Diabetes in Pregnancy:

Importance of Ethnicity, Timing of Onset and Modes of Prevention

Rochan Agha-Jaffar

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ABSTRACT

Gestational diabetes mellitus (GDM) is associated with adverse risks to mother and developing baby. The associated cost implications are substantial both in terms of resources required to adequately treat the condition as well as those needed to address secondary complications. Preventing GDM could therefore have benefits.

Factors contributing to GDM development and the associated complications, most notably fetal macrosomia, are explored. An analysis of 4562 women demonstrated variations in baseline maternal demographics, measures of glycaemia, fetal birth weight and adjusted birth weight centile across five ethnic groups. An ethnic group dependent effect on the interaction between glucose and fetal birth weight was identified that persisted following adjustment for maternal body mass index.

The impact of the degree of glycaemia and length of exposure to hyperglycaemia on risk is discussed. Unlike women with Type 1 diabetes, markers of glycaemic variability were not associated with fetal overgrowth in women with Type 2 diabetes. Length of exposure to glucose did adversely affect outcomes as illustrated by the results of a separate case control study of 200 pregnant women presented. However, despite identification and treatment of women who develop hyperglycaemia early in pregnancy, increased premature delivery rates and higher still birth rates were observed implicating the importance of preventing hyperglycaemia in pregnancy.

One of the principle risk factors identified for early hyperglycaemia, was a previous

pregnancy complicated by GDM. The final part of this thesis focuses on the rationale for an original trial designed to establish if metformin therapy, commenced early pregnancy, could prevent GDM recurring in women with previously affected pregnancies. While the impact of metformin in mitigating risk cannot be commented on, the low macrosomia rates and postnatal complication rates observed in this trial relative to pregnant women with previous GDM, continue to suggest the importance of addressing factors early in pregnancy.

DECLARATION OF ORIGINALITY

The author (Rochan Agha-Jaffar) performed the majority of the work presented in this thesis. Where appropriate, additional information from third parties has been referenced. Any collaboration and assistance provided was as follows:

Chapter 3:

Data analysed for the purpose of this Chapter was in part collected during the pre-gestational antenatal audit, which Dr Melina Kostoula, Endocrinology SpR contributed to.

Chapter 5:

Screening participants and blood sampling during study visits were performed with assistance from Mrs Cathy Turner (Research Nurse).

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CHAPTER 1: INTRODUCTION

1.1. Background

Gestational diabetes mellitus (GDM) is a common medical complication of pregnancy and is associated with increased risks to mother and baby. It is defined as "hyperglycaemia first detected in pregnancy that is not clearly overt diabetes" (1). The term was initially introduced to describe women with poor obstetric outcomes who had high glucose levels in subsequent pregnancies. Early diagnostic criteria were based on values that best predicted later development of maternal Type 2 Diabetes Mellitus (T2DM) (2). Since then understanding of the implications of hyperglycaemia in pregnancy have developed considerably. The mother whose pregnancy is complicated by hyperglycaemia in pregnancy has an increased risk of pre-eclampsia, caesarean section and development of T2DM later in life. The infant born to the mother with GDM is more likely to be macrosomic (birth weight ≥4000g) or large for gestational age (adjusted birth weight ≥90th centile) and encounter mechanical issues, physiological or metabolic abnormalities such as neonatal hypoglycaemia, respiratory distress syndrome, neonatal hyperbilirubinaemia (2-4). In the longer term, the infant exposed to in utero hyperglycaemia is at increased risk of obesity and insulin resistance in young adulthood.

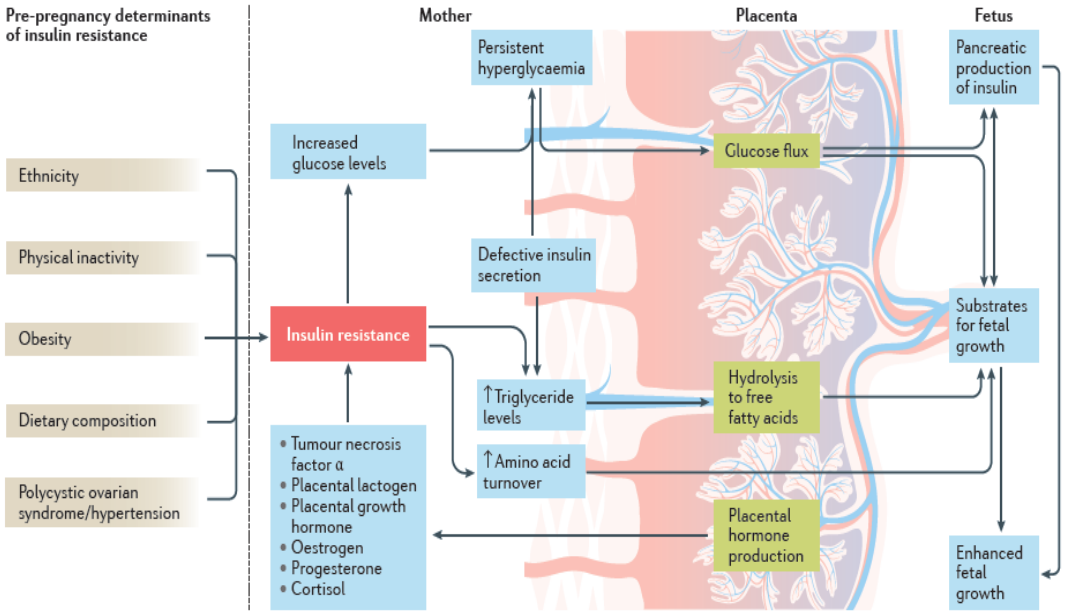
1.2. Epidemiology

The historic absence of universally agreed screening strategies and diagnostic criteria has rendered it difficult to establish the true incidence of GDM. In addition, prevalence varies considerably according to the ethnic group and geographical location examined and mirrors variations in prevalence of T2DM. Rates are thought to be highest in the Middle Eastern and North African region with a median estimate of 12.9% (range 8.4-24.5%) of pregnancies complicated by the condition and lowest in Europe, where the proportion of pregnancies affected is estimated at 5.8% (range 1.8-22.3%) (5). More recently, the World Health Organisation endorsed reductions to the glycaemic thresholds for diagnosing GDM. Adoption of these criteria has corresponded with incidences as high as 22% in some populations (6).

1.3. Pathophysiology

Pregnancy is characterised by an insulin resistance that increases with advancing gestation. The feto-placental unit is primarily responsible for driving this and while the mechanisms are not fully understood, placental production of tumour necrosis factor alpha, placental lactogen, growth hormone, and increased cortisol and progesterone levels are all thought to contribute (7, 8) (Figure 1). To maintain maternal normoglycaemia, beta-cell production of insulin increases (9). The resultant changes in maternal carbohydrate and lipid metabolism ensure the continuous and adequate delivery of substrate required for fetal development.

Figure 1: Factors contributing to maternal resistance and fetal growth



R Agha-Jaffar *et al* Nature Reviews Endocrinology (10)

Women unable to adapt to these pregnancy-induced physiological changes develop GDM. A significant overlap exists in the pathophysiology of GDM and T2DM such that GDM could be seen to reflect an early stage of T2DM expressed under the conditions of pregnancy. Consistent with this is the observed increased rate of progression to T2DM in those with a history of GDM (11). An early prospective study supporting this was conducted in Boston by O'Sullivan: 49.4% women who had pregnancies complicated by GDM developed T2DM within 22-28 years compared to an incidence rate of 7% in parous women without a history of GDM (12). A meta-analysis of 28 studies confirmed this increased risk of T2DM and demonstrated a cumulative incidence ranging from 2.6% to over 70% in women that were assessed anywhere between 6 weeks and 28 years postpartum (13).

T2DM is a heterogeneous condition with an aetiology relating to an individual's genetic predisposition interacting with both intrauterine and adult environmental factors (14). An insulin resistance that is present through adult life existing prior to the development of hyperglycaemia is one of the factors contributing to its pathophysiology (15). Subtle defects in insulin secretion, which include abnormal pulsatile insulin profiles, a higher proportion of immature insulin, and later adequate first phase insulin secretion failure are associated with the development of the initial postprandial hyperglycaemia observed in the early stages of T2DM (14). Eventually, beta cell failure is more complete and there is defective second phase insulin secretion leading to fasting hyperglycaemia.

In keeping with the pathogenesis of T2DM, the majority of women who develop GDM exhibit defective beta cell function leading to initial postprandial, and later fasting, hyperglycaemia (16) (17). Higher levels of insulin resistance either in the pre-conception period or early in pregnancy may accelerate this process (18). Subsequent increases in glucose levels provide substrates for enhanced fetal growth. This is in part stimulated by fetal hyperinsulinaemia. Elevated amino acid levels and non-esterified fatty acid concentrations contribute further to the development of macrosomia (19).

1.4. Risk Factors For Developing Gestational Diabetes

A large degree of heterogeneity exists across the epidemiological studies designed to establish the risk factors associated with developing GDM (20). Despite this certain consistent predictors have emerged, which mirror those associated with an increased risk of developing T2DM in non-pregnant adults (Table 1). Non-modifiable risks include non-Caucasian ethnicity, advancing maternal age, underlying polycystic ovary syndrome (PCOS), previous pregnancies complicated by GDM and a family history of T2DM.

Table 1: Risk factors associated with developing gestational diabetes

Non modifiable risk factors	Modifiable risk factors
Ethnicity	Dietary factors Carbohydrate intake; fat; cholesterol; haem iron; processed meats; eggs
Maternal age	Levels of physical activity
Previous pregnancies complicated by gestational diabetes	Pre-gravid obesity or excess maternal adiposity
Family history Type 2 Diabetes Mellitus	
Polycystic ovary syndrome	

Well-established modifiable risk factors include pre-gravid or early pregnancy excess adiposity and obesity (21). The evidence regarding the impact of sedentary lifestyle or physical inactivity, excess gestational weight gain and dietary composition on glucose tolerance varies depending on the study. Data exist demonstrating that a

higher intake of fat, cholesterol, haem iron, processed meats or eggs in either the pre-conception period or in early pregnancy, increases GDM risk: in contrast, a lower intake of carbohydrates mitigates this risk (22). Physical activity has also been shown to be of benefit with moderate physical activity reducing GDM risk by 55% in the pre-conceptual period and by 25% in early pregnancy (23).

1.5. The Clinical Problem

Treatment has consistently been shown to reduce the immediate adverse outcomes associated with the development of hyperglycaemia in pregnancy, such as shoulder dystocia and the requirement for neonatal intensive care (24) (25). In the first instance dietary modification and a moderate increase in physical activity are advised (26). When this fails to adequately achieve target glycaemic control, metformin and/or insulin are recommended depending on the clinical situation.

Despite effective treatment, the economic burden related to the condition remains substantial with one model predicting a 34% increase in the overall cost to the care of women affected by GDM compared to those who are not affected (27). In addition, longitudinal studies have demonstrated that risks of childhood obesity and metabolic dysfunction persist despite effective treatment (28). When considering these factors, preventing hyperglycaemia in pregnancy could potentially have considerable benefits.

The next section of this chapter examines the evidence relating to both lifestyle prevention strategies and dietary supplementation in preventing GDM in women with and without risk factors. Direct comparison of the interventions is problematic. This is in part due to the heterogeneity of the trials. Considerable variations in study population demographics exist as do widespread variations in the methodologies used to screen for and diagnose GDM, rendering it difficult to draw firm conclusions (10). Few trials have been adequately powered and assessing changes to lifestyle is in itself challenging. In the studies able to determine adherence to protocols, compliance was variable. In addition to consider the effect on GDM risk reduction, the impact of the interventions of factors beyond glycaemia will be considered in more detail below.

1.6. Lifestyle Intervention Strategies

1.6.1. Women with no defined risk factors

Only one trial has investigated the potential benefits of modifying diet to improve GDM risk in a randomly selected cohort of women (Table 2). The "Low-GI Diet in Pregnancy" study randomised 62 pregnant women to either a low glycaemic index diet (LGI) or to a high fibre, moderate to high glycaemic index diet (HGI) (29). No differences were demonstrated in either incidence of GDM or fetal birth weight. The proportion of infants born large for gestational age (LGA, birth weight ≥ 90th centile) in the LGI group was significantly lower indicating a potential benefit associated with the diet: however, this finding should be interpreted with caution given that the study was not adequately powered for this outcome.

Trials investigating the impact of physical activity programmes in reducing GDM risk in women with no defined risk factors have yielded conflicting results (Table 2). In a trial conducted in Spain, 272 women were randomised to either three supervised exercise sessions per week or to routine antenatal care (30).

Women in the intervention arm were more likely to adhere to gestational weight gain guidelines (as defined by the Institute of Medicine) and the intervention was associated with a significant reduction in GDM incidence (National Diabetes Data Group criteria) (31, 32). In a trial conducted in Norway, 855 women between 18 and 22 weeks gestation were randomised to either routine antenatal care or to a 12-week program consisting of three exercise sessions per week (33). A physiotherapist supervised one of these sessions; the second two were self-directed. No differences were recorded in the incidence of GDM as defined by the 1999 WHO criteria (34).

The reasons for the conflicting results are likely multifactorial: all the sessions were supervised in the Spanish trial thereby potentially impacting on compliance, which was documented to be higher compared to the Norwegian one (80% versus 55%). Furthermore, no differences were recorded in gestational weight gain in the latter trial. In contrast, women in the Spanish intervention group gained significantly less gestational weight than the control arm.

Table 2: Summary of the randomised-controlled trials investigating the effects of dietary, physical activity and combined lifestyle interventions on reducing gestational diabetes and adverse materno-fetal outcomes in women with no defined risk factors

Trial	Population Characteristics	Intervention and GDM Diagnostic Criteria	Group Characteristics			Outcomes	
				n	BMI (kg/m²)	% GDM cases	Significant Outcomes
"Low GI in pregnancy study" Moses *et al* ***(29)***	N=62 No control group **GA:** 12-16 weeks **Country:** Australia	**Randomised to either:** LGI diet **OR** High fibre – low sugar (HGI) diet. 5 dietary education sessions. **GDM criteria:** ND	LGI	32	**24.4 [±0.7]***	0.0	Following results significantly lower in LGI group: 1. GI index 2. Proportion infants born LGA 3. Neonatal ponderal index [III]
			HGI	30	**26.6 [±0.9]***	3.3	
Cordero *et al* ***(30)***	N=272 **GA:** 10-12 weeks **Country:** Spain	2 supervised sessions in gym (60 minutes moderate intensity) and 1 supervised session pool based activity (50 minute moderate intensity) **GDM criteria:** NDDG [I]	I	101	22.5 [±3.2]	**1.0***	Significant reduction in proportion of women with excess gestational weight gain in intervention group (22.8% versus 34.8%, p=0.040) [II].
			C	156	23.6 [±4.0]	**8.8***	
Stafne *et al* **(33)**	N=855 **GA:** 18-22 weeks **Country:** Norway	12 week exercise program (3 days/ week). 1 session supervised by physiotherapist; 2 home-based un-supervised sessions. **GDM criteria:** WHO (1999) [III]	I	429	24.7 [±3.0]	7	
			C	239	25.0 [±3.4]	6	
Asbee *et al* (35)	N=100 **GA:** 6-16 weeks **Country:** USA	Focused dietary counselling, unsupervised moderate-intensity exercise (3-5 times/ week) and education regarding target weight gain (IOM[II]), weighed at each visit: if not in target, lifestyle reviewed. **GDM criteria:** ND	I	57	25.5 [±6.0]	NSD	Significantly less GWG in intervention arm: 13.0 ±5.68kg versus 16.1 ±7.05 kg, p=0.01. GDM incidence similar on sub-analysis of those who adhered to IOM guidelines.
			C	43	25.6 [±5.1]	NSD	

Footnotes:
Continuous data are expressed as mean [± standard deviation]. Results denoted in bold and marked with an * are significant (p<0.05).

Abbreviations: ***BMI*** Body mass index (at study inclusion); ***GDM*** Gestational diabetes mellitus; ***GWG*** Gestational weight gain (defined as gain in weight from early pregnancy/ at study inclusion, to 36 weeks gestation/ prior to delivery); ***GI*** Glycaemic Index; ***GA*** Gestational Age (at inclusion); ***LGI*** Low Glycaemic Index; ***HGI*** High Glycaemic Index; ***LGA*** Large for gestational age (birthweight ≥90th centile); ***I*** Intervention group; ***C*** Control group; ***NSD*** No Significant Difference; ***GWG*** Gestational weight gain (defined as gain in weight from early pregnancy/ at study inclusion, to 36 weeks gestation/ prior to delivery).
I. National Diabetes Data Group (NDDG) criteria (32). II. As defined by Institute of Medicine Guidelines (IOM) (31). III. World Health Organisation (WHO) criteria 1999 (34). III Ponderal Index = (fetal weight (g) x 100)/ (fetal length (cm))^3

A combined approach to diet and physical activity in minimising gestational weight gain and preventing GDM has been evaluated by one randomised controlled trial (35). This study was powered to detect gestational weight gain as the primary outcome.

Though the intervention, which consisted of focused dietary counselling, encouragement to increase physical activity and advice regarding appropriate weight gain, was associated with significantly less gestational weight gain, no differences were recorded in the incidence of GDM.

1.6.2. Women With Risk Factors

Obesity

The relationship between maternal BMI and GDM risk is well described. An increase in category of early pregnancy BMI is associated with an increased odds ratio (OR) of developing GDM: BMI 25-30kg/m^2 OR 1.86, BMI 30-35kg/m^2 OR 3.34 and BMI ≥35kg/m^2 OR 5.77 (21). Excess gestational weight gain has also been implicated in GDM risk (36). Indeed, maternal weight has been identified as the strongest predictor of macrosomia, emphasising the detrimental effect of an enhanced insulin resistant state despite normal glucose tolerance (37, 38). In view of this, and the increasing incidence of obesity amongst women of childbearing age, considerable focus has been placed on preventing associated adverse outcomes.

In a feasibility study conducted in Denmark, 50 obese women without diabetes, who were of White ethnicity, were randomised to either active dietary intervention (consisting of 10 one hour educational sessions with a trained dietician) or to standard dietary advice (Table 3) (39). No women were diagnosed with GDM in the intervention arm: 10% were diagnosed in the control group and were subsequently excluded from the analysis given the potential for confounding (significance not commented on).

Since this, two randomised controlled trials have demonstrated reductions in GDM risk with dietary modification.

In a study in the USA powered to detect gestational weight gain as the primary outcome, 257 obese women were randomised to either routine antenatal care or to an active nutritional and behavioural intervention from 12 weeks gestation (40). Those in the intervention arm gained significantly less weight during pregnancy and a reduction in GDM incidence was noted. This reduction reached significance after a post-hoc analysis of those who adhered to the programme.

In an Australian trial that was powered to detect a difference in GDM incidence as the primary outcome, 132 overweight and obese pregnant women were randomised to either a four-step multidisciplinary antenatal care approach or to routine antenatal care (41). Those in the intervention arm attended specialised antenatal clinics where they were weighed and reviewed by an obstetrician, food technician and a clinical psychologist. The intervention was associated with significant reductions in mean gestational weight gain and GDM incidence. In this study, there was a high background incidence of GDM (29% in the control group) indicating that this was a particularly at risk population.

Table 3: Summary of the randomised-controlled trials investigating the effects of dietary, physical activity and combined lifestyle interventions in reducing gestational diabetes and adverse materno-fetal outcomes exclusively in overweight and obese pregnant women

Trial	Population Characteristics	Intervention and GDM Diagnostic Criteria	Group Characteristics			Outcomes	
				N	BMI (kg/m^2)	% GDM cases	Significant Outcomes
Wolff *et al* **(39)**	N= 50 BMI ≥30kg/m^2 and Caucasian **GA:** 12-18 weeks **Country:** Denmark	10 x1 hour dietary consultations with aim of restricting GWG to 6-7kg. **GDM criteria:** Not defined	I	23	34.9 [±4]	0	Reduction in GWG observed in the intervention arm: 6.6[±5.5] kg versus 13.3 [±7.5] kg, p=0.002.
			C	27	34.6 [±3]	10	
Thornton *et al* **(40)**	N = 257 BMI ≥30kg/m^2 **GA:** 12-28 weeks **Country:** USA	Active nutritional and behavioural intervention: 18-24kcal/kg from inclusion until delivery **GDM criteria:** Not defined	I	116	37.4 [±7.0]	9.5	Intervention associated with 1. Reduced GWG: 5.0 [±6.8] kg versus 14.1 [±7.3] kg, p<0.001. 2. Reduced GDM incidence on sub-analysis of group that complied with intervention: 2.2% versus 34.6%, p<0.01.
			C	116	38.2 [±7.5]	16.4	
Quinlivan *et al* **(41)**	N=124 BMI ≥ 25kg/m^2 **GA:** Not specified **Country:** Australia	4 step approach; continuity obstetric care provider, regular weight assessment, food technician and clinical psychology input **GDM criteria:** WHO (1999) [I]	I	63	58% Obese [II]	**6.0***	Reduction in GWG observed in the intervention arm: 7.0 [±0.7] versus 13.8 [±0.7] kg, p<0.001.
			C	61	51% Obese [II]	**29.0***	
"LIMIT Trial" Dodd *et al* (42)	N=2212 BMI ≥25kg/m^2 **GA:** 10-20 weeks **Country:** Australia	Individualised meal plans provided by dietician with advice regarding lifestyle at six different time points. **GDM diagnostic criteria:** 75g OGTT: FPG 5.5mmol/L, 2 hour 7.8mmol/L	I	1108	31.0 (28.1-35.9)	14	Lower rates macrosomia (>4000g) in intervention group (15% versus 19%, p=0.04)
			C	1104	31.1 (27.7-35.6)	11	
Callaway *et al* (43)	N= 50 BMI ≥30kg/m^2 **GA:** ≤12 weeks **Country:** Australia	Individualised exercise programme. Targeted energy expenditure 900kcal/ week. No supervised sessions. Monthly physiotherapy review. **GDM criteria:** ADIPS [III]	I	25	36% ≥35kg/m^2	16	
			C	25	36% ≥35kg/m^2	23	

Trial	Population Characteristics	Intervention and GDM Diagnostic Criteria	Group Characteristics			Outcomes	
				N	BMI (kg/m²)	% GDM cases	Significant Outcomes
"Fit for 2" Trial Oostdam *et al* (44)	N= 121 BMI ≥25kg/m² **And** 1 or more of: previous GDM or macrosomic infant; 1st degree relative T2DM **Country:** Finland	60 minutes aerobic/strength work twice per week from 15 weeks gestation until 6 weeks postpartum. All sessions supervised. **GDM criteria:** Not defined	I	49	33.0 [±3.7]	14.6	
			C	52	33.9 [±5.6]	21.6	
" UPBEAT" trial Poston *et al* **(45)**	N=1555 BMI ≥30kg/m² **GA:** 15-19 weeks. **Country:** UK	Weekly health trainer sessions for 8 weeks. Diet and physical activity addressed. **GDM criteria:** 5th IADPSG [IV]	I	783	36.3 [±5.0]	25%	Intervention associated with 1. Reduced GWG: 7.19 [±4.6] kg versus 7.76 [±4.6] kg, p=0.041 and 2. Increased incidence NNH [4 vs 2%, p=0.02].
			C	772	36.3 [±4.6]	26%	
"RADIEL" Trial Koivusalo *et al* **(46)**	N=293 Previous GDM +/-BMI ≥30kg/m² **GA:** <20 weeks gestation **Country:** Finland	3 lifestyle sessions provided targeting 1. No weight gain in 1st + 2nd trimesters and 2. ≥150 minutes moderate intensity activity/ week. **GDM criteria:** HAPO [V].	I	144	32.3 [±4.9]	13.9	Significant reduction in incidence GDM and GWG following adjustment for confounders.
			C	125	32.6 [±4.5]	21.6	
Mc Giveron *et al* **(47)**	N=178 BMI ≥35kg/m² **GA:** 16-18 weeks **Country:** UK	2-4 weekly educational sessions for healthy diet and maintaining physical activity 3-times/ week. **GDM criteria:** Not defined	I	89	38.4 [±3.2]	NSD	Reduced GWG in intervention group: 4.5 [±4.6] kg versus 10.3 [±4.4] kg, p<0.001.
			C	89	39.4 [±4.1]	NSD	
Simmons *et al* (48)	N=150 BMI ≥29kg/m² **GA:** <20 weeks **Countries:** 9 European countries	Randomisation to one of 3 arms: Healthy eating (HE), physical activity (PA) or HE+PA. Interventions were delivered by 5 face to face and 4 optional telephone coaching sessions. GWG<5kg targeted.	HE	50	34.8 [±5.9]	28	Significant reductions in GWG and fasting glucose demonstrated in HE versus PA group: 3.5±3.9 versus 5.2±3.1kg/m², p=0.03 and 4.3±0.4 versus 4.6±0.4mmol/L, p=0.01.
			PA	50	34.5 [±4.5]	42	
			HE+ PA	50	34.1 [±4.7]	31	
Renault *et al* **(49)**	N=425 BMI ≥30kg/m² **Country:** Denmark	Randomised to one of 3 arms**:** Physical activity + diet (PA + D), PA alone or routine care (C). **PA:** Pedometer to assess step count on 7 consecutive /month (target 11 000). **D:** 2-weekly review regarding hypo-caloric diet (1200-1675kcal) and target GWG (<5kg). **GDM criteria:** Not defined	PA+ D	142	34.4 [±4.2]	3.8	Reduction in 1. GWG across the groups: 8.6 (9.6-34.1)kg, 9.4 (-3.4-28.2)kg and 10.9 (-4.4-28.7)kg, p=0.1. 2. Emergency/ unplanned caesarean-section rate in women randomised to PA+D: 11%, 22% and 24%, p=0.015.
			PA	142	34.1 [±4.4]	1.6	
			C	141	33.7 [±3.5]	5.2	

Trial	Population Characteristics	Description of intervention	Group Characteristics			Outcomes	
				N	BMI (kg/m^2)	% GDM cases	Significant Outcomes
Vinter *et al* **(50)**	N=304 BMI 30-45 kg/m^2 **GA:** 10-14 weeks **Country:** Denmark	**Intervention with aim of limiting GWG to 5kg:** 1. 4 dietary sessions.. 2. 1 exercise session/ week with physiotherapist: encouraged moderate physical daily activity (30-60mins). **GDM criteria:** 75g oral glucose tolerance test (OGTT) 120 minute value ≥9.0mmol/L.	I	150	33.4 [31.7-36.5]	6.0	Reduction in GWG in intervention group: 7.0 (4.7-10.6) kg versus 8.6 (5.7-11.5) kg, p=0.01.
			C	154	33.3 [31.7-36.9]	5.2	
Harrison *et al* **(51)**	N=228 **Entry criteria:** 1. BMI ≥25kg/m^2 2. Risk of GDM **GA:** 12-15 weeks **Country:** Australia	Lifestyle sessions at 4 points provided by health coaches to address healthy dietary patterns and physical activity. **GDM criteria:** ADIPS [III]	I	121	30.4 [±5.6]	22.3	Reduction in GWG in intervention arm: 6.0 [±2.8] kg versus 6.9 [±3.3] kg, p<0.05.
			C	107	30.3 [±5.9]	32.7	
Hui *et al* **(52)**	N=116 **GA:** <20 weeks gestation Categorised by BMI: 1. Groups I1/ C1: ≤24.9kg/m^2 2. Group I2/ C2: ≥25.0kg/m^2 **Country:** Canada	1. Physical activity: attended either community based weekly exercises or used DVD (30-45 minute sessions, 3-5 times/week). 2. Dietary intervention: Individual dietary sessions at baseline and 2 months later. **GDM criteria:** CDA [VI]	I1	30	21.6 [±2.2]	0	Group 1: Women in the intervention arm were significantly more likely to achieve target GWG (37% versus 10%, p=0.03) as defined by the IOM guidelines [VII].
			C1	27	22.6 [±1.9]	0	
			I2	29	29.5 [±5.1]	4	
			C2	27	29.7 [±1.3]	10	

Footnotes: Continuous data are expressed as mean [± standard deviation] or median [interquartile range]. Results denoted in bold and marked with an * are significant (p<0.05).

Abbreviations: ***BMI*** Body mass index (at study inclusion); ***GDM*** Gestational diabetes mellitus; ***GWG*** Gestational weight gain (defined as gain in weight from early pregnancy/ at study inclusion, to 36 weeks gestation/ prior to delivery); ***GA*** Gestational Age (at inclusion); ***I*** Intervention group ***C*** Control group; ***OGTT*** Oral Glucose Tolerance Test; ***FPG*** Fasting Plasma Glucose; ***T2DM*** Type 2 Diabetes Mellitus, ***NNH*** Neonatal hypoglycaemia; ***NSD*** No significant difference.

I. World Health Organisation (WHO) criteria 1999 (34). II. BMI ≥25 kg/m^2. III. Australasian Diabetes in Pregnancy Society (ADIPS) criteria (53). IV. Criteria proposed by the 5th International Association of Diabetes and Pregnancy Study Groups (IADPSG) (54). V. Criteria adapted from the Hyperglycaemia and Adverse Pregnancy Outcomes Study (HAPO) [2]. VI. Canadian Diabetes Association (CDA) diagnostic criteria (55). VII Institute of Medicine (IOM) Guidelines (31)

A meta-analysis conducted in 2012 evaluated the effect of life-style interventions on pregnancy-related outcomes (56). When specifically looking at dietary intervention and GDM risk, the authors analysed the results from the last three trials. They determined that dietary intervention in any form resulted in a 61% risk reduction in GDM (RR 0.39, 95% CI 0.23-0.69, I^2 21%). However, since this meta-analysis, the findings from the LIMIT trial have been published (42). In this trial, 2212 overweight women were randomised to receive either dietary advice with individualised meal plans, or to routine antenatal care. No differences were detected in gestational weight gain, GDM incidence or in the proportion of infants born large for gestational age (≥90^{th} centile). The trial did however demonstrate significant reductions in the proportion of infants born macrosomic (birth weight ≥4000g) to the intervention group. This is an important consideration given that this intervention could be easier to replicate in the wider antenatal setting compared to the more intensive strategies employed in the trials demonstrating reductions in GDM incidence.

Trials investigating the impact of exercise have been unsuccessful in demonstrating a reduction in GDM incidence (Table 3). A feasibility study conducted in Australia targeted an energy expenditure of 900kcal per week using individualised exercise programmes in 50 obese pregnant women (43). Increases in physical activity in the intervention arm were demonstrated by 28 weeks gestation. While this in turn was associated with reduced fasting glucose and fasting insulin levels at 36 weeks gestation, no differences were observed in insulin resistance or GDM incidence as defined by the Australasian Diabetes in Pregnancy Society criteria (53). In the FitFor2 Trial, 121 women with an early

pregnancy BMI ≥25kg/m^2 and a second risk factor for developing GDM, were randomised to either attending two 60-minute group exercise sessions per week or to no lifestyle intervention (44). There were no significant differences in measures of glycaemia (fasting plasma glucose and HbA1c), insulin sensitivity or GDM incidence between the two groups. The background incidence of GDM in the control groups in both these trials (21.6% and 23% respectively) indicates that these cohorts were at particular risk. By nature of being overweight or obese, the women recruited were more likely to have entered pregnancy with higher levels of insulin resistance. The interventions may not have been sufficient to overcome this, particularly in the context of the profound increase in insulin resistance associated with pregnancy. Another factor to consider is that in the FitFor2 study, the required sample size was not achieved and only 16% of the women attended at least 50% of the exercise sessions, rendering it difficult to draw any firm conclusions from the data.

The two trials that have investigated combined lifestyle approaches in obese pregnant women and that were powered to detect reduction in GDM as the primary outcome, have yielded conflicting results (Table 3). In the UPBEAT trial conducted in the UK, 1440 obese pregnant women were randomised to either routine antenatal care or to lifestyle intervention (45). Women in the intervention arm were reviewed weekly, either individually or in group-led sessions, for a total of eight weeks from study inclusion. Recommended dietary intake and physical activity were discussed. Despite self-reported improvements in the glycaemic index of foods and the level of physical activity achieved, as well as a reduction in gestational weight gain in the intervention

arm, no differences were demonstrated in GDM incidence. In contrast, the RADIEL trial, a trial conducted in Finland, demonstrated that input from research midwives and dieticians at three different time points throughout pregnancy, reduced the risk of developing GDM following adjustment for confounding factors (age, pre-pregnancy BMI, a history of previous GDM and gestational age) (46). Women in the intervention arm were encouraged to maintain their weight in the first two trimesters and to engage in 150 minutes of moderate level physical activity. Differences in the baseline demographics of the UK and Finnish cohorts might account for the Finnish strategy being successful in reducing GDM: mean baseline BMI was lower compared to the British trial (32.3kg/m^2 versus 36.3kg/m^2). Ethnicity may also have impacted on the results: while not commented on in the Finnish trial (presumably therefore most women were of white European origin), 40% of those in the British trial were of non-European ethnicity. It is also important to note that the glucose threshold for diagnosing GDM was lower in the UPBEAT trial.

Four other trials have evaluated the benefits of combined lifestyle interventions in obese pregnant women and while all have been associated with reduced gestational weight gain, none have improved GDM risk (Table 3). However, two have shown positive effects on alternative materno-fetal outcomes indicating a potential benefit associated with the intervention even in the absence of improving glycaemia. One trial demonstrated an improvement in hypertensive disorders in pregnancy: "The Bumps and Beyond" intervention program randomised 178 obese women (BMI ≥35kg/m^2 at inclusion) to either routine antenatal care or to 2-4 weekly educational sessions provided by health advisors and specialist midwives. During these sessions,

the participants were encouraged to engage in physical activity at least three times per week and to follow dietary advice (47). The intervention was associated with a lower incidence of hypertensive disorders. The TOP study, a study conducted in Denmark randomised 452 obese women to one of three groups: combined physical activity and dietary intervention, intervention with physical activity alone or to routine antenatal care (49). Both the dietary and physical activity interventions were delivered via educational sessions with the encouragement to maintain a hypo-caloric diet and to increase daily physical activity as appropriate: those randomised to the combined intervention group had a significantly lower rate of unplanned or emergency caesarean-section rates.

Two remaining trials have evaluated combined lifestyle measures in obese pregnant women (Table 3). The LiP trial demonstrated a significant increase in median fetal birth weight with an intervention that consisted of one session with a physiotherapist and self-directed physical activity for 30-60 minutes/day (50). The intervention was not associated with an increase in the proportion of infants born LGA. The authors postulated that this unexpected finding could relate to improved placental function in those randomised to the intervention arm. A trial conducted in Australia that randomised women to either standard antenatal care or to lifestyle sessions provided at four time points throughout pregnancy found no improvements in materno-fetal outcomes (51).

One trial conducted in Canada, compared methodologies in 116 women according to category of BMI (Table 3) (52). The intervention in this trial combined two dietary

educational sessions with either a home-based DVD exercise programme or community-based weekly exercise programmes. Women with an early pregnancy BMI within the normal range gained less gestational weight in the intervention arm and the mean fetal birth weight born to this sub-group was significantly lower: no differences were recorded in outcomes in women with a suboptimal BMI.

Other at risk groups

The National Institute for Health and Clinical Excellence (NICE) in the UK recommends screening all pregnant women to ascertain if they have risk factors for developing GDM (26). In addition to the recommendation that women with a BMI exceeding 30kg/m^2 are tested for GDM, women with a family history of T2DM, a previous pregnancy complicated by GDM and a previous delivery of a macrosomic infant are advised to undergo a 75g oral glucose tolerance test (OGTT).

The effects of either dietary intervention or lifestyle counseling in reducing the incidence of GDM in women with the aforementioned risk factors have been evaluated in three randomised controlled trials (Table 4).

Table 4: Summary of the randomised-controlled trials investigating the effects of dietary, physical activity and combined lifestyle interventions on reducing gestational diabetes and adverse materno-fetal outcomes

Trial	Population Characteristics	Description of Intervention	Group Characteristics			Outcomes	
				N	BMI (kg/m^2)	% GDM cases	Significant outcomes
NELLI Trial Luoto *et al* (57)	N=399 **Entry criteria:** 1. 8-12 weeks gestation 2. One risk factor for GDM[I] **Country:** Finland	Lifestyle educational sessions at 5 points regarding achieving 800 MET minutes/ week and dietary counseling. **GDM criteria:** 5th IADPSG [II]	I	219	26.3 [±4.9]	15.8	Fewer infants born LGA in intervention arm (12.1 versus 19.7, p=0.042).
			C	180	26.4 [±4.3]	12.4	
ROLO Study Walsh *et al* (58)	N=800 **Entry criteria:** 1. Previous macrosomic infant (≥4000g) **Country:** Ireland	Education regarding low GI diet in one 2hr session at 15 weeks. Meetings with dietician at 28 and 34 wks. **GDM criteria:** Carpenter-Coustan [III]	I	383	26.8 [±5.1]	2.0	Significantly less GWG intervention group: 12.2 ±4.4kg versus 13.7 ±4.9kg, p=0.01.
			C	398	26.8 [±4.8]	2.0	
The GI Baby 3 Study Markovich *et al* (59)	N=139 **Entry criteria:** 1. One risk factor GDM[IV] **Country:** Australia	Randomised to either 1. LGI diet or 2. HF diet **GDM criteria:** Modified ADPS criteria [V]	LGI	65	25.2 [±5.2]	13.8	
			HF	60	25.2 [±5.2]	15.0	

Footnotes:
Continuous data are expressed as mean [± standard deviation]. Results denoted in bold and marked with an * are significant (p<0.05).

Abbreviations: ***BMI*** Body mass index (at study inclusion); ***GDM*** Gestational diabetes mellitus; ***MET*** Metabolic equivalent task; ***I*** Intervention group ***C*** Control group; ***LGA*** Large for gestational age (birthweight ≥90th centile); ***GI*** Glycaemic Index; ***GWG*** Gestational weight gain (defined as gain in weight from early pregnancy/ at study inclusion to weight at 36 weeks gestation/ prior to delivery).

I. BMI ≥25 kg/m^2, previous pregnancy complicated by GDM or macrosomia (≥4500g), family history of diabetes or age ≥40 years. II. Criteria proposed by the 5th International Association of Diabetes and Pregnancy Study Groups (IADPSG) 2010 (54). III. Carpenter-Coustan criteria (60). IV. BMI ≥30 kg/m^2, family history T2DM, previous history GDM/ glucose intolerance/ delivery macrosomic infant (≥4000g), high-risk ethnic group. V. Modified Australasian Diabetes in Pregnancy Society 1998 (61).

The NELLI trial, a trial powered to detect a reduction in GDM incidence as the primary outcome, recruited 399 women with at least one of the risk factors (57). Those in the

intervention arm received lifestyle counseling at five different time points during pregnancy. Gestational weight gain was similar in the two groups. GDM incidence was also similar. Post hoc analyses of those who adhered to the programme demonstrated reductions in GDM incidence and a significant reduction in the proportion of infants born LGA. However, the potential impact of confounding factors is not known as no data regarding baseline maternal demographics within the sub-groups were provided.

The ROLO trial evaluated the effects of an LGI diet in reducing neonatal birth weight in 800 women who had previously delivered a macrosomic infant (birth weight ≥4000g) (58). Those in the intervention arm received a 2-hour educational session on the components of the diet at inclusion. Refresher sessions were provided at 28 and 34 weeks gestation. The incidence of GDM (as defined by the Carpenter-Coustan criteria), mean fetal birth weight and the proportion of infants born macrosomic were similar in the intervention and control arms (60).

The GI Baby 3 Study randomised 139 women at high risk for developing GDM to either an LGI diet or to a high-fibre moderate glycaemic-index diet (HF) diet (59). No differences were recorded in GDM incidence (as defined by modified Australasian Diabetes in Pregnancy Society criteria), gestational weight gain, fetal birth weight or neonates born large for gestational age (61).

1.7. Potential For Future Strategies

The US Diabetes Prevention Programme (DPP) demonstrated that lifestyle intervention in a non-pregnant population with impaired glucose tolerance reduced progression to T2DM by 58% when compared to placebo (62). On average participants in the lifestyle intervention arm lost 7% in weight over a three-year period. Losing a similar amount in early pregnancy would be difficult and while it could be argued that women who are overweight should aim to achieve this in the pre-conception period, population-based surveys in the UK have demonstrated that 45% of pregnancies are unplanned and that only 48% of the women who plan pregnancies are taking the appropriate supplementation (63, 64). This indicates that in the vast majority of cases adequate medical advice is neither being sought nor given. A key factor in the DPP's success was the provision of personal trainers, which would require considerable financial resources. Indeed, when considering the trials that demonstrated reductions in GDM incidence, the majority of the intervention programmes utilised significant resources in terms of either the supervision required for physical activity or in the frequency of the dietary counseling sessions.

1.7.1. Non-pharmacological prevention

The potential for dietary supplements in reducing the risk of GDM have been explored (Table 5). Observational studies have demonstrated a reduction in insulin resistance with poly-unsaturated fatty acids found in fish oils (65).

Table 5: Summary of the randomised-controlled trials investigating the effects of dietary supplementation in preventing gestational diabetes and adverse materno-fetal outcomes.

Trial	Population Characteristics	Randomisation groups and protocol	Group Characteristics			Outcomes	
				N	BMI (kg/m^2)	% GDM cases	Significant Outcomes
"Domino" Trial Zhou *et al* (66)	N=2399 **GA:** <20 weeks gestation **Country:** Australia	**Double blind RCT** DHA-enriched fish oil capsules versus vegetable oil capsule (1500mg). **GDM criteria:** 50g GCT [I]	IMP	1197	26.2 (23.2-30.5)	13.8	Higher proportion macrosomia DHA group: 16.3 versus 12.8%, p=0.02.
			C	1202	26.3 (22.9-30.8)	13.8	
Luoto *et al* (67)	N=256 **Entry criteria:** No metabolic diseases **Country:** Finland	**Open label RCT** Randomisation to one of 3 groups: diet and probiotics (D+IMP), diet and placebo (D+P) or placebo only (C). **GDM criteria:** 4th International Workshop-Conference on GDM [II]	D+ IMP	85	ND	**13***	
			D+P	86	ND	**36***	
			C	85	ND	**34***	
D'Anna *et al* *(68)*	N=220 **Entry criteria:** 1st degree relative T2DM; Caucasian; BMI≤30kg/m^2 **Country:** Italy	**Open-label RCT** 4g myo-inositol + 400mcg folic acid (IMP) versus 400mcg folic acid (C). **GDM criteria:** 5th IADPSG [III]	IMP	99	22.8 [±3.1]	**6.1***	Fewer infants born macrosomic (≥4000g) in myo-inositol group: 0% versus 7%, p=0.007.
			C	98	23.6 [±3.1]	**15.3***	
Materelli *et al* (69)	N=75 **Entry criteria:** 1. Fasting glucose: 5.1-7.0 mmol/L. 2.BMI≤35kg/m^2. **Country:** Italy	**Double blind RCT** of myoinositol + folic acid (4000mg +400mcg) versus folic acid only (400mcg). **GDM criteria:** 5th IADPSG [III]	IMP	35	23.5 [±3.4]	**6.0***	Myo-inositol associated with lower: GWG (2.3 ±1.1 kg/m^2 versus 3.8 ±2.4kg/m^2, p=0.001), requirement for insulin therapy (3.0% versus 21.0%, p=0.053), **i**ncidence NNH (0% versus 26%, p=0.038) and BWC (42 versus 57, p=0.001).
			C	38	24.7 [±4.2]	**71.0***	

Trial	Population Characteristics	Randomisation groups and protocol	Group Characteristics			Outcomes	
				N	BMI (kg/m^2)	% GDM cases	Significant Outcomes
Santamaria *et al* (70)	N=220 **Entry criteria:** 1. BMI 25.0-30.0kg/m^2 2. Caucasian	**Open-label RCT** 4g myo-inositol + 400mcg folic acid (IMP) versus 400mcg folic acid (C). **GDM criteria:** 5th IADPSG (III)	IMP	95	26.9 [±1.3]	**11.6***	
			C	102	27.1 [±1.3]	**27.4***	
Myo-Inositol in Obese Pregnant Women D'Anna *et al* (71)	N=220 **Entry criteria:** 1.BMI≥30.0kg/m^2 **Country:** Italy	**Open-label RCT** 4g myo-inositol + 400mcg folic acid (IMP) versus 400mcg folic acid (C). **GDM criteria:** 5th IADPSG (III)	IMP	107	33.8 [30.0-46.9]	**14.0***	Myo-inositol associated with reduction in GWG (5.9±4.7 versus 4.6±4.5kg, p=0.04), HOMA-IR, PIH (0 versus 6%, p=0.02), and admission to NICU (0 versus 5%, p=0.03).
			C	107	33.8 [30.0-46.0]	**33.6***	
Farren *et al*	N=240 **Entry criteria:** 1. Family history T2DM **Country:** Ireland	**Double blind RCT** 1100 mg *myo*-inositol, 27.6 mg *D*-chiro-inositol, and 400 µg folic acid (IMP) versus 400 µg folic acid (C).	IMP	120	ND	23.3	
			C	120	ND	18.3	

Continuous data are expressed as mean [± standard deviation] were results provided. Results denoted in bold and marked with an * are significant [p<0.05].

Abbreviations: ***BMI*** Body mass index (at study inclusion); ***GDM*** Gestational diabetes mellitus; ***GA*** Gestational Age (at inclusion); ***RCT*** Randomised-controlled trial; ***DHA*** Docosahexaenoic acid; ***IMP*** Investigational medicinal product; ***C*** Control group; ***ND*** Not described; ***T2DM*** Type 2 diabetes mellitus; ***GWG*** Gestational weight gain (defined as gain in weight from early pregnancy/ at study inclusion to weight at 36 weeks gestation/ prior to delivery); ***NNH*** Neonatal hypoglycaemia; ***BWC*** Birthweight Centile; ***HOMA-IR*** Homeostasis model assessment of insulin resistance; ***PIH*** Pregnancy induced hypertension; ***NICU*** Neonatal intensive care unit.

I. 50g GCT; 50g Glucose Challenge Test. If ≥7.7mmol/L for diagnostic OGTT: FPG >5.5mmol/Land 2hr 8.0mmol/L. II. 4th International Workshop-Conference on GDM (72). III. Criteria proposed by the 5th International Association of Diabetes in Pregnancy Study Group criteria (54).

In the Domino trial, 2399 women were randomised to either docosahexaenoic acid (DHA) enriched fish oil capsules (1500mg daily) or to matched vegetable oil capsules from inclusion at less than 21 weeks gestation through to delivery (66). The study was adequately powered but failed to detect a significant reduction in either of the two primary outcomes: incidence of pre-eclampsia or GDM.

Probiotics have also been advocated based on the beneficial effects on insulin sensitivity observed in non-pregnant adults and evidence that maternal gut microbiota could impact offspring metabolic programming (73, 74). The "Probiotics and Pregnancy outcome" study conducted in Finland, randomised 256 women in the first trimester of pregnancy to one of three groups: dietary intervention with probiotic supplementation, dietary intervention with placebo or routine antenatal care (67). Supplementation (Lactobacillus rhamnosus GG and Bifidobacterium lactis Bb12) was associated with both reduced insulin resistance in the ante- and post-partum periods as well as a reduction in GDM incidence: 13% of women assigned to the probiotic group developed GDM compared to 36% in the diet/placebo and 34% in the control groups as defined by the criteria recommended by the 4th International Workshop – Conference on Gestational Diabetes Mellitus (72). Though these results are important, they need to be interpreted with caution. Baseline demographics in each of the three groups were not defined and therefore, confounding factors may have existed. Furthermore, the study was not adequately powered and looking at the background incidence of GDM, it is clear that a particularly at-risk group was recruited. Further research is therefore needed to ensure the results are replicable in the wider antenatal setting.

Myo-inositol, a B complex vitamin, has been shown to improve insulin resistance in women with established GDM (75). The effects of myo-inositol in preventing GDM in cohorts with a single defined risk factor (first degree relative with T2DM, fasting hyperglycaemia in early pregnancy, early pregnancy BMI 25-30.0kg/m^2 or ≥30.0kg/m^2) were initially evaluated in four randomised controlled trials of myo-inositol (4g + 400mcg folic acid) versus matched placebo (400mcg folic acid alone) (Table 5) (68-71). Preliminary data from these trials were encouraging in that they demonstrated significant reductions in the incidence of GDM (diagnosed by the criteria proposed by the 5th International Association of Diabetes and Pregnancy Study Groups (IADPSG)) with myo-inositol supplementation (54). Additionally, reductions in the proportion of macrosomic infants, incidence of pregnancy-induced hypertension and incidence of neonatal hypoglycaemia were associated with myo-inositol supplementation. However, the effects of myo-inositol in overweight non-obese pregnant women, obese pregnant women and those with a family history of T2DM were investigated in an open label manner and only the studies in women with fasting hyperglycaemia were performed in a double blinded manner. The women included in the latter cohort were a particularly high-risk group (background incidence of GDM in the control group 71%) and the inclusion criteria meant that participants already had a diagnosis of GDM at randomisation. Subsequent to these trials, a fifth trial evaluated the effects of myo-inositol in 240 women with a family history of T2DM. Women were randomised to combined myo-inositol, D-chiro-inositol and folic acid (doses 1100mg, 27.6mg and 400µg respectively) or 400µg folic acid alone (control group). In contrast to the earlier

trials, differences in GDM incidence were not significant: 23.3% in the intervention group and 18.3% in the control group (p=0.34).

1.8. Conclusions

The incidence of GDM and its associated complications are increasing, reflecting the increasing prevalence of maternal obesity globally. Preventing pathological hyperglycaemia during pregnancy has several potential benefits: reduction of associated immediate maternal and fetal adverse outcomes, potential improvements in the risk of long-term sequelae and reductions in the economic burden to healthcare systems worldwide.

Population studies of dietary or combined lifestyle measures have not demonstrated improvements in GDM risk and trials involving physical activity strategies have yielded conflicting results. In obese women, dietary modification may improve GDM risk and macrosomia: low compliance and no significant reductions in the incidence of GDM have been observed in trials investigating physical activity. Combined lifestyle measures have been associated with significant reductions in gestational weight gain in obese pregnant women: however, improvements in GDM incidence have been reported in only one trial following adjustment for multiple baseline covariates. Fish oil supplementation has failed to prevent GDM and probiotics have shown benefit to women at very high GDM risk. While preliminary data in open labeled trials demonstrated a potential positive impact of supplementation with myo-inositol,

these findings have not been replicated in a double blind randomised controlled trial of myo-inositol versus placebo.

1.9. Discussion

The conflicting evidence regarding intervention with lifestyle strategies in preventing gestational diabetes in women with and without risk factors probably relates to the large degree of heterogeneity across the trials. Differences existed both in the demographics of the cohorts recruited as well as the criteria used to diagnose the condition. These inconsistencies have rendered it difficult to identify a clear lifestyle intervention strategy to prevent hyperglycaemia in pregnancy. However, important signals have emerged, one of which relates to the benefits of combined lifestyle interventions in improving other pre-defined materno-fetal outcomes e.g. fetal macrosomia. Reductions in these complications were observed even in the absence of preventing GDM, indicating that factors beyond maternal glucose are implicated in the pathogenesis of fetal overgrowth. In addition, variations in glucose could exist within certain populations. Both these points will be considered in more detail in Chapters 2 and 3.

The gestational age at which interventions were implemented probably had an additional impact on the effectiveness of the strategies. The length of exposure to glucose could have a detrimental effect on pregnancy and this will be further explored in Chapter 4. Furthermore, observational data have indicated that pre-conception factors including a healthy body weight, adherence to a healthy dietary pattern,

regular exercise of 150 minutes per week and abstinence from smoking are each significantly and independently associated with a reduced risk of developing hyperglycaemia in pregnancy (76). This suggests that a strategy in the pre-conception period could be effective. No trials have investigated this to date.

Another point to consider are the considerable resources required to implement the majority of the lifestyle strategies investigated to date. This in itself would provide a cost burden to healthcare systems. Preliminary data have demonstrated the potential benefits of dietary supplementation. Another potential avenue to explore would be metformin, the biguanide agent that has a strong evidence base for safe use in pregnancy and has been shown to prevent progression to T2DM in non-pregnant adults with impaired glucose tolerance. The evidence relating to this will be covered in more detail in Chapter 5 and preliminary data from the Preventing Recurrent Gestational Diabetes with Early Metformin Intervention trial (PRoDroME trial) will be presented in Chapter 6.

CHAPTER 2: MATERNAL METABOLIC FACTORS CONTRIBUTING TO FETAL OVERGROWTH AND ETHNICITY-BASED VARIATIONS IN FETAL GROWTH

2.1. Introduction

Complex interactions between genotypes, maternal metabolism and the intrauterine environment contribute to the final fetal weight at birth. Intrauterine fetal development relies on a continuous supply of nutrients and metabolites, predominantly glucose, amino acids and triglycerides, crossing the placenta. This continuous delivery is facilitated by pregnancy-induced physiological changes in insulin resistance, which increases as gestation advances.

2.1.1. Glucose

The major source of energy for both the placenta and developing fetus is glucose. The fetus has a limited ability to generate its own glucose and consequently almost all the required amount is derived from the mother (77). Outside of pregnancy, glucose transport occurs via a family of nine facilitated glucose transporters, otherwise known as GLUTs (78). GLUT 1, is the predominant transporter involved in the transfer of glucose across the placenta. The three-to-four-fold increase in GLUT 1 observed on the microvillous membrane (maternal synctiotrophoblast) relative to the basal membrane (fetal interface), creates a concentration gradient ensuring the net transportation of glucose from the mother to the developing baby. Though GLUT 3

and GLUT 4 have also been identified as potential transporters in pregnancy, data relating to their expression is conflicting.

2.1.2. Amino acids

The transport of amino acids, which are required for the formation of fetal proteins and nucleic acids, is a tightly regulated process that is facilitated by placental carrier proteins (77). In contrast to glucose, transport occurs against a concentration gradient. Fifteen amino acid transport systems have been described with those specifically implicated in the pathogenesis of fetal overgrowth being described in more detail below.

2.1.3. Triglycerides

Quantitatively, glucose is the most important nutrient that crosses the placenta followed closely by amino acids. However, despite limited placental transfer, lipids, in particular triglycerides, play an important role in fetal development with the latter independently correlating with fetal birth weight in women with normal glucose tolerance (79). Though triglycerides do not cross the placenta directly, they are hydrolysed to free fatty acids (FFAs) at the microvillous membrane. FFAs are able to cross the placenta in turn freely down their concentration gradient. In normal pregnancy, a modest increase in maternal cholesterol levels is observed early in gestation (80). Later, triglyceride concentrations are substantially elevated with phospholipids and cholesterol also being increased but to a lesser extent (Table 6).

Table 6: Changes in cholesterol profiles in lean pregnant women with normal glucose tolerance observed at baseline to term

Triglycerides	200-400% increase
Total cholesterol	25-50% increase
Low density lipoprotein	50% increase
High density lipoprotein	30% increase by mid-pregnancy Slight decrease at term
Free fatty acids	Increase

2.1.4. Changes in substrate availability in obesity and diabetes

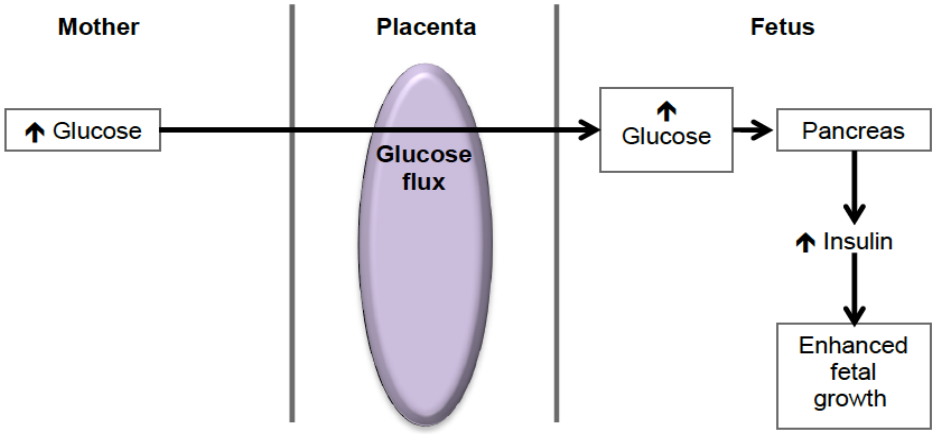
In the 1950s, that is even prior to the availability of an insulin assay, Pederson hypothesised that in Type 1 diabetes, maternal hyperglycaemia would result in fetal hyperglycaemia. He suggested that this in turn would serve two functions:

1. The glucose would act as a nutrient for enhanced fetal growth and

2. With the fetal pancreas able to respond to glucose from the second trimester, fetal hyperglycaemia would stimulate fetal hyperinsulinaemia, in turn further enhancing fetal growth (Figure 2) (81).

As the understanding of insulin and its mechanisms of actions increased, Pederson's original hypothesis was extended to conditions such as gestational diabetes and obesity. The enhanced insulin resistance observed in these and other related conditions is associated with an overall increase in maternal substrate availability including glucose, amino acids and triglycerides all of which drive fetal hyperinsulinaemia leading to enhanced fetal growth and an increase in neonatal adiposity (82).

Figure 2: Figure representing original Pedersen hypothesis

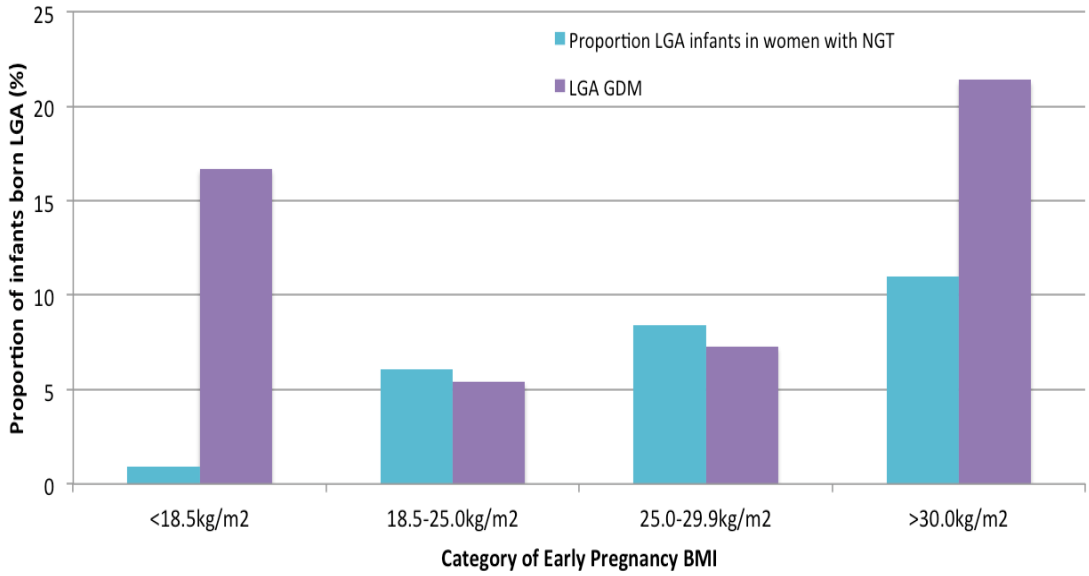


In addition to the increased circulating maternal metabolites observed, increases in placental expression of GLUT1 and alterations in amino acid transporters Systems A (involving alanine, serine and glutamine) and L (leucine and phenylanine) are observed in women with pregnancies complicated by either GDM or obesity compared to pregnancies in lean pregnant women with normal glucose tolerance (77).

Indeed, maternal obesity is an independent predictor of fetal macrosomia and neonatal adiposity. When maternal obesity and intolerance to glucose metabolism coexist the effects of enhanced substrate delivery is only compounded (83). This is further illustrated by data from 4562 women who delivered at Imperial College NHS Healthcare Trust over a one-year period (2014-2015: Figure 3). In this data set, the proportion of large for gestational age neonates (LGA: ≥90th adjusted birth weight centile) born to mothers who developed GDM (as defined by the modified WHO criteria (1999)) was compared to the proportion of LGA neonates born to mothers who were normoglycaemic on assessment of glucose tolerance at 24-28 weeks

gestation by category of maternal early pregnancy body mass index. Twenty-one per cent of neonates were born LGA to obese pregnant women with GDM compared to the 6% born LGA to women with a normal body mass index and normal glucose tolerance.

Figure 3: Bar chart illustrating the proportion of infants born large for gestational age (i.e. ≥90th birth weight centile) to women according to category of early pregnancy body mass index and diagnosis of gestational diabetes



Abbreviations: *LGA* Large for gestational age (≥90th fetal birth centile); *NGT* Normal glucose intolerance; *GDM* Gestational Diabetes (diagnosed according to modified WHO 1999 criteria).

2.2. Ethnicity

As demonstrated by a cross sectional survey of 276 436 births in 23 developing countries that was conducted by WHO, incidence of fetal macrosomia varies according to ethnicity (84). Non-White ethnicity is a known risk factor for developing GDM (6).

The prevalence of adverse materno-fetal outcomes, most notably fetal macrosomia in women diagnosed with GDM, additionally varies according to ethnicity (85). There are data demonstrating that ethnicity is an independent risk factor for adverse materno-fetal outcomes, with the risk of delivering large for gestational age infants being higher at lower grades of obesity in African American women (86).

Though the relationship between maternal obesity and glucose are well documented, few studies have explored the impact of ethnicity on the relationship between glycaemia and fetal birth weight.

2.3. Hypothesis and Aims

In non-pregnant adults, greater insulin resistance has been observed in non-White populations compared to those of White ethnicity (87). This is independent of variation in adiposity. Fetal overgrowth can be seen as a complication of enhanced insulin resistance. With these points in mind, a hypothesis is advanced that the proportion of infants born large for gestational age (LGA ≥90th centile) would vary according to ethnicity and that the impact of glucose and maternal adiposity on fetal growth would additionally vary according to ethnicity. To investigate this hypothesis, a retrospective analysis of a multiethnic cohort will be used to answer the following questions:

1. Do glucose and early pregnancy BMI vary with ethnicity in this multi-ethnic cohort?
2. Does the proportion of neonates born large for gestational age vary with ethnicity?

3. Does the predicted relationship between glucose and fetal birth weight vary with ethnicity?

4. Is there a relationship between early pregnancy BMI and fetal birth weight that varies with ethnicity?

2.4. Methodology

A retrospective analysis of pregnant women at risk of developing GDM who attended Imperial College NHS Trust over a one-year period (2014-2015) was conducted (n=4562). All women were screened for GDM risk factors at their initial antenatal visit. Risk factors were adapted from the National Institute of Health and Clinical Excellence (NICE) guidelines and consisted of any one of the following: non-White ethnicity, early pregnancy body mass index (BMI) ≥30kg/m^2, family history of Type 2 Diabetes (T2DM) and previous delivery of a macrosomic infant (fetal birth weight ≥4000g) (26). Those at risk were offered a diagnostic 75g oral glucose tolerance test at 24-28 weeks gestation. The modified 1999 WHO GDM diagnostic criteria were in use prior to 2016 i.e. fasting plasma glucose (FPG) ≥6.0mmol/L and/ or 120-minute post 75g glucose load ≥7.8mmol/L. Only women with singleton pregnancies who delivered above 24 weeks gestation and for whom complete information relating to fetal birth weight was available, were included in the final analysis (Figure 4).

For the purpose of this analysis, ethnicity was broadly classified into one of five ethnic groups: White, Black African-Caribbean, South Asian, Mixed/ Other Asian and Other/ Unknown (Figure 5). In this cohort ethnicity was self-reported and recorded at the

initial antenatal visit by the midwife performing the assessment who then selected one of eighteen options that most appropriately defined the ethnic origin.

Figure 4: Flow diagram illustrating data extraction from downloaded data

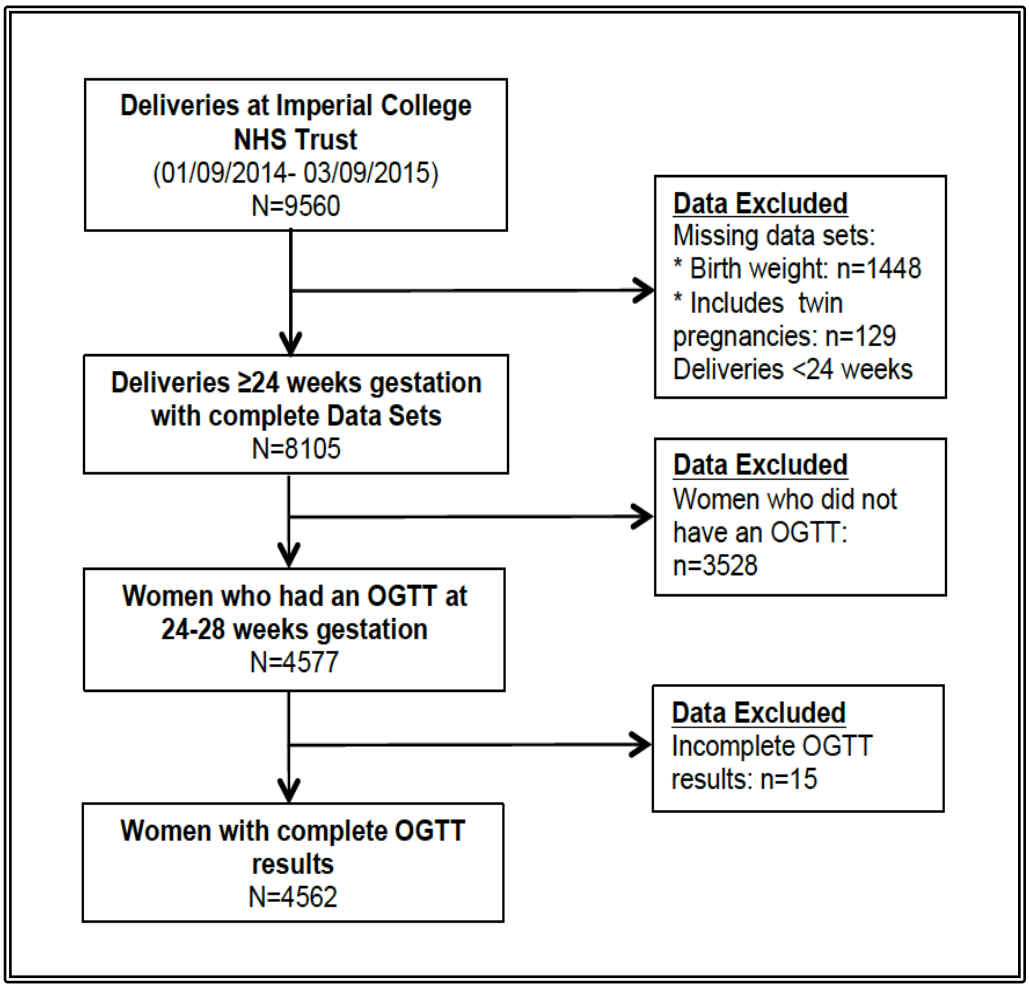
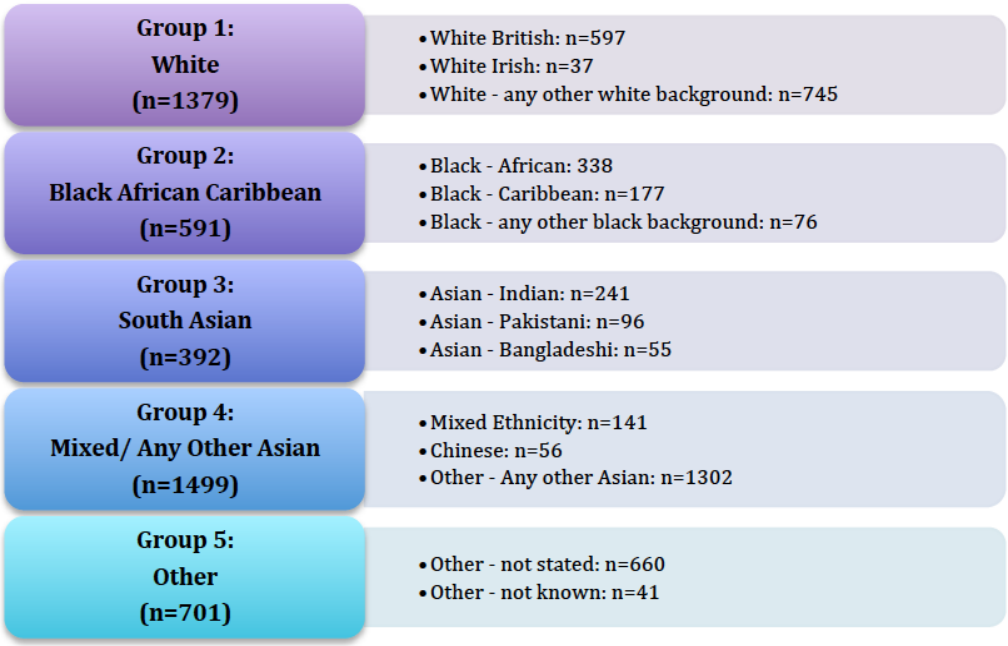


Figure 5: Categorisation of ethnicity



Baseline maternal demographics, glycaemia and fetal birth weight were compared across the five ethnic groups. Customised birth weight centiles were calculated using the GROW gestation network, which adjusts fetal birth weight for maternal height, weight, ethnicity and parity as well as fetal gestational age at birth and gender (88). Large for gestational age (LGA) infants were defined as infants with an adjusted birth weight ≥90th centile: small for gestational age (SGA) as those with a birth weight <10th centile.

2.4.1. Statistical Analysis

Continuous data are expressed as mean (±standard deviation) or median (interquartile range) depending on the distribution of the data. Analysis of variance or Kruskal-Wallis tests were used as appropriate to detect significant variation in continuous variables between the groups. Categorical data are expressed as

proportions and variation between groups tested by Chi-squared tests, with Fisher exact tests used where the expected cell frequency was less than five. Multivariable regression analyses with interaction terms were used to determine if relationships between glucose, early pregnancy BMI and fetal birth weight varied with ethnicity (with adjustment for gestational age and diagnosis of GDM). A p value <0.05 was accepted as statistically significant.

2.5. Results

In this cohort of 4562 women, mean (SD) age was 32.6 (±5.6) years and mean (SD) BMI 25.8 (±5.8) kg/m^2: 69.8% of women were of non-White ethnic origin. There was significant variation in baseline maternal demographics across the five ethnic groups. Women of White ethnic origin had the highest mean age and those of Black African-Caribbean ethnicity the highest mean early pregnancy BMI (Table 7). Significant variation also existed in parity, with the highest proportion of primigravida women observed in the Other/ Ethnicity Unknown group. The proportion of women with a previous pregnancy complicated by GDM also varied significantly as did 28 week oral glucose tolerance test results, with the highest proportion of previous pregnancies complicated by GDM and the highest mean fasting plasma glucose (FPG) and 120 minute glucose values demonstrated in women of South Asian ethnicity.

There was also significant variation in mode of delivery across the ethnic groups (Table 8). A sub-analysis demonstrated significant variation with ethnicity in both the proportion of women who had spontaneous vertex deliveries and emergency

caesarean sections (p<0.001 and p=0.016 respectively). These differences persisted on adjusting for parity.

Table 7: Maternal Demographics and 75g Oral Glucose Tolerance Test Results Across the Five Different Ethnic Groups

	White	Black African Caribbean	South Asian	Mixed / Other Asian	Other/ Ethnicity Unknown	Significance
Number (%)	1379 (30.2)	591 (13.0)	392 (8.6)	1499 (32.9)	701 (15.3)	
Mean (SD) Age (Years)	34.1 (±5.3)	30.8 (±6.0)	31.9 (±4.2)	32.0 (±5.5)	32.2 (±5.6)	<0.001
Mean (SD) BMI (kg/m)	25.5 (±6.1)	28.1 (±6.0)	25.4 (±4.9)	25.4 (±5.8)	25.5 (±5.3)	<0.001
Primigravida % (n)	53.4 (736)	40.4 (239)	49.8 (195)	54.5 (817)	60.6 (425)	<0.001
Proportion with GDM % (n)	5.1 (70)	8.1 (48)	13.0 (51)	9.3 (140)	8.0 (56)	<0.001
Mean (SD) FPG (mmol/L)	4.33 (±0.46)	4.35 (±0.66)	4.47 (±0.69)	4.39 (±0.60)	4.30 (±0.52)	<0.001
Mean (SD) 120 min glucose (mmol/L)	5.42 (±1.36)	5.54 (±1.52)	6.09 (±1.74)	5.70 (±1.61)	5.61 (±1.60)	<0.001

Abbreviations: *BMI* Body mass index, *GDM* Gestational diabetes, *FPG* Fasting plasma glucose

Mean (SD) fetal birth weight and birth weight adjusted for maternal and fetal characteristics varied significantly across the five ethnic groups, with neonates born to the White ethnic group having the highest median birth weight and adjusted birth weight centile and those born to the South Asian group the lowest (Table 8, Figures 6 and 7).

Table 8: Delivery modalities, fetal birth weight and adjusted fetal birth centile in the five different ethnic groups

	White	Black African Caribbean	South Asian	Mixed / Other Asian	Other/ Ethnicity Unknown	Significance
Delivery modality						
% SVD	49.9	61.3	43.7	51.1	50.4	
(n)	(685)	(361)	(171)	(762)	(351)	
% AVD	18.0	9.0	23.3	18.2	20.6	
(n)	(247)	(53)	(91)	(271)	(143)	<0.001
% ELCS	18.6	12.4	14.6	12.0	11.5	
(n)	(255)	(73)	(57)	(179)	(80)	
% EmCS	13.6	17.3	18.4	18.8	17.5	
(n)	(186)	(102)	(72)	(280)	(122)	
Mean (SD) fetal birth weight (g)	3391 (±562)	3266 (±551)	3074 (±552)	3269 (±546)	3294 (±524)	<0.001
Median (IQR) fetal birth weight centile	47 (25-75)	35 (16-62)	25 (10-49)	37 (16-64)	36 (16-62)	<0.001
Birth weight centile categorisation:						
% SGA	10.0	16.4	24.7	16.1	16.3	
(n)	(138)	(97)	(97)	(241)	(114)	
% AGA	77.7	76.5	70.2	77.9	78.3	<0.001
(n)	(1072)	(452)	(275)	(1167)	(549)	
% LGA	12.3	7.1	5.1	6.1	5.4	
(n)	(169)	(42)	(20)	(91)	(38)	

Abbreviations: *SVD* Spontaneous vertex delivery, *AVD* Assisted vaginal delivery, *ELCS* Elective caesarean section, *EmCS* Emergency caesarean section, *SGA* Small for gestational age (<10th birth weight centile), *AGA* Appropriate for gestational age (10th-90th birth weight centile), *LGA* Large for gestational age (>90th centile).

Figure 6: Distribution of fetal birth weight in the five ethnic groups

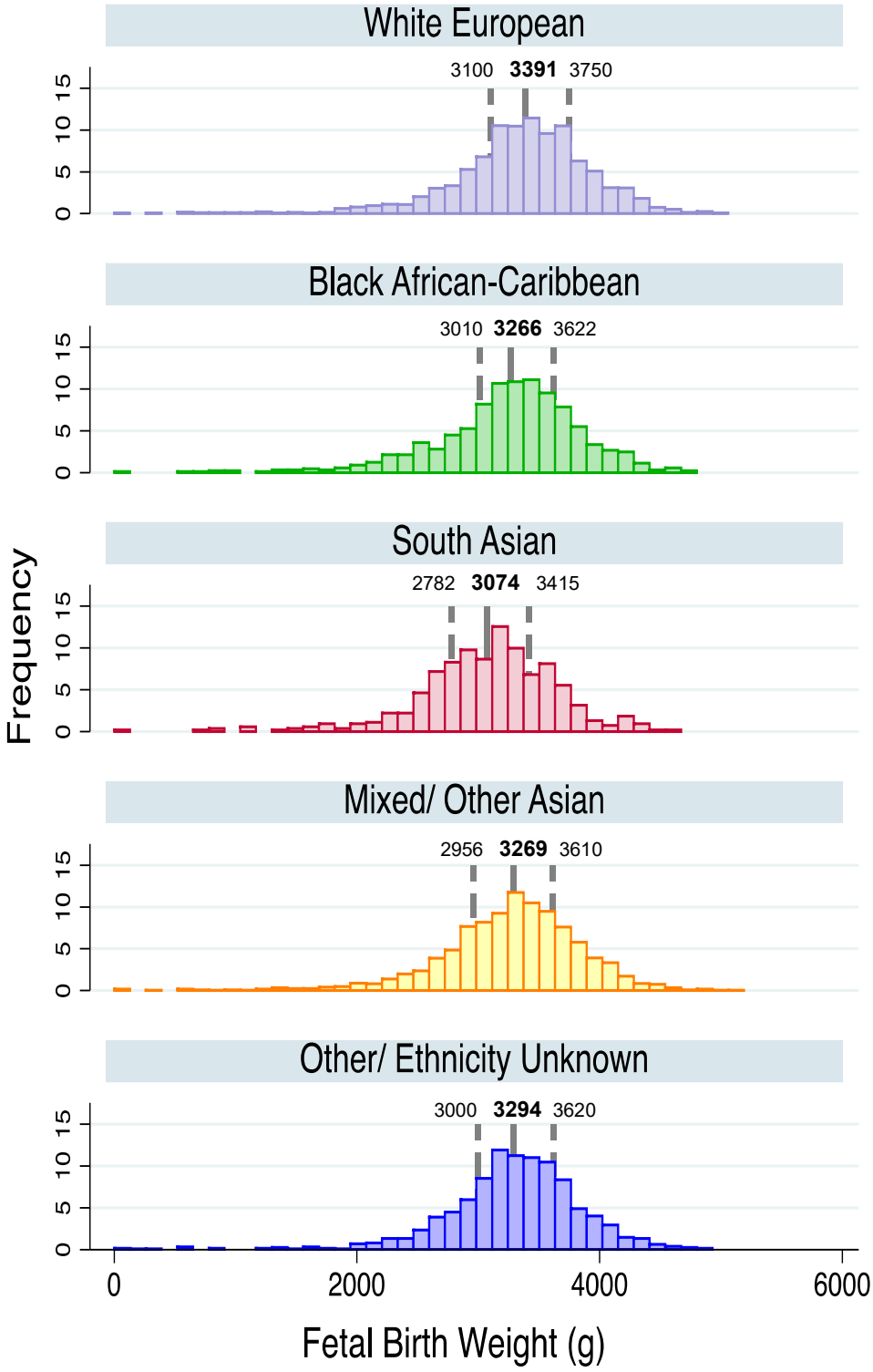
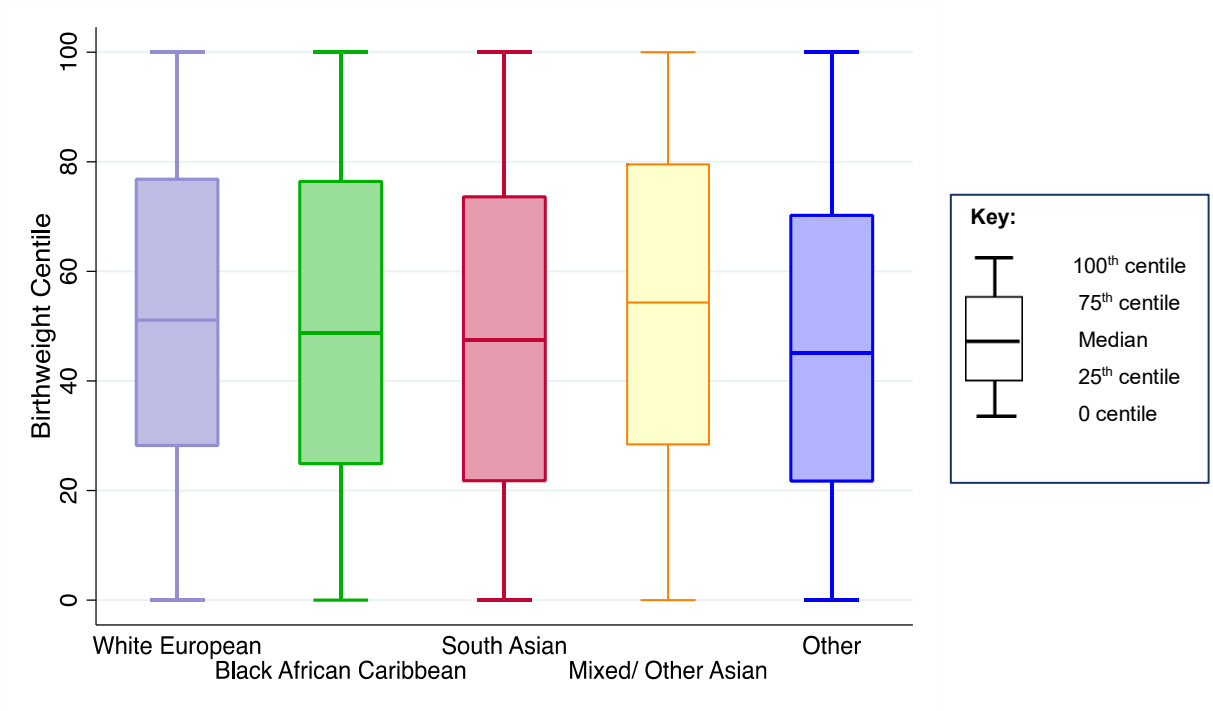


Figure 7: Distribution of fetal birth weight adjusted for maternal and fetal characteristics across the five ethnic groups



2.5.1. Variations in large-for-gestational-age infants according to glycaemia and ethnicity

Overall, 7.9% of neonates were born large for gestational age (LGA ≥90th centile). Variations in the proportion of LGA infants by category of glycaemia were examined in each ethnic group, with the diagnostic thresholds for GDM defining the highest category of glycaemia (Figures 8a and b). Multiple-way chi-squared tables were generated to examine statistical significance in the proportions of LGA neonates across the seven categories of glycaemia for each ethnic group. Fisher exact tests used where the expected cell frequency was less than five.

Figure 8a: Proportion of infants born large for gestational age (≥90th centile) in each ethnic group by category of fasting plasma glucose

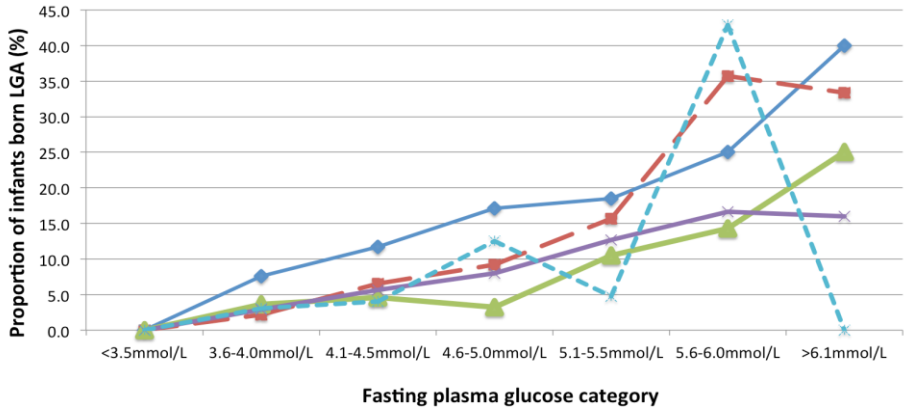


Figure 8b: Proportion of infants born large for gestational age (≥90th centile) in each ethnic group by category of 120 minute glucose

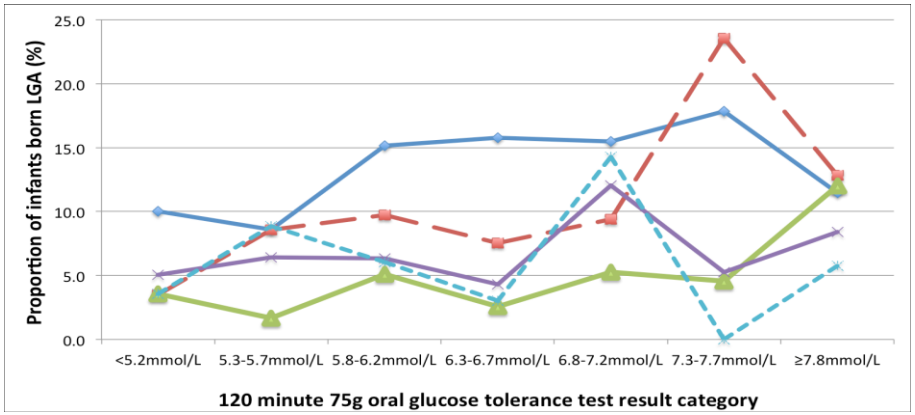


Figure 9a: Proportion of infants born large for gestational age (≥90th centile) in each ethnic group by early pregnancy body mass index category

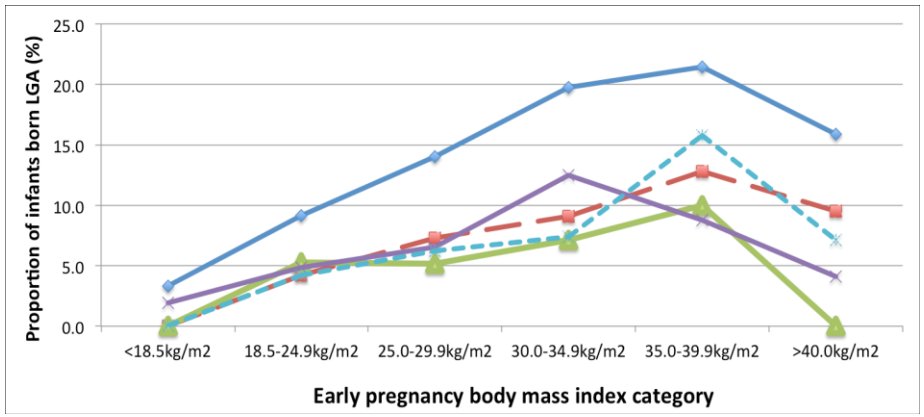
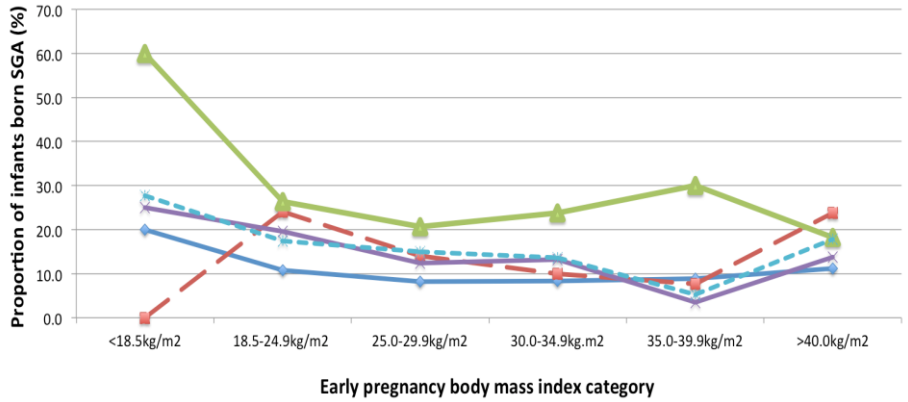


Figure 9b: Proportion of infants born small for gestational age (<10th centile) in each ethnic group by early pregnancy body mass index category



White European Black African Carribean South Asian Mixed/ Other Asian Other/ Unknown

A general trend towards an increasing proportion of infants born LGA with each 0.4mmol/L increment in FPG was observed in four of the ethnic groups. Women of White ethnicity delivered the highest proportion of LGA infants in the highest category of glycaemia with 40% of neonates born LGA when maternal FPG exceeded 6.1mmol/L at 28 weeks gestation (Figures 8a and 8b). Neonates born to the Other/ Unknown group did not demonstrate the same pattern. Variations in the incidences of LGA neonates by category of FPG were significant within each ethnic group at $p<0.001$ with the exception of the South Asian group where significance was borderline at 0.05.

In contrast, no clear pattern emerged in the proportion of infants born LGA by category of 120-minute glucose values within the five ethnic groups. Variation in the incidence of LGA infants by category of 75g OGTT glycaemia was significant only in women of Black African Caribbean and Other/ Unknown ethnicity ($p=0.010$ and 0.046 respectively). Variation in LGA incidence with 75g OGTT glucose was non-significant in women of White ($p=0.08$), South Asian ($p=0.3$) and Mixed/ Other Asian ethnicity ($p=0.2$).

The proportions of neonates born LGA by category of BMI in each ethnic group were also examined and multiple-way chi-squared tables generated to test for significance (Figure 9a). Overall, a general trend towards an increase in the proportion of infants born LGA with each 5.0kg/m^2 increment in early pregnancy BMI was demonstrated. This pattern persisted until the BMI exceeded 40.0kg/m^2, following which a reduction in LGA incidence was noted in each ethnic group. Variation in the proportion of LGA infants by category of BMI was significant in women of White ethnicity and Mixed/

Other Asian ethnicity: p<0.001 and p=0.03 respectively. In contrast, variation in LGA incidence according to BMI category was non-significant in women of Black African Caribbean, South Asian and Other/ Unknown ethnic origin (p=0.2, 0.9 and 0.2 respectively).

The lower proportion of LGA infants born to the women in the highest category of early pregnancy body mass index reflects data from the HAPO study (89). Though this pattern appears paradoxical, there are data to suggest that obesity is associated with placental insufficiency and consequently, intrauterine growth restriction (90). To explore this possibility, the incidence of small for gestational age infants (SGA <10th centile) by category of BMI was determined (Figure 9b). Overall, the incidence of infants born SGA was 15.1% in the cohort. In women of White European, Mixed/ Other Asian and Other/ Unknown ethnicity, the proportion of neonates born SGA was highest in either the lowest category of BMI (<18.5kg/m^2) or the highest (>40.0kg/m^2). Variation in the incidence of infants born SGA according to BMI category was significant in women of Black African-Caribbean ethnicity (p=0.01), South Asian ethnicity (p=0.04) and Mixed/ Other Asian ethnicity (p<0.001: White ethnicity p=0.2, Other/ Unknown p =0.4).

2.5.1. Impact of ethnicity on the relationship between glucose and fetal birth weight

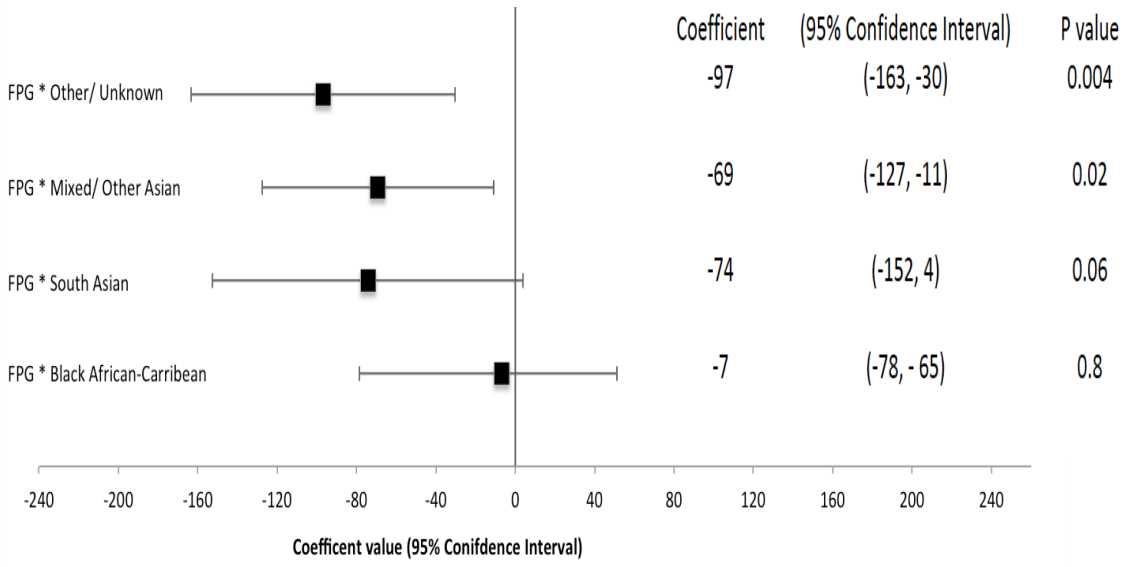
To explore whether the relationship between maternal FPG and fetal birth weight varied significantly according to ethnicity, a regression model was constructed with fetal birth weight as the dependent variable and, as independent variables, maternal FPG, dummy variables for each ethnic group (with White ethnicity as the reference

group), interaction terms for FPG*ethnicity (again with White ethnicity as the reference), gestational age and early pregnancy BMI. As expected, gestational age and early pregnancy BMI were significant, positive, independent predictors of fetal birth weight (Table 9). Maternal FPG was also a positive independent predictor with a 1mmol/L increase in FPG associated with a 157g increase in birth weight ($p<0.001$). Though the Other/ Not known group showed a positive effect at borderline significance ($p=0.06$), no ethnicity per se was significantly associated with fetal birth weight. However, some ethnicities significantly interacted with the relationship between FPG and fetal birth weight. In the Mixed/Other Asian, Other/Not Known and, at borderline significance, South Asian groups, the effect of FPG on fetal birth weight was reduced relative to the White ethnic group, as suggested by the profiles illustrated in Figure 8a (Figure 10).

Table 9: Independent effects on birth weight of FPG, ethnicity (relative to White ethnicity), FPG*ethnicity interaction (relative to White ethnicity), gestational age and early pregnancy BMI

	Coefficient [95% CI]	**Significance**
FPG	157 (108, 206)	<0.001
Black African-Caribbean	-114 (-427, 200)	0.5
South Asian	58 (-291, 407)	0.7
Mixed/ Other Asian	172 (-84, 427)	0.2
Other/ Not Known	280 (-10, 569)	0.06
FPG*Black African-Caribbean	-7 (-78, 65)	0.8
FPG*South Asian	-74 (-152, 4)	0.06
FPG*Mixed/ Other Asian	-69 (-127, -11)	0.02
FPG*Other/ Not Known	-97 (-163, -30)	0.004
Gestation weeks	203 (196, 210)	<0.001
Early pregnancy BMI	9 (7, 11)	<0.001
Abbreviations: *FPG* Fasting plasma glucose, *BMI* Body mass index		

Figure 10: Effect of fasting plasma glucose (FPG) on fetal birth weight in each ethnic group relative to the effect of FPG on fetal birth weight in women of white ethnic origin, independent of gestational age and ethnicity per se



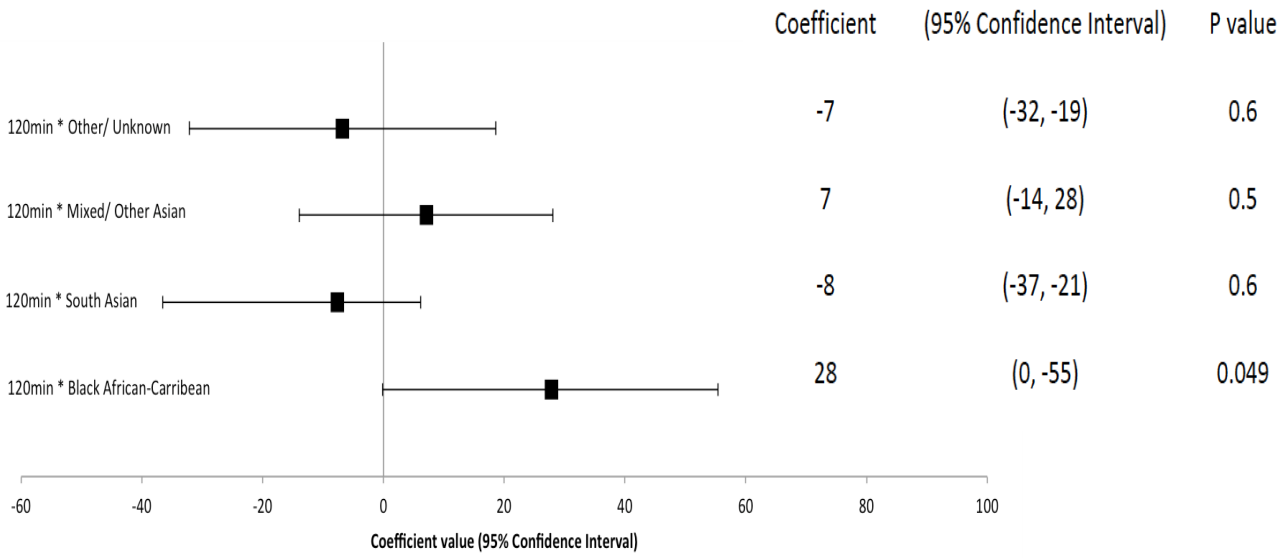
Using the same regression model structure, possible interactions of ethnicity with the relationships between 120-minute glucose and fetal birth weight and early pregnancy BMI and fetal birth weight were also explored. A 1mmol/L increase in 120-minute glucose was independently associated with an increase in birth weight of 16.9g (p=0.04) (Table 10). This relationship was significantly and independently modified by Black African-Caribbean ethnicity with a stronger effect of 120-minute plasma glucose on fetal birth weight apparent in Black African-Caribbean women relative to women of White ethnicity (Figure 11).

Table 10: Independent effects on birth weight of 120 minute glucose, ethnicity (relative to White ethnicity), 120 minute glucose*ethnicity interaction (relative to White ethnicity), gestational age and early pregnancy BMI

	Coefficient [95% CI]	**Significance**
120 minute glucose	17 (1, 33)	0.043
Black African-Caribbean	-294 (-451, 137)	<0.001
South Asian	-211 (-388, -34)	0.019
Mixed/ Other Asian	-168 (-288, -48)	0.006
Other/ Not Known	-107 (-252, 39)	0.2
120min*Black African-Caribbean	28 (0, 55)	0.049
120min*South Asian	-8 (-37, 21)	0.6
120min*Mixed/ Other Asian	7 (-14, 28)	0.5
120min*Other/ Not Known	-7 (-32, 19)	0.6
Gestation weeks	203 (196, 210)	<0.001
Early pregnancy BMI	9 (7, 11)	<0.001

Abbreviations: *FPG* Fasting plasma glucose, *BMI* Body mass index

Figure 11: Effect of 120-minute glucose on fetal birth weight in each ethnic group relative to the effect of 120-minute glucose on fetal birth weight in women of white ethnic origin, independent of gestational age and ethnicity per se

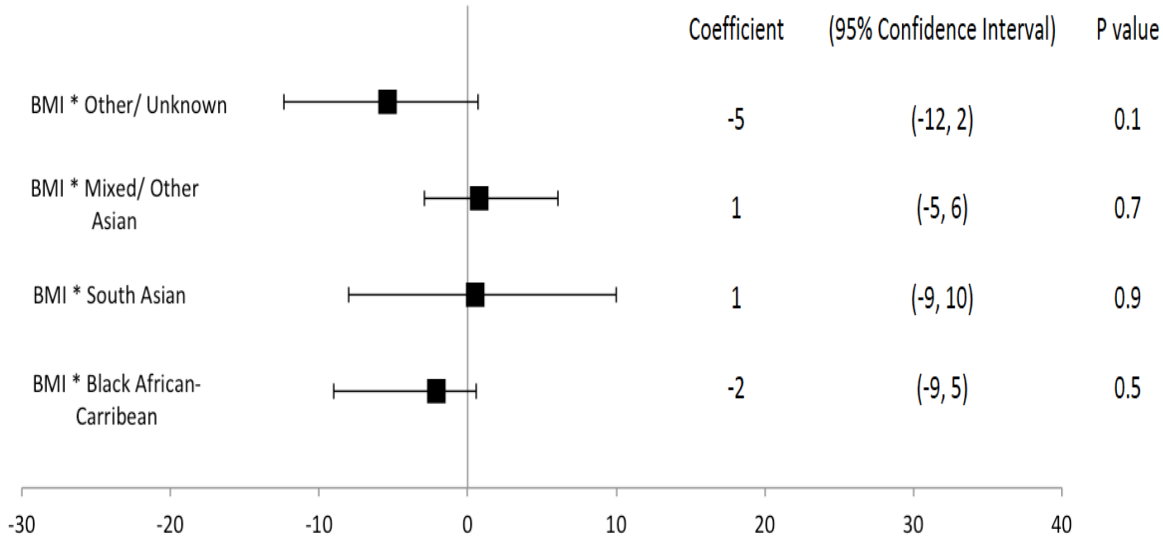


Ethnicity did not interact with the relationship between early pregnancy BMI and fetal birth weight (Table 11, Figure 12).

Table 11: Independent effects on birth weight of early pregnancy body mass index (BMI), ethnicity (relative to White ethnicity), FPG*ethnicity interaction (again relative to White ethnicity), gestational age

	Coefficient [95% CI]	**Significance**
Early pregnancy body mass index (BMI)	11 (8, 15)	<0.001
Black African-Caribbean	-103 (-297, 91)	0.3
South Asian	-258 (-504, -13)	0.04
Mixed/ Other Asian	-140 (-280, -1)	0.048
Other/ Not Known	-5 (-187, 177)	0.9
BMI*Black African-Caribbean	-2 (-9, 5)	0.5
BMI*South Asian	1 (-9, 10)	0.9
BMI*Mixed/ Other Asian	1 (-5, 6)	0.7
BMI*Other/ Not Known	-5 (-12, 2)	0.1
Gestation weeks	203 (196, 210)	<0.001

Figure 12: Effect of early pregnancy BMI on fetal birth weight in each ethnic group relative to the effect of early pregnancy BMI on fetal birth weight in women of white ethnic origin, independent of gestational age and ethnicity per se



2.5.2. Sub-analysis of the baseline demographics in the Mixed/ Other Asian or Other/ Unknown Groups

In view of the interaction of the Mixed/ Other Asian and Other/ Unknown groupings in the relationship between FPG and fetal birth weight, these two groups were investigated in greater detail. Given the number of sub-groups included within women of Mixed/ Other Asian ethnicity and the broad categorising terms included in the Other/ Unknown Groups, a large degree of heterogeneity probably existed within these groups (see Section 2.4. Figure 5). The baseline demographics and biochemical parameters differed were therefore analysed (Tables 12 and 13).

Table 12: Baseline demographics in the sub-groups included in women of Mixed/ Other Asian ethnicity

	Mixed Ethnicity	Chinese	Other or Any other Asian	Significance
Number (%)	141	56	1302	
Mean (SD) Age (Years)	31.3 (±5.7)	32.2 (±4.5)	32.1 (±5.6)	0.4
Mean (SD) BMI (kg/m)	26.2 (±5.1)	22.7 (±4.2)	25.6 (±5.0)	<0.001
Mean (SD) FPG (mmol/L)	4.3 (±0.5)	4.4 (±0.4)	4.4 (±0.8)	0.1
Mean (SD) 120 min glucose (mmol/L)	5.3 (±1.3)	5.7 (±1.3)	5.7 (±1.7)	0.01

Abbreviations: *SD* Standard deviation, *BMI* Body mass index, *FPG* Fasting plasma glucose

Table 13: Baseline demographics of the sub-groups included in women of Other/ Unknown ethnicity

	Other – not stated	Other –not known	P value
Number (%)	660	41	
Mean (SD) Age (Years)	32.2 (±5.7)	31.3 (±5.3)	0.3
Mean (SD) BMI (kg/m)	25.5 (±5.3)	24.7 (±3.2)	0.3
Mean (SD) FPG (mmol/L)	4.3 (±0.7)	4.3 (±0.5)	0.9
Mean (SD) 120 min glucose (mmol/L)	5.6 (±1.6)	5.6 (±1.5)	0.8

Abbreviations: *SD* Standard deviation, *BMI* Body mass index, *FPG* Fasting plasma glucose

Early pregnancy BMI and 120-minute glucose values differed significantly in the sub-groups in the Mixed/ Other Asian category, with women of Chinese ethnicity having

the lowest BMI and women of Mixed ethnicity having the lowest mean 120-minute glucose values. No differences in baseline demographics existed between the two sub-groups in women broadly classified as Other/ Unknown ethnicity.

2.6. Conclusions

This analysis has demonstrated ethnicity-based variations in maternal body mass index, measures of glycaemia at 24-28 weeks, fetal birth weight and birth weight centile in five different ethnic groups. In addition, significant variations existed in the proportion of infants born either small (SGA; <10th adjusted birth weight centile) or large (LGA; ≥90th centile) for gestational age, with women of White ethnic origin delivering the highest proportion of LGA infants and women of South Asian ethnicity the lowest: the reverse was true for SGA incidence.

Each 0.4mmol/L increment in fasting plasma glucose (FPG) was associated with an increase in the proportion of infants born LGA in each ethnic group with the exception of women categorised in the Other/ Unknown ethnic group. The relationship was particularly pronounced in women of White ethnic origin: 45% of infants were born LGA in the highest category of glycaemia (threshold exceeding 6.1mmol/L). In contrast, no clear relationship existed between the proportion of infants born LGA and 120 minute OGTT glucose category.

Additionally, an ethnic group dependent effect on the interaction between glucose and fetal birth weight was demonstrated that persisted following adjustment for

maternal BMI. In women in the Mixed/Other Asian, Other/Not Known and, at borderline significance, South Asian groups, the effect of FPG on fetal birth weight was reduced relative to the White ethnic group. The association of 120-minute glucose with fetal birth weight was smaller than the association of FPG with fetal birth weight: an increase of 1mmol/L in the former was independently associated with an increase of 16.9g in birth weight compared to the 157g increase observed with a 1mmol/L increase in FPG. The relationship between 120-minute OGTT glucose was significantly and independently modified by Black African-Caribbean ethnicity with a stronger effect of 120-minute plasma glucose on fetal birth weight apparent in Black African-Caribbean women relative to women of White ethnicity

An increase in category of early pregnancy body mass index was associated with an increase in the proportion of infants born LGA in all ethnic groups up to the point at which BMI exceeded 40kg/m^2. At this threshold, the proportion of LGA infants decreased. Again, this pattern was most pronounced in women of White ethnicity: women originating from this ethnic group with an early pregnancy BMI of 35.0-39.9kg/m^2 delivered the largest proportion of LGA infants (21.0%). The interaction between BMI and fetal birth weight was not dependent on ethnicity.

2.7. Discussion

The term Gestational Diabetes Mellitus (GDM) was first introduced to describe women with poor obstetric outcomes who had high glucose levels in subsequent pregnancies (12). Initial diagnostic criteria were based on values that best predicted

later development of maternal T2DM. GDM can be viewed as an early phase of T2DM, the metabolic stress of pregnancy demonstrating a predisposition to glucose intolerance. The main fetal complication, macrosomia, encompasses a spectrum from normality to hyperglycaemia and is predicted by factors other than hyperglycaemia such as maternal weight. Importantly, maternal obesity predicts macrosomia, a common complication of untreated GDM, with numerically more macrosomic babies born to obese mothers compared to those born to women whose pregnancies are complicated by GDM (37). Maternal dyslipidaemia also correlates with fetal growth and in certain groups, hypertriglyceridaemia is a stronger predictor of macrosomia than glucose. Excess gestational weight gain is additionally emerging as an important factor in determining risk of fetal overgrowth, with an increase in total gestational weight gain adding to the relative risk of LGA infants in this groups (36) (91). Finally, as is illustrated by the original data in this chapter, ethnicity plays an important role.

The data presented in this chapter suggest that ethnic specific glucose thresholds may be warranted for the diagnosis of gestational diabetes mellitus. This reflects findings from the Born in Bradford study, a prospective study that recruited pregnant women attending the antenatal centre at Bradford Royal Infirmary from 2007 to 2011. In total, 10,353 pregnancies were included in the analysis: 4088 of these women were of White ethnic origin and 5408 were South Asian (92). The 857 women who were classified as "Other" ethnicity were not included. Relative to women of White ethnicity, glucose (both post 75g load and to a lesser extent fasting) had a less pronounced effect on the proportion of infants born large for gestational age compared to women of South Asian origin. However, glycaemia had a stronger impact

on neonatal adiposity as assessed by skin fold measurements in the neonates born to women in the South Asian group compared to those of White ethnic origin.

In contrast to the Born in Bradford study, the analysis presented in this chapter suggests that FPG has a greater impact on fetal birth weight compared to 120-minute glucose values. This finding initially appears paradoxical particularly when considering both the pathophysiology of GDM, where defects in first phase insulin secretion are an initial feature, and the studies demonstrating an increased risk of adverse materno-fetal outcomes relating to the degree of post-prandial, not fasting, hyperglycaemia (93, 94). However, data from the Hyperglycaemia and Adverse Pregnancy Outcome study, a prospective cohort study of 25 505 women does emphasise fasting hyperglycaemia in contributing to excess fetal growth, supporting the findings from the analysis presented in this chapter (2). The HAPO study additionally demonstrates a continuum between glucose and the proportion of infants born LGA further supporting the findings presented.

There are limitations to this retrospective analysis, the main one relating to the classification of ethnicity, which in the first instance was self-reported. Subsequent categorisation relied on the healthcare professional registering the pregnancy to accurately select one of the 18 classifications on the electronic patient record. Consequently, a degree of heterogeneity within each of the five ethnic groups probably exists. the Mixed/ Other Asian or the Other/ Unknown groups. The analysis of the sub-groups classified under the Mixed/Other Asian heading demonstrated significant variations in maternal BMI and 120-minute glucose values between the

sub-groups, thus confounding the results. No separate categories for women of Middle-Eastern or North African ethnic origin existed on the electronic patient record system. This is an important limitation when considering the high proportion of women originating from these ethnic groups in the Imperial College NHS Trust catchment area (audit data: 15-20% in 2014) and the high incidence of GDM reported in these respective regions (5).

While the infant born LGA is at increased risk of obesity and insulin resistance in young adulthood, a birth weight above the 90th centile does not necessarily signify an increase in fetal adiposity. Data relating to the complications of fetal macrosomia/ pathophysiological overgrowth such as shoulder dystocia, neonatal hypoglycaemia and neonatal hyperbilirubinaemia would have more accurately determined the true impact of glucose and maternal adiposity in each ethnic group. Unfortunately these data were not coded for at the time of the data download and were therefore not available for analysis.

All women included in this study at risk of developing gestational diabetes. As previously stated, these risk factors were adapted from the NICE guidelines (26). The analysis presented here has demonstrated that the impact of glucose and body mass index on fetal birth weight is greatest in women of White ethnic origin. By definition, all pregnant women of non-White ethnic origin should have had an oral glucose tolerance test at 24-28 weeks gestation. Given the problems with defining ethnicity in London and potential knowledge gaps in the need to screen for GDM risk, this was not necessarily the case. Imperial College NHS Trust audit data from the same time

period demonstrated that only 64% of these women did undergo glucose tolerance testing. It would therefore be useful to ascertain the proportion of LGA infants across the entire cohort of women over a one-year period, both in those classified with and without risk. Furthermore, given the lesser contribution of glucose relative to women of White ethnicity, factors beyond glycaemia could play a greater role in excess fetal growth in other ethnic groups perhaps suggesting that a less glucose-centric approach could be adopted when addressing risk.

Future work could also explore the most appropriate glucose threshold to define LGA risk in specific ethnic groups. However, prior to altering GDM diagnostic thresholds, studies and even randomised controlled trials, would be needed to ensure that treating below revised criteria would indeed mitigate the risk of fetal overgrowth and any associated adverse materno-fetal outcomes.

The further points that this thesis will address relating to the impact of glucose on fetal outcomes are glycaemic variability and the length of exposure to hyperglycaemia. In the context of Type 1 and Type 2 diabetes, poor glycaemic control early in pregnancy is associated with an increased risk of congenital malformations and later development of fetal macrosomia (95). In non-pregnant adults, increased glycaemic variability has been implicated in the pathogenesis of complications. However, the evidence relating to the impact of variability on materno-fetal outcomes in pregnancy is limited. A study investigating this will be presented in Chapter 3. Metabolic imprinting is a feature of the second trimester and infants exposed to an adverse intrauterine environment in this phase of the pregnancy are more likely to be

obese or insulin resistant in young adulthood (96). This has clearly been demonstrated in women with pre-gestational diabetes and to a lesser extent, in women whose pregnancies are complicated by obesity. Chapter 4 will explore the risks associated with prolonged exposure to hyperglycaemia in women without established pre-gestational diabetes.

Chapter 3: Glycaemic variability and its impact on fetal growth in pregnant women with Type 1 Diabetes and Type 2 Diabetes

3.1. Background

Suboptimal glycaemic control contributes to adverse outcomes in pregnancies complicated by diabetes. Longitudinal data demonstrate the relationship between progressively deteriorating glycaemic control and the increasing risk of major congenital cardiac malformations in neonates born to women with Type 1 diabetes (T1DM) (97). Infants born to mothers with diabetes mellitus are additionally more likely to be large-for-gestational age (LGA: >90thcentile) and are at greater risk of birth-related injuries, physiological, and metabolic sequelae.

Rates and possible causes of macrosomia differ in Type 1 diabetes (T1DM) and Type 2 diabetes (T2DM) and the relative impact of glycaemic control on complications vary (98). A prospective cohort study in East Anglia demonstrated that despite better glycaemic control in women with Type 2 diabetes compared to those with Type 1 diabetes (HbA1c 52mmol/mol versus 63mmol/mol, p <0.0001), rates of congenital malformations and perinatal death were similar. Women with Type 2 diabetes delivered a lower proportion of large for gestational age infants and the incidence of preterm deliveries and neonatal care admissions was lower.

In the aforementioned studies, HbA1c was used as the main marker of glycaemic control. HbA1c has limitations in pregnancy: red cell turnover is increased and the increased incidence of iron deficiency observed in pregnant adults is in itself known to increase HbA1c values independent of associated changes in glucose indices (99). Furthermore individuals with similar glycated haemoglobin levels can have dramatically different glucose profiles, with the changes over time (either within a twenty-four period or between days) reflecting the concept of glycaemic variability.

In non-pregnant adults, there is an evidence base to suggest that increased glycaemic variability could be associated with an increased risk of developing complications (100). There exists little evidence on the association between glycaemic variability and fetal overgrowth in women with pre-gestational diabetes.

3.2. Aims

This analysis aims to:

1. Investigate if markers of glycaemic variability correlate with fetal growth

2. Investigate if markers of glycaemic variability are associated with fetal overgrowth in women according to type of diabetes.

3.3. Methods

Thirty-three women with pre-gestational diabetes (Type 1 DM n=14, Type 2 DM n=19) who underwent 72-120 hours of blinded continuous glucose monitoring (iPro2 sensors) as part of routine clinical care in the 2^{nd}-3^{rd} trimesters of pregnancy at Imperial College Healthcare NHS Trust were retrospectively reviewed. Baseline maternal demographics (age, early pregnancy body mass index and ethnicity) and fetal birth weight were analysed in these women. Customised birth weight centiles were calculated using the GROW gestation network, which adjusts fetal birth weight for maternal height, weight, ethnicity and parity as well as fetal gestational age at birth and gender (88). Large for gestational age (LGA) infants were defined as infants with an adjusted birth weight ≥90^{th} centile.

Absolute measures of glycaemia (mean glucose, standard deviation), intraday glycaemic variations (MAG, CV) and hypo-/hyperglycaemic risks (LBGI, HBGI) were calculated from the continuous glucose monitoring sensor data using EasyGV software v9.0 (Table 14).

3.3.1. Statistical Analysis

Continuous data are expressed as mean (±SD) or median (interquartile range) depending on the distribution of the data: ANOVA or Kruskal-Wallis tests were used as appropriate to detect significant variation in continuous variables between the groups. Categorical data are expressed as proportions and variation between groups tested by Chi-squared tests, with Fisher exact tests used where the expected cell

frequency was less than five. Correlation coefficients (r^2 values) were calculated to determine the relationship between continuous variables. A p value <0.05 was accepted as statistically significant. All analyses were performed with STATA v13.1 (StataCorp, Texas, USA).

Table 14: Metrics for Assessment of Measures of Glycaemia, Intraday Glycaemic Variations and Hypo-Hyperglycaemic Risk with Normal Reference Ranges quoted where applicable

Absolute Measures of Glycaemia	
Mean	Mean glucose
Standard Deviation (SD)	Calculated from the standard variance of the mean glucose (0.0-3.0mmol/L)
Intraday Glycaemic Variation	
MAG	Mean absolute glucose (MAG) calculates the sum of the differences between successive glucose values divided by the total time measured in hours (0.5-2.2)
CV	Coefficient of variation for glucose is the measure of short-term within-day glucose variability. Calculated as %= (SD/mean glucose)*100. Stable glucose levels indicated by CV < 33%.
Hypo-/ Hyperglycaemic Risk	
LGBI	Risk index for predicting hypoglycaemia (0.0-6.9)
HBGI	Risk index for predicting hyperglycaemia (0.0-7.7)
Normal reference ranges quoted are those from seventy eight subjects without diabetes in different ethnic groups (101)	

3.4. Results

Women with T2DM were older, had a higher early pregnancy BMI and a larger proportion were of non-white ethnicity compared to those with T1DM (Table 15). Median fetal birth weight, birth weight centile and the proportion of infants born large for gestational age (LGA ≥90th centile) were similar in women with T1DM and T2DM (Table 15).

Table 15: Baseline demographics, fetal birthweight and birthweight centile in women with Type 1 Diabetes (T1DM) and Type 2 Diabetes (T2DM)

	T1DM (n=14)	**T2DM (n=19)**	**p value**
Mean (SD) Age (years)	**29.5 (±1.14)**	**34.8 (±1.19)**	**0.004**
Mean (SD) BMI (kg/m^2)	**25.1 (±0.92)**	**32.5 (±1.36)**	**0.002**
Proportion Non-White ethnicity % (n)	**28.6 (4)**	**73.7 (14)**	**0.012**
Mean (SD) HbA1c (mmol/mol)	56.4 (±2.97)	58.8 (±4.06)	0.7
Median (IQR) fetal birthweight (g)	3500 (3000-3875)	3060 (2920-3770)	0.3
Median (IQR) fetal birthweight centile	88 (60-99)	68 (27-97)	0.2
Proportion of infants born LGA % (n)	42.9% (6)	31.6% (6)	0.4

Abbreviations: *SD* standard deviation; *BMI* Body mass index; *IQR* Interquartile range; *LGA* Large for gestational age (> 90th centile).

Mean first trimester HbA1c was similar in women with Type 1 and Type 2 diabetes (Table 16). In total, 29 144 glucose measurements derived from the continuous glucose monitoring data were analysed. With the exception of HBGI, which was higher in women with Type 1 diabetes, markers of absolute glycaemia (mean glucose and standard deviation) and glycaemic variability (CV and MAG) were similar in the two groups (Table 16).

Across the cohort, HbA1c and mean glucose did not correlate with either fetal birth weight or birth weight centile (Table 17). SD, and at borderline significance, HBGI correlated with fetal birth weight centile (r2 0.134 p =0.04 and r2 0.116 p=0.05, Figures 13 and 14 respectively).

Table 16: Markers of glycaemia and glycaemic variability in women with Type 1 diabetes and Type 2 diabetes

	T1DM (n=14)	**T2DM (n=19)**	**p value**
Mean (SD) HbA1c (mmol/mol)	56.4 (±2.97)	58.8 (±4.06)	0.7
Mean glucose	7.9	7.1	0.09
SD	3.1	2.4	0.09
MAG	2.7	2.3	0.9
CV	37.4	27.4	0.3
LBGI	5.2	4.1	0.3
HBGI	**7.8**	**4.4**	**0.03**

Abbreviations with normal reference ranges: *SD* standard deviation *BMI* Body mass index; *SD* Standard error of variation (0.0-3.0mmol/L); *MAG* Mean absolute glucose (0.5-2.2); *CV* Correlation of variation for glucose (<33%); *LBGI* Low blood glucose index (0.0-6.9); *HBGI* High blood glucose index (0.0-7.7).

Table 17: Correlation values for markers glycaemia and glycaemic variability

	Fetal Birth Weight		**Fetal Birth Weight Centile**	
	r^2 value	p value	r^2 value	p value
HbA1c (mmol/mmol)	0.025	0.4	0.069	0.1
Mean glucose (mmol/L)	0.054	0.2	0.072	0.1
SD	0.075	0.1	**0.134**	**0.04**
MAG	0.061	0.2	0.009	0.6
CV	0.026	0.4	0.055	0.2
LBGI	0.004	0.7	0.007	0.6
HBGI	0.033	0.3	**0.116**	**0.05**

Abbreviations with normal reference ranges: *SD* standard deviation *BMI* Body mass index; *SD* Standard error of variation (0.0-3.0mmol/L); *MAG* Mean absolute glucose (0.5-2.2); *CV* Correlation of variation for glucose (<33%); *LBGI* Low blood glucose index (0.0-6.9); *HBGI* High blood glucose index (0.0-7.7).

Figure 13: Scatter plot demonstrating relationship between the standard variance from the mean glucose and adjusted birth weight centile

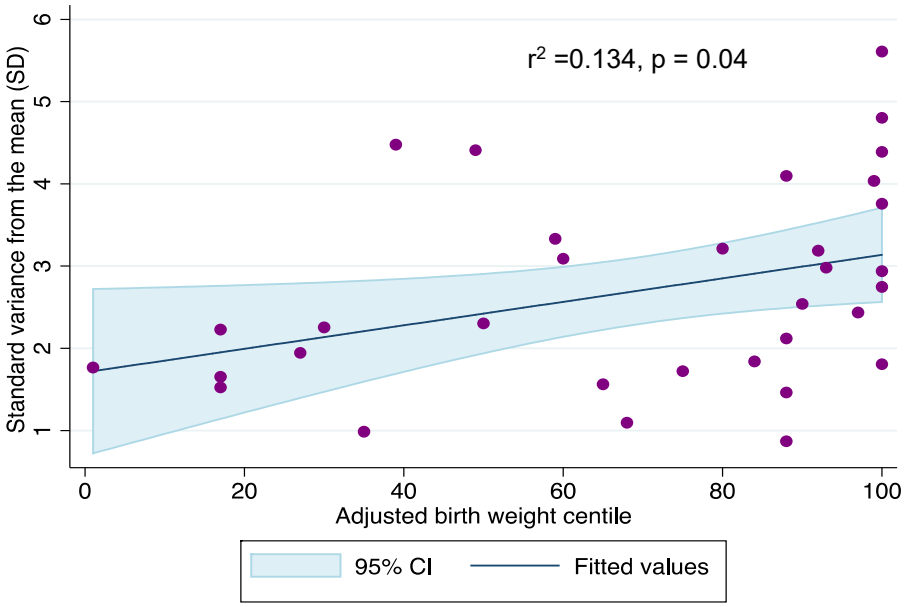
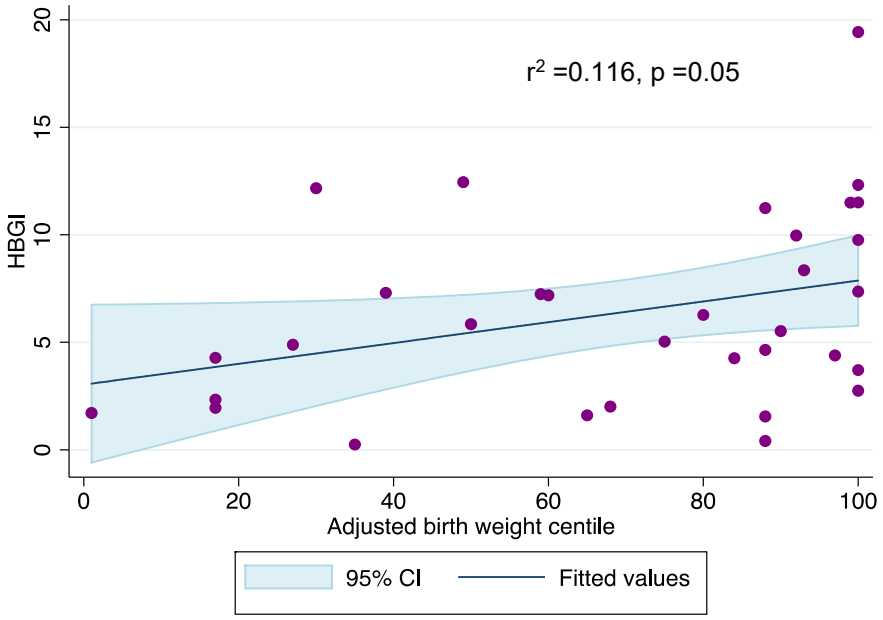


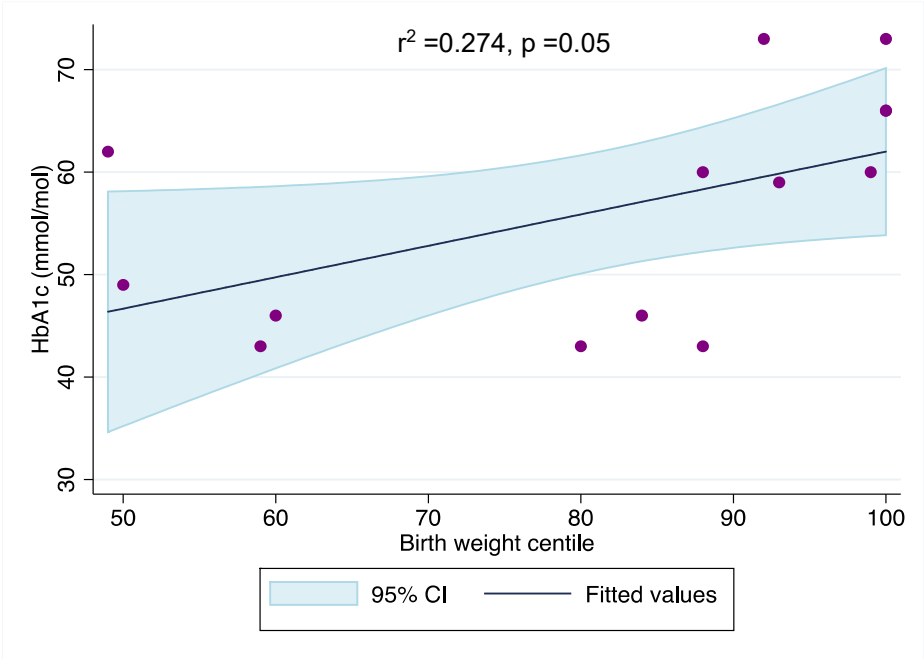
Figure 14: Scatter plot demonstrating the relationship between HBGI (high blood glucose index) and adjusted birth weight centile



3.3.1. Glycaemic control and glycaemic variability in women with Type 1 and Type 2 diabetes

In women with Type 1 diabetes, HbA1c correlated with fetal birth weight centile at borderline significance (r^2 =0.274 p=0.05, Figure 15) but not fetal birth weight: HbA1c did not correlate with either fetal birth weight or adjusted birth weight centile in women with Type 2 diabetes. Further markers of glycaemic variability did not correlate with fetal birth weight or birth weight centile in women with Type 1 or with Type 2 diabetes (Appendix 2).

Figure 15: Scatter plot demonstrating the relationship between HbA1c and adjusted birth weight centile in women with Type 1 diabetes



Women with Type 1 diabetes who delivered LGA infants demonstrated significantly elevated markers of glycaemia (HbA1c, mean glucose and at borderline significance,

SD) and hyperglycaemia risk (HBGI) compared with women who delivered infants in the normal birth weight centile range (10^{th}-90^{th} centile): markers of intraday glycaemic variability or hypoglycaemic risk did not differ according to category of adjusted fetal birth weight (Table 18). HbA1c, absolute glycaemia and glycaemic variability were similar in women with Type 2 diabetes who delivered LGA and appropriate for gestational age infants (Table 18, Figures 16-19).

Table 18: Differences in overall glycaemic control and markers of glycaemic variability in women according to category of birth weight centile in Type 1 Diabetes (T1DM) and Type 2 Diabetes (T2DM)

	T1DM (n=14)			**T2DM (n=19)**		
	Non-LGA infants (n=13)	LGA infants (n=6)	p value	Non-LGA infants (n=8)	LGA infants (n=6)	p value
Mean (SD) HbA1c (mmol/mol)	**49.0 (±2.73)**	**66.2 (±2.47)**	**<0.001**	58.4 (±5.46)	59.7 (±5.67)	0.9
Mean (SD) BMI (kg/m^2)	26.0 (±1.56)	23.8 (±0.17)	0.3	33.1 (±1.47)	31.3 (±3.10)	0.6
Mean glucose (mmol/L)	**7.00**	**9.13**	**0.005**	6.82	7.29	0.5
SD (mmol/L)	**2.56**	**3.86**	**0.05**	2.11	3.01	0.09
MAG	2.43	2.96	0.6	2.26	3.52	0.1
CV	33.38	41.79	0.4	25.59	35.03	0.07
LBGI	6.86	6.47	0.9	4.72	4.10	0.8
HBGI	**5.65**	**12.18**	**0.007**	4.57	5.58	0.6

Abbreviations with normal reference ranges: *BMI* Body mass index; *SD* Standard error of variation (0.0-3.0mmol/L); *MAG* Mean absolute glucose (0.5-2.2); *CV* Correlation of variation for glucose (<33%); *LBGI* Low blood glucose index (0.0-6.9); *HBGI* High blood glucose index (0.0-7.7).

Figures 16 and Figure 17: Differences in HbA1c and mean glucose in women who delivered infants in either the normal centile range or the large for gestational age infants by type of diabetes

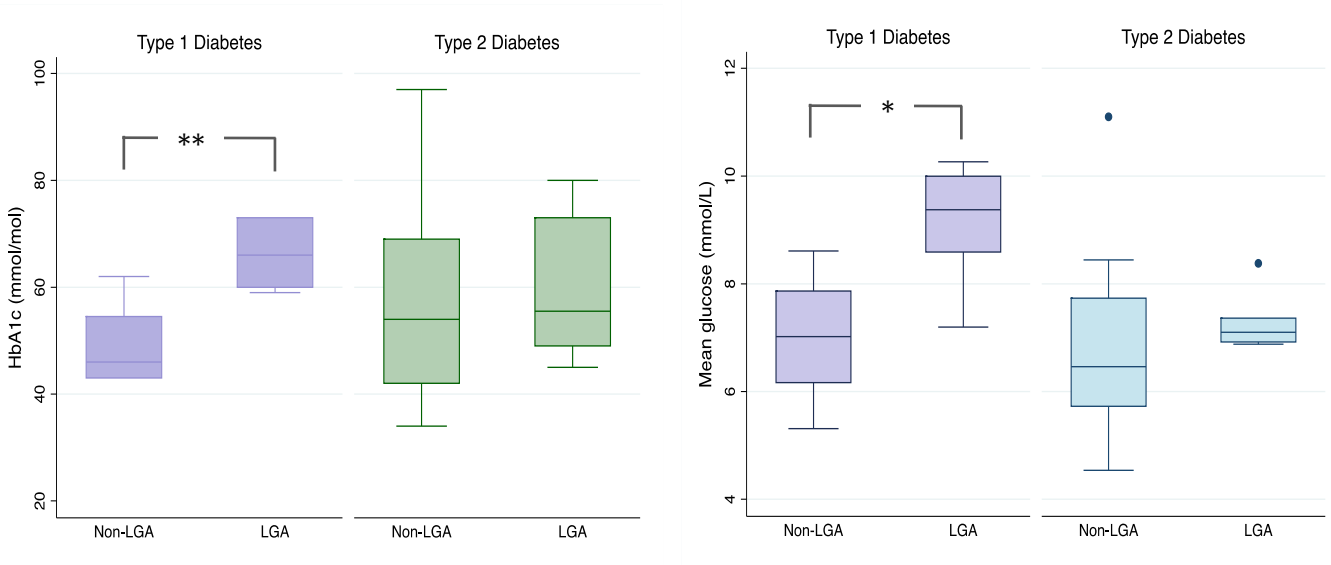


Figure 18: Differences in standard deviation from the mean glucose in women who delivered infants in the normal centile range and the large for gestational age infants by type of diabetes

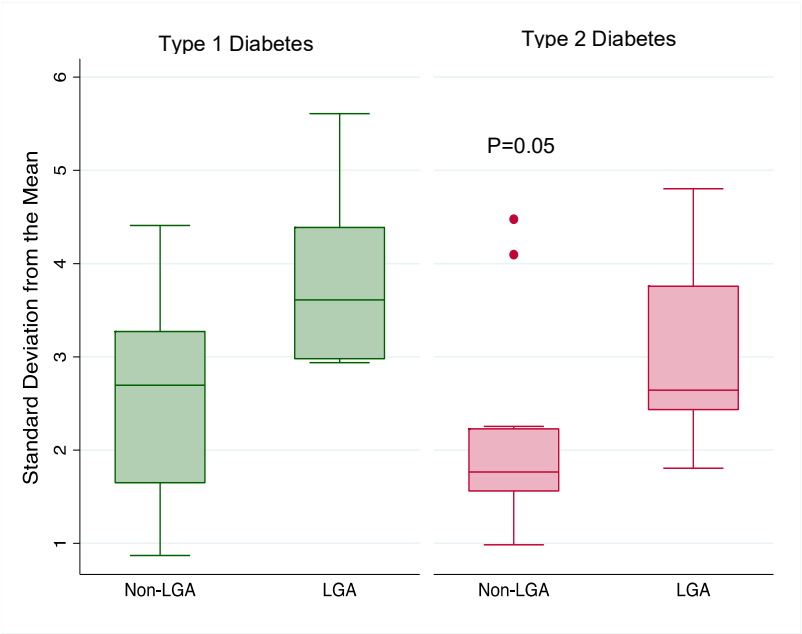
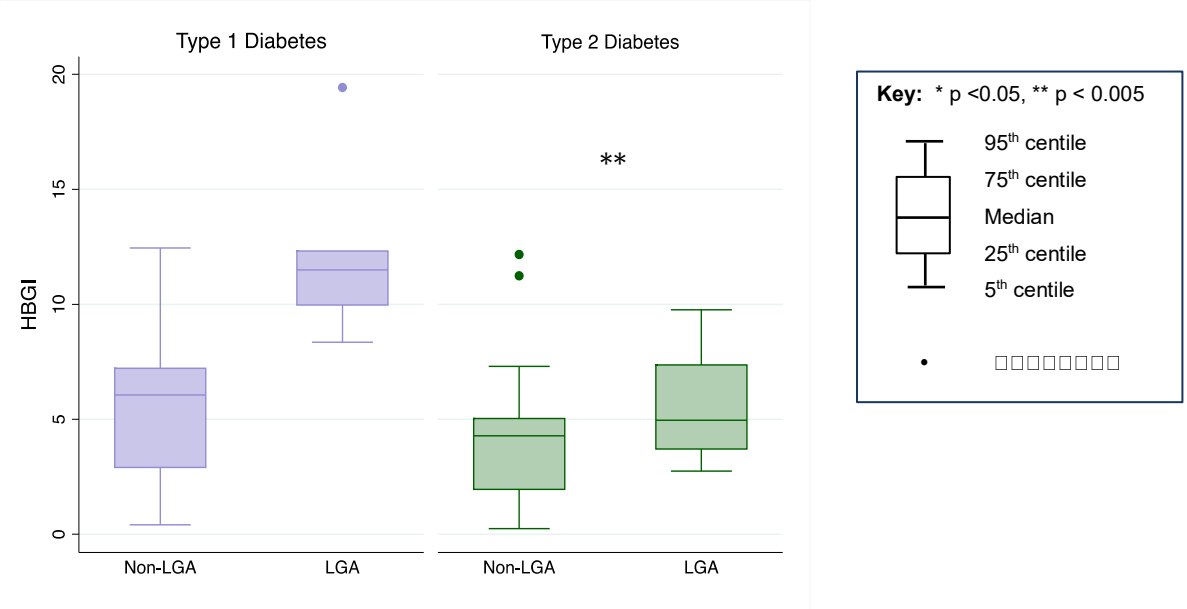


Figure 19: Differences in hyperglycaemia risk (HBGI) in women who delivered infants in the normal centile range and large for gestational age infants by type of diabetes



3.5. Conclusions

In this analysis of 33 women with Type 1 and Type 2 diabetes, glycaemia as indicated by HbA1c and mean glucose, derived from blinded continuous glucose monitoring data, did not correlate with either absolute fetal birth weight or birth weight adjusted for maternal and fetal characteristics. Though SD and HBGI correlated with fetal birth weight centile, the relationship was not strong as demonstrated by the distribution of data.

Women with Type 1 diabetes that delivered large for gestational age infants had higher markers of absolute glycaemia (HbA1c, mean glucose and at borderline significance, SD) and hyperglycaemic risk compared to those who delivered

appropriate for gestational age neonates. Markers of glycaemic control and variability were similar in women with Type 2 diabetes who delivered LGA and appropriate for gestational age infants.

3.6. Discussion

This analysis provides insight into the potential role that glucose excursions could play in fetal growth. A relationship between markers of glycaemic variability (SD and hyperglycaemic risk) and adjusted fetal birth weight was demonstrated in the overall cohort. The data additionally suggested that markers of glycaemic variability together with overall glycaemic control, are associated with fetal overgrowth in women with Type 1 diabetes but not Type 2 diabetes.

This is the first study to investigate the impact of glycaemic variability in fetal growth according to phenotype of diabetes. One large-scale study applied functional data analysis to 1.68 million glucose measurements to evaluate the relationship between trimester-specific mean glucose and incidence of large for gestational age infants (102). The continuous glucose monitoring data from women with Type 1 diabetes (n=89) and Type 2 diabetes (n=28) were combined in this study. Women who delivered LGA infants had higher mean glucose values in all three trimesters compared to those who delivered infants within the normal birth weight centile range (7.0 versus 7.1 mmol/L $p < 0.01$, 7.0 versus 6.7 mmol/L, $p < 0.001$ and 6.5 versus 6.4 mmol/L, $p< 0.01$ across the three trimesters respectively). Functional data analysis demonstrated that temporal variations also existed when analysing women who delivered large for

gestational infants, with lower midmorning and early evening glucose values in this subgroup in the first trimester, higher early morning and afternoon values in the second and higher evening values in the third trimester.

Rather than investigate the specific times of day at which glucose varied, the study presented in this chapter sought to investigate overall markers of glycaemia including glucose excursions and quality of glycaemic control. The finding that glucose contributes to different degrees in pregnancies complicated by Type 1 and Type 2 diabetes is a potentially important one and could have clinical implications. Mean glucose measured 2.13mmol/L higher (p=0.005) in women with Type 1 diabetes who delivered LGA infants relative to the mean 0.1mmol/L difference observed in the aforementioned study, which in day to day clinical practice would be easier to address. With respect to Type 2 diabetes, fetal overgrowth was not associated with increased markers of glycaemia or glycaemic variability, implicating factors beyond glycaemia in pathogenesis. Though the numbers presented here are small, the baseline demographics in the cohort of women included is reflective of national data as indicated by both the both the baseline demographics of women with Type 1 and Type 2 diabetes, as well as the proportion of neonates born large for gestational age (42.9% and 31.6% respectively)(98). Nonetheless, larger studies are needed to ensure that the signals relating to the impact of absolute glycaemia and variability persist.

Further limitations include a potential selection bias as resource limitations were such that at the time of data collection (2015), not all women with Type 1 and Type 2 diabetes were offered continuous glucose monitoring, rather those in which clinical

concerns existed were prioritised. Furthermore, continuous glucose monitoring data from both the second and third trimesters were combined. Differences in physiology at these respective times may have led to differences in glycaemia. A future analysis is planned with larger numbers to address this.

This Chapter has investigated the relationship between both degrees and variations of glycaemia in the development of fetal growth. A relationship has been demonstrated in women with Type 1 diabetes but not Type 2 diabetes. In the next Chapter, the impact of length of exposure to hyperglycaemia on materno-fetal outcomes will be evaluated.

CHAPTER 4: HYPERGLYCAEMIA RECOGNISED IN EARLY PREGNANCY AND THE IMPACT OF LENGTH OF EXPOSURE TO MATERNAL HYPERGLYCAEMIA ON FETAL OVERGROWTH

4.1. Introduction

Gestational diabetes mellitus (GDM) was traditionally defined as "hyperglycaemia first detected in pregnancy" (103). As such, the definition encompassed a wide range of clinical phenotypes with women with underlying genetic variants, autoimmunity or Type 2 Diabetes (T2DM) potentially labeled with the diagnosis if they presented with hyperglycaemia in pregnancy. However, as detailed in Chapter 1, GDM was first described to detect women at an increased risk of developing T2DM later in adult life. Furthermore, GDM has generally been seen as a temporary disturbance in glucose tolerance. Indeed, the majority of cases resolve post-partum: one large-scale prospective cohort of 5939 women whose pregnancies were complicated by GDM demonstrated that 82.6% women had normal glucose tolerance within a six month period of delivery (104).

The concerns relating to the increasing incidence of obesity and the subsequent potential for un-recognised T2DM amongst women of childbearing age prompted international authorities to revise the definition to the following: "diabetes first recognised in the second or third trimester that is not clearly overt diabetes" (105). The timeframe for diagnosing GDM was narrowed in view of the revisions to glycaemic

thresholds used to define the condition. These revised diagnostic criteria are based on findings from the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study, the previously described observational study that demonstrated a continuum between glucose and the proportion of infants born large for gestational age in 23,316 women who underwent 75g oral glucose tolerance testing (OGTTs)(2). Women with higher degrees of glycaemia (fasting plasma glucose (FPG) ≥5.8mmol/L; 120 minute value ≥11.1mmol/L) were un-blinded and excluded from the subsequent analysis. Diagnostic testing in this study was specifically carried out at 24-28 weeks gestation. The two preceding landmark trials that demonstrated a reduction in materno-fetal adverse outcomes when "mild hyperglycaemia" was treated additionally tested for GDM at 24-28 weeks gestation (24, 106). However, though the risk of partial insulin secretory deficits being exposed in predisposed individuals is greatest when insulin resistance is at its peak i.e. in the third trimester, defective beta cell function is often evident prior to this (17, 18). The optimal time to test for gestational diabetes is therefore unclear: sufficient time is needed for the hyperglycaemia to develop while simultaneously allowing an adequate treatment period for effective adverse outcome risk modification. Maternal glycaemia should additionally be considered early in pregnancy to detect pre-existing un-recognised T2DM given the theoretical risks associated with prolonged exposure to hyperglycaemia e.g. the potential teratogenic effects associated with hyperglycaemia during the period of organogenesis (95).

There is an emerging evidence base demonstrating that women with hyperglycaemia detected early in pregnancy have an increased risk of adverse materno-fetal outcomes when compared to those individuals with GDM diagnosed on routine testing (107). As

has previously been outlined, maternal obesity is an independent predictor of adverse materno-fetal outcomes and the results from these studies, which will be discussed in greater detail in Section 3.7, were confounded by significant variations in maternal weight across the groups analysed.

To investigate whether the length of exposure to glucose adversely affects materno-fetal outcomes independent of adiposity, a case control study was designed, the methodology and results for which are presented in this chapter.

4.2. Hypothesis

- Length of exposure to glucose will adversely affect materno-fetal outcomes independent of maternal weight
- Women diagnosed with hyperglycaemia early in pregnancy will phenotypically resemble those with T2DM in terms of outcomes rather than those with GDM diagnosed on routine testing.

4.3. Aims and Study Design

To investigate these hypotheses a case control study was designed to compare women with hyperglycaemia detected in early pregnancy (prior to 20 weeks gestation) to two separate control groups matched for early pregnancy body mass index (BMI): one with recognised pre-gestational T2DM and the second with GDM diagnosed on routine testing i.e. 24-28 weeks gestation.

4.4. Methodology

Pregnant women with hyperglycaemia detected prior to routine diagnostic testing, who attended the multidisciplinary antenatal clinic at St Mary's Hospital, Imperial College NHS Trust in London, were retropsectively reviewed. Acquisition of data originally began in 2010 and continued over a five-year period to 2015. Forty consecutive women were identified and these comprised the early hyperglycaemia (eGDM) group. These women had been identified to be at risk of developing hyperglycaemia prior to 20 weeks gestation either by nature of having had a previous pregnancy complicated by gestational diabetes, or due to an incidental finding of glycosuria in the first or early second trimester. All women meeting the former criteria had been provided with a home glucometer and standard dietary advice at 16 weeks gestation, unless a random plasma glucose (RPG) and or an HbA1c was elevated, in which case the intervention and subsequent follow up in the multidisciplinary diabetes antenatal clinic were expedited. The same intervention was provided for those with glycosuria and hyperglycaemia confirmed on HbA1c or 75g OGTT testing. Where dietary measures failed to adequately achieve target capillary blood glucose (CBG) values (fasting CBG <6.0mmol/L or 1 hour postprandial CBG <8.0mmol/L) metformin and/or insulin, were commenced as appropriate.

Two separate control groups, frequency-matched for early pregnancy BMI, were retrospectively identified from the cohort attending the same multidisciplinary antenatal clinic. The first control group consisted of 80 consecutive pregnant women with a clinician-assigned diagnosis of Type 2 Diabetes Mellitus that had been

established at least 3 months prior to conception (T2DM group); the second consisted of 80 women with GDM who had been diagnosed on routine testing at 24-28 weeks gestation (rtGDM group). The individuals within the rtGDM group were identified from the antenatal cohort attending the same multidisciplinary antenatal clinic over a one-year period (September 2014 to September 2015). In accordance with the National Institute for Clinical Health and Excellence (NICE) guidelines, all women at Imperial College NHS Trust are screened for GDM risk factors at their initial antenatal assessment (26). The risk factors adapted from the NICE guidelines, defining the need for a diagnostic 75g OGTT at 24-28 weeks gestation consisted of any one of the following: non-White ethnicity, an early pregnancy BMI ≥30kg/m^2, a previous pregnancy resulting in the delivery of a macrosomic infant (≥4000g) and a first-degree relative with T2DM. The modified 1999 WHO gestational diabetes diagnostic criteria were in use at the time of this study: FPG ≥6.0mmol/L, 75g OGTT 120 minute ≥7.8mmol/L (34). All women with a new diagnosis of GDM were immediately referred to the multidisciplinary antenatal clinic with an HbA1c measured prior to their attendance. Women whose results fulfilled diagnostic criteria for overt diabetes, either on the 75g OGTT or HbA1c, were excluded from this second control group. In addition, the baseline demographics, fetal birth weights and adjusted birth weight centiles of the 365 women diagnosed with GDM in 2014-2015 were reviewed to ascertain whether the rtGDM control group accurately reflected routinely diagnosed women (see Results Section 3.5.4.).

Prospectively collected maternal antenatal and delivery records were examined to establish baseline maternal demographics including self reported ethnicity, obstetric

history, parity (categorised as primagravida, parous 1-3 births, or multiparous 4 or more births ≥24 weeks gestation), anthropometric measurements (height, weight and BMI) and biochemical data. Hypertension at baseline was defined as either a blood pressure measuring ≥140mmHg systolic or ≥90mmHg diastolic and or the use of anti-hypertensive agents at the initial antenatal visit. Maternal outcomes evaluated included the following: initiation of insulin therapy at any point during the course of pregnancy, mode of delivery and development of pregnancy related hypertensive disorders. The latter was recorded as a composite outcome and a score of 1 applied if either of the following developed: pregnancy induced hypertension (PIH) i.e. hypertension developing after 20 weeks gestation (≥140/90mmHg or either a rise in systolic BP ≥30mmHg or ≥15-20mmHg in diastolic BP) and or pre-eclampsia (PET) defined as new hypertension presenting after 20 weeks gestation together with significant proteinuria (random urine protein to creatinine ratio >30). The final maternal outcome assessed was postpartum glycaemia: all women diagnosed with hyperglycaemia in pregnancy were invited to a 6-week postpartum FPG assessment and a subsequent 75g OGTT and HbA1c measurement was planned in those with impaired fasting glucose values (6.1 - 6.9mmol/L).

Delivery and neonatal records were examined to establish fetal birth weight, gender, gestational age at delivery and incidence of neonatal complications. The GROW gestation network calculator was used to determine customised birth weight centiles through adjusting fetal birth weight for maternal height, weight, ethnicity, parity, fetal gestational age and gender (88). Large for gestational age (LGA) infants were defined as infants with an adjusted birth weight ≥90th centile: small for gestational age (SGA) as those with a birth weight <10th centile. Preterm delivery was defined as delivery

prior to 37 weeks gestation i.e. 36 completed weeks. Neonatal complications were recorded as a composite outcome with a score of 1 applied if one or more of the following adverse events were recorded: shoulder dystocia, neonatal hypoglycaemia requiring treatment, respiratory distress syndrome requiring either oxygen therapy or continuous positive airway pressure, admission to the neonatal intensive care unit, or hyperbilirubinaemia requiring phototherapy.

4.4.1. Statistical Analysis

Continuous data are expressed as mean (±SD) or median (interquartile range) depending on the distribution of the data: ANOVA or Kruskal-Wallis tests were used as appropriate to detect significant variation in continuous variables between the groups. Categorical data are expressed as proportions and variation between groups tested by Chi-squared tests, with Fisher exact tests used where the expected cell frequency was less than five. A p value <0.05 was accepted as statistically significant. When significant variation between groups was detected, hypothesis driven post-hoc between-group comparisons were undertaken using t-test, Mann-Whitney test or proportions test as appropriate. All analyses were performed with STATA version 13.1 (StataCorp, Texas, USA).

4.5. Results

In this cohort of 200 women, mean (SD) age was 33.9 (±4.5) years and BMI 31.7 (±5.3) kg/m^2: overall 81% were of non-White ethnic origin with women of South Asian

ethnicity forming the largest ethnic sub-group (27.5%). Maternal baseline demographics in each of the three groups are tabulated (Table 19). In addition to the control groups being matched for early-pregnancy BMI, both mean age and the proportion of women of non-White ethnicity were similar in the three groups.

There was a significant variation in HbA1c at initial identification of hyperglycaemia between the groups (Table 19), with no difference in median HbA1c between the eGDM group and T2DM control group on post hoc testing ($p=0.1$), and a significantly higher HbA1c in the eGDM compared with the rtGDM group ($p<0.001$). Analysis by HbA1c category at baseline showed that 80.0% in the eGDM group and 18.7% in the rtGDM group had an HbA1c ≥43mmol/mol. In the eGDM group, 37.5% had an HbA1c ≥48mmol/mol: by definition, no women in the rtGDM group had an HbA1c diagnostic of type 2 diabetes i.e. ≥48mmol/mol. In the type 2 diabetes control group, 56.3% of women had an initial HbA1c exceeding the national recommendation for pre-conceptual planning i.e. ≥48mmol/mol (26). On average, women with T2DM had been diagnosed 3.0 (2.0-6.5) years prior to pregnancy.

Table 19: Maternal baseline demographics and biochemical data in women with hyperglycaemia diagnosed prior to 20 weeks gestation (eGDM), women with recognised pre-gestational Type 2 Diabetes (T2DM) and women with Gestational Diabetes diagnosed on routine testing (rtGDM)

	eGDM (n=40)	**T2DM (n=80)**	**rtGDM (n=80)**	**Significance**
Mean (SD) Age (years)	33.9 (±4.5)	34.2 (±5.1)	33.7 (±5.5)	0.4
Mean (SD) Height (cm)	161.7 (±7.3)	161.5 (±7.2)	160.8 (±6.0)	0.8
Mean (SD) Weight (kg)	83.6 (±15.8)	84.1 (±19.2)	78.8 (±12.5)	0.1
Median (IQR) BMI (kg/m^2)	32.0 (27.0-35.0)	31.0 (28.0-35.9)	30.4 (27.9-33.9)	0.5
Non-White ethnicity % (n)	80.0 (32)	86.2 (69)	76.3 (61)	0.3
Black African-Caribbean	25.0 (10)	26.2 (21)	22.5 (18)	
Arab/ North African	20.0 (8)	15.0 (12)	7.5 (6)	
South Asian	25.0 (10)	37.5 (30)	18.8 (15)	
Other	10.0 (4)	7.5 (6)	27.5 (22)	
Parity				
Primigravida % (n)	17.5 (7)	18 (22.5)	37 (46.3)	<0.001
Multiparous %(n) [I]	25.0 (10)	11 (13.8)	4 (5.0)	
History previous pregnancy complicated by GDM % (n)	71.8 (28)	38.5 (30)	0.0 (0)	<0.001
Diagnosis Hypertension %(n) [II]	20.0 (8)	23.4 (18)	3.8 (3)	0.001
Median (IQR) HbA1c (mmol/mol)	46 (43-56)	51 (43-62)	38 (34-40)	<0.001

Abbreviations: *IQR* Interquartile range; *BMI* Body mass index (measured at initial antenatal visit); *GDM* Gestational diabetes.

I. Defined as four or more deliveries after 24 weeks gestation
II. On anti-hypertensive medications and/ or blood pressure measured ≥140/90mmHg at initial antenatal visit.

There was significant variation in parity status across the three groups (Table 19). In post hoc analysis, primigravida status was lower in the eGDM compared to the rtGDM

group (p=0.002) and multiparity status higher (p=0.001): parity status did not differ significantly between the eGDM and type 2 diabetes groups.

Additionally, significant variation between the groups in the proportion of women with a previous pregnancy complicated by GDM existed. No women in the rtGDM group had a previous pregnancy complicated by GDM, whereas in the eGDM group the proportion was 71.8% (p<0.001). This was also higher than the 38.5% in the T2DM group (p<0.001). There was significant variation in the proportion of women with essential hypertension at baseline, with a higher proportion in the eGDM compared with the rtGDM group (p=0.004) but no difference between the eGDM versus the T2DM group (p=0.6) on post-hoc testing.

4.5.1. Maternal outcomes

There were significant differences in both the proportion of women requiring insulin treatment and those developing pregnancy-related hypertensive disorders in the three groups, with, on post hoc testing, no significant differences in proportions between the eGDM and T2DM groups (p=0.1 and p=0.2 respectively) and significantly higher proportions in the eGDM compared to the rtGDM group (p<0.001 and p<0.001 respectively) (Table 20).

No differences existed in the incidence of postpartum haemorrhage, either moderate (500-1000ml) or severe (≥1000ml).

Differences existed in delivery modality in the three groups (Table 20). However, a

sub-analysis demonstrated that emergency caesarean delivery rates were similar (22.5%, 27.5% and 21.3% in the eGDM, T2DM and rtGDM groups respectively, p=0.2).

Table 20: Outcomes in women with hyperglycaemia diagnosed prior to 20 weeks gestation (eGDM), women with recognised pre-gestational Type 2 Diabetes (T2DM) and women with Gestational Diabetes diagnosed on routine testing (rtGDM)

	eGDM (n=40)	**T2DM (n=80)**	**rtGDM (n=80)**	**Significance**
Proportion requiring insulin treatment % (n)	88.6 (31)	77.0 (57)	8.1 (6)	<0.001
Hypertensive disorders of pregnancy % (n) [I]	42.5 (17)	37.5 (26)	12.5 (5)	<0.001
Delivery modality %(n) SVD AVD Elective Caesarean Section Emergency Caesarean Section	27.5 (11) 12.5 (5) 37.5 (15) 22.5 (9)	31.3 (25) 5.0 (4) 36.3 (28) 27.5 (22)	46.3 (37) 16.3 (13) 16.3 (13) 21.3 (17)	0.01
Postpartum Haemorrhage Moderate (500-1000ml) % (n) Severe (≥1000ml) % (n)	 35.9 (14) 10.3 (4)	 32.5 (26) 8.8 (7)	 31.3 (25) 8.8 (7)	0.9
Median (IQR) postpartum fasting glucose (mmol/L)	5.7 (4.9-6.8)	NA	5.0 (4.6-5.3)	0.03

Abbreviations: *SVD* Spontaneous vertex delivery, *AVD* Assisted vaginal delivery, *LGA* Large for gestational age (adjusted birth weight ≥90th centile), *SGA* Small for gestational age (adjusted birth weight <10th centile). I. Hypertensive disorders of pregnancy recorded as a composite outcome if one of more of the following developed: Pregnancy induced hypertension (PIH: development of blood pressure ≥140/80 or increase in systolic blood pressure by 20mmHg from 20 weeks gestation) or Pre-eclampsia (PET: defined as development proteinuria and hypertension).

4.5.2. Fetal outcomes

In total, eight stillbirths were recorded in this cohort: 4 in the eGDM group, 3 in the T2DM group and 1 in the rtGDM group (10.0%, 3.8%, 1.3% stillbirth rate in the three groups respectively, p=0.069) (Table 21). Data from the stillbirths were excluded from the subsequent fetal outcome analyses. No congenital malformations or neonatal deaths were recorded in the remaining cohort. Median fetal birth weight and adjusted birth weight centile were similar in the three groups (Table 21 and Figure 20).

Table 21: Fetal outcomes in women with hyperglycaemia diagnosed prior to 20 weeks gestation (eGDM), women with recognised pre-gestational Type 2 Diabetes (T2DM) and women with gestational diabetes diagnosed on routine testing (rtGDM)

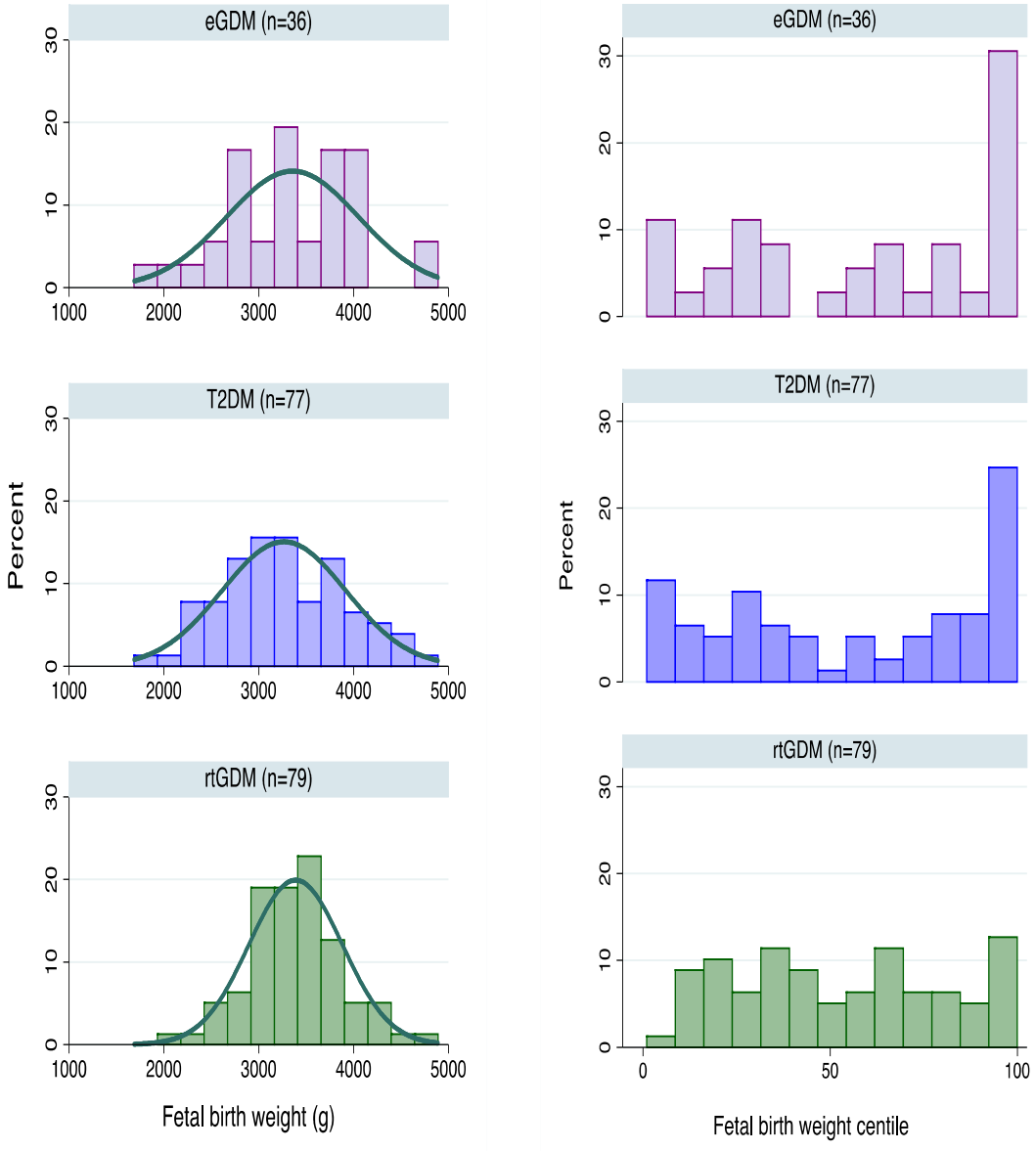
	eGDM (n=40)	**T2DM (n=80)**	**rtGDM (n=80)**	**Significance**
Stillbirth % (n)	10.0 (4)	3.8 (3)	1.3 (1)	0.07
Median (IQR) fetal birthweight (g)	3350 (2820-3840)	3225 (2855-3735)	3370 (3090-3670)	0.3
Median (IQR) birthweight centile [I]	63.6 (26.0-98.1)	61 (26.0-91.4)	50 (29.0-76.7)	0.5
Infants born LGA % (n)	30.6 (11)	27.3 (21)	17.7 (14)	0.2
Infants born macrosomic (≥4000g) % (n)	19.4 (7)	15.6 (12)	11.4 (9)	0.5
Infants born SGA % (n)	11.1 (4)	13.0 (10)	2.5 (2)	0.05
Preterm delivery (<37 weeks gestation) % (n)	30.0 (12)	20.0 (16)	3.8 (2)	<0.001
Neonatal complications % (n) [II]	11.1 (4)	13.0 (10)	11.4 (9)	0.9

Abbreviations: *LGA* Large for gestational age (adjusted birth weight ≥90th centile), *SGA* Small for gestational age (adjusted birth weight <10th centile).

I. Fetal birth weight centile is equal to fetal birth weight adjusted for maternal height, weight, ethnicity, fetal gender and gestational age at delivery.

II. Composite outcome with score of 1 applied if one of the following occurred: shoulder dystocia, neonatal hypoglycaemia requiring treatment, neonatal hyperbilirubinaemia requiring phototherapy, respiratory distress requiring either oxygen or continuous positive airway pressure and requirement for neonatal intensive care.

Figure 20: Fetal birth weight and adjusted birth weight distribution born to women with hyperglycaemia diagnosed prior to 20 weeks gestation (eGDM), women with recognised pre-gestational type 2 diabetes (T2DM) and women with gestational diabetes diagnosed on routine screening (rtGDM).



No differences existed in the proportion of infants born either large for gestational age (LGA; ≥90th birth weight centile) or macrosomic (fetal birth weight ≥4000g). Significant variations existed in the proportion of infants born small for gestational age

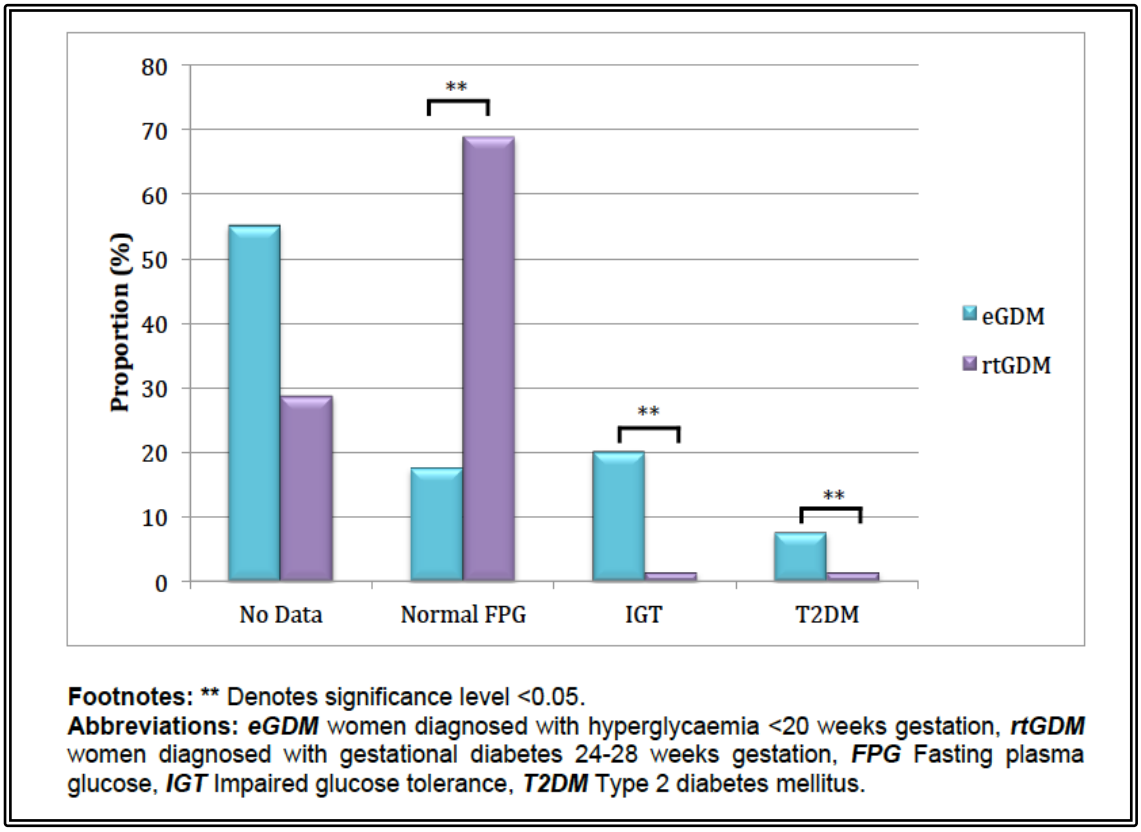
(SGA: ≤10th centile): post hoc analysis demonstrated no significant difference in the proportion between the eGDM and T2DM group (p=0.7) but a higher proportion in the eGDM compared with the rtGDM group at borderline significance (p=0.05).

There was significant variation in the proportion of neonates born preterm, with women with eGDM significantly more likely to deliver prior to 37 weeks gestation compared to those with rtGDM on post hoc analysis: there was no significant difference in the preterm delivery rate between the eGDM and T2DM group (p=0.2) (Table 16). Infants born to women in the eGDM groups had the highest observed rate of neonatal complications. However, no significant difference existed in the neonatal complication rates in the three groups (16.7%, 13.3% and 5.0%, p=0.4).

4.5.3. Postpartum Glucose Assessments

Postpartum glucose assessments were offered to all women diagnosed with hyperglycaemia in pregnancy: 18 women with eGDM (45.0%) and 57 women with rtGDM (71.3%) attended. Median (IQR) fasting plasma glucose levels were higher in the latter group: 5.7 (4.9-6.8) mmol/L versus 5.0 (4.6-5.3) mmol/L, p=0.02 (Table 20). Individuals with impaired fasting plasma glucose were invited to attend for a 75g OGTT. Overall, a higher proportion of women in the eGDM group were diagnosed with either impaired glucose tolerance or type 2 diabetes within a 3 month period of assessment: 20.0% versus 1.3% and 7.5% versus 1.3% respectively, p<0.001 (Figure 21).

Figure 21: Bar chart demonstrating postpartum glucose assessments in women with hyperglycaemia diagnosed in pregnancy



Footnotes: ** Denotes significance level <0.05.
Abbreviations: ***eGDM*** women diagnosed with hyperglycaemia <20 weeks gestation, ***rtGDM*** women diagnosed with gestational diabetes 24-28 weeks gestation, ***FPG*** Fasting plasma glucose, ***IGT*** Impaired glucose tolerance, ***T2DM*** Type 2 diabetes mellitus.

4.5.4. Baseline demographics and fetal birth weight centile in women diagnosed with GDM

To ascertain if the rtGDM group reflected the cohort of women diagnosed with GDM at Imperial College Healthcare NHS Trust, the baseline demographics and fetal birth weight and birth weight centile born to women whose pregnancies were complicated by GDM over a one-year period (2014-2015) were examined (Table 22). Women selected to the rtGDM group had a higher early pregnancy BMI than the cohort of 365 women who were treated for GDM. Variations additionally existed in the median fetal birth weight and birth weight centile in the two groups (Table 22).

Table 22: Baseline demographics in women in the rtGDM group and the entire cohort of women with routinely diagnosed GDM (2014-2015)

	rtGDM	GDM	Significance
N	80	365	
Mean (SD) Age (years)	33.7 (±5.5)	33.4 (±5.9)	0.6
Median (IQR) BMI (kg/m^2)	30.4 (27.9-33.9)	26.1 (22.7-30.3)	<0.001
Non-White ethnicity % (n)	76.3 (61)	80.8 (295)	0.4
Median (IQR) fetal birthweight (g)	3370 (3090-3670)	3170 (2800-3520)	<0.001
Median (IQR) birthweight centile [I]	50 (29-76)	38 (19-70)	0.02

Abbreviations: *IQR* Interquartile range; *BMI* Body mass index (measured at initial antenatal visit); *GDM* Gestational diabetes.

I. Fetal birth weight centile is equal to fetal birth weight adjusted for maternal height, weight, ethnicity, fetal gender and gestational age at delivery.

4.6. Conclusions

These data suggest that women with hyperglycaemia detected early in pregnancy resemble women with established Type 2 Diabetes (T2DM) in terms of maternal outcomes, with a similar proportion of women requiring insulin treatment and developing hypertensive disorders in pregnancy compared to those diagnosed with Gestational Diabetes (GDM) diagnosed on routine testing i.e. 24-28 weeks gestation. Furthermore, risk of glucose intolerance persisting postpartum was heightened in women with early hyperglycaemia. These findings were independent of maternal age and adiposity.

In terms of fetal outcomes, a similar proportion of neonates were born preterm (<37 weeks gestation) in women with early hyperglycaemia and with T2DM: the rate in the former group was significantly higher than among women with routinely diagnosed GDM. Variations in the stillbirth rate and the proportion of infants born small for gestational age in the three groups were also observed, with the lowest rate being demonstrated in women with routinely diagnosed GDM (rtGDM). These differences were non-significant on post-hoc testing.

Both the control groups i.e. the T2DM and rtGDM groups were frequency matched for early pregnancy body mass index. The baseline demographics of the rtGDM group were compared to the 365 women whose pregnancy was complicated by GDM and who were treated for GDM over the same one-year period. Mean age was similar in the two groups. However, women in the rtGDM group had a higher early pregnancy body mass index and the neonates born to this group had a higher median birth weight and birth weight centile, reflecting the fact that this selected control group did not accurately reflect those women routinely diagnosed with GDM and were in fact, a higher risk group.

4.7. Discussion

The original data presented in this chapter suggest that the length of exposure to glucose may adversely affect materno-fetal outcomes independent of maternal adiposity and age.

These data complement those described by a large retrospective study conducted in Australia (107). In this study, data from 4873 women attending an antenatal centre over a ten-year period were examined. Women were categorised into one of 4 groups: pre-existing diabetes, hyperglycaemia detected at <12 weeks gestation, hyperglycaemia detected at 12-23 weeks and lastly, hyperglycaemia diagnosed ≥24 weeks gestation. Requirement for insulin therapy, hypertensive disorders, preterm delivery, and caesarean sections were all more prevalent in women with pre-existing diabetes and early gestational diabetes. Both maternal age and obesity are independent risk factors for adverse materno-fetal outcomes including macrosomia and pregnancy-related hypertensive disorders (37, 108). Significant variations in the mean pre-pregnancy BMI existed across the four groups in the Australian study with women in the pre-existing diabetes group having the highest BMI (mean 30.2 SD ±6.2kg/m^2), and those in the group diagnosed at 24 weeks gestation the lowest (mean 24.2 SD ±5.3kg/m^2): in those diagnosed at <12 weeks, mean (SD) BMI measured 28.0 ±6.9kg/m^2. In contrast, BMI was matched across the three groups in our case control study, removing the potential for confounding and clearly demonstrating that the spectrum of GDM to T2DM is defined not only by the degree of glycaemia as illustrated in the HAPO study, but also by the length of exposure to hyperglycaemia (2).

In contrast to the Australian study, no significant variations existed in the proportion of infants born macrosomic (≥4000g) in our study. However, the distribution of birth weight was wider in the eGDM and T2DM groups compared to the rtGDM group and variations existed in the proportion of infants born small for gestational age (<10th centile). This study was not designed to demonstrate the cause of small for gestational age neonates. The observational finding could relate to placental insufficiency associated with the increased incidence of hypertensive disorders observed in these groups. A non-significant higher rate of stillbirths was observed in women with eGDM. Both Type 1 and Type 2 Diabetes Mellitus are associated with an increased risk of congenital malformations, which is mediated by the teratogenic effects of hyperglycaemia in addition to the increased substrate delivery and the oxidative stress observed during the period of organogenesis (95). The higher stillbirth rate demonstrated in the eGDM group could indeed indicate developmental malformations secondary to early pregnancy hyperglycaemia and similar pathophysiological features. However, this could not be determined as the necropsy reports were not available for review.

The case-control study in this chapter has further limitations. The diagnosis of T2DM was clinician assigned. In addition, there were no antibody results available for women in the eGDM group and theoretically, given the earlier presentation, the physiological stress associated with pregnancy could unmask T1DM or even disclose women who have undiagnosed genetic variants.

The proportion of women of non-White ethnicity was similar in the three groups in

this study. As the original data in Chapter 2 demonstrates, baseline demographics and materno-fetal outcomes vary across five different ethnic groups. Therefore, residual confounding on sub-ethnicity classifications may have existed: however, the numbers were too small to assess for this in the case-control study presented in this Chapter.

Decisions to intensify treatment during the antenatal period were generally based on home capillary blood glucose monitoring values. Laboratory-based assessments of glucose control during the antenatal period are not available for all the women in this cohort rendering it difficult to compare the effectiveness of glycaemic control strategies across the three groups, which in turn may have been a confounding factor.

Finally, postpartum glucose assessments were dependent on subsequent clinic attendance, which was incomplete: 37.5% of women diagnosed with hyperglycaemia in pregnancy did not attend their postpartum follow up appointments. Though 72.5% of the women who attended in the eGDM group had normoglycaemia postpartum, the length of follow-up may have been insufficient, particularly when considering the evidence suggesting that breast-feeding is associated with a delay in progression to T2DM in women whose pregnancies are complicated by GDM (109).

This was an exploratory, observational study examining whether there might be an issue relating to the length of exposure to hyperglycaemia independent of variation in adiposity. Appreciably larger studies will be needed to confirm that the signals observed persist.

In addition, future work should focus on viable screening strategies for high-risk groups of women and the appropriate diagnostic criteria for hyperglycaemia in early pregnancy. In this study, the majority of women were screened for hyperglycaemia early in pregnancy due to a history of a previous pregnancy complicated by gestational diabetes (71.8%), indicating that this is an important risk factor. Differences in parity status were also found with women with early hyperglycaemia being less likely to be in their first pregnancy and more likely to be multiparous. Data from the Australian study indicate that a higher incidence of a family history of T2DM is found in women with early hyperglycaemia compared to those with GDM diagnosed ≥24 weeks gestation.

The most appropriate criteria to diagnose hyperglycaemia in early pregnancy are the subject of considerable debate. In the case control study presented in this chapter, significant variations existed in HbA1c at initial diagnosis. However, twenty per cent of individuals in the eGDM group had an HbA1c that would be considered normal in a non-pregnant population i.e. <43mmol/mol indicating that there are limitations to the use of HbA1c as a diagnostic tool. The reasons for these limitations probably relate to the increased red cell turn over observed in pregnancy and the increased incidence of iron deficiency, which in itself is known to elevate HbA1c values independent of associated changes in glucose indices (99). In addition, ethnicity based variations in HbA1c exist, further rendering it difficult to clearly define an appropriate threshold at which GDM should be diagnosed (110, 111).

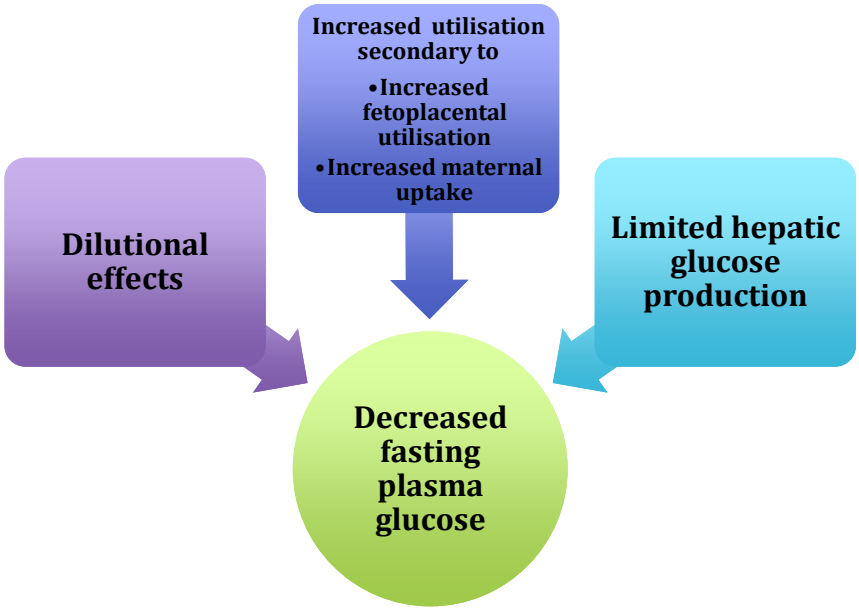
One prospective study conducted in New Zealand demonstrated that women with an

HbA1c above 41mmol/mol prior to 20 weeks gestation had a positive predictive value of 52.9% for developing GDM later in the pregnancy. In women with an HbA1c that exceeded this threshold, relative risk of a major congenital anomaly, preeclampsia, shoulder dystocia and perinatal death were all increased (112). The potential for ethnicity-based variations in HbA1c were addressed in a subsequent prospective cohort study conducted in Barcelona, which demonstrated that an HbA1c exceeding a 41mmol/mol threshold was associated with a significantly increased risk of macrosomia and development of pre-eclampsia in a multi-ethnic cohort following adjustment for confounding factors (113).

In relation to the most appropriate diagnostic fasting plasma glucose threshold, international authorities including the ADA recommend that a value equal or greater to 5.1mmol/L should be used to confirm gestational diabetes in the early stages of pregnancy (54, 103). However, a substantial fall in glucose values is demonstrated in early pregnancy both in women with normal glucose tolerance and those who later develop GDM. This is in part related to the increase in feto-placental utilisation of glucose and the increase in maternal uptake relative to limited hepatogluconeogenesis (Figure 22). The haemodilutional state observed in pregnancy further contributes to the decrease in glucose levels relative to the non-pregnant state. Prospective cohort studies conducted in China and Italy have suggested that an early pregnancy threshold of 5.1mmol/L is poorly predictive of development of GDM in the second or third trimester even in those individuals who go on to exhibit hyperglycaemia later in their pregnancy (114, 115). One further avenue to explore would be practical methods for screening for insulin sensitivity changes in high-risk

groups in early pregnancy, given that reductions in indices are observed prior to the onset of hyperglycaemia (5).

Figure 22: Changes in fasting plasma glucose during pregnancy



The case control study described in this chapter has demonstrated that women with hyperglycaemia detected early in pregnancy represent a separate clinical entity to those diagnosed with hyperglycaemia on routine diagnostic testing, implicating the importance of length of exposure to hyperglycaemia in addition to the degree of intolerance to glucose in contributing to adverse materno-fetal outcomes. Indeed, an overlap has been demonstrated between women with early hyperglycaemia and those with established and recognised T2DM in terms of materno-fetal outcomes signifying the importance of screening in high-risk population groups. Practical considerations in this group include effective screening strategies and early identification and prevention of risk.

Addressing factors other than glycaemia could address the heightened risk of adverse materno-fetal outcomes observed in women with early hyperglycaemia. One consideration would be exploring the benefit of aspirin as a means of preventing or delaying pre-eclampsia. The second could relate to considering early delivery in the same way that delivery of neonates prior to 39 weeks gestation is recommended for women with pre-gestational diabetes to avoid the risk of still birth (26). The use of high dose folic acid could be explored in relation to reducing risk of congenital malformations. This would however have practical implications as the most effective time to start folic acid in women with pre-gestational diabetes is in fact in the pre-conceptual period (116).

Given the difficulties in establishing clear diagnostic thresholds for hyperglycaemia in pregnancy and the impact of factors such as maternal obesity, dyslipidaemia and ethnicity on adverse materno-fetal outcomes and fetal overgrowth, it is worth considering the benefits of a less glucose-centric approach when targeting particular at risk groups. In addition, preventing hyperglycaemia in pregnancy is theoretically of great importance particularly as despite early intervention and management, the women with hyperglycaemia detected prior to 20 weeks gestation had an increased risk of adverse outcomes compared to those diagnosed routinely with GDM at 24-28 weeks gestation. Furthermore, data is emerging that effective management of GDM does not mitigate the risk of obesity or metabolic dysfunction at age 5-6 or 7-10 years in the offspring exposed to hyperglycaemia in utero, regardless of the fact that immediate adverse outcomes were reduced with treatment (28). This adds further weight to the argument that preventing hyperglycaemia in pregnancy could be of

paramount importance.

CHAPTER 5: PREVENTING RECURRENT GESTATIONAL DIABETES MELLITUS WITH EARLY METFORMIN INTERVENTION (PRoDroME Trial)

5.1. Introduction

As illustrated by the original data described in the preceding chapter, the spectrum of gestational diabetes mellitus (GDM) to type 2 diabetes (T2DM) is defined not only by the degree of glycaemia but also by the length of exposure to maternal hyperglycaemia. This is independent of maternal adiposity. In the multi-ethnic cohort analysed in Chapter 4, one major risk factor for developing hyperglycaemia early in pregnancy (i.e. prior to 20 weeks gestation) was a previous pregnancy complicated by GDM with 71.8% of these women having had such a history. Indeed, once one pregnancy is complicated by GDM, subsequent pregnancies are more likely to be affected by the same condition (117). This reported risk of recurrence is thought to range between 35 and 80%, with non-White ethnicity being the strongest predictor (118). A history of gestational diabetes complicating previous pregnancies is associated with an increased risk of adverse fetal outcomes in subsequent pregnancies as demonstrated by a retrospective study conducted in the United States (119). This analysis of 62 013 women who had delivered at least twice demonstrated that GDM in a previous pregnancy increased the risk of infants being born large for gestational age (LGA: ≥90th fetal birth weight centile) in the subsequent pregnancy (RR 1.20; 95% CI 1.05-1.38) even in the absence of hyperglycaemia. If the pregnancy was complicated by recurrent GDM, the risk of an infant born LGA was compounded further (RR 1.76; 95% CI, 1.56-1.98). Risks of shoulder dystocia and preterm birth were

similarly increased (RR 1.98; 95% CI 1.46-2.70 and RR 1.68; 95% CI 1.44-1.96 respectively). Furthermore, there is a suggestion that cumulative GDM pregnancies are associated with an increased risk to the mother in the longer term, with exposed women more likely to have evidence of metabolic syndrome (120).

Pregnant women with a previous pregnancy complicated by GDM are therefore a particularly at risk group. However, as is illustrated by the case control study described in the previous chapter, identification of hyperglycaemia early in pregnancy and immediate treatment does not necessarily mitigate the risks that are associated with prolonged exposure to hyperglycaemia.

Preventing recurrence of hyperglycaemia could therefore be beneficial. However, preventing pathological hyperglycaemia during pregnancy is not without difficulties as illustrated by the literature review described in Chapter 1. Intervention with dietary strategies did not reduce the risk of developing GDM in unselected cohorts of women and the evidence for physical activity was conflicting in this group. In contrast, modifying dietary factors improved the risk of developing GDM in obese pregnant women but the evidence relating to the effectiveness of physical activity was not consistent. Finally, preliminary data have shown that supplementation with probiotics could improve the incidence of GDM in groups at high risk of developing the condition e.g. obese pregnant women or those with a family history of T2DM. While some trials suggested that myo-inositol could do the same, results from a randomised controlled trial of myo-inositol versus placebo in women with a family history of T2DM did not replicate the earlier findings (Chapter 1.7.).

5.2. Metformin in Pregnancy

Outside of pregnancy, there exists an evidence base describing the potential for pharmacological therapies in preventing T2DM in predisposed individuals. Metformin, an insulin-sensitising biguanide agent, has been shown to reduce progression to T2DM in non-pregnant individuals with impaired glucose tolerance (62). Lifestyle intervention proved more effective than metformin at achieving this until a sub-analysis of women with previous pregnancies complicated by GDM was conducted. Intensive lifestyle measures and metformin therapy each reduced the incidence of T2DM by approximately 50% in parous women with a history of GDM: this reduction was 49% and 14% respectively in parous women with pregnancies uncomplicated by hyperglycaemia (121). The TRIPOD study demonstrated the benefits of troglitazone in preventing T2DM in Hispanic parous women with previous GDM (5.4% incidence versus 12.2% in the placebo group) (122). The benefits with troglitazone were associated with a reduction in endogenous insulin secretion illustrating the importance of reducing insulin resistance in disease process modification.

Metformin has been in use since the 1950s. Its glucose lowering effects are principally thought to be mediated by reductions in hepatic gluconeogenesis and activation of the AMP kinase pathway, which in turn assists in translocation of GLUT4, the key receptor in glucose uptake. Though metformin is currently not licensed in pregnancy, there is a strong evidence base demonstrating the safety of metformin both pre-

conceptually and in the ante-partum period (123). Furthermore, there are potential advantages associated with its use, with consistently less gestational weight gain, higher levels of maternal satisfaction and a reduced incidence of neonatal hypoglycaemia being observed in pregnant women treated with metformin rather than insulin (124).

Polycystic ovary syndrome (PCOS) is a disorder characterised by hyperandrogenism, hyperinsulinaemia and polycystic ovaries (125). Women affected by PCOS tend to enter pregnancy with higher levels of insulin resistance, predisposing them to developing GDM (126). 60-80% of women with PCOS are obese, only compounding this risk further. Furthermore, affected pregnancies are more likely to be complicated by early term miscarriage, pre-eclampsia and stillbirth even in the absence of developing hyperglycaemia. Prospective cohort studies have demonstrated that in the context of PCOS, metformin reduces the risk of developing GDM and pre-eclampsia (127, 128). In contrast, in a Norwegian randomised controlled trial of metformin versus matched placebo commenced prior to 12 weeks gestation in 257 pregnant women affected by PCOS, there was no difference in GDM or pre-eclampsia incidence (Table 23) (129).

Table 23: Summary of the randomised-controlled trials investigating the effects of pharmacological agents in preventing gestational diabetes and adverse materno-fetal outcomes.

Trial	Population Characteristics	Description of Intervention	Group Characteristics			Outcomes	
				N	BMI (kg/m^2)	% GDM cases	Significant outcomes
Vanky *et al* (129)	N=273 **Entry criteria:** 1. PCOS [I] **GA:** 5-12 weeks **Country:** Norway	Double blind RCT Metformin versus placebo. 7-day "washout period" if taking metformin pre-conceptually prior to randomisation. **GDM criteria:** WHO (1999) [II]	IMP	135	29.5 [±7.0]	17.6	Less GWG in metformin group: -2.2kg, p=0.001. Per-protocol analysis: reduced preterm delivery in metformin group: 2.8% versus10.2%, p=0.03.
			C	138	28.5 [±7.2]	16.9	
Chiswick *et al* (130)	N= 449 **Entry criteria:** 1. BMI ≥30kg/m^2 2. Caucasian **GA:** 12-16 weeks **Country:** UK	Double blind RCT: Metformin versus placebo: maximum daily dose 2500g. Randomisation stratified by study site and BMI category. **GDM criteria:** 5th IADPSG criteria [(III)]	IMP	226	37.8 [±4.9]	18.0	Metformin associated with reduction in CRP and IL-6. Fewer neonates required admission to neonatal unit in the metformin group: 7.0% versus 13.0%, p=0.02.
			C	223	37.7 [±5.6]	24.0	
Syngelaki *et al* (131)	N=400 **Entry criteria:** 1. BMI ≥35kg/m^2 GA: 12-16 weeks **Country:** UK	Double blind RCT Metformin versus placebo: maximum daily dose 3.0g. GDM criteria: WHO (1999) [II]	IMP	202	38.6 (36.5-41.5)	12.4	Metformin associated with significant reductions in: 1. GWG: 4.6 (1.3-7.2) versus 6.3 (2.9-9.2) kg, p<0.001 and 2. Pre-eclampsia incidence: 3.0% versus 11.3%, p=0.001.
			C	198	38.4 (36.3-41.9)	11.3	

Abbreviations: *BMI* Body mass index (at study inclusion); *GDM* Gestational diabetes mellitus; *GWG* Gestational weight gain (defined as gain in weight from early pregnancy/ at study inclusion to weight at 36 weeks gestation/ prior to delivery); *PCOS* Polycystic Ovary Syndrome; *GA* Gestational Age (at inclusion); *RCT* Randomised control trial; *IMP* Investigational medicinal product; *C* Control group; *LGA* Large for Gestational Age (Birth weight ≥90th centile).

I. PCOS diagnosed by Rotterdam criteria (125). II. World Health Organisation (WHO) criteria 1999 (34). III. 5th International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria (54).

The disparity in the findings could relate to the time at which the intervention was started. In the studies yielding positive results, women had been on metformin pre-conception: in contrast, metformin was commenced in the first trimester in the latter trial.

The impact of metformin in potentially improving outcomes in obese pregnant women has additionally been evaluated in two randomised controlled trials (Table 23). The "Effect of metformin on maternal and fetal outcomes in obese pregnant women" (EMPOWaR) trial, a trial that was powered to detect differences in fetal birth weight, randomised 449 obese pregnant women of White ethnic origin to either metformin or matched placebo (2500mg in divided doses) prior to 16 weeks gestation (130). Mean (SD) fetal birth weight was similar in the two groups (3462 ±548g versus 3463 ±660g in the metformin and placebo groups respectively). No differences were detected in either the proportion of women who developed GDM (IADPSG diagnostic criteria: metformin arm 18.0% incidence GDM and placebo arm 24.0%, p=0.3) or in the proportion of infants born large for gestational age (LGA; ≥90th centile: metformin arm 14.0%, placebo arm 17.0%) (54). The women recruited to the EMPOWaR trial had a mean early pregnancy BMI ≥35.0kg/m^2 (37.8kg/m^2 in the metformin group: 37.7kg/m^2 in the placebo) and no differences existed in gestational weight gain in the two groups. The "Metformin in obese pregnant women" trial (MOP) was similarly powered to detect differences in fetal birth weight as the primary outcome (Table 23) (131). A total daily dose of 3.0g metformin versus matched placebo was used in this trial. No differences in the incidence of GDM (1999 WHO criteria: 12.4% incidence metformin arm versus 11.3% in the placebo group, p=0.7), fetal birth weight z-score (0.05 (-0.71 to 0.92) versus 0.17 (-0.62 to 0.89), p=0.7) or in the proportion of infants born LGA were observed (16.8 versus 15.4, p=0.8) (34).

Though metformin did not reduce either of the primary outcomes in the trials described above, important findings were nonetheless demonstrated. In the MOP trial, metformin was associated with significantly less gestational weight gain and a lower incidence of pre-eclampsia when compared to women randomised to the placebo arm (Table 23). The EmPOWAR trial demonstrated improved insulin sensitivity at 28 weeks gestation in women randomised to metformin and a reduction in markers of inflammation. In addition, a lower proportion of infants born to the women in the metformin group required admission to neonatal intensive care.

Given the increasing incidence of GDM, the high risk of recurrence in subsequent pregnancies and the increasing risk of adverse materno-fetal outcomes associated with recurrence of GDM, it is important to identify suitable prevention strategies. Metformin is now widely used in the treatment of GDM and has been shown to reduce the incidence of GDM in the context of PCOS and improve certain outcomes in obese pregnant women with normal glucose tolerance. With these factors in mind, a randomised control trial was designed to investigate the effects of metformin in preventing recurrent gestational diabetes, the protocol for which is described in this chapter.

Study Name: Preventing **R**ecurrent Gestational **D**iabetes Mellitus with Early **Me**tformin Intervention

Study Acronym: PRoDroME Trial

5.3. Study Hypothesis, Design and Aims

Hypothesis: Intervention with metformin therapy early in pregnancy prevents gestational diabetes mellitus recurring in women with previous pregnancies complicated by GDM.

Design: Phase 4 double blind randomised controlled trial of metformin versus matched placebo in early pregnancy.

Aims (Figure 23)

Primary: To ascertain for the first time, if early intervention with metformin prevents recurrence of GDM.

Secondary: To evaluate the effect of early metformin intervention on the following:

- Maternal factors (weight gain, requirement for insulin therapy, post-partum glucose measurements and levels of health and satisfaction).
- Neonatal features (fetal birth weight, birth weight centile and a composite of outcomes related to complications of gestational diabetes)
- Cost effectiveness of early intervention with metformin therapy

Tertiary: To ascertain if early metformin intervention has an effect on the following:

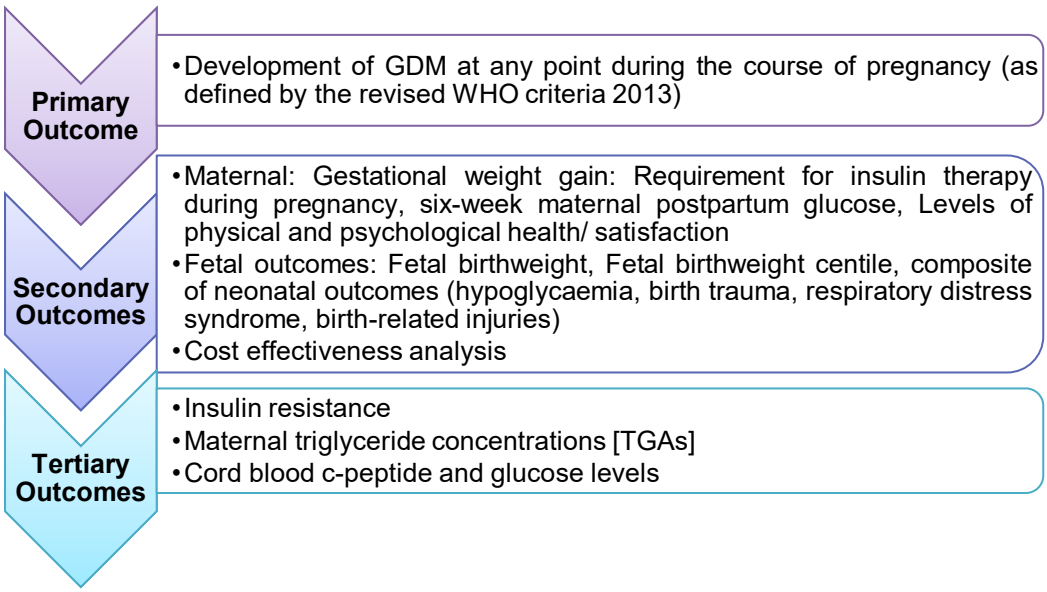
- Insulin resistance: Using the Homeostasis Model Assessment method (HOMA), insulin resistance will be characterised in both the metformin and placebo arms. Preliminary studies have shown a correlation when HOMA-IR is measured against the current gold standard, the euglycaemic

hyperinsulinaemic clamp in pregnancy (early pregnancy r^2= 0.52, p=0.002; late pregnancy r^2=0.61, p=0.0001) (132).

- Maternal fasting triglycerides concentrations [TGAs]: As described in Chapter 2, fasting triglyceride concentrations correlate independently with fetal growth (79). In non-pregnant populations, metformin has been shown to have a beneficial impact on lipid metabolism (133). Fasting triglycerides will be determined in both the metformin and placebo arms as per the schedule of assessments.

- Fetal hyperinsulinaemia: To determine this, cord blood c-peptide and insulin levels will be measured to provide surrogate markers of fetal hyperinsulinaemia as in previous large scale studies (134).

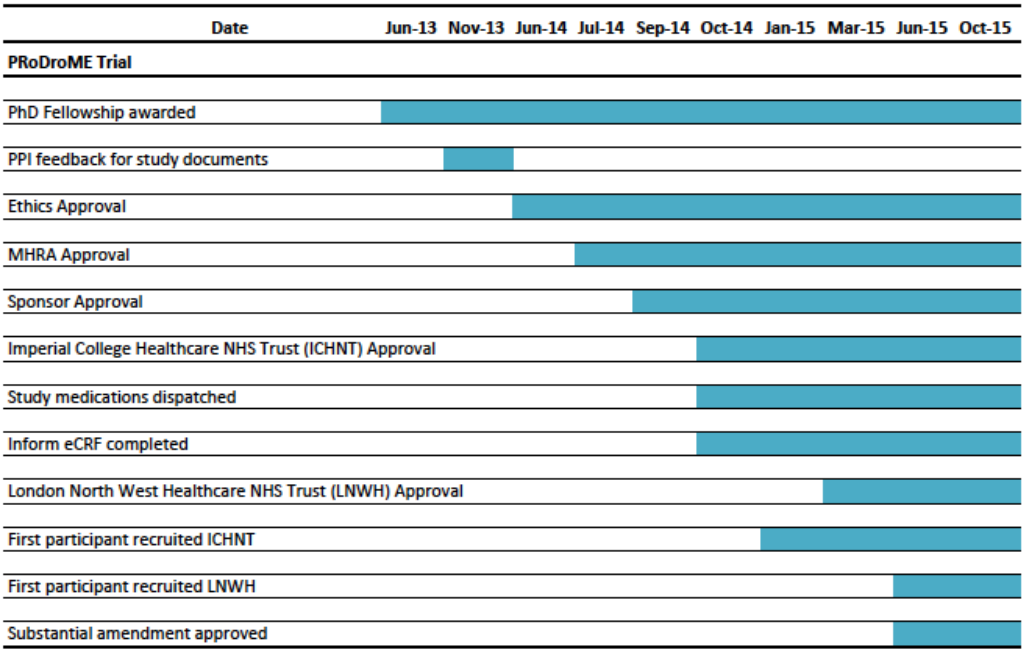
Figure 23: Summary of outcomes assessed



5.4. Study Approvals

The Westminster National Research and Ethics Committee approved the study in April 2014 and the Medicines and Healthcare Regulatory Agency (MHRA) in July 2014. Imperial college sponsor approval was obtained in September 2015 with site-specific approvals for Imperial College NHS Healthcare Trust (ICHNT) granted in October 2015 and for London North West NHS Healthcare Trust (LNWH) in February 2015 (Figure 24).

Figure 24: Timeline illustrating relevant milestones for the PRoDroME Trial



Prior to obtaining the necessary approvals, the participant related documents were circulated to members of the public via the National Institute and Health Research (NIHR) Diabetes Research Network to ensure that the information clearly explained the purpose of the study.

5.5. Sample Size and Power Calculation

Depending on the cohort examined, the risk of GDM recurring in a subsequent pregnancy varies considerably and ranges anywhere between 35 and 80% (135). Pilot data from the ICHNT antenatal cohort in 2012 demonstrated that the pre-trial estimate of GDM recurrence, defined as the need for pharmacological anti-glycaemic therapy in a pregnancy following a previous pregnancy complicated by GDM, was 50%. Though this probably under-estimated the true recurrence rate as women able to achieve target glycaemic control with diet alone were not included, accurately determining the incidence of recurrent GDM was problematic: all women at ICHNT are routinely provided with a home glucometer at 16-18 weeks gestation and dietary advice i.e. they are effectively managed as if they have GDM.

The predicted response to metformin therapy at the time of designing this trial was 50%. This value was based on findings from the Metformin in Gestational Diabetes trial (MiG trial), a randomised controlled trial of metformin versus insulin in the treatment of GDM in which 47.9% of the women who had been randomised to metformin required supplemental insulin therapy to control hyperglycaemia (136).

A logistic regression analysis is planned for the end of the trial. The predictors of the principal outcome, GDM recurrence at any time prior to 36 weeks gestation (or delivery if occurs prematurely) will be evaluated using treatment with metformin as the primary predictor. Based on this planned statistical analysis, 47 women were

determined to be required in each group to achieve 80% power at 5% significance levels. To allow for a 20% drop out rate, a sample size of 112 was planned.

5.6. Participant Characteristics with Relevant Justifications

Women were eligible for inclusion if all of the following selection criteria were fulfilled:

- Singleton viable pregnancy
- Between 8 and 22 weeks gestation
- Previous pregnancy complicated by GDM

If any of the following existed, women were subsequently excluded:

- Established pre-existing diabetes (including un-recognised diabetes defined as FPG ≥ 7.0mmol/L and/ or HbA1c ≥ 48mmol/mol)
- Contraindications to metformin therapy
    - Renal impairment: creatinine ≥ 130µmol/L
    - Impaired liver function (ALT ≥ 2.0 x upper limit normal)
- Previous intolerance to metformin
- Planned continued antenatal care or delivery at a centre not included in the trial
- Planned fast for cultural/ religious reasons e.g. Ramadan

At the study outset, the upper window of recruitment was defined at 16 weeks gestation with a plan to randomise a consented participant by completion of the 18th week of pregnancy. However, due to the problems identifying suitable women within

such a narrow time frame following the point at which they registered their pregnancy, a protocol amendment was submitted in March 2015 to extend the recruitment window from 16 to 22 weeks gestation with the upper limit of randomisation set at completion of the 22nd week. In view of the increased risk of developing GDM associated with a multiple pregnancy, only women with a singleton pregnancy were invited to participate (137). The specific screening strategy or diagnostic criteria used to establish GDM in the previous pregnancy did not preclude an invitation to participate in the trial. This was due to the variations in the screening and diagnostic strategies across the sites.

As described in Chapter 4, the thresholds to diagnose pathological hyperglycaemia in early pregnancy are a source of considerable debate. Therefore, women with a fasting plasma glucose (FPG) and or an HbA1c diagnostic of T2DM were excluded from the study on the basis that this group would clearly need immediate and intensive management. In view of the absence of evidence relating to what threshold would constitute the need for treatment at the time of writing this protocol, lower glucose and HbA1c values were not used to define exclusion. Relating to the renal threshold at which metformin therapy would be contraindicated, an absolute creatinine value was defined rather than changes in the glomerular filtration rate as physiological changes in the latter occur in pregnancy. Both the absolute creatinine value and the alanine transaminase threshold that defined exclusion from the trial were based on the EmPOWAR study, the protocol of which was available at the time of designing the study (130).

Once randomised, development of any of the following resulted in the participant being withdrawn from the study:

1. Development of a contra-indication to metformin therapy i.e.
    - Hepatic impairment (ALT > 2.5 upper limit normal)
    - Renal impairment with deterioration in creatinine by 25% **or** increase in creatinine by 30µmol/L above baseline
2. Hypersensitivity to metformin
3. An inability to tolerate study medications

Participants have been free to discontinue trial medications at any point during the study. Where possible, follow up has continued and maternal/ fetal outcomes have been recorded. If a participant chose to withdraw consent, data up to the point of self-withdrawal were included unless the participant chose otherwise. Subjects who were either withdrawn from the trial or who had chosen to no longer participate, were not replaced.

5.7. Participating Sites and Participant Identification

To date, the trial has recruited from three sites in the North West London region (St. Mary's Hospital, Queen Charlotte's Hospital and Northwick Park Hospital) with plans to open to a fourth NHS trust in 2018 (Chelsea and Westminster Hospital: West Middlesex site). In 2012, the three hospitals from which recruitment was planned at

the outset of this trial cared for a total of 755 women whose pregnancies were complicated by GDM.

The strategy used to identify participants differed according to the hospital site. In all instances, effective identification relied heavily on midwifery support and or other members of the multidisciplinary team involved in the care of these women.

Imperial College Healthcare NHS Trust: At both the St Mary's Hospital (SMH) site and the Queen Charlotte's and Chelsea Hospital (QCCH) site, midwives involved in the initial registration of the pregnancy were informed of the study either during departmental meetings or at educational days. Clipboards were left at each of the booking clinics, which enabled the midwives to record details of eligible participants and provide study information where appropriate. However, identification was most effective at the point of contact with the specialist diabetes midwife at both sites. In accordance with trust policy, the majority of women with a previous pregnancy complicated by GDM are provided with a home glucometer at 16-18 weeks gestation together with standard dietary advice consistent with that recommended for the management of hyperglycaemia in pregnancy. Following these appointments, the midwives would inform a member of the research team of the patient's attendance. This patient would then either be contacted prior to their next attendance or reviewed at the antenatal clinic by an individual familiar with the trial. Information relating to the study would be provided at this stage and screening visits organised if appropriate.

London North West Healthcare: In contrast to Imperial College Healthcare NHS Trust, pregnant women with a previous history of GDM attending this Trust are not routinely

provided with a home glucometer at 16-18 weeks gestation. Instead, the midwife registering the pregnancy organises a 75g oral glucose tolerance test (OGTT) at 16 weeks gestation. The specialist diabetes midwife only subsequently reviewed women if GDM was diagnosed by the 1999 WHO criteria: FPG ≥7.0mmol/L, 120-minute glucose ≥7.8mmol/L. Those with a normal OGTT would have a repeat one organised for 26-28 weeks gestation. A member of the research team would review the list of women awaiting the initial OGTT and contact them prior to their appointment to ascertain their level of interest. Where appropriate, an appointment to discuss the study in more detail, or a screening visit would then be organised.

5.8. Screening, Assessments and Follow Up

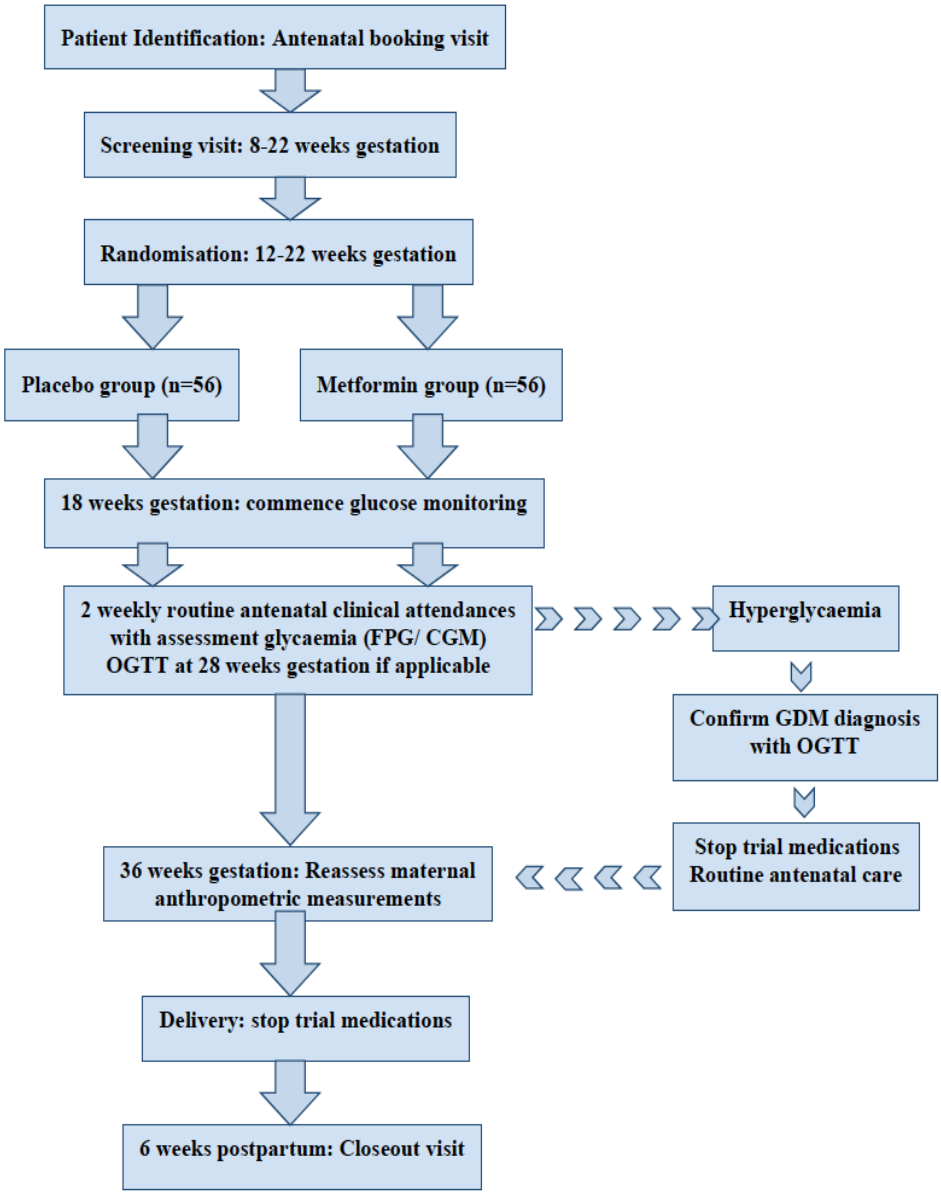
All study visits took place either in the antenatal clinic or the diabetes centre. When possible, these were performed in conjunction with routine antenatal attendances and a member of the research team was present to facilitate data collection. Figure 25 summarises the study follow up schedule.

5.8.1. Screening

To date, screening visits have occurred between 8 and 22 weeks gestation. Women have been instructed to attend following an overnight 8-hour fast (no food or drink except water). In addition, where applicable, women have been asked to refrain from smoking for a minimum of twelve hours prior to the visit. Designated members of the research team have conducted this visit.

Following the informed consent process, a medical and obstetric history was recorded, including information relating to the diagnosis and the management of the previous pregnancy complicated by GDM, together with any adverse outcomes. Concomitant medications at the time of recruitment, including any nutritional supplements were noted.

Figure 25: Participant identification, screening and study visit outline



The following anthropometric data were measured at the screening visit:

- Height and weight
- Blood pressure
- Body composition as determined by bioelectrical impedance analysis (BIA)

A B240 SMA Tanita device was used to ascertain body composition by analysing bioelectrical impedance. This estimates body composition by measuring the impedance or resistance to a small electrical current (<1mA) passed across body tissues, with fatty tissues offering the greatest resistance and lean tissues and water offering the least. As such, the distribution of fat, which has been shown to be an important predictor of clinical outcomes, could be easily estimated (138). Bioelectrical impedance analysis (BIA) has been validated in pregnancy (139).

In addition to the above, women were asked to complete two health surveys, the Spielberger State-Trait Anxiety Inventory (SS-TAI) and the EQ-5D-5L, both of which have been validated in pregnancy (24) (136) (Appendix 3). Where necessary, a member of the research team was available to help explain the questions.

Samples for the following were collected and processed at the local laboratories (Table 24):

- Fasting plasma glucose (FPG) and HbA1c
- Liver function/ renal function/ full blood count
- Fasting plasma insulin (FPI): this was immediately placed on ice and transported to the laboratory within thirty minutes of collection

- Fasting triglyceride concentrations [TGAs]
- Vitamin D
- Vitamin B12

Table 24: Approximate blood sample requirements screening visit

Tube Number	Preservative	Volume	Tests
2 x yellow	SST / gel separator	2 ml each	Renal function, Lipid profile, Liver function, Vitamin B12, Vitamin D Insulin: immediately placed on ice and transferred to laboratory within 30 minutes
2 x small purple	EDTA	2 ml each	Full blood count HbA1c
1 x grey	Fluoride oxalate	1 ml	Glucose
Samples for storage			
1 x green	Lithium heparin	5-7 ml	Save for metabolomics analysis
1 x yellow	Plain tube	2ml	Save for lactate, vitamin B12 binding globulin and vitamin D

Though not routinely measured in pregnancy, Vitamin D levels were determined due to the documented association of vitamin D deficiency with increased insulin resistance (140). Similarly, measurement of vitamin B12 is not performed routinely in pregnancy but was introduced as a safety measure for the purpose of this trial: non-pregnant adults treated with metformin have an increased incidence of either vitamin B12 insufficiency or deficiency when compared to age and weight matched controls with diabetes who are not treated with metformin (141).

Lactate has been measured in large-scale randomised control trials of metformin versus placebo due to concerns regarding the association of one of the older generation biguanides, phenformin, with severe lactic acidosis. However, the evidence relating to metformin as a cause of lactic acidosis is conflicting and ranges from 1 to 47 cases per 100 000 patient years, with most cases reported occurring in the context of severe renal dysfunction, sepsis or critical illness (142). In view of this, together with the paucity of data relating to longitudinal changes in lactate levels during pregnancy, a decision was made to perform post-hoc analyses of lactate levels. To this effect, two samples (one in a lithium heparinised tube and one in a plain tube) were collected and immediately placed on ice (see section 4.9. for instructions regarding omission of metformin during inter-current illness). Following completion of the screening visit, samples were centrifuged and aliquots of serum were stored at -80°C. In addition to the post-hoc analysis of lactate, analysis of vitamin D binding globulin and Vitamin B12 binding globulins were planned.

A subsequent follow up appointment was arranged to coincide with either the next antenatal scan or midwifery appointment.

5.8.2.Randomisation procedures and study medication supply

Following a review of the results from the screening visit, subjects who met the relevant criteria were randomised to either metformin or placebo in a 1:1 ratio. Randomisation occurred in blocks of ten and was stratified according to treatment centre rather than NHS Trust. The randomisation lists were generated by a member of the Statistical Support department at Imperial College and were distributed to each

of the three sites at the initial opening of the trial. These lists were built in to a purpose designed electronic case reporting form used to store data for this trial. An automatic randomisation process was therefore available once all the necessary inclusion and exclusion criteria were fulfilled.

Women were provided with the study medications at the closest possible opportunity to the screening visit, with completion of the 22nd week of gestation defining the upper limit at which the medications could be supplied. Women were advised to start taking one tablet of 500mg metformin / matched placebo with their evening meal and to titrate the dose by 500mg increments every five days until the maximum dose of 1000mg twice daily (breakfast and evening meal) was achieved. They were provided with a dosing card, which outlined the instructions for this (Appendix 4).

All randomised participants were provided with a Patient Alert Card indicating the name of the investigational medicinal product (IMP), the study number, the Principal Investigator's name and a 24-hour emergency contact number. The date of the medication supply was recorded in their antenatal records. Further details regarding the IMP and pharmacovigilence are outlined in section 4.9.

5.8.3. Capillary blood glucose monitoring

By this stage, the majority of women recruited had been issued with a home glucometer. However, if this was not the case, a glucometer together with the necessary education was provided. The glucometers were not standardised across the trusts due to existing differences in agreements with Primary Care Trusts

regarding the type of glucose testing strip. Instructions as to the frequency of monitoring was site specific i.e. fasting value and one hour post breakfast, lunch and dinner at QCCH and Northwick Park Hospital (NPH); pre and one hour post one meal per day at SMH. Participants were advised to target a fasting capillary blood glucose (CBGs) value of less than 5.5mmol/L and one-hour post-prandial levels of less than 7.8mmol/L (values recommended by NICE criteria 2008). If a participant had three or more elevated CBGs in one week, they were instructed to contact a member of the research team.

5.8.4. Follow-up visits

The expected date of delivery (EDD) defined by the dating scan was used to accurately time the subsequent visits following randomisation. The remainder of the visits took place in conjunction with routine antenatal clinic attendances.

As well as the assessments outlined below and summarised in Table 25 (found at the end of this section) for specific visits, all women had a routine clinical review conducted by the obstetric and diabetes specialists in the clinic. Broadly, this included a review of CBG values in the preceding 2-4 weeks and a clinical examination consisting of a blood pressure recording, urinalysis, abdominal examination and fetal heart rate assessment. Fetal growth scans were also reviewed: in accordance with NICE guidance these are routinely performed at 28, 32 and 36 weeks gestation. In addition, where applicable, adherence to study medications was recorded.

At 28 weeks gestation, a 75g oral glucose tolerance test was organised. Participants were advised to attend this after an 8 hour overnight fast as per the instructions for the screening visit. During the visit, the following was also measured:

- Fasting plasma insulin
- Lipid profile (for maternal triglyceride concentrations)
- Vitamin B12 and Vitamin D
- Renal and liver function
- Full blood count

In addition two further samples were collected and immediately placed on ice. Sample processing was as per described for the screening visit. Bioimpedance analysis (BIA) was also conducted and the participants were asked to complete the EQ-5D-5L and STAI questionnaires (Appendix 3).

Women were advised to omit their study medications on the morning of the 28-week visit in preparation for the oral glucose tolerance test. This was done in accordance with the protocol outlined by the US Diabetes Prevention Program, the largest interventional trial involving metformin that has been conducted to date (62). A follow up to this study determined that the pharmacological effect of metformin accounted for 26% of the observed reduction in progression to T2DM in individuals with impaired glucose tolerance following a two-week period in metformin omission. However, despite metformin withdrawal, the incidence of diabetes in the metformin group was still reduced when compared to the placebo arm of the DPP trial: Odds Ratio (OR) 0.75, 95% CI 0.62-0.92, p=0.005 versus 0.66, 0.54-0.82, p <0.001 on

completion of the initial 3 year trial period. Discontinuing study medications for two weeks prior to an OGTT in pregnancy was considered impractical given the short time frame that metformin was being studied for.

In the event that a participant had hyperglycaemia as evidenced by three or more elevated CBG values in the preceding week, this OGTT together with the associated measurements collected at the 28-week visit, was expedited.

Women were asked to continue the study medications pending the results of the OGTT, which were communicated as soon as available (generally within 24 hours).

For the purposes of this trial, the International Association of Diabetes in Pregnancy Study Group (IADPSG) criteria and the recently revised World Health Organisation criteria i.e. fasting plasma glucose (FPG) ≥ 5.1mmol/L and/or 2 hour post glucose load ≥ 8.5mmol/L, were used to diagnose GDM. These criteria were opted for as at the time of writing this protocol the NICE diagnostic criteria were under revision: no preliminary statements had been issued as to which glycaemic thresholds NICE would be recommending for the diagnosis of hyperglycaemia in pregnancy.

If a diagnosis of GDM was confirmed, the study medications were discontinued and a case-by-case decision was made regarding treatment modality following discussion with a member of the multidisciplinary team and relevant participant. A decision was made to discontinue trial medications completely rather than commencing supplemental insulin on the basis that metformin has certain advantages when

compared to insulin monotherapy e.g. reduced incidence of neonatal hypoglycaemia (see section 5.2.).

If the OGTT at 28 weeks gestation was consistent with normal glucose tolerance, then the participant was instructed to continue the study medications until delivery. Follow up continued as per routine with visits timed every two to four weeks according to the antenatal guidelines at the specific hospital for the individual participant. At any stage, if there were three or more elevated CBG values in the preceding week to the visit, a random plasma glucose (and preferably FPG) was measured and the study medications discontinued. Treatment in the form of metformin and/or insulin was then commenced. An increase in fetal abdominal circumference (FAC) to above the 95th centile on a growth scan or a 25% increase in the FAC above the previous assessment necessitated careful review of maternal glycaemia with a low threshold for discontinuing study medications. In these instances, a repeat 75g OGTT was planned.

In addition to ensuring that glucose was addressed at the 28-week visit, changes in vitamin B12 from baseline were also noted. In the event of a 50% reduction in vitamin B12 values or a decrease to below the lower limit of normal, vitamin B12 supplementation was commenced.

Unless otherwise necessitated by the event of an acute illness or need for emergency treatment, ceasing treatment rather than un-blinding was encouraged as far as

possible. For un-blinding, a member of the on call pharmacy team would be contacted for assistance.

At 36 weeks gestation, all women originally recruited to the study underwent a repeat assessment of their anthropometry (weight and BIA). In addition, they were asked to complete the two questionnaires (EQ-5D-5L and STAI). The following was also recorded at this point:

- Blood pressure
- Treatment required for GDM (if any)
- Treatment required for hypertension (if any)
- Development of pre-eclampsia
- Planned mode of delivery

Women who had not developed hyperglycaemia by this point of time and who had continued the study medications were advised to discontinue them at the onset of labour.

All women initially recruited to the study were provided with two blood collection tubes to keep with them i.e. a fluoride oxalate tube for cord blood glucose and a plain tube for c-peptide. They were instructed to give these to the midwives at the time of labour. At the point of delivery, midwives routinely collect cord blood samples for acid-base assessment. An instruction sheet at the front of the handheld antenatal notes advised the attending midwife to collect an additional 7ml cord blood: 2ml for the fluoride oxalate (grey topped) tube and 5ml for the plain (red topped) tube. The

latter was stored on ice and urgently sent to the laboratory within 30 minutes for centrifuging at the local laboratory. The serum for this was decanted prior to freezing at - 80°C and then transported to the Charing Cross laboratory in batches for analysis. In contrast, the cord blood glucose collected was processed locally.

Details regarding the delivery including mode of delivery, fetal birth weight and any materno-fetal complications were collected retrospectively. The GROW gestation network calculator was used to determine customised birth weight centiles through adjusting fetal birth weight for maternal height, weight, ethnicity, parity, fetal gestational age and gender (88). Large for gestational age (LGA) infants were defined as infants with an adjusted birth weight ≥90th centile: small for gestational age (SGA) as those with a birth weight <10th centile. Preterm delivery was defined as delivery prior to 37 weeks gestation i.e. 36 completed weeks. Neonatal complications were recorded as a composite outcome with a score of 1 applied if one or more of the following adverse events developed: shoulder dystocia, neonatal hypoglycaemia requiring treatment, respiratory distress syndrome requiring either oxygen therapy or continuous positive airway pressure, admission to the neonatal intensive care unit, or hyperbilirubinaemia requiring phototherapy.

5.8.5. Post-partum data collection

All subjects originally recruited to the trial were asked to attend for the final assessment at a minimum period of 6 weeks postpartum. Women were asked to fast for 8-hours prior to their attendance.

The following was measured in the mother:

- Weight and body composition (BIA)
- Fasting plasma glucose, lipid profile (for maternal triglyceride concentrations) and fasting plasma insulin
- Full blood count
- Liver function, renal function, Vitamin D and Vitamin B12
- Two additional samples were collected for storage and processed as per the protocol in the screening visit for post hoc analysis of lactate, vitamin D binding globulin and vitamin B12 binding globulin

In addition, women were asked to complete the EQ-5D-5L and STAI questionnaires.

The following was assessed in the infant:

- Weight was measured using calibrated electronic scales or recorded from that measured at the six-week health visit
- Length was measured on a standardised plastic length board
- Head and abdominal circumference
- Skin fold thickness: using skin fold calipers, skin fold thickness was determined at the following three sites
    - Flank skin fold: measured above the iliac crest on the diagonal skin fold in the mid-axillary line (left side).
    - Triceps skin fold: measured on the vertical fold over the triceps muscle at a point equidistant between the acromion process and olecranon.
    - Subscapular skin fold: measured just below the lower angle of the scapula at approximately a 45° angle to the spine.

To ensure the accuracy, reliability and consistency of the anthropometric data across the three centres, the number of assessors was minimised as far as possible.

5.8.6. Additional aspects to the study and long-term follow up

Women who met the inclusion criteria but were not suitable for randomisation were invited to provide consent for a separate non-interventional arm to the study that is being conducted to identify clinical predictors of developing recurrent GDM. In addition to the routine blood tests normally collected at 32 weeks pregnancy, an FPG, FPI and lipid profile were also measured in this group of individuals. At 36 weeks gestation, weight and body composition was recorded. Where possible, these women also attended the 6-week postpartum fasting visit where, in addition to the routine assessments normally carried out (measurement of maternal weight, blood pressure and FPG), the assessments defined in the postpartum study visit were performed.

Table 25: Schedule of assessments for the PRoDroME Trial

Visit	S	R	28 week	36	Delivery	Postnatal check
Weeks Gestation	**10-12**	**12-16**	**28**	**36**		**6-12 weeks postpartum**
Height	X					
Weight	X			X		X
Waist Circum.	X					X
BIA	X			X		X
FPG	X			X		X
HbA1c	X					
FBC						X
FRP	X					
LFT	X					
75g OGTT	X		X			
Vitamin B12	X					X
Lactate	X		X	X		X
Vitamin D +Vitamin D binding globulin	X		X	X		X
FPI	X		X	X		X
TGA	X		X	X		X
Aliquots for storing	X		X	X		X
Health surveys	X		X	X		X
Cord blood glucose					X	
Cord blood C-Peptide					X	
Neonatal measurements						X
						X
						X

Abbreviations: *S* Screening, *R* Randmisation, *BIA* Bioelectrical impedance analysis, *FPG* Fasting plasma glucose, *FBC* Full blood count, *FRP* Full renal profile, *LFT* Liver function, *OGTT* Oral glucose tolerance test, *FPI* Fasting plasma insulin, *TGA* Triglyceride concentrations

During the screening visit women were specifically asked if they would provide consent to members of the research team contacting them in the future (up to 5 years following trial closure) for information relating to the state of their own health (with particular reference to the incidence of T2DM and hypertension) and to the growth and development of their child.

5.9. Investigational Medicinal Product and Pharmacovigilence

The Investigational Medicinal Product (IMP) is metformin 500mg or matched placebo tablets. Participants were instructed to start at a dose of 500mg metformin / placebo (one tablet) with the evening meal and to then titrate the dose by 500mg increments until the desired maximum dose was achieved (1000mg twice daily).

Individuals interested in participating were informed that metformin is generally well tolerated. However, approximately 30-40% of those prescribed metformin outside of pregnancy experience gastrointestinal side effects with 5% of individuals needing to discontinue therapy due to these side effects. Taste disturbance, light-headedness, dyspnoea, myalgia and rashes are less commonly reported side effects (<1%). If the participant was unable to tolerate the full dose, they were encouraged to take the highest dose possible. Those unable to tolerate even the lowest possible dose were withdrawn from the trial. All participants were advised to withhold the trial medications during times of inter-current illness due to the increased risk of dehydration during such episodes. Participants would be advised to inform a member of the research team if this occurred.

The Pharmacy Manufacturing Unit at Guy's and St Thomas NHS Foundation Trust manufactured the study medications. Rather than over-encapsulate the metformin and placebo, matched placebo tablets were manufactured. The medications were stored in their original containers below 25°C in a secure location within the pharmacy department at each of the relevant sites. The lead pharmacist kept a record of study

medication movements to maintain accountability. Only authorised personnel (as designated by the study delegation log) were allowed to distribute the drugs.

Two bottles of 120 tablets were supplied at the following points: randomisation, 20 weeks gestation (if randomised at or prior to 16 weeks gestation), 26 weeks gestation and if required, 34 weeks gestation.

Adverse events (AEs) were recorded, notified, assessed, reported, analysed and managed in accordance with the Medicines for Human Use (Clinical Trials) Regulations 2004. Events were counted from the time of inclusion until 30 days following the final postpartum visit.

The following adverse events were of medical interest and were therefore recorded and reported to the Sponsor:

- **Gastrointestinal:** Nausea, vomiting, diarrhoea, abdominal pain or loss of appetite leading to the requirement to either discontinue therapy or reduce medication doses.
- **Metabolic:**
    - Abnormal ALT result 2.5 times upper limit of normal
    - Deterioration in creatinine by 25% **or** increase in creatinine by 30µmol/L above baseline
    - Reduction in vitamin B12 levels by 50% or development of vitamin B12 deficiency
- **Other:** Hypersensitivity reaction to metformin

- **Complications relating to pregnancy or delivery:** Development of pregnancy-induced hypertension, preterm labour, preterm delivery in maternal interest, preterm delivery in fetal interest, and delivery complications such as unplanned requirement for caesarean section or post partum haemorrhage.

At all study visits, subjects were questioned regarding the details of any illnesses, unexpected hospital admissions or attendances and the expected adverse reactions or events listed above. Full details were recorded in the patient notes and all adverse events were followed up until satisfactory resolution.

In accordance with Good Clinical Practice guidelines, serious adverse events (SAEs) were defined as any adverse event or reaction that

- Was life threatening
- Required hospitalisation or prolongation of existing hospitalisation
- Resulted in significant disability or incapacity
- Consisted of a congenital anomaly or birth defect
- Miscarriage
- Admission of the baby to the neonatal unit for a period of up to fourteen days
- Resulted in still birth or maternal death
- Was otherwise considered medically significant by the investigator

All serious adverse events were recorded in the patient records and reported to the Principal Investigator with 24 hours of becoming aware of the event. The Chief Investigator and the Sponsor were notified at the same time.

A suspected unexpected serious adverse reaction (SUSAR) was defined as any severe adverse reaction that was not in keeping with the information regarding the medicinal product.

5.10. Statistical Analysis Plan

An Intention To Treat (ITT) analysis is planned for the end of the trial process. Dropouts will be retained in their initial randomisation group and their outcomes based on the information from their routine antenatal follow up and delivery data. Predictors of the principal outcome, recurrence of GDM prior to delivery or 36 weeks gestation, will be evaluated by cox regression analysis, with metformin treatment as the primary predictor.

5.11. Governance and Data Monitoring

Each subject was assigned a unique identification number determined by the electronic case reporting form. A pro-forma was completed during the screening visit, 28-week visit, 36-week visit and postpartum visit. The data on this pro-forma was anonymised and stored within locked designated filing cabinets at the respective sites. Only members of the research team had access to the filing cabinets.

Members of the research team transferred the anonymised data to the GCP accredited electronic Case Reporting form (InForm). This case reporting form had

been constructed in line with the Sponsor requirements for controlled trials of investigational medicinal products (CTIMPs). Database specifications had been agreed with members of the research team and the Inform programmers.

To obtain a username and password, members of the research team had to complete appropriate training in how to use the database.

An independent Data Monitoring Committee (DMC) was established to oversee the safety of the participants in the trial. They met within one year of the first participant being recruited. The committee consists of a consultant diabetologist (Chair person), consultant obstetrician and an independent statistician. The minutes from the meeting were stored in the trial management folder and the suggestions and comments put forward by the committee will be outlined in the next Chapter.

In addition, there have been three trial monitoring visits conducted since the study first opened to recruitment: two annual visits for the Imperial College Healthcare NHS Trust sites and one for the London North West Healthcare site. Reports have also been submitted to both the NRES ethics committee and the MHRA.

An analysis of the screening data and the outcome data for the participants successfully randomised is outlined in the next chapter. Data from women who consented to the prospective non-interventional arm of the trial have also been included.

CHAPTER 6: RECRUITMENT TO THE PRoDroME TRIAL, SCREENING DATA AND PRELIMINARY RESULTS

6.1. Introduction

The "Preventing Recurrent Gestational Diabetes Mellitus with Early Metformin Intervention" (PRoDroME) trial, the methodology of which is outlined in the preceding chapter, is recruiting participants at the time of writing. The progress with recruiting and screening data will be presented in this Chapter. In addition, the baseline demographics for the women who were randomised and the materno-fetal outcomes in those who have delivered will be reviewed. Any adverse events recorded during the trial will be discussed. An analysis of the outcomes in those who could not be randomised due to interim development of hyperglycaemia and in those who participated in the prospective observational arm of the trial will be presented. Finally, an update as to the progress of the trial following completion of this thesis is included at the end of this Chapter.

6.2. Participant Identification, Recruitment and Screening

As outlined in Chapter 5, the Ethics committee approved the PRoDroME trial in June 2014, the MHRA in July 2014 and Imperial College Healthcare NHS Trust (ICHNT) in October 2014 (Chapter 5: Figure 24). Following delivery of the study medications, the

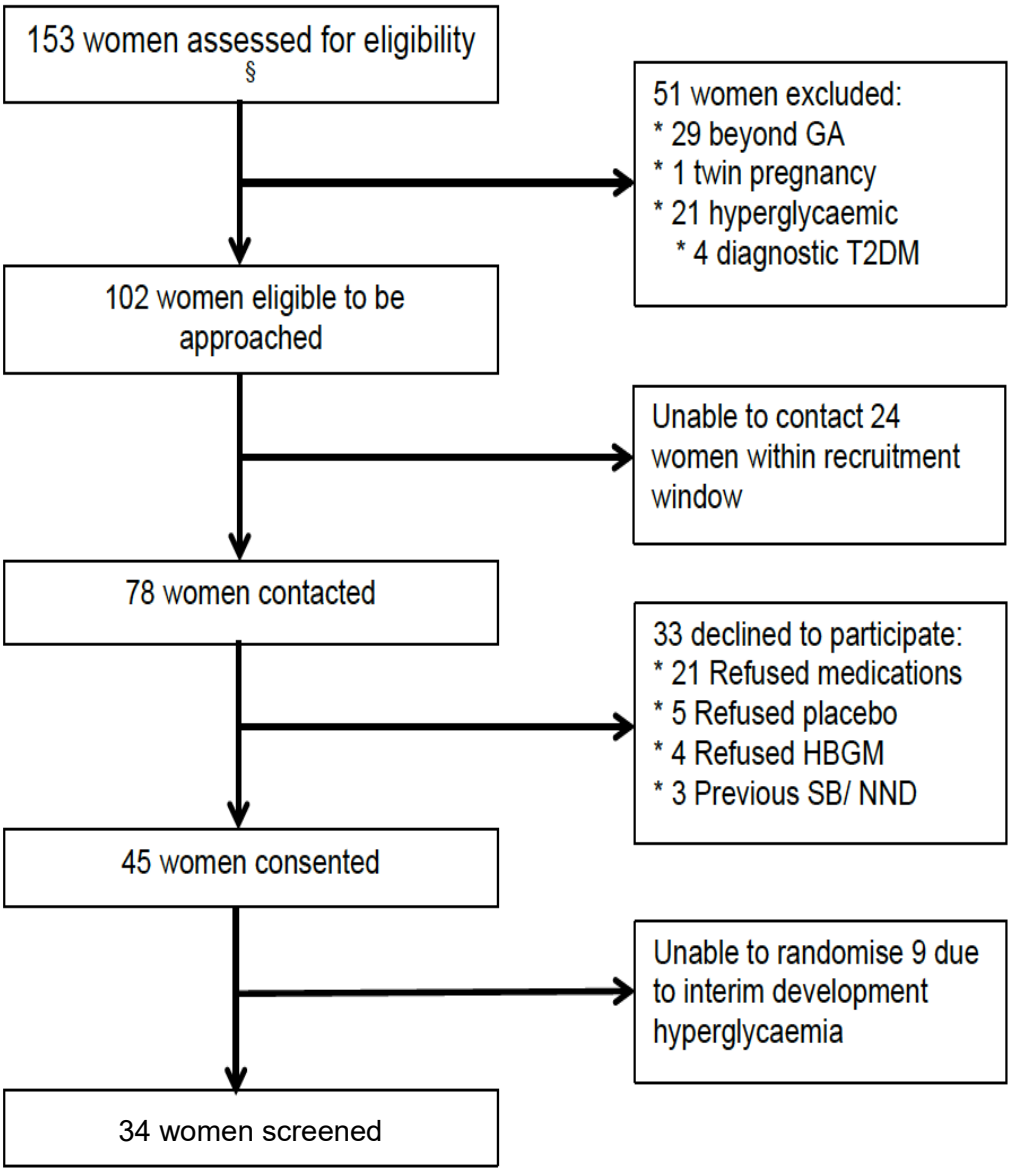
first participant was recruited at ICHNT in January 2015 and at Northwick Park Hospital in March 2015.

6.2.1. Participant identification

In total, 153 pregnant women with a previous pregnancy complicated by gestational diabetes were identified from November 2014 through to December 2016 (Figure 26). At the time of identification, 19.0 % were outside the recruitment window i.e. 29 women were beyond 16 weeks gestation. Following the approval to the substantial amendment, in which the inclusion criteria were extended from 8-16 weeks gestation to 8-22 weeks gestation, eligible participant identification rates improved. A further 21 individuals (13.7%) could not be approached as pre-screening identified that they had been commenced on pharmacotherapy for hyperglycaemia.

In total, pre-screening identified 102 eligible women. The research team was unable to contact 24 individuals. Of the 78 individuals who could be contacted, 45 (57.7%) expressed an interest in participating and were screened subsequent to the informed consent process. Reasons for declining to participate included not wishing to take any medications in pregnancy (n= 21, 26.9% of those who were eligible), not wishing to take placebo tablets (n=5), not wishing to monitor their glucose values (n=4) and adverse outcomes in their previous pregnancy presenting a personal barrier to taking part in any type of research (n=3).

Figure 26: Women assessed for eligibility for the PRoDroME trial (November 2014-December 2016 inclusive)



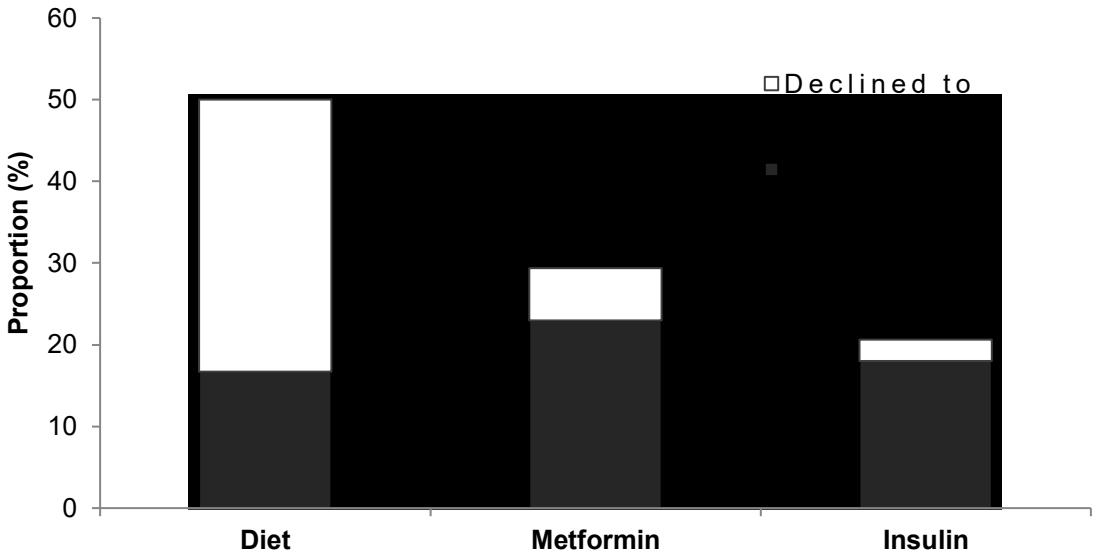
Footnotes

§ Excludes women who attended an antenatal booking visit but did not subsequently re-attend the service

Abbreviations: *GA* Gestational age, *T2DM* Type 2 diabetes mellitus, *HBGM* Home blood glucose monitoring, *SB* Still birth, *NND* Neonatal death.

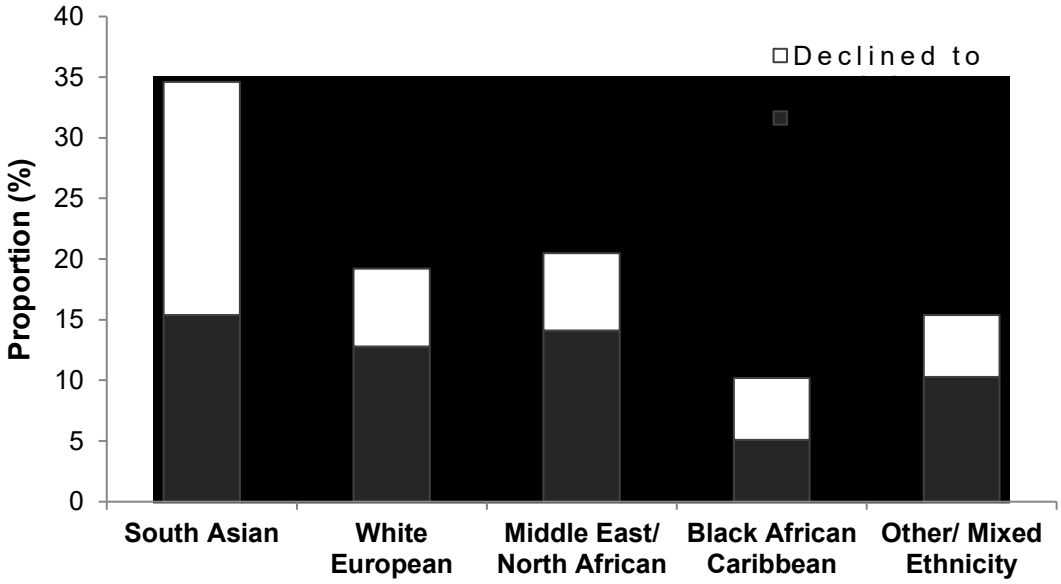
Of the 78 eligible women who were successfully contacted within the recruitment window, 39 (50.0%) had been managed with diet in their previous pregnancy complicated by GDM (Figure 27). The majority within this subgroup declined to participate (n=26, 78.8% of those managed with dietary modification only). The proportion of women expressing interest was higher in those previously treated with either metformin and/ or insulin (Figure 27).

Figure 27: Expression of interest in participating in the randomised control trial according to the mode of treatment required to control hyperglycaemia in the previous pregnancy across the three sites (November 2014 – December 2016 inclusive)



Expression of interest according to ethnicity was also reviewed (Figure 28). Overall, the largest proportion of eligible women who were contactable, were of South Asian ethnicity (34.6%): 55.5% of those within this ethnic group declined to participate. The majority of women of White-European, Middle east/ North African and Other/ Mixed ethnicity expressed an interest in participating (66.7%, 68.8% and 66.9% respectively).

Figure 28: Expression of interest in participating in the randomised control trial according to ethnicity across the three sites (November 2014 – December 2016 inclusive)



Differences in recruitment rates varied across the trusts with recruitment rates being higher in Imperial College Healthcare NHS Trust. This may have related to differences in practice. As outlined in Chapter 5 Section 5.1, women attending the London North West Healthcare NHS Trust were offered a 16-week oral glucose tolerance test if they had had a previous pregnancy complicated by GDM. By definition, those with hyperglycaemia diagnostic of GDM were not eligible to participate in the PRoDroME trial and were provided with a home glucometer together with dietary advice. In general, women with normoglycaemia at their 16-week assessment felt they would "not need to worry about [diabetes] now" and many declined on the basis that they did not wish to take tablets when their glucose values were normal. Furthermore, the proportion that had been managed with diet alone in their previous pregnancy was substantially higher in Northwick Park Hospital compared to ICHNT (37.2% versus 12.8% of eligible women who were successfully contacted). This probably had an

additional impact on the low recruitment rates observed at this trust particularly when considering the lower uptake amongst those who had been previously managed without pharmacological therapy (Figure 27).

6.2.2. Screening

From 30/11/2014 to 01/11/2016, 45 women expressed interest. Nine women developed hyperglycaemia prior to their screening visits and therefore required immediate treatment. Consent was obtained to prospectively collect data regarding the latters' materno-fetal outcomes as well as to collect data at a postnatal visit to assess the maternal and fetal characteristics defined for the postnatal study visit.

To date, 34 women have been consented to the ProDroME trial. Median (IQR) gestational age at recruitment was 16 (13.6-22) weeks, median (IQR) age 34 (30-36) years and mean (SD) body mass index (BMI) 28.7 (±5.1) kg/m^2 (Table 26). Overall, 79.4% were of non-White ethnicity with women of South Asian ethnicity forming the largest sub-group (26.5%) and those of Arab and North African origin the second largest (23.4%). Seven individuals were unable to read English. Assistance with reading the participant information sheet had either been provided at home by their partners or by their general practitioner. Only one participant was unable to speak English: a formal NHS accredited translator provided translation during the consenting process.

Table 26: Baseline demographics, obstetric history and anthropometry in the participants screened for the PRoDroME Trial

	Participants screened for ProDroME Trial
N	34
Median (IQR) Gestational age at screening (weeks)	16 (13.6-21)
Median (IQR) Age (years)	34 (30-36)
Proportion Non-White Ethnicity % (n)	79.4 (27)
Proportion able to read English % (n)	79.4 (27)
Family history T2DM % (n) - Proportion with 1st degree relative - Proportion with 2nd degree relative	 73.4 (25) 8.8 (3)
Parity - Proportion multiparous % (n)	 6.2 (2)
Mean (SD) Height (cm)	160.5 (±5.6)
Median (IQR) Weight (kg)	69.9 (64.2-85.6)
Mean (SD) BMI (kg/m^2)	28.7 (±5.1)
Median (IQR) Water mass (kg)	32.1 (29.8-35.8)
Median (IQR) Water percentage	44.1 (41.3-47.8)
Median (IQR) Fat mass (kg)	29.3 (20.5-34.4)
Median (IQR) Fat percentage	39.5 (33.1-43.8)
Median (IQR) Systolic blood pressure (mmHg)	106 (100-113)
Median (IQR) Diastolic blood pressure (mmHg)	70 (62-71)
Footnotes: Multiparous defined as delivery of four or more neonates ≥ 24 weeks gestation	

By definition, all women had delivered at least one neonate ≥24weeks gestation: 62.5% of the recruited cohort had had only one child prior to recruitment. The majority of the women who consented to the trial had had only one pregnancy complicated by GDM (74.2%). Of the 34 recruited participants, 10 (29.4%) had required insulin (with or without metformin) in their previous pregnancies to achieve

target glycaemic control. Seven (20.6%) individuals achieved target glycaemia with dietary modification alone. The remainder had been prescribed metformin alone. Biochemical measurements including glycaemia and assessments of insulin resistance and insulin sensitivity as determined by homeostatic model assessment tools, are detailed in Table 27.

Table 27: Biochemical data in those screened for participation in the PRoDroME Trial

	Participants screened for ProDroME Trial
N	34
Median (IQR) FPG (mmol/L)	4.6 (4.4-5.2)
Mean (SD) HbA1c (mmol/mol)	35 (±5)
Median (IQR) Triglycerides (mmol/L)	1.31 (1.14-2.08)
Median (IQR) Insulin (pmol/L)	9.6 (5.5-12.8)
Median (IQR) HOMA-IR	1.7 (1.0-2.6)
Median (IQR) HOMA-β (%)	134.5 (97.8-196.4)
Median (IQR) Vitamin B12 (pmol/L)	257 (194-376)
Median (IQR) Vitamin D (nmol/L)	37 (26.9-54.3)

At screening, women were provided with two questionnaires to complete (Appendix 3). Of the 34 individuals screened, 14 were unable to complete the questionnaires fully due to language restraints (20.6% of the women were unable to read English) or time constraints (Figures 29-31).

Figure 29: EQ-5D-5L Questionnaires at Screening for the PRoDroME Trial (n=21)

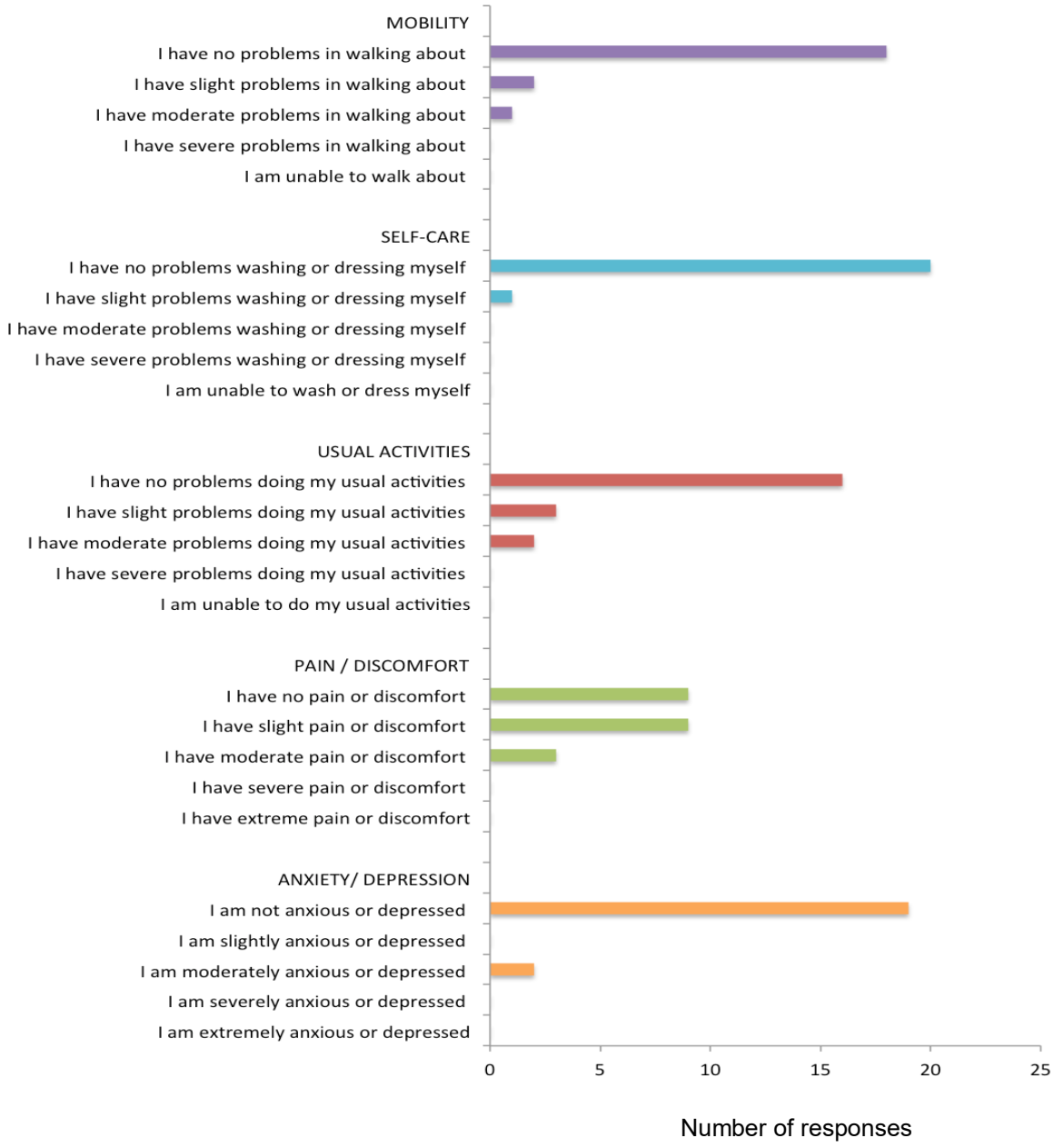


Figure 30: EQ-5D-5L Health Scale completed at screening visit (n=21)

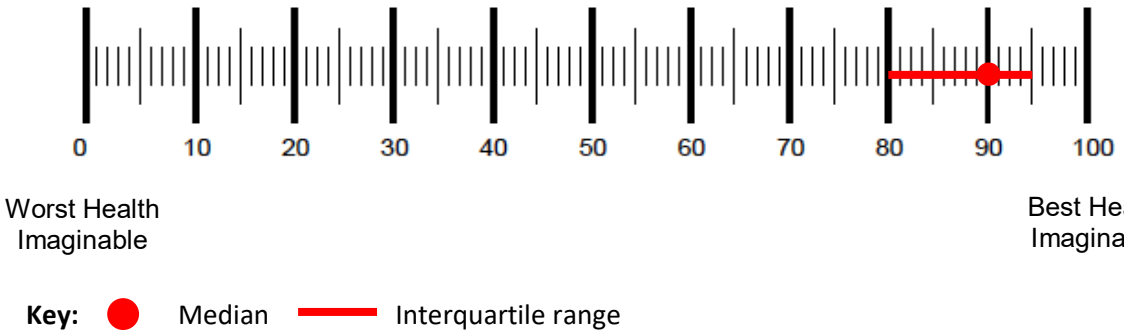
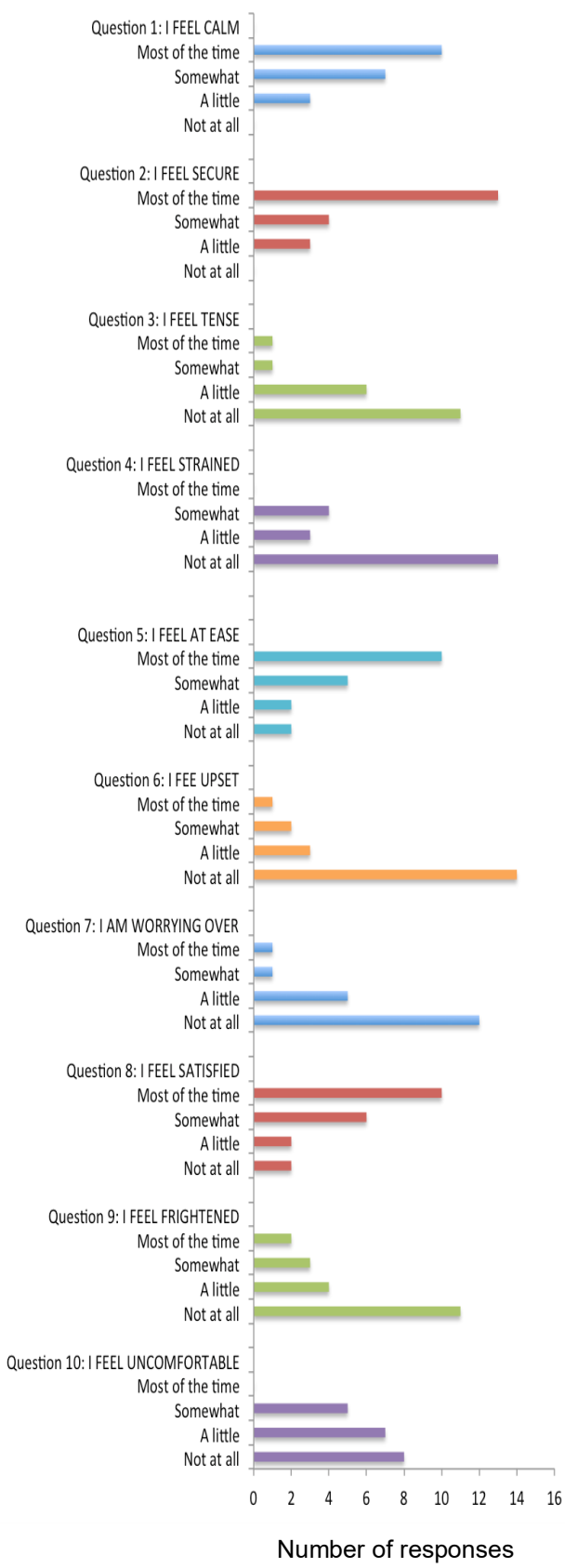
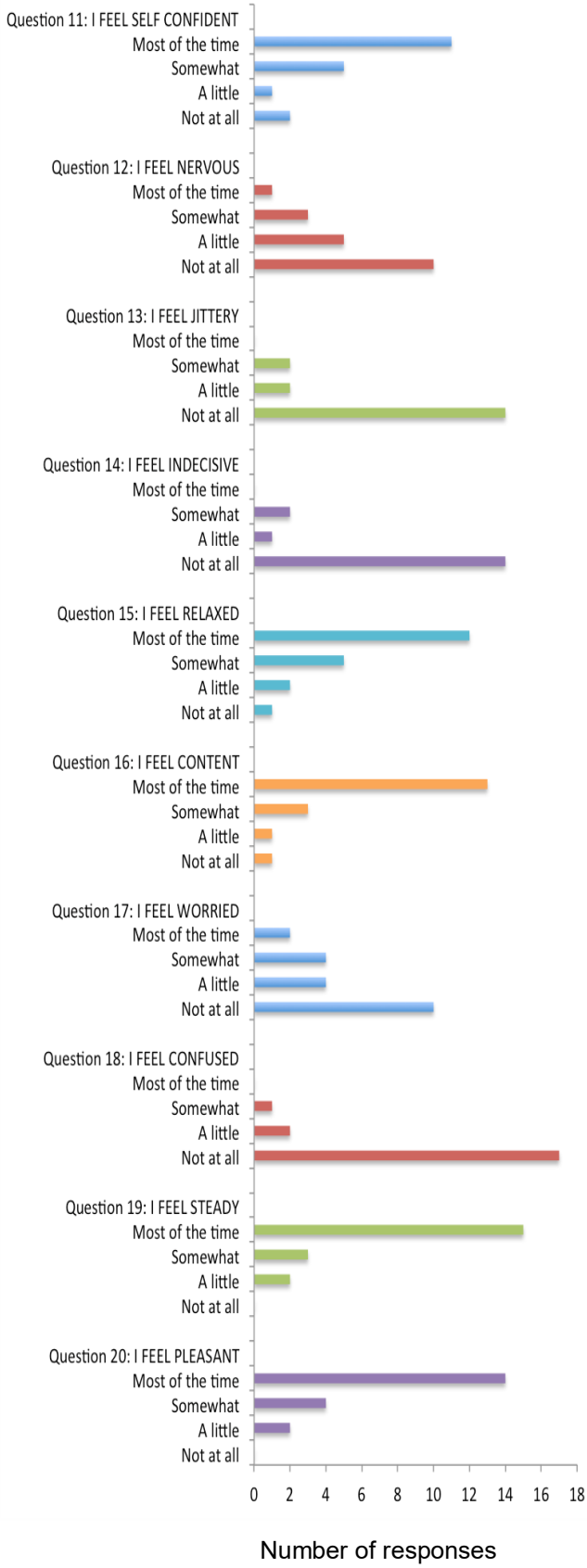


Figure 31: Stait-Trait Anxiety Inventory Questionnaire Results at Screening for the PRoDroME Trial (n=20)





6.3. PRoDroME Trial: Study Progress

6.3.1. Randomisation

Of the 34 women screened, 22 were successfully randomised to the study medications. Two of the 12 individuals could not be randomised due to laboratory evidence of hyperglycaemia either by fasting plasma glucose (FPG) criteria or HbA1c. Ten developed hyperglycaemia as evidenced by their home capillary blood glucose values prior to being randomised (defined as three or more elevated readings in one week). Importantly, this was despite an early pregnancy HbA1c ≤43mmol/mol and an FPG measuring <6.1mmol/L. These women were consented to the prospective arm of the trial and data relating to their baseline demographics, gestational weight gain, delivery information and postnatal glucose assessments were collected.

6.3.2. Differences in demographics, anthropometry and biochemistry at screening in individuals who could and could not be randomised

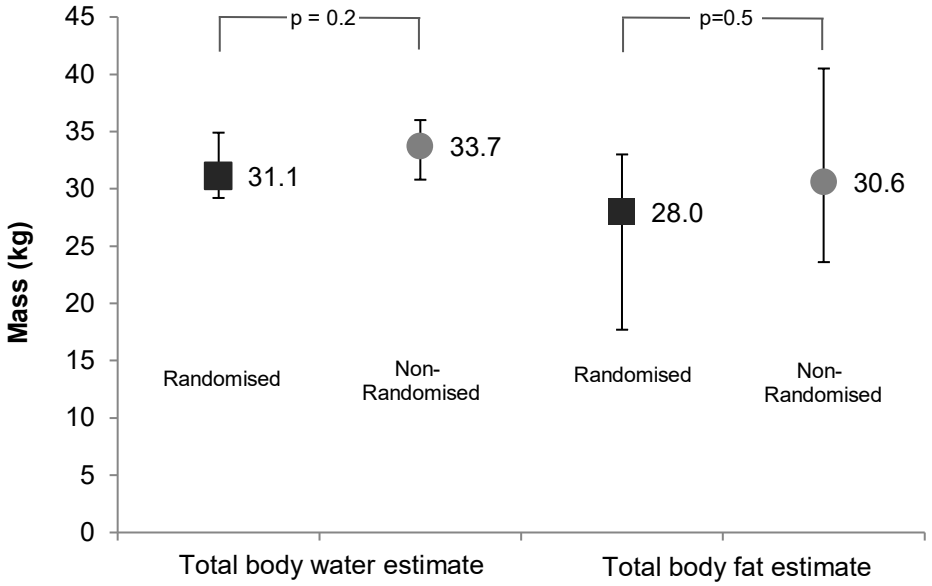
Women who were successfully randomised had a higher gestational age at screening than those who could not be randomised (Table 28). Median age, the proportion of women of non-White ethnicity and the proportion of those requiring insulin treatment in their previous pregnancy were similar in the randomised and non-randomised individuals. Anthropometric data including height, weight, body mass index and total body fat and water were similar in these two sub-groups (Table 28, Figures 32 (a) and (b)).

Table 28: Baseline demographics, obstetric history, anthropometry and laboratory investigations in women randomised to the study medications and in women who did not fit criteria fro randomisation

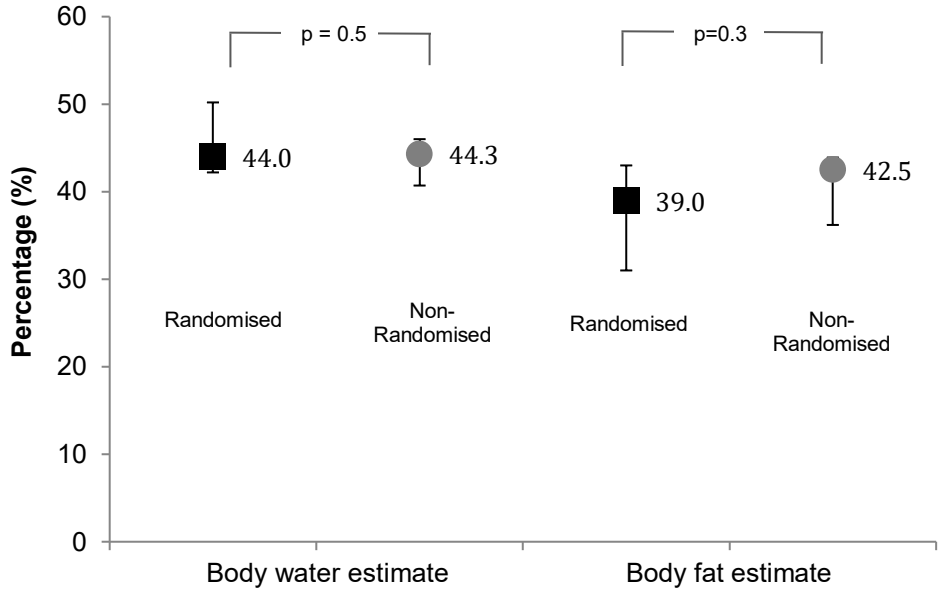
	Randomised participants	**Non-randomised participants**	**Significance**
N	22	12	
Median (IQR) Gestational age at screening (weeks)	18.2 (15.5-21.6)	13.6 (12.0-17.5)	0.012
Median (IQR) Age (years)	33.5 (29-36)	35 (33-37.5)	0.1
Proportion Non-White Ethnicity % (n)	81.8 (18)	75.0 (9)	0.6
Family history T2DM % (n) - Proportion with 1st degree relative - Proportion with 2nd degree relative	90.9 (20) 77.3 (17) 13.6 (3)	66.7 (8) 66.7 (8) 0.0 (0)	0.08
Proportion requiring insulin in previous pregnancy % (n)	31.8 (7)	25.0 (3)	0.4
Mean (SD) Height (cm)	160.3 (±6.7)	160.9 (±3.3)	0.8
Median (IQR) Weight (kg)	69.9 (61-84.8)	74.4 (65.3-91.9)	0.5
Mean (SD) BMI (kg/m^2)	28.1 (±4.7)	29.8 (±5.7)	0.4
Median (IQR) Systolic blood pressure (mmHg)	106 (101-113)	104 (98-110)	0.4
Median (IQR) Diastolic blood pressure (mmHg)	70 (62-71)	67 (64-70)	0.7

Figures 32 (a) and (b): Bioimpedance analysis results at screening in randomised and non-randomised individuals screened for the PRoDroME Trial

(a) Total body water and fat mass

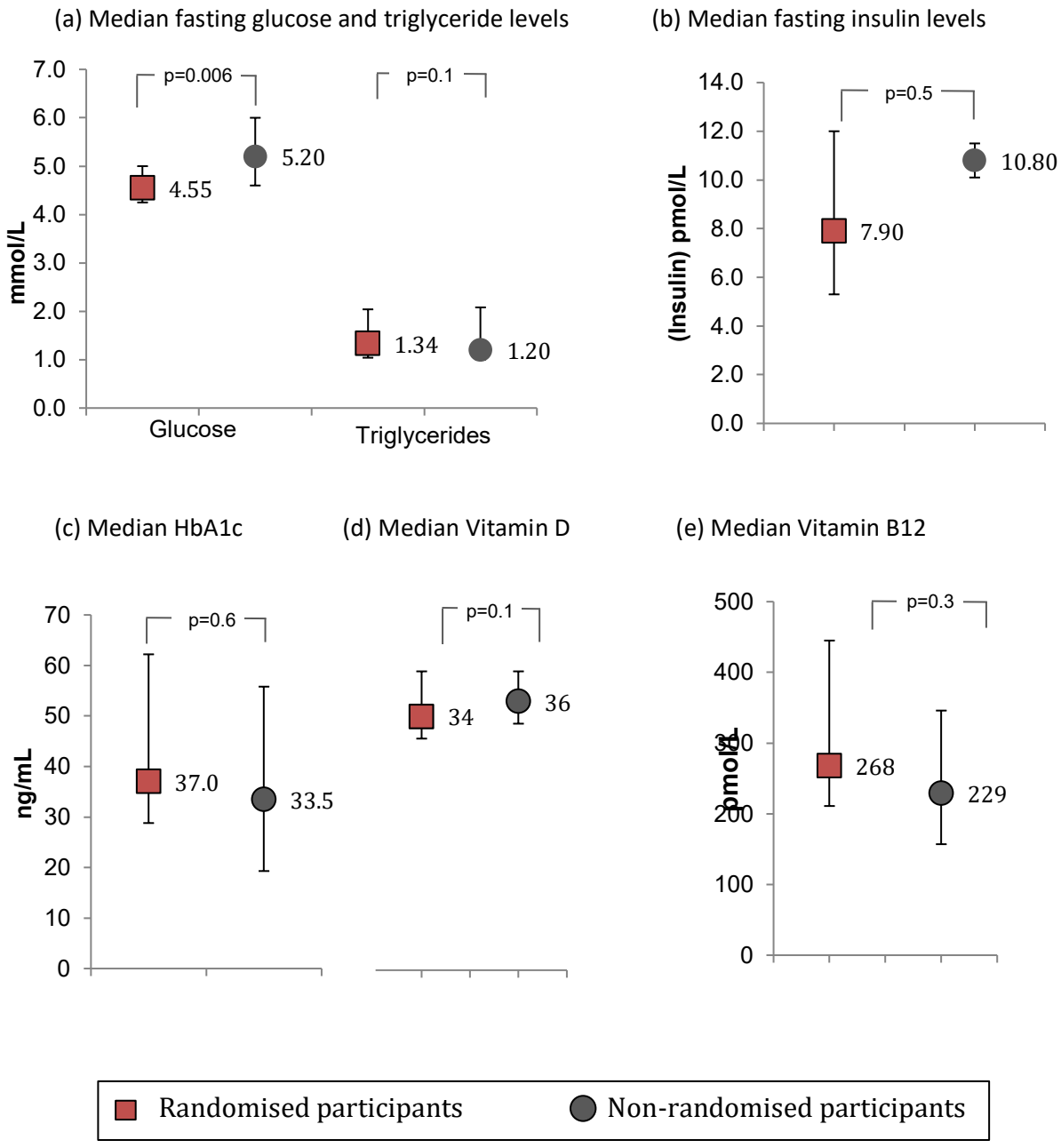


(b) Total body water and fat percentage

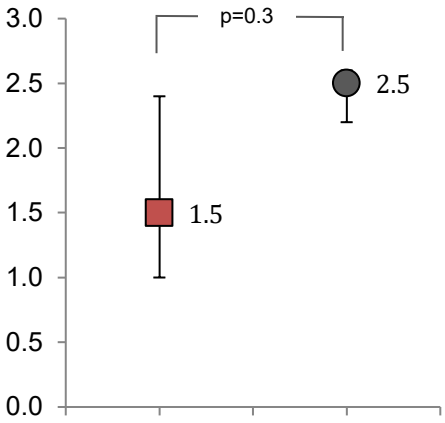


Median fasting plasma glucose was significantly lower in the women who were randomised (Figure 33(a)). Median triglyceride concentrations, plasma insulin levels, HbA1c, vitamin D, vitamin B12, insulin resistance and insulin sensitivity were similar in the two groups (Figures 33 (b-g)).

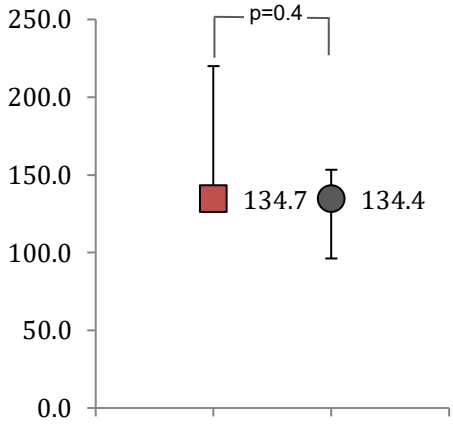
Figures 33 (a-g): Screening biochemical data

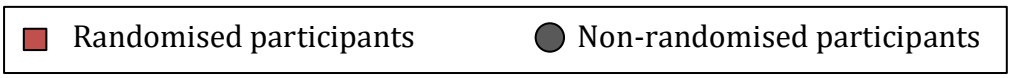


(f) Median HOMA-IR



(g) Median HOMA- β





6.3.3. Study Medication Concordance

Following randomisation, 19 women (86.4%) were able to appropriately up-titrate the study medications and tolerate the maximum prescribed dose of 1g twice daily. Two individuals were unable to titrate the dose beyond 500mg twice daily due to associated gastro-intestinal side effects and one individual discontinued the medications within 5 days of commencing treatment due to the development of lower pelvic pain. Concordance was good and measured 76.8%.

6.3.4. Adverse Events, Serious Adverse Events and Protocol Deviations

Adverse events, serious adverse events and protocol deviations were reported to the Sponsor as outlined in Chapter 5. In total, six adverse events, two serious adverse events and four protocol deviations were recorded the details of which are included in Table 29.

Table 29: Adverse events, serious adverse events and protocol deviations of the PRoDroME Trial (27/01/2015-01/11/2016)

	Participants randomised to metformin/ matched placebo
Adverse Events	
Unplanned hospital attendances	4
Gastrointestinal side effects §	2
Serious Adverse Events	
NICU admission 14/7: premature delivery	1
Shoulder dystocia	1
Protocol Deviations	
Accidental increase in tablets	2
Incorrect labeling of study medications	1
Incorrect randomisation number allocated	1

§ Defined as side effects resulting in either discontinuing study medications permanently or withholding them for a minimum of five days.

6.3.4. Development of GDM

In total, eleven randomised participants developed gestational diabetes: four developed hyperglycaemia prior to the scheduled 75g oral glucose tolerance test (OGTT) at 28 weeks gestation, three were diagnosed with GDM at the 28 week OGTT results and a further 4 developed hyperglycaemia after 30 weeks gestation. Eleven women continued the study medications for the duration of their pregnancy until the onset of labour i.e. the GDM recurrence rate in the women recruited to date is 50% (Table 30).

6.3.5. Maternofetal outcomes recorded in randomised participants

At 36 weeks gestation, randomised participants had gained a median of 5.1 (IQR 0.8-7.6) kg in weight (Table 30). Of the 11 randomised participants who developed GDM, 81.8% (n=9) participants required treatment with insulin (i.e. 40.0% of the overall cohort). One individual developed pre-eclampsia: there were no further reported incidences of pregnancy-related hypertensive disorders. One individual required an emergency caesarean section (4.8%): 66.7% neonates were born by spontaneous vertex delivery.

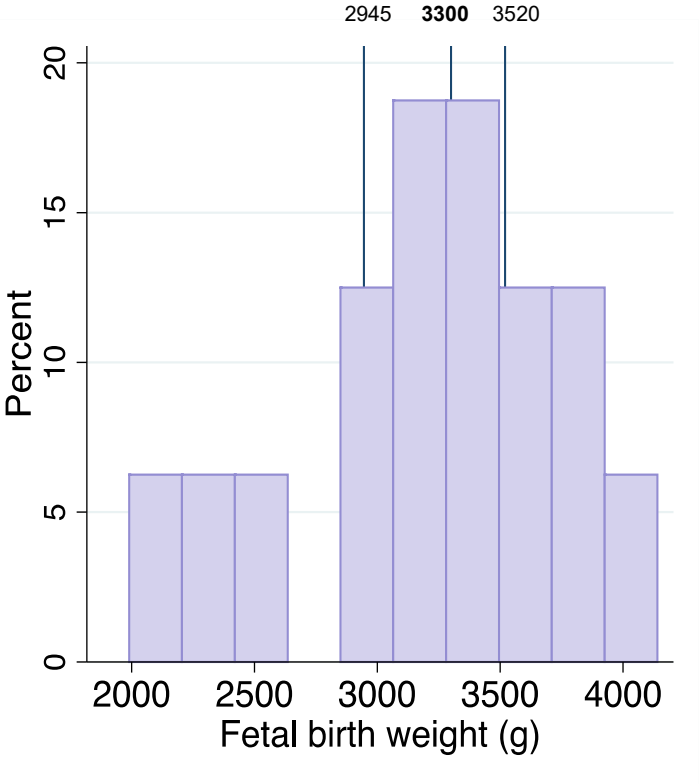
Table 30: Maternal outcomes and delivery modalities in women randomised to metformin or matched placebo in the PRoDroME Trial

	Participants randomised to metformin/ matched placebo
N	22
Median (IQR) Weight at 36 weeks gestation (kg)	80.1 (65.2-86.4)
Median (IQR) Gestational weight gain [I] (kg)	5.1 (0.8-7.6)
Proportion developed GDM % (n)	50.0 (11)
Proportion requiring insulin treatment % (n)	40.9 (9)
Proportion with pre-eclampsia % (n)	4.5 (1)
Proportion with PIH % (n)	0 (0)
Delivery Modality	
Spontaneous vertex delivery % (n)	66.7 (14)
Assisted vertex delivery % (n)	4.8 (1)
Elective caesarean section % (n)	23.8 (5)
Emergency caesarean section % (n)	4.8 (1)
Incidence postpartum haemorrhage	
Moderate (500-100ml) % (n)	28.6 (4)
Severe (≥1000ml)	7.1 (1)

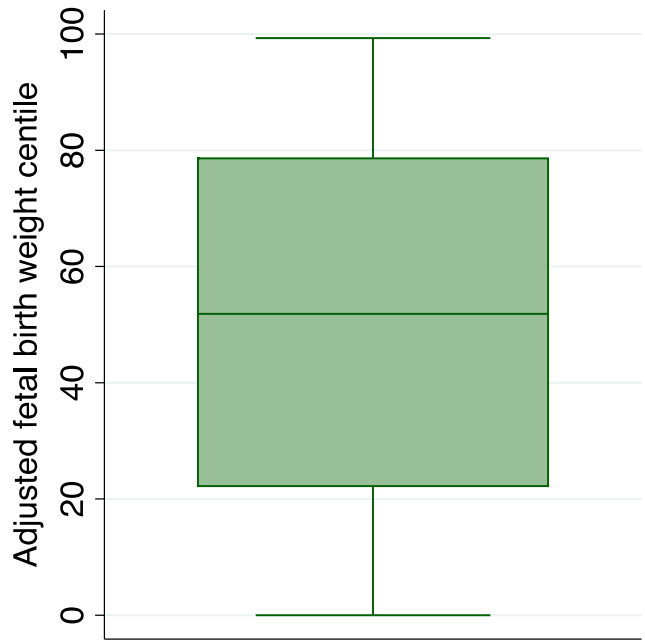
Median (IQR) fetal birth weight measured 3300 (2945-3520) g and mean (SD) adjusted fetal birth weight centile 47.9 (±28.4) (Figures 34 (a) and (b)). One neonate was born macrosomic (i.e. ≥4000g): however, overall, no neonates were born large for gestational age (i.e. ≥ 90th birth weight centile). Two neonates (9.1%) were born small for gestational age. Three (13.6%) neonates experienced postnatal complications: there was one case of shoulder dystocia, one of respiratory distress requiring 30 minutes of oxygen therapy and one neonate was born prematurely at 32 weeks gestation following which a fourteen day admission to the neonatal intensive care unit was required. There were no concerns relating to developmental milestones in these neonates (followed for up to one year postpartum).

Figures 34 (a) and (b): Distribution fetal birth weight and fetal birth weight centile in the neonates born to randomised participants

(a): Fetal birth weight



(b): Fetal birth weight centile



Cord blood collection was performed in only a small number at delivery (n=8): mean (SD) cord blood glucose measured 4.4 (±1.2) mmol/L and cord blood c-peptide 379 (± 61) pmol/L.

6.3.6. Postnatal visits

Final study visits were conducted at a median (IQR) of 9 (6-10) weeks postpartum. Median (IQR) fasting plasma glucose measured 4.7 (4.5-4.9) mmol/L. All randomised participants had a normal FPG postpartum.

6.3.7. Changes in weight and biochemical data at screening, 28 weeks gestation and postpartum in randomised participants

Changes in weight throughout the course of pregnancy and postpartum were non-significant (Table 31). Fasting plasma glucose was also similar at these three time points. Absolute insulin values and assessment of both insulin resistance (as determined by HOMA-IR) and insulin sensitivity (as determined by HOMA- β), varied significantly at these three time points, with the highest values being at 28 weeks gestation mirroring the physiological changes observed in pregnancy (Chapter 1).

Differences in vitamin B12 values were also significant with the highest levels being demonstrated at the postnatal visit (Table 31). In contrast, Vitamin D levels were similar throughout gestation and at the postnatal in the randomised participants.

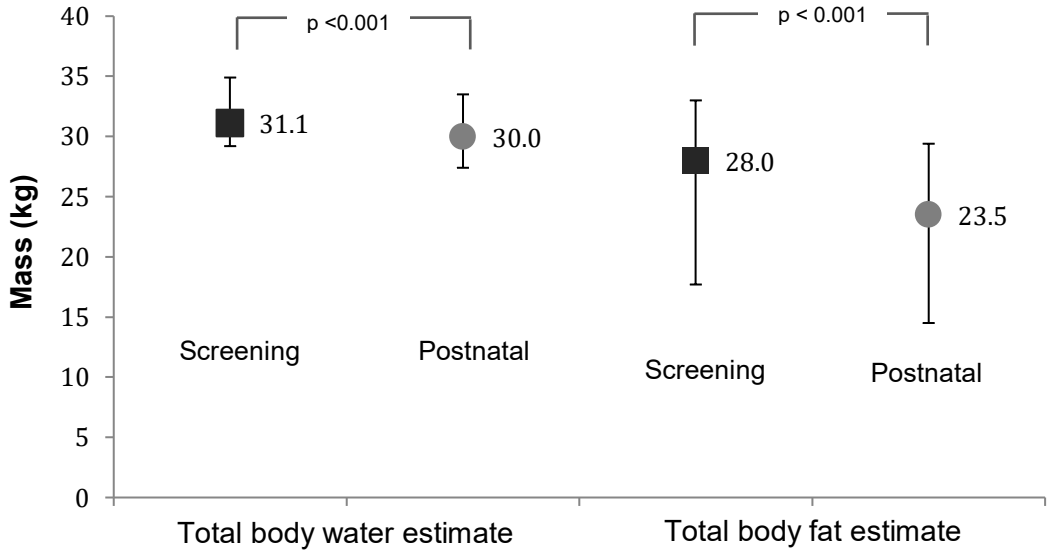
Table 31: Maternal weight and biochemical data at screening, 28 weeks gestation and postnatal visit

	Screening	28 weeks gestation	Postnatal Visit	P value
Median (IQR) Weight (kg)	69.9 (61-84.8)	70.8 (65.5 -74.4)	64.7 (53.2-64.7)	0.9
Median (IQR) FPG (mmol/L)	4.6 (4.3-4.7)	4.6 (4.2-4.7)	4.7 (4.5-4.9)	0.9
Median (IQR) Triglyceride (mmol/L)	1.34 (1.19-2.04)	1.9 (1.5-2.1)	0.98 (0.72-1.32)	0.02
Median (IQR) Insulin (pmol/L)	7.9 (5.3-12.0)	8.8 (5.7-10.0)	5.6 (4.1-7.2)	0.004
Median (IQR) HOMA-IR	1.5 (1.0-2.4)	1.6 (1.07-2.09)	0.89 (0.8-1.5)	0.049
Median (IQR) HOMA-β (%)	134.7 (127.3-220.0)	170.6 (103.6-240.0)	115.0 (75.3-136.7)	0.009
Median (IQR) Vitamin B12 (pmol/L)	268 (211-388)	321 (257-374)	453 (311-634)	0.007
Median (IQR) Vitamin D (nmol/L)	37.0 (28.8-54)	37.1 (34.5-75.6)	40.2 (29.3-69.6)	1.0

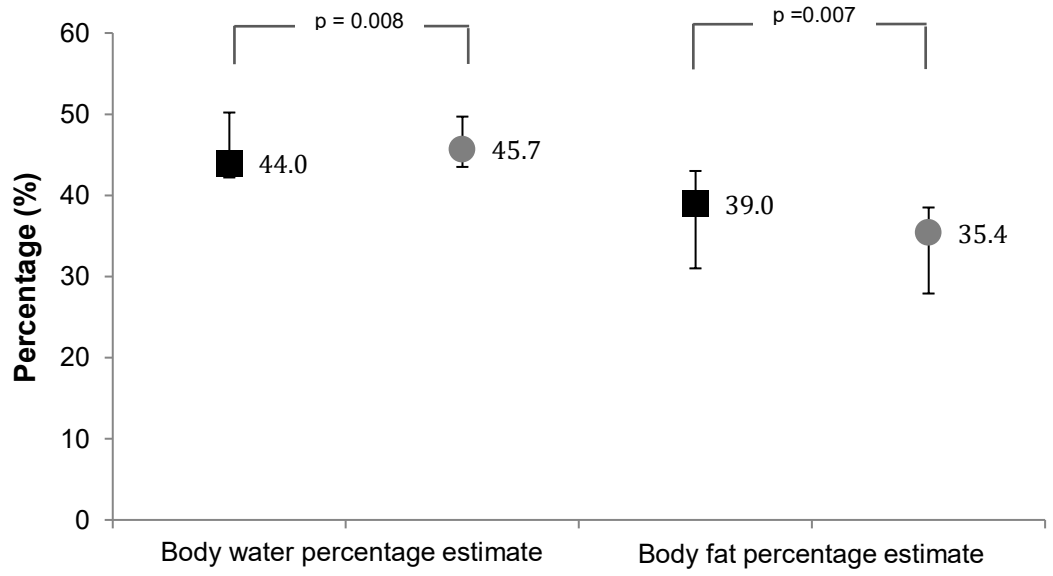
Bioimpedance analysis demonstrated that total body water and fat estimates significantly reduced postnatally from the original values at their screening visits (Figures 35 (a) and (b)).

Figure 35 (a) and (b): Changes in bioimpedance analysis at screening and at the postnatal assessment in randomised participants

(a) Changes in total body water and total body fast estimates



(b) Changes in total body water and total body fast estimates



6.4. Clinical Predictors for Recurrent Gestational Diabetes

6.4.1. Baseline demographics and biochemical data in women participating in the prospective arm of the ProDroME trial

To ascertain if clinical predictors for recurrent gestational diabetes mellitus could be identified, data relating to women who consented to the prospective arm were examined (n=58). Mean age in this group was 34.5 (± 4.3) years old, median (IQR) height measured 160cm (157-163) and median (IQR) weight 69.4 (61.5-83.2) kg. Median BMI measured 27.4 (23.9-31.6) kg/m^2. 76.8% were of non-White ethnicity.

Due to the differences in practice across the sites with women at St Mary's Hospital being offered home blood glucose monitoring from 16-18 weeks gestation and those at Northwick Park Hospital being offered a 75g oral glucose tolerance test at 16 weeks gestation (individuals at Queen Charlotte's and Chelsea Hospital were offered the option of either of the latter two), recurrence of gestational diabetes was defined as either an abnormal oral glucose tolerance test or the need for pharmacotherapy to maintain normoglycaemia. 77.6% of the women developed hyperglycaemia as defined by either of the latter criteria. Of the 22.4% of women who maintained normoglycaemia, 3.5% had a normal OGTT result i.e. 2 women.

Women who developed hyperglycaemia requiring pharmacological treatment had a higher weight and a body mass index at their initial antenatal assessment (Table 32). A higher proportion had a family history of type 2 diabetes (either affecting a first or second degree relative). Mean age was similar in the two groups as were the

proportion of women of non-White ethnicity. A higher proportion of women who developed hyperglycaemia required insulin in the previous pregnancy (24%), though this was non-significant (p=0.2).

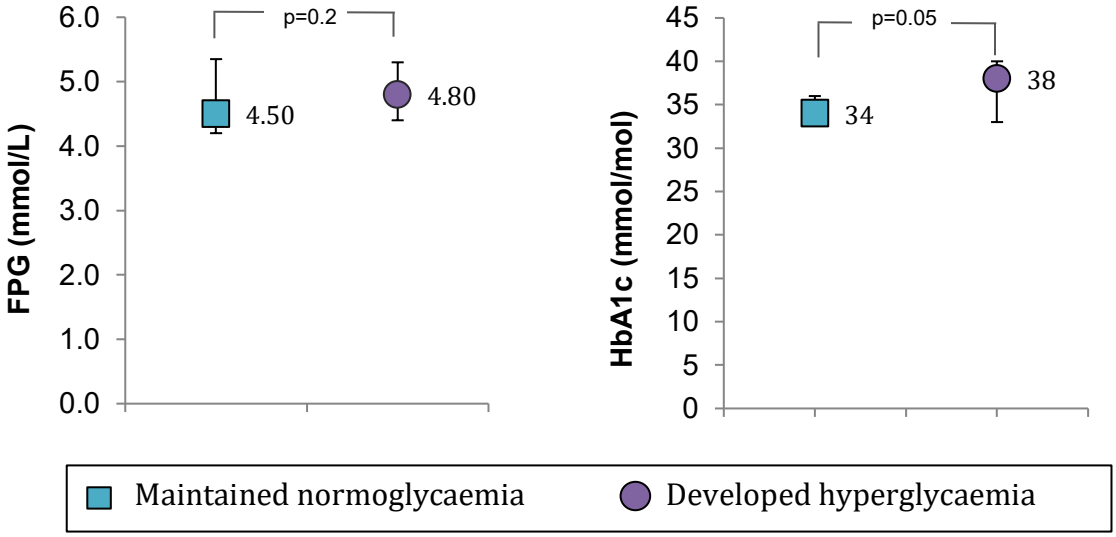
Table 32: Baseline demographics, obstetric history, anthropometry and laboratory investigations in women participating in the prospective non-interventional arm of the trial

	Maintained normoglycaemia	**Required pharmacotherapy**	**Significance**
N	13	45	
Median (IQR) gestational age (weeks)	10 (6-11)	9 (6-12)	0.4
Median (IQR) Age (years)	35.8 (±4.6)	34.2 (±4.2)	0.2
Proportion Non-White Ethnicity % (n)	76.9 (10)	77.8 (35)	0.9
Family history T2DM % (n) - 1st degree relative - 2nd degree relative	38.4 (5) 77.3 (17) 13.6 (3)	68.9 (31) 66.7 (8) 0.0 (0)	<0.001
Proportion requiring insulin in previous pregnancy % (n)	0 (0)	24.4 (11)	0.2
Mean (SD) Height (cm)	160.0 (157-163)	160.0 (157-163)	0.8
Median (IQR) Weight (kg)	62.2 (58.9-69.0)	72.7 (64.3-85.6)	0.03
Mean (SD) BMI (kg/m^2)	23.9 (22.7-26.9)	28.7 (24.7-32.9)	0.01

There was no difference in median fasting plasma glucose in early pregnancy in women who did and did not later develop hyperglycaemia (Figure 36 (a)). Women who required treatment in their pregnancy had a higher HbA1c in early pregnancy:

median HbA1c 38 (33-40) mmol/ mol versus 34 (33-35) mmol/mol in those who maintained normoglycaemia (p=0.05) (Figure 36(b)).

Figure 36 (a, b): Baseline biochemical screening data in randomised and non-randomised participants



6.4.2. Materno-fetal outcomes in women with a previous history of gestational diabetes mellitus

The majority of women who developed hyperglycaemia required insulin treatment (71.1%, n=32) (Table 33). Though a higher proportion of neonates were born premature in women who developed hyperglycaemia, this was non-significant (15.6% versus 0.0% in women who maintained normoglycaemia, p=0.1).

Median fetal birth weight and adjusted birth weight centile were significantly higher in women who maintained normoglycaemia compared to those who did not (Table

33). The proportion of infants born macrosomic (i.e. ≥4000g) and large for gestational age (≥ 90th adjusted birth weight centile) were similar in the two groups. 15.6% of neonates were born small for gestational age (SGA) in women who developed hyperglycaemia: no neonates were born SGA in women who maintained normoglycaemia (p=0.5).

Table 33: Materno-fetal outcomes in women participating in the prospective arm of the trial

	Maintained normoglycaemia	**Required pharmacotherapy**	**Significance**
N	13	45	
Proportion requiring insulin treatment % (n)	NA	71.1 (32)	NA
Proportion born <37 weeks gestation % (n)	0 (0)	15.6 (7)	0.1
Median (IQR) fetal birth weight (g)	3560 (3370-3920)	3220 (2750-3510)	0.007
Median (IQR) fetal birth weight centile	60.4 (41.7-87.7)	43.4 (19.2-69.9)	0.02
Proportion infants born macrosomic % (n)	15.4 (2)	13.3 (6)	0.9
Proportion infants born LGA % (n)	23.1 (3)	24.4 (11)	0.6
Proportion infants born SGA % (n)	0 (0)	15.6 (7)	0.5
Postnatal complications % (n)	7.7 (1)	26.5 (9)	0.1

LGA Large for gestational age (adjusted birth weight ≥90th centile), *SGA* Small for gestational age (adjusted birth weight <10th centile)

A non-significant increase in postnatal complications was also observed in women who developed hyperglycaemia (26.5% versus 7.7%, p=0.1). Of the 10 incidences of postnatal complications observed, 5 were attributable to neonatal hypoglycaemia,

two to neonatal hyperbilirubinaemia requiring phototherapy and one to respiratory distress syndrome requiring oxygen therapy. One neonate required admission to the neonatal intensive care unit and one stillbirth was recorded (fetus aged 26 weeks gestation: mother had developed hyperglycaemia at 20 weeks gestation).

6.5. Future Work and Recruitment

Future work will focus on continued recruitment to the PRoDroME trial. While the planned target sample size is 112 women, this does include a drop out rate of 20%. To date, no women have dropped out of the trial and only one individual has self-discontinued study medications within a four week period. A smaller sample size to include a drop out rate of 10% may therefore be adequate in being able to determine the primary outcome of this trial. An extension to the recruitment period is planned and a fourth site is considering opening to the trial.

6.6. Conclusions

The Preventing Recurrent Gestational Diabetes with Early Metformin Intervention trial (PRoDroME) is currently actively recruiting participants. From the time of all the necessary regulatory approvals to the pre-defined end point for data collection for this work, 153 pregnant women with a previous history of gestational diabetes were identified across the three participating sites.

Difficulty in contacting the women and interim development of hyperglycaemia in pregnancy meant that the trial was discussed with only 78 participants. Of these, 45

expressed an interest in participating: the majority of those who declined did so on the basis of not wishing to take medications. A higher proportion of those who had previously been treated with either metformin or insulin expressed an interest in participating compared to those who had only been managed with diet in their previous pregnancy complicated by hyperglycaemia.

The ability to randomise interested participants was impacted by the interim development of hyperglycaemia either prior to or following screening in 22 individuals. In total, 22 individuals have been randomised. Baseline demographics, anthropometry and biochemical data for these individuals have been presented in this chapter. Medications have generally been well tolerated as demonstrated by the majority being able to up-titrate the dose appropriately and concordance with the study protocol has also been good.

Gestational diabetes mellitus has recurred in 50% of the women who have been randomised to metformin or matched placebo. Of the women, who developed hyperglycaemia, 81.8% (40.9% of total cohort), required insulin therapy. One incidence of pregnancy associated hypertensive disorders was recorded (pre-eclampsia). One neonate was born macrosomic (birth weight ≥4000g): none were born large for gestational age and two were born small for gestational age. Immediate postnatal complications occurred in three deliveries: one incidence of premature delivery at 32 weeks gestation requiring a neonatal care admission for 14 days, one incidence of shoulder dystocia and one incidence of respiratory distress syndrome requiring oxygen therapy. All these incidences were reported as serious adverse

events and discussed at the data monitoring committee: none were deemed related to the study medications.

The recurrence rate for gestational diabetes mellitus in the prospective cohort was 77.9%. Women who maintained normoglycaemia were more likely to have a lower early pregnancy weight and body mass index than those who developed hyperglycaemia and required treatment. Importantly, these individuals were matched for gestational age when their demographics were recorded. Prior treatment with insulin was also similar in the two groups. A lower proportion of those who maintained normoglycaemia had a family history of T2DM. Of those who later developed hyperglcaemia, 71.1% required treatment with insulin. Mean birth weight and adjusted birth weight centile were higher in those born to the women who maintained normoglycaemia. However, proportions of neonates born macrosomic or large for gestational age were similar in the two groups (15.4% versus 13.3% and 23.1% versus 24.4% respectively). Non-significant increases in the proportion of neonates born small for gestational age or with postnatal complications were observed in infants born to the mothers who required treatment for hyperglycaemia (15.6% versus 0% and 26.5% versus 7.7% respectively).

6.7. Discussion

The PRoDroME trial is currently actively recruiting participants. Where women have successfully been identified within the correct gestational window, levels of interest

have been good with 61.6% of eligible participants expressing interest. This is consistent with previous metformin intervention studies in pregnancy: 50% in Metformin in Obese Pregnant (MOP) women and 33% in EmPoWAR (130, 131).

To date, 50% of the individuals randomised have developed GDM. The recurrence rate in the non-interventional arm is higher (77.9%). The latter rate is indeed reflective of data relating to GDM recurrence in multiethnic cohorts (135). Due to the blinded nature of the trial and the small numbers randomised to date, the effects of metformin in mitigating the risk of GDM recurring cannot currently be established. Factors beyond metformin therapy could be playing a part in the lower rates of GDM observed in the cohort participating in the interventional arm. In the first instance, the women who were randomised were in regular contact with members of the research team. Regular review by the same care-giver has already been demonstrated to be an important factor in preventing index gestational diabetes (41). Furthermore not only was glycaemic control discussed but gestational weight gain was also addressed. While no targets relating to the latter were implemented, the opportunity to discuss this did probably have a benefit as women on average gained no more than 5.0kg in weight throughout the course of their pregnancy: figures similar to this were observed in cohorts in whom gestational diabetes was successfully prevented in index pregnancies (see Chapter 1.6.2). In addition, the majority of the women recruited to PRoDroME were highly motivated individuals and were keen on implementing lifestyle strategies as well as taking the study medications. A strong evidence base does in fact exist demonstrating that outcomes in individuals taking part in randomised controlled trials are improved compared to the relevant general population (143). One key

limitation to this trial will be the absence of data collection relating to dietary patterns or measures of physical activity in randomised participants rendering it difficult to assess the individual impact of this.

Overall, 40.9% of individuals participating in the interventional trial required treatment with insulin: 71.1% required this in the prospective arm of the trial. No data exist relating to requirement for insulin therapy in women with recurrent GDM. When considering women with either recurrent GDM or an index pregnancy complicated by GDM, 70% need pharmacotherapy overall with 30% estimated to need insulin therapy to achieve target glycaemia (26).

In the randomised cohort, only one neonate has been born macrosomic and none have been born large for gestational age. This is in direct contrast to the prospective arm of the trial were approximately 15% of the neonates born to women who did or did not develop overt hyperglycaemia were born macrosomic and 24% large for gestational age. No data exist relating to incidence of LGA infants in women with recurrent GDM though the incidence of adverse outcomes e.g. shoulder dystocia is notably higher in individuals with more than one GDM pregnancy (ref).

When considering the prospective cohort in isolation, the fact that similar rates of fetal overgrowth were observed in women who maintained normoglycaemia in pregnancy once again implicates factors beyond glycaemic control. However, the finding that the women in the former group had a lower postnatal complication rate suggests an absence of pathophysiological complications secondary to the excess fetal

weight. The findings from the prospective cohort re-iterate those presented in Chapter 4, which implicated the detrimental effect of the length of exposure of glucose on materno-fetal outcomes.

6.8. Trial Update

Subsequent to the period of data collection that took place for this thesis, a further six women were recruited and successfully randomised to metformin/ matched placebo. Expiry of the study medications prevented further recruitment. Due to limitations in both funds to manufacture medication replacements and study personnel, recruitment for the trial has been put on hold.

In order to facilitate future recruitment, reduce the trial-related burden on participants and achieve the target sample size, the study protocol has been simplified as follows:

1. While the biochemical data will still be collected for the screening visit, follow up samples will be minimised and only collected at two further time points: at 28 weeks gestation, when the oral glucose tolerance test is planned, and at the postnatal visit (Table 34).

2. Study follow up visits will only take place at the following time points:

- Screening (12-22 weeks gestation)
- Randomisation visit to coincide with routine attendance e.g. at either 16-week midwife follow up appointment or 20-22 week anomaly scan

- 28-week glucose tolerance test to coincide with routine fetal growth scan (assuming glucoses have remained normal and participant has not developed hyperglycaemia in the interim).

As per routine clinical care, members of the research team will review home blood glucose levels every four weeks when women routinely attend for their antenatal follow up visits. If more than three glucose values are above target in the preceding week, i.e. ≥5.3mmol/L fasting or ≥7.8mmol/L, then the study medications will be discontinued and further management decided on by the clinical team i.e. initiation of open label metformin or insulin. Though repeat fasting plasma glucoses will not be measured (this has proved burdensome for both participants and study personnel) the integrity of the primary outcomes is maintained.

3. Cord blood will no longer be collected

- As demonstrated by the small number of samples collected in women recruited to date, this has been resource dependent and challenging to complete.

4. Neonatal skin folds will no longer be assessed

- Minimising variations in the measurements has also been challenging and the time points at which women have attended have varied substantially thereby rendering it difficult to draw firm conclusions from the data.

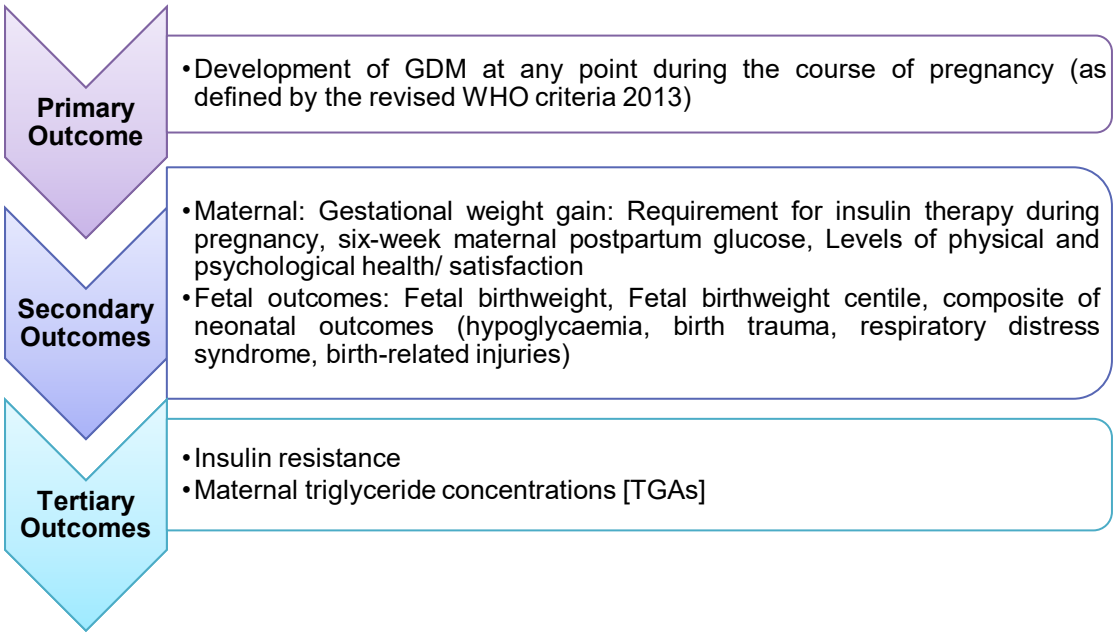
In addition to the above, bioimpedance analysis will only be assessed in centres, which have easy access to the Tanita machines.

Table 34: Revised schedule of assessments for PRoDroME Trial

Visit	S	28 week	Postnatal check
Weeks Gestation	**10-22**	**28**	**6-12 weeks postpartum**
Height	X		
Weight	X	X	X
Bioimpedance analysis	X	X	X
Fasting plasma glucose	X		X
HbA1c	X		
Full blood count	X		
Renal function	X		
Liver function	X		X
75g OGTT	X	X	
Vitamin B12	X	X	
Vitamin D	X	X	
Fasting plasma insulin	X	X	X
Triglycerides	X	X	X
Aliquots for storing	X	X	X
Health surveys	X	X	X
Neonatal weight and length			X

While sample collection has been minimised and the study protocol simplified, the integrity of the primary outcome has been maintained. Indeed, the primary and secondary outcomes are unaltered as a result of the above (Figure 37). Only the tertiary outcomes are affected, as assessing fetal hyperinsulinaemia will no longer be possible.

Figure 37: Figure illustrating revised outcomes



A joint collaborative Diabetes In Pregnancy Working Group has been established across the North West London region, which is supported by the Clinical Research Network. This Group has agreed to support provision of study personnel and commit to recruitment at sites within NWL including West Middlesex, Hillingdon and Chelsea and Westminster. Funding sources for the medication supply are currently being sought with an aim to resume recruitment in September 2019.

CHAPTER 7: DISCUSSION AND CONCLUSIONS

Gestational diabetes (GDM) is defined as "hyperglycaemia recognised in the second or third trimester that is not clearly overt diabetes" (1). The condition represents a significant risk to both the mother and developing baby: for the former, this includes an increased risk of developing pre-eclampsia or later development of type 2 diabetes (T2DM) and for the latter, birth-related injuries and metabolic as well as physiological sequleae both in the immediate post-natal period and in young adulthood e.g. neonatal hypoglycaemia and in adolescence, insulin resistance and obesity (2-4).

The incidence of GDM and its complications are increasing, reflecting changing pre-gravid female demographics. Managing GDM has significant cost implications both in terms of the resources required to adequately treat the condition as well as in terms of treating the complications associated with its development. Though treatment has consistently been shown to reduce the economic burden, particularly when considering the reduction in requirement for neonatal intensive care, it nonetheless remains substantial (27). Furthermore, long term follow up of infants born to mothers whose pregnancies are complicated by hyperglycaemia suggest that adiposity remains a significant problem despite effective treatment and management.

Preventing pathological hyperglycaemia during pregnancy therefore has several theoretical benefits: reduction in associated immediate maternal and fetal adverse outcomes and the potential for improvements in the risk of long-term sequelae as well as reductions in the economic burden to healthcare systems worldwide.

The randomised control trials, which have investigated the impact of lifestyle interventions in preventing GDM, have yielded conflicting results. This in part relates to the large degree of heterogeneity across the trials both in terms of the baseline demographics of the cohorts recruited as well as the screening strategies and diagnostic criteria used to define the condition (10). However, despite the absence of identifying a clear preventive strategy, important signals from these trials have emerged. One of these relates to the benefits of interventions in improving pre-defined materno-fetal outcomes e.g. fetal macrosomia even in the absence of preventing maternal hyperglycaemia. This in turn indicates that factors beyond maternal glucose are implicated in the pathogenesis of fetal overgrowth.

These factors have been explored in this thesis. As described in Chapter 2 complex interactions between genotypes, maternal metabolism and the intrauterine environment contribute to the final fetal weight at birth. Fetal development relies on a continuous supply of nutrients and metabolites, predominantly glucose, amino acids and triglycerides, crossing the placenta. The enhanced insulin resistance observed in pre-gestational conditions such as obesity are associated with an overall increase in maternal substrate availability including glucose, amino acids and triglycerides all of which drive fetal hyperinsulinaemia leading to enhanced fetal growth and an increase in neonatal adiposity (82).

The analysis presented in Chapter 2, demonstrated ethnicity-based variations in maternal body mass index, measures of glycaemia at 24-28 weeks, fetal birth weight

and birth weight centile in five different groups: White, Black African-Caribbean, South Asian, Mixed/ Other Asian and Other/ Unknown. Significant variations existed in the proportion of infants born either small (SGA; <10th adjusted birth weight centile) or large for gestational age (LGA; ≥90th centile), with women of White ethnic origin delivering the highest proportion of LGA infants and women of South Asian ethnicity the lowest: the reverse was true for SGA incidence. An increase in the category of fasting plasma glucose (FPG) at 28 weeks gestation was associated with an increase in the proportion of infants born LGA in each ethnic group with the exception of women categorised as Other/ Unknown ethnicity. This pattern was particularly pronounced in women of White ethnic origin with 45% of neonates being born LGA to women with the highest FPG category. In contrast, no clear relationship existed between 120-minute OGTT glucose values and the proportion of infants born LGA. While the absence of a clear pattern in post glucose load is unexpected given the pathophysiology of gestational diabetes (defects in first phase insulin are the initial feature of the condition), women who had GDM by modified WHO 1999 criteria were treated. This would have therefore mitigated the risk of fetal overgrowth associated with postprandial hyperglycaemia. An ethnic group dependent effect on the interaction between glucose and fetal birth weight was demonstrated that persisted following adjustment for maternal early pregnancy body mass index. Interactions between early pregnancy body mass index and fetal birth weight was not dependent on ethnicity. These findings have suggested ethnicity-based glucose thresholds may be warranted. In addition, they have implicated the importance of ensuring that any potential prevention strategy is applicable to a multi-ethnic cohort in view of probable

underlying pathophysiological differences contributing to fetal overgrowth in different ethnic groups.

The impact of glucose on fetal growth is considered in more detail in Chapter 3. Using blinded continuous glucose monitoring data from women with Type 1 and Type 2 diabetes (n=14 and 15 respectively), the impact of markers of absolute glycaemia (mean glucose, standard deviation from the mean glucose), glycaemic variability (mean absolute glucose, correlation variation) and low/ high blood glucose indices on fetal growth were analysed. 29 144 data points were examined. Overall, SD and HBGI correlated with adjusted fetal birth weight. When examining women who delivered large for gestational age neonates, differences in glycaemic variability were observed in women with Type 1 diabetes but not Type 2 diabetes. Large for gestational age infants were associated with a higher HbA1c, mean glucose, HGBI (high blood glycaemic index) and at borderline significance, standard deviation, in the former group. Glucose was not associated with fetal overgrowth in women with Type 2 diabetes implicating factors beyond glycaemia in its pathogenesis.

In considering implementing an intervention to prevent gestational diabetes, the time point at which the intervention is started is an additional important consideration. At the initial antenatal assessment, considering maternal weight and the appropriate associated adverse impact of this would be important. In addition, ethnicity and culturally sensitive approaches to improve risk would be best addressed early in pregnancy. As illustrated by the original data presented in Chapter 4, the spectrum of GDM to type 2 diabetes (T2DM) is not only defined by the degree of glycaemia but

also by the length of exposure to maternal hyperglycaemia. The case control study presented in Chapter 4 compared women with hyperglycaemia detected in early pregnancy (prior to 20 weeks gestation) to two separate control groups matched for early pregnancy body mass index (BMI): one with recognised pre-gestational T2DM and the second with GDM diagnosed on routine testing at 24-28 weeks gestation. The results demonstrated that women in the early hyperglycaemia group resembled those with established T2DM in terms of maternal outcomes, with a similar proportion of women requiring insulin treatment and developing hypertensive disorders in pregnancy compared to those diagnosed with routinely diagnosed GDM. Furthermore, the risk of glucose intolerance persisting postpartum was heightened in women with early hyperglycaemia. These findings were independent of maternal age and adiposity. Length of exposure to hyperglycaemia additionally adversely affected fetal outcomes: a higher proportion of neonates were born preterm to women with early hyperglycaemia and variations in still birth rates existed implicating the detrimental effect of prolonged exposure to maternal high glucose levels. The data suggest that implementing a prevention strategy early in the pregnancy is therefore an important consideration.

One clear risk factor for developing early hyperglycaemia that emerged from the analysis in Chapter 4 was a previous history of GDM: 71.8% of the women who had evidence of hyperglycaemia prior to 20 weeks gestation had had a previous pregnancy complicated by GDM. This suggested that women with a previous pregnancy complicated by GDM would be an important group to focus on. In considering means of preventing recurrence of hyperglycaemia in such a cohort, resources are an

important factor. Indeed lifestyle strategies that had successfully prevented GDM in previous randomised controlled trials all utilised considerable resources (Chapter 1 section 1.6.2), which in itself would provide a cost burden to healthcare systems.

Preliminary data have demonstrated the potential benefits of dietary supplementation in preventing GDM in index pregnancies (67). Another potential avenue that has been suggested is metformin, the biguanide agent that has a strong evidence base for safe use in pregnancy, which has been shown to prevent progression to T2DM in non-pregnant adults with impaired glucose tolerance (62). With the hypothesis advanced that intervention with metformin therapy early in pregnancy could prevent gestational diabetes mellitus recurring in women with previous pregnancies complicated by GDM, the "Preventing Recurrent Gestational Diabetes Mellitus with Early Metformin Intervention" trial (PRoDroME) was designed, the original methodology for which was presented in this thesis. This double blind randomised controlled trial of metformin versus placebo in early pregnancy is actively recruiting at the time of writing. Women with a singleton viable pregnancy, who are between 8 and 22 weeks gestation and who have had a previous pregnancy complicated by GDM are eligible for inclusion. Those with established pre-existing diabetes (including unrecognised diabetes defined as FPG ≥ 7.0mmol/L and/ or HbA1c ≥ 48mmol/mol) and or contraindications to metformin therapy have been excluded. Randomisation has occurred prior to the 22nd week of gestation and women are followed regularly throughout their pregnancy (every 2-4 weeks) with the final study visit occurring 6 weeks postpartum.

During the data collection period defined for this thesis, 153 women were identified across the participating sites. Difficulties in contacting potential participants have represented a barrier to recruitment, as has development of hyperglycaemia necessitating treatment early in pregnancy. However, uptake amongst eligible women has been good with 57.7% consenting to participation. Limited data exist relating to individuals with recurrent GDM and to this end, prospectively collected data from women participating in the observational arm of the PRoDroME trial has provided valuable information in terms of treatment modalities required to control hyperglycaemia (with particular reference to insulin treatment), fetal overgrowth as determined by either incidence of macrosomia or large for gestational age incidence and postnatal complication rates. These data have further reflected the heightened risk of women with a previous pregnancy complicated by GDM and early development of hyperglycaemia. While it is impossible to comment on whether metformin mitigates the risk of gestational diabetes recurring due to the blinded nature of the PRoDroME trial, the lower risks of macrosomia identified in those women participating in the intervention arm compared to those in the prospective arm (with or without recurrent GDM) signify the potential for early intervention in preventing fetal overgrowth.

Gestational diabetes is an important clinical problem, which represents substantial risks to both the mother and baby and is associated with a significant economic burden. However, as illustrated by the work presented in this thesis, there are many facets to consider when looking at the impact of glucose on complications including diagnostic thresholds, degree of hyperglycaemia and length of exposure to

hyperglycaemia. Furthermore, factors beyond glycaemia are implicated in the pathogenesis of the priniciple complication, macrosomia, with both ethnicity and maternal adiposity contributing. Identifying one clear strategy to prevent gestational diabetes has proved challenging. Perhaps rather than preventing hyperglycaemia per se, strategies should focus on avoiding hard outcomes such as macrosomia, which has implications on future generations. Though treating hyperglycaemia would be an important factor in this, a less glucose-centric approach may help address maternal risk as a whole mitigating development of complications.

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APPENDIX

Appendix 1: Relevant Publications, Presentations and Prizes

Peer Reviewed Publications

R. Agha-Jaffar, N Oliver, M Kostoula, IF Godsland, C Yu, J Terry, D Johnston, D Gable and S Robinson. Hyperglycaemia recognized in early pregnancy is phenotypically Type 2 diabetes not Gestational diabetes. *Journal of Maternal-Fetal Neonatal Medicine.* DOI 10.1080/14767058.2019.1593959.

R Agha-Jaffar, N Oliver, D Johnston & S Robinson. Preventing Gestational Diabetes: Does an effect prevention strategy exist? *Nature Reviews Endocrinology*. 2016; 12: 533-546.

Correspondence

R Agha-Jaffar & S Robinson. Re: Gestational diabetes: new criteria may triple the prevalence but effect on outcomes is unclear. *BMJ*. 2014; 348:g1567.

Oral Presentations

R Agha-Jaffar, S Robinson, N Oliver, C Yu, A McCarthy, B Jones, J Terry, D Gable, A Dornhorst and H Shaikh. The Impact of Revised NICE Gestational Diabetes Diagnostic Criteria on an Inner-City Multi-Ethnic Cohort. *Diabetic Medicine*, 2016: 33: S1.

R Agha-Jaffar, M Reddy, M Kostoula, A Sackey, J Terry, C Yu, TG Teoh, H Shaikh, D Gable, N Oliver and S Robinson. Glycaemic Variability and its Impact on Fetal Growth in Pregnant Women with Type 1 Diabetes and Type 2 Diabetes. 8th International Diabetes in Pregnancy Symposium. 2015.

R Agha-Jaffar, NS Oliver, M Reddy, TG Teoh, L Phelan, C Yu, D Gable, and S Robinson. Diabetes Recognised in Early Pregnancy is Phenotypically Type 2 Diabetes Mellitus (T2DM) not Gestational Diabetes Mellitus (GDM). Diabetic Medicine, 2014; 31: s1.

Poster Presentations

R Agha-Jaffar, S Misra, N Oliver, J Terry, A McCarthy, C Yu, B Jones, D Johnston, D Gable, H Shaikh, A Dornhorst, I Godsland, KGMM Alberti & S Robinson. Ethnic variations in glucose and the interaction with fetal growth in a multi-ethnic inner city antenatal cohort. *Diabetologia* 2016. 59:1.

R Agha-Jaffar, A Sackey, M Reddy, TG Teoh, D Gable, S Robinson, NS Oliver. Potential Benefits of Continuous Glucose Monitoring in Predicting Fetal Outcomes in Pregnant Diabetic Women. DTT 2014; 16: s1.

Oral Presentations at National Conferences

National Diabetes in Pregnancy Conference, London 10/11/2016

Title: Hyperglycaemia Recognised in Early Pregnancy is Phenotypically Type 2 Diabetes Mellitus (T2DM) not Gestational Diabetes Mellitus (GDM).

RANK Nutrition Symposium, Lake District 20/10/2016

Title: The Impact of Maternal Obesity on Pregnancy Outcomes.

Prizes

1. Oral Presentation Prize: DUK National Diabetes In Pregnancy Conference (2016)

2. RANK Nutrition Symposium Prize for Presentation: Mini Symposium on Nutrition and Obesity, Rank Prize Funds (2015)

3. Best Abstract Award: 8th International Diabetes in Pregnancy Symposium (2015)

Appendix 2: Correlation coefficients for markers of glycaemia and glycaemic variability in women with Type 1 and Type 2 diabetes

Table 1: Correlation coefficients for markers of glycaemia and glycaemic variability with Type 1

	Fetal Birthweight		Fetal Birth Weight Centile	
	r^2 value	p value	r^2 value	p value
HbA1c	0.212	0.09	0.274	0.05
Mean Glucose	0.150	0.2	0.092	0.3
STDEV	0.001	0.9	0.010	0.7
MAG	0.017	0.7	0.019	0.6
CV	0.064	0.4	0.010	0.7
LBGI	0.072	0.4	0.017	0.7
HBGI	0.045	0.5	0.061	0.4

Table 2: Correlation coefficients for markers of glycaemia and glycaemic variability and fetal growth in women with Type 2 diabetes

	Fetal birth weight		Fetal birth weight centile	
	r^2 value	p value	r^2 value	p value
HbA1c	0.005	0.7	0.067	0.3
Mean Glucose	0.013	0.6	0.023	0.5
STDEV	0.164	0.08	0.163	0.08
MAG	0.143	0.1	0.045	0.4
CV	0.123	0.2	0.096	0.2
LBGI	0.001	0.9	0.005	0.7
HBGI	0.013	0.6	0.077	0.3

Appendix 3: Questionnaires Used for the PRoDroME Trial

EQ-5D-5L Questionnaire: Page 1

Under each heading, please tick the ONE box that best describes your health TODAY

MOBILITY

I have no problems in walking about	❑
I have slight problems in walking about	❑
I have moderate problems in walking about	❑
I have severe problems in walking about	❑
I am unable to walk about	❑

SELF-CARE

I have no problems washing or dressing myself	❑
I have slight problems washing or dressing myself	❑
I have moderate problems washing or dressing myself	❑
I have severe problems washing or dressing myself	❑
I am unable to wash or dress myself	❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities	❑
I have slight problems doing my usual activities	❑
I have moderate problems doing my usual activities	❑
I have severe problems doing my usual activities	❑
I am unable to do my usual activities	❑

PAIN / DISCOMFORT

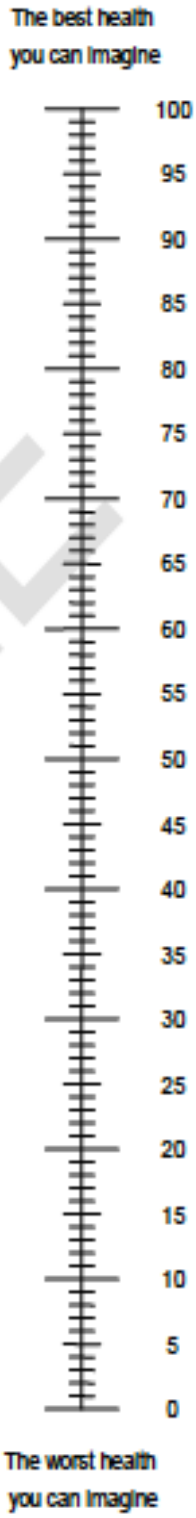
I have no pain or discomfort	❑
I have slight pain or discomfort	❑
I have moderate pain or discomfort	❑
I have severe pain or discomfort	❑
I have extreme pain or discomfort	❑

ANXIETY / DEPRESSION

I am not anxious or depressed	❑
I am slightly anxious or depressed	❑
I am moderately anxious or depressed	❑
I am severely anxious or depressed	❑
I am extremely anxious or depressed	❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



Strait-Trait Anxiety Inventory (STAI) Questionnaire

SELF-EVALUATION QUESTIONNAIRE STAI Form Y-1

Please provide the following information:

Name_____Date_____S_____

Age_____ Gender (*Circle*) **M** **F** T_____

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

STAIP-AD Test Form Y
www.mindgarden.com

Appendix 4: Dosing card provided to recruited participants for the PRoDroME Trial

Instructions for Taking Study Tablets

	Total Daily Dose	Morning Meal	Evening Meal
Day 1 (for 5 days)	Metformin 500mg or placebo each day	----	ONE tablet
Day 6 (for 5 days)	Metformin 1000mg or placebo each day	ONE tablet	ONE tablet
Day 11 (for 5 days)	Metformin 1500mg or placebo each day	ONE tablet	TWO tablets
Day 16 **FOR THE REMAINDER OF PREGNANCY**	Metformin 2000mg or placebo each day	TWO tablets	TWO tablets

If you experience side effects when you increase the number of tablets, please go back to the smallest dose that you are able to manage without problems and contact the study team for further advice.

Sample patient dosing card Version 1.0 21st January 2014

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