnesaemia*		0	0	-
Duration of hospital stay (days)**		4.0 (3.0 – 5.0)	4.5 (3.0 – 10.25)	0.124

*Values for continuous variables presented as median (interquartile range); Values for categorical variables presented as n (%); **Mann Whitney U test used for continuous variables; *Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.*

Figure 3.10: Reason for NICU admission in LGA and AGA infants.

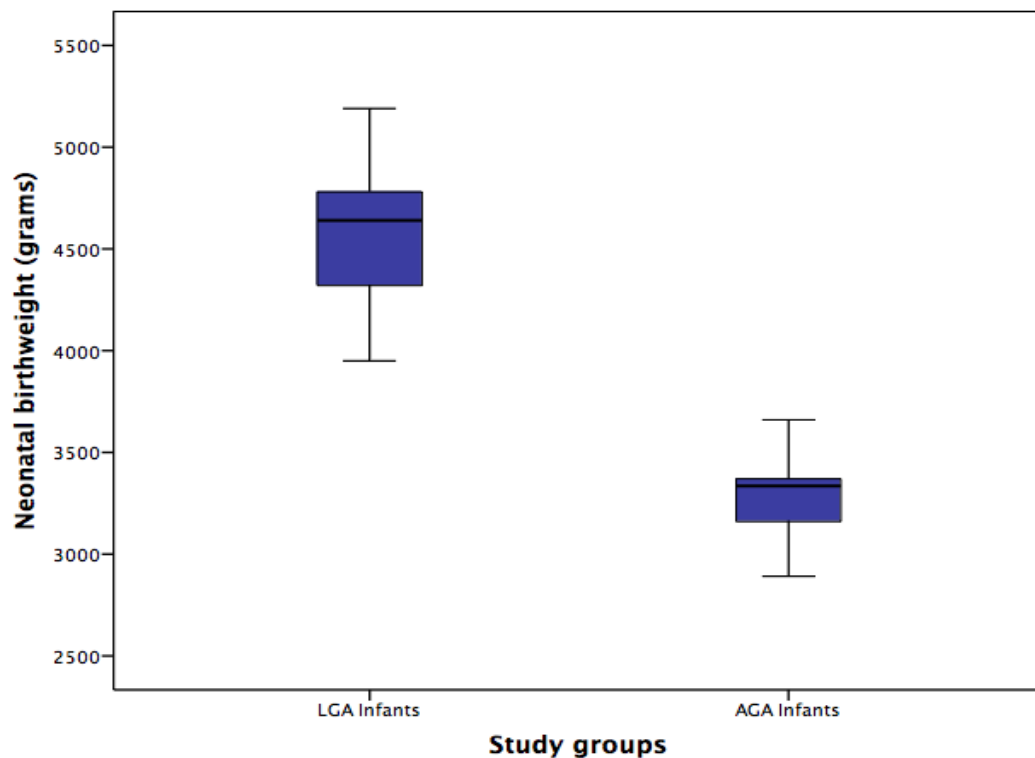


The LGA and AGA infants admitted to NICU had a combination of one or more postnatal complications. The LGA infants had a significantly higher risk of admission to NICU 10.6% (21/199) as compared to 5.0% (10/200) in the AGA infants ($p=0.029$). The reasons for admission to NICU in 21 LGA infants were: presumed sepsis (9.0%), respiratory distress (7.0%), hyperbilirubinaemia (2.5%), hypoglycaemia (2.0%), congenital anomaly (1.5%), poor feeding (1.0%) and shoulder dystocia (0.5%).

The reasons for admission to NICU in the 10 AGA infants were: presumed sepsis (5.0%), hyperbilirubinaemia (3.0%), respiratory distress (1.5%) and congenital anomaly (1.5%).

Mann – Whitney U test revealed that there was a significant difference in the birthweight of the LGA infants admitted to NICU (median = 4640.0 grams, IQR = 4300.0–4840.0 grams, $N = 21$) as compared to AGA infants needing NICU admission (median = 3335.0, IQR = 3152.5–3412.5 grams, $N = 10$), $p < 0.001$, $Z = -4.44$, $r = 0.8$ (large effect) (fig 3.11).

Figure 3.11: Comparison of the birthweight of LGA and AGA infants admitted to the NICU.



Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values.

LGA infants admitted to NICU had a tendency to be of a slightly lower median (IQR) gestational age as compared to the AGA infants, 39+6 (39+0 – 40+6) vs. 40+3 (39+3 – 41+2) weeks. However Mann – Whitney U testing revealed that there was no significant difference in the gestational age of the LGA infants (median = 39+6 weeks, IQR = 39+0 – 40+6 weeks, N = 21) and of the AGA infants (median = 40+3 weeks, IQR = 39+3 – 41+2, N = 10), $p = 0.217$.

Mann – Whitney U testing revealed that once admitted to NICU there was no difference in the duration of the hospital stay between the LGA infants (median = 4.0 days, IQR = 3.0 – 5.0 days, N = 21), and AGA infants (median = 4.5 days, IQR = 3.0 – 10.25 days, N = 10), $p = 0.124$.

3.8.5.2 Comparison of neonatal hypoglycaemia between the two groups

Table 3:15: Comparison of hypoglycaemia between LGA and AGA groups.

		LGA Infants N = 35	AGA Infants N = 4
Hypoglycaemia		6 (3.0)	0
Hypoglycaemia	NICU	4 (2.0)	0
	PNW	2 (1.0)	0

Values for categorical variables presented as n (%);

Chi-square test for categorical variables; Fisher's exact test, 2-sided exact significance.

University Hospitals of Leicester have a local policy to test pre-feed blood sugar in infants born with a birthweight ≥ 4500 grams (which roughly corresponds to a 97th centile for male infants born at 40 weeks gestation) to identify and treat hypoglycaemia. However the other infants included in the LGA study group i.e. infants born with a birthweight $> 97^{\text{th}}$ centile for sex, gestation and ethnicity but did not exceed the 4500 grams threshold did not routinely receive blood sugar testing. Blood sugar was tested in these infants only if they were symptomatic for hypoglycaemia or if they needed blood test for any other reason.

Table 3:16: Characteristics of LGA, hypoglycaemic infants.

	Admitted to NICU	Managed on PNW	P-value
Gestation (weeks)	39+6 (39+3 – 40+4)	39+3 (38+2 – 40+3)	0.740
Birthweight (grams)	4812.5 (4640 – 5190)	4745.0 (4690 – 4800)	0.650
Blood glucose (mmol/L)	1.65 (1.0 – 2.0)	1.75 (1.5 – 2.0)	0.484

Values for continuous variables presented as median (interquartile range);

***Mann Whitney U test used for continuous variables*

The pre-feed blood sugar was tested in 35 LGA infants and four AGA infants. Of the entire cohort, only six LGA infants (3%) developed hypoglycaemia (defined as blood sugar < 2.0 mmol/L) as compared to none in the AGA group. Of the six LGA infants who developed hypoglycaemia, two were managed on the postnatal ward

(PNW) and four were admitted to NICU. The two infants who were managed on the PNW had median birthweight of 4745.0 (4690 – 4800) grams. Only one infant had symptomatic hypoglycaemia and the other had biochemical hypoglycaemia detected on routine pre-feed blood sugar monitoring for LGA infants. Hypoglycaemia resolved in both these infants with breast feeding and additional top-up feeding with term formula milk. The four hypoglycaemic LGA infants who were admitted to the NICU had a median birthweight of 4812.5 (4640 – 5190) grams. They were not symptomatic and their biochemical hypoglycaemia was detected on routine pre-feed blood sugar monitoring. Of these infants, hypoglycaemia resolved in three infants with additional term formula milk feeds on the NICU. Only one infant (gestational age of 40+3 weeks and birthweight 4640kg) needed a 10% intravenous dextrose bolus and maintenance intravenous infusion to maintain normoglycaemia. There was no difference in the gestational age, birthweight or blood glucose levels amongst the LGA hypoglycaemic infants who needed NICU admission or those who were managed on the postnatal ward (table 3.16).

3.8.5.3 Comparison of presumed sepsis resumed sepsis

The most common reason for admission to the NICU was presumed sepsis. It was noted in 18/21(85.7%) of LGA infants and 10/10 (100%) of AGA infants admitted to NICU. None of the infants had a positive blood or CSF culture. The median (IQR) duration of antibiotic treatment in the LGA infants was 3.33 (2.0 – 5.0) days and 3.8 (2.0 – 7.0) days in the AGA infants ($p = 0.132$) (table 3.17).

Table 3:17: Comparison of LGA and AGA infants admitted to NICU with presumed sepsis.

	LGA Infants N = 18	AGA Infants N = 10	P-value
CRP	< 5	<5	0.350
Positive blood /CSF culture	0	0	-
Duration of antibiotics	3.33 days (2 – 5 days)	3.80 days (2 – 7 days)	0.132

Values for continuous variables presented as median (interquartile range),

***Mann Whitney U test used for continuous variables*

3.8.5.4 Respiratory distress

Respiratory distress was the second commonest reason for admission to NICU amongst the LGA infants. 14/21 (62.5%) LGA infants admitted to NICU had respiratory distress as compared to 3/10 (30%) of the AGA infants ($p = 0.037$). Amongst LGA infants and the AGA infants, the reasons for respiratory distress were: transient tachypnoea of newborn (TTN) in 12 vs. 2 infants and congenital pneumonia in 2 vs. 1 infants respectively. The median (IQR) FiO₂ requirement was 23% (21 – 37%) in the LGA infants and 33% (24 – 44%) in the AGA infants ($p = 0.058$). Only one control infant needed ventilation for just one day. He was born at a gestation of 40+1 weeks with birthweight of 3130 grams. He had a postnatal diagnosis of Trisomy 9 with multiple congenital anomalies and his total duration of hospital stay was 48 days.

3.8.5.5 Other neonatal morbidities

There was no difference in neonatal hyperbilirubinaemia between the LGA and the AGA infants. None of the infants in the LGA or the AGA group had symptoms of other potential postnatal complications such as hypocalcaemia, hypomagnesaemia, and polycythaemia. As all of these infants were not routinely screened for these complications there is a possibility that these infants might have had subclinical biochemical abnormalities, which were not identified.

3.8.6 Comparison of readmission

25 (12.6%) LGA infants and 17 (8.5%) control infants were readmitted to the hospital after initial discharge after birth. There was no statistical difference in the readmission rate between LGA infants and control infants ($p = 0.196$). The median (IQR) duration of days after birth at which readmission occurred was 12 (6 – 21) days for LGA infants and 10 (7 – 24) days for the control infants ($p = 0.954$). The median duration of the readmission was less than one day for both the groups ($p = 0.533$).

3.8.7 Regression analysis

Univariate analysis showed that there was a significant difference in the adverse composite outcome between LGA and AGA infants born to mothers without diabetes in pregnancy. LGA infants had a significantly higher risk of adverse composite outcome 22% vs. 12.5% ($p = 0.032$), OR 2.31 (1.269 – 4.19). Various maternal, intrapartum and neonatal confounding factors could have also contributed to this difference in the neonatal outcome seen in the two groups. The various maternal factors that could have influenced adverse neonatal outcome were maternal age, pre-pregnancy weight, height, pre-pregnancy BMI, smoking and parity. The various intrapartum and neonatal factors that could have influenced adverse neonatal outcome were caesarean section, neonatal sex and being LGA. A binary logistic regression model for multilevel analysis was used to study the influence of various confounding factors stated above on composite adverse outcome in LGA and AGA infants born to mothers without diabetes in pregnancy. A binary logistic regression was used as it predicts the probability of an observation to fall into one of two categories of a dichotomous dependent variable based on one or more independent variables that can be either continuous or categorical.

3.8.7.1 Assumptions for binary logistic regression

Assumption 1: The dependent variable (adverse composite outcome) was measured on a dichotomous scale.

Assumption 2: The independent variables were either continuous or categorical.

Assumption 3: There was independence of observations and the dependent variable had mutually exclusive and exhaustive categories.

Assumption 4: There was a bare minimum of 15 cases per independent variable.

Assumption 5: Two or more independent variables were not highly correlated with each other and the data did not show multicollinearity.

Assumption 6: The continuous independent variables were linearly related to the logit of the dependent variable. Linearity of the continuous variables with respect to the logit of the dependent variable was assessed via the Box-Tidwell (1962) procedure. This assessment confirmed that as the interaction term for the four continuous independent variables (maternal age, maternal weight, maternal height and maternal BMI) was not statistically significant, the original continuous independent variable was linearly related to the logit of the dependent variable as shown in table 2.24.

Table 3:18 Box-Tidwell (1962) procedure for the assessment of the linearity of the continuous variables with respect to the logit of the dependent variable

Variables in the Equation								
Step 1 ^a	B	S.E.	Wald	d f	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
Maternal weight	6.799	4.509	2.273	1	.132	897.020	.130	618119 3.131
Maternal height	-7.343	5.414	1.839	1	.175	.001	.000	26.273
Maternal BMI	-15.418	10.135	2.314	1	.128	.000	.000	85.303
Smoking (1)	.132	.304	.189	1	.663	1.142	.629	2.073
Parity			2.269	2	.322			
Parity(1)	-.398	.324	1.506	1	.220	.672	.356	1.268
Parity(2)	-.780	.594	1.727	1	.189	.458	.143	1.468
Caesarean section (1)	-.044	.322	.019	1	.891	.957	.509	1.799
Neonatal sex (1)	-.841	.304	7.673	1	.006	.431	.238	.782
LGA (1)	.930	.331	7.892	1	.005	2.534	1.325	4.849
IN_Maternal_Age by Maternal_Age	-.002	.006	.124	1	.725	.998	.986	1.010
IN_Maternal_weight by Maternal_Weight	-1.071	.711	2.269	1	.132	.343	.085	1.381
IN_Maternal_height by Maternal_height	1.040	.820	1.609	1	.205	2.828	.567	14.103
IN_Maternal_BMI by Maternal_BMI	2.877	1.892	2.312	1	.128	17.763	.436	724.382
Constant	342.145	215.378	2.524	1	.112	3.906E +148		

Assumption 7: During regression analysis, the casewise diagnostic identified that there were 11 infants with studentized residuals greater than ± 2 standard deviations. These infants were reviewed in further detail and decision was made to include them in the analysis.

3.8.7.2 Results of regression analysis

3.8.7.2.1 Data coding

Case processing summary (table 3.19) showed that 399 cases (99.8%) were included in the analysis and 1 case (0.3%) was missing.

Table 3:19: Summary of the cases included in the analysis

Case Processing Summary			
Unweighted Cases ^a		N	Percent
Selected Cases	Included in Analysis	399	99.8
	Missing Cases	1	.3
	Total	400	100.0
Unselected Cases		0	.0
Total		400	100.0
a. If weight is in effect, see classification table for the total number of cases.			

3.8.7.2.2 Baseline analysis (Block 0, beginning block)

The beginning block was the first step of binary regression analysis. This step of the model just included the constant without any independent variables. It provided the best guess for the outcome without the influence of the independent variables. This information was later used as a comparison to the model with all the independent variables added. The model at this stage correctly identified 83.2% of the cases (table 3.20). Table 3.21 shows that only constant was included in the model at this stage and table 3.22 shows the list of independent variables not included in the baseline analysis.

Table 3:20: Classification table to show the prediction of the outcome without any independent variables.

Classification Table ^{a,b}					
	Observed		Predicted		
			COMPOSITE		Percentage Correct
			.00	1.00	
Step 0	COMPOSITE	.00	332	0	100.0
		1.00	67	0	.0
	Overall Percentage				83.2
a. Constant is included in the model.					
b. The cut value is .500					

Table 3:21: Inclusion of constant in the model (without any independent variables).

Variables in the Equation							
		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	-1.600	.134	142.797	1	.000	.202

Table 3:22: Independent variables not included in the model.

Variables not in the Equation					
			Score	df	Sig.
Step 0	Variables	Maternal_Age	.721	1	.396
		Maternal_Weight	.024	1	.876
		Maternal_height	.207	1	.649
		Maternal_BMI	.000	1	.999
		Smoking (1)	.056	1	.813
		Parity	1.471	2	.479
		Parity (1)	.466	1	.495
		Parity (2)	.397	1	.529
		Caesarean section (1)	.553	1	.457
		Neonatal sex (1)	8.608	1	.003
		LGA(1)	5.287	1	.021
	Overall Statistics		17.900	10	.057

3.8.7.2.3 Model fit

Omnibus Tests of Model Coefficients provided the overall statistical significance of the model. Table 3.23 shows that the model was statistically significant with p value of 0.046. Another way of assessing the adequacy of the model was to analyse how poor the model was at predicting the categorical outcomes. This was done using Hosmer and Lemeshow test. Table 3.24 shows that the Hosmer and Lemeshow test was not statistically significant, $p = 0.988$, indicating that the model was not a poor fit.

Table 3:23: Omnibus Tests of Model Coefficients.

Omnibus Tests of Model Coefficients				
		Chi-square	df	Sig.
Step 1	Step	18.551	10	.046
	Block	18.551	10	.046
	Model	18.551	10	.046

Table 3:24 : Hosmer and Lemeshow Test.

Hosmer and Lemeshow Test			
Step	Chi-square	df	Sig.
1	1.721	8	.988

3.8.7.2.4 Variance in the model

The explained variation in the dependent variable based on the model ranged from 4.5% to 7.6%, depending on whether the Cox & Snell R² or Nagelkerke R² methods were used, respectively.

Table 3:25: Model summary.

Model Summary			
Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	342.602 ^a	.045	.076
a. Estimation terminated at iteration number 5 because parameter estimates changed by less than .001.			

3.8.7.2.5 Category prediction

The earlier classification table in section 3.8.7.2.2, which did not include any independent variables showed that 83.2% of cases overall could be correctly classified. With the independent variables added, the model still classified 83.2% of cases (table 3.26). That is, the addition of the independent variables did not improve the overall prediction of cases into their observed categories of the dependent variable.

Table 3:26: Classification table to show prediction of the outcome with the independent variables.

Classification Table ^a					
	Observed		Predicted		
			COMPOSITE		Percentage Correct
			.00	1.00	
Step 1	COMPOSITE	.00	332	0	100.0
		1.00	67	0	.0
	Overall Percentage				83.2
	a. The cut value is .500				

3.8.7.2.6 Variables in the equation

A binary logistic regression was performed to ascertain the effects of maternal age, pre-pregnancy weight, height, BMI, smoking, parity, caesarean section, neonatal sex and LGA on the likelihood of adverse composite outcome in their newborn infants. The logistic regression model was statistically significant, $\chi^2(4) = 18.5\%$, $p = 0.046$. The model explained 4.5% (Nagelkerke R²) of the variance in heart disease and correctly classified 83.2% of cases. Of the abovementioned independent confounding factors only being LGA and male sex were statistically significant. LGA and male sex increased the risk of adverse composite outcome, adjusted OR (95% CI) 2.415 (1.282 – 4.549) and 2.236 (1.243 – 4.020) respectively.

Table 3:27: Results of binary logistic regression.

Variables in the Equation								
Step 1 ^a	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
Maternal_Age	-.017	.026	.416	1	.519	.983	.935	1.035
Maternal_Weight	.000	.102	.000	1	1.000	1.000	.818	1.222
Maternal_height	-.018	.090	.041	1	.839	.982	.824	1.170
Maternal_BMI	-.011	.278	.001	1	.970	.990	.574	1.705
Smoking (1)	.065	.302	.046	1	.830	1.067	.590	1.930
Parity			1.793	2	.408			
Parity (1)	-.352	.317	1.233	1	.267	.703	.377	1.309
Parity (2)	-.665	.581	1.312	1	.252	.514	.165	1.606
Caesarean section (1)	.024	.315	.006	1	.939	1.024	.553	1.898
Neonatal sex (1)	.805	.299	7.225	1	.007	2.236	1.243	4.020
LGA (1)	.882	.323	7.440	1	.006	2.415	1.282	4.549
Constant	1.430	14.711	.009	1	.923	4.177		

3.9 Summary of the results

1. Mothers of LGA infants were older, had higher weight, height and BMI at booking and higher parity as compared to AGA infants.
2. LGA infants had a significantly higher risk of being delivered by elective caesarean section before term or undergoing induction of labour before term. There were also at a significantly higher risk of delivering by emergency caesarean section as compared to AGA infants.
3. LGA infants had a significantly higher risk of adverse composite outcome and NICU admission as compared to AGA infants.

4 CHAPTER

4.1 Introduction

Fetal growth and development is primarily determined by the genetic potential of the fetus. However, this genetic potential is influenced by environmental factors, which can exert either a stimulatory or inhibitory effect. The fetus is highly dependent on the nutritional status of the mother and on the ability of the placenta to transport nutrients to the fetus. The fetus also has its own growth factors, which influence growth and differentiation. Normal fetal growth is the result of a carefully controlled equilibrium between these different maternal and fetal factors. Any imbalance between these factors can result in fetal growth restriction or fetal overgrowth (macrosomia). Glucose, amino-acids and lactate have been reported as the principal energy substrates during fetal life, with glucose alone providing about half the total energy requirements. In the absence of fetal pathways for gluconeogenesis, the fetus is crucially dependent on the placental transfer of glucose for its growth (268). Glucose crosses the placenta by facilitated diffusion along a concentration gradient between maternal and fetal plasma with fetal plasma concentrations being about 75% (about 1.5mmol/L lower) than maternal venous plasma levels (269, 270). Net fetal glucose consumption on average is about 5mg/kg/min, which is similar to the rate of endogenous glucose production after birth. Fetal insulin is the main anabolic hormone and it dominates the fetal endocrine milieu. Insulin does not cross the placenta due to its molecular weight. Fetal insulin secretion is influenced by the concentration of the fetal plasma glucose level and is independent of maternal insulin levels (explained in detail in later sections). Insulin promotes anabolism in the fetus by stimulating uptake of glucose into muscles and adipose tissue and the accumulation of glycogen in the liver in preparation for birth. Thus, the last trimester of pregnancy is a period of rapid fetal growth particularly deposition of adipose tissue(167). At birth newborns have to make an abrupt transition from a state of net glucose uptake and glycogen synthesis to independent glucose production. This occurs by a decline in insulin production and secretion and by utilisation of glycogenolytic and gluconeogenetic pathways, which, although present during fetal life, are mainly dormant. The endocrine

pathways that help in release of glucose and mobilisation of fat include a steady drop in insulin and an increase in secretion of glucagon and adrenaline (adrenaline levels are higher after birth than any other time of life; indicating the vital role this hormone plays in metabolic and cardiorespiratory adaptation in the immediate perinatal period). During this endocrine transition in the initial few hours after birth, healthy newborn infants experience a physiological nadir in their blood glucose levels (271) . However in healthy newborns within a few hours after birth, the metabolic transition from glucose deposition to glucose production is established and the availability of alternative fuels (free fatty acids and ketone bodies) helps maintain normoglycaemia and prevent the development of hypoglycaemia (272, 273) . It is in the macrosomic and non-macrosomic infants of diabetic mothers and mothers with glycaemic derangements during pregnancy (which in its most severe form leads to GDM) that the pancreatic B-cell – insulin axis is over-stimulated due to maternal and fetal hyperglycaemia. After birth this pathway takes slightly longer than usual to down-regulate and switch-off thereby resulting in transient hyperinsulinism. As insulin is an anabolic hormone it also prevents gluconeogenesis and glycogenolysis and hence these infants also lack an alternative fuel supply.

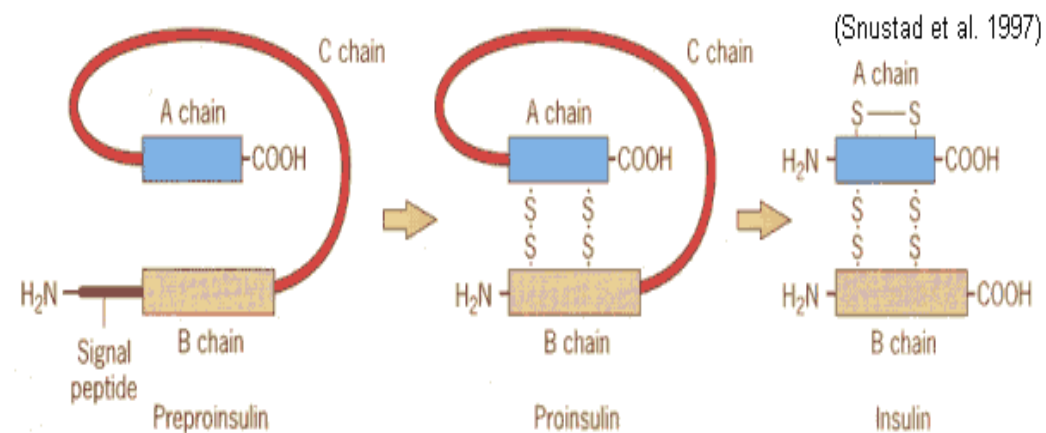
Most of the neonatal complications seen in infants of diabetic mothers are thought to be due to fetal hyperinsulinism. The aim of the literature review in this chapter is to examine the evidence to support this belief and to review the relationship between C-peptide (which is a surrogate marker of endogenous insulin) and neonatal outcomes.

4.2 Background and Literature review

4.2.1 Insulin structure and synthesis

The B - cells in the pancreatic islets produce insulin. Its synthesis involves stepwise cleavage of its two precursor molecules, preproinsulin (98 amino acids) and proinsulin (86 amino acid). The gene encoding preproinsulin is located on the short arm of chromosome 11. Following its synthesis, the preproinsulin molecule undergoes enzymatic cleavage, which involves rapid removal of a peptide with 12 amino acids to form proinsulin (272, 274) . The single chain proinsulin folds back onto itself, thereby aligning the A-chain (21 amino acids) and B-chain (30 amino acids) of insulin and creating two disulphide bonds in this process. The A-chain and B-chain remain connected by the connecting peptide (C-peptide – 35 amino acids).

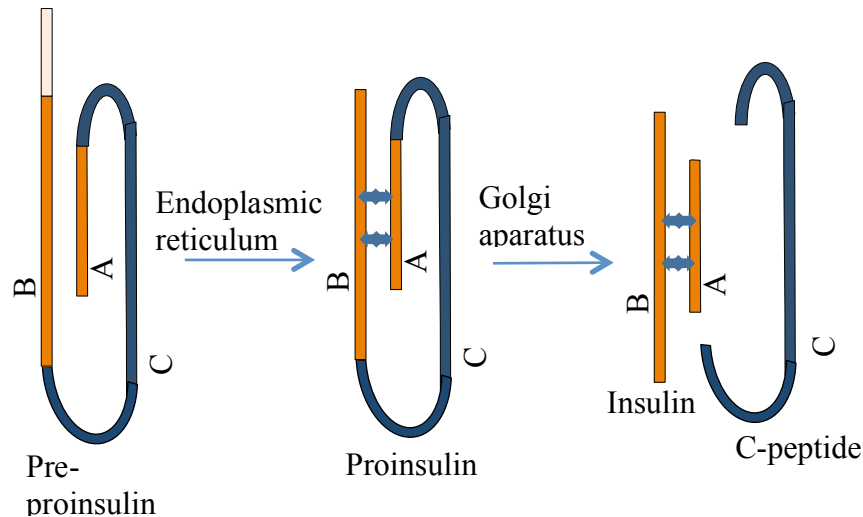
Figure 4.1: Structure and formation of insulin from preproinsulin.



In the Golgi complex of B - cells, proinsulin is stored in so-called beta-granules. These contain the proteolytic enzymes that will cleave and remove the C-peptide from proinsulin, resulting in equimolar amounts of insulin and C-peptide in the mature beta-granule.

4.2.1.1 Storage and release of insulin and C-peptide into circulation

Figure 4.2: Storage and release of insulin and C-peptide from mature B-granules in B-cells of pancreas.



The mature beta-granules form a large storage pool for insulin, well in excess of the daily requirement. Following an increase in blood glucose levels, insulin is released into the circulation by fusion of the granules with the beta-cell membrane and exocytosis. Insulin and C-peptide are released into the circulation in equimolar concentrations along with small amounts of other molecules like proinsulin and its metabolites. Human C-peptide in circulation has 31 amino acids. C-peptide had been historically used in adults as a measure of endogenous insulin production in patients with T1DM and T2DM. C-peptide, which was initially thought to be an inert hormone, has more recently been shown to have an influence on human muscle microcirculation and renal function and hence may play a role in the renal complications seen in the diabetics (272) .

4.2.1.2 Insulin metabolism and degradation

Insulin has a short half-life of 4-6 minutes, allowing minute-by-minute regulation of glucose metabolism (272, 275-277) . The liver is the primary site for insulin clearance (277, 278) . Approximately 50% of portal insulin is removed during first-pass transit through the liver. This means that portal levels of insulin are higher than those in the systemic circulation. The kidney is largely responsible for insulin clearance (279) , and delayed insulin clearance may cause problems with blood sugar control in those with kidney disease. Some degradation occurs within the insulin granule, and insulin is degraded in other tissues like muscles and adipose tissues after binding to the insulin receptor.

4.2.1.3 C-peptide metabolism and degradation

After secretion of C-peptide from the pancreatic B-cells in equimolar concentration to insulin, it enters portal circulation. Unlike insulin, the liver extracts only a very minor fraction of C-peptide. Hence the systemic levels of C-peptide reflect the amount secreted by the B-cells and act as a surrogate marker for insulin. The half-life of C-peptide is about 30 minutes. C-peptide is mainly excreted by the kidneys (280) .

4.2.2 Difficulty in measurement and assessment of serum insulin

1. The liver rapidly clears about 50% of the total insulin secreted by pancreas as it passes through the portal circulation – first pass metabolism. Hence serum levels of insulin do not reflect the pancreatic B-cell secretory capacity (281, 282) .
2. The half-life of insulin is only 4 – 6 minutes (272, 275-277) .
3. The degradation of insulin is even faster in the presence of haemolysis. An insulin-degrading enzyme found in red blood cells as well as in other tissues is responsible for this (283) .
4. Administration of exogenous insulin leads to varying degrees of antibody production and this makes measurement of insulin using radioimmunoassay difficult.
5. Proinsulin and its metabolites may cross-react with insulin in some insulin radioimmunoassays. This can be significant, especially because the half-life of proinsulin is at least three times as long as that of insulin.

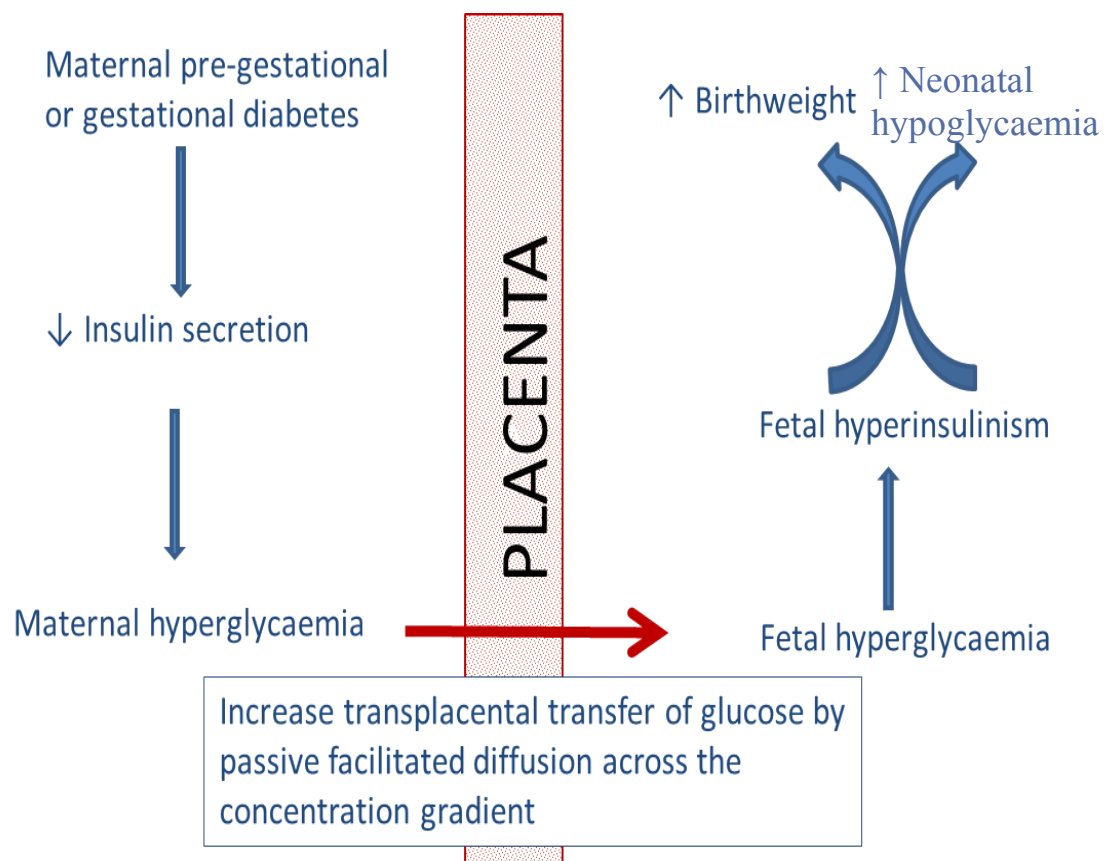
4.2.3 Benefits of measuring C-peptide

1. C-peptide is secreted in equimolar concentration from the pancreas and has been used as a surrogate marker for endogenous insulin secretion in patients with T2DM for a long time (284, 285) .
2. Extraction of C-peptide by the liver during the first pass is negligible compared to insulin (286) .
3. Half-life of C-peptide is approximately 30 minutes as compared to 4-6 minutes for insulin (272) .
4. C-peptide is not affected by the presence of haemolysis in the sample (287) .
5. C-peptide does not bind to insulin antibodies and hence it is easier to reliably measure serum levels.

4.2.4 Role of maternal hyperglycaemia in inducing fetal hyperinsulinism

It was in the 1960s when Jorgen Pedersen formulated the hyperglycaemia-hyperinsulinaemia hypothesis more commonly known today as Pedersen's hypothesis. This stated "maternal hyperglycaemia results in fetal hyperglycaemia and hence in hypertrophy of B-cells of fetal pancreas and fetal hyperinsulinism. This results in greater fetal utilisation of glucose and overgrowth of insulin sensitive tissues. This phenomenon explains several abnormal changes found in neonates born to mothers with diabetes in pregnancy"(137). Several studies have since been conducted in animals and humans to support this concept. Fig 4.3 shows the schematic representation of Pedersen's hypothesis.

Figure 4.3: Schematic representation of influence of maternal hyperglycaemia on the fetus.



4.2.4.1 Histopathological evidence

Fee et al in 1963 described infants of diabetic mothers, as 'infants that are physically large in a manner that is inconsistent with gestational age, appear at first glance to be oedematous, plethoric and have a peculiar facial appearance akin to that seen in patients with Cushing Syndrome'. Autopsy examination of stillborn infants of diabetic mothers had consistently shown visceral enlargement and pancreatic islet cell hyperplasia with accumulation of excessive fetal fat rather than lean mass resulting in fetal macrosomia(210).

Steinke et al studied fetal pancreas at the time of autopsy in 9 infants of diabetic mothers and 28 control infants of mothers without diabetes. They found functional fetal pancreas between 10 – 14 weeks of gestation(167). The mean extracted insulin in the control group increased from 6.3 ± 1.1 units/gram of pancreas for infants born at 20 – 32 weeks of gestation to 12.7 ± 3.2 units/gram of pancreas for infants born at 34 – 42 weeks of gestation. These insulin levels are significantly higher as compared to reported insulin levels of 2.15 ± 0.33 units/gram of pancreas in adults (288) . The mean extracted insulin in infants of diabetic mothers was 21.1 ± 5.2 units/gram of pancreas which was significantly higher than the infants of controls ($p < 0.01$) and these levels correlate with insulin levels seen in functioning islet cell tumours (289) . Histological examination of the pancreas of infants of diabetic mothers showed evidence of marked B – cell hypertrophy and hyperplasia with increased B – cell granulation(167).

Cardell D.S et al studied various organs during post-mortem examination in 25 infants of diabetic mothers. In 18 infants of diabetic mothers, the pancreatic islet tissue ranged from 1.8 – 9.9% of total pancreatic tissue, compared to a normal range of 0.7 – 2.6% in normal newborn infants born to non-diabetic mothers. 72% showed an increase in islet tissue, which was mainly due to an increase in B-cells. There was a close correlation between the amount of islet tissue and the body weight of the infant(166).

Naeye et al conducted a quantitative morphological study to compare the weights and measurements of various organs from 30 infants of diabetic mothers who were stillborn or who had a neonatal death and compared them to 14 gestational age and postnatal age matched control infants born to non-diabetic mothers with birthweight within 20% of the predicted weight. Of the 30 infants of diabetic mothers 21 had a birthweight more than 20% above the predicted value, 4 had birthweight less than 20% below the predicted value and 5 infants had a normal birthweight. Weight of the body, heart, lungs, liver, thymus and adrenal gland were all significantly higher for the macrosomic infants and significantly lower for the underweight infants as compared to the controls (290) .

The pancreatic weight in the overweight infants was 110% of the normal weight of the organ; however a striking difference was noted in the endocrine part of the gland. The islet cells constituted 10.8% of the gland in the overweight infants and 5.4% in the underweight infants of the diabetic mothers as compared to only 3.5% in the controls.

Increase in the endocrine part of the pancreas in the overweight and underweight infants of diabetic mothers was reported to be due to an increase in the number and size of the islet cells (290) .

These experiments show that maternal hyperglycaemia due to diabetes in pregnancy leads to chronic fetal exposure to increased blood sugar levels and this in turn results in excessive stimulation of the fetal pancreas. This leads to hypertrophy and hyperplasia of the B-cells of the fetal pancreas. This in the antenatal period results in fetal macrosomia due to the anabolic action of insulin, which results in overgrowth of insulin sensitive tissues and enlargement of various internal organs due to excessive glycogen deposition. The persistence of hyperinsulinism in the early postnatal period is most likely responsible for fetal hypoglycaemia.

4.2.4.2 Biochemical evidence

Baird et al, in their study measured glucose tolerance in 6 infants of diabetic mothers and 8 infants of non-diabetic mothers after a rapid single intravenous glucose injection of 0.5g/kg of 20% dextrose. Blood glucose levels were measured immediately before and at 10 minutes intervals up to 60 minutes after the initial glucose bolus. There was no difference in the fasting blood sugar levels at birth between infants of diabetic mothers and controls 3.2 vs. 3.1 mmol/L respectively with blood sugar levels rising to the highest level at 5 minutes, 9.5 and 8.3mmol/L respectively. After this there was a declining trend with the blood sugars reaching the lowest level at 60 minutes, 7.1 and 2.5mmol/L respectively.

The glucose disposal rate was much faster in infants of diabetic mothers as compared to controls. The fasting level of insulin was not statistically different between the two groups 200 vs. 149 μ U/L however at 5 mins after the glucose load, insulin levels in infants of diabetic mothers were 10 times higher at 700 μ U/L as compared to 72 μ U/L in control infants ($p < 0.01$). The pancreas of infants of diabetic mothers had a significantly heightened response to the glycaemic stimulus (291) .

Similarly Jorgensen et al have reported that the insulin levels were higher at birth in 15 infants of diabetic mothers as compared to 13 infants of non-diabetic mothers. After a glucose injection, a rapid increase of the insulin concentration was noted in infants of diabetic women, whereas the rise was slow in infants of non-diabetics, thus indicating an increased baseline insulin levels and a greater reactivity of insulin secretion in infants of diabetic women (292) .

Obenshain SS et al conducted two experiments to assess fetal pancreatic responsiveness to a sustained glucose stimulus. The first experiment was conducted at early gestation (between 12–20 weeks – fetuses being delivered by hysterotomy) and at term. Maternal hyperglycaemia was maintained by infusion of glucose at 6mg/kg/min for at least 180 minutes before delivery in normal mothers and they were compared to control mothers at similar gestation who were infused with

normal saline before delivery. At term, maternal glucose levels were 8.3 ± 0.8 vs. 5.1 ± 0.4 mmol/L and insulin levels were 84 ± 29 vs. 8.8 ± 1.6 μ U/ml in glucose infused mothers as compared to saline infused mothers. However there was no statistically significant difference in the umbilical cord insulin levels between infants of glucose and saline infused mothers 6.9 ± 1.2 vs. 6.4 ± 1.3 μ U/ml. This suggests that maternal insulin, which was raised in glucose infused mothers, did not cross the placenta and glucose infusion lasting for 180 minutes before delivery was not long enough to stimulate the fetal pancreas, which requires chronic fetal exposure to hyperglycaemia. There was a direct correlation between fetal blood glucose and plasma insulin levels with a correlation co-efficient of 0.75 ($p < 0.001$) at all gestation. However, the insulin response to a similar glucose stimulus was attenuated at early gestation suggesting gradually increasing sensitivity of the fetal pancreas to hyperglycaemic stimulus as gestation progresses.

In the second experiment, they studied term infants of mothers with GDM and compared them to term infants of control mothers without diabetes. There was no difference in blood glucose levels between the diabetic women and the controls 5.9 ± 0.6 vs. 5.1 ± 0.39 mmol/L respectively but the diabetic women had significantly raised insulin levels 22 ± 5 vs. 8.8 ± 1.6 μ U/ml ($p < 0.05$) that suggested insulin resistance in diabetic mothers. Infants of gestational diabetic mothers had increased umbilical plasma insulin levels of 23.3 ± 6.9 μ U/ml as compared to 6.4 ± 1.3 μ U/ml in normal controls even though there was no difference in the umbilical blood glucose levels in these two groups 5.0 ± 0.5 vs. 4.6 ± 0.3 mmol/L. This would suggest fetal hyperinsulinism secondary to maternal hyperglycaemia during pregnancy (293) .

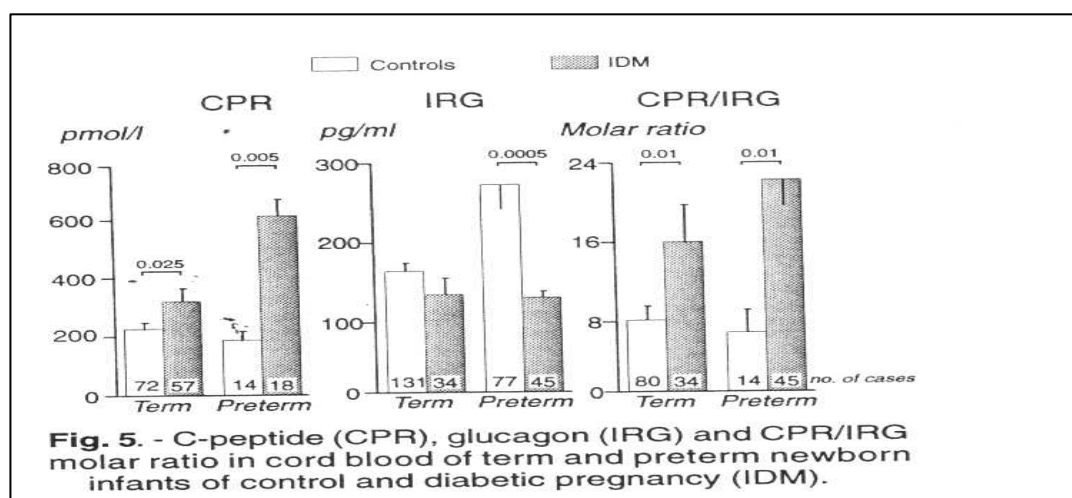
Heding et al compared 20 pre-gestational diabetic women (T1DM and T2DM) without insulin antibodies (as antibodies may give a falsely high estimation of free immune reactive insulin - IRI and proinsulin) and 13 non-diabetic women to establish the relation between:

- B-cell function of the mother and the offspring,
- B-cell function between diabetic and non-diabetic pregnant women and
- To seek correlations between neonatal B-cell activity and neonatal complications.

Their results showed that in spite of higher blood sugars, mean (IQR) in diabetic mothers 6.4 (2.6 – 15.7) vs. 4.3 (2.9 – 6.4) mmol/L, there was no significant difference in the serum levels of insulin 25 (0 – 42) vs. 21 (6 – 103) μ U/L and C-peptide 1180 (50 – 3000) vs. 945 (280 – 2400) pmol/L as compared to non-diabetic women i.e. pre-gestational diabetic women who had long standing diabetes had poor endogenous insulin production. Contrary to this, the newborn infants of diabetic mothers had significantly higher umbilical cord blood insulin 47 (3 – 205) vs. 13 (4 – 32) μ U/L ($p < 0.01$) and C-peptide 963 (250 – 4000) vs. 394 (200 – 680) pmol/L ($p < 0.001$) at more or less similar blood sugar levels of 4.5 (1.4 – 8.6) vs. 4.0 (2.0 vs. 7.3) mmol/L as compared to infants of non-diabetic mothers suggesting fetal hyperinsulinism. Cord blood insulin and C-peptide correlated positively with each other and birth weight and negatively with neonatal hypoglycaemia ($p < 0.01$, $p < 0.01$, $p < 0.01$ respectively). In another group of diabetic women, treated with exogenous insulin and had insulin antibodies, they reported similar levels of insulin - antibody complexes in the mothers and their offspring suggesting transplacental transfer of IgG insulin-antibodies but not of maternal free endogenous insulin or C-peptide (294) .

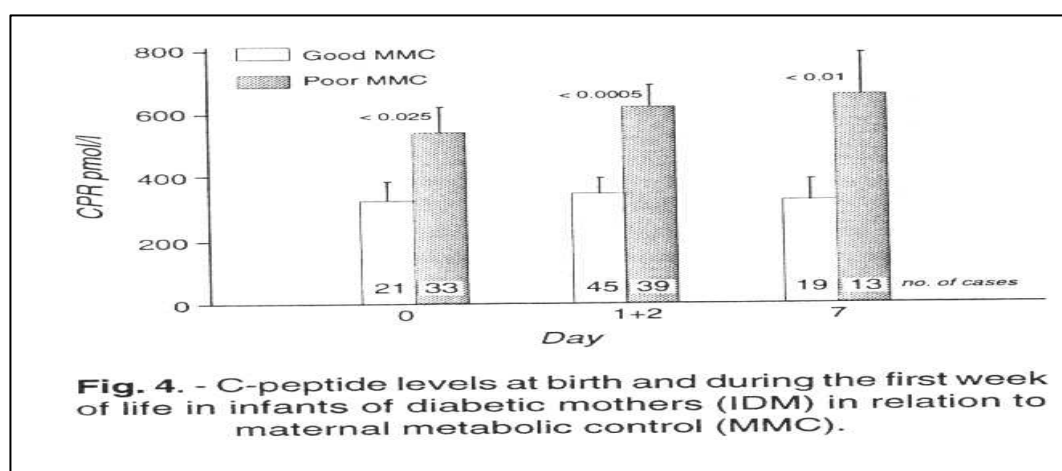
Fallucca et al reported that insulin and C-peptide secretion was higher throughout pregnancy at early gestation (16 – 24 weeks) and at late gestation (34 – 36 weeks) in diabetic pregnant women as compared to non-diabetic pregnant women (fig 4.6) (295) . This difference was even greater when the metabolic control of the diabetic mothers was poor (fig 4.7). This difference persisted even in the first week after birth and was associated with significant neonatal morbidity (295-297)

Figure 4.4: C-peptide, glucagon secretion and C-peptide glucagon ratio in preterm and term infants born to diabetic and control mothers.



'Pathology of diabetes in pregnancy' by Francesco Fallucca, Ann. Ist. Super Sanita, Vol 33, n.3 (1997), p 353-360.

Figure 4.5: Difference in C-peptide levels during the first week after birth in infants of mothers with good and poor metabolic control.



'Pathology of diabetes in pregnancy' by Francesco Fallucca, Ann. Ist. Super Sanita, Vol 33, n.3 (1997), p 353-360.

4.2.5 Placental permeability to maternal insulin and C-peptide:

The high insulin and C-peptide levels noted in the umbilical cord blood, amniotic fluid and neonatal blood in infants of diabetic mothers are of fetal origin and not a result of placental transfer of maternal hormones. The placenta acts as a barrier and it prevents the transfer of maternal insulin and C-peptide to the fetus. The human

placenta is impermeable to polypeptide hormones as they have a large molecular weight 1100 – 30000 daltons (298) .

Table 4:1: Placental permeability (298) .

The placenta is impermeable to the following polypeptide hormones	
Growth hormone	Adrenocorticotrophic hormone
Thyroid stimulating hormone	Follicle stimulating hormone
Luteinizing hormone	Oxytocin
Vasopressin	Glucagon
Insulin	C-peptide
Parathyroid hormone	Calcitonin

Table 4:2: Placental hormone transfer in relation to molecular weight.

Hormone	Approximate molecular weight	Placental transfer
Polypeptide hormones	1100 – 30000	No
Thyroid hormone	800	No
Steroid hormone	350	Yes
Catecholamines	180	Yes

There have been animal and human experiments to prove the placental impermeability to pancreatic hormones. However maternal and exogenous (bovine insulin more than porcine insulin) insulin bound to insulin antibodies (IgG) in the form of insulin-antibody complexes have been reported to cross placenta. However the use of less antigenic forms of exogenous insulin (human insulin, synthetic insulin rather than animal insulin) has led to a decrease in antibody formation.

Buse et al undertook a study to investigate the role of the human placenta in the transfer and metabolism of insulin in vivo in 28 pregnant non-diabetic women. 24 women received either a single intravenous bolus injection of insulin - I^{131} (N = 15), Na - I^{131} (N = 7) and human albumin - I^{131} (N = 2) about 7 – 274 minutes before delivery. Four women received a slow infusion of insulin - I^{131} . In 19 patients who received insulin - I^{131} the maternal levels of trichloroacetic acid (TCA) precipitable and TCA soluble levels of insulin - I^{131} increased progressively with time while

counts of insulin - I^{131} in cord plasma remained consistently low. However I^{131} rapidly equilibrated across the placenta (90% in 80mins after a single dose of intravenous Na - I^{131}). This suggests that I^{131} crossed the placenta and the low count of cord insulin - I^{131} was due to placental impermeability to insulin rather than I^{131} . During the study of the placenta in 7 patients who received insulin - I^{131} ratio of placental counts to maternal counts was significantly higher than after Na - I^{131} and human albumin - I^{131} . This suggests that the placenta actively traps and degrades the hormone (299) .

Adam et al studied pregnant women scheduled for therapeutic abortions by abdominal hysterotomy at 15 – 20 weeks of gestation. In two different experiments, they assessed

- The fetal insulin response to infused glucose in 8 insitu fetuses and
- Insulin transfer across the placenta following continuous insulin- I^{131} infusion in 8 pregnant women via peripheral vein for 4 – 6 hours.

In the fasting state maternal and fetal glucose and insulin levels were similar. Following glucose infusion directly into the fetus, an increase in fetal glucose levels was demonstrated without an increase in maternal glucose level or fetal insulin level at 5 or 10 minutes. This suggests that a shorter duration of hyperglycaemia is not sufficient to stimulate the fetal pancreas. Following infusion of human insulin- I^{131} in the mother there was no transfer or sequestration of insulin- I^{131} in the placenta suggesting that the placenta acts as a barrier to human insulin- I^{131} .

In another study, Wolf et al studied women in labour at term to assess the influence of maternal glucose and bovine insulin infusion on cord insulin levels to assess transplacental passage of insulin. The insulin in cord blood was nearly always elevated after maternal infusions (glucose $\pm$ insulin) compared with controls (without glucose infusion). Even an 18-fold increase in the maternal insulin level did not affect the cord insulin level. This study shows placental impermeability to maternal insulin in spite of high maternal insulin levels and the need for a sustained hyperglycaemic stimulus to induce the fetal pancreas (300) .

These above studies, using labelled insulin demonstrate that human placenta acts as a physical barrier and prevents transplacental passage of free insulin from the mother to the fetus. It is unclear whether it plays any active role in entrapment and degradation of insulin. There is a possibility that a small amount of maternal insulin bound to IgG insulin antibodies may be transferred to the fetus. Whether transfer of such antibody bound insulin plays any role in fetal overgrowth is not clear. The use of human insulin has eliminated the problem of insulin antibodies that was more commonly seen with the use of bovine or porcine insulin. The insulin levels measured in the cord blood and the neonatal venous blood reflect endogenous insulin production in these neonates. It is important to acknowledge that these studies were conducted about 5 decades ago in a very small number of mother-neonate dyads. They have provided valuable information and it would not be ethically correct now to conduct such studies using labelled insulin in a large number of pregnant women.

4.2.6 Cord blood C-peptide as a surrogate marker for fetal insulin

C-peptide has been historically used as a surrogate marker for endogenous insulin production in adults with T1DM and T2DM (301) . Measurement of C-peptide levels helps in distinguishing between T1DM and T2DM and in assessing endogenous insulin production and need for exogenous insulin supplementation.

Phelps et al. assessed basal values for C-peptide and the responses to intravenous glucose at 2 - 4 hours after birth in 9 control infants and 9 infants of diabetic mothers. The mean basal glucose levels were 3.9 mmol/L in the control group and 2.2 mmol/L in the diabetic group infants. In spite of this, mean C-peptide concentration tended to be higher and peaked earlier along with insulin following a glucose bolus in the infants of diabetic mothers. This study suggests augmented basal β cell function in the offspring of insulin-treated diabetic mothers at birth and during the first few hours after birth (302) .

Ogata et al assessed cord blood C-peptide as a marker for the impact of maternal hyperglycaemia on fetal growth and development. They compared 17 diet controlled GDM (fasting <5.8 mmol/L), 18 insulin treated GDM (fasting >5.8 mmol/L), 13 insulin treated pre-gestational diabetic women and 22 control women without diabetes.

Table 4:3: Comparison of birthweight, cord blood C-peptide and glucose in pre-gestational and gestational diabetic mothers and control mothers (303) .

Groups	N	Birth weight (grams)	Cord blood	
			C-peptide (pmol/L)	Glucose (mg/dl)
Diet controlled GDM	17	3638 $\pm$ 160	801 $\pm$ 142*	7.0 $\pm$ 0.42
Insulin controlled GDM	18	3707 $\pm$ 180	711 $\pm$ 106*	5.2 $\pm$ 0.40
Insulin controlled DM	13	3780 $\pm$ 186	847 $\pm$ 156*	6.7 $\pm$ 0.85
Control	22	3170 $\pm$ 74	404 $\pm$ 40	5.3 $\pm$ 0.21

(* p < 0.01)

Cord blood C-peptide levels were not significantly different in infants of diabetic mothers; however these levels were significantly higher and almost double compared to a control group ($p < 0.01$). They also measured amniotic fluid insulin and glucose levels in infant of diabetic mothers during amniocentesis between 36 – 40 weeks, which also showed significantly raised amniotic fluid insulin levels compared with controls (amniotic fluid collected before planned caesarean section). This study shows that the fetal pancreatic B-cell response is enhanced and is operative in utero during the third trimester and this B-cell functional enhancement is even seen in offspring of mothers with the mildest form of diabetes during pregnancy i.e. diet controlled mothers with GDM (303) .

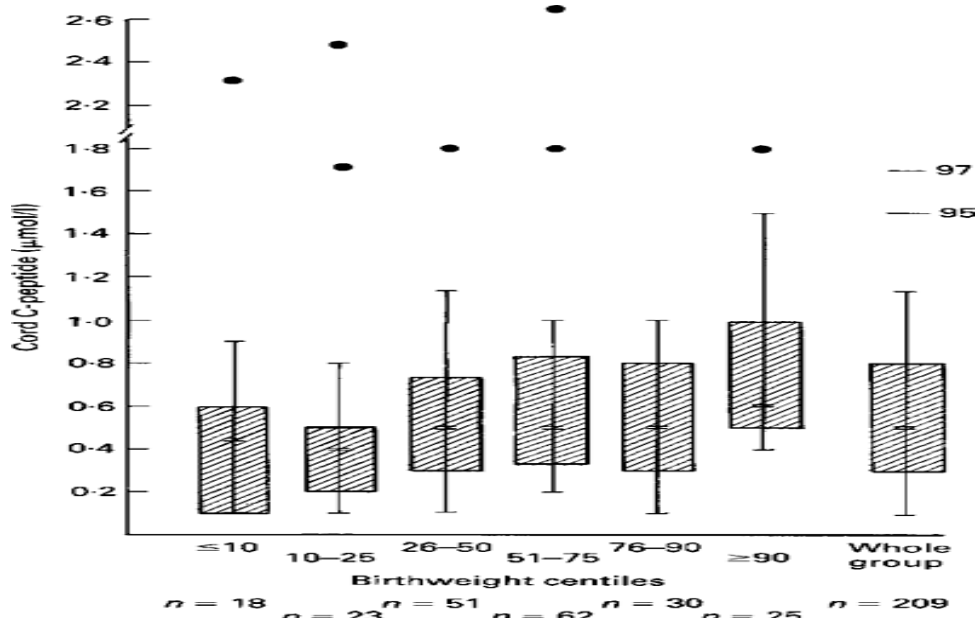
Burke et al compared 44 infants of insulin requiring diabetic mothers and 32 infants of non-diabetic mothers. Mean cord blood glucose levels were lower and mean cord blood C-peptide levels were higher in infants of diabetic mothers 3.9 ± 1.0 mmol/L vs. 4.7 ± 3.6 mmol/L and 460 ± 440 pmol/L vs. 180 ± 100 pmol/L respectively than in normal infants. When the diabetic group was re-examined after subdivision on the basis of maternal diabetic control there was a significant difference in mean cord blood C- peptide of infants of well controlled diabetic mothers 280 ± 300 pmol/L as compared with a mean of 560 ± 500 pmol/L for infants of poorly controlled mothers (304) . The cord blood C-peptide levels reflected the severity of fetal hyperinsulinism that in turn appeared related to the severity of maternal glycaemic derangement.

De Villers TJ et al compared 8 insulin treated overt diabetic (OD), 12 diet controlled GDM and 12 non-diabetic pregnant women. They showed that infants of women with GDM as compared to infants born to insulin treated OD women and non-diabetic women had significantly higher mean cord blood C-peptide levels 679 ± 92 pmol/L vs. 466 ± 58 pmol/L vs. 412 ± 50 pmol/L ($p < 0.002$). These values in infants of women with GDM correlated with the increased birthweight ratios (BWR - actual birthweight / 50th percentile weight for that gestation) ($p < 0.05$). There was no significant difference in cord glucose levels between the three study groups and there was no significant difference in cord C-peptide, C-peptide/glucose ratio and BWRs between OD and control women suggesting adequate maternal glycaemic

control in the women with OD. Increased birthweight, cord blood C-peptide levels and increased cord blood C-peptide/glucose ratio seen in women with GDM may reflect glycaemic untreated derangements during pregnancy (305) .

Stanley et al, in their study of 209 unselected singletons between 34 – 42 weeks showed a positive correlation between umbilical cord blood insulin and C-peptide levels and the positive relation of both to birthweight.

Figure 4.6: The relation between cord C-peptide and birthweight centile for gestational age showing the birthweight centiles of hyperinsulinaemic babies. (median; interquartile range; 10th–90th birthweight centile) (306) .



They also found increased insulin levels in babies belonging to all birthweight centile groups rather than just LGA group. In other words, even babies with normal birthweight (10 – 90th centile) could be hyperinsulinaemic and hence at risk of complication, the most important being neonatal hypoglycaemia (306) .

The above experiments suggest that fetal hyperglycaemia results in fetal hyperinsulinism and a parallel rise in fetal C-peptide levels. There is a direct correlation between increased fetal insulin and C-peptide levels to fetal overgrowth and the risk of neonatal complications in the early postnatal period in infants of mothers with diabetes in pregnancy.

4.2.7 Relation of fetal insulin and C-peptide to neonatal complications

4.2.7.1 Animal Studies

In an experiment using in utero insulin infusion via an osmotic pump in fetal rhesus monkeys, Susa et al induced primary hyperinsulinaemia in the fetal rhesus monkey during the last trimester, with insulin levels similar to those observed in human infants of diabetic mothers at delivery. This physiologically relevant hyperinsulinaemia, in the absence of hyperglycaemia, resulted in a 23% increase in total body weight with no effect on skeletal growth. Low-grade hyperinsulinaemia resulted in only cardiomegaly, whereas high-grade hyperinsulinaemia resulted in cardiomegaly, hepatomegaly, and splenomegaly (307) .

Phillipps et al conducted a corollary experiment by injecting streptozocin into fetal sheep, which resulted in B-cell destruction and subsequent hypoinsulinaemia. Fetal body weight was decreased by 21% (308) .

Both these animal experiments support Pedersen's hypothesis that fetal insulin is a powerful growth stimulator and plays a predominant role in controlling normal, as well as augmented fetal growth.

4.2.7.2 Cord blood C-peptide and insulin levels in infants of diabetic mothers

Sosenko et al measured cord serum C-peptide levels in 79 infants of diabetic mothers and 62 infants of non-diabetic mothers. In control infants the cord blood C-peptide levels increased with gestation. In infants of diabetic mothers, cord blood C-peptide levels were significantly higher than controls even when they were born at less than 34 weeks of gestation ($p < 0.001$) and were directly proportional to the severity of maternal diabetes. This difference between the two groups narrowed near term gestation. Mean cord blood C-peptide levels in hypoglycaemic infants of diabetic mothers were 2454 ± 410 vs. 1302 ± 230 pmol/L ($p < 0.025$) and with macrosomia were 2500 ± 486 vs. 1392 ± 200 pmol/L ($p < 0.001$) in comparison to those who did not develop these complications (309) .

Fallucca et al compared cord blood C-peptide levels, insulin antibodies and fetal morbidity in 54 infants of diabetic mothers and 20 control infants born to non-diabetic mothers. The mean cord blood C-peptide levels in the control infants was 183 ± 25 pmol/L vs. 406 ± 50 in infants of diabetic mothers ($p = 0.01$). Mean C-peptide levels in infants of diabetic mothers who developed hypoglycaemia (blood sugar < 1.4 mmol/L) was 681 ± 142 pmol/L as compared to those who did not develop hypoglycaemia 351 ± 69 pmol/L ($p = 0.025$). Infants of mothers with poorer glycaemic control in pregnancy had a significantly higher risk of hypoglycaemia ($p < 0.01$) as compared to those with good control. The authors have also suggested that maternal IgG insulin antibodies following transplacental passage bind to fetal insulin. This further prolongs the fetal exposure to hyperglycaemia and further pancreatic stimulation (270) .

This group also measured C-peptide levels in 57 infants of insulin dependent T1DM mothers and 27 control infants at birth and on the 1st, 2nd and 7th day of life. C-peptide levels were higher in infants of diabetic mothers 374 ± 77 , 660 ± 117 , 399 ± 76 , 401 ± 87 vs. 183 ± 25 , 146 ± 28 , 74 ± 24 and 23 ± 33 pmol/L on each of these days respectively. These findings provide evidence that fetal B-cell hyperfunction is present at birth and even after 1 week of life in neonates born to diabetic mothers. This again supports Pederson's hypothesis that maternal hyperglycaemia leads to precocious stimulation of the fetal pancreas leading to fetal hyperinsulinism and complications (296, 297) .

Abdel Halim Badr El Din studied 30 infants of insulin treated diabetic mothers who were compared with 15 infants of non-diabetic mothers matched for gestational age, mode of delivery and one and five minute Apgar score. Infants of poorly controlled diabetic mothers had higher mean cord blood C-peptide levels 1076 ± 622 pmol/L as compared to infants of well controlled diabetic mothers 818 ± 275 pmol/L and both these values were higher than mean cord blood C-peptide levels 351 ± 169 pmol/L in control infants born to non-diabetic mothers. Although it did not reach a significant level, mean cord blood C-peptide was higher in macrosomic infants of diabetic mothers 1125 ± 665 pmol/L as compared to non-macrosomic infants of diabetic mothers 828 ± 288 pmol/L. Mean cord blood C-peptide was

higher in hypoglycaemic infants of diabetic mothers (hypoglycaemia defined as term baby, blood sugar <1.9 mmol/L and preterm baby, blood sugar <1.4 mmol/L) 1139 ± 543 pmol/L as compared to non-hypoglycaemic infants of diabetic mothers 927 ± 569 pmol/L. The cord blood C-peptide levels correlated well with the birthweight and birthweight indices and inversely with blood sugar levels at 1-2 hours of age in infants of diabetic mothers (310) .

Hagbard et al in their study on LGA infants born to women without overt diabetes, showed that the fetal hyperglycaemia – hyperinsulinaemia pathway was activated even in the presence of mild chronic hyperglycaemia as these mothers did not have overt diabetes noted during OGTT but had higher fasting and postprandial peaks as compared to control women (311) . This suggests that even milder forms of maternal glycaemic derangement leads to higher rather than normal fetal insulin production and secretion. The absolute amount of insulin production in the fetus is directly proportional to the duration and the severity of maternal and hence fetal hyperglycaemia.

Dube et al measured cord blood insulin and C-peptide levels in 18 infants born to mothers with GDM (8 diet controlled and 10 insulin controlled) and 23 infants born to non-diabetic mothers. As compared to control infants, infants of mothers with GDM tended to have higher cord blood glucose 5.0 ± 1.0 vs 4.5 ± 0.9 mmol/L ($p=0.09$) and higher C-peptide levels 652 ± 324 vs. 511 ± 166 pmol/L ($p=0.08$). Three macrosomic infants born to diabetic mothers had significantly higher cord blood C-peptide 1213 ± 136.4 vs. 482 ± 118.1 pmol/L ($p=0.009$) and cord blood insulin 229.7 ± 35.4 vs. 97.3 ± 30.6 pmol/L ($p=0.04$) as compared to four macrosomic control infants. The researchers showed a correlation between cord blood C-peptide level and birthweight ($p<0.05$). They did not find any relation between cord blood C-peptide levels and hypoglycaemia as they had only 5 hypoglycaemic infants (312) .

Knip et al studied 35 infants of diabetic mothers, 35 infants of non-diabetic mothers and 20 healthy non-diabetic adults. Infants of diabetic mothers had a significantly higher mean birthweight of 3560 grams as compared to 2861 grams in the controls

($p < 0.001$) and they were more likely to be delivered by caesarean section ($p < 0.001$). They also had a 3-fold increase in mean C-peptide levels 830pmol/L as compared to 270pmol/L in the controls and 390pmol/L in the adults ($p < 0.001$). 45% of the infants of diabetic mothers were macrosomic and they had higher mean free IRI of 160mU/L and mean C-peptide levels of 1190pmol/L as compared to the levels of 72mU/L and 530pmol/L respectively in the non-macrosomic infants of diabetic mothers. 17 infants of diabetic mothers who developed hypoglycaemia (blood sugar < 1.7 mmol/L in term and < 1.2 mmol/L in preterm infant) had higher mean free IRI level of 186mU/L vs. 42mU/L ($p < 0.001$) and mean C-peptide level of 1210pmol/L vs. 480pmol/L ($p < 0.01$) as compared to those who did not. None of the infants in the control group developed hypoglycaemia (313) .

Schwartz et al compared 95 non-diabetic pregnant women with 155 insulin treated pregnant women.

(Group 1: non-diabetic women with AGA infants,

Group 2: non-diabetic subjects with LGA infants,

Group 3: GDM and insulin treated White class A and B (refer to definitions and glossary of terms for White's classification),

Group 4: White class C and D and

Group 5: White class F and R).

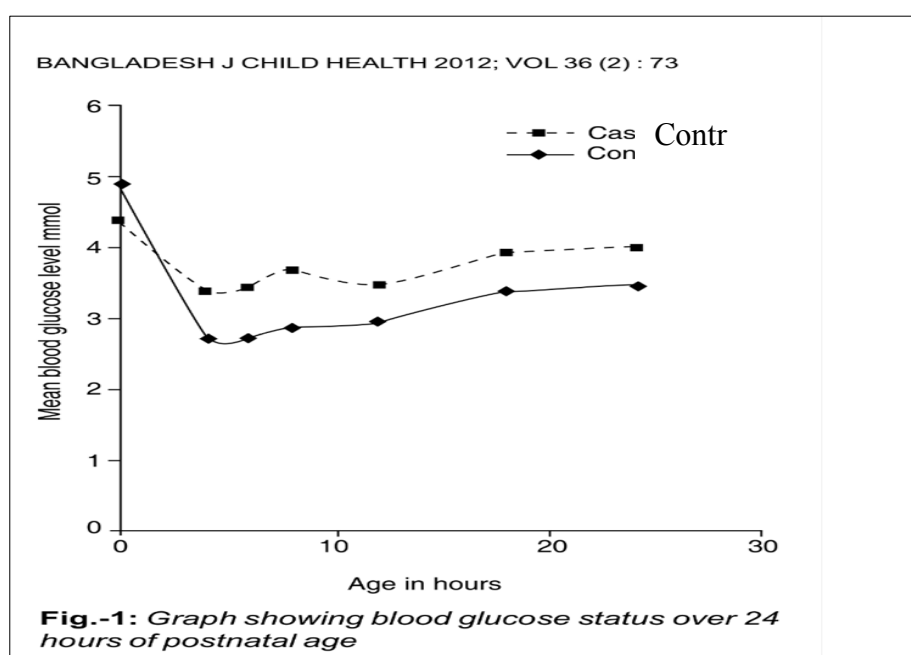
The mean cord blood C-peptide concentration was 320 ± 110 (130 – 620) pmol/L in group 1 as compared to 1240 ± 940 (140 – 5480) pmol/L in group 4. The other groups were intermediate. Even diet controlled women with GDM showed evidence of elevated fetal insulin 203 ± 192.5 pM and C-peptide levels, 680 ± 600 pmol/L (260 – 3000) (more than two times higher than controls). 10-27% infants of diabetic mothers were macrosomic and there was no correlation of infant birthweight to maternal weight, BMI or HbA1c but it correlated significantly with umbilical total insulin, free insulin and C-peptide levels. Such a co-relation to birthweight was not seen in the control groups(213).

In last decade, Hyperglycaemia and Adverse Pregnancy Outcome Study was undertaken to establish the relationship between maternal glycaemia, cord blood C-peptide and maternal and neonatal outcomes (314) . 17,094 pregnant women and their infants from 15 centres in 9 counties were enrolled in the study. Cord blood C-peptide level increased in direct proportion to increasing maternal blood glucose levels (315) . A blood sugar level from a heel prick blood sample was measured from all the infants at 1 – 2 hours of age and in 5% a second sample was repeated after 4 hours. 9.6% of infants had a birthweight more than 90th centile, 9.1% infants had biochemical hypoglycaemia (blood sugar <1.7mmol/L) while only 1.8% had clinical hypoglycaemia. Frequency of biochemical and clinical hypoglycaemia increased with increasing maternal blood glucose levels.

The frequency of biochemical hypoglycaemia increased progressively from 5.5% in the lowest category of C-peptide levels to 36.9% in the highest category. Clinical hypoglycaemia progressively increased from 2.1% in the lowest category to 7.4% in highest category. Infants with a birthweight more than 90th centiles had an increased risk of biochemical hypoglycaemia 12.3% vs. 8.9% ($p<0.001$) and clinical hypoglycaemia 3.4% vs. 1.9% ($p<0.001$) as compared to infants with birthweight less than the 90th centile. In infants with a birthweight and cord blood C-peptide level more than the 90th centile 38.7% vs. 16.8% ($p<0.01$) developed biochemical hypoglycaemia and 31.7% vs. 19.3% developed clinical hypoglycaemia. Macrosomic infants who developed biochemical hypoglycaemia and clinical hypoglycaemia had a mean cord blood C-peptide level of 584 vs. 405pmol/L ($p<0.01$) and 554 vs. 429pmol/L ($p<0.01$) respectively as compared to those who did not develop hypoglycaemia. Hence in conclusion the HAPO study showed that the cord blood C-peptide levels increased in direct proportion to maternal glycaemic control and there was a linear relationship between cord blood C-peptide levels and neonatal hypoglycaemia. However they measured their first blood sugar level at 1 – 2 hours after birth and hence they have a significantly higher incidence of biochemical hypoglycaemia as compared to clinical hypoglycaemia due to the physiological nadir in blood sugar levels after birth (316, 317) .

Begum NN et al carried out a case control study in 60 infants of diabetic mothers (21 mothers had GDM, 37 had T2DM and 2 had T1DM). In accordance with their existing guideline, infants were screened for hypoglycaemia (defined as a blood glucose $< 2.6\text{mmol/L}$) at 4, 6, 8, 12, 18 and 24 hours. 30 infants developed hypoglycaemia based on this definition and the remaining 30 were considered as control infants.

Figure 4.7: Graph showing blood glucose status over 24 hours of postnatal age (318).



The above graph shows a trend in the blood sugar level in the cases and the controls (both being infants of diabetic mothers) in the first 24 hours after birth highlighting a more dramatic decline in the blood sugar levels in infants who developed hypoglycaemia. 50% infants developed hypoglycaemia in the first 4 hours, further 23.3% by 6 hours, 13.3% by 8 hours and a further 13.3% by 12 hours of age. There were no episodes of hypoglycaemia beyond 12 hours after birth. In infants who developed hypoglycaemia, the mean cord blood C-peptide level was $1508 \pm 825\text{pmol/L}$ vs. $923.3 \pm 696.3\text{pmol/L}$ in comparison to infants who did not develop hypoglycaemia ($p < 0.005$) (318).

All these studies support the fact that maternal hyperglycaemia leads to an increased supply of glucose to the fetus resulting in fetal pancreatic stimulation and fetal hyperinsulinism. Fetal insulin is a powerful growth stimulator and results in excessive fetal growth and visceromegaly. Persistence of this fetal hyperinsulinism in the early neonatal period while the pancreatic B-cell–insulin axis is undergoing down regulation results in neonatal hypoglycaemia. Fetal hyperinsulinism can potentially be detected at birth by measuring cord blood C-peptide which is a surrogate marker for insulin and a more reliable biochemical marker to measure as compared to insulin.

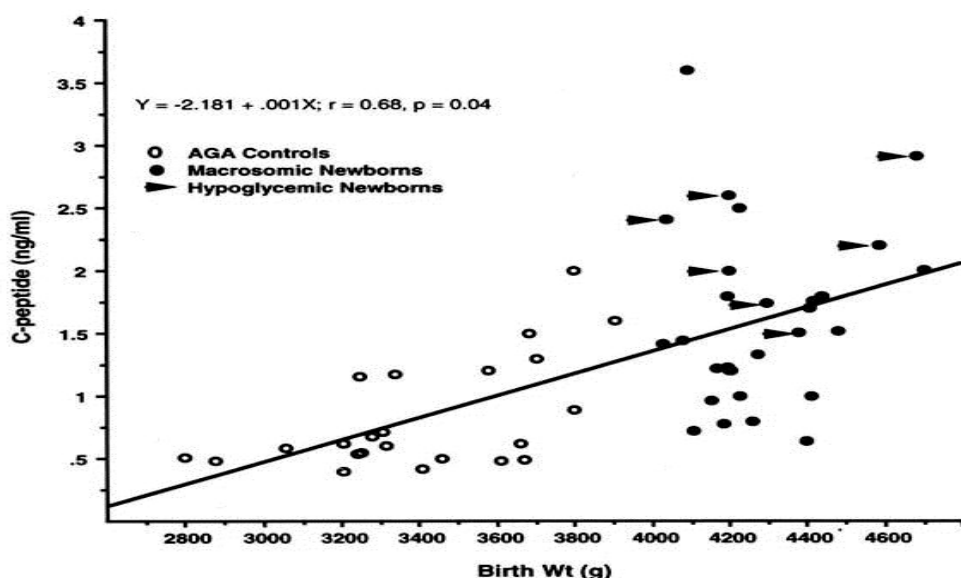
4.2.7.3 Cord blood C-peptide levels in LGA infants of non-diabetic mothers

Aygun et al measured cord blood insulin and C-peptide levels in 15 small for gestational age (SGA, birthweight < 10th centile), 15 appropriate for gestational age (AGA, birthweight 10th -90th centile) and 15 LGA (birthweight > 90th centile) term infants. All these infants were born to mothers without diabetes. They reported that there was no difference in cord blood C-peptide levels and log insulin in LGA and AGA infants. They found a positive co-relation between cord blood insulin and C-peptide levels ($r = 0.57$, $p < 0.001$) (319) .

Results of the study from Aygun et al are similar to those of Schwartz et al who showed that LGA infants of non-diabetic mothers had similar cord blood C-peptide and insulin levels of 390 ± 210 vs. 320 ± 110 and 116 ± 54.8 vs. 81.2 ± 63.9 respectively as compared to AGA infants of non-diabetic mothers. This might suggest that these LGA infants were constitutionally big and did not have a pathological reason for being LGA (213).

The results from the study conducted by Akinbi et al were contrary to the above-mentioned two studies. They measured cord blood C-peptide levels at birth in 29 LGA term infants and 23 AGA term infants, born to non-diabetic mothers.

Figure 4.8: Scatter plot to show the relationship between birthweight and cord blood C-peptide levels in infants with and without hypoglycaemia (285) .



The LGA infants had significantly elevated cord blood C-peptide levels 422.9 ± 233.1 vs. 286.4 ± 266.4 pmol/L as compared to AGA infants ($p=0.01$) (fig 4.11). They also had a higher risk of being delivered by caesarean section 29% vs. 4% ($p=0.04$) and there was a significant correlation between birthweight and cord blood C-peptide level ($r=0.68$, $p=0.04$). 20% (6/29) LGA infants, four of which had cord blood C-peptide level of 2SDs above mean for the whole study group, developed hypoglycaemia. None of the babies in the control group developed hypoglycaemia (285) . It is difficult to extrapolate more from this study as there does not seem to have been a clear threshold level of cord blood C-peptide above which the risk of hypoglycaemia increased. Information about feeding methods in these infants might have helped to clarify the occurrence of hypoglycaemic complications.

In another prospective study, Rou-Lin Hou et al measured cord blood C-peptide, insulin, HbA1c and lipids in 2873 term infants born to non-diabetic mothers who delivered in Zhejiang province, China. The LGA infants (nearly 20%) had higher mean cord serum C-peptide and insulin levels of 360 (270 - 490) pmol/L and 6.05 (3.42-9.71) mIU/L as compared to 280 (180 – 340) pmol/L and 2.81 (1.26 – 5.22) mIU/L respectively in the SGA infants and 310 (230 – 420) pmol/L and 4.31 (2.32 – 7.13) mIU/ respectively in the AGA group ($p<0.05$) (320) .

The above mentioned three studies involving LGA infants born to non-diabetic mothers showed conflicting results in relation to cord blood C-peptide levels, birthweight and neonatal hypoglycaemia. This could be attributed to the very small number of infants included in the studies. In addition to this LGA infants are a heterogeneous group of infants who are big due to either pathological overgrowth or constitutional make-up. The above studies show that cord blood C-peptide may play a role in early identification of LGA infants at risk of neonatal hypoglycaemia. However further adequately powered studies involving LGA infants are needed to answer questions about whether cord blood C-peptide can be used as a reliable predictive marker for neonatal hypoglycaemia in this group of infants.

4.2.8 Current management in infants of mothers with diabetes and LGA infants

The infants of mothers with pre-gestational (T1DM and T2DM) and gestational diabetes are at increased risk of complications in the early neonatal period and hence UK NICE guidelines suggests that these infants should be delivered in a centre with the facility to provide advanced neonatal resuscitation and neonatal intensive care support. It suggests that newborns should be monitored in hospital for at least 24 hours after birth(65). As routine practice, all infants born to mothers with diabetes are screened for hypoglycaemia by bedside monitoring of pre-feed blood glucose levels. In some hospitals in the UK, a similar protocol of pre-feed blood glucose monitoring is also followed for LGA infants born to mothers without diabetes in pregnancy. However the current guideline subjects a large number of infants to unnecessary investigation and medicalisation of their care as only a very small proportion of these infants develop postnatal complications. In those who develop postnatal complications, problems are identified only after they arise.

Another important factor from the point of view of service provision is the fact that in 2013 nearly 17% of pregnancies were complicated with diabetes and this figure is expected to rise markedly(80). The proportion of LGA infants has also increased by 15-25% in the past 2-3 decades and parallels the increase in diabetes and obesity in the community(216, 217). Providing routine monitoring to all these infants in hospital for 24 hours is already putting a significant strain on midwifery and neonatal services. Hence it is important to consider how the care of these infants can be streamlined. Currently there is no clinical assessment tool or biochemical test that has been shown to be useful in predicting infants of diabetic mothers and LGA infants who are at risk of early neonatal complications. Cord blood C-peptide has the potential to be used as a biochemical predictor for early neonatal complications enabling intensive monitoring and intervention to be applied effectively

4.2.9 Conclusion

Maternal hyperglycaemia in women with both pre-gestational and gestational diabetes in pregnancy leads to chronic fetal hyperglycaemia which in turn leads to overstimulation of the fetal pancreas resulting in B – cell hypertrophy and hyperplasia and an increase in B – cell function. Infants born to diabetic mothers have high basal insulin and C-peptide levels and these correspond directly with the severity of dysglycaemia experienced during pregnancy. Infants with high basal levels of insulin and C-peptide at birth have increased B – cell reserves of insulin and increased responsiveness to a glucose load. Cord blood insulin and C – peptide levels correlate well with each other and hence C – peptide can be used as a surrogate marker for fetal insulin. Cord blood C-peptide has the potential to be used as a biochemical marker at birth to identify infants of diabetic mothers and LGA infants of non-diabetic mothers at risk of neonatal complications.

This raises two questions:

1. Is it feasible to measure cord blood C-peptide at birth to inform clinical management of infants of diabetic mothers and LGA infants of non-diabetic mothers?
2. Can cord blood C-peptide reliably identify at birth the infants of diabetic mothers and LGA infants at high risk of adverse neonatal outcomes?

In order to answer these questions a prospective pilot study was undertaken which is discussed in more detail in the next section.

4.3 Study methodology for prospective Pilot Study – C-peptide Study

A prospective pilot study was undertaken to evaluate the potential of cord blood C-peptide to identify, at birth, infants of diabetic mothers and LGA infants of non-diabetic mothers at high risk of adverse neonatal outcomes.

4.3.1 Research question

Can cord blood C-peptide identify infants of diabetic (T1DM, T2DM and GDM) mothers and LGA infants of non-diabetic mothers who are at risk of developing neonatal complications?

4.3.1.1 Research aims

1. To evaluate the potential of cord blood C-peptide to identify at birth the infants of diabetic mothers and those LGA infants of non-diabetic mothers at high risk of adverse neonatal outcomes.
2. To test the feasibility of measuring cord blood C-peptide and to gain baseline information for use in a power calculation prior to a definitive study.

4.3.2 Selection of study subjects

Approaching pregnant women and/or mothers to take part in perinatal research is a highly sensitive issue. In order to establish a method for approaching the women for the study the clinical lead obstetricians, lead midwives, diabetes specialist midwives and the obstetricians with special interest in diabetes in pregnancy at both Leicester Royal Infirmary and Leicester General Hospital were contacted and their advice sought in designing the study. A copy of the study protocol summary, patient information leaflet, summary of cord blood collection process and contact number for the research fellow was provided to them.

4.3.2.1 Patient and public involvement

During the study design two focus groups were conducted, one involving five pregnant women with GDM and the second involving three women with previous history of giving birth to LGA infant (one of the risk factor for screening for GDM in the current pregnancy). Women in both these focus groups were keen to engage with healthcare services, wanted to know more about their condition and implications for their pregnancy and their baby. Most of the women were not aware about the clinical entity of GDM, which can occur during pregnancy. They were not aware that they had risk factors for developing GDM before they were screened and diagnosed and hence did not take any precautions to reduce their risk factors. Most of them were surprised that they were diagnosed with GDM and felt reassured that it was a transient condition that would resolve after pregnancy. They were not aware of the long-term risk of T2DM and metabolic syndrome. They wanted to know if their baby would also get diabetes after birth and what other complications can occur in the baby. When explained about the C-peptide study, they all seemed keen, commented that they would be happy to participate and help find a blood test that can identify babies at risk of complications so that steps can be taken to prevent it. As it was cord blood test (collected from cord attached to placenta that is usually discarded), not blood test from mother or baby they all commented that there should not be any reason not to participate especially when it might help to find a useful test.

4.3.2.2 Awareness amongst clinical staff

The midwives on the delivery suites and the specialist diabetes midwives in the antenatal clinics at both Leicester hospitals (Leicester Royal Infirmary – LRI and Leicester General Hospital - LGH) were introduced to the study by the research fellow through twice weekly sessions in February 2012 i.e. in the month before the start of the study. The midwives and medical staff on the delivery suite were also informed about the C-peptide study, the process of recruitment, cord blood collection and obtaining consent. These sessions were planned with the aim to target education and awareness in most of the staff working on the delivery suite.

They had practice sessions to correctly identify newborns with a birthweight more than the 97th centile using ethnic specific birthweight cut offs. For infants born to mothers with diabetes in pregnancy (T1DM, T2DM and GDM), staff were asked to collect cord blood sample before the placenta was discarded irrespective of the birthweight. For LGA infants and control infants, if the birth weight was above the 97th centile and between 10th – 90th centile respectively for gestation, sex and ethnicity, they were asked to collect cord blood before the placenta was discarded. They were requested to inform the research fellow immediately so that arrangements could be made to provide patient information leaflets to mothers of eligible infants and to later obtain consent. Further analysis of the collected sample took place only after informed written consent was obtained (the consent process is explained in more detail in section 4.4.3). Study posters were displayed in the staff room, main clinical areas and treatment rooms to remind midwives about the study, eligible participants, the process of cord blood collection and the research fellow's contact details (appendix 6 in section 6.6). The research fellow also regularly visited the delivery suites at both LRI and LGH to ensure that the study maintained a high profile and to encourage and boost recruitment of eligible infants, as this was heavily reliant on the attending midwife. Midwives who successfully collected the cord blood sample and contacted the research fellow to inform about an eligible infant were provided with £10 gift voucher from Marks and Spencer as a small token of appreciation for their help and contribution towards the study in spite of their busy clinical commitment.

4.3.2.3 Awareness amongst parents

Posters describing the study were displayed in antenatal clinics and delivery suites to inform parents about the study (appendix 6 in section 6.6).

4.3.3 Recruitment of study subjects

The specialist diabetes midwives, delivery suite midwives, medical staff on the delivery suite or the research fellow identified eligible infants for the C-peptide Study. The study participants and controls were recruited from two hospitals: Leicester Royal Infirmary and Leicester General Hospital, which are part of the University Hospitals of Leicester NHS trust.

4.3.3.1 Recruitment of infants of mothers with diabetes in pregnancy

Live born infants of WB and SA diabetic mothers (T1DM, T2DM and GDM) born between 37+0 and 41+6 weeks of gestation were eligible for the C-peptide Study. Only these ethnicities were included in the C-peptide Study as ethnic specific birthweight charts were only available for WB and SA infants. Infants were excluded if the baby was from a multiple pregnancy, had a congenital abnormality or a known metabolic problem. The specialist diabetes midwives helped to identify mothers with pre-gestational diabetes during the antenatal period. The research fellow contacted them during the antenatal follow-up clinics in their last trimester of pregnancy. They were contacted during the last trimester (around 32 – 36 weeks) to ensure the mothers would have already had the opportunity and time to discuss the diagnosis, management, planning for delivery and postnatal management with the obstetricians. This was especially important in women with GDM as they would normally have been diagnosed in late second trimester or early third trimester. Having this background information also helped them to understand the study better. The research fellow introduced them to the study and gave study information leaflet. However written consent for involvement in the study was only obtained during admission for delivery. If an eligible mother with diabetes in pregnancy was not provided information in the antenatal period then this was done during labour (after confirming with the delivery suite clinical team that it was appropriate and safe to do so).

4.3.3.2 Recruitment of LGA infants born mothers without diabetes in pregnancy

Live born large for gestational age (LGA) infants (birthweight > 97th centile) born to WB and SA non-diabetic mothers between 37+0 and 41+6 weeks were recruited to the study. SA ethnic specific customised birthweight charts and WHO-UK birthweight charts were used to identify LGA infants in the SA and WB ethnic groups respectively (see section 3.6.3). Their exclusion criteria were similar to infants of mothers with diabetes in pregnancy (as detailed in section 4.3.3.1). In some cases, antenatal scans detected LGA fetuses (with estimated fetal weight of more than the 97th centile) however the definitive diagnosis in all cases was made only after birth. As some of the LGA infants were identified only at birth, cord blood was collected and stored immediately after birth before the placenta was discarded while awaiting parental consent (retrospective consent). The mothers of eligible LGA infants were then approached in the postnatal period at the earliest opportunity after discussion with the obstetric and midwifery team to ensure that it was appropriate to do so. Only after the informed consent was obtained, the collected cord blood sample was analysed for C-peptide levels.

4.3.3.3 Recruitment of control infants

Live born infants between 37+0 and 41+6 weeks of gestation with a birthweight between the 10th and 90th centiles to SA and WB mothers without diabetes in pregnancy, with a normal antenatal period and antenatal scans born by normal vaginal delivery or by elective caesarean section were eligible to be recruited as control infants. The control infant was the next available eligible infant to be born after the recruitment of the study infant from the above-mentioned study groups. Their exclusion criteria were similar to infants of mothers with diabetes in pregnancy (as detailed in 4.3.3.1). Their recruitment and consent process to the study was very similar to LGA infants.

4.3.4 Consent

During the consent process, mothers of the eligible infants of the above mentioned study and control groups once identified were introduced to the study and the study was explained to them in detail. The mothers were provided with a written study information leaflet explaining the research. The written information leaflet was left with the mothers for them to read through and to discuss with their partner/husband/family (appendix 3 in section 6.3). The research fellow gave the parents opportunity to ask questions and seek clarification. If the mother was willing to participate in the study, informed consent was obtained by signing an approved consent form (appendix 4 in section 6.4). The informed consent was obtained at least 12hrs after providing study information to the mother. The parents were given a copy of the consent form and the patient information leaflet and requested to retain them for future reference. Two further copies of the consent form were made: one copy was retained in the maternal medical records and the second copy in the research files.

Consent was obtained for the following:

- Measurement of C-peptide levels.
- Retention and analysis of maternal and neonatal data.

For all women cord blood could only be obtained immediately after birth before the placenta was discarded and in many cases this was before consent was obtained. As a result, ethical permission was granted to collect the sample and store it and then proceed with its analysis only if parental consent to participate in the study was obtained. For any who declined to participate in the study, it was mandatory that the collected sample would be discarded. In this study, all the mothers who were approached were willing to participate in the study and therefore no collected cord blood sample had to be discarded. A similar method of obtaining consent retrospectively was used in the Epicure 2 (321) .

When the eligible infants were born at a time when the research fellow was not available, nominated specialist registrars in neonatal medicine at Leicester General

Hospital (Dr. Durga Herath) and at Leicester Royal Infirmary (Dr. Hazel Clargo) were nominated to undertake recruitment and obtain informed consent. These investigators were added to the study by Ethics and by the local R&D committee.

The research fellow could speak English, Gujarati, Hindi and Marathi. This helped to communicate with mothers with limited understanding of English. They were encouraged to write down information about the study if they wished to in their own language. The two largest ethnic groups in Leicester are WB and SA. All the SA women recruited to the study who could not communicate in English were Gujarati or Hindi speaking and so the research fellow was able to communicate with them. It was decided before the start of the study that if there was a SA woman who spoke any other language that the research fellow could not speak, then their infants would not be recruited into this pilot study.

4.3.5 Collection of venous cord blood

2-5mls of venous cord blood was collected in lithium heparin bottles in the same way as it was routinely collected in clinical practice for biochemical analysis by trained midwives. The samples were transferred to the biochemistry laboratory urgently on ice. Serum was separated from the cord blood sample by centrifugation and the separated serum was stored at -70°C. This process was carried out in the local pathology laboratory at Leicester Royal Infirmary. The stored serum was further analysed after parental consent was obtained. The stored serum samples were then sent to the pathology laboratory at Nottingham City Hospital (Nottingham University Hospitals NHS Trust) in batches in frozen state (using dry ice) for the analysis of C-peptide levels. C-peptide was measured using Siemens 1000 Immunoassay System. This analyser used a chemiluminescence immunoassay (CLIA) detection method. The centrifuged serum samples were added to C-peptide antibody coated microtiter wells (solid phase). Then anti-C-Peptide antibody labelled with horseradish peroxidase (enzyme linked antibodies) was added. If C-peptide was present in the specimen then it was sandwiched between two antibodies. After an hour of incubation the wells are washed to remove excess unbound labelled antibody. A solution of chemiluminescent substrate was then

added. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of C-peptide in the sample. The centrifuged serum samples remain stable when stored for up to 3 months at temperatures $< 20^{\circ}\text{C}$ and can be analysed later without any degradation or alteration of C-peptide levels.

4.3.6 Sample size

As this was a pilot study with the aim of gaining information about the normal variation of cord blood C-peptide in the various groups of infants mentioned in section 4.4.2 the intention was to recruit a total of 50 infants.

There were about 300 women with diabetes in pregnancy (all types) annually at the Leicester hospitals (as per information obtained from the database of pregnant diabetic women over two years i.e. 2009 and 2010) and of these 40% were WB, 50% SA and 10% belonged to other ethnic minority groups. 30 infants of diabetic mothers (15 infants born to WB and SA mothers with pre-gestational diabetes and 15 infants born to WB and SA mothers with GDM) were recruited to the study. The study period for the cord blood C-peptide Study extended from 1st March 2012 to 30th November 2012.

During the same period, there were about 410 infants born between 37+0 to 41+6 weeks of gestation with a birthweight of more than the 97th centile. Of these, 10 LGA infants born to WB and SA mothers without diabetes were also recruited.

10 control infants born between 37+0 to 41+6 weeks of gestation born to WB and SA mothers without diabetes in pregnancy with a normal antenatal period, normal antenatal scans and born by normal vaginal delivery or elective caesarean section were also recruited.

All mothers of eligible infants who were approached for study participation consented to participate in the study. Hence the recruitment for C-peptide Study was 100%.

4.3.7 Study Outcomes

4.3.7.1 Primary outcome

The primary outcome of this pilot study was:

1. To check feasibility of measuring cord blood C-peptide at birth and its use to guide clinical management.
2. To establish range of cord blood C-peptide levels in the different study groups.
3. To review the relationship between cord blood C-peptide levels and neonatal morbidity such as neonatal macrosomia and neonatal hypoglycaemia.

As per the local policy, pre-feed blood sugar was routinely monitored in infants of diabetic mothers and LGA infants with a birthweight of more than 4500 grams. Other infants recruited to the C-peptide study (control infants and infants identified as LGA for gestational age, sex and ethnicity but with a birthweight < 4500grams) had their blood sugar measured only if there was another clinical indication to do a blood test. The definitions for the various outcome measures are as detailed in retrospective study 1 in section 2.9.4.

4.4 Results of the C-peptide Study

In the C-peptide Study, 50 mother - infant dyads were prospectively recruited at birth after obtaining informed consent. These term infants were born at the University Hospitals of Leicester between 1st March 2012 and 30th November 2012. 10 infants were born to mothers with a low risk pregnancy – control infants, 9 infants were LGA born to mothers without diabetes in pregnancy, 16 infants were born to mothers with GDM (12 – diet controlled GDM mothers and 4 insulin controlled GDM mothers) and 15 infants were born to mothers with pre-gestational diabetes mellitus (5 mothers with T1DM and 10 mothers with T2DM). Cord blood C-peptide levels were available in 49 infants (cord blood C-peptide level could not be measured in one infant due to an unsuitable dilution)

4.4.1 Characteristics of the mothers in the study groups

Characteristics of the mothers of the infants included in the C-peptide study are summarised in table 4.5. Mothers with GDM and pre-gestational diabetes were older (median (IQR) 31.00 (28.00 – 34.75) years and 33.00 (30.00 – 40.00) years vs. 27.00 (24.75 - 30.50) years, had a higher BMI 31.10 (26.85 – 37.39) and 32.00 (26.89 – 35.82) vs. 24.80 (20.87 – 27.07) at the time of booking as compared to the control group. The mothers of the control infants had a tendency to have lower systolic and diastolic BP and hence a lower incidence of hypertensive complications in pregnancy as compared to the diabetic mothers. Mothers of LGA infants were similar to the mothers in the control group except their median booking weight and BMI, which were significantly higher (median (IQR) 79.00 (65.50 – 94.50) kg vs. 70.50 (59.25 – 78.00) kg and 29.70 (25.67 – 34.24) vs. 24.80 (20.87 – 27.07) respectively. There was no difference in smoking and alcohol consumption rates at booking between the groups. Gestational and pre-gestational mothers were more likely to be multigravida or grand multigravida as compared to mothers of control and LGA infants.

Table 4:4: Comparison of maternal demographic characteristics.

	Control infants (N = 10)	LGA infants (N = 9)	Infants of GDM mothers (N = 16)	Infants of T1DM/T2DM mothers (N = 15)
Maternal age (years)	27.0 (24.8 - 30.5)	26.0 (22.5 – 31.0)	31.0 (28.0 – 34.8)	33.0 (30.0 – 40.0)
Maternal weight (kg)	70.5 (59.25 – 78.00)	79.0 (65.5 – 94.5)	80.5 (66.0 – 103.8)	78.0 (69.0 – 93.0)
Maternal height (cm)	166.5 (161.5–171.5)	166.0 (163.0–169.0)	165.0 (154.0-168.8)	163.0 (159.5-165.0)
Maternal BMI	24.8 (20.9 – 27.1)	29.7 (25.7 – 34.2)	31.1 (26.9 – 37.4)	32.0 (26.9 – 35.8)
Systolic BP (mmHg)	111.5 (109.0-120.0)	113.0 (104.0-120.5)	127.0 (108.0-139.0)	118.0 (112.0-128.0)
Diastolic BP (mmHg)	70.0 (60.8 – 72.5)	73.0 (63.0 – 78.0)	76.0 (60.00– 87.0)	75.0 (67.0 – 80.0)
Smoking, n(%)	2 (20.0)	2 (22.2)	1 (6.3)	1 (6.7)
Alcohol, n(%)	None	1 (11.1)	2 (12.5)	1 (6.7)
Primigravida, n(%)	5(50.0)	None	4 (25.0)	3 (18.8)
Multigravida, n(%) ⁰	5 (50.0)	9 (100)	8 (50.0)	8 (53.3)
Grand multigravida, n(%) ⁰⁰	None	None	4 (25)	3 (18.8)
PIH, n(%)	None	None	3 (18.6)	3 (20)

⁰ Multigravida - Gravida 2 – 4

⁰⁰ Grand multigravida – Gravida more than 4

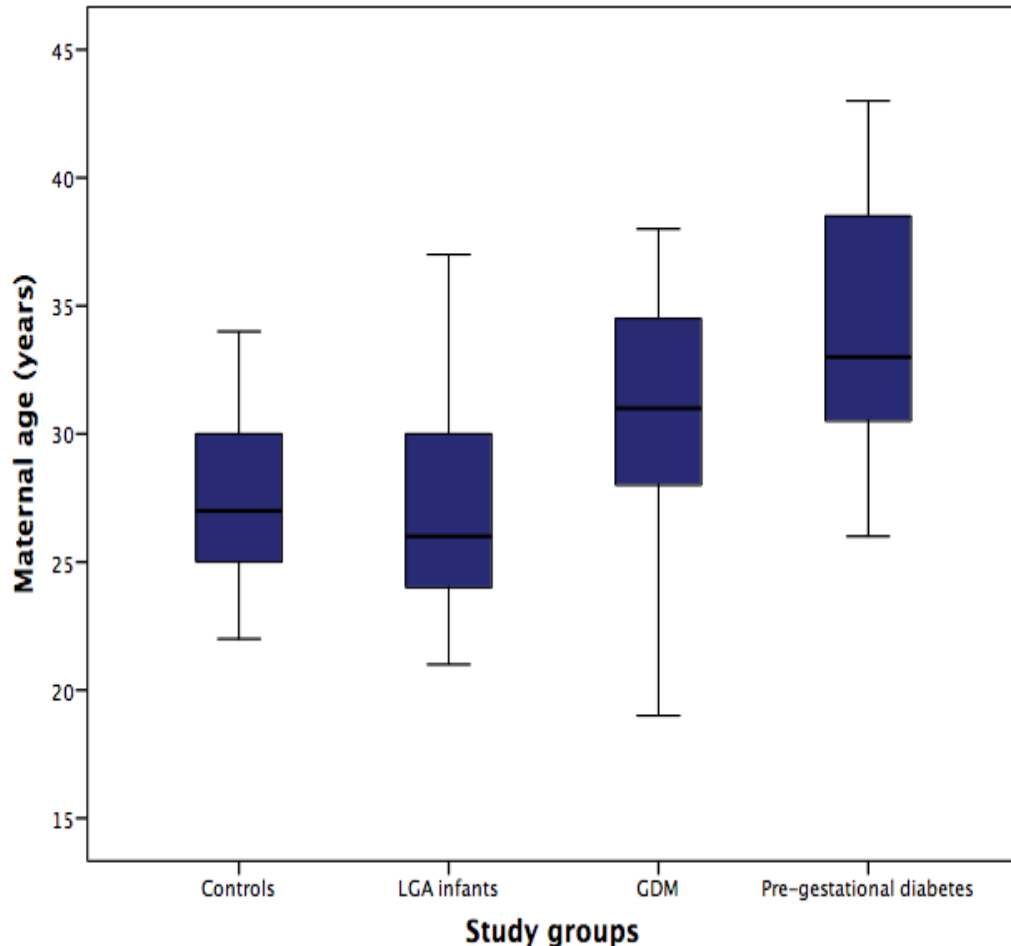
PIH – Pregnancy Induced Hypertension

Values for continuous variables presented as median (interquartile range);

Values for categorical variables presented as n (%).

4.4.1.1 Comparison of maternal age in the study groups

Figure 4.9: Box plot to show comparison of maternal age in different study groups.



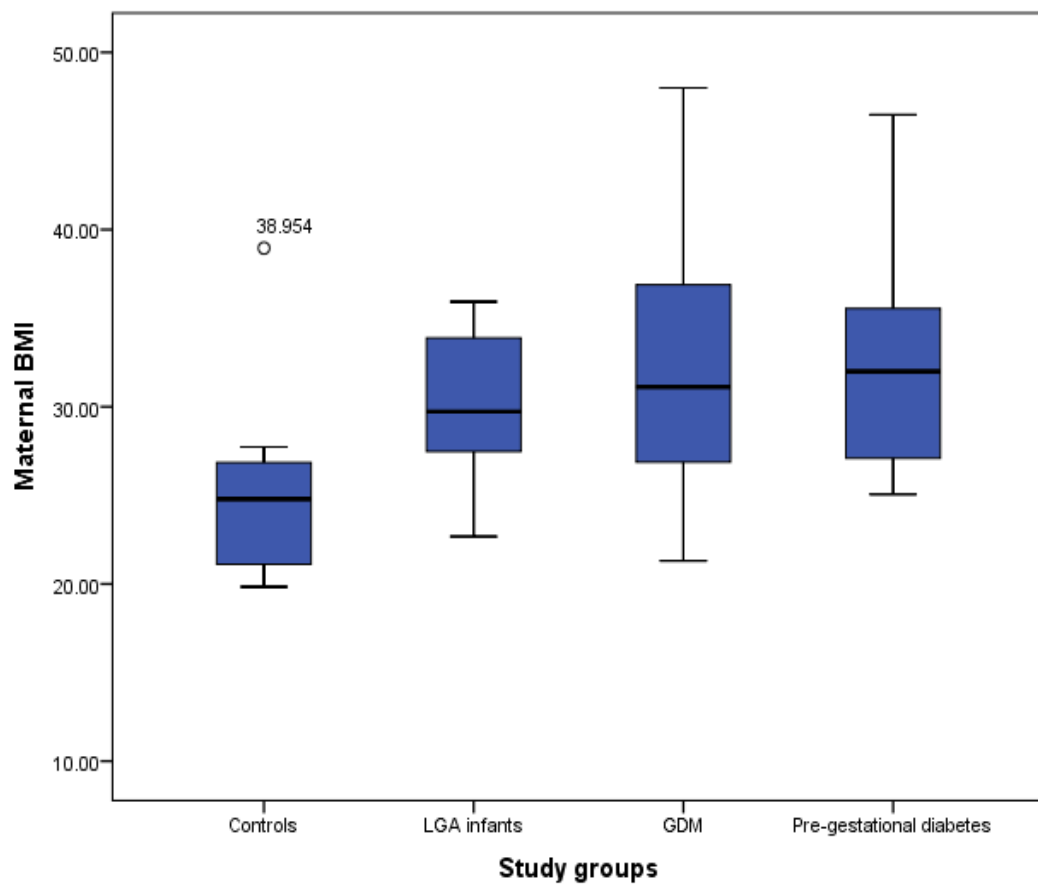
Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values.

There was no difference in the median maternal age between the mothers of LGA and control infants 26.0 vs. 27.0. However mothers with GDM and pre-gestational diabetes (T1DM and T2DM) were older as compared to the mothers from the control group 31.0 vs. 27.0 years and 33.0 vs. 27.0 years respectively. Fig 4.12 compares the median (interquartile range) maternal age for the mothers in the different study groups.

4.4.1.2 Comparison of maternal BMI in the study groups

The mothers in the control group had a median BMI of 24.8, which was within the normal BMI range for adult women. The median (IQR) BMI in mothers with GDM was 31.10 (26.85 – 37.39) vs. 24.80 (20.87 – 27.07), in mothers with pre-gestational diabetes was 32.0 (26.89 – 35.82) vs. 24.80 (20.87 – 27.07) and in mothers of LGA infants was 29.70 (25.67 – 34.24) vs. 24.80 (20.87 – 27.07) in the control infants. Women with diabetes in pregnancy and those that delivered LGA infants were of much higher BMI than the control mothers.

Figure 4.10: Box plot to show comparison of maternal BMI in the different study groups.



Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ^o represents outlier value.

4.4.2 Comparison of mode of delivery in the study groups

Table 4:5: Mode of delivery in the different study groups.

	Control infants (N=10)	LGA infants (N=9)	Infants of GDM mothers (N=16)	Infants of T1DM/T2DM mothers (N=15)
Before term caesarean section	None	3 (33.3)	2 (12.5)	5 (33.3)
Before term IOL	None	6 (66.7)	13 (81.5)	9 (60.0)
Spontaneous vaginal delivery	8 (80.0)	3 (33.3)	7 (43.8)	4 (26.7)
Ventouse delivery	1 (10.0)	1 (11.1)	None	2 (13.3)
Forceps delivery	None	1 (11.1)	None	None
Emergency caesarean section	1 (10.0)	1 (11.1)	7 (43.75)	4 (26.7)

Values for categorical variables presented as n (%);

Almost all the mothers of LGA infants and mothers with GDM and pre-gestational diabetes were either booked for elective caesarean section or induction of labour before term (table 4.6). 80.0% of the control infants were delivered by normal vaginal delivery as compared to only 33%, 43.8% and 26.7% of LGA infants, infants of mothers with GDM and pre-gestational diabetes respectively. Infants of mothers with GDM and pre-gestational diabetes also had a higher risk of being delivered by emergency caesarean section 43.75% and 26.7% respectively as compared to only 10% in the control infants.

4.4.3 Comparison of neonatal outcomes in the study groups

Table 4:6: Comparison of neonatal demographic features and neonatal outcomes.

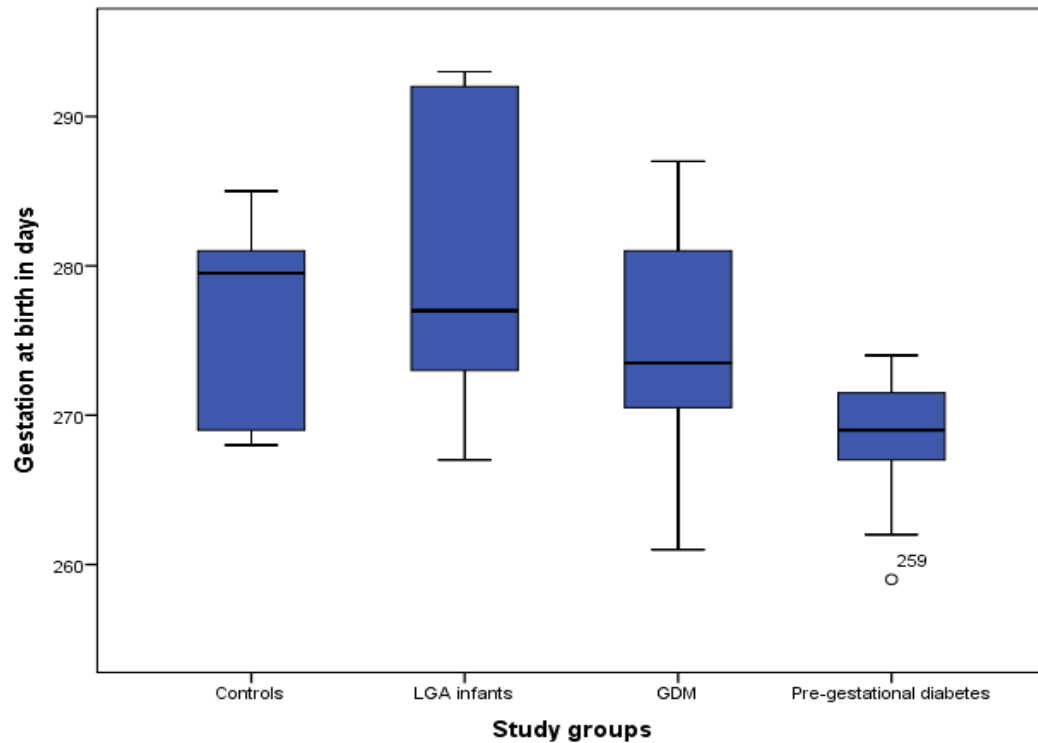
		Control infants (N = 10)	LGA infants (N = 9)	Infants of GDM mothers (N = 16)	Infants of T1DM/T2D M mothers (N = 15)
Sex Male, n (%)		7 (70)	9 (30)	8 (50)	6 (40)
Gestation (weeks)		40+0 (38+3 – 40+1)	39+4 (38+6 – 41+6)	39+1 (38+4 – 40+1)	38+3 (38+1 – 39+0)
Birthweight (grams)		3665.0 (3450.5–3890.0)	4480.0 (4323.0–4639.0)	3470.0 (3218.5–4015.0)	3740.0 (3020.0–3940.0)
Head circumferenc		35.0 (34.1 – 35.5)	37.4 (36.9 – 38.2)	35.2 (34.2 – 36.2)	33.5 (32.7 – 35.1)
Time of first feed (hr)		1.20 (0.54 – 1.37)	1.11 (0.58 – 2.00)	1.16 (0.56 – 1.28)	1.26 (1.05 – 2.01)
First feed n (%)	Breast	6 (60)	5 (55.6)	8 (50)	7 (46.7)
	Bottle	4 (40)	4 (44.4)	6 (37.5)	7 (46.7)
	Both	-	-	2 (12.5)	1 (6.7)
Neonatal hypoglycaem		None	None	None	2
Cord blood C-peptide (pmol/L)		263.50 (255.25–353.75)	473.00 (283.50–533.00)	367.50 (317.00–500.00)	751.50 (446.50–950.25)
Blood sugar (mmol/ L)		-	2.5 (2.1 – 2.9)	3.1 (2.1 – 3.5)	2.6 (2.1 – 3.4)
Admission to NICU, n		1	0	3	1
Respiratory distress, n		1	0	2	0
Sepsis, n		1	0	3	0
Duration of hospital stay (days)		1 (0 – 5)	2 (1 – 4)	2 (0 – 10)	2 (0 – 5)
Readmission (%)		3 (30)	2 (8.0)	5 (22.2)	4 (31.3)

Values for continuous variables presented as median (interquartile range);

Values for categorical variables presented as n (%).

4.4.3.1 Distribution of gestational age in the different study groups

Figure 4.11: Box plot showing the difference in the gestation at birth in infants born in different study groups.

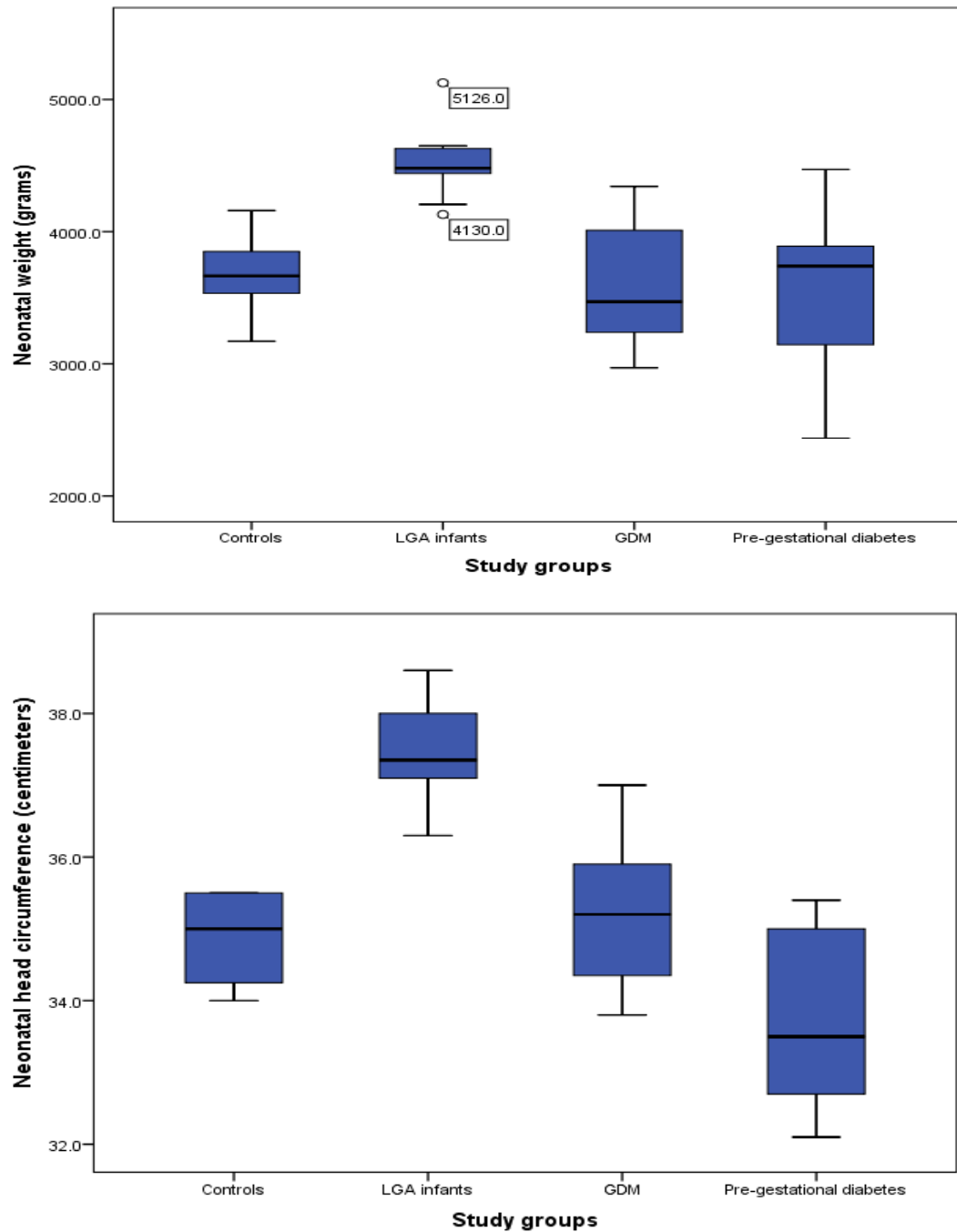


Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ^o represents outlier value.

The median (IQR) gestation at birth for the control infants was 40+0 (38+3 – 40+1) weeks. There was no difference in the median gestation at birth amongst control infants and LGA infants, which were 40+0 (38+3 – 40+1) and 39+4 (38+6 – 41+6) weeks respectively. Median gestational ages at birth in infants born to mothers with GDM and pre-gestational diabetes were about 1 week and 2 weeks earlier as compared to the control infants 39+1 (38+4 – 40+1) weeks vs. 40+0 (38+3 – 40+1) weeks and 38+3 (38+1 – 39+0) vs. 40+0 (38+3 – 40+1) weeks respectively.

4.4.3.2 Distribution of birthweight and head circumference in the different study groups

Figure 4.12: Comparison of the birthweight and head circumference of infants in the different study groups.

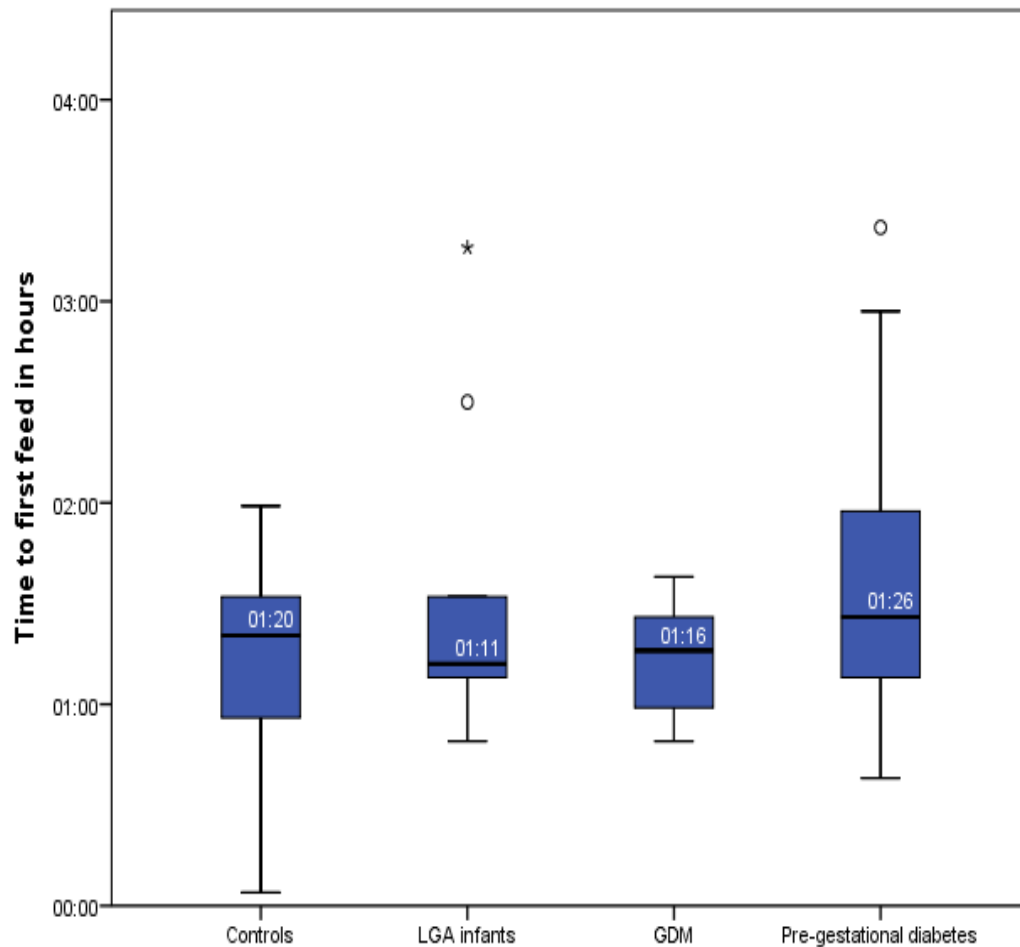


Top and bottom of the boxes represent 25th and 75th centile; bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values and ^o represents outlier values.

LGA infants had a higher median (IQR) birthweight and head circumference, 4480.0 (4323.0 – 4639.0) grams vs. 3665.0 (3450.5 – 3890.0) grams and 37.4 (36.9 – 38.2) cm vs. 35.0 (34.1 – 35.5) cm respectively as compared to the control infants. This suggests symmetrical growth in these infants. There was no significant difference in birthweight and head circumference between the control infants and infants born to mothers with GDM (fig 4.15) However infants of mothers with pre-gestational diabetes had notably lower median (IQR) head circumference of 33.5 (32.7 – 35.1) cm as compared to 35.0 (34.1 – 35.5) cm in the control infants in spite of a slightly higher median birthweight 3740.0 (3020.0 – 3940.0) grams vs. 3665.0 (3450.5 – 3890.0) grams suggesting asymmetrical growth in these infants due to maternal diabetes.

4.4.3.3 Time and type of first feed in the different study groups

Figure 4.13: Box plot to show time to first feed in the different study groups.

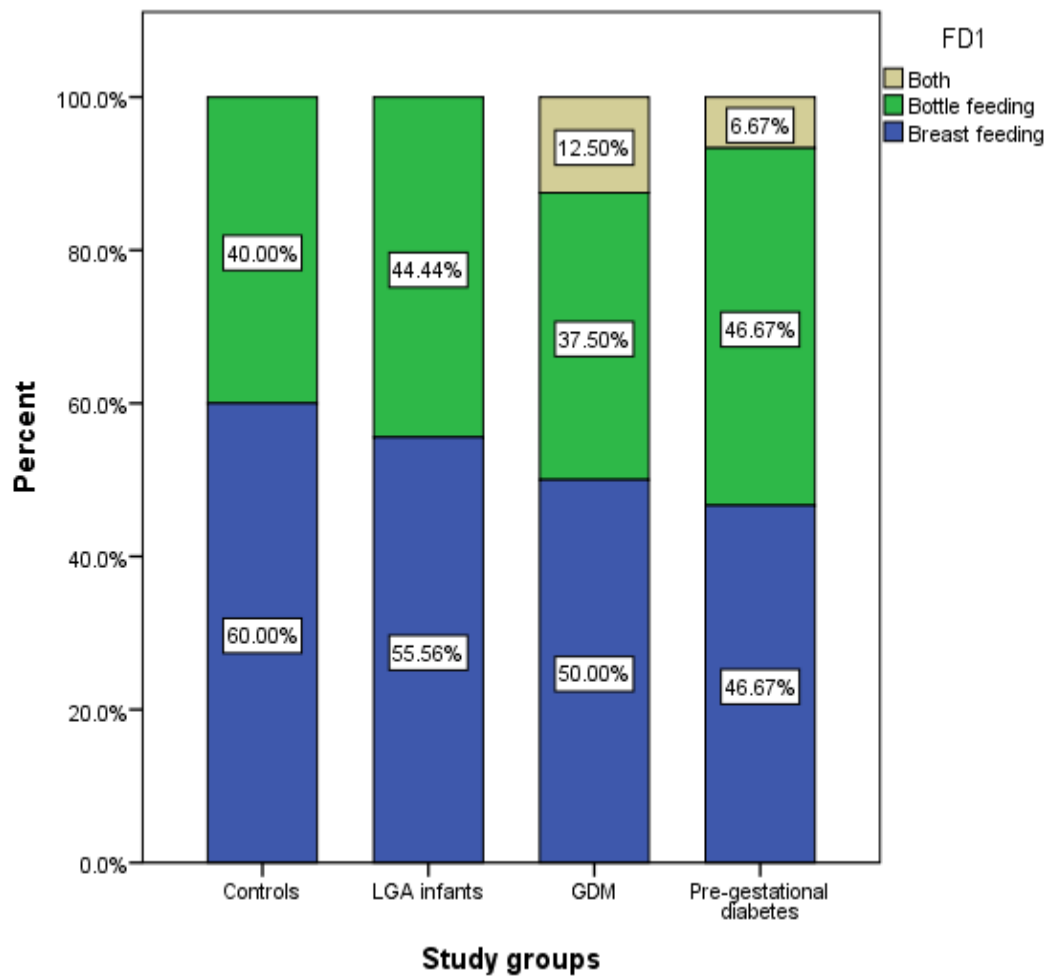


*Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values.*

There was no difference in the median time for the first feed after birth in the various study groups (fig 4.16).

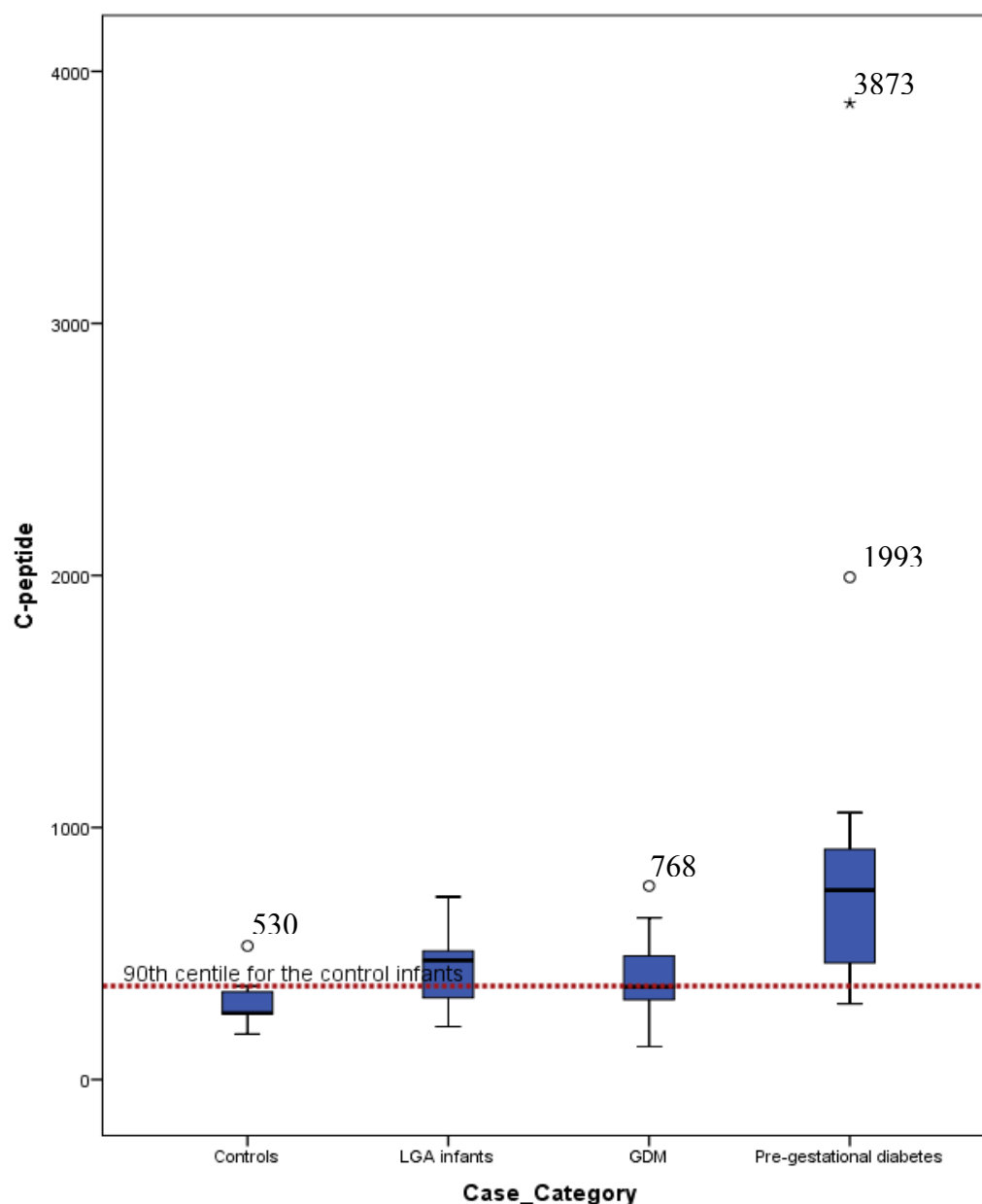
In this cohort of infants, the highest rates of exclusive breast feeding were seen in the control infants (60.0%) followed by LGA infants (55.56%), followed by infants of GDM mothers (50.0%) and the lowest rates were noted in the infants of mothers with pre-gestational diabetes (46.67%). There was no difference in the exclusive formula feeding rates amongst the study groups at the time of discharge (fig 4.17).

Figure 4.14: Stacked bar chart to show the type of first feed in the different study groups.



4.4.3.4 Distribution of cord blood C-peptide in the different study groups

Figure 4.15: Box plot showing the C-peptide levels in the different groups.



*Top and bottom of the boxes represent 25th and 75th centile; Bars represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values; ° represents outliers and * represents extreme values.*

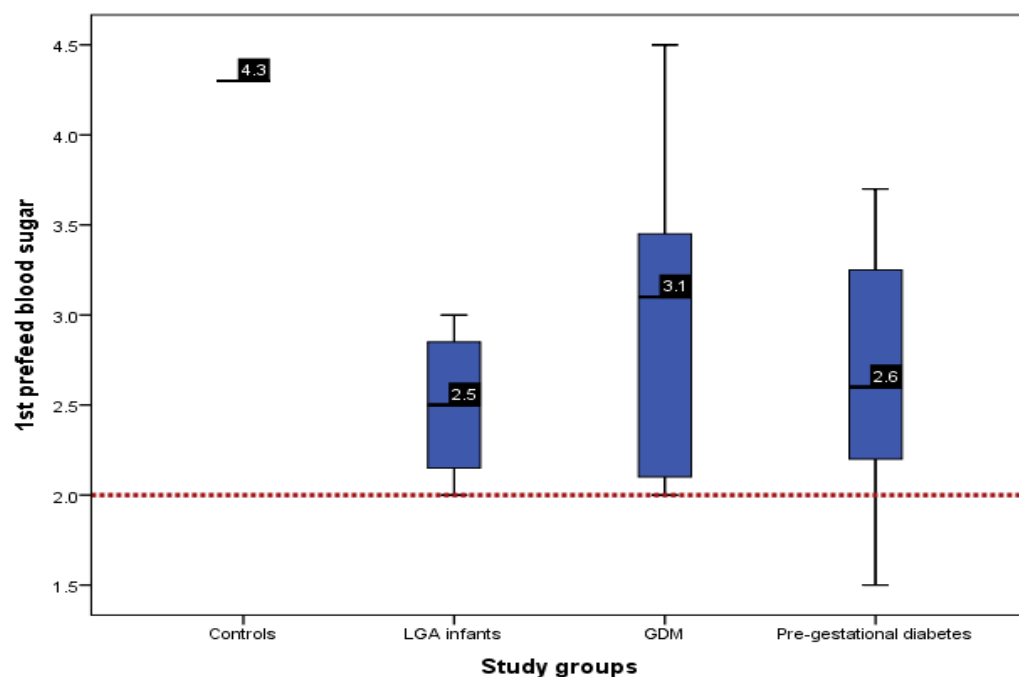
The median (IQR) cord blood C-peptide level for the control group was 263.50 (255.25 – 353.75) pmol/L, for the LGA infants was 473.00 (283.50 – 7533.00) pmol/L, for infants of mothers with GDM was 367.50 (317.0 – 500.0) pmol/L and for infants of mothers with pre-gestational diabetes was 751.0 (446.50 – 950.25) pmol/L. The median cord blood C-peptide level for infants of mothers with GDM

corresponded to the 90th centile for the control infants (371.0pmol/L) but the median cord blood C-peptide levels for the infants of mothers with pre-gestational diabetes was nearly three times higher than the median for the control infants. The median cord blood C-peptide levels for the LGA infants were nearly two times higher than the median for the control infants (see fig 4.18).

The subgroup analysis of mothers with GDM, showed that the infants of mothers with diet controlled GDM had a higher mean cord blood C-peptide level of 420.25 (172.0 – 768.0) pmol/L vs. 325.75 (131.0 – 510.0) pmol/L and mean neonatal birthweight of 3607.67grams vs. 3522.50grams as compared to infants of insulin controlled mothers with GDM.

4.4.3.5 Range of blood sugar levels in the different study groups

Figure 4.16: Box plot to show comparison of the range of blood sugar in the different groups.



Top and bottom of the boxes represent 25th and 75th centile; Bar represents median value; Whiskers represent the greatest and the smallest values that are not outliers or extreme values.

Only two infants in this cohort developed significant hypoglycaemia (blood sugar < 2.0mmol/L). Both of these infants were born to mothers with pre-gestational diabetes and had C-peptide levels, which were more than 3 times and 10 times higher than the 90th centile (371.0pmol/L) for the control group.

The first infant was a female infant born to a mother with T1DM at 38+1weeks of gestation with birthweight of 3818.0grams (95th centile for birthweight). She was delivered by emergency caesarean section following failure to progress for an induced labour. Her cord blood C-peptide level was 3873pmol/L. Her first feed was breast feed at 1hr and 53mins (median time to first feed was 1hr and 26mins). She developed asymptomatic biochemical hypoglycaemia with blood sugar of 1.5mmol/L at around 10 hours of age. She needed admission to the neonatal unit and her hypoglycaemia was treated with provision of extra oral feeds: breast feeding with additional term formula milk. Her subsequent pre-feed blood sugars were within the normal range. She was discharged home on day 5 after birth, demand breast feeding with bottle top up feeds.

The second hypoglycaemic infant was a female infant born to a mother with T2DM at 39+1weeks of gestation with a birthweight of 3940grams (95th centile for birthweight) by emergency caesarean section following failure to progress. Her cord blood C-peptide level was 1059pmol/L. Her first breast feed was at 1hour and 20mins (median time to first feed was 1hr and 26mins). She developed symptomatic hypoglycaemia with a blood sugar of 1.7mmol/L at four hours of age. Her hypoglycaemia was also treated with additional oral feeds: breast feeding with top up cup feed using term formula milk. She did not require admission to NICU and was discharged home on day 2 after birth, demand breast feeding with stable blood sugars.

Another male infant born to mother with T2DM diabetes at 38+3 weeks gestation by elective caesarean section with a birthweight of 3020 grams also had a elevated cord blood C-peptide level of 1993pmol/L. His first feed was a bottle feed as per maternal preference which was at 1hr and 5mins. His pre-feed sugar was 3.7 and he did not develop hypoglycaemia in spite of elevated cord blood C-peptide level.

4.4.3.6 Admission to NICU

Table 4:7: Comparison of NICU admission in the different groups.

	Control infants (N = 10)	LGA infants (N = 9)	Infants of GDM mothers (N = 16)	Infants of T1DM/T2DM mothers (N = 15)
Admission to NICU	1 (10%)	None	3 (18.8%)	1 (6.7%)
Reason for NICU admission	Meconium aspiration syndrome		2 presumed sepsis, 1 pneumonia	Hypoglycaemia
Cord blood C-peptide (pmol/L)	180	-	340	3873

In this cohort, five infants were admitted to NICU after birth with one or more clinical reasons. Details of the reason for admission and the cord blood C-peptide levels have been summarised in table 4.8. One infant (10.0%) was admitted from the control group with meconium aspiration syndrome, 3 infants (18.8%) born to mothers with GDM were admitted (two with presumed sepsis and one with congenital pneumonia) and one infant (6.7%) born to a mother with pre-gestational diabetes was admitted for neonatal hypoglycaemia. The two infants who developed hypoglycaemia had very high C-peptide levels as compared to the control infants. There was no relation between other adverse neonatal outcomes and cord blood C-peptide levels.

4.4.4 Comparison of readmission

There was no significant difference in the readmission rates in the different study groups. The commonest reason for readmission was neonatal hyperbilirubinaemia, presumed sepsis and excessive weight loss.

4.5 Summary of results

1. It was feasible to measure cord blood C-peptide levels at birth in infants of mothers with diabetes and LGA infants born to mothers without diabetes.
2. Two infants that developed hypoglycaemia had very high cord blood C-peptide levels as compared to control infants.
3. As it was a feasibility study cut point analysis could not be carried out but this study had provided useful information regarding feasibility and some preliminary information about the range of cord blood C-peptide levels in different groups which would help in power calculation for a bigger prospective study.

5 CHAPTER

5.1 Discussion

The aim of this project was to review the influence of maternal hyperglycaemia during pregnancy on neonatal outcomes. The project consisted of two retrospective studies and one prospective study, all designed with the aim of streamlining care of infants born to mothers with diabetes in pregnancy. The first retrospective study was intended to test the hypothesis that the risk of adverse neonatal outcomes is lower in South Asian (SA) infants as compared to White British (WB) infants both born to mothers with diabetes in pregnancy. The second retrospective review was designed to test the hypothesis that large for gestational age infants are at increased risk of adverse neonatal outcomes as compared to appropriate for age infants both born to mothers without diabetes during pregnancy. The third element of the study was a prospective pilot study to test the hypothesis that elevated cord blood C-peptide at birth is positively associated with adverse neonatal outcomes in the infants of diabetic mothers and large for gestational age infants born to mother without diabetes. The aim of the prospective study was also to assess the feasibility of measuring cord blood C-peptide at birth in clinical practice. This chapter includes a review of the study design and its impact on the study findings followed by interpretation and discussion of the results of the three elements of the study.

5.2 The study design

5.2.1 Choice of the study design

5.2.1.1 Retrospective study 1

A cohort study design was selected to assess the influence of diabetes in pregnancy on neonatal outcomes in the SA and the WB ethnic groups. It is already well established that infants born to mothers with T1DM, T2DM and GDM have increased risks of adverse neonatal outcomes. Hence the currently recommended clinical care pathway for infants of diabetic mothers was widely implemented following the Confidential Enquiry into Maternal and Child Health's report of

pregnancy in women with T1DM and T2DM. What has not been previously established is if this risk of adverse neonatal outcomes varies in the different ethnic groups to such an extent to justify a different care pathway, which would be more appropriate to their needs. The SA ethnicity is of particular interest as they have an eleven times higher risk of developing GDM and a six times higher risk of developing T2DM as compared to the WB ethnic group (322) . The NICE guideline on diabetes in pregnancy has identified the need for further research focusing on the impact of diabetes during pregnancy on the management and outcome of newborn infants as a priority. Similarly the 5th international conference on GDM highlighted that there was a lack of information about neonatal outcomes from the various ethnic groups, which is needed to guide clinical care (323) . Diabetes UK and the South Asian Health Foundation have also identified that research related to maternal hyperglycaemia and neonatal outcomes in the SA women is a priority (322) . Hence a cohort study to compare neonatal outcomes between the infants of SA and WB mothers with diabetes during pregnancy was undertaken to establish if SA infants of mothers with diabetes have significantly different outcomes to justify a different care pathway. As the SA ethnic group is the second largest ethnic group (30%) in Leicester where the study was conducted, comparisons were made between the SA and the WB infants. At the time of study design, it was decided that the neonatal outcomes in the other ethnic minority groups would not be compared as the numbers in these would be small and at the time that the study was designed, there was no evidence of any ethnic differences in neonatal outcome to justify a more extensive comparison. It was decided that if the current study showed statistically and clinically significant ethnic differences in the neonatal outcomes, then future studies including other ethnic groups would be important.

It was felt that a case control study, using mothers without diabetes as a control group would not be appropriate, as pregnant women are not universally screened for diabetes in pregnancy. Hence it would not be possible to identify women in the control group who were truly unexposed to diabetes in pregnancy.

5.2.1.2 Retrospective Study 2

Here a retrospective case - control study was chosen as the study aimed to compare the neonatal outcomes between LGA infants (birthweight > 97th centile) and AGA infants. This study design allowed for the required number of cases to be studied without the need to collect data for the total population of births. One of the important issues related to the case - control study methodology is the clear definition of case and control infants. Care was taken to ensure that the cases and the controls along with their inclusion and exclusion criteria were clearly defined (see section 3.6.3).

The combined data collection for both of the first two elements of this project represented 720 mother-neonate dyads with 331 data variables for each dyad. As there was funding available for only one researcher and in the interest of cost and time it was decided that a retrospective study design was the most appropriate. All the data variables were clearly defined at the start of the study and only one researcher collected the data, which allowed consistency in data collection, definition and completeness of the data. In order to maintain the highest level of data quality and data completeness a system of meticulous data checking was carried out. Following collection, the data was double entered onto a computer database. This study database had built in checks to identify outliers or extreme values for various variables and double entry checked for any inconsistencies between the first and second data collection. Any errors in data or data coding were identified during this process and immediately checked and resolved. Once the data entry was complete data cleaning was carried out on all the variables especially quantification of any missing data which was clarified and entered thereby minimising the possibility of missing or incorrect data. However, in spite of this through checking at various stages, inevitably with any large data collection, minor errors may have been missed.

5.2.1.3 Prospective pilot study

It was decided to undertake a pilot study (N = 50) to evaluate the feasibility of measuring cord blood C-peptide immediately after birth and to gather information about the normal range of cord blood C-peptide in the normal infant population to inform power calculation for a bigger more definitive prospective study to evaluate the potential role of cord blood C-peptide as a screening tool to identify infants of diabetic mothers and LGA infants at risk of adverse neonatal outcomes. The case definitions of the eligible study infants for the collection of cord blood C-peptide and the pathway for processing the samples were clearly established before the start of the study and the clinicians and the midwives who would play a crucial role in the identification of the study infants and cord blood sample collection were informed about the pathway. In some cases the informed written consent was obtained prospectively but if the infant delivered before the consent could be obtained or if an eligible infant was identified only after birth (e.g. LGA infant), then informed written consent was obtained retrospectively (a similar approach to that undertaken in the Epicure Study).

5.2.2 Limitation of the study

The main limitation of the study design in the first two elements of the work undertaken was its retrospective nature. This has major limitations as compared to prospective case – control or cohort studies. The most crucial issue is that the data collected was originally for clinical purposes rather than for research. In addition the researcher has to make assumptions regarding the accuracy and completeness of the clinical records. Incomplete data can result in bias in the results depending upon the reasons for which the data is missing. In this study, all the maternal and neonatal medical records were available for data collection. There was a delay in obtaining some medical records especially if they were booked to a clinic, undergoing a medico-legal investigation or stored at an off-site medical record storage site. Clinical entries in the medical records were considered to be complete unless there were unusual entries, which were then cross checked against other clinical entries or discussion with the clinicians.

The cohort and the case – control studies were not population-based. Although such an approach would have been ideal it was considered that it would not have been feasible due to both financial and time constraints. Hence the study participants were selected from a single NHS Trust. This might have introduced some selection bias mainly related to the systematic difference/preference in clinical approach in the one centre and the differences in the maternal and infant population due to cultural, ethnic, genetic, socio-economic and environmental disparities. The study centre followed the relevant national guideline recommended for screening women with / at risk of diabetes in pregnancy and there was no change in the maternal or neonatal screening or treatment guideline during the study period.

As risk factor based selective screening was used for the diagnosis of GDM, there is a possibility that all the eligible pregnant women during the study period may not have received the screening OGTT as referral of the eligible women for the screening test was at the discretion of the attending community midwife. However this would have not led to any selection bias, as there was an equal chance for women in both the ethnic groups to be missed. In addition to this there was a difference in the way pregnant women were offered screening for GDM in the two ethnic groups due to risk factor based selective screening. All SA women are at high risk and would have been offered screening for GDM. While in the WB ethnic group, only women with BMI > 30 kg/m² or with previous history of an LGA infant were offered screening for GDM. This would potentially introduce a selection bias in the two groups.

Last, but not least, is the issue of the influence of confounding factors that limit conclusions that can be drawn from retrospective studies. Attempts were made to obtain information about all potential confounding factors. These retrospective studies, at most, provide information about the associations between the independent variables and the outcome variables but such associations cannot prove causality. They do, however, provide valuable information to support current practice, suggest areas for clinical development and guide future research.

5.3 Retrospective study 1

The UK has a diverse ethnic population and in recent years Asians have been one of the fastest growing ethnic groups in various parts of the UK especially in Leicester where this study was conducted. The impact of race and ethnicity on obstetric, maternal and perinatal care and outcomes has been of great interest in recent years. In the general population, significant differences have been reported in perinatal outcomes amongst women belonging to different racial and ethnic groups (324-327) . The reason for such differences may be multifactorial: variation in access and engagement with healthcare services, maternal education, socio-economic background and cultural beliefs.

Amongst women in the reproductive age group, diabetes in pregnancy has been a rapidly growing problem worldwide. This includes women who already have a diagnosis of diabetes (T1DM or T2DM) at the start of the pregnancy and those who develop diabetes during pregnancy called gestational diabetes mellitus (GDM). Wide variations have been reported in the prevalence of diabetes in pregnancy amongst the different ethnic groups with the SA ethnic group reported to have the highest risk for developing this condition. A systematic review conducted in 2002 by Scott et al which included 135 studies concluded that risk factors for GDM were obesity, advanced maternal age, family history of diabetes, belonging to an ethnic minority group, increased weight gain in adulthood and current history of smoking (66). A UK based study, which was included in this systemic review, also showed that women who developed GDM were older, had a higher BMI and were more likely to be from an ethnic minority group. Some recent studies mainly conducted in the US involving Caucasians, African-Americans, Indo-Asian, Pacific Islander and Hispanic women have reported conflicting results regarding the influence of race and ethnicity on maternal and neonatal outcomes in pregnancies complicated with diabetes (326, 328) . The findings of these studies varied from no difference in perinatal and neonatal outcomes between Asian and Caucasian women to a significant difference in the outcomes between Asian and Pacific Islander women with pre-gestational and gestational diabetes in pregnancy. These studies were conducted in the US and hence the inferences that can be drawn from the study

findings are limited by the study population of pregnant diabetic women residing mainly in the US. There have been no studies based on ethnicity conducted in the UK to compare maternal, perinatal and neonatal outcomes in pregnancies complicated by diabetes. If perinatal and neonatal outcomes indeed differ according to ethnicity, it is vital to establish this as it may have important implications for counselling and management of these patients. More importantly it would provide important information about alterations in the care pathway needed and hence help in service planning and development. In the UK, as is the case in the rest of the world, diabetes in pregnancy is growing exponentially and is said to have reached epidemic levels with almost 17% of pregnancies complicated by diabetes globally in 2013.

Hence as the first step to answer the question regarding the ethnic differences in the perinatal and neonatal outcomes in infants of mothers with diabetes in pregnancy, a retrospective review was undertaken to compare maternal, perinatal and neonatal outcomes between SA and WB mothers with diabetes in pregnancy. At the University Hospitals of Leicester (East Midlands) where the study was conducted, the WB ethnic group forms the largest proportion of the population (65.4%) and SAs are the second largest ethnic group (19.4%). Only these two ethnic groups were included in the retrospective review as the number of women belonging to the other ethnic minority groups was very small and it would not have been practically possible to get the required number of women with diabetes in each ethnic group to confidently compare their outcomes. The SA ethnic group included women from Indian, Pakistani, Bangladeshi and Sri Lankan ethnic background. Although these women were clubbed together under the umbrella of SA ethnic group, it was acknowledged that they were ethnically diverse and come from a very wide geographical area. But as this was the first study in the UK to look at ethnic differences in neonatal outcome in infants of mothers with diabetes in pregnancy it was decided to group them together with the aim to look at each ethnic group separately if the study identifies statistically significant ethnic differences in the neonatal outcomes.

5.3.1 Comparison of the SA and WB women with diabetes in pregnancy

5.3.1.1 Comparison of maternal age and weight

Comparison of the demographic characteristics between SA and WB mothers revealed that the SA women who developed diabetes in pregnancy were younger, had a lower weight and BMI at the time of booking of their pregnancy as compared to the WB women. This finding is in keeping with the differences in maternal demographic characteristics between the SA and the WB ethnic groups reported by the other studies. Gunton JE et al, in a prospective study from New South Wales, Australia, reported that both South Asian and Caucasian women had similar fasting glucose and insulin levels with similar levels of insulin resistance and B-function. Hence both these ethnic groups had similar aetiology for developing GDM. However SA women developed GDM at a lower BMI and at a younger age (329) . Similarly, in a UK based prospective study involving 312 women with diabetes in pregnancy, Dunne et al reported that SA women had a significantly lower BMI 27.1 vs. 29.8 ($p<0.01$) as compared to the Caucasian women (328) .

In the current study the median (IQR) BMI for SA women was 26.95 (23.16 – 31.82) vs. 31.64 (26.37 – 36.89) in the WB women. Although the median BMI for SA women was significantly lower than for WB women, they were above the recommended BMI range for adult women. In this cohort, 30.1% of SA women and 20.6% of WB women diagnosed with diabetes in pregnancy were overweight and 32.1% of SA women and 59.4% of WB women were obese. SA women diagnosed with diabetes in pregnancy had a lower median BMI and fewer women were classed as overweight or obese as compared to WB women. However, the standard definitions for BMI for adult women were used to define overweight and obese women. If accurate and validated ethnic specific BMI cut-offs were available then it is more likely that this would decrease the difference in the number of women classed as overweight or obese between the two ethnic groups. Higher weight and BMI at booking is known to be one of the most important pre-disposing factors for developing diabetes in pregnancy. In a retrospective analysis of prospectively collected data, Steer et al reported that after controlling for confounding factors,

maternal booking BMI was a strong predictor of fetal macrosomia especially in women who developed GDM during pregnancy (330) . Hence higher maternal weight and BMI at the time of booking increases the risk of developing GDM during pregnancy and these women have a higher risk of adverse neonatal complications. More and more women each year are entering pregnancy at higher weights than in the past(41). This is an area that would benefit from public health intervention, as there is potential for both short term and long term health implications for the mother and her offspring.

5.3.1.2 Comparison of the weight gain during the pregnancy

Information about weight gain in pregnancy was available for 113 SA women and 96 WB women with diabetes in pregnancy. SA women had a median (IQR) weight gain of 11.0 (6.5 – 14.0) kg, which was higher as compared to 8.0 (4.0 – 13.0)kg ($p = 0.0033$) in the WB women. 32.7% of SA women and 32.3% of WB women had weight gain above the RCOG recommended range for their BMI at the time of booking. In addition to higher weight and BMI at the time of booking, accelerated weight gain is also a strong predictor of the development of higher insulin resistance during pregnancy and hence the development of GDM. In a retrospective review in Florida, Kim et al reported that in terms of risk factors for delivering a LGA infant for all the races/ethnicities studied by this group, GDM contributed only 2.0 – 8.0% whereas excessive gestational weight gain contributed to 33.3 – 37.7% towards LGA infants (331) . A retrospective Korean study, which included 2,789 women, reported that early gestational weight gain increased the risk of GDM, OR 2.00 (1.26 – 3.16) and PIH, OR 2.34 (1.11 - 4.93) and macrosomia, OR 3.20 (1.52 – 6.71) compared to women with normal weight gain (332) . Women with higher weight gain in pregnancy have a higher residual weight gain after delivery and this increases the risk of development of T2DM and metabolic syndrome in the future (333-335) . One of the important issues related to gestational weight gain is the lack of awareness amongst the pregnant women in the general population regarding the recommended range of weight gain in pregnancy by the Institute of Medicine and the Royal College of Obstetrics and Gynaecology. In addition to this, cultural beliefs in some ethnic groups include higher weight gain in

pregnancy being linked to healthier newborns. This belief might contribute to excessive weight gain in pregnancy. In malnourished mothers better nutrition during pregnancy would certainly lead to healthier offspring. However in developed nations where maternal malnutrition is rare and in developing nations where maternal malnutrition is becoming rare, excessive weight gain in pregnancy is counterproductive. If these women with excessive weight gain develop GDM, they are increased risk of adverse pregnancy, perinatal and neonatal outcomes. In addition to this there are severe long-term health implications for the mother (increased risk of developing T2DM, cardiovascular complications and metabolic syndrome) and her offspring (increasing evidence of epigenetic influence of intrauterine environment in early development of chronic non-communicable diseases such as T2DM, cardiovascular diseases and metabolic syndrome)

5.3.1.3 Comparison of history of smoking and alcohol history

SA women had a significantly lower reported history of smoking 6.4% vs. 43.4% ($p<0.001$) and alcohol consumption 3.8% vs. 25.8% ($p<0.001$) as compared to WB women at the time of the initial booking visit. This information was collected at the time of booking and more detailed information regarding the number of cigarettes smoked per day or units of alcohol consumed each week were not available. Most women would try to decrease or stop smoking and alcohol consumption during pregnancy. Hence there is a possibility that although WB women had a very high reported rate of smoking and alcohol consumption at the time of booking these numbers would have decreased when the pregnancy was confirmed. This finding of ethnic variation in smoking and alcohol consumption rates are in keeping with the findings from national population surveys (336). A UK based report from 2011 on ethnic variation in smoking rates revealed that smoking rates were as low as 2% amongst SA women as compared to 37% in WB women (337). Similarly a UK based literature review reported significantly lower alcohol consumption in SA women as compared to WB women and SA women were also less likely to exceed the recommended weekly alcohol allowance as compared to WB women (338). SA women with a low prevalence of smoking have a higher reported prevalence of diabetes outside and during pregnancy and hence lower smoking rates do not seem

to provide a protective effect. Other factors such as genetic predisposition, lifestyle choices and environmental factors seem to play a more important role in the development of diabetes in pregnancy. However, increased rates of smoking in the WB women who also had a higher risk of being overweight and obese might have contributed to the development of GDM. However this causality could not be tested as it was not the primary aim of the study and the study was not adequately powered to answer this question.

5.3.1.4 Previous obstetric history

There was no statistically significant difference between the two ethnic groups for the previous miscarriage and stillbirth rates which were 39.8% vs. 47.1% and 8.1% vs. 3.4% respectively between SA and WB women respectively. However the risk for previous miscarriage in both ethnic groups was much higher than the risk of 15 - 20% reported in the general population (339) . Similarly their rate of previous stillbirth was much higher than the reported rate of 0.5% in the general population (340) . 11.4% of SA women and 18.5% of WB women reported previous preterm birth. Again there was no statistically significant difference between the two groups but this risk was higher than the reported rate of preterm birth of 7.3% in the general population, which has been static in the UK since 2009 (341) . The reason for adverse previous pregnancy outcomes in this cohort of women with diabetes in the index pregnancy might be undetected subclinical metabolic derangement, which predated the pregnancy. In the current cohort, significant numbers of women in both the ethnic groups had a booking BMI above the recommended range and obesity has been well proven to increase the risk of metabolic syndrome. In a retrospective review of prospectively collected data, Lashen et al concluded that the risk of early miscarriage and recurrent early miscarriages was nearly one and a half times higher in the overweight women and three and a half times higher in the obese women as compared to normal weight women (342) . Wang et al reported that in women undergoing infertility treatment, the risk of miscarriage significantly increased in obese women as compared to women with a normal BMI. The fecundity (probability of achieving at least one pregnancy during infertility treatment) of women with a normal BMI was 60% higher than obese women (343) .

The findings of the current study are in keeping with the above-mentioned studies. It can be postulated that the increased reported rates of previous pregnancy losses in the women in the current study might be due to undetected metabolic derangements and the high prevalence of obesity in this cohort would only worsen this risk.

Another important result to highlight is the higher rate of previous stillbirth and neonatal deaths in the SA women as compared to the WB women. This could either be an incidental finding or possibly because of two reasons: poorer metabolic control in the SA mothers and limited access to healthcare services. SA women diagnosed with GDM in pregnancy may have been investigated for impaired glucose tolerance for the first time in pregnancy. Some of these SA women may have had an impaired or an altered glucose tolerance from the start of the pregnancy or it may even have predated pregnancy especially as the SA ethnic group has a significantly higher background prevalence of T2DM (SA women have a six times higher risk of T2DM as compared to WB women (322) . In other words some of these women may have undetected T2DM and hence would require more intensive treatment using insulin to control their glycaemic derangements. Another important issue is to educate SA women with a diagnosis of diabetes in pregnancy regarding the glycaemic index of their food and the need to control their carbohydrate intake. Empowering women from the ethnic minority groups with a language barrier (limited understanding of English) to understand their diabetes, its control and its treatment poses a major clinical challenge and it might not be possible to achieve this resulting in suboptimal control in these women. This would compound the higher risk of adverse pregnancy outcomes and pregnancy losses. Another factor that contributes to poorer outcomes in women of ethnic minority groups is the perception of health and a lack of understanding of the severity of the disease and poor access to healthcare facilities. A study from the US reported a significant racial/ethnic discrepancy and variation amongst women with a history of GDM in their attitude towards health care access, risk factors and perception of health in the postnatal period. Studies from developed nations, including those from the UK have reported late initiation of antenatal care, lower engagement with the antenatal and post natal care programmes and increased infant mortality in women belonging to ethnic minority groups (344-349) . Further adequately powered studies are

needed to evaluate if there is a real increase in late pregnancy losses and neonatal deaths in SA women with diabetes in pregnancy and then evaluate the possible reasons and the interventions needed.

5.3.1.5 Comparison of antenatal fetal well-being

Women with diabetes in pregnancy underwent regular antenatal ultrasound assessment for fetal wellbeing between 28 - 32 weeks, 32 - 36 weeks and with the last assessment between 36 – 40 weeks. The most striking finding was the significantly lower risk of carrying a macrosomic fetus in the SA women as compared to the WB women. This difference between the two ethnic groups, based on the ultrasound estimate, was noted throughout the pregnancy, 8.5% vs. 29.8% ($p = 0.019$) between 28 – 32 weeks of gestation, 13.9% vs. 44.8% ($p = 0.012$) between 32 – 36 weeks gestation and 21.8% vs. 39.39% ($p = 0.015$) between 36 – 40 weeks of gestation. There was no statistically significant difference in the severity of diabetes between the two groups as suggested by lack of difference in their blood sugar control and the need for insulin treatment. Hence the higher risk of macrosomia in the WB women cannot be explained by glycaemic control in the pregnancy. The study by Steer et al mentioned earlier in section 8.2.1.1 reported a higher risk of macrosomia in women with GDM who were overweight or obese at the start of the pregnancy. This could be the possible reason for increased risk of macrosomia in the WB women as 80% of the WB women in this cohort had a booking BMI above the normal BMI recommended for adult women. There was no difference in any other antenatal complications such as polyhydramnios, poor growth/growth restriction or Doppler abnormalities between the two groups. There was no statistically significant difference in the different modes of delivery between the two groups.

5.3.2 Comparison of mode of delivery

SA women were less likely to be delivered by caesarean section as compared to WB women, 39.4% vs. 49.4%. ($p = 0.431$). Although not statistically significant SA women had lower odds of delivering by caesarean section OR (95% CI) 0.66

(0.42 – 1.04) but had significantly higher odds of perineal trauma 2.23 (1.24 – 4.24) associated with the higher tendency to deliver vaginally. This difference may not be statistically significant but would be clinically significant. Dune et al, in their UK based prospective cohort study reported that 60% of SA women with diabetes in pregnancy delivered vaginally as compared to 38% of WB women ($p < 0.001$). WB women had double the risk of being delivered by caesarean section as compared to SA women and this difference was most significant in women with T2DM. The higher prevalence of caesarean section amongst the WB women may be due to the increased rate of macrosomia detected in WB fetuses, which would almost certainly lower the clinician's threshold for caesarean section. Esakoff et al in their retrospective review of 26,411 women with GDM in California between 2001 and 2004 similarly reported that SA women had lower adjusted odds of delivering by caesarean section aOR of 0.86 (0.77-0.96) as compared to Caucasian women. In another similar study in the US, Nguyen et al compared the neonatal outcomes amongst 32,193, Black, White, Asian and Hispanic women with GDM. They reported that the Asian women had the lowest adjusted odds of primary cesarean delivery aOR (95% CI), 0.75 (0.69-0.82).

5.3.3 Comparison of neonatal outcomes

There are only two studies comparing neonatal outcomes between infants of SA and WB mothers with diabetes in pregnancy. Esakoff et al in their retrospective review of 26,411 women with GDM in California between 2001 and 2004, reported that SA women had lower odds of delivering a macrosomic infant (defined as birth weight < 4000 grams) 4.2% vs. 10.3% ($P < 0.05$) with OR (95% CI) of 0.58 (0.48-0.70) as compared to Caucasian women. There was no difference in their risk for preterm birth, intrauterine death and need for NICU admission between the two ethnic groups (350) . In another similar study in the US, Nguyen et al compared the neonatal outcomes amongst 32,193 Black, White, Asian and Hispanic women with GDM. They reported that the Asian women had lower adjusted odds of delivering a LGA infant adjusted OR, 0.40 (0.33-0.48), and developing neonatal respiratory distress syndrome adjusted OR, 0.54 (0.40-0.73) as compared to White mothers (351) .

The findings of the current study are similar to those reported from the studies in the US. SA infants had fewer adverse outcomes as compared to WB infants. There was no difference in the median gestation at birth between the two ethnic groups. The median (IQR) birthweight of SA infants was 3260 (3825 – 3670) grams as compared to 3535 (3055 – 3975) grams in WB infants ($p < 0.001$). SA infants had significantly reduced risk of being born LGA (birthweight $> 97^{\text{th}}$ centile) as compared to WB infants 28.8% vs. 39.4% ($p = 0.032$), OR (95%CI) 0.62 (0.39 – 0.98).

SA infants also had a significantly lower risk of being born preterm (defined as < 37 weeks gestation at birth) 9.6% as compared to 17.5% ($p = 0.049$) OR 0.50 (0.26 – 0.98). This risk remained unchanged even after adjusting for confounding factors like maternal age, weight and BMI. It is likely that the higher rate of preterm deliveries in both the ethnic groups in this study as compared to the reported background rate of 7% in the UK may be iatrogenic. The clinicians are likely to have a lower threshold to deliver diabetic women before term especially if there is a risk that continuation of the pregnancy may be detrimental to fetal well-being. For example, one of the possible explanations for a higher rate of preterm delivery in the WB mothers may have been early induction of labour or early elective caesarean section before term in WB women with evidence of fetal macrosomia. This finding of fewer preterm births in SA women with diabetes is different from the Californian study by Esakoff et al who did not find any difference in the rate of preterm birth between the Asian and the Caucasian study groups.

There was a significant difference in the composite adverse outcome (NICU admission, neonatal hypoglycaemia, birth trauma, need for readmission within the first 28 days and neonatal death) between the SA and the WB infants 18.6% vs. 31.9%, $p = 0.009$. SA infants had nearly half the risk of an adverse composite neonatal outcome, OR (95% CI) 0.488 (0.289 – 0.823) as compared to WB infants. This risk remained unchanged after controlling for various confounding factors such as maternal age, pre-pregnancy weight, height, BMI, parity, smoking, alcohol consumption, need for insulin treatment for GDM, caesarean section and

prematurity OR (95% CI) . SA infants also had less than half the risk of NICU admission OR 0.49 (0.26 – 0.91), respiratory distress OR 0.40 (0.19 – 0.98) and neonatal hypoglycaemia OR 0.49 (0.24 – 1.02). There was no difference in rate of congenital anomalies, sepsis, hyperbilirubinaemia, duration of hospital stay and readmission rates between the two groups.

5.3.4 Conclusion

I believe this is the first study in the UK to compare the neonatal outcomes of infants born to SA and WB mothers with diabetes in pregnancy. The infants born to SA mothers had a significantly lower risk of adverse neonatal outcomes as compared to WB infants in spite of the higher prevalence of diabetes amongst SA mothers. This risk remained low even after controlling for various confounding factors that could influence the risk of complications in these infants. The difference in neonatal outcomes might have been influenced by the striking differences in other attributes noted between mothers in the two groups. WB mothers had a significantly higher weight/BMI at booking and more advanced maternal age as compared to SA mothers. SA mothers developed GDM at a lower BMI and lower age suggesting that their development of diabetes was probably triggered by their genetic predisposition and they had a lower metabolic derangement. On the other hand, 80% of WB mothers who had a BMI above the normal range for adult women might have had more severe long-standing metabolic derangement. They had a higher tendency to require insulin to control their diabetes during pregnancy and they had a significantly higher risk of delivering a macrosomic infant at a more preterm gestation and with a higher need for a caesarean delivery. All these factors in combination will have contributed to the increased risk of adverse neonatal outcomes seen in the infants of WB mothers.

There are numerous studies conducted nationally and internationally to address the issues of screening methods for diabetes and the blood sugar thresholds to be used for the diagnosis of GDM, and the best treatment modality for GDM. However all these studies focus on secondary and tertiary prevention of the complications of diabetes in pregnancy. None of the studies have been designed to address primary

prevention of gestational diabetes focusing on public awareness and education of women in the fertile age group and those planning a family. Such measures would include education of women regarding the importance of ideal pre-pregnancy weight and BMI, healthy lifestyle and dietary choices during pregnancy and weight gain during pregnancy. As diabetes in pregnancy is increasing rapidly in parallel to obesity and as diabetes begets diabetes, large public health intervention programmes focusing on primary prevention of diabetes in pregnancy are urgently needed.

5.4 Retrospective study 2

Over the last two to three decades, there has been a rise in the prevalence of LGA infants in both developed and developing nations. An overall increase of 15 – 25% in the proportion of LGA infants has been reported in the USA, Canada, Australia, Germany, Scotland, and Denmark (218, 220-222, 352, 353) . LGA infants constitute a heterogeneous group of physiologically and pathologically LGA infants. There is wide variation in the management of LGA infants ranging from intensive postnatal management similar to infants of diabetic mothers to usual postnatal care as in normal infants born following a low risk pregnancy. This variation in practice exists as there is clinical equipoise regarding the management of LGA infants born to non-diabetic mothers due to the lack of good quality studies to review the postnatal outcome of these infants and lack of evidence for best practice. There has been no UK based study to address this issue. Hence as a first step a retrospective review was undertaken to compare the neonatal outcome of LGA infants born to non-diabetic mothers as compared to appropriate for gestational age infants born following a low risk pregnancy.

5.4.1 Comparison of maternal characteristics of LGA and AGA infants

There was a significant difference in the maternal age, weight, height, BMI and parity between the mothers of LGA and AGA infants. Mothers of LGA infants were older (30years vs. 28years, $p < 0.001$), had a higher weight (76kgs vs. 64kgs, $p < 0.001$), height (168cm vs. 163cm, $p < 0.001$) and BMI (27.7 vs. 23.9 kg/m², $p < 0.001$) at booking and a higher parity as compared to AGA infants. The median birthweight of infants born to primiparous women was 3542 grams, which increased to 3905 grams in multiparous women, and this further increased to 4285 grams in grand-multiparous women. There was a direct relationship between maternal age, pre-pregnancy weight, BMI and parity with birthweight for the entire cohort of infants. Infant birthweight increased as a continuum with increasing maternal age, pre-pregnancy weight, BMI and parity. There was no threshold effect above, which the risk of delivering a LGA infant increased significantly.

Li G et al in a 14 provinces based cross-sectional survey of 101,723 Chinese term infants reported that the possibility of delivering a macrosomic infant was moderately increased with maternal age > 35 years and significantly increased with higher maternal height, weight gain during pregnancy and parity (354) .

Similarly Li Yi et al in a retrospective review from China reported that the healthy mothers of macrosomic infants were nearly 2 years older than mothers of normal infants ($p < 0.001$). Maternal age (OR (95%CI) = 1.09 (1.03 – 1.15), weight gain in pregnancy (OR = 1.14 (1.10 – 1.19) and gestational age at birth (OR = 1.62 (1.31 – 1.99) were significantly associated with macrosomia(231).

Kramer et al in their analysis of temporal trends of birthweight in Canada over an 18 year period reported that the increased prevalence of LGA infants from 8% to 11.5% was associated with a decrease in teenage pregnancies from 4.4% to 1.0% and an increase in maternal age above 35 years from 7.8% to 20.1%, an increase in tall stature from 24.4% to 32.7%, an increase in overweight and obese women from 7.0% to 9.0% and 4.7% to 10.6% respectively, an increase in more education from 17.0% to 36.6% and decrease in smoking from 12.7% to 5.6%. After adjusting for various confounding factors, maternal pre-pregnancy BMI, gestational weight gain, gestational diabetes and decrease in maternal smoking remained significantly associated with LGA infants(216).

Gaudet L et al in a systematic review and meta-analysis of 30 studies reviewed the influence of maternal obesity on fetal macrosomia. They reported that maternal obesity defined as a BMI ≥ 30 kg/m² was associated with an increased risk of fetal overgrowth: birthweight ≥ 4000 grams OR (95%CI) 2.17 (1.92 – 2.45), birthweight ≥ 4500 grams OR 2.77 (2.22 – 3.45) and birthweight > 90th centile for gestation OR 2.42 (2.16 – 2.72) (355) .

Gyselaers et al in Belgium reported that the prevalence of macrosomia from 1991 to 2010 had increased from 7.3% to 8.63% and one of the three important factors responsible for this rise had been the proportion of pregnant women aged 35 years and more which had increased from 6.1% to 14.3%, an increase in overweight and

obese women by 4% each and an increase in maternal height by 10cm during the same time period (219).

Worldwide there has been a gradual increase in maternal age at the time of first and subsequent pregnancies. This has been due to an increase in maternal education at all levels and subsequent engagement in a vocation leading to family postponement due to conflict between employment and motherhood. Older women are at higher risk of adverse pregnancy outcomes including GDM and LGA and associated maternal, pregnancy and neonatal complications (356-361) . There has also been a global increase in obesity resulting in more and more women entering pregnancy being overweight or obese (362) .

Another important factor is the excessive gestational weight gain during pregnancy, which had been reported by several studies as an independent factor for delivering LGA infant (231, 363, 364) .

Two areas that have been of great concern to the various obstetric societies across the world have been increase in pre-pregnancy weight and BMI in women in the fertile age group and the gestational weight gain (365, 366) . Large national and international campaigns to spread awareness amongst overweight and obese women regarding the importance of weight reduction before pregnancy, optimum weight gain during pregnancy and the pregnancy related complications associated with higher BMI are needed (366) .

5.4.2 Comparison of mode of delivery

In the current study, LGA infants had a significantly higher risk of being delivered by elective caesarean section before term 23.0% vs. 9.0% ($p < 0.001$), OR (95% CI) 2.9 (1.63 – 5.29) or undergoing induction of labour before term 15.5% vs. 6.0% ($p = 0.003$), OR 2.87 (1.4 – 5.7) as compared to AGA infants. LGA infants were also at a significantly higher risk of being delivered by emergency caesarean section 18.5% vs. 8.0% ($p = 0.003$), OR 2.6 (1.4 – 4.88) and hence consequently a lower chance of delivering by spontaneous vaginal delivery 55.5% vs. 75% ($p < 0.001$), OR 0.41 (0.27 – 0.63). The reason for higher caesarean sections could be due to combination of anticipated difficulty in delivering vaginally due to cephalo-pelvic disproportion resulting in either a prolonged or obstructed labour. This approach is inevitably linked to the risk of short term and long-term maternal and fetal injury and medico-legal liability.

Wide variation exists in the management of fetuses who are expected to be LGA on antenatal scanning with regards to the timing of delivery and the mode of delivery. This is due to the inability to accurately predict the estimated fetal weight from pre-pregnancy and antenatal risk factors and antenatal ultrasound scanning. There is also a lack of consensus regarding the definition of LGA and macrosomia (367, 368) . This leaves the management of these infants at the discretion of the individual clinicians and their interpretation of limited evidence for best practice. In addition to this, the perinatal management of this group of infants is further complicated by associated maternal factors like obesity, advance maternal age and unidentified GDM due to the selective screening for diabetes in pregnancy. In this study after adjusting for various confounding factors, LGA OR 2.95 (1.77 – 4.90) and maternal age OR 1.09 (1.04 1.14) were two factors that predicted the risk for caesarean section.

Information regarding the perinatal management of LGA infants of non-diabetic mothers comes mainly from two landmark papers. The first being an American study by Rouse et al for the evaluation of cost effectiveness of three policies: management without antenatal scanning, elective caesarean section for fetuses with

estimated fetal weight of 4000 grams and elective caesarean section for fetuses with estimated fetal weight of 4500 grams. They reported that additional 2345 caesarean sections would have to be performed for every case of permanent brachial plexus injury prevented with the 4500 grams policy and 3695 caesarean sections with every 4000 grams policy. They concluded that for 97% of pregnant women who are non-diabetic, the policy of elective caesarean section was not medically or economically sound (369) . The second paper that extensively supported this recommendation was a bulletin published by the American College of Obstetricians and Gynaecologists (ACOG) which concluded that the antenatal assessment of fetal weight is imprecise and to consider elective caesarean section only in women with an estimated fetal weight ≥ 5000 grams (367) .

In the UK, the Royal College of Obstetricians and Gynaecologists (RCOG) acknowledge the Rouse paper and the ACOG recommendations but do not provide any practice guidance(181). The RCOG/NICE guidelines on antenatal care (2008) recommend that routine estimation of fetal weight in low risk populations should not be undertaken (368) and the RCOG/NICE guideline on caesarean section does not include fetal macrosomia as an indication for elective caesarean section (370) . At the same time, ACOG and RCOG recommend elective caesarean section in infants of diabetic mothers with an estimated birthweight > 4500 grams thereby suggesting that the antenatal ultrasound estimation of fetal birthweight is not entirely valueless and that this group of infants are at high risk.

Vendittelli et al in a French study of 3077 non-diabetic pregnant women have reported that even induction of labour in women expected to deliver a LGA infant does not decrease the risk of maternal morbidity (371) . Hence it can be concluded that further studies are needed to evaluate the best perinatal management for the LGA infants of non-diabetic mothers.

In this study, the higher rate of caesarean sections in the LGA infants decreased the risk of instrumental delivery 5.8% vs. 25.0% ($p < 0.001$) OR 0.19 (0.07 – 0.05) and the risk of all grades of perineal trauma 31.7% vs. 45.7% ($p = 0.003$), OR 0.55 (0.36 – 0.83) in their mothers as compared to AGA mothers. However it did not

reduce the risk of birth trauma and NICU admissions in the LGA infants which is discussed in the next section.

5.4.3 Comparison of neonatal outcome of LGA and AGA infants

Wide variation exists in the definition of LGA infants and this terminology is wrongly used interchangeably with macrosomia. Some clinicians and authors define LGA as a birthweight ≥ 4000 grams (which corresponds to the 90th centile for a male infant born at 40 weeks of gestation), some as a birthweight ≥ 4500 grams (which corresponds to the 97th centile for a male infant born at 40 weeks of gestation) and others as a birthweight ≥ 5000 grams. Birthweight cut offs are arbitrary and do not take into account other factors that influence birthweight such as the gestation at which these infants are born, their sex or their ethnicity. Such a definition would fail to truly identify all the at risk infants. Another way, which is a more appropriate way of defining LGA infants, is according to their birthweight centile. Again there exists a difference of opinion as some clinicians consider birthweight $\geq 90^{\text{th}}$ centile as significant, while others use the 95th birthweight centile or the 97th birthweight centile to define LGA. In addition to this LGA infants constitute a heterogeneous group of physiological LGA and pathological LGA infants. There are a plethora of small studies comparing neonatal outcomes in LGA/macrosomic infants. But information from these studies cannot be easily interpreted as they have used different definitions of LGA and some have included mothers with diabetes in pregnancy. Some of this lack of consistency is probably due to the fact that there are no large, good quality, prospective population based studies to address the question of neonatal outcomes in LGA infants in relation to their birthweight centiles. Macrosomic infants of mothers with diabetes in pregnancy have been extensively studied and most of the clinical management of LGA infants born to non-diabetic mothers has been extrapolated from this. Hence wide variation exists in clinical practice with some clinicians treating them as infant of diabetic mothers and others as normal infants.

In the current study, 200 LGA infants (defined as birthweight $\geq 97^{\text{th}}$ centile for gestation, sex and ethnicity) were compared to 200 AGA infants (defined as

birthweight between 10th to 90th centile for gestation, sex and ethnicity). LGA infants had a significantly higher risk of adverse composite outcome 22% vs. 12.5% ($p = 0.032$), adjusted OR 2.24 (1.24 – 4.02) and NICU admission 10.6% vs. 5.0% ($p = 0.029$) OR 2.74 (1.168 – 6.36). LGA infants had a significantly higher risk of respiratory distress OR 4.94 (1.4 – 17.48).

Only 35 LGA infants had their blood sugar tested in the postnatal period of which six infants developed hypoglycaemia and there were none in the AGA group. However, due to small numbers it is difficult to comment on this outcome. There was no difference in other outcomes like congenital anomalies, hyperbilirubinaemia and readmission after initial discharge. LGA infants had more than double the risk of adverse neonatal outcomes as compared to AGA infants.

Linder et al from Israel, in their retrospective comparison of LGA and AGA infants reported that the LGA infants had a higher risk of prolonged hospitalisation mainly due to maternal caesarean section, adverse composite outcome, NICU admission, hypoglycaemia, transient tachypnoea of newborn and birth trauma. Asymmetric LGA infants had a significantly higher risk of neonatal hypoglycaemia as compared to the control and symmetrical LGA infants. Neonatal hypoglycaemia progressively increased with increasing birthweight category. However, in this study there is a possibility that all the infants of diabetic mothers would not have been truly excluded as in their report, they did not provide information about the diabetes-screening programme during the 11 year study period (259).

Aranha et al in a small retrospective study from Australia reported a significant increase in the risk of neonatal hypoglycaemia in macrosomic infants as compared to normal weight infants both born to non-diabetic mothers. However they used a rather liberal definition of hypoglycaemia (blood sugar ≤ 3.0 mmol/L). Macrosomic infants had a higher however statistically non-significant risk of respiratory distress. There was no difference in other maternal and neonatal complications. (372) .

Araz N et al from Turkey, in their prospective study reported that macrosomic infants of non-diabetic mothers had a significantly increased risk of neonatal

hypoglycaemia (blood sugar < 2.2 mmol/L) 16.7% vs. none in the control group ($p < 0.001$). However as the authors have not clearly mentioned how they identified women with diabetes in pregnancy to exclude them, this cohort might have women with undiagnosed diabetes and hence such a high rate of hypoglycaemia (373) .

Ute M. Schaefer-Graf et al from Germany, in a 5 year retrospective review reported a direct relationship between maternal blood sugar during pregnancy and neonatal hypoglycaemia even in mothers who were not diagnosed with diabetes in pregnancy. 16.0% of LGA infants developed hypoglycaemia within the first 24 hours. The hypoglycaemia rate was 5.9% in infants of mothers with a normal OGTT (control infants), 12.2% in infants of mothers with one elevated blood glucose value on OGTT and 17.7% in infants of mothers without antenatal glucose testing suggesting undiagnosed GDM in these women(258).

Brand PL et al assessed the neurodevelopmental outcome of neonatal hypoglycaemia in term LGA infants born to non-diabetic mothers at 4 years of age. They reported that after controlling for various confounding factors there was no difference in the Denver developmental score and child behaviour checklist score between LGA infants with normoglycaemia and those with hypoglycaemia, suggesting that the hypoglycaemia in LGA infants in the neonatal period did not have a significant long-term impact. However this was a very small study of only 75 LGA infants (374) .

5.4.4 Conclusion

The current study and the studies summarised above show that LGA infants are at higher risk of neonatal complications such as need for NICU admission, neonatal hypoglycaemia, respiratory distress and birth trauma. However the entire issue of LGA infants and their management is complicated by the lack of certainty that this cohort does not include infants of unidentified diabetic mothers due to the selective screening for diabetes in pregnancy that is currently used. In addition to this majority of LGA infants born to truly non-diabetic mothers are constitutionally big rather than due to metabolic complications and hence at very low risk of postnatal complications. From the current evidence available it is difficult to come to a conclusion regarding the postnatal management of LGA infants born to mothers without diabetes in pregnancy.

Further large, multicentre, population based studies are needed to answer the questions regarding the best definition of LGA based on outcome measures and the best perinatal and postnatal management of LGA infants of non-diabetic mothers. Further long-term studies are also needed to assess the long-term impact of being LGA at birth. Currently there is no clinical tool available to identify at birth constitutionally large LGA infants from pathologically large LGA infants. The availability of a biochemical tool to identify at birth, the LGA infants at risk of adverse neonatal complications may help to streamline the postnatal management of these infants. This brings us to the prospective pilot study, which was conducted to assess the potential of cord blood C-peptide to identify the infants of diabetic mothers and LGA infants of non-diabetic mothers at risk of adverse neonatal complications. This is discussed in detail in the next section.

5.5 Prospective study

5.5.1 Feasibility of measuring cord blood C-peptide at birth

The pilot study showed that it was possible to collect cord blood samples for the estimation of C-peptide levels immediately after birth. The venous cord blood samples were collected by the midwife delivering the baby using the same method used to collect cord blood samples for biochemical analysis.

Two important issues were:

1. Identification of the study infants and
2. Sending the samples on ice to the pathology laboratory immediately to ensure that the samples were suitable for analysis.

Before the pilot study started, the research fellow took several sessions to spread awareness amongst the midwives and the other clinical staff on the maternity units at Leicester Royal Infirmary and Leicester General Hospital. They were given information about case identification and the procedure for sending cord blood samples for C-peptide levels. In addition to this study posters with details of the inclusion criteria and sampling process were displayed in clinical areas. On a day-to-day basis the research fellow was involved in the identification of cases and ensuring that the cord blood samples were collected and sent to the pathology laboratory in a timely manner.

Recruitment to the C-peptide study was 100% i.e. all mothers approached to participate in the study consented to take part. The reason for this might have been the fact that only cord blood was being collected for analysis and not mother's or their baby's blood. Patient and public involvement work during the study design, revealed that mothers were keen to know if a biochemical marker could be used to identify the newborns at risk of neonatal complications before those complications arise.

At the beginning of the study there were two cases where mothers were consented for collection of cord blood samples but the samples were not collected as both these mothers delivered at night and were very unwell. In total, 50 cord blood samples were collected and none were reported to be unsuitable for analysis. In only one case the result could not be obtained due to a dilution error in the pathology laboratory. Hence it appears feasible to collect cord blood samples for estimation of C-peptide levels.

During this pilot study, serum was separated from the cord blood sample by centrifugation and stored at -70°C at the local pathology laboratory at Leicester Royal Infirmary. The frozen samples were sent in batches to the pathology laboratory at Nottingham City Hospital (Nottingham University Hospitals NHS Trust) in a frozen state (using dry ice) for the analysis of C-peptide levels. This was because at the time of the study the local pathology laboratory in Leicester was at the beginning of the process for setting up a C-peptide assay. It is clear from my discussion with the local pathology team that, more and more hospitals now have C-peptide assays available, they are not expensive and the analysis time is about one hour. Hence a result could be available within 90minutes of birth. The median time to first feed for the entire cohort in the pilot study was 86 minutes after birth. The results of cord blood C-peptide levels would not be used to decide the timing of the first feed which should be given to all the infants at the earliest available opportunity and ideally within the first hour. Cord blood C-peptide levels could help to identify infants with hyperinsulinism who would benefit from more intensive management while freeing the vast majority of infants from unnecessary medicalisation of their care. In conclusion, it is feasible to collect cord blood samples for estimation of C-peptide levels after birth in infants of diabetic mothers and LGA infants of non-diabetic mothers and it is possible to get the results back from this analysis to influence clinical decisions. Further studies would be needed to assess the safety of basing management on these results.

5.5.2 Potential of cord blood C-peptide to identify at risk infants at birth

As this was a pilot study the number of infants in each study category was small and hence it is not possible to derive a robust conclusion. The mothers of the LGA infants and the mothers with gestational and pre-gestational diabetes had a median BMI within the obese range for BMI and they were nearly 10kgs heavier than the mothers of the control infants who had normal BMIs at the time of the booking. Mothers with diabetes were older by 4 – 6 years and had an increased tendency to be either multigravida (gravida 2 – 4) or grand-multi gravida (gravida $\geq$ 5) as compared to the mothers of LGA and control infants.

Almost all the diabetic mothers and mothers of LGA infants were either offered induction of labour before term or elective caesarean section. In diabetic mothers, this is due to the NICE recommendations to offer induction of labour or caesarean section to diabetic women beyond 38 weeks of gestation and to avoid continuation of pregnancy beyond term due to the increased risk of stillbirth (65). In this study, the most common reasons for early induction of labour or caesarean section in women with diabetes were poor maternal blood sugar control with hypertensive complications, fetal macrosomia and previous caesarean section. However there is no evidence to support iatrogenic early delivery in LGA infants of non-diabetic mothers as discussed in section 5.4.2. Due to the lack of evidence for best practice for the management of infants with an antenatal diagnosis of LGA, there is a tendency for clinicians to treat them as macrosomic infants of diabetic mothers. This is due to the increased risk of maternal and fetal birth trauma and associated litigation. In this study, 80% of the control infants delivered by normal vaginal delivery. This figure dropped down to 43.8% in the infants of mothers with GDM, to 33.3% in the LGA infants and the lowest vaginal delivery rates of 26.7% were noted in infants of mothers with pre-gestational diabetes. There were no complications of birth trauma noted amongst the study infants.

There was no difference in the gestational age at birth between the control infants, LGA infants and the infants of mothers with GDM during pregnancy. They were born between 39 – 40 weeks of gestation. However the infants of mothers with pre-

gestational diabetes were born nearly a week earlier at a median age of 38+3 weeks as compared to the other three study groups. This might have been due to the need for early delivery in these infants due to maternal, pregnancy and fetal complications. The LGA infants as expected had a higher median birthweight of 4480grams and head circumference of 37.4cm as compared to 3665grams and 35cm respectively in the control infants. The infants of mothers with GDM had similar birthweight and head circumference to the control infants. However the infants of mothers with pre-gestational diabetes, although they were born at the earliest median gestational age, their median birthweight of 3740grams was higher than the control infants with the lowest head circumference of 33.5cm, suggesting asymmetrical growth in these infants.

There was a higher tendency for the infants of diabetic mothers to receive mixed feeds (breast and bottle) as compared to the control and the LGA infants. This could be explained by the combination of higher rates of caesarean sections in the mothers with diabetes in pregnancy as compared to the control infants and the need to ensure adequate milk intake in their infants to prevent neonatal hypoglycaemia. The control infants had the shortest median time to first feed of 71 minutes while the infants of mothers with diabetes had the longest median time to first feed of 86 minutes. This difference in the time to first feed might not be clinically very significant but just reflects the fact that the delay might have been due to more maternal medical needs requiring midwifery attention in the early postnatal period in this group of patients.

Only two babies developed neonatal hypoglycaemia. Both these babies were born to mothers with pre-gestational diabetes (i.e. with more metabolic derangement), at term gestation with birthweight on the 95th centile and were exclusively breast-fed. The cord blood C-peptide levels were more than 1000pmol/L (90th centile for cord blood C-peptide in control infants was 371pmol/L). The hypoglycaemia occurred in the first 12 hours and resolved with additional feeds with term formula milk. The elevated cord blood C-peptide levels and maternal type of diabetes correlated well with neonatal hypoglycaemia. There was no correlation between birthweight or gestation at birth with hypoglycaemia. There was no correlation of cord blood C-

peptide levels to other NICU admission. An additional baby with elevated C-peptide level did not develop hypoglycaemia but he received bottle feeds (sufficient amounts of milk) since birth. None of the infants in the control group, LGA group or the GDM group had hypoglycaemia.

There is no doubt that breast-feeding is important for mothers with diabetes in pregnancy. WHO and NICE recommends that breast-feeding is beneficial for women with diabetes in pregnancy as it not only offers the benefits of breast milk to the newborn but it also decreases the risk of T2DM in mothers with GDM (375, 376) . Women with diabetes in pregnancy should be given adequate support and education to help initiate and continue breast-feeding. At the same time it is important to acknowledge that infants of diabetic mothers have not had a completely normal intrauterine environment. They have been exposed to high blood sugar levels throughout their fetal life, which results in up regulation of their pancreatic B-cell – insulin axis and higher insulin secretion per unit glucose load. At birth, the steady flow of blood glucose is abruptly cut off and the pancreas is still in a state of hyper secretion, which take a few days to adjust (296) . Diabetic mothers are also at high risk of other co-morbidities like hypertension, sepsis, post partum haemorrhage, birth trauma and increase risk of caesarean section, which can make commencement and establishment of breast-feeding difficult. Hence while breast-feeding is being established it is important to assess the amount of milk intake and top up with additional formula milk feeds until breast-feeding is well established. As was the case in this study, the hypoglycaemia noted in two infants resolved with additional oral feeds. This complication could have been totally prevented if their elevated C-peptide levels were known at birth.

The findings of this study are in keeping with other studies. Sosenko et al have shown that the mean cord blood C-peptide levels in symptomatic infants of diabetic mothers with hypoglycaemia and macrosomia were double as compared to control infants who did not have these complications ($p<0.001$) (309) . Similarly, Fallucca et al showed that infants of diabetic mothers had elevated cord blood C-peptide levels as compared to control infants. Amongst the infants of diabetic mothers the

ones who developed hypoglycaemia had higher levels than those who did not (296)

Abdel Halim Badr El Din et al reported that there was a direct relationship between maternal blood glucose control during pregnancy and cord blood C-peptide levels. The cord blood C-peptide levels were higher in poorly controlled diabetic mothers as compared to well controlled mothers and both these levels were significantly higher than the control mothers. Infants with hypoglycaemia and macrosomia had higher cord blood C-peptide levels when compared to those who did not develop the complication (310) . Knip et al reported similar findings to Halim Badr El Din et al (377) .

Schwartz et al showed that although cord blood C-peptide increased in direct relation to the severity of maternal diabetes during pregnancy, with elevated levels seen in macrosomic infants of diabetic mothers as compared to the non-macrosomic infants, such a rise was not seen in LGA infants of the control mothers(213). Similar results were echoed by the HAPO Study, which showed that the macrosomic infants of diabetic mothers had higher cord blood C-peptide levels and the macrosomic infants with elevated cord blood C-peptide levels were at a significantly higher risk of neonatal hypoglycaemia (315) .

Aygun et al and Rou-Lin Hou et al similar to Schwartz et al have reported that the cord blood C-peptide levels were not significantly elevated in the LGA infants of non-diabetic mothers as compared to control infants suggesting that these infants may be constitutionally big rather than due to pathological overgrowth as seen in infants of diabetic mothers (319, 320) .

5.5.3 Conclusion

In summary, the current study and the other smaller studies done in the past have shown that cord blood C-peptide levels are elevated in the infants of mothers with diabetes in pregnancy and the increase in the C-peptide level directly corresponds to the severity of maternal diabetes and its control. The cord blood C-peptide levels in LGA infants of non-diabetic/control mothers are not elevated to the same extent

as the LGA/macrosomic infants of diabetic mothers suggesting a different antenatal developmental mechanism. Amongst infants of diabetic mothers, the infants who develop complications of hypoglycaemia have significantly elevated levels as compared to those who do not. So cord blood C-peptide has the potential to be used as a biochemical marker to identify infants of diabetic mothers and LGA infants of non-diabetic mothers (not identified as diabetic due to the antenatal selective screening programme) at risk of complications. Once identified at birth they could be more intensely monitored and managed thereby avoiding hypoglycaemia and its short-term and long-term complications. This provides a potentially huge benefit over current practice, as currently, the high-risk infants are identified only after the complication of hypoglycaemia has occurred. The vast majority of infants of mothers with diabetes are not at risk of postnatal complications and do not need extra monitoring. The estimation of cord blood C-peptide levels would help to identify these infants thereby freeing them from unnecessary medicalisation of their postnatal care.

One big question that faces us at this stage is: what is a safe level of cord blood C-peptide and what is the threshold level above which the risk of adverse postnatal complications increase? Large multicentre, prospective studies are needed with both short-term and long-term follow-up to answer this question. The current pilot study has been useful as a first step as it has shown that it is feasible to measure cord blood C-peptide levels at birth. It has also provided with some important information about the C-peptide normal range in the various study groups, which would help in power calculations for a bigger prospective study. Finally long-term follow-up at two and five years following a big study would be useful to be confident about the reliability of this test before it can be brought into clinical practice.

5.6 Recommendation for future research studies

1. To study the benefits of educational interventions in pregnant women to improve awareness about GDM and its complications to prevent development of GDM and its associated maternal, perinatal and neonatal complications. Experiences from DESMOND (Diabetes Education and Self Management for Ongoing and Newly Diagnosed) Programme can be used to design educational intervention in the pregnant women.
2. To study whether ethnic specific educational interventions in pregnant women from the ethnic minority groups help to improve maternal, perinatal and neonatal outcomes in women with diabetes in pregnancy.
3. To study the benefits of postnatal longer-term follow-up and ongoing educational interventions in women with a history of GDM during pregnancy to avoid or delay progression to T2DM. A follow-on study would be to review the cost effectiveness of such interventions.
4. To conduct a multicentre trial to understand the feasibility of measuring cord blood C-peptide levels in newborns and the benefits of cord blood C-peptide in the management of infants of mothers with diabetes during pregnancy and LGA infants born to non-diabetic mothers.

6 APPENDICES

6.1 Appendix 1: Retrospective Study 1 and 2

Study questionnaire for retrospective study 1 and retrospective study 2

<b style="color: red;">HENS maternal H yperglycaemia and E thnicity on N eonatal outcome S tudy																								
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☐ Other																								

Medical problems during pregnancy

☐ Diabetes ☐ Type I ☐ Type II

Treatment

☐ Pregnancy induced hypertension

☐ Pre-eclampsia

☐ Eclampsia

Treatment

Gestation at the time of OGTT + Wks + days

Diagnosis of GDM ☐ Yes ☐ No

Fasting blood sugar . mmols/L

Post prandial blood sugar mmols/L

Treatment

☐ Diet & exercise ☐ Insulin ☐ Oral hypoglycaemics

28– 32 weeks

Weight (kg)

Blood sugar control ☐ Good ☐ Moderate ☐ Poor

Treatment

☐ Diet & exercise ☐ Insulin ☐ Oral hypoglycaemics

HbA1c (gm%) .

Urine

Ultrasound at 28 – 32 weeks

☐ Normal

☐ Congenital anomaly

☐ Fetal macrosomia ☐ Symmetrical ☐ Asymmetrical

☐ Growth restriction

☐ Polyhydramnios

☐ Umbilical artery Doppler ☐ N ☐ Abs ☐ Rev

☐ Other

Estimated fetal weight (grams)

32– 36 weeks

Weight (kg)

Blood sugar control ☐ Good ☐ Moderate ☐ Poor

Treatment

☐ Diet & exercise ☐ Insulin ☐ Oral hypoglycaemics

HbA1c (gm%) .

Urine

Ultrasound at 32 – 36 weeks

☐ Normal

☐ Congenital anomaly

☐ Fetal macrosomia ☐ Symmetrical ☐ Asymmetrical

☐ Growth restriction

☐ Polyhydramnios

☐ Umbilical artery Doppler ☐ N ☐ Abs ☐ Rev

☐ Other

Estimated fetal weight (grams)

36 wks– Delivery

Weight (kg)

Blood sugar control ☐ Good ☐ Moderate ☐ Poor

Treatment

☐ Diet & exercise ☐ Insulin ☐ Oral hypoglycaemics

HbA1c (gm%) .

Urine

Ultrasound at 36wks to delivery

☐ Normal

☐ Congenital anomaly

☐ Fetal macrosomia ☐ Symmetrical ☐ Asymmetrical

☐ Growth restriction

☐ Polyhydramnios

☐ Umbilical artery Doppler ☐ N ☐ Abs ☐ Rev

☐ Other

Estimated fetal weight (grams)

Labour and Delivery

Date of admission to the hospital

Date of birth (dd/mm/yyyy)

Time of birth (hh:mm)

Gestation at birth (weeks+days)

Sex

☐ Male ☐ Female ☐ Indeterminate ☐ NK

Place of delivery ☐ LRI ☐ LGH

Mode of delivery

☐ Caesarean section before term

☐ Maternal reason ☐ Fetal reason

Reason

☐ Induction of labour before term

☐ Maternal reason ☐ Fetal reason

Reason

Delivery

☐ Normal vaginal delivery

☐ Forceps

☐ Ventouse

☐ Assisted breech

☐ Emergency CS

☐ NK

Length of first stage hours minutes

Length of second stage hours minutes

Date of rupture of membranes

Time (hh:mm)

Condition at birth

Apgar score

1 minute 5 minutes Status at delivery ☐ Live born ☐ Still born**Cord bloods**Cord bloods done ☐ Yes ☐ No

	Arterial	Venous
pH	.	.
pCO ₂	.	.
pO ₂	.	.
BE	.	.

CTG Monitoring

☐ Normal	☐ Early deceleration
☐ Fetal tachycardia	☐ Late deceleration
☐ Variable deceleration	☐ Fetal bradycardia
☐ Other	

Doppler abnormality	☐ Yes	☐ No	☐ N/K
Abnormal scalp pH	☐ Yes	☐ No	☐ N/K
Meconium present	☐ Yes	☐ No	☐ N/K
Other (specify) e.g. Fetal distress	☐ Yes	☐ No	☐ N/K

Postnatal history

Perineal tear

☐ 1st degree
☐ 2nd degree
☐ 3rd degreeEpisiotomy required ☐ Yes ☐ NoBlood sugar control ☐ Good
☐ Moderate
☐ PoorBlood Pressure (mmHg) /

Date of maternal discharge(dd/mm/yyyy)

 / / **Neonatal details**

Surname

First name

Unit number

Address

Postcode

Date of birth (dd/mm/yyyy)

 / /

Time of birth

 : Birth weight (grams) Head circumference (cms) . **Birth injury**

Shoulder dystocia	☐ Yes	☐ No
Clavicular fracture	☐ Yes	☐ No
Erb's palsy	☐ Yes	☐ No
Hypoxic ischaemic injury	☐ Yes	☐ No

Postnatal history

Baby stayed with mother on postnatal ward

☐ Yes ☐ No

Baby admitted to NNU

☐ Yes ☐ No

☐ Hypoglycaemia	☐ Hypocalcaemia
☐ Hyperbilirubinaemia	☐ Hypomagnesaemia
☐ Transient tachypnoea	☐ Polycythaemia
☐ Congenital Infection	☐ Congenital anomaly
☐ RDS	☐ PPHN
☐ MAS	☐ HIE
☐ Other	

1st feed after birth☐ Breast feed ☐ Bottle feed ☐ BothTime of first feed :

Blood sugar testingTime of the first blood sugar test Neonatal hypoglycaemia ☐ Yes ☐ No ☐ N/KFirst blood sugar < 2.0 : Time of lowest blood sugar : Action taken ☐ Breast feed given on the postnatal ward

(Can be more than one)

☐ Bottle feed given on the postnatal ward☐ Admitted to NNU nasogastric tube feed☐ Admitted to NNU 10% dextrose bolus☐ Admitted to NNU 10% dextrose infusion☐ Higher concentration of dextrose infusion☐ Other **Symptoms of hypoglycaemia**☐ Jitteriness ☐ Apnoea☐ Hypothermia ☐ Respiratory distress☐ Poor feeding ☐ Bradycardia☐ Lethargy ☐ Irritability☐ Hypotonia ☐ Seizure**Venous access required**☐ Canula ☐ Long line☐ UVC ☐ Central line**Complications of Venous access**☐ Difficult access, multiple attempts☐ Infection☐ Extravasation injury☐ NoneFeed intolerance ☐ Yes ☐ No

Date when on full enteral feeds

 / /

Date when on full sucking feeds

 / / **Feed on discharge**☐ Demand breast feeding☐ Demand bottle feeding☐ Breast & bottle feeds☐ Nasogastric tube feeds**Neonatal hypocalcaemia** ☐ Yes ☐ NoLowest Ca level . Treatment required ☐ Yes ☐ NoNo of days if treatment required **Respiratory distress**☐ Yes ☐ No☐ At birth ☐ LaterIntervention required ☐ Yes ☐ No☐ Oxygen requirement Days ☐ NCPAP Days ☐ Ventilation Days ☐ Required nitric oxide Days ☐ ECMO Days Highest FiO₂ in first 24hours after resuscitation Surfactant required ☐ Yes ☐ NoNo of doses of surfactant required Inotropic support required ☐ Yes ☐ No**Final diagnosis**☐ Persistent pulmonary hypertension☐ Pulmonary haemorrhage☐ Congenital pneumonia☐ Meconium aspiration syndrome☐ Other **Concerns with sepsis** ☐ Yes ☐ NoAntibiotics required ☐ Yes ☐ NoBlood culture result ☐ Positive ☐ NegativeLumbar puncture done ☐ Yes ☐ NoCSF culture ☐ Positive ☐ NegativeHighest CRP Chest X ray

Start date of antibiotics

 / /

End date of antibiotics

 / /

Neonatal hypomagnesaemia ☐ Yes ☐ No

Lowest Mg level .

Treatment required ☐ Yes ☐ No

No of days if treatment required

Congenital anomaly

Is the condition lethal?

☐ Yes ☐ No

Hypoxic ischaemic injury

☐ Yes ☐ No

Initial blood gas

☐ Arterial ☐ Venous ☐ Capillary

pH .

BE

Lactate

Clinical e/f encephalopathy ☐ Yes ☐ No

CFM started ☐ Yes ☐ No

CFM results ☐ Normal ☐ Abnormal

Cooling required ☐ Yes ☐ No

Neonatal seizures ☐ Yes ☐ No

Treatment required ☐ Yes ☐ No

Other system involvement ☐ Yes ☐ No

Cranial USS Date

☐ Normal ☐ Abnormal

Brain MRI Date

☐ Normal ☐ Abnormal

Polycythemia ☐ Yes ☐ No

Highest Hb .

Highest hematocrit (%)

Treatment required ☐ Yes ☐ No

Symptoms of polycythemia ☐ Yes ☐ No

Complication of polycythemia ☐ Yes ☐ No

Neonatal hyperbilirubinaemia ☐ Yes ☐ No
(above phototherapy line)

On day of life

Bilirubin above exchange transfusion line ☐ Yes ☐ No

Treatment required ☐ Yes ☐ No

☐ Single phototherapy

☐ Double phototherapy

☐ Multiple phototherapy

☐ Exchange transfusion

Number of days phototherapy treatment required

Baby's blood group

Direct coombs test ☐ Positive ☐ Negative ☐ N/K

Discharge details

Date of discharge from NNU Time of discharge

/ / :

Date of discharge from hospital Time of discharge

/ / :

Discharged to (name of hospital, home, death, etc)

☐ Home

☐ Cardiac care

☐ Specialist care

☐ Palliative care

☐ Surgical care

Readmission to the hospital

☐ Yes ☐ No

Date of readmission Time of discharge

/ / :

Reason for readmission

Date of discharge Time of discharge

/ / :

6.2 Appendix 2: C-peptide Study questionnaire

C-peptide Study																								
Surname First name Address Postcode Hospital number Date of birth (dd/mm/yyyy) Ethnicity <table style="width: 100%;"> <tr> <td>☐ White British</td> <td>☐ Asian Indian</td> </tr> <tr> <td>☐ White Irish</td> <td>☐ Asian Pakistani</td> </tr> <tr> <td>☐ White</td> <td>☐ Asian Bangladeshi</td> </tr> <tr> <td></td> <td>☐ Asian Sri Lankan</td> </tr> </table> Consanguineous Marriage ☐ Yes ☐ No Did the mother smoke during pregnancy ☐ Yes ☐ No Did the mother drink alcohol during pregnancy ☐ Yes ☐ No Did mother take recreational drug during pregnancy ☐ Yes ☐ No ☐ Cannabis ☐ Other	☐ White British	☐ Asian Indian	☐ White Irish	☐ Asian Pakistani	☐ White	☐ Asian Bangladeshi		☐ Asian Sri Lankan	Family History <table style="width: 100%;"> <tr> <td>Diabetes in family</td> <td>☐ Yes</td> <td>☐ No</td> </tr> <tr> <td>FH of hypertension</td> <td>☐ Yes</td> <td>☐ No</td> </tr> <tr> <td>FH of PIH</td> <td>☐ Yes</td> <td>☐ No</td> </tr> <tr> <td>Congenital anomalies</td> <td>☐ Yes</td> <td>☐ No</td> </tr> <tr> <td>Neonatal deaths</td> <td>☐ Yes</td> <td>☐ No</td> </tr> </table> Reason for neonatal death Diabetes in previous pregnancy ☐ Yes ☐ No ☐ NK Treatment ☐ Diet & exercise ☐ Insulin ☐ Oral hypoglycaemics Any neonatal complication of diabetes 	Diabetes in family	☐ Yes	☐ No	FH of hypertension	☐ Yes	☐ No	FH of PIH	☐ Yes	☐ No	Congenital anomalies	☐ Yes	☐ No	Neonatal deaths	☐ Yes	☐ No
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Medical problems during pregnancy☐ Diabetes ☐ Type I ☐ Type II

Treatment

☐ Pregnancy induced hypertension☐ Pre-eclampsia☐ Eclampsia

Treatment

Gestation at the time of OGTT + Wks + daysDiagnosis of GDM ☐ Yes ☐ NoFasting blood sugar . mmols/LPost prandial blood sugar . mmols/L

Treatment

☐ Diet & exercise ☐ Insulin ☐ Oral hypoglycaemics**36 wks- Delivery** Weight (kg) Blood sugar control ☐ Good ☐ Moderate ☐ Poor
Treatment☐ Diet & exercise ☐ Insulin ☐ Oral hypoglycaemicsHbA1c (gm%) . Urine **Ultrasound at 36wks to delivery**☐ Normal☐ Congenital anomaly☐ Fetal macrosomia ☐ Symmetrical ☐ Asymmetrical☐ Growth restriction☐ Polyhydramnios☐ Umbilical artery Doppler ☐ N ☐ Abs ☐ Rev☐ Other

Estimated fetal weight (grams)

28- 32 weeks Weight (kg) Blood sugar control ☐ Good ☐ Moderate ☐ Poor

Treatment

☐ Diet & exercise ☐ Insulin ☐ Oral hypoglycaemicsHbA1c (gm%) . Urine **Ultrasound at 28 - 32 weeks**☐ Normal☐ Congenital anomaly☐ Fetal macrosomia ☐ Symmetrical ☐ Asymmetrical☐ Growth restriction☐ Polyhydramnios☐ Umbilical artery Doppler ☐ N ☐ Abs ☐ Rev☐ Other

Estimated fetal weight (grams)

Labour and Delivery

Date of admission to the hospital

Date of birth (dd/mm/yyyy)

Time of birth (hh:mm)

Gestation at birth (weeks+days)

 +

Sex

☐ Male ☐ Female ☐ Indeterminate ☐ NKPlace of delivery ☐ LRI ☐ LGH**Mode of delivery**☐ Caesarean section before term☐ Maternal reason ☐ Fetal reason

Reason

☐ Induction of labour before term☐ Maternal reason ☐ Fetal reason

Reason

Delivery

☐ Normal vaginal delivery☐ Forceps☐ Ventouse☐ Assisted breech☐ Emergency CS☐ NKLength of first stage hours minutesLength of second stage hours minutes

Date of rupture of membranes

Time (hh:mm)

Condition at birth

Apgar score

1 minute 5 minutes Status at delivery ☐ Live born ☐ Still born**Cord bloods**Cord bloods done ☐ Yes ☐ No

	Arterial	Venous
pH	.	.
pCO ₂	.	.
pO ₂	.	.
BE	.	.

CTG Monitoring

☐ Normal ☐ Early deceleration
☐ Fetal tachycardia ☐ Late deceleration
☐ Variable deceleration ☐ Fetal bradycardia
☐ Other

Doppler abnormality ☐ Yes ☐ No ☐ N/K
Abnormal scalp pH ☐ Yes ☐ No ☐ N/K
Meconium present ☐ Yes ☐ No ☐ N/K
Other (specify)
e.g. Fetal distress ☐ Yes ☐ No ☐ N/K

Postnatal history

Perineal tear

☐ 1st degree
☐ 2nd degree
☐ 3rd degree

Episiotomy required ☐ Yes ☐ NoBlood Pressure (mmHg) /

Date of maternal discharge(dd/mm/yyyy)

 / / **Neonatal details**

Surname

First name

Unit number

Address

Postcode

Date of birth (dd/mm/yyyy)

 / /

Time of birth

 : Birth weight (grams) Head circumference (cms) . **Birth injury**

Shoulder dystocia ☐ Yes ☐ No
Clavicular fracture ☐ Yes ☐ No
Erb's palsy ☐ Yes ☐ No
Hypoxic ischaemic injury ☐ Yes ☐ No

Postnatal history

Baby stayed with mother on postnatal ward

☐ Yes ☐ No

Baby admitted to NNU

☐ Yes ☐ No

☐ Hypoglycaemia	☐ Hypocalcaemia
☐ Hyperbilirubinaemia	☐ Hypomagnesaemia
☐ Transient tachypnoea	☐ Polycythaemia
☐ Congenital Infection	☐ Congenital anomaly
☐ RDS	☐ PPHN
☐ MAS	☐ HIE
☐ Other	

1st feed after birth☐ Breast feed ☐ Bottle feed ☐ BothTime of first feed :

Blood sugar testingTime of the first blood sugar test Neonatal hypoglycaemia ☐ Yes ☐ No ☐ N/KFirst blood sugar < 2.0 : Time of lowest blood sugar : Action taken ☐ Breast feed given on the postnatal ward
(Can be more than one)☐ Bottle feed given on the postnatal ward☐ Admitted to NNU nasogastric tube feed☐ Admitted to NNU 10% dextrose bolus☐ Admitted to NNU 10% dextrose infusion☐ Higher concentration of dextrose infusion☐ Other **Symptoms of hypoglycaemia**☐ Jitteriness ☐ Apnoea☐ Hypothermia ☐ Respiratory distress☐ Poor feeding ☐ Bradycardia☐ Lethargy ☐ Irritability☐ Hypotonia ☐ Seizure**Venous access required**☐ Canula ☐ Long line☐ UVC ☐ Central line**Complications of Venous access**☐ Difficult access, multiple attempts☐ Infection☐ Extravasation injury☐ NoneFeed intolerance ☐ Yes ☐ No

Date when on full enteral feeds

 / /

Date when on full sucking feeds

 / / **Feed on discharge**☐ Demand breast feeding☐ Demand bottle feeding☐ Breast & bottle feeds☐ Nasogastric tube feeds**Neonatal hypocalcaemia**☐ Yes ☐ NoLowest Ca level . Treatment required ☐ Yes ☐ NoNo of days if treatment required **Respiratory distress**☐ Yes ☐ No☐ At birth ☐ LaterIntervention required ☐ Yes ☐ No☐ Oxygen requirement Days ☐ NCPAP Days ☐ Ventilation Days ☐ Required nitric oxide Days ☐ ECMO Days Highest FiO₂ in first 24hours after resuscitation Surfactant required ☐ Yes ☐ NoNo of doses of surfactant required Inotropic support required ☐ Yes ☐ No**Final diagnosis**☐ Persistent pulmonary hypertension☐ Pulmonary haemorrhage☐ Congenital pneumonia☐ Meconium aspiration syndrome☐ Other **Concerns with sepsis**☐ Yes ☐ NoAntibiotics required ☐ Yes ☐ NoBlood culture result ☐ Positive ☐ NegativeLumbar puncture done ☐ Yes ☐ NoCSF culture ☐ Positive ☐ NegativeHighest CRP Chest X ray

Start date of antibiotics

 / /

End date of antibiotics

 / /

Neonatal hypomagnesaemia ☐ Yes ☐ No

Lowest Mg level .

Treatment required ☐ Yes ☐ No

No of days if treatment required

Congenital anomaly

Is the condition lethal?

☐ Yes ☐ No

Hypoxic ischaemic injury

☐ Yes ☐ No

Initial blood gas

☐ Arterial ☐ Venous ☐ Capillary

pH .

BE

Lactate .

Clinical e/f encephalopathy ☐ Yes ☐ No

CFM started ☐ Yes ☐ No

CFM results ☐ Normal ☐ Abnormal

Cooling required ☐ Yes ☐ No

Neonatal seizures ☐ Yes ☐ No

Treatment required ☐ Yes ☐ No

Other system involvement ☐ Yes ☐ No

Cranial USS Date

☐ Normal ☐ Abnormal

Brain MRI Date

☐ Normal ☐ Abnormal

Polycythemia ☐ Yes ☐ No

Highest Hb .

Highest hematocrit (%)

Treatment required ☐ Yes ☐ No

Symptoms of polycythemia ☐ Yes ☐ No

Complication of polycythemia ☐ Yes ☐ No

Neonatal hyperbilirubinaemia ☐ Yes ☐ No
(above phototherapy line)

On day of life

Bilirubin above exchange transfusion line ☐ Yes ☐ No

Treatment required ☐ Yes ☐ No

☐ Single phototherapy

☐ Double phototherapy

☐ Multiple phototherapy

☐ Exchange transfusion

Number of days phototherapy treatment required

Baby's blood group

Direct coombs test ☐ Positive ☐ Negative ☐ N/K

Discharge details

Date of discharge from NNU Time of discharge

/ / :

Date of discharge from hospital Time of discharge

/ / :

Discharged to (name of hospital, home, death, etc)

☐ Home

☐ Cardiac care

☐ Specialist care

☐ Palliative care

☐ Surgical care

Readmission to the hospital

☐ Yes ☐ No

Date of readmission Time of discharge

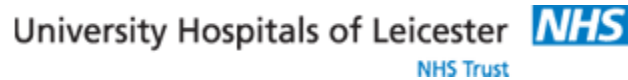
/ / :

Reason for readmission

Date of discharge Time of discharge

/ / :

6.3 Appendix 3: C-peptide Study, Patient information leaflet



PATIENT INFORMATION SHEET

C-peptide Study

Invitation

You are being invited to take part in a small research project called the C-peptide Study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. One of the members of our team will go through this information sheet with you and answer any questions you have. Please take time to read the following information carefully, and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

1. What is the purpose of the study?

Diabetes in pregnancy (Type I Diabetes, Type II Diabetes and Gestational Diabetes Mellitus - GDM) is a growing problem. About 2-5% of pregnancies are complicated by diabetes. It has been long known to increase the risk of problems for mother during pregnancy and delivery and for the baby immediately after birth. The most common problem is delivery of very large baby.

In addition to this, the current tests in pregnancy fail to identify about 40% of women with diabetes. These women with potentially undiagnosed diabetes and their babies do not get the extra attention that is part of the package of care planned for women known to have diabetes. These women are also at risk of delivering large babies.

It is only a proportion of these very large babies (whether from known diabetic mothers or non-diabetic mothers) who will develop complications such as low

blood sugar, poor feeding and need for admission to the baby unit. Not all large babies get problems as some babies are just meant to be big. However currently there is no available test to identify the at risk babies before the complications arise. Most of the complications in these babies are due to high levels of a hormone called insulin, which is released by the baby when their mother's blood sugar is high during the pregnancy. Insulin cannot be reliably measured but we can measure a related hormone called C-peptide, which gives an accurate estimate of how much insulin has been produced. In this study we want to see if the level of C-peptide in the umbilical cord blood (afterbirths that are discarded) at the time of birth tells us which large babies are at the highest risk of problems.

2. Why have I been chosen?

You have been chosen as your newborn baby fulfils the criteria for one of the study groups or control group stated below.

Study group 1: Term babies born to mothers with diabetes (any type) in pregnancy.

Study group 2: Large babies born to mothers without diabetes in pregnancy.

Control group: Term babies born with normal birthweight to mothers without diabetes in pregnancy

3. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form to confirm that you understand what is involved when taking part in this study. If you decide to take part you are free to leave the study at any time and without giving a reason.

A decision to withdraw at any time, or a decision not to take part, will not affect the quality of care you receive.

4. What will happen to me if I take part?

If you decide to participate in the study you will be asked to sign a consent form. This is to allow us to process the cord blood sample for C-peptide levels and to collect information about your pregnancy and your baby.

5. What do I have to do?

You only have to sign the consent form to give permission for your and your baby's participation in the study.

6. What is the drug / treatment / procedure* that is being tested?

After birth of the baby, the umbilical cord attached to the baby is clamped and cut off. Following this, the placenta delivers. The placenta and the attached umbilical cord are usually discarded after birth. The midwife taking care of you and your baby will have collected a teaspoon of blood from the discarded umbilical cord and stored it. If you decide to participate in the study and give your consent, we will analyse the stored cord blood sample for C-peptide level. We will also collect details of your pregnancy and your baby to see if any problems occur in the first day or so after birth. Just to be sure, we will collect information of any health problems in your baby up to the first 28 days after birth. We will compare C-peptide level to baby's outcome to see if C-peptide levels can be used to identify babies at risk of complications before they arise.

When the study is completed we will write to you to inform you about the study results.

If you decide not to participate in the study then the collected umbilical cord blood sample will be safely discarded. There will not be any difference in the care you or your baby receives.

7. What are other possible disadvantages and risks of taking part?

As a teaspoon of blood sample is collected from the afterbirths (umbilical cord and placenta) that are normally discarded after birth, there will NOT be any risk to the baby or the mother. There will not be any blood collection from the baby or the mother.

8. What are the possible benefits of taking part?

This study will not benefit you directly but the information we get regarding usefulness of C-peptide levels might help us in future to improve the management

of babies born to mothers with high blood sugars during pregnancy. It will help us to identify babies at risk of complications before they arise.

9. Who has reviewed the study?

This study was given favourable ethical opinion for conduct in the NHS by East Midlands - Leicester Research Ethics Committee.

10. What happens when the research study stops?

When the study stops we will write to you to let you know about the study results. We hope the information we get from this study will help us design a bigger study to confirm the usefulness of C-peptide levels. If the bigger study shows that the test is beneficial in identifying babies of mothers with diabetes and large babies at risk of complications before they arise then measures will be implemented for routine use of this test.

11. What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your question.

12. Will my taking part in this study be kept confidential?

Information about your participation in the study will be noted in your medical notes. A copy of the consent form signed by you will also be kept in your medical notes. After you consent to take part in this study, the information about your pregnancy and your baby will be collected and this will remain strictly confidential at all times. The information will be held securely on paper and electronically at your treating hospital and at University of Leicester under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the sponsor, who is not involved in the trial. Your records will only be available to people authorised to work on the study.

The information collected about you may also be shown to authorised people from the UK Regulatory Authority and Independent Ethics Committee; this is to ensure

that the study is carried out to the highest possible scientific standards. All will have a duty of confidentiality to you and your baby as research participants.

In line with Good Clinical Practice guidelines, at the end of the study, your data will be securely archived for a minimum of 21 years. Arrangements for confidential destruction will then be made.

13. Will the General Practitioner (GP) be informed?

We will not be informing your GP about your participation in the C-peptide Study.

14. Contact Details

Study Doctor

Name: Kamini Yadav

Tel. Number: 0116 252 5468

15. What will happen if I don't want to carry on with the study?

If at any stage you decide that you do not want to carry on with the study you can let us know and your decision will be respected. You will not be asked to give any explanation and your baby will be excluded from the study. Your decision not to continue in the study will not alter any clinical care that you or your baby receives.

If you withdraw from the study at a later date, unless you object, your data and cord blood sample results will remain on file and will be included in the final study analysis. If you object all records will be destroyed.

16. What will happen to the results of this clinical trial?

The results of the study will be available after it finishes and will usually be published in a medical journal and be presented at a scientific conference. The data will be anonymous and none of the patients involved in the trial will be identified in any report or publication. We will write to you to let you know the study results. Should you wish to see the publication, please contact the study doctor and it will be provided to you.

This is a brief summary of the C-peptide Study. If you have any questions please feel free to discuss them with your study doctor. If you decide you would like to take part then please read and sign the consent form. You will be given a copy of this information sheet and the consent form to keep. A copy of the consent form will be filed in your medical notes and one will be filed with the study records.

You can have more time to think this over if you are at all unsure.

Thank you for taking the time to read this information sheet and to consider this study.

6.4 Appendix 4: C-peptide Study, Consent form

 University of Leicester	University Hospitals of Leicester  NHS Trust	
CONSENT FORM		
Project: C-peptide Study (Pilot Study) Researcher: Dr. Kamini Yadav		
Maternal Details	Neonatal Details	
Name:	Name:	
Surname:	Surname:	
Hospital Number:	Hospital Number:	
Date of Birth:	Date of Birth:	
		Initial this box
1) I confirm that I have read and understand the information in the patient information leaflet (Version 1, dated 09/09/2011) for the above mentioned study. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily.		☐
2) I understand that my own and my baby's participation is voluntary and that participation in the study would not affect the medical care that I receive or my baby receives in any way.		☐
3) I am free to withdraw from the study at any time without giving reason, without my or my baby's medical care or legal rights being affected.		☐
4) I understand that the information relevant to the study about my and my baby's condition and hospital stay will be collected from the medical and nursing notes by the researcher. I understand that the information collected will be stored securely and kept confidential.		☐
5) I understand that relevant sections of my medical notes and/or study data may be looked at by responsible individuals from the study team, the sponsor, NHS Trust or from regulatory authorities where it is relevant to my taking part in the research. I give permission for these individuals to have access to my records up to 6 months after delivery.		☐
6) I give consent for the cord blood to be retained for the measurement of C-peptide levels.		☐
7) I give consent for my contact details to be safely retained by the research team to inform me about the results of the C-peptide Study.		☐
_____ Name of the mother	_____ Date	_____ Signature
_____ Name of the researcher	_____ Date	_____ Signature
C-peptide Study Version 2		Date: 02/01/2012

6.5 Appendix 5: C-peptide Study, Information document

C-peptide Study

Instructions for cord blood collection for C-peptide levels

Patient details:

_____ has consented to participate in the C-peptide Study.

(Consent form in the maternal medical notes).

When she delivers please follow the following steps:

1. Collect 5ml of venous cord blood as soon as possible after delivery of placenta (same way as venous cord blood is collected for venous cord gas – use normal syringe and not heparinised syringe). Transfer the collected sample in two orange top paediatric bottles provided.
2. Please keep the collected sample on **ice**.
3. Ensure that the bottles and request form are labelled correctly. Please check the following information on bottles and request form:
 - a. Name and surname for baby.
 - b. Baby's unit number (S – number).
 - c. Baby's date of birth and sex.
 - d. Date and time of sample collection.
4. Please call porter to take the sample to biochemistry laboratory. Samples need to reach the lab on ice within ½ an hour of sample collection.

C-peptide Study

Aim of the study:

To evaluate the potential of **cord blood C-peptide** to identify the infants of diabetic mothers and macrosomic infants of non-diabetic mothers at high risk of adverse neonatal outcomes.

Any woman with:

1. Type I Diabetes

2. Type II Diabetes

3. Gestational Diabetes Mellitus

4. Large for dates infant (Birthweight >4.5kg)

Is eligible to participate in the C-peptide Study.

If you are looking after any woman who is eligible for the study please contact:

Dr. Kamini Yadav on 07761800276.

Thank you for your support towards this study

C-peptide Study

When an eligible woman delivers please follow the following steps:

1. Collect 3-5ml of venous cord blood as soon as possible after delivery of placenta (same way as venous cord blood is collected for venous cord gas – use normal syringe and not heparinised syringe). Transfer the collected sample in two orange top paediatric bottles provided.
2. Please keep the collected sample on **ice**. Ice is kept in drug freezer on delivery suite.
3. Ensure that the bottles and request form are labelled correctly. Please check the following information on bottles and request form:
 - a. Name and surname for baby.
 - b. Baby's unit number (S – number).
 - c. Baby's date of birth and sex.
 - d. Date and time of sample collection.
4. Please call porter to take the sample to biochemistry laboratory. **Samples need to reach the lab on ice within ½ an hour of sample collection.**
5. Call the researcher **Dr. Kamini Yadav on 07761800276** and I shall let the biochemistry know about the sample.

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