Ethnic differences in gestational diabetes: Impact on South Asians

By

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List of Abbreviations

1hPG - 1 hour plasma glucose
2hPG - 2 hour plasma glucose
AAWT - anterior abdominal wall thickness
AC - Abdominal circumference
ACTH - Adreno-Cortico-Trophic hormone
ADA – American diabetes association
AUC - Area under the curve
BMI - Body Mass Index
BPD – Bi-parietal diameter
BW - Birth weight
CHD – Coronary heart disease
CAR - Cortisol awakening response
CBG - Cortisol binding globin
CRH - Corticotropin releasing hormone
CS - Caesarean Section
EFW - estimated fetal weight
FL - Femur length
FPG - Fasting plasma glucose
GC - Glucocorticoid
GCT - Glucose tolerance test
GDM - gestational diabetes
GROW - Gestation related optimal weight
HAPO - Hyperglycemia and adverse pregnancy outcomes
HbA1c - Hemoglobin A1c
HC - Head circumference
HPA - Hypothalamic pituitary adrenal axis
HSD - Hydroxy steroid dehydrogenase
IADPSG - International Association of the Diabetes and Pregnancy Study Groups
IFG - Impaired fasting glucose
IGT - Impaired glucose tolerance
IMD - Index of multiple deprivation
IL – Interleukin
IUGR - intra-uterine growth restriction
LBW - Low birth weight
LGA - Large for gestational age
Mmol/L – Milli-moles per litre
Mg/l – Milligrams per litre
NICE - National institute for health and care excellence
NPV - Negative predictive value

OGTT - Oral Glucose tolerance test
OR - Odds ratio
PAI – Plasminogen activator inhibitor
PPV - Positive predictive value
PMNS – Pune maternal nutrition study
PUFA – Polyunsaturated fatty acids
ROC - Receiver operating characteristics
RR - Risk Ratio
SA - South Asians
SGA - Small for gestational age
T2D - type 2 diabetes
THE - Tettra-hydro cortisone
THF - Tettra-hydro-cortisol
TNF – Tumor necrosis factor
WC - White Caucasians
WHO - World Health organization

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Declarations

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. I, Hema Venkataraman, declare that it has been composed by myself and has not been submitted in any previous application for any degree and all the research has been undertaken in accordance with University safety policy and Guidelines on Ethical Practice.

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Submitted papers

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Abstracts presented at conferences

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1 Chapter 1: Introduction

1.1 Abstract:

Background: GDM is a state of glucose intolerance first diagnosed in pregnancy. It is a pre-diabetes state, predisposing both the mother and offspring to future risk of diabetes. GDM is associated with increased risk to macrosomia, adiposity, Caesarean Section (CS) delivery, shoulder dystocia, and neonatal hypoglycaemia. SA have a greater than two fold risk of both GDM and future diabetes risk compared to WC. However, despite having higher levels of hyperglycaemia in pregnancy, SA babies are amongst the smallest babies in the world.

The mechanism behind this increased glycaemic risk in SA is complex, multifactorial and unclear. Disordered hypothalamic-pituitary-adrenal axis (HPA) has been linked to adult diabetes, obesity and metabolic syndrome in WC but has not been studied in SA.

The current management of GDM is largely based on evidence from studies in WC and has been extrapolated to other ethnic groups such as SA. This includes: diagnostic criteria to define GDM, postnatal screening methods for postpartum glucose abnormalities, effect of GDM on offspring birth weight (BW) and fetal growth in GDM. Through this research we aim to explore the ethnic differences between SA and WC in the applicability of diagnostic criteria, post partum screening methods, effect of GDM on BW, fetal growth patterns in GDM and also examine ethnic differences in HPA activity as a potential mechanism underlying the increased glycaemic risk in SA in pregnancy.

Methods:

i. Retrospective analysis of a routinely collected multicentre data (n=14477) over a 3-year period was used to study the applicability of various GDM diagnostic criteria and post partum screening methods. A subgroup analysis of the above data set was used to compare fetal growth between SA and WC (177 WC and 160 SA).

ii. A retrospective analysis of a large birth weight cohort (n=53,128) from Leicestershire between 1994 and 2006 was used to compare the effect of maternal diabetes and GDM on BW in SA and WC.

iii. To examine fetal growth in SA, a retrospective case control analysis of serial fetal biometry was performed between GDM and control population from India. (178 controls and 153 GDM)

iv. To explore underlying HPA dysfunction as a potential mechanism for increased glycemia in SA and ethnic differences in HPA behaviour a prospective cohort study comprising of high risk pregnant SA and WC women was performed. Diurnal salivary and urinary cortisol excretion was studied in relation to glycaemia in SA and WC (n=100, 50 SA, 50WC)

Results:

i. The newer IADPSG detects obese women with mild fasting hyperglycaemia. The benefits of treatment of hyperglycemia are not well established. The increase in detection rates of GDM with the new NICE and IADPSG criteria were uniform across ethnic groups in a selectively screened population.

ii. Postnatal screening with oral glucose tolerance test (OGTT) is associated with poor uptake in all ethnic groups, which improves substantially with using HbA1c. SA were more likely to attend postnatal screening with HbA1c compared to WC. Screening for postnatal diabetes using FPG is more likely to miss women of non-WC ethnicity owing to the larger proportion of post-load glucose abnormalities.

iii. The BW increase associated with maternal diabetes was lower in SA by 139g compared to WC.

iv. Important ethnic differences in fetal growth were noted. SA fetuses had overall smaller measures of head and abdomen circumferences, but with disproportionately smaller abdominal circumference compared to WC, signifying early evidence of a head sparing growth restricted pattern.

v. SA fetuses of GDM mothers showed early evidence of increased abdominal adiposity at 20 weeks with smaller measures of other fat free mass and skeletal growth compared to non-GDM controls

vi. SA had higher cortisol awakening responses compared to WC. First trimester waking cortisol was an independent predictor of glycaemia in the third trimester. Despite significantly lower BMI, SA had similar glucocorticoid (GC) excretion to WC. Urinary GC excretion was independently predicted by maternal adiposity and not BMI in SA.

Conclusion: This research addresses important gaps in the literature in gestational diabetes in SA. There are important ethnic differences in the impact of maternal diabetes and gestational diabetes on BW and fetal growth, and evidence of early

increase in adiposity at the expense of lean body mass in SA. This research provides novel evidence to support the argument for ethnicity tailored management of GDM. Our research also provides novel evidence for disordered HPA activity as a possible mechanism for the increased glycemic risk in SA. Larger randomized prospective studies incorporating offspring outcomes in relation to HPA are needed.

1.2 Gestational Diabetes Mellitus and ethnicity:

GDM is defined as glucose intolerance first diagnosed in pregnancy [3]. It can be regarded as a pre-diabetes state, which is unmasked by the relative diabetogenic hormonal milieu of pregnancy. Normal pregnancy is associated with about 60% decrease in insulin sensitivity, exaggerating the risk of clinical hyperglycemia/gestational diabetes in women with pre-existing undiagnosed metabolic dysfunction [4].

The prevalence of GDM is increasing at an alarming rate globally in line with the increase in prevalence of type 2 diabetes (T2D) [5, 6]. The prevalence of GDM varies between 2-22% in population based studies [7] depending on the screening criteria used and the population characteristics.

GDM increases the future risk of T2D by 7-8 fold in mothers and 2-4 fold in the offspring [8]. GDM has also been associated with offspring risks such as macrosomia, neonatal jaundice, increased C-Section rates, neonatal hypoglycaemia and shoulder dystocia [9]. There is strong evidence to show that treatment of mild GDM reduces offspring risk [10, 11].

While GDM has been associated with various traditional risk factors such as obesity and family history of T2D, ethnicity has been regarded as one of the independent risk factors in GDM and has several implications in GDM, in particular in South Asians (SA).

1. Risk of GDM: The prevalence of GDM vary widely with ethnicity [12]. Using the same criteria, the prevalence is more than double in South Asians (SA) compared to White Caucasians (WC) despite lower BMIs in SA [13, 14]. While the incidence of GDM is increasing globally, this increase is disproportionately

higher in SA compared to WC [15], paralleling the increase in T2D in SA. It is not entirely clear why SA are at higher risk of GDM than WC. Several theories have been postulated as discussed in the next section.

2. Diagnosis of GDM: Currently GDM is diagnosed between 24-28 weeks of pregnancy using various criteria across the world, with little consensus. The different criteria that have evolved since the 1960's are purely based on studies in WC. How these criteria perform in different ethnic groups especially SA is unclear. Some groups have called for the use of ethnic specific cut offs in the diagnosis of GDM [16, 17].
3. The effect of GDM on birth weight (BW): It is well known that GDM increases the risk of macrosomia[9]. Large for gestational age (LGA)/macrosomia has been used to derive glycaemic cut offs for defining GDM, assuming that the risk of LGA/macrosomia are uniform across ethnic groups [9]. However it has been shown that the effect of GDM may not be uniform across different populations. Studies among Blacks and WC show that the effect of GDM on BW is significantly modulated by ethnicity [18]. Literature comparing the effects of GDM on BW in SA and WC is sparse. If proven this further strengthens the argument for having ethnic specific diagnostic criteria for GDM.
4. Post-partum screening and risk of future GDM: The risk of post-partum diabetes and methods of post-partum screening practices vary significantly across populations. It has been reported that the prevalence of post-partum T2D after GDM varies with ethnicity. Blacks had higher post-partum T2D rates compared to WC, despite having a lower prevalence of GDM [19]. SA have up to a two

fold increased risk of developing T2D following GDM after adjusting for potential confounders [2, 20]. However characterisation of the type of postnatal abnormalities and applicability pf various postnatal screening strategies in the two ethnic groups has not been studied previously. Ethnicity tailored postnatal testing strategies may be indicated if these differences were observed.

1.3 Increased metabolic risk in SA and theories

South Asians (SA) are a diverse population originating from the Indian subcontinent, including India, Bangladesh, Nepal, Pakistan and Sri Lanka. According to the 2011 UK Census, South Asians were UKs largest ethnic minority and constituted around 4.9% of the population of UK and Wales.

It is well known that SA have disproportionately higher risk of gestational diabetes, type 2 diabetes, pre-diabetes, premature cardiovascular mortality and morbidity compared to WC. In the UK, the prevalence for T2D in SA is 4-6 times higher and develops 5-10 years earlier compared to WC [21-25]. Coronary heart disease [26] and stroke are more prevalent in SA with a 40-50% higher mortality than in WC [27, 28], in part secondary to the higher prevalence of diabetes. After adjusting for age, BMI, family history and smoking status, the hazard ratios for T2D in SA vs WC were over 4.07 for Bangladeshi women, 2.15 for Pakistani women and 1.71 for Indian women [29]. This adverse risk is also seen in pregnancy, which has metabolic implications both for the mother and offspring. The prevalence of GDM, which is a pre-diabetes state, using the same criteria are more than double in SA compared to WC despite lower BMIs in SA [13].

The reasons for this wide disparity are far from clear, although socio-economic and cultural influences on health have been implicated. A few studies using self-reported

physical activity reported lower activity in SA, compared to other ethnic groups.[30-32]. However objective evidence for this, using activity monitors is lacking in SA. Others reporting dietary trends in India in relation to insulin resistance report higher intakes of carbohydrates, saturated fatty acids, n6-poly-unsaturated fatty acids (PUFA) and lower n3 PUFA and fibre compared to other populations [33, 34].

Novel risk factors, including inflammatory markers such as highly sensitive-C-reactive protein, adipokines (e.g adiponectin and leptin), inflammatory cytokines like tumor necrosis factor (TNF) alpha, Interleukin (IL-6), and prothrombotic plasminogen activator inhibitor (PAI-1) may be related to the excess coronary heart disease [26] mortality rates in South Asians, but unfortunately have not been examined prospectively in South Asians [35-37]. Lipoprotein(a), has been reported in one study to confer an increased genetic predisposition to pre-mature CHD [37].

While above factors could play a role in adult metabolic risk, this risk is evident in early childhood and even at birth. Studies conducted in the UK and in the US report the incidence of T2D in children and adolescents to be at least 3 fold higher in SA compared to WC [38, 39], associated with increased prevalence of childhood obesity as seen in the reports of the National Child Measurement program [40]. SA children have higher % of body fat and are more insulin resistant for any given body mass than matched WC controls [41, 42]. Yajnik and his colleagues from the Pune Maternal Nutrition Study (PMNS) coined the term ‘thin-fat Indian baby’ to depict increased abdominal adiposity seen even at birth despite smaller size [43, 44]. It is believed that this pattern of central obesity that is closely linked to future adult metabolic risk is determined at birth.

Several theories have evolved to explain this high risk in SA seen at birth and early life.

1. *Thrifty genotype theory*: In 1962 James Neel described a "thrifty genotype" that modified insulin regulation and glucose and fat storage to provide a survival advantage during periods of famine in the hunter-gatherer days [45]. With calorie abundance, this genetic disposition became maladaptive resulting in adverse metabolic risk. However, it is an improbable assumption that South Asians would have been more vulnerable than other races.
2. *Thrifty phenotype theory*: In 1992 Barker and Hales reported that low birth weight (LBW) and thinness at birth was associated with high risk of T2D, proposing that intra-uterine nutritional insults induce foetal adaptations to confer a survival advantage in the short term, which became maladaptive when nutrition improved thereafter [46]. The 1998 Dutch Winter hunger study reported a high incidence of glucose intolerance in adults who were exposed to malnutrition in utero during the famine [47]. In 1999, Yajnik and Fall demonstrated that children who were smaller at birth were more insulin resistant with greater adiposity. [48]. In the majority of the studies low birth weight is used as a marker of under nutrition. India has both the largest number of diabetics in the world and this could be related to the fact that Indian babies are amongst the smallest [49].
3. *Fetal Insulin hypotheisis*: In 1999, Hattersley proposed the theory that the same underlying genetic factors result in both the phenotype of a small baby and an adult with insulin resistance [50]. The association seen between fetal thinness and insulin resistance is thus explained as two manifestations of the same underlying cause. Although monogenic diabetes supports this theory this is relative rare and cannot explain the epidemic of T2D in SA. Considering the

predominant role of adulthood obesity in combination with LBW, a gene-environment interaction in the peri-conceptional period is more a likely explanation.

4. *Thrifty Epigenotype*: Another possibility is that of a relatively recent heritable modification of the Asian Indian epigenome or "thrifty epigenotype," induced by a suboptimal intrauterine environment that could "program" the offspring for adult health outcomes [51]. These refer to changes in DNA other than gene sequence changes. Methylation of the CpG islands of DNA is a major epigenetic mechanism of gene silencing. The importance of methyl donors in nutritional programming is well studied in animals. Feeding Agouti mice with a methylating cocktail (B12 + Folate + Betaine + Choline) resulted in methylation of various candidate genes such as gluco-corticoid receptor and PPAR-γ genes involved in insulin and energy metabolism [52]**.** Another sheep model with periconceptional deficiency of methyl donors (methionine, vitamin B12 and folate) resulted in the methylation of a number of genes in the offspring leading to insulin resistance and obesity [53]. It has been shown that SA especially Indians have a high prevalence of B12 deficiency compared to WC, with studies reporting mean B12 levels in SA nearly half of that in WC [54]. The Pune maternal nutrition study of 675 women was the first clinical study to demonstrate the link between maternal vitamin B12 & folate status and offspring insulin resistance at 6 years [55]. It can therefore be hypothesized that SA have an altered epigenome at birth owing to a deficiency in various methyl donors especially Vitamin B12.
5. *Altered set points of regulatory systems*: Hypothalamic-pituitary-adrenal axis

(HPA) hyperactivity and increased activity of 11 beta-hydroxy-steroid-dehydrogenase-1 enzyme (HSD1) enzyme in adipose tissue resulting in hypercortisolemia has been linked to metabolic syndrome, diabetes and obesity [56, 57]. Exogenous maternal steroid administration during pregnancy has been linked to low BW in animal and human studies [58] and could be a possible explanation of the low birth size in SA. Variation in HPA and HSD1 activity has been described in different ethnic groups [59]. Reynolds reported lower 0900 cortisol in SA men living in the UK compared to their WC counterparts [60]. However detailed HPA behaviour in SA has not been studied before in relation to GDM risk.

In this study we will focus on the ethnic differences in GDM risk in particular between SA and WC. We will aim to study ethnic differences in applicability of different GDM diagnostic criteria, postnatal abnormalities after GDM, effects of GDM on foetal size and birth weight between SA and WC, and the role of the HPA in this risk in both ethnic groups.

2 Chapter 2: Diagnosis of GDM

2.1 Abstract

Background: There is intense debate surrounding the various defining criteria for gestational diabetes. There is reluctance worldwide to adopt the International Association of the diabetes and pregnancy study groups (IADPSG) guidelines despite endorsement from the WHO. The National Institute for Health and Care Excellence (NICE) 2015 guidelines recommend new criteria based on cost-effectiveness analysis using observational data. Both these criteria recommend lower Fasting Plasma Glucose (FPG) thresholds than current practice. Our aim was to compare the performance of IADPSG and NICE-2015 criteria in a risk-factor based selectively screened population consisting of WC and SA.

Methods: A retrospective analysis was performed for 14477 women undergoing antenatal oral-glucose-tolerance-test (OGTT) (2010-2012) across three UK centres that used NICE recommended risk-factor based selective screening. The IADPSG, NICE-2015 and the mWHO1999 diagnostic criteria were applied to this population and the outcomes assessed in the two ethnic groups.

Results: The prevalence of GDM was similar by NICE (10.5%) and IADPSG (10.6%). Both detected older women with higher BMI, higher multiparty and stillbirth rates compared to controls and had higher risk of large-for-gestational-age (LGA) infants despite adjustment for maternal characteristics including body-mass-index (BMI). Maternal BMI was a significant predictor of C-Section risk, in the IADPSG group. CS rates in the NICE group were comparable to controls. SA have higher OR of being detected by the IADPSG criteria compared to WC.

Conclusion: Both new criteria diagnose women with higher metabolic risk and adverse offspring outcome, with similar increases in prevalence of GDM. Maternal BMI appears to be an important contributor of adverse pregnancy outcome, especially CS. It is unclear if treating mild maternal hyperglycaemia in these obese women will reduce offspring risk. Until further interventional evidence is available, the change in practice leading to increased incidence of mild GDM cannot be justified.

2.2 Introduction

2.2.1 History and evolution of various criteria for GDM

Gestational Diabetes Mellitus is typically described as a state of glucose intolerance first recognised in pregnancy. The incidence of GDM is increasing rapidly in line with the increase in prevalence of type 2 diabetes (T2D) [5, 6]. The reported rates vary between 2-22% in population based studies [7] depending on the screening criteria used. It is associated with a multitude of maternal and offspring complications. The short-term risks include macrosomia, neonatal jaundice, neonatal hypoglycaemia and shoulder dystocia in the offspring [9] and preeclampsia [61] and higher likelihood of CS deliveries for the mother [62] [9]. In the long-term, the diagnosis of GDM is associated with at least 7 fold higher lifetime risk of T2D in the mothers and 2-4 fold higher risk of pre-diabetes and T2D in the offspring [8, 63, 64]. There have been two well-designed multi-centre randomised controlled trials, which have demonstrated proven benefit of treatment in reducing short-term adverse outcomes in GDM [10, 11]. However, there is still intense debate on what should be the screening and diagnostic strategy for GDM with little consensus across the world.

Historically O'Sullivan first defined GDM criteria in the 1964 using a 100g OGTT in a landmark study of 752 White Caucasian (WC) women in the second and third trimester. This criteria became the standard for GDM detection for the next decades although these threshold values were based on the predictive ability for subsequent development of maternal diabetes in the non-pregnant state among a second cohort of 1,013 women [65]. The O'Sullivan's criteria was subsequently modified to convert the blood to plasma glucose values and adopted by the American NDDG group in 1979 [66]. However in 1982, Carpenter and Coustan following a study in

381 predominantly WC women recommended slightly lower cut offs to account for the change in the enzymatic method used to measure glucose in plasma since the 1980s [67]. The evolution of various criteria for GDM diagnosis is shown in the table 1.

- The WHO in 1985 for the first time recommended that a 75g OGTT [68].
- The criteria for GDM diagnosis remained unchanged till this was further clarified in the fourth international workshop conference on GDM in 1998 [3]. For the first time there was mention of risk stratification and selective screening for high-risk women who included ethnic minority women such as SA. They recommended the Carpenter-Coustan criteria. They also recommended criteria for 75g OGTT based on a large study by Sachs in a high risk population of 3505 predominantly WC women in Los Angeles and the recommendation of the European association for the Study of Diabetes based on observation in diverse European women [69].
- Prior to HAPO other countries recommended slight variations of the NDDG criteria or the WHO criteria.
- WHO (1999) recommended using a 75g OGTT to diagnose GDM in line with non-pregnant adults using cut offs to define GDM as either diabetes or IGT in pregnancy. It was acknowledged that the role of IFG was not very clear [70].
- The HAPO was a landmark trial of 23,316 women across 9 countries between July 2000 and April 2006 [71]. 29.0% of women were termed "Asian or oriental" origin, however these participants were South East Asian from Honk Kong, Singapore and Thailand. There was no SA representation from India, Pakistan or Bangladesh where the prevalence rates are

disproportionately high. The HAPO study demonstrated an increased risk of macrosomia and increased cord c-peptide with increasing levels of glycaemia with no clear threshold at which risk escalates. The mean glucose values in this predominantly WC cohort were 4.5, 7.4 and 6.2 mmol/l at Fasting 1 and 2h respectively. The IADPSG met in 2008 to translate the HAPO study results to GDM criteria. It was decided that the diagnostic threshold would be the level of glycaemia at which the OR of adverse outcome such as LGA, increased cord C-Peptide and increased offspring body fat was 1.75. These levels corresponded to the IADPSG recommended criteria of GDM of FPG, 1hPG or 2hPG of 5.1, 10.0 or 8.5 respectively.

- Following publication of the HAPO results the American diabetes Association (ADA), Australasian Diabetes in pregnancy society (ADIPS) and the WHO adopted the IADPSG criteria [72-74].
- The ADA later reverted back to its original criteria due to lack of evidence of on reduction of C-Section rates [75].
- In Feb 2015, NICE UK 2015 proposed new GDM diagnostic criteria using 75g OGTT [76] (FPG: ≥5.6 and 2-hour: ≥7.8 mmol/l, one of two required for diagnosis) following a risk factor based selective screening process The recommendation was based on a health economic analysis that reported better cost-effectiveness for this new criteria. Their evidence was derived from routine observational data sets comprising of over 40,000 pregnant women across 14 centres, which included the HAPO centres in the UK and Australia, along with other centres in the UK. Majority of the centres used risk factor based selective screening with just over 12,000 women being screened by a universal screening process. NICE rejected the IADPSG

criteria deeming it not cost-effective based on health economic modelling in a subset (n=18,974) of these women for whom FPG, 1hPG and 2hPG values were available.

Table 1: Evolution of various GDM screening and diagnostic criteria

Criteria	Recommendation	Criteria
O'Sullivan – (1964) Study of 752 WC women	50g Glucose challenge followed by 100g OGTT for those with a 1hPG ≥7.8mmol/l. Universal screening	FPG ≥ 5.3, 1hPG ≥10, 2hPG ≥ 8.6 or 3hPG ≥ 7.7 mmol/L (2 or more values)
NDDG modification of O'Sullivan (1979) (to obtain plasma G from blood G values)	50g G challenge followed by 100g OGTT for those with a 1hPG ≥7.8mmol/l. Universal screening	FPG ≥ 5.8, 1hPG ≥10.6, 2hPG ≥ 9.2 or 3hPG ≥ 8.0 mmol/L (2 or more values)
Carpenter Couston (1982): Study of 381 WC women.	50g G challenge followed by 100g OGTT for those with a 1hPG ≥7.8mmol/l. Universal screening	FPG ≥ 5.3, 1hPG ≥10, 2hPG ≥8.6 or 3hPG ≥ 7.8 mmol/L (2 or more values)
Second International Workshop on Gestational Diabetes Mellitus (1984)	Recommended the NDDG criteria. Universal screening.[77]	
WHO (1985)	Recommended 75g OGTT as in non-pregnant. Selective screening	FPG ≥ 7 or 2hPG ≥ 7.8
Fourth international workshop conference on GDM (1998)	-Risk stratification -100g OGTT or 75gOGTT	- Carpenter Coustan criteria for 100gOGTT - FPG≥5.3, 1hPG≥10 or 2hPG≥8.6 mmol for 75gOGTT
International Association of Diabetes and Pregnancy Study Groups (IADPSG) formed in 1998		
ADIPS (1998)	A 50g G challenge and a 75g OGTT for those with a 1hPG ≥7.8. Universal screening	FPG ≥ 5.5 and 2hPG≥8.0mmol/l
New Zealand	Same as above	FPG ≥ 5.5 and 2hPG≥9.0mmol/l
CDA	Same as above	FPG ≥ 5.3, 1hPG≥10.6, 2hPG≥8.9mmol/l (Any 2 or more)
WHO (1999)	75g OGTT in line with non-pregnant diagnosis of IGT. Selective screening	FPG ≥ 7 or 2hPG ≥ 7.8
HAPO trial of 23,316 women across 9 countries between July 2000 and April 2006.		
ADA (2004)	Universal screening with 100 or 75gOGTT	Carpenter Coustan criteria for 100gOGTT - FPG≥5.3, 1hPG≥10 or 2hPG≥8.6 mmol for 75gOGTT
IADPSG (2008)	Universal screening with 75gOGTT	FPG, 1hPG or 2hPG≥5.1, 10.0 or 8.5 respectively
NICE (2008)	Selective screening with 75gOGTT- As per WHO - 1999	Same as WHO 1999
ADA (2011)	Adopted IADPSG	
ADIPS (2013)	Adopted IADPSG	
WHO (2013)	Adopted IADPSG	
CDA (2013)	50 g GCT followed by a 75 g OGTT using the glucose thresholds that result in an OR of 2.00. Universal screening	FPG ≥5.3 mmol/L, 1hPG ≥10.6 mmol/L, 2hPG ≥9.0 mmol/L (any 1) or 1-step 75 g OGTT IADPSG recommended criteria.
ADA (2014)	Recommends either 2 step using 50gOGTT -NDDG / or universal screening with IADPSG	NDDG or IADPSG.
NICE [78]	Selective screening using risk factors. Diagnosis by 75g OGTT	FPG ≥5.6 mmol/L or 2hPG ≥7.8 mmol/l

2.2.2 Applicability of GDM screening criteria among various ethnic groups

The decision to adopt the IADPSG criteria globally and in particular in SA has been contentious for several reasons.

1. Firstly the IADPSG is associated with significantly increased prevalence. The expected increase is to up to 17.8% varying across populations between 9.3-25% (22). This is thought to be secondary to the lowering of the FPG cut off in those countries where the WHO-1999 criteria is used and to the lack of the initial 50 g glucose challenge in regions using the Carpenter-Coustan criteria. In countries like India where the previous WHO criteria and the 2 step Carpenter and Coustan [79] criteria are prevalent, the impact of changing to IADPSG may be significant. Studies have reported upto a three times increase in the prevalence of GDM by the IADPSG criteria in Asians[80].
2. Secondly, without including SA in the HAPO study it is impossible to say whether the odds of LGA in SA are comparable to WC at a particular glycaemic level. Considering that the prevalence of LGA in SA is far lower than WC, the corresponding level of glycaemia to result in the odds ratio (OR) of LGA of 1.75 could in fact be higher. Also, it has been shown that the effect of glycaemia on birth weight varies with ethnic groups [18, 81]. One recent study that compared the effect of the IADPSG criteria on a large population including SA minority in fact recommended lower GDM diagnostic thresholds for SA than in WC[82].
3. Thirdly, there is evidence from the DECODE study that SA have a greater proportion 2hPG abnormalities compared to FPG abnormalities and it is possible that raising the 2hPG cut off might miss a significant proportion of

the SA minority group [83]. In the HAPO study 55% of women were diagnosed based on FPG alone, however this was as low as 24-26% in Bangkok and Hong Kong, and up to 47% in Singapore reflecting ethnic differences in the prevalence of fasting and post-prandial hyperglycaemia. The highest rates of diagnosis by FPG were in Barbados and America (74 and 73%). Although lowering the FPG cut off to 5.1 is expected to identify women who are likely to be missed by the increased 2hPG cut off of 8.5mmol/l, this has not been assessed systematically in mixed ethnic populations.

4. Fourthly, it is now increasingly recognised that maternal obesity is a more significant and independent player for LGA and C-Section rates than maternal glucose except at the highest glucose category [71, 84, 85]. In fact 75% of LGA in the HAPO study were born to mothers without with normal glucose tolerance. The long-term risks of obesity and glucose intolerance in offspring associated with GDM were lost when maternal BMI is factored into the analysis [86]. With significantly lower prevalence of obesity in SA, it is conceivable that the OR of foetal outcome, especially LGA in the different glycaemic categories would be very different.
5. Lastly, evidence of treatment benefit in this newly diagnosed additional population with mild fasting hyperglycaemia is lacking. Two intervention studies, the ACHOIS and MFMU trials published in 2005 and 2009 showed the benefit of treating mild GDM, using 75g OGTT [10] and 100g OGTT [11] respectively. It is important to note that neither of these intervention studies included the same outcomes used to define the IADPSG criteria as their composite primary outcome, except the C-peptide level in the MFMU

study [11]. The ACHOIS trial screened women using either risk factor based selective screening or 50g glucose challenge test (GCT) to select women with a 1hPG ≥ 7.8mmol/l for the 75g OGTT. They defined mild GDM as FPG <7.8mmol/L with 2hPG between 7.8 & 11.1mmol/l on 75g OGTT. 95% of women in the ACHOIS study had a fasting plasma glucose (FPG) levels between 3.4mmol to 6.2mmol, with median (IQR) 2-hour glucose value of 8.6 (8.1-9.3) mmol/l after a 75g OGTT [10]. The MFMU trial used an initial screening test of 50g GCT to select women with 1hPG between 7.5 and 11.1mmol/l to undergo a subsequent 100g OGTT. Mild GDM was defined as FPG<5.3mmol/l with one of 3 other thresholds that include 1hPG, 2hPG, and 3hPG values greater than 10.0, 8.6 and 7.8mmol/l respectively. In the MFMU trial 95% of the women in the intervention arm had a FPG between 4.5 and 5.1mmol/l with a 2-hour value ranging between 7.2 and 12.1mmol/l after a 100g OGTT [11]. Therefore, while there was some overlap in the glycaemic ranges with the proposed IADPSG criteria in both these trials, a direct extrapolation of the results from the available interventional studies is difficult given the differences in screening methods and selection criteria used. Again both these trials were conducted in a predominantly WC population.

With both the NICE and IADPSG criteria recommending lower FPG thresholds than the prevalent mWHO99 criteria there is increased pressure among care providers to adopt a change in practice [87]. If universal screening is followed, the incidence of GDM is expected to increase to up to 17.8% with the new IADPSG criteria [88], identifying a larger proportion of women with mild fasting glucose abnormalities. However, many countries and around 90% of the units in the UK still use risk factor

based selective screening, with a majority using the WHO / mWHO-99 criteria for diagnosis with a 75g OGTT [89]. Only 4% of UK centres adopted the IADPSG criteria [89].

Meek et al were the first to compare the likely impact of the NICE and IADPSG criteria in a retrospective cohort. The centre used a random blood glucose in early pregnancy, and subsequent glucose challenge test (GCT) at 26-28 weeks for screening followed by a diagnostic 75gOGTT at 26-28 weeks [90]. They reported the highest risk of LGA and CS in the group diagnosed by IADPSG and NICE criteria compared to control women who did not have OGTT. While this study added additional critical evidence for the diagnosis of GDM, this may not be applicable to the UK and other countries that follow risk factor based selective screening and not a GCT. The impact of the new NICE guidelines should ideally be studied on a selectively screened population, on which the evidence for the guideline is largely based.

2.3 Hypotheses and research questions

We hypothesised that moving to the new IADPSG criteria is likely to preferentially miss a larger number of SA with adverse foetal outcome compared to WC.

2.4 Aims

Our aim was to compare between SA and WC the impact of changing to the IADPSG and new NICE criteria in a risk factor based selectively screened multi-ethnic in UK.

2.5 Objectives

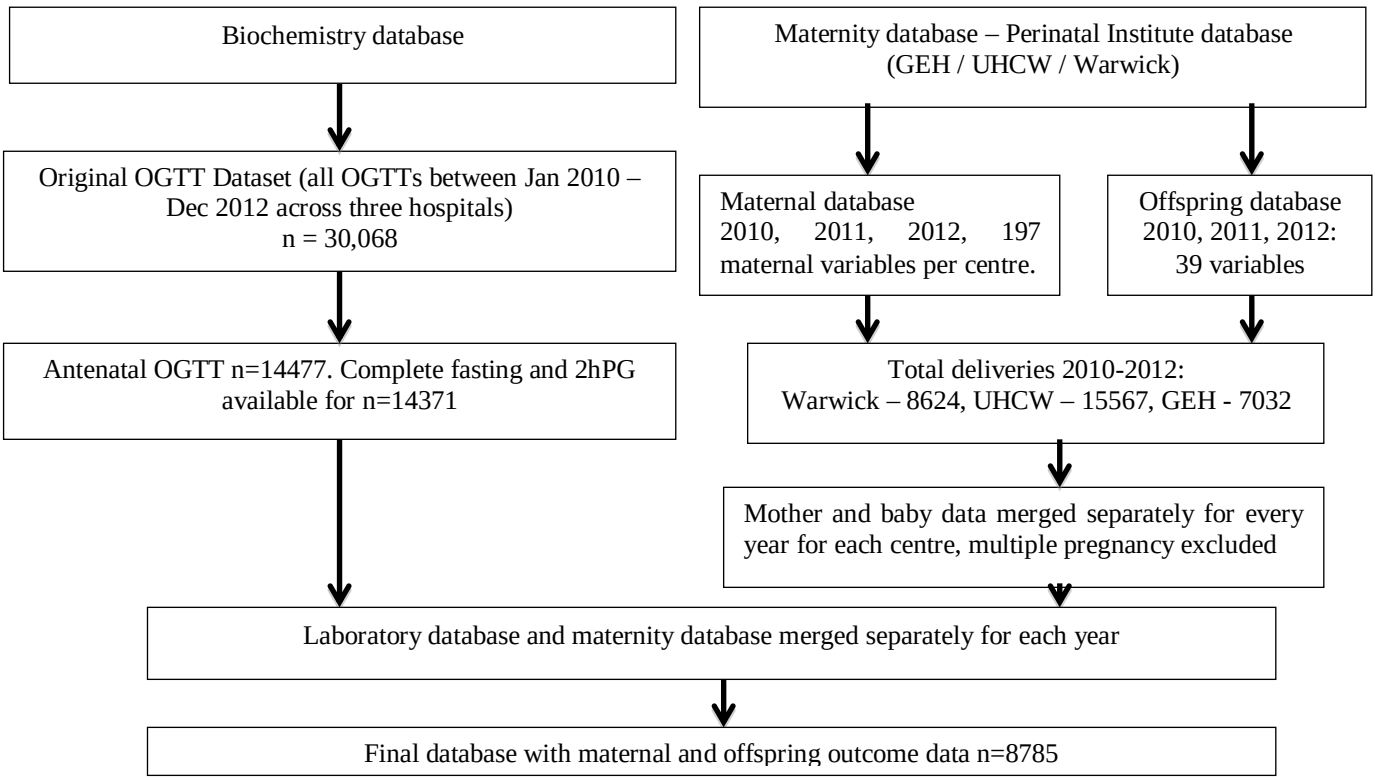
- Compare the prevalence of GDM by various criteria
- Compare short-term adverse pregnancy outcomes by various criteria

2.7 Methods

2.7.1 Subjects:

This was a retrospective study of all pregnant women who attended for antenatal OGTT across three National Health Service (NHS) hospitals in the West Midlands (Coventry, Nuneaton and Warwick) between Jan 2010 and Dec 2012. OGTT results were collected from the pathology database of Warwickshire. Maternal and offspring data were collected for these women from the Perinatal Institute database for each trust. The maternal, offspring and pathology databases were cleaned and merged to obtain data for all singleton pregnancies during this period. For the purpose of this study, ethnicity was grouped into South Asians (SA -Indian, Bangladeshi, Pakistani, Sri Lankan, Nepali), White Caucasian (WC - British / European) and others (Chinese, Black, Middle Eastern, Mixed).

2.7.2 Data cleaning and merging



2.7.3 GDM screening and criteria

All these centres used the selective screening based on at least one of the following risk factors recommended by NICE: BMI≥30, first-degree relative with diabetes, previous GDM, previous unexplained stillbirth, previous baby with birth weight ≥ 4.5 kg or women of ethnic minority origin. During this period, the centres used modified WHO-1999 criteria (mWHO-99) for the clinical diagnosis of GDM following a 75g OGTT: FPG ≥6.1 and/or 2hPG ≥7.8mmol/l, one of two readings sufficient for diagnosis. Treatment was based on the mWHO-99 criteria.

2.7.4 Statistical analysis

The new NICE criteria (FPG ≥5.6 or 2hPG ≥7.8mmol/l), mWHO-99 and the modified IADPSG criteria (FPG ≥5.1 or 2hPG ≥8.5mmol/l) were applied to this population. Several subgroups were identified as shown in the results section. Maternal characteristics and offspring outcomes were compared across the groups. Student t test, chi-square test and fisher exact test were used to compare means and proportions between any two groups. A significance level of $p \leq 0.001$ was considered significant, with $p \leq 0.01$ being considered a trend when making multiple comparisons. Logistic regression was used to assess the predictors of LGA and CS. SPSS version 22.0 was used for analysis.

2.7.5 Laboratory analysis

Venous samples were used for glucose testing. Venous blood was collected using fluoride oxalate tubes and the plasma glucose is analyzed using a hexokinase method in our laboratory accredited by UKAS.

*2.7.6 **Definitions***

Macrosomia was defined was birth weight >4000g. Multiparty was defined as having had 2 or more live births beyond 24 weeks gestation. Pre-eclampsia was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg on two or more occasions with proteinuria ≥1+ on dipstick. LGA and SGA were defined as BW ≥90^{th} and ≤ 10^{th} centile for gestational age respectively. WHO centile calculator using mean BW of 3,542 g (SD 437 g) at 40 weeks was used and available from the link below.

www.who.int/reproductivehealth/topics/best_practices/weight_percentiles_calculator.xls [91-93].

2.8 Results

Of the 14,477 pregnancies during this period, both FPG and 2hPG values were available for 14,371. Of these 8785 women had maternal and offspring data. The baseline maternal and offspring characteristics of all women undergoing OGTT split by ethnicity are shown in Table 2.

The incidence of GDM with the mWHO-99 criteria was 9.4% (1347/14371). The incidence with the IADPSG and new NICE would have been 10.6% (1525/14371) and 10.5% (1505/14371), respectively. Fig 1 shows the control population as well as the relative numbers of women diagnosed by each criterion.

In the WHO group, 7.1% of women were diagnosed by FPG alone, 81.6% by 2hPG alone, and 11.3% were diagnosed by both FPG and 2hPG readings. In the IADPSG group 52.6% of diagnosis was based on FPG alone, 26.2% on 2hPG alone and 21.2% on both abnormal readings. In the NICE group, these figures were 15.8%, 65.9% and 18.3% respectively.

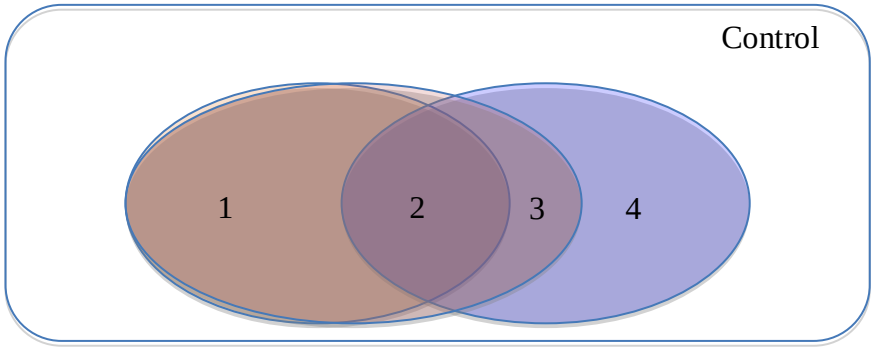
Out of the 1347 GDM women diagnosed by mWHO-99, the IADPSG criteria will miss 440 women (group 1) but diagnoses an additional 618 women (group 3 and 4). NICE diagnoses an additional 158 (group 3), in addition to the 1347 GDM women by the mWHO-99 criteria (total n=1505) but will miss 460 women diagnosed by IADPSG alone (group 4). We studied the maternal and offspring characteristics of women in the IADPSG (group 3+4), NICE (group 3) in comparison to the currently used mWHO-99 (group1) and controls to assess the impact of changing from the current mWHO-99. We also compared the untreated groups by NICE (group 3) with the IADPSG alone (group 4).

Table 2: Baseline maternal and offspring characteristics of the whole cohort of women undergoing antenatal OGTT.

	Total (n=14477)	**SA (n=1209)**	**WC (n=6060)**	**P***
Age in years: Mean(SD)	30.37(5.89)	31.01(4.7)	30.11(6.20)	<0.0001
Height in cm: Mean(SD)	163.81(7.49)	159.78 (6.39)	164.78 (7.25)	<0.0001
BMI (Kg/cm^2): Mean(SD)	28.53 (6.91)	25.26 (5.3)	29.52 (7.13)	<0.0001
Multiparity (≥2): % (n)	24.9 (2170/8708)	27.0 (324/1202)	23.4 (1412/6044)	0.008
Smoking: % (n)	13.8 (1202/8712)	2.1 (25/1209)	17.9 (1083/6040)	<0.0001
FPG in mmol/l: Mean(SD)	4.41(0.56)	4.48 (0.65)	4.41(0.52)	<0.0001
2hPG in mmol/l: Mean(SD)	5.78(1.49)	5.85 (1.64)	5.78 (1.53)	0.142
Pre-eclampsia: % (n)	6.7 (203/3024)	9.1 (25/232)	5.5 (142/2566)	0.028
Still birth	0.3 (24/ 6914)	0.6 (6/1049)	0.3 (14/4584)	0.405
Birth weight in grams: Mean(SD)	3360.70 (632.1)	3092.09(580.10)	3431.25 (630.98)	<0.0001
Gestational age (days): Mean(SD)	276.11(13.59)	274.39(13.54)	276.39(13.56)	<0.0001
Macrosomia: % (n)	13.6 (939/6890)	4.3 (45/1049)	16.5 (752/4561)	<0.0001
LGA: % (n)	12.2 (757/6208)	4.4 (43/984)	15.0 (612/4076)	<0.0001
SGA: % (n)	13.2 (933/7079)	25.1 (267/1062)	10.3 (487/4723	<0.0001
Congenital Anomalies: % (n)	1.9 (127/6680)	2.0 (2/1018)	1.9 (85/4435)	0.920
C-Section: % (n)	34.5 (2384/6903)	35.3 (370/1047)	33.9 (1552/4578)	0.376
Special care: % (n)	4.5 (81/1801)	2.2 (3/134)	4.7 (74/1559)	0.181
1 min APGAR score: <7 % (n)	6.8 (468/6861)	4.4 (46/1043)	7.2 (325/4544)	0.001

P* values represent differences between SA and WC.

Fig 1: Representation of populations detected by IADPSG, NICE and mWHO-99 criteria.



1: mWHO-99 positive-NICE positive-IADPSG negative (2HPG 7.8-8.4 and FPG < 5.1mmol/l): n=440 (3.06%), 100% treatment
2: mWHO-99 positive-NICE positive-IADPSG positive (2HPG ≥8.5 or FPG ≥ 6.1mmol/l): n=907 (6.3%), 100% treatment
3: mWHO-99 negative-NICE positive-IADPSG positive (FPG 5.6-6.0 and 2hPG <7.8mmol/l): n=158 (1.1%) , 0% treatment
4: IADPSG positive-NICE negative-mWHO-99 negative (FPG 5.1-5.5 and 2hPG<7.8mmol/l): n = 460 (3.2%) , 0% treatment
Control group. Risk factor positive but GDM negative by all criteria (FPG<5.1 and 2hPG<7.8mmol/l): n=12406 (86.3%), 0% treatment
Total population represents all women undergoing antenatal OGTT: n=14371

2.8.1 Comparison of mWHO-99, IADPSG and NICE criteria

Table 3 shows the key maternal and offspring outcomes in the various subgroups and the control population. Additional women who were detected with the IADPSG (group 3&4) and NICE criteria (group 3) had significantly higher BMI and rates of multiparity than controls and mWHO-99 groups (group 1). The offspring in both groups also had significantly higher crude birth weight, rates of macrosomia and LGA. CS rates in the untreated IADPSG group (group 3&4) were higher than that of the controls but similar to that of the treated GDM women (group 1). CS rates in the NICE group (group 3) were no higher than that in controls and mWHO-99 groups. Both NICE (group 3) and IADPSG (group 3&4) groups had significantly higher still birth rates compared to controls and the treated mWHO-99 groups, with the highest rates seen in the NICE group (4.1%, 3/73).

2.8.2 Comparison of NICE and IADPSG criteria

There were no significant differences in maternal or offspring risk characteristics between additional women diagnosed by NICE (group 3) (FPG 5.6-6.0 and 2hPG <7.8mmol/l) and IADPSG (group 4) (FPG 5.1-5.5 and 2hPG <7.8mmol/l) (table 2). Still birth rates were the higher in the additional women diagnosed by NICE (group 3) compared to the IADPSG group (group 4).

2.8.3 Risk of LGA and CS

Women in the IADPSG women (group 3&4) had significantly higher odds of CS and LGA rates compared to controls (table 4). The differences in rates of LGA persisted even after the adjustment for maternal age, smoking, parity, ethnicity and BMI. However, the differences in CS rates became non significant after the adjustment for maternal BMI. Although there were higher overall CS rates, there were no differences in the rates of emergency CS between the groups (group 3&4 vs

control: 7.4 vs 8.2%, p=0.486). Similar observations were seen in additional GDM women diagnosed by NICE (group 3; n=158) for LGA, however there were no differences in CS risk between controls and the NICE in any of the models. The odds of LGA and CS in the IADPSG group was no different from that in the NICE group both in adjusted and unadjusted models (table 5)

2.8.4 Impact of the newer criteria on SA

There were no significant differences in the ethnic composition of women detected by any criteria. The proportion of SA women in the mWHO-99 group (group 1&2) was significantly higher than in the control group. However there were no significant differences in ethnic composition between any other groups.

SA were more likely than WC (OR: 1.862 (1.152, 3.012), p = 0.011) to be in the IADPSG group (group 3&4) than in the WHO group (group 1) after adjustment for maternal BMI, smoking, parity and age. In the unadjusted model, ethnicity did not predict the risk of being in either groups (Table 6).

Ethnicity was not an independent predictor of being diagnosed by the new NICE criteria, i.e of being in group 3 compared to group 1 either in the adjusted or unadjusted model.

Table 3: Comparison of maternal characteristics and pregnancy outcomes by mWHO-1999, IADPSG and NICE Criteria.

	Normal (0% treatment) n=12406	**mWHO1999 positive - IADPSG negative (group 1) (100% treatment) n=440**		**IADPSG Positive -mWHO1999 negative (group 3+4) (0% treatment) (n=618)**			**mWHO1999 positive-NICE positive (100% treatment) (group 1 +2) n= 1347**		**mWHO1999 negative-NICE positive (0% treatment) (group 3) n= 158**			**IADPSG positive-NICE Negative (group 4) (0% treatment) (n=460)**		
	Mean or %	Mean or %	P* (vs control)	Mean or %	P* (vs control)	P** (vs group 1)	Mean or %	P*(vs control)	Mean or %	P* (vs control)	P*** (vs group 1+ 2)	Mean or %	P*	P¥ (vs group3)
Maternal Age	30.10 (5.89)	31.78 (5.58)	<0.0001	31.54 (5.67)	<0.0001	0.579	32.28 (5.63)	<0.0001	31.66 (6.15)	0.028	0.365	31.52 (5.56)	<0.0001	0.85
Height cm	163.91 (7.63)	161.89 (7.79)	<0.0001	164.30 (6.74)	0.218	<0.0001	162.53 (6.76)	<0.0001	162.99 (6.96)	0.207	0.561	164.60 (6.66)	0.048	0.05
BMI in Kg/cm^2	28.15 (6.91)	28.16 (7.25)	0.985	32.85 (7.19)	<0.0001	<0.0001	29.62 (7.04)	<0.0001	34.05 (7.11)	<0.0001	<0.0001	32.59 (7.20)	<0.0001	0.09
Multiparty	24.3 (1802/7403)	23.4 (62/265)	0.724	33.6 (160/476)	<0.0001	0.004	25.3(204/805)	0.675	42.5 (37/87)	<0.0001	0.001	31.6 (123/389)	0.001	0.05
FPG	4.9 (0.4)	4.5 (0.4)	<0.0001	5.4 (0.2)	<0.0001	<0.0001	5.2 (1.3)	<0.0001	5.7 (0.1)	<0.0001	<0.0001	5.3 (0.1)	<0.0001	<0.0001
2hPG	5.4 (1.1)	8.0 (0.2)	<0.0001	6.0 (1.1)	<0.0001	<0.0001	8.9 (1.7)	<0.0001	5.9 (1.1)	<0.0001	<0.0001	6.0 (1.1)	<0.0001	1.000
Smoking at booking	14.2 (1054/7403)	8.2 (22/265)	0.006	15.1 (72/476)	0.586	0.007	9.5 (76/803)	<0.0001	20.7 (18/87)	0.086	0.001	13.9 (54/389)	0.845	0.11
Ethnicity % (SA; WC; others)	13.4; 69.7; 16.9 (7404)	15.5; 67.4; 17.0 (264)	0.598	14.9; 68.6; 16.6 (477)	0.674	0.950	16.8; 66.4; 16.8 (804)	0.032	18.4; 69.0; 12.6 (87)	0.292	0.601	14.1; 68.5; 17.4 (390)	0.872	0.39
Pre-eclampsia	6.4 (161/2533)	6.1 (5/82)	0.925	9.3 (19/205)	0.106	0.381	8.4 (25/296)	0.213	10.0 (3/10)	0.446	0.772	9.1 (16/175)	0.149	0.88
Still birth	0.3 (17/5877)	0 (0/194)	1.000	1.2 (5/413)	0.012	0.049	0.3 (2/620)	0.702	4.1 (3/73)	0.002	0.01	0.6 (2/340)	0.271	0.041
BW in g	3366.12 (625.18)	3147.09 (625.30)	<0.0001	3542.36 (700.30)	<0.0001	<0.0001	3199.86 (611.618)	<0.0001	3463.54 (897.62)	0.416	0.017	3559.05 (651.34)	<0.0001	0.39
Gestational age in days	276.9(13.31)	269.70 (14.46)	<0.0001	274.31(14.93)	<0.0001	<0.0001	269.09 (13.479)	<0.0001	270.31 (19.16)	0.002	0.564	275.2 (13.69)	0.012	0.03
Macrosomia (>4000g)	13.7 (800/5858)	5.2 (10/191)	0.001	24.3 (100/412)	<0.0001	<0.0001	7.0 (43/616)	<0.0001	26.4 (19/72)	0.003	<0.0001	23.8 (81/340)	<0.0001	0.65
LGA	10.9 (570/5213)	11.1 (21/190)	0.959	27.1 (102/377)	<0.0001	<0.0001	14.2 (87/614)	0.017	26.1 (18/69)	<0.0001	0.009	27.3 (84/308)	<0.0001	0.84
SGA	13.6 (822/6030)	17.2 (33/192)	0.159	8.0 (34/423)	0.001	0.001	12.8 (79/619)	0.547	8.1 (6/74)	0.168	0.249	8.0 (28/249)	0.003	0.98
Congenital anomalies	1.9 (108/5)693	1.1 (2/183)	0.483	2.3 (9/393)	0.583	0.328	2.0 (12/586)	0.795	4.5 (3/67)	0.126	0.209	1.8 (6/326)	0.942	0.19
CS	33.3 (1954/5870)	39.1 (75/192)	0.09	40.0 (165/413)	<0.006	0.835	42.5 (262/616)	<0.0001	41.1 (30/73)	0.16	0.81	39.7 (135/340)	0.015	0.83
Special Care	4.7 (72/1534)	2.2 (1/46)	0.422	4.5 (5/111)	0.927	0.488	4.3 (7/162)	0.842	6.3 (1/16)	0.765	0.72	4.2 (4/95)	0.828	0.72
1 min APGAR <7	6.7 (391/5839)	6.3 (12/189)	0.851	8.6 (35/371)	0.137	0.339	7.4	0.538	8.5 (6/71)	0.557	0.74	8.7 (29/335)	0.166	0.96

P* Comparison between control and respective groups, P** comparison between group 1 and group (3&4), P***comparison between group 3 and group (1&2)
P¥ comparison between group 3 and group 4

Table 4: Risk of LGA and CS in IADPSG (group 3&4) and NICE (group 3) groups compared to controls

	Control	**IADPSG**		**NICE**	
		OR (CI)	p	OR (CI)	p
LGA*	1.00 (Ref)	2.989[a] (2.340, 3.818)	<0.0001	2.889 (1.668, 5.003)	<0.0001
		3.056[b] (2.374, 3.934)	<0.0001	3.123 (1.770, 5.513)	<0.0001
		2.989[c] (2.340, 3.818)	<0.0001	2.677 (1.508, 4.751)	0.001
CS	1.00 (Ref)	1.333[a] (1.087, 1.636)	0.006	1.398 (0.874, 2.236)	0.162
		1.262[b] (1.025, 1.555)	0.029	1.352 (0.838, 2.180)	0.217
		1.134[c] (0.917, 1.402)	0.245	1.198 (0.740, 1.937)	0.463

Table shows OR of LGA and CS in the IADPSG and the NICE group compared to women in the control group

Table 5: Risk of LGA and CS in IADPSG (group 4) compared to NICE groups (group 3)

	NICE	**IADPSG**	
	OR (CI)	OR (CI)	p
LGA*	1.00 (Ref)	1.043 (0.573, 1.897)[a]	0.892
		0.963 (0.519, 1.790)[b]	0.906
		0.985 (0.529, 1.834)[c]	0.985
CS	1.00 (Ref)	0.944 (0.564, 1.579)[a]	0.826
		0.917 (0.543, 1.548)[b]	0.745
		0.929 (0.550, 1.570)[c]	0.783

All OR are in comparison to controls. [a]Unadjusted, [b]Adjusted for age, ethnicity, parity, smoking, [c]Model 2+ BMI, *All models include offspring sex for LGA analysis

Table 6: Predictors of being in group (3 & 4) vs being in group 1

	OR (CI)[a]	p	OR (CI)[b]	p
SA (WC ref)	0.943 (1.443, 0.616)	0.786	1.862 (1.152, 3.012)	0.011
BMI	1.117 (1.086, 1.149)	<0.0001	1.128 (1.094, 1.163)	<0.0001
Age	0.988 (0.960, 1.017)	0.410	1.005 (0.972, 1.038)	0.785
Parity	0.663 (0.456, 0.964)	0.031	0.744 (0.496, 1.116)	0.153
Smoking	0.592 (0.353, 0.992)	0.047	0.591 (0.335, 1.041)	0.068

[a]Unadjusted model, [b]Multivariable regression after adjustment for age, parity, smoking, BMI and ethnicity in the same model.

2.9 Discussion

Our study reports the impact of the new NICE and IADPSG criteria on a large risk factor based selectively screened multi-ethnic British population. Considering that the majority of UK centres still practice risk factor based selective screening and use the mWHO-99 criteria [76, 89], our data provides crucial evidence for UK and other centres that follow the same. Compared to the mWHO-99 criteria, the NICE criteria detect an additional cohort of 158 women with isolated mild fasting hyperglycaemia. (Group 3: FPG 5.6-6.0mmol/l, and 2hPG<7.8mmol/l) The IADPSG detects a larger cohort of 618 women with isolated mild fasting hyperglycaemia (group 3&4: FPG 5.1-6.0 & 2hPG<7.8) at the expense of missing 440 women with mild 2-hPG abnormalities (group 1: 2hPG: 7.8-8.4 mmol/L & FPG <5.1mmol/l) Both the criteria resulted in similar increases in the incidence of GDM compared to the mWHO-99 criteria.

2.9.1 Impact of changing to NICE or IADPSG

Both NICE (group3) and IADPSG (group 3&4) groups diagnosed additional women with mild fasting hyperglycaemia with characteristics of higher maternal metabolic risk (higher BMI and multiparity) and higher rates of adverse pregnancy outcomes (LGA, still births) compared to controls and the currently practiced mWHO-99 group.

Women in the IADPSG but not the NICE group had a significantly higher risk of CS compared to controls. Despite higher overall CS rates, the proportion of emergency CS in the IADPSG group was similar to that in controls. Both the groups had similar CS rates to treated women in the mWHO-99 groups (group 1) despite not being labelled as GDM in routine care.

A comparison of the untreated NICE (group 3) and IADPSG (group4) groups revealed no differences in maternal characteristics, LGA or CS risk (table 3, table 6), with the exception of higher still birth rates in NICE group. However, the overall numbers of stillbirths were small to make any meaningful conclusions. The Cambridge study reported higher rates of LGA and CS in the IADPSG group when compared to the NICE group [90]. However it must be remembered that they compared untreated women in IADPSG group with the treated women in the NICE positive/IADPSG negative group, making treatment differences a likely explanation for their results.

The higher risk of CS and LGA with the IADPSG criteria were reported in two other observational studies in the UK: The ATLANTIC-DIP study using universal screening and a more recent retrospective study from Cambridge using 50gGCT screening [90, 94]. Other studies comparing the IADPSG to the Carpenter-Coustan criteria in the setting of a 50g GCT based screening [95-97] also depicted higher CS rates in the IADPSG group, but LGA rates were similar to non-GDM controls. The variation in these results is likely due to the differences in screening and hence detection of a different control population, against which these criteria were compared.

2.9.2 Predictors of higher CS and LGA risk

The higher LGA risk in both groups, persisted despite adjustment for maternal characteristics including BMI (table 4). It is difficult to say if this risk is entirely mediated by glycaemic differences or by the altered metabolic milieu of an obese pregnancy such as higher circulating free fatty acids which have been shown to independently correlate with LGA risk [98]. In our study, the difference in mean glycaemia between controls and the IADPSG group was 0.5 mmol/l with FPG and

0.6 mmol/l with 2hPG, which although statistically significant, could be argued not to be clinically significant to contribute to a three fold higher risk of LGA.

From our data, it also appears that the risk of CS in the IADPSG group is largely mediated by BMI. The higher risk of CS in the IADPSG group was no longer significant after adjustment for maternal BMI, signifying that maternal BMI played an important role in determining this risk (table 4). The higher rates of primary, as well as repeat CS in obese women has been reported previously, with obese women having difficulty completing the second stage of labour owing to soft tissue dystocia [99]. Our results are also in agreement with the HAPO post hoc analysis and other studies where maternal obesity has been shown to be a more significant predictor of CS rates than maternal glucose except in women with the highest degree of hyperglycaemia [71, 84, 85, 90, 100].

Despite detection of a higher risk cohort with more adverse offspring outcome, the question of whether treatment of glycaemia in the IADPSG or NICE group would indeed improve outcomes such as LGA or CS still remains unanswered. Two non-randomised observational studies observed a reduction in LGA rates with treatment in the IADPSG group, however only one study showed a reduction in CS rates [101, 102]. Both these trials compared the IADPSG groups (using universal screening) with the Carpenter-Coustan criteria, which uses a 50g GCT screening strategy, hence making it difficult to make direct comparisons. In fact women in the above studies had far lower BMI than seen in our study, owing to the risk factor based selective screening used in our study. Evidence form the MFMU trial showed that women who benefited from intervention in GDM gained significantly less weight in pregnancy than those in the control arm [11]. Hence it is conceivable that the benefit of treatment came from weight lowering rather than glucose lowering effects of the

intervention. While it may be prudent to risk stratify and intervene on women with high BMI to reduce these risks, the available intervention trials on the overweight and obese pregnant women have failed to show a benefit in reduction of perinatal risk, especially LGA [103, 104].

2.9.3 Effect of IADPSG and NICE on SA ethnicity

Contrary to our hypothesis SA were not more likely to be missed by the higher 2hPG cut off of the IADPSG group. The ethnic compositions of all groups were similar. Again BMI but not ethnicity was the most important predictor of being detected by the IADPSG criteria in univariate linear regression analysis (table 6). After adjustment for BMI, SA had a higher OR of being in the IADPSG group (group 4&5) compared to group 1, indicating that BMI was the main driver for mild FPG abnormalities in the WC ethnic group. It is possible that our selective screening strategy influenced the lack of ethnic differences between the groups and that a more significant ethnic difference might have been more apparent in a universally screened population. Only one other study compared the impact of the IADPSG criteria on a mixed ethnic group. Our results are in agreement with another study that reported that SA minority was an independent predictor of being diagnosed by the IADPSG criteria [86]. Some studies have reported up to a threefold increase in the prevalence in GDM using the new IADPSG criteria compared to the prevalent criteria in Asians [80]

In summary, further randomised interventional trials are therefore needed to investigate the real benefit of detection and treatment of mild fasting hyperglycaemia (whether by NICE or IADPSG) in reducing offspring risks. Such trials should not only include the cost effectiveness of treating GDM with respect to short-term adverse outcomes but also incorporate the increase in CS rates, which is

likely to accompany a diagnosis of GDM. We therefore believe that without such evidence to support the detection of milder forms of hyperglycaemia, the change in diagnostic criteria cannot be supported purely based on adverse offspring outcome in observational studies.

Our study has important limitations: it is retrospective in nature, lacks data on glycemic control or treatment during pregnancy and 1hPG values in the IADPSG criteria were not available. The key strength of our study is the real life data from a large multi-ethnic population in which the NICE recommended selective screening criteria were used. The selective screening method and our diagnostic criteria for GDM (mWHO-99) is still widely used in the UK and other centers across the world, making it more relevant to current practice.

2.10 Future directions and gaps in the evidence

- Prospective studies are needed to assess the risk of adverse offspring outcome using various criteria in SA
- Prospective randomised interventional studies are needed both in SA and WC to assess the real benefit of treating GDM based on the IADPSG criteria
- Above studies should incorporate cost effectiveness analysis
- GDM diagnostic criteria should be evaluated in the light of screening strategies. Studies are needed to assess the impact of different GDM criteria in universal vs selective screening settings.

3 Postnatal screening in GDM

3.1 Abstract

Background: The ideal test for postnatal screening after gestational diabetes mellitus is still under debate. Most international guidelines continue to recommend a postnatal oral glucose tolerance test (OGTT). In contrast, the new National Institute of health and Care Excellence (NICE), UK guidance recommends fasting plasma glucose (FPG) or HbA1c but *not* an OGTT for postnatal testing. Our aim was to compare the performance of FPG (using both the WHO and ADA definitions of IFG) with OGTT and the uptake rates of OGTT with HbA1c in SA and WC.

Research Design and Methods: Multicentre retrospective study of 1289 women with GDM referred for postnatal OGTT between 2008 and 2012. Subsequent data was collected from one centre for 339 women referred for postnatal HbA1c between Dec 2013 and Dec 2014. Sensitivity and ROC analysis was performed.

Results: 630 (48.8%) attended postnatal OGTT. FPG at the cut point of 6.1mmol/l (WHO criteria for impaired fasting glycaemia - IFG) would miss 88% of IGT and 16.7% of diabetes. The ADA criteria for IFG (FPG of ≥5.6 mmol/l) would detect 100% diabetes in both ethnic groups but would still miss 64% IGT. The uptake improved to 62.8% with subsequent use of postnatal HbA1c.

Conclusion: FPG at the ADA threshold of ≥5.6 mmol/l has high sensitivity to detect persisting postnatal diabetes even in SA ethnic minority groups. Postnatal HbA1c shows promising increase in uptake compared to OGTT. The new NICE recommendation for postnatal screening is therefore a welcome change to improve care of these high-risk women.

3.2 Introduction

3.2.1 Risk of post-natal diabetes – summary of literature and variation with ethnicity

Gestational Diabetes is a recognised 'pre-diabetes' state associated with a 7-8 fold higher lifetime risk of developing type 2 diabetes compared to those without GDM [8]. The risk of developing type 2 diabetes (T2D) increases rapidly within the first 5 years of index delivery, the incidence of which can range from 2.5 to 16.7% in the first year after delivery [105]. This risk is higher in South Asians (SA) who have up to a 5-fold higher risk of T2D compared to White Caucasians (WC) [106].

Literature comparing the risks of postnatal T2D incidence between SA and WC is sparse. A systematic review of 20 studies compared the relative risk (RR) of developing diabetes post GDM across different nationalities [8]. (Table 1). The RR of T2D in India was significantly higher than the pooled RR (22.7 (10.09, 51.08) vs 7.43 (4.79, 11.51)).

Table 1: Relative risk of postnatal diabetes following GDM.

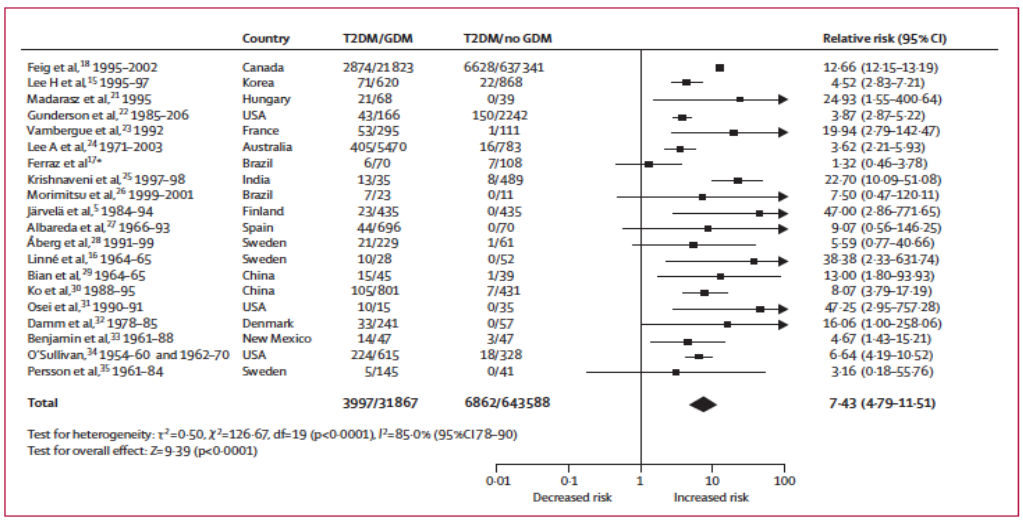


Table 1: Forest plot shows the Relative risk of postnatal diabetes following a GDM pregnancy across the world. Reproduced with permission from a systematic review by Bellamy et al [8]

Table 2: Summary of literature comparing the risk of post-partum T2D after GDM between SA and WC:

Study	Time of follow up post GDM	Results
Bellamy et al [8]	Systematic review of varying time periods post GDM	RR: India: 22.7 (10.09, 51.08), Cumulative: 7.43 (4.79, 11.51)
Sinha et al [106]	6-12 weeks post partum	Incidence: 7% in WC, 5% in Black, 35% in SA
Mukerji et al [2]	Median FU period 7.6 years post GDM	Cumulative incidence of diabetes was 16.5% for Chinese, 31.8% for SA and 25.7% for WC
Krishnaveni et al [79]	5 years follow up	Incidence of Diabetes: GDM vs control. 37% versus 2%

Figure 1: Cumulative incidence of diabetes following GDM:

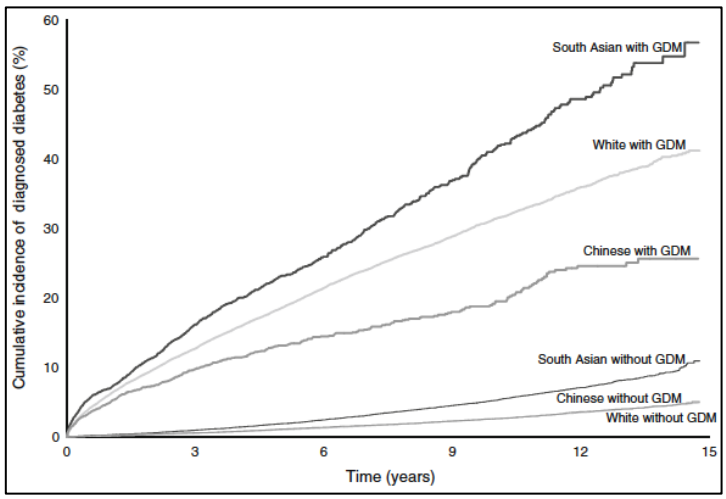


Figure 1 shows the cumulative incidence of diabetes following a diagnosis of GDM between SA, WC, and Chinese ethnic groups. Reproduced with permission from Mukerji et al [2]

A summary of other literature comparing the risk of post-partum diabetes after GDM is shown in table 2. All other studies apart from the systematic review reported incidence rates and not relative risk. However the incidence of post-partum T2D was significantly higher in SA compared to WC. Figure 1 shows the cumulative incidence of T2D in women with and without GDM, across SA, Chinese and WC ethnicities. This study reported that although the overall incidence of T2D

was higher in SA in both categories, the presence of GDM conferred a 13 fold higher risk in WC compared to 9-10 fold risk in SA [2].

In summary, postnatal screening in women with GDM provides a unique opportunity for early detection of diabetes, intervention to prevent T2D and for timely pre-conception care for subsequent pregnancies. This assumes greater importance in SA who have a significantly higher risk of postnatal diabetes compared to WC.

3.2.2 Poor uptake of postnatal screening:

Postnatal testing following GDM is poor with uptake rates of OGTT being between 23 and 58% [107]. This is in sharp contrast to high uptake (94%) of other postnatal screening programs such as cervical screening done at similar time interval post-delivery [108]. A few studies have examined the impact of ethnicity on postnatal uptake rates [109-111] (table 3). All the three studies show that SA and non-WC ethnicity was associated with higher rates of uptake for postnatal testing.

Table 3: Summary of relevant studies comparing uptake of postnatal screening between ethnic groups.

Studies	Type of test	Sample size	Uptake rates by ethnicity
Lawrence et al	FPG (79.1%) or OGTT (18.2%), both (2.7%).	n = 11,825,	Whole cohort 50.2% WC 47.7%; SA 59.0%; Black 27.2%; Hispanic 51.1%; Others 47.8 %
Ferrara et al	OGTT or FPG	n = 14,448	Whole cohort: 38.2 % WC 33.2%; SA 45.5%; Black 26.1%; Hispanic, 40.5%; Other/Unknown, 34.1%
Kwong et al	FPG (4.8%) or OGTT (95.2%)	n = 909	Overall 48.2 % WC 46.7%; Non-Caucasian 50.1%.

*3.2.3 **Various postnatal screening strategies***

OGTT, FPG and HbA1c are three tests that have been used for postnatal screening following GDM. The Fifth International Workshop Conference on GDM [112], Australasian Diabetes in Pregnancy Society (ADIPS) [73] and the Canadian Diabetes Association [113] recommend an OGTT as the gold standard at 6 weeks post-partum. The ADA guidelines recommend "post-partum screening at 6-12 weeks using non-pregnancy OGTT criteria" to detect diabetes and specifically mention not to do HbA1c due to the potential effects of antepartum treatment [114]. The American College of Obstetrics and Gynaecology (ACOG) guidelines are ambiguous and recommend either a FPG or OGTT at 6 weeks [62]. Although most international guidelines recommend an OGTT as the postnatal test of choice following GDM, there is considerable debate about replacing this with either FPG or HbA1c, due to poor uptake, cost, complexity and question of the real rationale for a OGTT [115, 116]. The latest NICE guidelines now categorically and unambiguously recommend either FPG or HbA1c *but not* an OGTT for postnatal screening[117].

The lack of consensus among recommendation is also reflected in clinical practice. A survey of ACOG fellows reported that only 50·8% of obstetricians used an OGTT for postnatal screening with 27·4% using FPG and the remaining relying on other tests [118]. Similarly, only 54% of practitioners in North Carolina reported using OGTT of the 27% of respondents who performed any form of postnatal screening for GDM [119]. A cross sectional survey of Australian women with a recent history of GDM women revealed use of various postnatal screening tests nationally, i.e. OGTT (56·4%), FPG (32·6%), capillary blood glucose (23.5%), random plasma glucose (6.1%), and HbA1c (2·4%) [120].

While there is no doubt that FPG or HbA1c are one-step alternatives to a cumbersome and more expensive OGTT, it is not known whether using these tests to simplify postnatal screening would indeed improve uptake. Several studies also depict poor sensitivity of FPG to detect overall postnatal abnormalities [107]. This could especially be a problem in SA who are known to have a higher prevalence of postprandial abnormalities compared to WC [121]. Only a few studies have examined the sensitivity of the lowered definition of normal FPG of 5.6mmol/l to detect diabetes and IGT in populations with SA and other ethnic minority groups [122].

3.3 Aims and objectives

The primary aim of this study is to compare the performance of FPG (according to WHO and ADA IFG criteria) with that of OGTT in the postnatal period, in a multi-ethnic British population. We also assessed the change in uptake rates with postnatal HbA1c compared to OGTT in a sequential retrospective observational cohort study.

3.4 Methods

3.4.1 Subjects

Routine clinical data from two independent cohorts of pregnant women were collected from across three NHS hospitals in the West Midlands (Coventry, Nuneaton and Warwick). A total of 14,477 pregnant women underwent OGTT as a part of their antenatal testing for GDM between 2009 and 2012. Of these, 8.9% of the women (n=1289) were diagnosed with GDM (OGTT cohort). Women in the OGTT cohort were requested to attend postnatal OGTT around 6 weeks postpartum. The second cohort (HbA1c cohort) contained 339 women with history of GDM, who delivered between Nov 2013 and Dec 2014. These women were recommended

to undergo a postnatal HbA1c instead of OGTT, following a change in clinical practice in one of the hospitals (Coventry).

3.4.2 GDM screening and diagnostic criteria

All these centres used the selective screening based on the risk factors recommended by NICE: BMI ≥ 30Kg/cm^2, first-degree relative with diabetes, previous GDM, previous unexplained stillbirth, previous macrosomia (≥ 4.5 kg) or women of ethnic minority origin. Women meeting any one of the above criteria underwent a 75 g OGTT between 26-28 weeks. During this period all centres used the modified WHO 1999 criteria for the diagnosis of GDM following a 75g OGTT: FPG ≥6.1mmol/l and/or 2-hour plasma glucose (2hPG) ≥7.8mmol/l. Obstetric and neonatal characteristics were obtained for all women undergoing an OGTT. BMI was measured at the booking visit.

3.4.3 Definitions

For the purpose of this study, ethnicity was grouped into South Asians (SA -Indian, Bangladeshi, Pakistani, Sri Lankan and Nepali), White Caucasian (WC - British / European) and Other (Chinese, Black, Middle Eastern, Mixed).

Multiparity was defined as ≥2 live previous pregnancies that progressed beyond 24 weeks gestation. Macrosomia was defined as BW≥4000g.

3.4.4 Definitions of postnatal glucose abnormalities

Impaired glucose tolerance (IGT) was defined as a 2hPG ≥7.8 but <11.1mmol/l, with FPG <7.0mmol/l. IFG was defined either as FPG ≥5.6 or ≥6.1mmol/L as per the ADA and WHO definitions, respectively with 2hPG <11.1mmol/L. Isolated IFG (iIFG) was defined as IFG with normal 2hPG (<7.8mmol/L). Isolated IGT (iIGT) was defined as IGT with a normal FPG (ADA <5.6 or WHO <6.1mmol/L). Diabetes

was defined as either FPG ≥ 7.0 or 2hPG ≥ 11.1 mmol/l or both. We applied both the WHO and the ADA cut points for IFG to evaluate the performance of FPG compared to OGTT to detect postnatal abnormalities.

3.4.5 Statistical methods

Student's t test and Chi square test were used to compare means and proportions respectively. Post hoc testing and Bonferroni adjustment was performed to enable comparisons between multiple groups. Receiver operating characteristics and sensitivity analysis were used to assess the performance of FPG to detect IGT and diabetes as diagnosed by OGTT. SPSS version 22.0 was used for analysis.

3.5 Results

3.5.1 Uptake rates with OGTT and HbA1c

Baseline characteristics of the women who were invited for OGTT (OGTT cohort) are shown in table 4.

630 (48.9%) women attended postnatal OGTT. 627 had full OGTT, 2 women had FPG >14mmol (hence OGTT was abandoned) and one had only FPG but did not complete the OGTT. The mean timing of postnatal OGTT was 9.39 ± 4.17 weeks post-delivery.

In Coventry, 213 (62.8%) women attended for postnatal HbA1c tests, compared to only 48.7% (381/783) for OGTT testing ($p<0.0001$). The mean timing of post-partum HbA1c testing was 16.21±9.65 weeks post-delivery.

Comparisons of characteristics of women who attended and did not attend postnatal testing by OGTT and HbA1c are shown in the table 5. Women who failed to attend postnatal OGTT were younger and more likely to be smokers, multiparous and have macrosomic offspring compared to those who attended. There were no differences in

characteristics between women who attended and failed to attend postnatal HbA1c except a significantly higher proportion of SA compared to WC among the attenders.

Table 4: Baseline characteristics of women invited for postnatal OGTT

Mean (SD) or % (n) for proportions	
Baseline characteristics	Total n=1289
Age in years	31.97 (5.50) (1288)
BMI (kg/cm^2)	29.64 (6.93) (n=761)*
Height (cm)	162.45 (6.72) (n=761)*
Multiparity (parity≥2)	26.3 (200/761)
Smoking	9.2 (70/759)
Macrosomia (>4 Kg)	9.2 (70/757)
Ethnicity (WC; SA; others)	65.5; 27.2; 7.3 (n=1281)*

*Available data on variables in indicated by (n) for continuous variables and as a proportion for categorical variables.

¥ Post hoc testing revealed significant differences in proportions of WC and other minority ethnic groups only but not of SA.

Table 5: Characteristics of women who attended and did not attend post-natal testing

	Mean (SD) or % (n) for proportions					
	OGTT cohort			**HbA1c cohort**		
	Attended (n=630)	Not attended (n=659)	P value	Attended (n=213)	Not attended (n= 126)	P value
Age	32.35 (5.25)	31.61 (5.7)	0.016	33.19 (5.09)	31.99 (6.18)	0.069
BMI	29.64 (6.61)*	29.63 (7.26)¥	0.974	29.04 (6.79)[a]	29.66 (7.99)[a]	0.516
Height	161.87 (7.02)*	163.07 (6.33)¥	0.013	161.96 (7.24)[b]	162.29 (6.03)[b]	0.671
Smoking	4.9 (19/390)	13.8 (51/369)	<0.0001	5.3 (11/207)	8.5 (10/118)	0.265
Ethnicity (WC; SA; others)	64.4; 27.8; 7.8 (627)	66.5; 26.8; 6.7 (654)	0.654	43.5; 33.8; 22.7 (207)	58.7; 21.5; 19.8 (121)	0.02[c]
Multiparity (Parity ≥ 2)	21.0 (82/391)	31.9 (118/370)	0.001	46.4 (97/209)	49.2 (60/122)	0.626
Macrosomia (≥4000g)	5.9 (23/391)	12.8 (47/366)	0.001	8.6 (18/210)	8.0 (10/125)	0.855

Baseline maternal characteristics obtained at booking and offspring birth weight of women who attended and failed to attend postnatal testing by both HbA1c and OGTT.

* Data was available for 392 (62.2%) women. ¥ Data was available for 369 (55.9%) women.

[a] BMI was available for 182 (85.4%) and 98 (77.7%) of women who attended and did not attend respectively

[b] Height was available for 187 (87.7%) and 107 (84.9%) of women who attended and did not attend respectively

[c] Post-hoc testing revealed significant differences in the proportions of SA and WC only.

*3.5.2 **Postnatal abnormalities with OGTT by ethnicity***

Table 6 shows the relative proportions of isolated FPG, isolated 2hPG and concomitant FPG and 2hPG abnormalities using both the ADA and WHO definitions of IFG.

By WHO criteria of IFG: Seventy-eight (12.4%) had abnormal OGTT results. The overall prevalence of diabetes was 1.9%. iIGT was the most common abnormality accounting for 56.4% (44/78) of the abnormal results. 88% (44/50) of IGT and 16.7% (2/12) of diabetes had normal FPG. On post-hoc testing, WC had significantly lower proportion of overall abnormalities (10% vs 19.2%, $p<0.05$) and isolated 2hPG abnormalities compared to the other minority ethnic group (5.0 vs 15.4%, $p<0.05$). SA tended to have higher overall abnormalities ($p=0.032$) and also isolated 2hPG abnormalities ($p = 0.02$) compared to WC however on applying the Bonferroni correction this difference ceased to be significant.

By ADA criteria of IFG: One hundred and fourteen (18.1%) had abnormal OGTT results. iIFG was the most common abnormality accounting for 45.6% (52/114) of the abnormal results. 64% (32/50) of women with IGT but none of those with diabetes had normal FPG. Again, WC had lower proportions of 2hPG abnormalities compared to the other minority ethnic group (3.2 vs 15.4%, $p<0.05$). SA tended to have higher overall abnormalities ($p = 0.03$) compared to WC but had similar proportion of 2hPG abnormalities to WC.

Table 6: Categories of postnatal abnormalities based on Fasting and 2hPG abnormalities

Category of abnormality	Total population (629) n (%)	SA (174) n (%)	WC (403) n (%)	Others (52) n (%)	p value
WHO					
Normal	551 (87.6)	146 (83.9)	363 (90.0)*	42 (80.8)*	0.027
Isolated FPG abnormalities (FPG≥6.1 & 2HPG<7.8)	17 (2.7) (1d)	4 (2.3) (0d)	12 (3.0) (1d)	1 (1.9) (0d)	
Isolated 2hPG abnormalities (2HPG≥7.8 & FPG<6.1)	46 (7.3) (2d)	18 (10.4) (0d)	20 (5.0)* (2d)	8 (15.4) * (0d)	
Both FPG and 2hPG abnormalities (2HPG≥7.8 & FPG≥6.1)	15 (2.4) (9d)	6 (3.4) (4d)	8 (2.0) (5d)	1 (1.9) (0d)	
ADA					
Normal	515 (81.9)	134 (77.1)	341 (84.6)	39 (75.0)	0.001
Isolated FPG abnormalities (FPG≥5.6 & 2HPG<7.8)	53 (8.4) (1 d)	16 (9.2) (0d)	34 (8.4) (1d)	4 (7.7) (0d)	
Isolated 2hPG abnormalities (2HPG≥7.8 & FPG<5.6)	32 (5.1) (0d)	11 (6.3) (0d)	13 (3.2)* (0d)	8 (15.4)* (0d)	
Both FPG and 2hPG abnormalities (2HPG≥7.8 & FPG≥5.6)	29 (4.6) (11 d)	13 (7.5) (4d)	15 (3.7) (7d)	1 (1.9) (0d)	

All categories also include diabetes (d) based on FPG, 2hPG values

* Indicate significant difference between WC and other ethnic group in respective categories on post-hoc testing.

Sensitivity analysis

We conducted a ROC analysis to assess the ability of FPG to detect diabetes and IGT as defined by OGTT (figure 2). ROC analysis revealed that postnatal FPG was an excellent predictor of overall postnatal diabetes (AUC=0.983, CI: 0.966-1.00, $p<0.0001$) and in both SA and WC. FPG was a moderate predictor of IGT (AUC=0.697, CI: 0.620-0.773, $p<0.0001$). ROC curves with AUC for SA and WC are presented in figure 3 and 4 for IGT and diabetes respectively.

The Sensitivity, specificity, positive predictive value (PPV) and negative predictive Value (NPV) of various postnatal FPG cut offs to predict postnatal IGT and diabetes are shown in table 7. FPG of 6.1mmol/l will miss up to 88% of IGT and 16.7% of diabetes. While FPG of 5.6mmol/l will detect 100% diabetes, it would miss 64% of IGT. Only 13% of women had a FPG≥5.6mmol/l. Therefore performing confirmatory OGTT only in those women with FPG ≥5.6mmol/l will reduce the need for OGTT by 87%. In order to obtain 100% sensitivity for detection of IGT, the FPG threshold will have to be lowered to 4 mmol/l, with over 98% of women needing a second confirmatory OGTT.

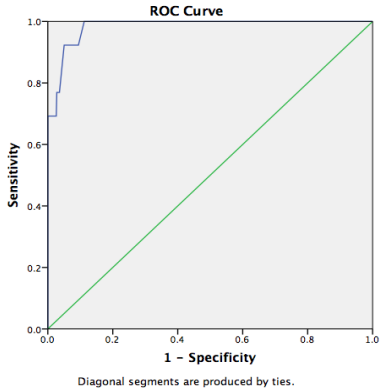
Table 7: Sensitivity analysis for various thresholds of FPG to detect IGT and diabetes

FPG threshold (mg/dl)	IGT				Diabetes				% meeting criteria*
	Sensitivity	**Specificity**	**PPV**	**NPV**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	
FPG ≥4.0	100.0	2.3	8.3	100.0	100.0	2.1	1.9	100.0	97.9
FPG ≥4.5	94.0	24.3	9.9	97.9	100.0	22.9	2.5	100.0	77.5
FPG ≥5.0	63.8	70.0	14.6	96.0	100.0	61.1	4.8	100.0	40
FPG ≥5.6	36.0	90.8	25.7	94.1	100.0	88.7	14.6	100.0	13
FPG ≥6.1	12.0	97.2	27.3	92.6	83.3	96.4	31.3	99.7	5.1

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of various thresholds of FPG to detect diabetes and IGT.

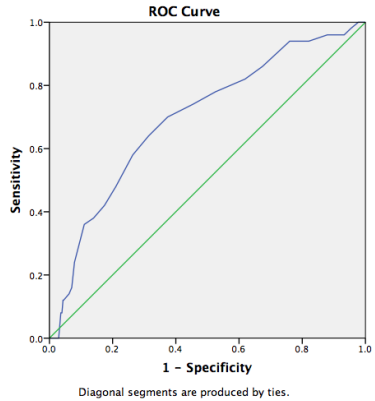
* The proportion of women in the respective FPG ranges.

Figure 2: Receiver operating characteristics curve for fasting plasma glucose concentrations to identify persistent postnatal glucose abnormalities



a: FPG as a predictor of postnatal IGT

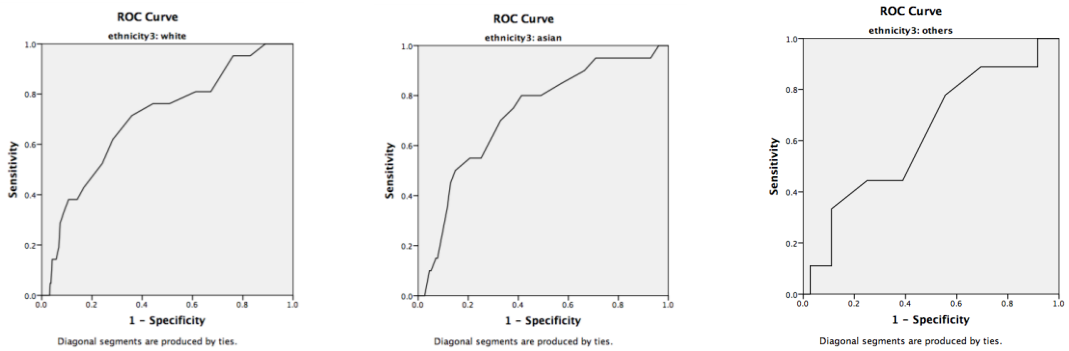
AUC=0.697, CI: 0.620-0.773, p<0.0001



b: FPG as a predictor of postnatal diabetes

AUC=0.983, CI: 0.966-1.00, p<0.00

Figure 3: ROC curves for FPG to detect IGT by ethnicity

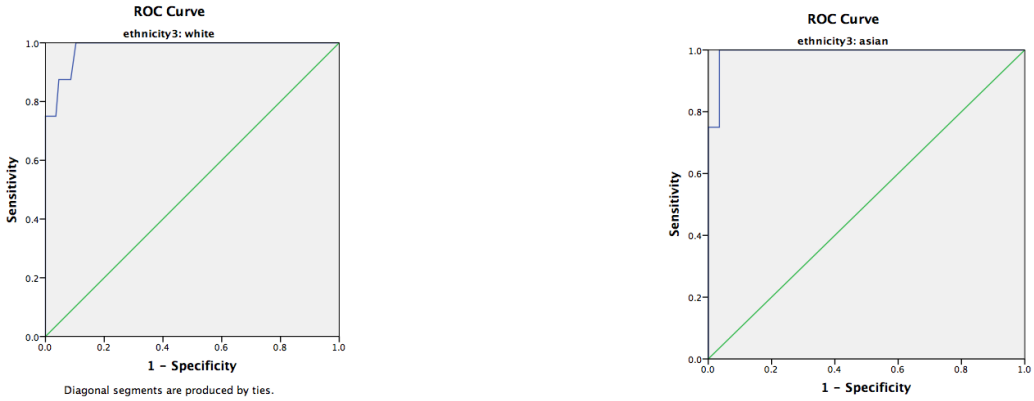


a) WC: AUC: 0.705 (0.591, 0.819) p=0.002

b) SA: AUC: 0.726 (0.610, 0.841) p=0.001

c) Others: AUC: 0.623 (0.418, 0.829) p=0.256

Figure 4: ROC curves for FPG to detect diabetes by ethnicity



a) WC: AUC 0.983 (0.960, 1.000) p<0.0001

b) SA: AUC 0.991 (0.974, 1.000) p=0.001

3.6 Discussion

3.6.1 Uptake rates with HbA1c and OGTT

Our study involving a large multi-ethnic population of high-risk women with GDM showed that the uptake for OGTT was just under 50%, in agreement with world-wide reported rates [107, 123]. Although reasons for the poor attendance for OGTT have not been not widely studied, time constraints of the OGTT were the most commonly cited reasons in surveys of GDM women [124]. It is possible that simplifying the postnatal test to a one-step FPG or HbA1c might improve uptake although direct evidence for this is lacking. The first 12-month data from one of our centres showed an encouraging increase in uptake with HbA1c. To our knowledge, we are the first to show that uptake rates for postnatal HbA1c were higher than that for OGTT. Women who failed to attend postnatal OGTT also had features of higher metabolic risk such as smoking, macrosomia and multiparity, despite a younger age. It was reassuring to note that unlike with postnatal OGTT, women who failed to attend HbA1c testing had similar metabolic risk characteristics to those who attended. SA were more likely to attend postnatal HbA1c than WC. This is in agreement with previously summarised studies that non-WC ethnicity, especially SA were more likely to attend postnatal screening than WC.

3.6.2 FPG as a postnatal test to detect diabetes

Our study showed that FPG at the ADA definition of IFG of FPG≥5.6mmol/l had 100% sensitivity to detect postnatal diabetes even in populations with SA and other ethnic minority groups. However, the NICE and WHO recommended definition of IFG at FPG ≥6.1mmol/l would miss 16.7% of diabetes. Whilst our data is in agreement with some studies reporting a high sensitivity of FPG at 5.6 mmol/l of

98-100% to detect diabetes [122, 125, 126], other studies have reported a lower performance of FPG [105, 127]. This variation in sensitivity is probably due to the variation in the screening and diagnostic criteria used to diagnose GDM, and hence in the population characteristics of the women screened. In our study about 4% of women with FPG between 5.6 and 7.0 mmol/l had 2hPG values diagnostic of diabetes (≥11.1mmol/l). Both the ADA and the WHO recommend that the diagnosis of diabetes in asymptomatic, high-risk population should not be based on a single test alone [128, 129]. Therefore we recommend that women with postnatal FPG ≥ 5.6mmol/l, should have a confirmatory OGTT for diagnosis of diabetes. This will substantially reduce the need for OGTT by 87% (Table 7).

3.6.3 FPG as a postnatal test to detect IGT

On the other hand, despite the high sensitivity to detect diabetes, our study showed that FPG threshold of ≥5.6mmol/l and ≥6.1mmol/l would miss 64% and 88% of IGT, respectively. Such low sensitivity of FPG to detect IGT has been confirmed in other studies [107, 130]. In addition, our data also showed that iIGT was seen in a higher proportion of ethnic minority groups who were therefore more likely to be missed if FPG alone used for postnatal testing (Table 6). Older studies in non-postnatal adults using a FPG cut off of ≥7.1mmol/l showed that 2hPG abnormalities were more common in SA than WC thereby limiting the utility of using FPG to detect concomitant IGT in SA at these thresholds [121]. Although SA had a greater proportion of 2hPG abnormalities this did not reach statistical significance probably owing to the small numbers in the groups.

Do we really need to worry about missing isolated postnatal IGT following GDM? It has been argued that the real purpose of postnatal screening is to detect women with overt diabetes and not those “at risk of diabetes” such as IGT [115, 125]. The

diagnosis of GDM already places these women in the category of those "at risk" of future diabetes thereby making postnatal detection of IGT redundant practice.

On the contrary, it could be argued that a further risk stratification of GDM women in the postnatal period might help to identify a sub-group of women who may benefit more from lifestyle intervention and/or metformin. The post-hoc analysis of the DPP showed that both intensive lifestyle intervention and metformin therapy significantly reduced the risk of future diabetes risk in GDM women with postnatal IGT [131] compared to those without GDM. However this conclusion warrants closer inspection. Women in the DPP were at an average of 12 years post partum and did not have *isolated IGT*. Their mean FPG and HbA1c were 5.9 (SD: 0.5) mmol/l and 5.87 (SD: 0.5) % (29.7-51.6mmol/l), respectively, implying a significant overlap between IGT, IFG and pre-diabetes by HbA1c criteria. Therefore, this evidence cannot be extrapolated to assume direct benefit for isolated IGT in the immediate postpartum period. Additionally, such risk stratification can also be also performed using FPG or HbA1c. Thus both the ADA and the NICE guidance for the prevention of diabetes post GDM recommend metformin [132] and intensive lifestyle intervention [132, 133] for women with IFG (FPG 5.6-6.9mmol/l) or pre-diabetes using HbA1c (5.7-6.4%) (39 - 47 mmol/mol).

Furthermore, the argument for detection of postnatal IGT for the purpose of intervention should be taken in context with the poor uptake of OGTT across the world. Not having any postnatal test would mean that a large number of women would miss the opportunity for postnatal risk stratification and hence engagement in any form of preventive intervention that has proven benefit. Therefore, we believe that persevering with a complex test with poor uptake rates for the sole purpose of detecting postnatal IGT is not justified.

The key strength of the study is the large sample size, and real-world evidence in a mixed ethnic population. Our study has important limitations. The newly proposed IADPSG or NICE criteria were not used for diagnosing GDM. Data from our study therefore cannot be extrapolated to populations where these criteria are already adopted for diagnosis GDM. However, several centres in Europe and most of the UK still follow the old WHO criteria for diagnosis [134] where our results will be relevant. Our study is observational and retrospective and therefore prone to missing data. Prospective randomised trials are needed to assess the real effect of FPG or HbA1c on uptake of postnatal screening and to inform the ideal timing of postnatal HbA1c in the context of antepartum treatment for GDM, postpartum anaemia and volume shifts during pregnancy.

In conclusion, we welcome the new NICE recommendation to replace postnatal OGTT by FPG or HbA1c. Simplifying postnatal testing may improve uptake, enable further risk stratification and hence better engagement in further preventive strategies. However, the FPG threshold of ≥6.1mmol/l recommended by NICE should be lowered to the ADA defined threshold of ≥5.6mmol/l to improve detection of postnatal diabetes. FPG at this lowered cut off is a highly sensitive test to detect diabetes even in a population with SA.

3.7 Future directions

- Prospective randomised studies comparing the uptake rates of HbA1c and FPG with OGTT in a mixed ethnic population
- Cost effectiveness analysis for long term follow up of women with GDM using HbA1C

- Exploring effectiveness of other interventions to improve uptake of postnatal testing – currently in the process of conducting a systematic review to apply for funding for Health-Technology-Appraisal (HTA) project.

4 Effect of maternal diabetes on offspring birth weight (BW) in SA and WC

4.1 Abstract

Background: Both gestational and pre-gestational diabetes are associated with adverse offspring outcomes particularly macrosomia, with a continuous relationship between glucose and birth weight (BW). This evidence from studies in White Caucasians (WC) is extrapolated to other ethnic groups, under the assumption that the relationship between maternal glycaemia and BW is uniform across ethnic groups. We compared the impact of maternal diabetes on BW between South Asians (SA) and WC.

Methods: A Retrospective analysis was conducted for all SA and WC singleton, live births across Leicester from 1994-2002 (n=53,128). Ethnic specific BW z-scores and centiles were derived from this dataset. The increase in BW and the odds-ratio of large-for-gestational-age with maternal diabetes was compared between SA and WC using regression analyses with interaction terms.

Results: SA had a higher prevalence of both GDM (SA vs WC: 2.9% vs 0.8%, $p<0.0001$) and pre-gestational diabetes (SA vs WC: 0.6% vs 0.4%, $p<0.0001$) than WC. Both gestational and pre-gestational diabetes were significant predictors of offspring BW and LGA after adjustment for confounding maternal characteristics. The increase in offspring BW with pre-gestational diabetes was 139.24g lower in SA compared to WC ($p=0.034$ for interaction). Similar results persisted with ethnic specific BW z-scores. ($p=0.013$ for interaction). The effect of maternal GDM on BW and LGA was similar across both ethnic groups.

Conclusion: The relationship between maternal diabetes and BW differs with ethnicity. The increase in BW with pre-gestational diabetes was significantly lower in SA. Our results emphasize the need for ethnic specific glycaemic targets in pregnancy.

4.2 Introduction

The prevalence of diabetes among women of childbearing age and the incidence of gestational diabetes mellitus is increasing alongside the epidemic of type 2 diabetes (T2D) and obesity[5] [135] [12] [13]. This is especially higher in certain ethnic minority populations[136]. South Asians (SA) have a 2-4 fold higher risk of T2D and GDM compared to White Caucasians (WC) [12] [137] [138] [139]. While the prevalence of T2D doubled in the USA over 40 years[140], this has tripled in Indian populations in just 14 years[141]. Studies in the UK also show an alarming increase in the incidence of T2D in young people with a three-fold higher prevalence in SA compared to WC [38].

Poorly controlled maternal diabetes and GDM are known to be associated with a number of perinatal complications such as macrosomia, congenital malformations and increased perinatal mortality[9, 142] with evidence of a continuous relationship between maternal glucose and adverse offspring outcome[9, 143]. Most evidence for this risk comes from studies in WC[9, 142, 143]. In fact, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) based the definition of GDM on glycaemic thresholds of increased risk of large for gestation age (LGA), increased cord C-Peptide and adiposity observed in the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study[11]. Despite their significantly higher metabolic risk, SA were poorly represented in the HAPO group, except for a cohort of South East Asian women from Singapore[9]. The IADPSG criteria to define GDM were therefore based on the questionable assumption that the effect of glucose on birth weight is uniform across different ethnic groups. Furthermore, similar glycaemic targets are set in pregnancy (both GDM and pre-gestational diabetes) for all ethnic groups under the same premise.

However, there is emerging evidence that the magnitude of the impact of maternal glycaemia on offspring size may differ with ethnicity. Maternal diabetes results in a larger increase in birth weight (BW) and increased odds of LGA in Blacks despite their overall lower mean BW[18, 81, 144, 145] when compared to non-Hispanic Whites. While the odds of macrosomia in WC was 2.5 fold higher in mothers with diabetes compared to those without, the corresponding odds were 6 fold higher in Blacks[144], suggesting that ethnicity significantly modified the relationship between maternal glycaemia and BW.

Conversely, despite higher levels of maternal glycaemia [146-148], SA offspring are amongst the smallest babies in the world [13, 149-151]. A few studies have compared the impact of GDM on BW between SA and WC with conflicting results. Table 1 shows a summary of all the relevant studies comparing the impact of maternal diabetes on BW across ethnic groups. These studies lacked appropriate adjustment for maternal characteristics [148, 152], failed to use ethnic specific BW centiles [137, 145, 149] or included a heterogeneous 'Asian' population that included both SA and South East Asians [14, 15, 137]. Only one study examined the effects of pre-gestational diabetes on offspring BW in SA and WC, but used the same population centiles for both ethnic groups and included a heterogeneous Asian population[149].

Our aim was to compare the impact of both pre-gestational diabetes and GDM on offspring BW between SA and WC in a large population based, bi-ethnic pregnancy cohort from Leicestershire, UK

Table 1: Summary of literature comparing the impact of maternal diabetes on offspring birth weight

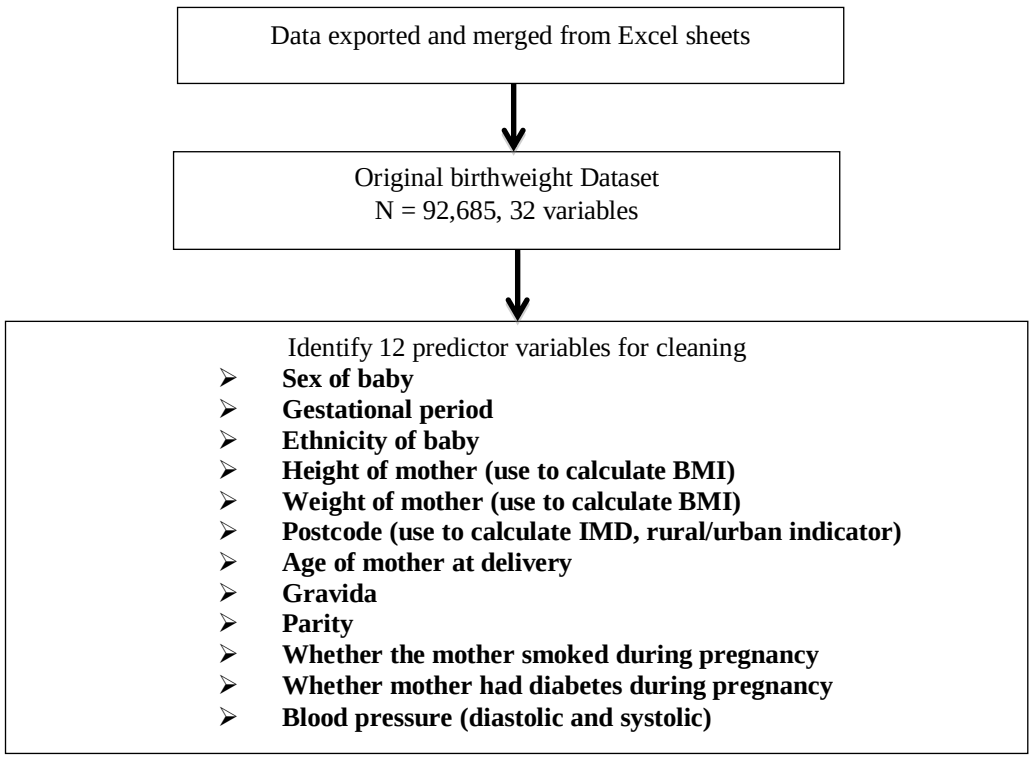
	Subjects	Maternal diabetes type	Key results	Confounding variables adjusted for	SA included	Ethnic centiles	Controls
Xiang [153]	29,544 women (9 ethnic groups)	GDM	LGA in Asians*: RR: 1.24 (0.98, 1.58), p =0.07 (WC reference) *Heterogeneous group including SA	Maternal age, education, insurance type, presence of comorbidity, pre-eclampsia/eclampsia, anti-hyperglycemic drug use, smoking, BMI, gestational weight gain.	Yes*	Yes	No
Hedderson[14]	n =7648 (SA & WC)	GDM	Odds of LGA in Asian* 1.19 (0.98, 1.46) (Ref WC) *Heterogeneous group including SA	Parity, age, gestational age, education, BMI, sex, medication	Yes*	Yes	No
Mocarski[145]	536,084, 4 Ethnic groups	GDM	Adjusted OR of macrosomia SA: 1.1 (0.9, 1.2), WC: 1.1 (1.0, 1.2).	Maternal age, being foreign born, insurance, education, parity, tobacco use during pregnancy, and pre-pregnancy weight.	Yes	No	Yes
Makgoba [154]	130 549 (SA, Blacks WC)	GDM	Prevalence of LGA in SA vs WC 9.2% vs 18.8% (p<0.001)	None. (Adjustment made only for analysis of effects of BMI)	Yes	No	Yes
Rosenberg[149]	329 988 (4 ethnic groups)	GDM and pre-gestational Diabetes	Adjusted OR for low birth weight (LBW) in Asian* vs WC: Pregestational 2.28 (1.42, 3.68) vs 1.59 (1.01, 2.05) GDM: 1.17 (0.99, 1.39) vs 1.06 (0.87, 1.28). Only LBW was reported. *Heterogeneous group including SA	Maternal age, marital status, mothers education, birth place, prenatal care, parity, social risk, pre-pregnancy weight, pregnancy weight gain, hypertension and pre-eclampsia	Yes	No	Yes
Dunne[152]	312 WC and 128 Indo-Asian	GDM, T2DM, T1DM	Prevalence of 25% LGA in SA vs 37% in WC. No OR/RR reported.	None	Yes	No	No
Nguyen [137]	32,193 – North California	GDM	OR for LGA in Asian* compared to WC 0.40 (0.33, 0.48). *Heterogeneous group including SA	Maternal age, obesity, education, prenatal care, nulliparity and hypertension.	Yes	No	No
Wong[148]	5 Ethnic groups (n=869)	GDM	Prevalence of LGA: SA vs WC- 11.0 vs 13.9 (p: non significant)	None	Yes	Yes	No
Dalfra[155]	Native Italian and immigrant	GDM	Adjusted OR for LGA. Immigrants vs Native Italian 1.63 (0.97, 2.74), p=0.06	Age, pre-pregnancy BMI, Insulin therapy, Weight gain in pregnancy,	No	Yes	No
Homko [156]	103 African and 36 Latino	GDM	Higher risk of macrosomia in Latino compared to African (RR = 2.68)	BMI, weight gain, Glycaemic control, therapy.	No	No	No
Kieffer[144]	Blacks and Whites: 111,044 infants of diabetic mothers and 5,008,970 infants of non-diabetic mothers	GDM and T2D and T1D	Offspring weight gain with maternal diabetes: Black vs Whites: 220g vs 96g, adjusted OR of macrosomia: Blacks: 3.24 (95% CI 3.09–3.38), WC: 1.66 (95% CI 1.63–1.68).	Gestational age, maternal hypertension status, prenatal care use, and maternal socio-demographic characteristics. No BMI adjustment.	No	No	Yes
Hunt [18]	92,233 NHB and 151,957 NHW births	GDM and pre-gestational Diabetes	Adjusted RR for LGA: GDM: NHW vs NHB: 1.21 (1.15, 1.29) vs 1.94 (1.77, 2.13). Pre-gestational diabetes: NHW vs NHB: 1.61 (1.48, 1.76) vs 2.22 (1.98, 2.49)	Maternal age, offspring sex, BMI, maternal tobacco use, hypertension status, education and prenatal care	No	No	Yes
Sandana[81]	1190 WC and 865 Blacks	GDM and IGT	Adjusted OR for LGA: WC vs Blacks: 1.4 (0.7–2.7) vs 1.6 (0.6–4.2)	Pre-pregnancy BMI, age and height	No	Yes	Yes
D Simmons[157]	529 European, 540 Maori, 916 Pacific Islanders	Normal Glucose tolerance	Population attributable fraction (PAF) for relationship of macrosomia with maternal glycaemia: Europeans vs Maori vs Pacific: 16.7 vs 17.0 vs 18.7%, p = 0.567.	Gestational age, smoking and maternal weight tertiles	No	No	NA
Scholl et al[158] et al	African-American (n = 1,040), Hispanic (n = 750), White (n = 282)	Normal Glucose tolerance	Adjusted β for relationship between BW & Maternal glucose level (mg/dl) African American: 1.56 (0.63 0.34), Hispanic: 2.19 (0.66 0.89), White: 1.98 (0.95 0.11).	Age, parity, smoking (cigarettes/day), pregravid BMI, gestational weight gain, clinic payment status, a prior low birth weight infant, and duration of gestation	No	No	NA
Farrar et al [82]	9509 women (4821 SA and 3888 WC)	Normal Glucose tolerance	Relationship between BW LGA and glycaemia not significantly different between SA and WC (interaction term 0.90)	Gestational age at oral glucose tolerance test, family history of diabetes, family history of hypertension, previous GDM, previous macrosomia, smoking status, alcohol consumption during pregnancy, maternal age and BMI, maternal education, baby sex, and parity.	Yes	No	NA

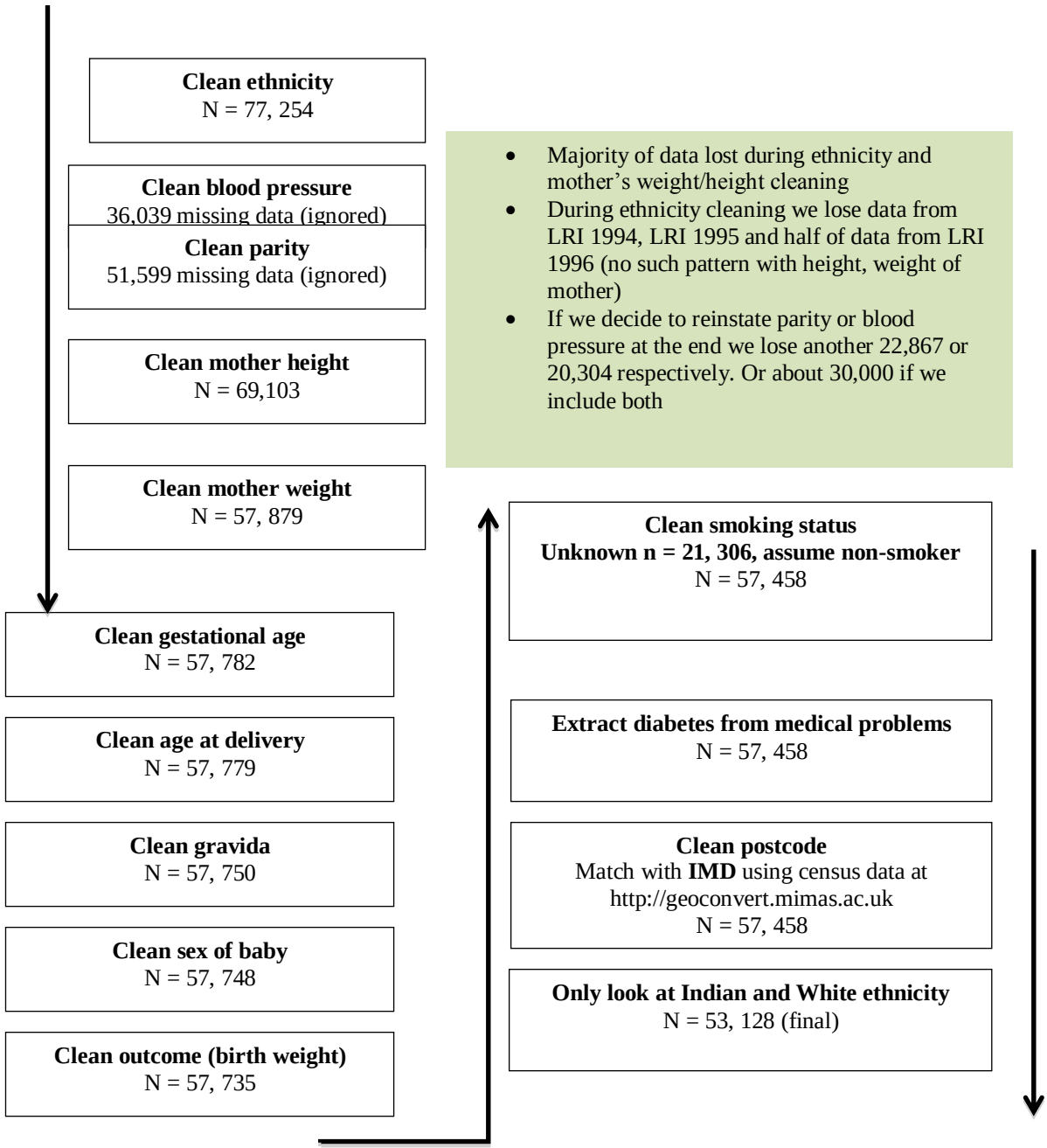
4.3 Methods

A retrospective analysis of all deliveries between 1994-2006 was conducted across Leicester Royal Infirmary (1994-2002) and University Hospitals of Leicester (2002-2006). Detailed maternal demographic and offspring data was extracted from routinely collected electronic booking and delivery records respectively. Only term, singleton, live births from WC and SA pregnancy were included for this analysis. Pre-term deliveries (less than 37 weeks of gestation) were excluded because of the possibility of alternate underlying pathology. Pregnancies coded as "diabetes developed in pregnancy" were considered as GDM and "diabetes before pregnancy" as pre-gestational diabetes.

4.3.1 Data Cleaning

The original data set comprising of 92, 685 deliveries from the original excel file containing 32 variables was cleaned as explained in the flow chart below.




Clean ethnicity
N = 77, 254
Clean blood pressure
36,039 missing data (ignored)
Clean parity
51,599 missing data (ignored)
Clean mother height
N = 69,103
Clean mother weight
N = 57, 879
Clean gestational age
N = 57, 782
Clean age at delivery
N = 57, 779
Clean gravida
N = 57, 750
Clean sex of baby
N = 57, 748
Clean outcome (birth weight)
N = 57, 735
Clean smoking status
Unknown n = 21, 306, assume non-smoker
N = 57, 458
Extract diabetes from medical problems
N = 57, 458
Clean postcode
Match with IMD using census data at
http://geoconvert.mimas.ac.uk
N = 57, 458
Only look at Indian and White ethnicity
N = 53, 128 (final)
Majority of data lost during ethnicity and mother's weight/height cleaning
During ethnicity cleaning we lose data from LRI 1994, LRI 1995 and half of data from LRI 1996 (no such pattern with height, weight of mother)
If we decide to reinstate parity or blood pressure at the end we lose another 22,867 or 20,304 respectively. Or about 30,000 if we include both

*4.3.2 **Definitions***

SA were defined as women of Indian, Pakistani, Sri Lankan, Nepali and Bangladeshi origin. LGA and SGA were defined as birth weight ≥90th centile and ≤10th centile respectively using gestational age, ethnicity and sex standardised z scores. L, M and S values derived from this dataset were used to calculate ethnicity specific BW z scores as described previously[159]. In brief the L (Box-Cox power), M (median) and S parameters were estimated using the GAMLSS package in R version 2.9 as recommended by the World Health Organization (WHO). This method uses smoothed values of L, M and S to transform the observed distribution of birth weights to a standard normal distribution [160].

Multiparity was defined as parity more than two, i.e. two or more live births beyond 24 weeks gestation. Data on parity was incomplete hence gravidity was used to calculate parity, when parity was not available, under the assumption that previous pregnancies were singleton and progressed beyond 24 weeks. Obesity was defined was BMI ≥30kg/cm^2. Ethnicity specific obesity definitions were also used where appropriate[161], depicted by obesity^e. Obesity^e was defined as BMI ≥27.5kg/cm^2 for SA and BMI ≥30kg/cm^2 for WC.

Relative levels of deprivation scores were calculated using the The English Indices of Deprivation 2004 from the Department for Communities and Local Government[162] . IMD ranks were split into standard quintiles for the purpose of all analysis according to national standards with quintile 5 being the most deprived and 1 being the least deprived.

*4.3.3 **Screening and definition of GDM***

During this period risk factor based selective screening was performed using any one of the following criteria: Previous macrosomia (offspring BW ≥4.5kg), booking

weight ≥85kg, family history of diabetes, previous GDM or a diagnosis of polycystic ovarian syndrome. The diagnostic criteria varied during the study period: 1994-1999: FPG ≥5.8mmol/l or 2hPG ≥8.5mmol/l, 2000 - 2006: FPG ≥5.5mmol/l or 2hPG ≥ 8.0mmol/l.

4.3.4 Statistical analysis

Student-t test and Chi^2 test were used to compare continuous variables and proportions between ethnic groups. Multivariable linear and logistic regression models were used with birth weight and LGA as outcome variables to study the predictors of birth weight. Interaction terms between ethnicity and diabetes were calculated to assess the differential role of diabetes on birth weight in the two ethnic groups. Statistical significance was set at the 5% level. SPSS version 22.0 was used for analysis.

4.4 Results

A total of 88,606 deliveries were recorded during this time period. We limited our analysis to the two main ethnic groups i.e SA and WC, because of small numbers in other ethnic groups. A complete dataset on all variables of interest was available for a total of 53,128 singleton, term and live births of WC or SA origin over a period of 13 years. SA and WC comprised of 15.9% and 84.1% of the whole population.

There were significant differences between SA and WC with respect to most maternal and offspring characteristics with the exception of offspring sex and multiparity (Table 2). Overall SA were younger, shorter, had lower BMI, less likely to be smokers, had a higher prevalence of maternal diabetes (GDM and pre-gestational diabetes), had lower offspring BW with lower proportion of macrosomia

and higher proportion of LBW. SA were more likely to live in deprived neighbourhoods compared to WC.

The baseline characteristics of women with GDM and pre-gestational diabetes split by ethnicity are shown in Table 3. The proportion of LGA and SGA infants did not differ between the SA and WC in either in GDM or pre-gestational diabetes. Ethnic differences in height, BMI, smoking, obesity and deprivation seen in the general pregnant population persisted even among women with diabetes.

Table 4 depicts the predictors of BW, LGA and SGA in both ethnic groups using simple linear and logistic regression analyses. After adjustment for age, height, BMI, year of birth, multiparity, smoking and deprivation maternal diabetes was an independent predictor of BW, associated with a significant increase in BW and the odds of LGA in both SA and WC. However, the increase in BW and odds of LGA with maternal diabetes differed in the two ethnic groups. To investigate this further, we subdivided maternal diabetes into GDM and pre-gestational diabetes, and studied their interactions with ethnicity (Table 5).

Table 5 shows the increase in BW and odds of LGA with both GDM and pre-gestational diabetes in both ethnic groups. Both GDM and pre-gestational diabetes was associated with an increase in offspring weight in both ethnic groups. The effect of pre-gestational diabetes on offspring BW was more marked than that of GDM.

4.4.1 Interactions of maternal diabetes with ethnicity

Compared to pregnancies without diabetes, the BW increase seen with pre-gestational diabetes was 139.24g lower in SA compared to WC, after adjusting for year of birth, maternal age, BMI, height, IMD rank, multiparity, smoking status and offspring gestational age and sex (Table 5, p=0.034 for interaction effect). A similar

difference was seen in the effect of pre-gestational diabetes on BW z scores between the two ethnic groups (0.378 points lower in SA; p=0.013 for interaction effect; Table 5). The OR for LGA in SA with pre-gestational diabetes was 1.7 fold lower than the corresponding OR in WC (3.69 vs 5.49, Table 5). However, this interaction term did not reach statistical significance (p=0.103 for interaction effect).

The analysis of BW in the GDM group showed that the BW gain was 31.9g lower in SA after adjustment as above. Similar, trends were seen with BW z scores and LGA rates in SA with GDM, but the interaction did not reach statistical significance in any of these analyses (Table 5).

Table 2: Baseline maternal and offspring characteristics by ethnicity

		SA (n = 8471)	WC (n = 44657)	P
Age		28.1 (5.08)	28.6 (5.806)	<0.0001
Height		158.2(6.38)	164.3(6.78)	<0.0001
BMI		23.5(4.64)	24.9(5.3)	<0.0001
Obesity		9.1 (775)	14.1 (6291)	<0.0001
Obesity[e]		17.2 (1457)	14.1 (6291)	<0.0001
IMD	1 (least deprived)	11.4 (964)	29.8 (13299)	
	2	10.3 (875)	20.9 (9327)	
	3	13.3 (1129)	15.0 (6687)	
	4	32.5 (2755)	14.8 (6611)	
	5 (most deprived)	32.4 (2748)	19.6(8733)	
Smoking		1.6 (139)	19.2 (8580)	<0.0001
Gestational Diabetes		2.9 (247)	0.8 (375)	<0.0001
Pre-existing diabetes		0.6 (55)	0.4 (160)	<0.0001
Multiparity		34.7 (5528)	35.4 (28866)	0.275
Birth weight		3102.4 (453.36)	3444.6 (487.66)	<0.0001
Gestational age		277.2 (8.1)	279.6 (8.28)	<0.0001
Sex of baby m:f %		50.6: 49.4	51.6: 48.4	0.700
Macrosomia (>4000g)		2.9 (249)	12.6 (5639)	<0.0001
Low birth weight (LBW) (<2500g)		8.5 (722)	2.6 (1154)	<0.0001

Data are presented as mean (SD) or % (n) for continuous and categorical variables respectively. Comparisons between ethnicities are made using the Student t-test for continuous variables and the Chi-Squared test for categorical variable

Table 3: Baseline maternal and offspring characteristics by type of maternal diabetes in SA and WC

		SA (247)	**WC (375)**	**p**	**SA (55)**	**WC (160)**	**p**
GDM mean (SD) or %					**Pre-gestational diabetes**		
Age		31.7 (4.9)	31.3 (5.8)	0.391	30.58 (5.51)	29.77(5.27)	0.342
Height		156.9 (5.94)	163.4(6.80)	<0.0001	157.63 (5.92)	164.13 (6.59)	<0.0001
BMI		27.8 (5.9)	30.3 (7.3)	<0.0001	27.27 (5.89)	28.43 (6.22)	0.219
Smoking		1.6	10.9	<0.0001	1.8	16.9	0.004
Multiparity		46.6	43.2	0.410	40	40	1.000
Obesity		34.0	47.2	0.001	27.3	31.3	0.580
Obesity[e]		47.8	47.2	0.889	45.5	31.3	0.057
BW		3309.6 (540.4)	3634.8 (591.5)	<0.0001	3270.9(599.5)	3671.1(577.1)	<0.0001
Gestational age		274.2 (6.9)	273.2 (7.5)	0.090	270.9 (5.7)	269.4 (6.7)	0.103
LGA		26.3	31.5	0.168	27.3	40.6	0.077
SGA		3.2	5.9	0.134	3.6	3.8	0.969
IMD	**1**	10.5	30.7	<0.0001	7.3	23.1	0.016
	2	8.5	21.6		14.5	21.9	
	3	12.1	12.0		14.5	13.8	
	4	32.0	16.0		29.1	14.4	
	5	36.8	19.7		34.5	26.9	

Data are presented as mean (SD) or % (n) for continuous and categorical variables respectively. Comparisons between ethnicities are made using the Student t-test for continuous variables and the Chi-Squared test for categorical variable

Table 4: Predictors of birth weight by ethnicity

	Birth weight Z scores				LGA				SGA			
	SA		WC		SA		WC		SA		WC	
	β coeff (95% CI)	p	β coeff (95% CI)	p	OR	p	OR	p	OR	p	OR	p
Year	-0.006 (-0.12, 0.0)	0.06	0.001 (-0.001,0.003)	0.454	0.992 (0.970,1.14)	0.456	0.997 (0.989, 1.006)	0.513	1.011 (0.989, 1.033)	0.340	0.995 (0.987, 1.004)	0.279
Age	0.004 (0.0-0.08)	0.109	0.004 (0.003, 0.006)	<0.0001	1.024 (1.007, 1.041)	0.005	1.019 (1.012, 1.025)	<0.0001	1.007 (0.990,1.024)	0.400	1.005 (0.999, 1.11)	0.110
Height	0.032 (0.28, 0.35)	<0.0001	0.031 (0.029, 0.032)	<0.0001	1.071 (1.058, 1.084)	<0.0001	1.066 (1.061, 1.071)	<0.0001	0.946 (0.934, 0.957)	<0.0001	0.945 (0.940, 0.949)	<0.0001
BMI	0.042 (0.37, 0.46)	<0.0001	0.036 (0.035, 0.038)	<0.0001	1.095 (1.079, 1.111)	<0.0001	1.080 (1.074,1.086)	<0.0001	0.916 (0.897, 0.935)	<0.0001	0.943 (0.936, 0.950)	<0.0001
Multiparity	0.198 (0.245, 0.151)	<0.0001	0.160 (0.180, 0.140)	<0.0001	1.423 (1.206, 1.679)	<0.0001	1.385 (1.293, 1.483)	<0.0001	0.701 (0.584, 0.842)	<0.0001	0.780 (0.725, 0.840)	<0.0001
Diabetes in pregnancy	0.486 (0.372, 0.601)	<0.0001	0.661 (0.578, 0.774)	<0.0001	2.660 (1.988,3.559)	<0.0001	3.413 (2.812, 4.141)	<0.0001	0.496 (0.268, 0.918)	0.026	0.671 (0.456, 0.989)	0.044
Smoking	-0.267 (-0.429,-0.106)	0.001	-0.323 (-0.347,0.299)	<0.0001	0.682 (0.357, 1.30)	0.245	0.571 (0.516, 0.632)	<0.0001	1.451 (0.849, 2.482)	0.174	2.131 (1.975, 2.300)	<0.0001
IMD	-0.017 (-0.001, 0.033)	0.035	-0.044 (-0.037, -0.05)	<0.0001	1.0 (0.768, 1.303)	0.999	0.701 (1.284, 1.585)	<0.0001	1.394 (1.053, 1.845)	0.020	1.59 (1.43, 1.75)	<0.0001

Table shows results of multivariable linear (BW z scores) and logistic (LGA, SGA) regression for studying the predictors of BW, LGA and SGA. Age, height and BMI were treated as continuous variables. Diabetes is any diabetes in pregnancy including GDM and pre-gestational. IMD was divided into quintiles as described in methods section. In linear regression: β coefficient represents change in BW per quintile change in IMD. In logistic regression IMD quintile 1 (least deprived quintile) was used as reference category. OR represents the Odds of LGA and SGA in IMD quintile 5 (most deprived) compared to IMD quintile 1 (least deprived).

Table 5: Effect of GDM and pre-existing diabetes on BW and LGA in SA and WC

	Controls (mothers without diabetes)	Pre-existing diabetes		Gestational diabetes	
		SA	WC	SA	WC
BW*: β (95% CI)	0.00 (ref)	263.436 (153.665, 373.208)	402.677 (338.245, 467.109)	212.232 (159.582, 264.883)	240.133 (197.672, 282.594)
BW z score: β (95% CI)	0.00 (ref)	0.610 (0.354, 0.866)	0.987 (0.837, 1.137)	0.466 (0.343, 0.589)	0.571 (0.472, 0.669)
LGA: OR (95% CI)	1.00 (ref)	3.69 (1.660-5.672)	5.488 (3.926, 7.670)	2.697 (1.984, 3.667)	2.825 (2.231, 3.577)

Table shows results of multivariable linear (BW and BW z scores) and logistic regression (LGA) using GDM and pre-gestational diabetes as covariates in separate models.
β and OR represent adjusted β coefficients and Odds Ratios of outcome in women with diabetes compared to controls as reference. All models included adjustment for year of birth, maternal age, height, BMI, IMD rank, multiparity and smoking status. LGA are >90th centile for gestational age, sex and ethnicity.* Adjusted for above and gestational age and sex of baby. All values are significant with p <0.0001.

4.5 Conclusion

Our study depicts important differences in characteristics of SA and WC pregnant women. Overall SA were younger, shorter, had lower BMI, less likely to be smokers, had a higher prevalence of maternal diabetes (GDM and pre-gestational diabetes), had lower offspring BW with lower macrosomia rates and higher rates of LBW. In line with literature especially in the UK and the midlands[163], it was noted that SA with and without diabetes tended to live in more deprived neighbourhoods than WC. More than a third of SA lived in the most deprived neighbourhoods compared to just under a fifth of WC.

4.5.1 Pre-gestational diabetes

Pre-gestational diabetes was associated an increase in BW and LGA risk in both ethnic groups. Our study shows that ethnicity significantly modulates the impact of maternal diabetes on offspring BW. The increase in BW attributable to pre-gestational diabetes was lower in SA compared to their WC counterparts after adjustment for likely confounding maternal variables including deprivation (Table 5). This corresponds to 11.7% and 8.5% of the mean BW in WC and SA, respectively. This result was also confirmed using ethnic specific BW z scores. The odds of LGA with pre-gestational diabetes also tended to be lower in SA compared to WC, however this difference did not reach statistical significance (Table 5). We believe that our results from the linear analysis of BW and BW z scores reflect a true difference between ethnic groups and the lack of differential effect on LGA could simply be the result of thresholds chosen to define LGA.

Only two other studies have compared the effects of pre-gestational diabetes on offspring BW, with only one including SA. Both were conducted in American population. Hunt et al showed a higher risk of LGA with pre-gestational diabetes in Blacks compared to WC, (RR of LGA compared to controls: WC vs Blacks: 1.61 vs 2.22)[18]. Rosenberg et al reported higher odds of LBW in a heterogeneous population of 'Asian' women compared to WC[149]. Both these studies used crude BW and did not use ethnic specific centiles (Table 1).

To the best of our knowledge our study was the first to systematically compare between SA and WC, the impact of pre-gestational diabetes on birth weight and LGA risk, using ethnic specific centiles. Contrary to what was seen in Blacks in the literature presented above[18], our study showed that pre-gestational diabetes had a lower effect on BW in SA after adjusting for important maternal variables including deprivation. The reasons for this differential effect of diabetes on BW in different ethnic groups are unclear. We believe that these are independent effects of maternal ethnicity on BW, not mediated by smaller maternal size or deprivation. Other less studied factors such as inflammation or vascular dysfunction in diabetes that occur more commonly in SA [164] could in addition play a role in determining the effect of diabetes on BW.

4.5.2 GDM

The effect of GDM on BW or LGA did not differ between SA and WC. The overall BW increase and odds of LGA in offspring of GDM mothers was lower than that seen with pre-gestational diabetes in both ethnic groups.

Our results are in agreement with three of four studies that reported that the odds of LGA in SA [14, 145, 153] was similar to WC. Nguyen [137] et al reported lower

odds of LGA but in a heterogeneous 'Asian' population compared to WC, using unadjusted BW centiles.

We present a summary of the literature examining the effects of GDM, pre-gestational diabetes on BW across ethnic groups and the associated mechanistic flaws associated with them, to summarise why our study provides unique complementary data to support previous observations (Table 1). Firstly, they failed to adjust for maternal size [148, 152], which is a well-recognised predictor of offspring BW. Secondly, several studies used crude BW and did not use ethnic specific centiles[137, 145, 149]. Thus they failed to account for the constitutional "smallness" or "largeness" of certain races, wrongly attributing this to pathological influences such as diabetes. Finally, many lacked a control population without diabetes making it was difficult to compare the BW increase attributable to diabetes across ethnic groups[14, 137]. The two studies that adjusted for maternal characteristics and used ethnic specific centiles included a heterogeneous "Asian" group without clear separation of South East Asians from South Asians [14, 153]. Of the three studies [158, 165] that examined the effect of maternal glycaemia on BW in women with normal glucose tolerance between ethnicities, only one recent cohort included women of SA ethnicity [82]. There was no significant interaction between ethnicity and maternal glycaemia with respect to BW as an outcome. It is likely this difference was not apparent in GDM because of the milder degrees of glucose intolerance and a shorter duration of hyperglycaemia compared to diabetes.

We believe that ours is the first study to examine the effects of both GDM and pre-gestational diabetes separately in SA and WC, accounting for all above-mentioned mechanistic issues.

The strengths of our study are its large size and long period of study spanning over 12 years, in a region of the UK with a large SA population. Limitations of our study include its retrospective nature, hence its liability to coding errors and incomplete data. There was no data on type of pre-gestational diabetes (type 1 or type 2), glycaemic control, details of treatment or maternal weight gain in pregnancy. Data on BP was incomplete and hence was omitted form analysis. In addition, the country of birth of the SA women was not available, which would have helped understand the effect of migration on adverse metabolic risk better.

Implications of these findings are several. The results of our study question the traditional glucocentric goal of management of maternal diabetes, wherein intensive glycaemic control is advocated to all mothers. There is evidence to show that such intensive glycaemic control in pregnancy may increase the risk of SGA [166]. Early induction and tight control of maternal diabetes may further increase SGA risk and adversely impact future offspring health in SA, who are already known to be smaller at birth. Ethnic specific glycaemic goals should be considered based on the impact of maternal glycaemia on offspring BW. Our study provides further support to the argument of taking into account fetal size [167] when optimising glycaemic control in pregnancy.

4.6 Future Directions

- Further randomized prospective studies are needed to assess the impact of glycaemia of offspring BW in SA and WC, to guide management targets in the two ethnic groups
- The above studies could examine not only overall BW but also objective measures of infant body composition and adiposity eg densitometry.

- The impact of insulin on metformin on offspring body weight and adiposity should be studied in different ethnic groups to individualize treatment goals.

5 Ethnic differences in fetal growth in GDM:

5.1 Abstract

Background: The effect of maternal diabetes on offspring BW is not uniform across ethnic groups. BW is however a crude composite outcome of fetal growth. The growth of individual fetal parameters in GDM has not been compared between ethnic groups. We aimed to study differences in fetal growth between SA and WC in GDM pregnancies.

Methods: A retrospective study of pregnancies with GDM between 2009 & 2012 of White Caucasian or South Asian ethnicity from University Hospital Coventry & Warwickshire in the West Midlands of the United Kingdom. Fetal growth parameters i.e. Head Circumference [168], Abdominal Circumference (AC), and Femur Length (FL) at 28, 32 and 36 weeks were recorded for all women.

Results: 177 WC and 160 SA were included in the analysis. SA mothers were shorter had lower BMI and multiparous compared to WC. SA had lower AC at 28, 32 and 36 weeks compared to WC, but other skeletal parameters such as FL and HC remained similar to WC. SA also had higher HC/AC and FL/AC ratios, with HC/AC remaining > 1 even at 36 weeks.

Conclusions: It appears that SA fetuses of mothers with GDM had features of asymmetric growth when compared to WC. The SA offspring displayed head and femur sparing phenotype with smaller abdomens suggesting a possible asymmetric intrauterine growth restriction. Standard intensive glucocentric treatment may need to be tailored to ethnicity based on differences in fetal growth.

5.2 Introduction

Estimated fetal weight (EFW) and hence prediction of macrosomia has been used as a part of management of GDM pregnancies and mothers with GDM are advised serial growth scans to monitor fetal growth [78]. Both the Shepard's [169] and the Hadlock's formula [170] for estimating fetal weight using a combination of Abdominal Circumference (AC), Femur Length (FL) and Biparietal diameter (BPD), were derived from populations of healthy WC women. However, there is evidence of a disproportionate and accelerated growth in diabetic pregnancies [171] [172] and therefore this relationship between various fetal parameters and EFW may not be the same. Wong found that there was underestimation of BW in 26.3 % of pregnancies with diabetes by more than 15 %, compared to 5.4% in the control group. Studies in type 1 diabetes show that EFW at term could be in error by up to 900g [173].

This estimation is especially erroneous in babies with macrosomia [174, 175].

Hence there is increasing interest in studying individual components of fetal body composition rather than EFW. Special focus has been on the measurement of the AC, which was found to be a better predictor of macrosomia than EFW [176] and associated complications such as shoulder dystocia [177]. Altered HC/AC trajectories with reduced HC/AC ratio was also noted in offspring of maternal pre-gestational diabetes and GDM [171].

It is to be remembered that this link between GDM and altered fetal growth, i.e. disproportionate growth and altered growth velocities been explored predominantly in WC [172] and extrapolated to other ethnic groups for clinical practice.

Despite having greater degrees of hyperglycaemia in pregnancy [146-148], SA babies are amongst the smallest in the world [13, 149-151]. There is evidence to

show that the effect of maternal glycaemia on BW may vary with ethnicity [137, 149, 153]. Sparse evidence in SA suggests that the effect of maternal diabetes on BW may be smaller in SA compared to WC [137]. In Chapter 4, we reported that the effect of maternal diabetes on offspring BW was lower in SA compared to WC. Therefore it is possible that GDM can affect fetal growth differently in SA and WC. Despite the high risk of GDM in SA, studies examining fetal growth in GDM in SA are sparse [178]. In chapter 6, we report disproportionate fetal growth in SA living in India. There are no studies comparing fetal growth between SA and WC.

5.3 Hypotheses

SA foetuses have different growth patterns compared to WC in GDM pregnancies

5.4 Aims

To study the differences in fetal growth between SA and WC in GDM pregnancy

5.5 Objectives

To compare the growth of fetal growth parameters i.e HC, AC and FL in SA and WC in GDM pregnancies

5.6 Methods

This was a retrospective study of all pregnancies with GDM between 2009 & 2012 of White Caucasian or South Asian ethnicity from University Hospital Coventry & Warwickshire in the West Midlands of the United Kingdom.

All these centres used the selective screening based on the risk factors recommended by NICE: BMI $\geq$ 30Kg/cm^2, first-degree relative with diabetes, previous GDM, previous unexplained stillbirth, previous macrosomia (BW $\geq$ 4.5 kg) or women of ethnic minority origin. Women meeting any one of the above criteria underwent a 75

g OGTT between 24-28 weeks. During this period all centres used the modified WHO 1999 criteria for the diagnosis of GDM following a 75g OGTT: FPG ≥6.1mmol/l and/or 2-hour plasma glucose (2hPG) ≥7.8mmol/l. Obstetric and neonatal characteristics were obtained for all women undergoing an OGTT. BMI was measured at the booking visit. For the purpose of this study, ethnicity was grouped into South Asians (SA -Indian, Bangladeshi, Pakistani, Sri Lankan), White Caucasian (WC - British / European). Multiparity was defined as ≥2 live previous pregnancies that progressed beyond 24 weeks gestation.

Routine fetal ultrasound was performed at 28 weeks, 32 weeks and 36 weeks gestation for all GDM women. Head circumference, abdomen circumference (AC) and femur length (FL) were recorded for all women electronically from View-point software, version 2.0.

Statistical analysis: Mean and SD and student t tests were used to compare maternal and offspring characteristics between the two groups. Linear regression was used to compare the fetal biometry parameters between the two ethnic groups after adjustment for maternal age, BMI, gestation, sex of the baby and plasma glucose. A repeated measures ANOVA was performed to examine the differences in the trends of growth of individual fetal parameters. SPSS version 22.0 was used for analysis.

LGA and SGA centiles at birth were calculated based on the customised Gestation Related Optimum weight (GROW) centiles obtained from the Bulk centile calculator available online at www.gestation.net. These centiles are ethnic specific centiles for gestational age and sex of baby, adjusted for maternal height, weight and parity.

5.7 Results

Initial data for WC was obtained from pregnancies of GDM mothers between March

2011 and 2013. Of total 212 babies born during this period, complete sonographic data was available for 177 WC women, after excluding 8 sets of twins, 2 sets of triplets.

To obtain a similar number of SA women, this time period was extended back to April 2009. Of 192 babies born to SA mothers with GDM, 160 had complete scan data after excluding women with multiple pregnancy.

Table 1 shows baseline characteristics of the two ethnic groups. SA were significantly leaner, shorter, had a higher prevalence of multiparity, and had significantly higher FPG and 2hPG at OGTT compared to WC.

Table 2 shows the comparison of fetal biometry between the two groups. SA had significantly lower AC at all time points despite similar skeletal measures of FL and HC, with the exception at 32 weeks when HC was also lower in SA. FL/AC was significantly higher in SA compared to WC at all times. H/C was also higher in SA than WC at 28 weeks and at 36 week. As expected the BW in SA was significantly lower than in WC, however on using the customized GROW centiles the two groups had similar proportion of LGA and SGA.

Table 1: Baseline characteristics of WC and SA women

	WC: Mean (SD): n=177	**SA: Mean (SD): n=160**	**p**
Age in years	31.27 (6.01)	31.87 (4.87)	0.131
BMI (Kg/cm^2)	32.21 (7.42)	28.57 (6.14)	<0.0001
Height (cm)	164.35 (6.48)	158.90 (6.55)	<0.0001
Multiparity (≥ 2)	28.2 %	42.8 %	<0.0001
FPG (mmol)	5.11 (0.76)	5.27 (0.71)	0.003
2hPG (mmol)	7.64 (1.89)	8.07 (2.13)	0.007

Table 1 shows the differences in baseline characteristics between SA and WC

Table 2: Differences in fetal biometry between SA and WC according to gestation

	WC: Mean (SD)	SA: Mean (SD)	p
AC1 (28 weeks) mm	248.80(18.46)	242.67(19.29)	0.016
HC1 (28 weeks) mm	269.04 (16.108)	267.21(15.247)	0.381
FL1 (28 weeks) mm	53.25(3.57)	53.89(4.06)	0.197
HC/AC	1.08 (0.06)	1.10 (0.06)	0.008
FL/AC	0.21 (0.01)	0.22 (0.01)	<0.0001
Gestation 1 (28 weeks)	28.38 (1.06)	28.32 (1.16)	0.653
AC2 (32 weeks) mm	297.17 (21.21)	288.11 (21.03)	0.001
HC2 (32 weeks) mm	307.86 (14.25)	301.82 (14.78)	0.001
FL2 (32 weeks) mm	63.05 (4.17)	62.35 (4.45)	0.199
HC/AC	1.04 (0.06)	1.05 (0.08)	0.154
FL/AC	0.21 (0.01)	0.22(0.02)	0.031
Gestation scan 2	32.77 (1.06)	32.50 (1.09)	0.048
AC 3 (36weeks) mm	330.47 (20.09)	322.62 (19.08)	0.002
HC 3 (36 weeks) mm	327.20 (29.48)	325.08 (10.77)	0.46
FL 3 (36 weeks) mm	68.68 (2.77)	68.60 (3.10)	0.199
HC/AC	0.99 (0.098)	1.01 (0.05)	0.057
FL/AC	0.21 (0.01)	0.21(0.01)	0.001
Gestation at scan 3	35.97 (0.80)	35.98 (1.00)	0.957
Birth weight (g)	3419.41 (630.28)	3223.55 (586.58)	<0.0001
Gestation at birth [179]	38.81 (1.81)	38.68 (1.82)	0.369
LGA (GROW centiles)	14.8 %	14.1%	0.829
SGA (GROW centiles)	10.9 %	8.8 %	0.449

Table 2 shows the differences in fetal biometry at the various gestational ages along with the birth weight data in SA and WC

A mixed, between subjects ANOVA (repeated measures ANOVA) was conducted to assess the main effect of ethnicity on the growth of AC, HC and FL with gestational age and also the interaction of ethnicity with gestational age.

Figure 1 and table 3 show the results of the repeated measures ANOVA. There was a significant main effect of time on AC, HC and FL, showing as expected a significant change of the above with time. Only AC but not HC and FL showed a significant main effect of ethnicity. The trend in the change of AC, HC and FL with time was similar between both ethnic groups, with no significant effect of interaction between ethnicity and gestational age. Figure 2 and table 4 shows the results of repeated measures ANOVA for HC/AC and FL/AC ratio in SA and WC.

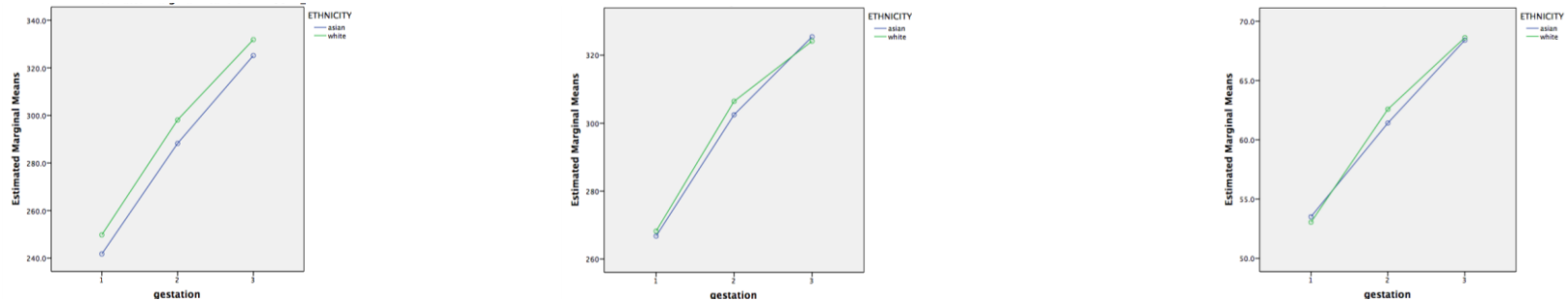
Finally a multivariable linear regression showed that the differences in AC, HC/AC and HC/FL ratio between the two ethnic groups that was seen on descriptive analysis persisted following adjustment for maternal age, BMI, FPG at OGTT, gestational age, and baby's sex all time points (Table 5). HC/AC ratio at 32 weeks was similar between the two ethnic groups as seen previously in descriptive analysis. Table 6 showed that AC was independently determined by FPG

Table 3 Trends of change of AC, HC and FL with time and ethnicity

	AC	HC	FL
Gestational age	Wilks' Lambda = 0.054, F (2, 122) = 1069.67, p <0.0001	Wilks' Lambda = 0.107, F (2, 119) = 493.99, p <0.0001	Wilks' Lambda =0.062, F (2, 122) = 920.516 p < 0.0001
Ethnicity	F (1, 123) = 8.45, p = 0.004	F (1, 120) = 0.279, p =0.599	F (1, 123) = 0.377, p = 0.540
Interaction of Gestational age with ethnicity	Wilks' Lambda = 0.988, F (2, 122) = 755, p = 0.472	Wilks' Lambda = 0.983, F (2, 119) = 1.023 p = 0.363	Wilks' Lambda = 0.964, F (2, 122) = 2.274, P=0.107

A mixed between-within subject's analysis of variance (repeated measures ANOVA) shows that the change in AC, HC and FL with time was similar across both ethnic groups. While HC and FL were not affected by ethnicity, the main effect of ethnicity on AC was significant, indicating a significant ethnic difference in AC.

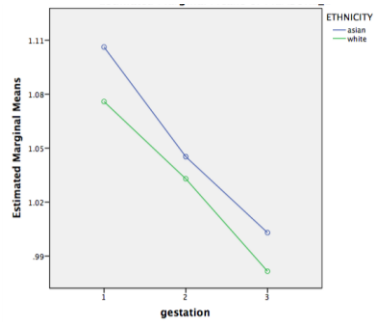
Figure 1: Trend of change of fetal biometry: AC, HC and FL in SA and WC



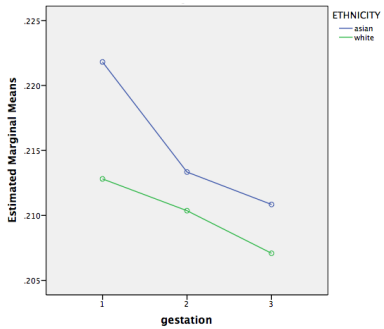
1a) AC 1b) HC 1c) FL

Figure 1 depicts graphical representation of the repeated measures ANOVA showing trend of change of fetal biometry i.e AC, HC and FL in SA and WC**.** Green line represents WC, Blue line represents SA. In line with table 3 only the trend of AC with was significantly different between the two groups.

Figure 2: Trend of change of fetal biometry: HC/AC and FL/AC in SA and WC



2a) HC/AC ratio



2b) FL/AC ratio

Figure 2 depicts graphical representation of the repeated measures ANOVA showing trend of change of HC/AC and FL/AC in SA and WC**.** Green line represents WC, Blue line represents SA. In line with table 4, the trend of HC/AC but not FL/AC was significantly different between the two groups.

	HC/AC ratio	FL/AC ratio
Gestational age	Wilks' Lambda = 0.054, F (2, 122) = 1069.67, p <0.0001	Wilks' Lambda = 0.107, F (2, 119) = 493.99, p <0.0001
Ethnicity	F (1, 123) = 8.45, p = 0.004	F (1, 120) = 0.279, p =0.599
Interaction of Gestational age with ethnicity	Wilks' Lambda = 0.988, F (2, 122) = 755, p = 0.472	Wilks' Lambda = 0.983, F (2, 119) = 1.023, p = 0.363

Table 4: Trends of change of HC/AC and FL/AC with time and ethnicity

A mixed between-within subject's analysis of variance (repeated measures ANOVA) shows that the change in HC/AC and FL/AC with time was similar across both ethnic groups. While FL/AC was not independently affected by ethnicity, the main effect of ethnicity on HC/AC was significant, indicating a significant ethnic difference in HC/AC.

Table 5: Ethnic differences in fetal growth after adjustment for confounders

	28 weeks	32 weeks	36 weeks
	β coefficient (95% CI)		
AC	1.964 (0.488, 3.440), p=0.009	2.585 (0.945, 4.226), p=0.002	2.468 (0.864,4.071), p=0.003
HC/AC	-0.007 (-0.013, -0.002), p=0.006	-0.004 (-0.010, 0.002), p=0.157	-0.002 (-0.003, -0.001), p=0.002
FL/AC	-0.003 (-0.004,-0.002), p<0.0001	-0.002 (-0.004, -0.001), p=0.002	-0.002 (-0.003, -0.001), p=0.002

Multivariable linear regression was performed with AC as dependent variable and ethnicity as independent variable, after adjustment for maternal age, BMI, FPG, gestational age at scans and sex of baby. SA were the reference group: SA had a significantly lower AC at all gestations after full adjustment as shown below.

Table 6: Effect of Fasting plasma glucose on AC in SA and WC

	Whole group	SA	WC
	β coefficient (95% CI)		
28	0.267 (-2.57, 3.107), p = 0.853	-1.41 (-5.69, 2.87), p=0.515	1.90 (-2.09, 5.88), p=0.348
32	4.99 (1.76, 8.23), p = 0.003	3.21 (-1.29, 7.70), p = 0.160	7.38 (2.48, 12.29), p=0.003
36	5.31 (2.27, 8.34), p = 0.001	3.83 (-0.006, 7.665), p=0.05	7.79 (2.64, 12.95), p=0.003

Multivariable linear regression was performed with AC as dependent variable and FPG as independent variable, after adjustment for maternal age, BMI, ethnicity, gestational age at scans and sex of baby. FPG was an independent predictor of AC at 32 and 36 weeks after adjustment as shown. When analysed separately in the two ethnic groups, this relationship between FPG and AC was only significant in WC, not SA.

5.8 Discussion

Our study is the first to compare patterns of fetal growth between SA and WC in GDM pregnancies. We report several important findings.

Firstly we showed that SA with GDM have significantly smaller AC but largely similar skeletal growth (HC, FL) than WC foetuses despite having significantly lower maternal BMI and height than WC. The lower AC is seen as early as 28 weeks despite the higher FPG and 2hPG in SA.

These findings agree with the Intergrowth data in healthy women [180] and to the Sparks theory [181] that show that skeletal growth is largely conserved across race. The intergrowth study is a large study of fetal growth in healthy women without GDM of different ethnic groups, which explored ethnic differences in patterns of fetal growth. This study observed that skeletal parameters are fairly similar across ethnic groups and are unaffected by ethnicity. Country of origin explained only 1.9-3.5% of variance in skeletal growth, i.e. crown-rump length, fetal head circumference, and newborn birth length in healthy pregnancies [180]. This study however did not report on AC, which is very closely related to Birth Weight (BW) especially in diabetic pregnancies.

Our results are also partly in line with other observations in healthy SA and WC new-borns, where SA were observed to have a significantly lower AC at birth [43, 182]. However, the Pune study also reported smaller skeletal parameters such as HC in contrast to our data that only showed differences in AC, with relative sparing of HC and FL. The Pune study differed from our study in two respects, which could contribute in this disparity in results. One: The Pune Maternal nutrition study only recruited healthy women without diabetes. Two: Offspring of SA women living in

India in the Pune study were overall smaller with lower BW compared to SA living in the UK in our study.

This difference in AC appears to be an independent effect of ethnicity and persists despite adjustment for maternal age, BMI and FPG. It appears that this difference in AC largely drives the difference in offspring BW in the two ethnic groups. FPG but not 2hPG appears to be an independent predictor of AC at 32 and 36 weeks in WC but not in SA. Again, in line with our results in chapter 10, maternal glycaemia appears to have a less significant impact on fetal size in SA than WC.

Furthermore, the growth in SA fetuses was asymmetric compared to WC. Increased HC/AC and FL/AC ratios with head and femur sparing and smaller abdomen, have been used in traditional sonographic fetal biometry to describe asymmetric fetal growth [183-185]. It has been shown previously that in healthy fetuses HC/AC exceeds 1.0 before 32 weeks, is approximately1.0 at 32 to 34 weeks, and is less than 1 beyond 34 weeks [184]. An elevated HC/AC ratio, which remains greater than 1 after 32-34 weeks is regarded as a sign of asymmetric intrauterine growth restriction (IUGR) [185]. HC/AC and FL/AC ratios were markedly higher in SA compared to WC. SA with GDM displayed higher HC/AC ratios, which remained >1 even at 32, and 36 weeks depicting a picture of asymmetric IUGR.

The significance of smaller abdomen in SA fetuses of mothers with GDM is unclear. The lower AC is a cumulative effect of subnormal liver and adipose tissue growth [184]. However it has been shown that SA neonates had higher intra-abdominal and subcutaneous abdominal adipose tissue than WC [44], despite overall lower birth weight. Hence it is plausible that the lower AC is the result of poor visceral i.e. liver growth in SA. Whether this disproportionate growth is simply an effect of ethnicity

or the difference in effect of maternal GDM is unknown. Whether lower AC is an indicator of future metabolic risk akin to lower BW needs to be assessed.

Lastly, these findings have important implications on the management of GDM in SA. We have shown that FPG has an insignificant effect on AC and also on BW as shown in chapter 6. Hence it could be speculated that standard intensive treatment goals may aggravate disproportionate IUGR in SA. These findings raise the question of whether ethnic specific treatment goals are required for the management of GDM.

5.9 Future directions

- Larger randomised studies in both ethnic groups incorporating GDM and control groups to study the effect of GDM on fetal growth in the two ethnic groups.
- Studies incorporating other measures of fetal growth including fetal adiposity and liver length.
- Above studies to add evidence to the argument for the incorporation of ethnic specific treatment targets.
- Long term follow up of offspring of these mothers to study the link between lower AC and future metabolic risk.

6 Early impact of GDM on fetal adiposity in SA

6.1 Abstract

Background: Increased fetal growth is an important complication of GDM leading to both short-term delivery complications and long-term risks of obesity in adulthood. Intensive glycaemic management regimens in GDM aim to reduce overall offspring BW. Recent evidence in WC points to altered body composition and asymmetric fetal growth in infants of GDM mothers, with preferential increase in fetal adiposity. Despite having the highest risk of GDM and future metabolic risk evidence of fetal growth abnormalities in GDM is largely lacking in SA. We aimed to assess changes in fetal body size and adiposity in GDM in a SA population.

Methods: A retrospective study of 153 GDM and 178 controls from an obstetric centre in Chennai, India. Serial scans data were obtained at 11, 20 and 32 weeks for all women along with maternal and offspring demographic data. Traditional biometry including fetal HC, AC and FL at 11, 20 and 32 weeks and anterior abdominal wall thickness (AAWT) was measured as a measure of central adiposity at 20 and 32 weeks in all women.

Results: Offspring of GDM women had significantly higher AAWT at both 20 (2.63 (0.51) vs 2.39 (0.41) mm, $p<0.0001$) and 32 week (4.67 (0.81) vs 4.37 (0.67) mm, $p=0.001$) scan despite lower measures of AC, HC and FL at 32 weeks. Both groups had similar BW at term. FPG was an independent predictor of AAWT at both 20 and 32 weeks after adjustment for age, maternal BMI, parity and gestational age.

Conclusion: We present novel evidence of the early origins of adult obesity in offspring of SA women with GDM. Foetuses of GDM mothers show evidence of disproportionate fetal growth with increased adiposity and reduced lean body mass as early as 20 weeks gestation. Increased abdominal wall thickness could serve as

an early marker of GDM. Therapy in GDM should be guided by fetal body composition.

6.2 Introduction

6.2.1 GDM and offspring risk

Gestational Diabetes Mellitus is typically described as a state of glucose intolerance first recognised in pregnancy. The incidence of GDM is increasing rapidly in line with the increase in prevalence of type 2 diabetes (T2D) [5, 6]. GDM is associated with a multitude of offspring complications that include macrosomia, neonatal jaundice, neonatal hypoglycaemia and shoulder dystocia [9] for the offspring and preeclampsia [61] in the mother. Offspring of mothers with GDM have a 2-4 fold higher future risk of T2D and adult onset obesity [63, 64, 186]. Macrosomia, is the most important consequence of the altered metabolic milieu of GDM pregnancies [187, 188] occurring in about 10-20% of GDM pregnancies[189, 190]. Fetal macrosomia is closely related to several other neonatal complications such as shoulder dystocia[191], caesarean delivery, stillbirth, neonatal mortality, neonatal asphyxia and birth injury [192]. Hence most international guidelines recommend serial fetal US at 28, 32 and 36 weeks gestation to monitor for increased foetal growth [76, 193].

However macrosomia is a crude composite outcome of the growth of both fat mass and fat free mass, determined by a variety of genetic, maternal and environmental factors and it is to be remembered that the infant of a diabetic mother has significantly different body proportion from the general population [175]. Offspring with disproportionate macrosomia have been shown to have greater morbidity with higher risk of hypoglycaemia and hyperbilirubinemia compared to those with proportionate macrosomia [194]. Furthermore, the common formulae [170] derived for the estimated fetal weight (EFW) were derived from normal populations and are poor predictors of fetal weight in diabetic pregnancies and especially in in LGA

babies[174, 175]. Therefore there is renewed interest in studying individual components of fetal body composition, and fetal growth trajectories in GDM.

6.2.2 Normal fetal growth

The intrauterine growth trajectory is the composite outcome of substrate availability, genetic make up and hormonal milieu of the intrauterine environment. Changes in the intra-uterine environment largely determine the growth of fetal fat, while the fat free mass is determined by genetic factors [181, 195]. Fetal fat and hence fetal weight increases exponentially in the second half of pregnancy [196]. The differences in body composition between SGA and LGA neonates are largely determined by differences in adiposity rather than that of fat free mass, with fetal adiposity contributing to upto 50% of variance in birth weight [197, 198].

6.2.3 Changes in fetal growth in GDM

As a result of increased nutrient bioavailability and the permissive environment of fetal hyperinsulinism there is accelerated fetal growth of both fat and lean mass in later pregnancy [172]. However, more recently evidence points towards a preferential growth of insulin sensitive adipose tissue mass compared to growth of fat free lean tissues [199] with higher total fat mass and fat/lean mass ratio[172] in GDM offspring compared to normal. Preferential increase in fetal adiposity has been reported in several studies in offspring of women with both pre-existing and gestational diabetes [199, 200]. Abdominal subcutaneous obesity, often measured as anterior abdominal wall thickness (AAWT) could in-fact be an early marker of gestational diabetes and its associated fetal risk [201]. However all the above studies examined fetal adiposity predominantly in the third trimester or at birth [172].

Our aim was to assess the difference in fetal body composition and abdominal wall thickness between GDM and controls in early pregnancy to enable early pregnancy prediction of GDM and risk to the offspring.

6.3 Hypothesis

1. GDM is associated with altered fetal body composition

6.4 Aims

1. To compare differences in fetal biometry i.e. head circumference [168], abdominal circumference (AC) and femur length (FL) between GDM and control population
2. To compared difference in fetal adiposity between GDM and control population

6.5 Materials and methods

This is a retrospective case control study of all pregnant women diagnosed with GDM and controls booked at Seethapathy clinic & hospital, Chennai, India from September 2011 to December 2013.

All pregnant women had OGTT with 75 g glucose 22-26 weeks. GDM was diagnosed based on the IADPSG criteria i.e. Fasting plasma glucose (FPG) ≥ 5.1mmol/l, 1hour PG (1hPG) ≥ 10.0 or 2hour PG (2hPG) ≥ 8.5 mmol/l. Diet and lifestyle advice was given to all women with GDM. The optimal targets was a FPG < 5.1 and a 1hPG < 7.8mmol/l. If glycaemic control was not achieved with diet, insulin was started.

Ultrasound scans were performed at two centres, Mediscan and Seethapathy hospital. All patients underwent a dating scan at 11 weeks, a detailed anomaly scan at 20 weeks where fetal biometry (BPD, HC, AC and FL) was documented. A subsequent scan was done for assessing fetal growth at 28 and 32 – 34 weeks for

GDM women. For Control women a routine growth scan was performed at 32 weeks. Measurement of the abdominal wall thickness (AAWT) was done retrospectively, on images archived from Sonocare, an ultrasound reporting and imaging software. The AAWT was measured at both the 20^{th} week and at 32 weeks, by two operators using standardised technique as described previously[202].

The plane of the abdominal circumference was taken, with optimum gain control, as per standard described protocol. The abdominal wall was identified and the thickness of the echogenic rim was measured. Care was taken not to include the hypo echoic area between the abdominal wall and the liver. In addition traditional biometry measures such as bi-parietal diameter (BPD), AC, HC, and FL were measured at every time point.

Patients with GDM on insulin were induced at 38 weeks. Patients with GDM on diet with good glycaemic control were induced at 40 weeks. Control women were induced at 40^{+5} weeks if spontaneous labour did not occur. The nature and mode of delivery, the gestational age, sex and weight of the baby were documented.

Statistical analysis: Parametric tests i.e student t test was used to compare means between the two groups. Multivariable linear regression was used to study differences in fetal growth parameters after adjustment for confounders. Repeated measures ANOVA were used to study the difference in trend of growth of individual parameters between the 2 groups. SPSS version 22.0 was used for analysis.

6.6 Results

A total of 178 controls and 153 GDM women were recruited (total n= 331). The first dating scan was performed at 11-14 weeks. The second fetal biometry scan was performed at a mean of 20.9 (SD: 1.1) (scan 2) and again at 32.5 (SD: 1.6) weeks (scan 3). For Scan 2 fetal biometry was available for 325 women, and for scan 3 for

316 women in total. AAWT was available for 322 and 324 women for scan1 and 2 respectively. GDM diagnosis was made at 24.7 (SD: 2.45) weeks.

Baseline characteristics of the two groups are shown in table 1. GDM women had higher BMI and maternal weight than controls. They had significantly higher measures of glycaemia at OGTT at all time points. Nearly half of the women had family history or maternal or paternal diabetes.

Table 2 shows the differences in fetal biometry and AAWT between the GDM and control groups. At 11-week scan both groups had similar measures of all biometry. At scan 2 GDM women had higher AAWT, but smaller HC and BPD. All other traditional fetal parameters were similar in both the groups. At Scan 3 the significant differences in AAWT persisted despite smaller measures of AC, HC, BPD and FL. Birth weight was similar in the two groups. With multivariable linear regression the above differences in traditional biometry and AAWT between the two categories persisted even after adjustment for maternal BMI, age, gestational age at scan and parity (table 3).

Table 1: Baseline characteristics of GDM and control groups

	GDM: Mean (SD)	Controls: Mean (SD)	p
Age	28.5 (3.8)	28.8 (4.2)	0.610
BMI (KG/cm^2)	25.9 (5.8)	23.7 (6.6)	0.002
Ht cm	155.2 (19.1)	150.8 (33.40)	0.142
Weight in Kg	65.9 (12.5)	60.6 (16.0)	0.001
FPG (mmol)	5.3 (0.7)	4.4 (0.5)	<0.0001
1hPG (mmol)	9.7 (1.9)	7.0 (1.5)	<0.0001
2hPG (mmol)	8.1 (1.8)	5.9 (1.2)	<0.0001
Multiparity (>1)	4.6 % (7 / 145)	1.7 % (3 / 175)	0.123
Previous GDM	3.9 (6 / 153)	0 (0/178)	0009
Family H/O diabetes	49.1 (75/153)	33.1 (59 / 178)	0.003

Table 1 shows the difference in baseline characteristics between GDM and control women.

Table 2: Fetal biometry in GDM and control groups

	GDM: Mean (SD)	Controls Mean: (SD)	p
AC (11-14 weeks) mm	52.3 (25.1)	51.7 (22.9)	0.820
HC (11-14 weeks) mm	66.2 (28.7)	65.5 (29.6)	0.824
FL (11-14 weeks) mm	7.5 (7.3)	7.5 (7.5)	0.930
BPD (11-14 weeks) mm	18.5 (7.9)	19.2 (9.9)	0.488
AC1 (20 weeks) mm	154.4 (11.1)	156.6 (11.6)	0.084
HC1 (20 weeks) mm	178.1 (11.7)	181.4 (11.2)	0.010
FL1 (20 weeks) mm	34.3 (4.3)	34.9 (2.8)	0.153
BPD1 (20 weeks) mm	48.9 (3.1)	49.9 (3.3)	0.004
AAWT1 (20 weeks) mm	2.6 (0.5)	2.4 (0.4)	<0.0001
Gestation at scan 2	21.1 (1.2)	20.7 (0.9)	0.007
AC 2 (32 weeks) mm	250.3 (66.3)	268.9 (49.9)	0.005
HC 2 (32 weeks) mm	265.6 (71.6)	289.3 (51.6)	0.002
FL 2 (32 weeks) mm	55.4 (16.9)	60.9 (11.2)	0.001
BPD 2 (32 weeks) mm	74.4 (22.4)	80.9 (14.6)	0.002
AAWT 2 (32 weeks) mm	4.7 (0.8)	4.4 (0.7)	0.001
Gestation at scan 3	32.1 (1.8)	32.9 (1.4)	<0.0001
Birth weight (Kg)	3.0 (0.5)	3.1 (0.4)	0.070
Gestational age at birth [179]	38.5 (1.5)	38.8 (1.1)	0.056

Table 2 shows the difference in fetal biometry between GDM and Control groups at various gestational ages.

Table 3: Differences in fetal biometry between GDM and controls after adjustment for maternal characteristics and gestational age

Fetal biometry parameters in mm	B coefficient (95% CI)	p
AAWT1 (20 weeks)	0.25 (0.15,0.35)	<0.0001
HC1 (20 weeks)	-4.47 (-6.46, -2.48)	<0.0001
BPD1 (20 weeks)	-1.31 (-1.91, -0.72)	<0.0001
AC 2 (32 weeks)	-3.50 (-6.78, -0.219)	0.037
HC 2 (32 weeks)	-8.64 (-15.53, -1.74)	0.014
FL 2 (32 weeks)	-1.94(-3.43, 0.46)	0.010
BPD 2 (32 weeks)	-2.11 (-4.05, -0.16)	0.034
AAWT 2 (32 weeks)	0.50 (0.35, 0.65)	<0.0001

Multivariable linear regression was performed with fetal biometry parameters as dependent variable and glycaemic category (GDM vs control) as independent variable after adjustments made for age, BMI, parity and gestational age at scan.

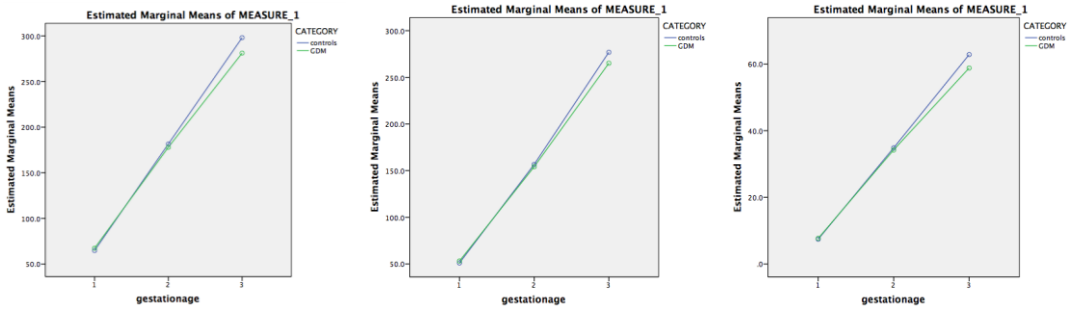
A repeated measures ANOVA was conducted to examine the differences in trend of change of AAWT, AC, HC and FL in the two groups across three time points of the gestational age: 11-14 weeks, 20 weeks and 32 weeks. Figure 1 shows the trend in change of AAWT, AC, HC and FL in the two groups. Table 4 shows the results of repeated measures ANOVA showing a significant effect of both gestational age and glycaemic category for all measures of fetal growth. The trend of change of HC, AC and FL with gestational age differed significantly between GDM and controls, with a significant interaction effect between ethnicity and category of glycaemia. However, the trend for AAWT was similar in both controls and GDM, with AAWT being greater in GDM group at all gestations.

Table 4: Fetal growth trends in GDM and controls: Repeated measures ANOVA

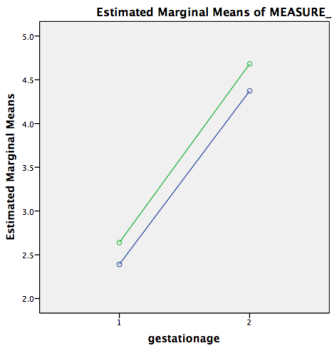
	HC	AC	FL	AAWT
Gestational age	Wilks' Lambda = 0.037, F (2, 301) = 3598.959, p = 0.001	Wilks' Lambda = 0.018, F (2, 301) = 8207.319, p <0.0001	Wilks' Lambda =0.036, F (2, 301) = 3982.09, p < 0.0001	Wilks' Lambda =0.119, F (1, 313) = 2306.40, p < 0.0001
Category (GDM vs controls)	F (2, 301) = 11.615, p = 0.001	F (1, 302) = 9.042, p =0.003	F (1, 302) = 11.067, p = 0.001	F (1, 313) = 24.853, p < 0.0001
Interaction of Gestational age with category	Wilks' Lambda = 0.951, F (2, 301) = 7.749, p =0.001	Wilks' Lambda = 0.941, F (2, 301) = 9.498, p <0.0001	Wilks' Lambda = 0.949, F (2, 301) = 8.116, p <0.0001	Wilks' Lambda = 0.998, F (1, 313) = 514, p = 0.474

A repeated measures ANOVA shows that there was a significantly different trend of change of AC, HC and FL with gestational age between GDM and control group. While the AAWT was different between the two groups at all time points, the change in AAWT with gestational age followed a similar pattern.

Figure 1: Differences in fetal growth between GDM and controls



1a) HC **1b) AC** **1c) FL**



1d) AAWT

Figure 1: Trend of AC, HC, FL, AAWT in GDM and controls. AC, HC and FL measured at 11, 20 and 32 weeks. AAWT measured between 20 and 32 weeks.
Blue lines indicate GDM
Green lines indicate Controls

There was a significant and independent relationship between glycaemia and fetal adiposity. Table 5 shows the relationship between fasting glycaemia at OGTT and measures of foetal adiposity at 20 and 32 weeks respectively. FPG at OGTT was significantly associated with AAWT at 20 and 32 weeks after adjustment for

maternal age, parity, BMI and gestational age at scan. 1 and 2hPG were not independent predictors of abdominal adiposity.

Table 5: Relationship between Fasting Glycaemia and abdominal adiposity

	B coefficient (95% CI)	p
AAWT1 (20 weeks) mm	0.145 (0.073, 0.217)	<0.0001
AAWT 2 (32 weeks) mm	0.248 (0.140, 0.356)	<0.0001

Multivariable linear regression was performed with FPG as dependent variable and fetal biometry parameters as independent variable after adjustments made for maternal age, BMI, parity and gestational age at scan.

6.7 Discussion

Our results provide novel evidence for the increased fetal adiposity seen in offspring of GDM mothers as early as 20 weeks gestation, in a South Asian population. Our most significant finding was that of increased AAWT in GDM fetuses despite similar or lower measures of other traditional fetal biometry in the second trimester, pointing towards disproportionate fetal growth. This increased adiposity persisted despite correction for age, BMI, gestational age and parity and was closely related to maternal FPG. We also noted that similar measures of fetal adiposity persisted at 32 weeks of pregnancy despite treatment of GDM. Several studies reported differences in AAWT in the third trimester, well beyond 26 weeks of pregnancy [172, 199-201, 203, 204] (Table 6). Ours is the first study to report early differences in adiposity at 20-22 weeks, even prior to diagnosis of GDM with OGTT. Larcipete [205] compared GDM women with high-risk controls at 20 weeks by serial scans but abdominal fat differences were not evident till 37 weeks. Hence sonographic measurement of AAWT at 20 week scan, which is routinely performed for anomaly screening and hence available for all pregnant women, could be incorporated into risk stratification, screening or early diagnostic process for GDM.

Table 6: Summary of the literature studying fetal biometry and fat mass in GDM

	Sample size	Gestational weeks	Adiposity	Other fetal biometry	BW	GDM diagnostic criteria
Larciprete[205]	85 GDM, 218 controls	Enrolled at 20-22 weeks	No difference seen prior to 31 weeks. Abdominal fat mass higher at 39-40 weeks, Mid-arm fat and Supra-scapular fat mass higher at 31 weeks, mid-thigh fat higher at 37 weeks in GDM	Mid-thigh lean mass higher at 20-22 weeks	Higher in GDM	NDDG criteria
Aksoy[201]	55 GDM, 69 controls	26-28 weeks	AAWT: 4.07 ± 0.46 vs 3.28 ± 0.37 (controls), p<0.0001	No difference	Higher in GDM	IADPSG criteria
Tantanasis[200]	20 GDM, 15 control	24 and 26 weeks	Increased Subcutaneous fat at Abdomen GDM: 5.30 (0.52) vs Controls: 2.94 (0.58)	Not reported	Not reported	(FPG: 7mmol/l & 2hPG:11.1mmol/l)
De-Santis[172]	43 controls, 171 GDM	20 – 38 week serial measurements (only 15 scans <22 weeks)	Significantly higher fat mass (abdominal, supra-scapular, arm and thigh fat)	HC and BPD similar. Faster growth of AC and FL	Higher BW z score, similar length	Carpenter Coustan
Catalano[199]	195 GDM, 220 controls	At birth	Restricting comparisons between infants appropriate for gestational age with GDM and controls: Fat mass in g: (371 ± 163 g vs 329 ± 150 g, p = 0.02) (GDM vs controls), p=0.0002: Body fat (%):11.4% ± 4.6% vs 9.9% ± 4.0%, p = .002 (GDM vs controls).	No difference in lean mass, HC, leg length, AC. (Whole GDM group vs controls)	Higher proportion of LGA in GDM group.	NDDG criteria
Enzi[203]	17 GDM, 17 controls	At birth	% body fat: GDM(17% ± 1.7%) vs controls (12.2% ± 0.5%). (newborn anthropometry)	Not reported	No difference	Whites Classification
Vedavathi [178]	30 GDM, 30 controls	32-40	Not Studied	Higher AC and HC	Higher in GDM	Carpenter Coustan
Hammoud [171]	99 GDM, 145 Controls	17 weeks, 37 weeks	Not studied	Similar AC, HC, FL	Not reported	100g GTT ADA
Nasrat [204]	51 GDM, 501 controls	At birth	Increased skim fold: Biceps, subscapular, suprailiac, sum of all skin folds.	Similar HC and AC	Higher in GDM	ADA

A point to note in our study is that these differences in adiposity were observed in the two groups despite similar overall BW at term, signifying an asymmetric growth pattern even in offspring of relatively well-controlled GDM. Most other studies that have reported increased fetal adiposity in utero have also reported overall larger BW in GDM offspring compared to controls (table 6). Our results provide further early evidence to Catalano's observation of increased adiposity at term in the two groups of comparable weight [199].

The other intriguing finding in our study is that fetuses of GDM mothers displayed decreased lean mass, as early as 20 weeks. Both HC and BPD were significantly lower in GDM at 20 weeks even after adjustment for maternal characteristics and gestational age. AC displayed a trend to be lower than the controls at 20 weeks but this did not reach statistical significance. At 37 weeks all traditional parameters including AC, HC and FL were significantly smaller in GDM fetuses, with the difference more marked than at 20 weeks. These findings are in contradiction to most previous studies that either showed an increase [172, 178] or no difference [171, 199, 201, 204] in traditional fetal parameters. However in all of the above studies the offspring of GDM were either macrocosmic or had significantly greater BW than control fetuses (see table 6). Therefore it is conceivable that if the comparison was restricted to fetuses of similar overall weight, the reduced parameters of other fetal biometry would be more apparent. In fact, a study from the United states [199] reported similar lean fetal mass between GDM and controls, but on restricting the comparison to GDM fetuses who were appropriate for gestational age (AGA), the latter had lower lean mass than controls. Such early growth delay with lower BPD has also been reported in fetuses of mothers with pre-gestational diabetes [174, 206]. It is therefore possible our SA women with GDM had more

severe hyperglycemia or undiagnosed pre-gestational diabetes and therefore display features of early growth delay akin to women with pre-gestational diabetes.

The smaller fetal biometry at 37 weeks in addition can be explained by the influence by treatment of GDM. Since the groups had similar BW, the increase in adiposity combined with decreased lean mass could be a consequence of tight maternal glycemic control coupled with the effect of medication such as insulin. Aggressive treatment of maternal glycaemia has been shown to increase the risk of overall small for gestational age neonates [166]. It has been shown that known that maternal treatment with insulin preferentially favors that increase of fetal adiposity over lean mass [199]. When compared to offspring of GDM mothers on diet alone, the offspring of GDM mothers on insulin have significantly greater skin fold thickness, greater total body fat mass and % body fat but similar measures of lean body mass [199]. Therefore it can be conceived that intensive treatment goals with insulin regimens may in fact reduce lean body mass and increase adiposity.

Other possible explanations of the smaller overall size could be the presence of underlying placental dysfunction impairing the growth of skeletal and visceral growth.

This is the first study examining early fetal growth in GDM along with measures of adiposity in SA. It is possible that this asymmetry in fetal growth is a feature that is more marked in glucose intolerant SA.

Our study had few important limitations. It was retrospective and hence liable to incomplete data. Detailed information of treatment and glycemic control, which affects late pregnancy fetal growth, was not available. However this could not have influenced our results at 20 weeks.

The implications of these results are several.

Firstly, fetal abdominal adiposity could be a useful marker of GDM in early pregnancy even prior to the traditional diagnosis using the OGTT. Hence a sonographic measure of AAWT could be used as an early marker. Further more AAWT measurements could be used to detect women who would benefit from early treatment of GDM. The effect of metformin in modulating fetal adiposity and hence disproportionate growth should be explored in future studies.

Secondly, this study is evidence for the early origins of adult adiposity and metabolic risk in infants of GDM, and that this is apparent even in fetuses with comparable BW to controls. Previous studies have shown that increased adiposity in term infants of GDM mothers is linked to future adult obesity and diabetes risk [186, 207]. Further studies are needed to extend this observation to measures of adiposity in early fetal life.

Thirdly, our results raise the question if intensive treatment regimens to reduce overall fetal size will truly reduce fetal adiposity or in fact do more harm to worsen the asymmetry between lean and fat tissue. There is also evidence to show that gestational age is the strong predictor of fat free mass [208]. Treatment of GDM with insulin, coupled with early induction, may increase fetal adiposity and reduce lean mass further. It may be that tight glycemic control especially in SA may do more harm. Finally our evidence therefore encourages a more detailed study of adiposity and fetal composition to tailor therapy in GDM and move away from the glucocentric or fetal weight based approach. Again our study could not examine the effect of treatment, such as metformin, however future studies are needed to investigate this role.

6.8 Future directions

- Need for larger prospective studies comparing early fetal body composition including AAWT between GDM and controls in SA and other ethnic groups and incorporation of this into early diagnosis, screening and risk stratification and early treatment of GDM.
- Need for examining the role of GDM therapeutic regimens including metformin, insulin and diet on fetal and neonatal body composition and usefulness of fetal body composition to guide therapy in GDM
- Long term follow up of neonates with increased fetal adiposity to assess future risk of childhood and adolescent obesity and insulin resistance
- Studies assessing the role of other non glycaemic factors – eg Amino acids, cytokines and Free fatty acids in fetal growth, adiposity and lean mass.

7 Differences in Hypothalamic Pituitary adrenal Axis (HPA) activity between SA and WC in relation to GDM

7.1 Abstract

Introduction: SA have more the double the prevalence of GDM even at lower levels of obesity. The mechanisms for increased risk of GDM in SA are still unclear. Hyperactive HPA with altered diurnal cortisol rhythms have been observed in metabolic syndrome, obesity and diabetes. We hypothesised that differences in HPA activity contribute to increased risk of GDM in SA.

Methods: A prospective multicentre study of high-risk SA and WC women was conducted (PRiDE-HPA study). All women of SA or WC origin less than 16 weeks of gestation, meeting selective screening criteria for GDM were recruited, after excluding topical, oral, inhaled steroids and multiple pregnancy.

Maternal data, anthropometry, blood are obtained with timed salivary collections and a 24 hour urine. Saliva is collected at waking, 30 min after, 4pm and bedtime twice in pregnancy. At oral glucose tolerance test (OGTT) blood samples fasting and 2 hour plasma glucose was estimated. The differences in salivary cortisol behaviour and urinary excretion were studied in SA and WC in relation to glycaemia in later pregnancy.

Results: SA had a significantly greater cortisone awakening response than WC and a more enhanced conversion of cortisol to cortisone because of increased renal HSD2 activity. Waking and peak cortisone in early pregnancy correlated independently with fasting plasma glucose at 24 weeks. While BMI was an independent predictor of total GC excretion in WC, adiposity but not BMI independently predicted total glucocorticoid excretion in SA.

Conclusion: There are distinct differences in HPA activity and cortisol clearance between SA and WC in early pregnancy. Early pregnancy waking salivary cortisone was an independent predictor of glycaemia in later pregnancy and could be used as an early predictor of GDM. It is possible that the differences in the HPA activity and cortisol clearance could in part explain the higher risk of GDM and overall metabolic risk in SA.

7.2 Introduction

7.2.1 Ethnic differences in GDM risk

GDM is a pre-diabetes state, associated with 7-8 fold increase in maternal risk and 2-4 fold increase in offspring risk of type 2 diabetes (T2D)[207, 209]. Currently GDM is diagnosed at 24-28 weeks of gestation with a 75g oral glucose tolerance test (OGTT). Early prediction of GDM is poor and the search for reliable early biomarkers in GDM is ongoing. It is well known that SA have more than double the prevalence of GDM and T2D than WC, even at lower levels of obesity [13, 14]. Mechanisms for this heightened susceptibility are not fully understood.

7.2.2 Cortisol and its metabolism

Cortisol is a steroid hormone produced by the zona-fasciculata of the adrenal gland. It has several functional roles which includes regulation of inflammatory and immune responses, energy metabolism i.e glucose and fat metabolism, and regulation of vascular function. Its secretion is controlled by the Adreno Cortico Trophic Hormone (ACTH), secreted by the anterior pituitary, which in turn is controlled by Corticotropin releasing hormone (CRH) from the hypothalamus. (Figure1).

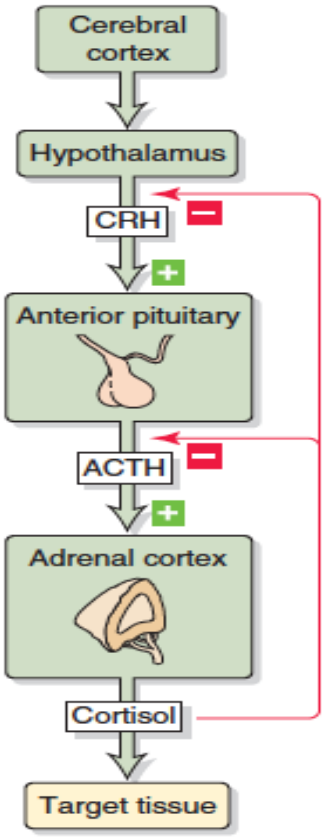


Figure 1: Feedback control of cotisol secretion. The Hypothalamic-pituitary-adrenal axis. HPA. (Adapted from Baron and Boulpaep, Medical physiology, 1st Edition Saunders, 2003)

CRH from hypothalamus stimulates the anterior pituitary to release ACTH from the anterior pituitary which in-turn stimulates the Zona-Fasciculata of the adrenal cortex to produce cortisol. Cortisol exerts negative feedback on both the pituitary and hypothalamus to inhibit both CRH and ACTH.

Cortisol in circulation is largely bound to cortisol binding globulin (CBG) and in basal conditions less then 5% of cortisol is unbound [210]. It is metabolized in the body to inactive Cortisone by the enzyme 11β hydroxy-steroid dehydrogenase (HSD 2) present in the kidneys [211] and the salivary glands and the female reproductive system including the placenta [212]. This enzyme assumes special importance in mineralocorticoid sensitive tissues such as the kidneys to prevent the excessive mineralocorticoid action of cortisol. In the renal tubules cortisol is also metabolised to tetra-hydro cortisol (THF) and αTHF by 5β and 5α reductase respectively. Cortisone is further metabolised by 5β reductase to tetra-hydro cortisone (THE). (Figure 2). 11β hydroxy-steroid dehydrogenase 1(HSD1) regenerates cortisol from cortisone and is largely present in the adipose tissue, liver, muscle and bone.

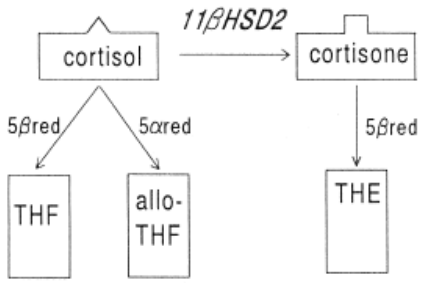


Figure 2: Cortisol metabolism in the kidneys. (Adapted from Van Uum Et al) [1]
11βHSD2 metabolises cortisol to inactive cortisone. Cortisol is also metabolised to tetra-hydro cortisol (THF) and αTHF by 5β and 5α reductase respectively. Cortisone is further metabolised by 5β reductase to tetra-hydro cortisone (THE).

7.2.3 Functions of cortisol

Circulating cortisol exerts its actions by binding to cytosolic corticoid receptors, which are a part of the nuclear steroid-thyroid-retinoid receptor superfamily [213] and influence transcription of target genes. Cortisol is an important hormone for cellular energy metabolism, and plays an important role in adaptive mechanisms during periods of stress. The acute metabolic effects of cortisol are largely adaptive in nature i.e increasing mobilization of glucose [214], free fatty acids [215] and amino acids [216] from endogenous stores for mitochondrial oxidation and energy production. In addition to its role in cellular growth and energy metabolism it also regulates blood pressure, immune function and fluid balance [215].

7.2.4 Cortisol and metabolic risk

As opposed to acute adaptive responses of cortisol, chronic increased cortisol exposure on the other hand is maladaptive, as seen in the typical example of Cushing's syndrome, which is associated with a number of morbidities such as central obesity, insulin resistance and dysglycaemia, osteoporosis, hypertension, and immune suppression [217]. Here, cortisol is largely catabolic leading to decreased lean body and muscle mass associated with increased central obesity and increased fat mass.

Subclinical hypercortisolism with raised plasma cortisol levels have been seen in insulin resistance states, obesity and metabolic syndrome [218] [219, 220].

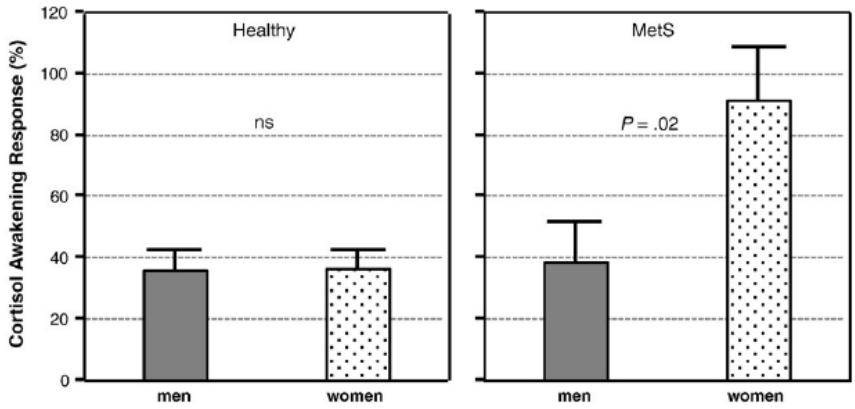
Individuals with diabetes have been shown to have higher 24-hour urine free cortisol and basal plasma cortisol compared to controls [221]. A picture of hypercortisolism along with increased urinary clearance suggests a hyperactive hypothalamic pituitary adrenal axis (HPA). Adults with glucose intolerance have increased activation of the HPA with higher fasting cortisol and exaggerated responses to the repeated stress of venepuncture [222, 223]. The causal relationship between HPA regulation and metabolic risk is still unclear [224]. While the peripheral hypothesis states that adiposity in obesity states cause altered cortisol metabolism, the central hypotheses believes that HPA dysregulation is the cause of adverse metabolic states such as obesity[224].

In addition to hypercortisolism, changes in the diurnal patterns of cortisol have been observed in conditions of adverse metabolic risk. In healthy adults, cortisol rises steeply from awakening up to 30 min (cortisol awakening response-CAR), a measure of HPA reactivity followed by a decline throughout the day [225, 226]. The decline is characterised by an initial steep phase (early decline) followed by a more gradual decline (late decline). The Multiethnic study of Atherosclerosis (MeSA) showed that individuals with diabetes had a trend towards a lower awakening cortisol and a significantly lower CAR than individuals without diabetes [57]. In another study, BMI negatively correlated with awakening salivary cortisol and a showed a greater decline of cortisol following the initial peak [227]. In contrast to what was seen in diabetes states, metabolic syndrome in women was associated with a higher CAR but lower awakening cortisol than those women without metabolic syndrome (figure 3)[228].

Whether HPA dysregulation is associated with hyperglycaemia of pregnancy or GDM, which predates diabetes is not known. One study of 23 women reported

higher 9am cortisol in GDM but no studies have looked at diurnal cortisol patterns [229].

Figure 3: Higher CAR in metabolic syndrome



Women with metabolic syndrome have greater CAR and lower awakening cortisol compared to those without This difference was not seen in men. Adapted from Bengtsson [228]

7.2.5 Cortisol patterns in normal pregnancy

Both total and free cortisol and cortisone increase about 2-3 fold in later pregnancy compared to the post-partum state [230]. Although there is a surge of both cortisol and CBG levels simultaneously [231, 232] the normal diurnal rhythm of cortisol is preserved in pregnancy [230]. The placental HSD2 inactivates cortisol to cortisone to prevent fetal exposure to excess cortisol.

7.2.6 Ethnic differences in cortisol patterns

Ethnic differences in diurnal cortisol in non-pregnant adults have been reported in studies between Blacks, Hispanics and WC [59]. Blacks were shown to have flatter diurnal cortisol patterns compared to WC [233]. It has been demonstrated previously that although morning cortisol concentrations were lower there was a stronger positive correlation of cortisol with components of metabolic syndrome (including glucose intolerance) in SA compared to WC [60]. There are no studies systematically studying the differences in diurnal or cortisol clearance patterns

between SA and WC. Studies examining ethnic differences in HPA activity are described below in table 1.

Table 1: Summary of literature examining cortisol patterns between ethnic groups

Studies	Ethnic groups	Sample analyzed	Result
DeSantis [234]	Blacks, Hispanic, WC	Diurnal Salivary Cortisol	Flatter curves in Hispanics and Blacks compared to WC
Hajat [59] (MeSA study)	Blacks, Hispanic, WC	Diurnal Salivary Cortisol	Lower Waking Cortisol and less steep decline in Blacks and Hispanics compared to WC
Reynolds [60]	SA and WC	Single Plasma Cortisol	SA had lower morning Cortisol than WC
Karlamangla [233]	WC and Blacks	Diurnal Salivary Cortisol	Blacks had lower waking and peak Cortisol but higher nadirs (i.e., flatter cortisol rhythms) compared to WC
Cohen [235]	WC and Blacks	Diurnal Salivary Cortisol	Blacks had a flatter evening declines compared to WC
Suglia [236]	Blacks and Hispanics	Diurnal Salivary Cortisol	In Black but not Hispanic women, cumulative stress was associated with lower morning cortisol levels.
Martin [237]	WC, Blacks and Latinos	Diurnal Salivary Cortisol	Blacks have flatter morning-to evening cortisol slopes and Latinos have lower evening cortisol levels than WC

7.2.7 Salivary cortisol

Measuring total serum cortisol, as a measure of bioavailable cortisol is fraught with problems especially in conditions associated with alterations in CBG (eg pregnancy and contraceptive use) where there is a rise in both total cortisol and CBG. Free serum cortisol assays are rarely used in practice because of cost and complexity involved. Saliva provides a filtrate, free of CBG and salivary cortisol has been found to have a good correlation with free serum cortisol, irrespective of CBG variability (17). Hence salivary cortisol has been used recently as a surrogate for free serum cortisol [238]. In addition salivary cortisol is a non-invasive test, can be performed at home and does not include the stress of venae puncture. In pregnancy and oestrogen excess states salivary cortisone is thought be a better predictor of free circulating cortisol than salivary cortisol and is unaffected by changes in CBG [230, 239].

7.3 Hypothesis and research question

- SA women have a higher cortisol exposure compared to WC in pregnancy
- Higher cortisol exposure contributes to higher risk of GDM.

7.4 Aims

The main aim is to study the differences in cortisol exposure between SA and WC

Secondary aims are to study the relationship between cortisol exposure and glycaemia of pregnancy

7.5 Outcomes

Primary outcome:

Difference in waking and peak cortisol between SA and WC in early pregnancy.

Secondary outcomes:

1. Ethnic differences in AUC (Area under the Curve), CAR, decline, bedtime and 24-hour urinary cortisol and metabolites.
2. Relationship of maternal cortisol indices with GDM risk

7.6 Methods: The PRiDE-HPA Clinical study

7.6.1 Subjects

This was a prospective cohort study of 100 pregnant women (50 SA and 50 WC), who were recruited from the ongoing, PRiDE (micronutrients in Pregnancy as a Risk factor for Diabetes and Effects on mother and baby) study. SA include women of Indian, Sri-Lankan, Nepalese, Pakistani and Bangladeshi origin. The two ethnic groups were matched for age, smoking status and BMI. Women were recruited in early pregnancy (<12 weeks) and followed up until OGTT at 24-28 weeks. Data and samples were collected at each visit along with additional data available from the parent PRiDE study as outlined in the table 2 below.

Table 2: Summary of clinical and biochemical data collected for the PRiDE-HPA study

	Data and samples collected		
Visit	**PRiDE study (available)**	**PRiDE-HPA study**	**Analytes**
Recruitment (<12 weeks) Visit - 1	History Questionnaires (quality of life, socio-economic, well-being, anxiety, depression, physical activity) Anthropometry Waist circumference, height, weight, skin fold thickness.	**24-hour urine** **Saliva (waking, 30min, 16:00 and bedtime)**	Urine – 24-hour Cortisol excretion Saliva- Cortisol
OGTT (24-28 weeks) Visit - 2	Anthropometry Questionnaires	**Blood samples at 30, 60, 90 min during OGTT** **Saliva as above**	Fasting and 120 min blood samples Saliva- Cortisol

7.6.2 Laboratory Analysis

Salivary cortisol (SalF) and cortisone were estimated form the saliva samples by Mass Spectrometry at the University of Manchester. Techniques used were as described previously [240].

Urine glucocorticoid (GC) analysis was performed at the university of Edinburgh: Urine GC metabolites were quantified in 24-hour urine collections by gas chromatography electron impact tandem mass spectrometry following solid phase extraction hydrolysis of conjugates and formation of their methoxime-trimethylsilyl derivatives, as previously described previously [241].

The list of metabolites include

1. Urinary Free cortisol (F)
2. Cortisol metabolites: 5β-tetrahydrocortisol (THF), 5α-tetrahydrocortisol (α-THF), α-cortol, and β-cortol
3. Free urinary Cortisone (E)
4. Cortisone metabolites: tetrahydrocortisone (THE), α-cortolone, β-cortolone
5. Total GC excretion: sum of all above

Enzyme activity was measured as previously described [242]:

1. F/E ratio: 11beta-hydroxysteroid-dehydrogenase-type 2 (HSD2) enzyme activity, which converts active cortisol into inactive cortisone
2. α-THF / cortisol ratio: a measure of 5α-reductase activity, which converts cortisol to α-THF
3. (THF+α-THF) / THE ratio: Whole body 11β-HSD1 and 11β-HSD2 activity

7.6.3 Sample size calculations

With no literature on salivary cortisol in SA in pregnancy, we used the accepted Cohen's estimate of d>0.5SD as a clinically meaningful difference. A sample size of

100 (50 SA and 50 WC) would detect a difference of 0.6SD in both waking and peak cortisol between SA and WC with 80% power and 5% significance, allowing for 10% incomplete data. We however expect the difference to be much higher based on studies which showed the difference in waking SC between Hispanics and WC to be at least twice SD[59]. Another study measuring a difference in awakening and peak salivary cortisol between WC and non-WC also showed a difference of >2SD[233]. Our sample size would therefore have even greater power (>95 %) to detect a larger difference as seen in the above studies.

7.6.4 Calculations and Statistical analysis

Cortisol indices such as AUC, awakening, peak, CAR, early, late decline and bed time cortisol were calculated from SC. CAR is defined as the cortisol increase form awakening to 30 min, early decline as the decrease from 30 min to 16.00, late decline as the decrease from 16.00 to bed time. AUC was calculated using the linear trapezoid method using the formula AUC = Σ ($\frac{1}{2}$*(C_{t1}+C_{t2})/(t_1-t_2)), where t1 and t2 represent the two consecutive time points and C represents the concentration of cortisol or cortisone at the specific time point. All indices were log transformed for all analysis.

7.6.5 Minimizing Bias

Age, BMI, waking time, and smoking status have been shown to affect cortisol levels. Therefore, adjustments were made for these covariates in all models.

Student t test was performed to study differences in cortisol and cortisone at different time points and the differences in CAR, early decline and late decline.

Linear multivariable regression was performed for all outcome measures after adjustment for aforementioned confounders.

- Primary outcome (differences in peak and waking cortisol between SA and WC): simple parametric student t-test and linear multivariate regression of peak and waking cortisol will be performed.
- Secondary outcomes:
    1. AUC, CAR, early, late decline and bed time cortisol will be used as dependent variables with ethnicity as the independent variable after appropriate adjustment.
    2. Ethnic differences in Urinary GC metabolites
    3. Relationship between maternal glucose at OGTT and early pregnancy cortisol indicators

Repeated measures ANOVA was performed to examine the differences in trend of diurnal salivary cortisol between the two groups

7.6.6 Plan of investigation

Participants for the current PRiDE-HPA study were recruited from the ongoing PRiDE study cohort.

PRiDE Study

Is a large, multicentre, MRC-funded prospective pregnancy cohort designed to recruit 4500 mothers in early pregnancy who have been identified to have a high risk of developing GDM according to the selective screening criteria. (Women must satisfy at least one of the following criteria: Obesity, previous history of GDM or unexplained still birth, 1st degree relative with T2D, previous history of macrocosmic babies or ethnic minority origin i.e South-Asian, Middle –Eastern or Afro-Caribbean). The study is funded to test the hypothesis that early pregnancy vitamin B12, folate and homocysteine levels independently predict the risk of GDM in WC and SA.

PRiDE HPA study

Inclusion: Pregnant women aged 18-45 years from the PRiDE cohort

Duration of pregnancy <16 weeks

Exclusion: Pre-existing diabetes

Ethnic groups other than SA or WC

Oral, inhaled or topical steroid use within last 3 months

Multiple pregnancy

Recruitment and study visits:

Participant Identification

- Eligible participants were identified in early pregnancy (<12 weeks) from dating scan clinics
- Study explained and consent obtained

Recruitment - Visit 1

- History, anthropometry details obtained
- Participants given salivettes for timed salivary collection and 24-hour urine collection bottles to return before 16 weeks
- Further salivettes given for saliva collection between 24-28 weeks.
- A mobile-phone text reminder system was used to alert women for saliva collection one day prior to sample collection date. Women were asked to record the exact time of waking and saliva collection.

OGTT - Visit 2 (24-28 weeks)

- Anthropometry, history, saliva and blood samples are obtained

7.7 Results

7.7.1 Differences in Salivary cortisol and metabolites – Unadjusted analysis

Table 3 shows the baseline characteristics of the women in SA and WC groups. Apart from differences in anthropometry, both groups were similar with respect to age, glycaemia and gestational age at recruitment. Despite significant differences in BMI, and abdominal circumference, SA had similar measures of subcutaneous adiposity at both triceps and subscapular skin folds.

Table 3: Baseline characteristics of women in PRiDE-HPA study in SA and WC

	WC: Mean (SD)	SA: Mean (SD)	P
Age in years	29.52 (5.19)	30.58 (5.49)	0.332
Gestational age at recruitment	13.08 (2.32)	13.69 (1.56)	0.128
Height cm	164. (7.41)	158.98 (5.65)	<0.0001
Waist circumference cm	101.20 (15.97)	86.29 (10.35)	<0.0001
Skin fold thickness (triceps) mm	25.23 (7.41)	23.35 (7.01)	0.201
Skin fold thickness (subscapular)mm	28.07 (9.45)	25.17 (9.03)	0.127
BMI kg/cm^2	29.89 (6.95)	24.56 (3.97)	<0.0001
FPG mmol/l	4.36 (0.34)	4.40 (0.51)	0.786
2hPG mmol/l	5.72 (1.02)	6.09 (1.18)	0.238
Smokers n (%)	3 (6.4%)	0	0.055

Table 3 depicts the differences in baseline characteristics between SA and WC.

Table 4 and 5 show differences in diurnal cortisol and cortisone patterns in the saliva. In the unadjusted analysis, SA had similar salivary cortisol and cortisone levels despite having significantly lower BMI than WC. CAR of cortisone tended to be higher in SA compared to WC with p=0.059.

Table 4: Ethnic differences in salivary cortisol

	WC: Mean (SD)	SA: Mean (SD)	p
Cortisol Waking (nmol/l)	8.43 (4.95)	7.68 (4.12)	0.342
Cortisol @30min (nmol/l)	9.19 (5.4)	8.90 (4.97)	0.946
Cortisol@4pm (nmol/l)	3.53 (6.53)	2.53 (1.32)	0.983
Cortisol@Bed (nmol/l)	2.77 (5.18)	2.21 (4.66)	0.651
CAR Cortisol (nmol/l)	1.51 (4.11)	1.17 (4.29)	0.932
Early decline Cortisol (nmol/l)	6.45 (4.63)	6.47 (4.21)	0575
Late-decline Cortisol (nmol/l)	0.56 (3.2)	0.39 (3.09)	0.751
AUC Cortisol (nmol/l)	249478.84 (203474.01)	229951.48 (126043.62)	0.91

Table 4 shows the salivary cortisol at different time points along with CAR, decline and AUC. p values indicates difference in $\log_{10}$(cortisol) using the student t test.

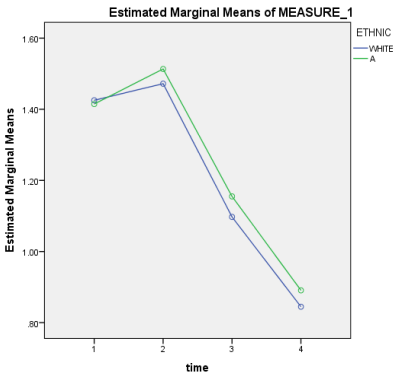
Table 5: Ethnic differences in salivary cortisone

Cortisol metabolites (nmol/l)	WC: Mean (SD)	SA: Mean (SD)	p
Cortisone Waking	29.42 (10.77)	28.35 (8.65)	0.658
Cortisone (30min)	32.91 (12.19)	35.62 (12.99)	0.254
Cortisone (4pm)	14.84 (7.84)	14.97 (5.48)	0.743
Cortisone (Bed)	10.68 (9.82)	9.04 (4.43)	0.838
CAR Cortisone	3.49 (12.39)	7.29 (12.03)	0.059
Early decline Cortisone	17.51 (11.63)	20.73 (14.07)	0.582
Late decline Cortisone	4.67 (8.30)	5.73 (8.17)	0.959
AUC Cortisone	1,023,217.21 (492669.7)	1,028,613.5 (294076.2)	0.514
salE/salF waking	3.98 (1.21)	4.27 (1.51)	0.316
salE/salF @30 min	4.16 (1.27)	4.54 (1.37	0.176
salE/salF @ 4pm	6.60 (1.97)	6.58 (2.06)	0.962
salE/salF @ bedtime	5.79 (1.83)	6.33 (2.21)	0.207

Table shows the salivary cortisone at different time points along with CAR, decline and AUC. p values indicates difference in $\log_{10}$(cortisone) using the student t test. SalE = salivary cortisone, SalF = salivary cortisol

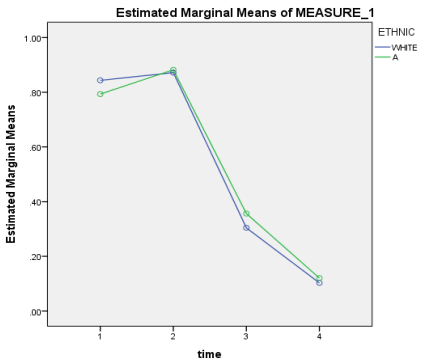
Analysis of the diurnal trends of Cortisol and Cortisone between the 2 ethnic groups is shown in Fig 4 & 5. A repeated measures or Mixed between subjects ANOVA was conducted for both cortisone and cortisol between SA and WC ethnicities. In line with previous results there was no significant interaction between ethnicity and time or independent effect of ethnicity on either of the two analytes.

Figure 4: Diurnal trends of cortisone in SA and WC



- A mixed between subjects ANOVA (repeated measures) was performed for log cortisone at 4 time points for SA and WC
- There was no significant interaction between ethnicity and time: Wilks' Lambda = 0.965, F (3, 81) = 0.967, p=0.413.
- There was a significant main effect for time: Wilks' Lambda = 0.108, F (3, 81) = 223.43, p < 0.0001, partial eta squared = 0.892 (large effect size), with both ethnic groups showing a significant change in cortisone across all 4 time points.
- The main effect comparing the two ethnic groups was not significant, F (1, 83) = 2.710, p = 0.103, partial eta squared =0 .032, suggesting no difference in cortisone between SA and WC.

Figure 5: Diurnal trends of cortisol in SA and WC



- A mixed between subjects ANOVA (repeated measures) was performed for log cortisol at 4 time points for SA and WC
- There was no significant interaction between ethnicity and time, Wilks' Lambda = 0.964, F (3, 81) = 1.007, p=0.394.
- There was a significant main effect for time, Wilks' Lambda = .116, F (3, 81) = 205.652, p < 0.0001, partial eta squared = 0.884, with both ethnic groups showing a significant change in cortisone across all 4 time points.
- The main effect comparing the two ethnic groups was not significant, F (1, 83) = 0.079, p = 0.779, partial eta squared =0 .001, suggesting no difference in cortisone between SA and WC.

7.7.2 Adjusted analysis

To account for the significant differences in maternal characteristics between the two ethnic groups, linear regression was conducted after adjustment for covariates such as gestational age, maternal age, maternal age, smoking, waking time and BMI (Table 6). SA had significantly higher CAR of cortisone compared to WC after adjustment for the above characteristics. Similar analysis of salivary cortisone: cortisol ratio (which could be regarded as a surrogate of HSD2 activity in the saliva)

showed significantly higher ratio at 30 min in SA compared to WC after adjustment for BMI. (table 7)

Linear regression analysis of other cortisol and cortisone variables did not yield significant ethnic differences.

Table 6: Relationship between Ethnicity and CAR (Cortisone)

	Log CAR (Cortisone) (β coefficient (95% CI))
Model 1	0.272(-0.11, 0.55), p=0.059
Model 2	0.27 (-0.39, 0.58), p=0.086
Model 3	0.401 (0.031, 0.771), p=0.034

Multivariable linear regression analysis using CAR (Cortisone) as dependent variable and ethnicity as a independent variable and addition of covariates as below.
Model 1: Ethnicity only (0 = WC, 1 = Asian)
Model 2: Ethnicity + gestational age + waking time + smoking
Model 3: Model 2 + BMI

Table 7: Association between peak cortisone: cortisol ratio and ethnicity

	Log (cortisone: cortisol @30 min) (β coefficient (95% CI))
Model 1	β = 0.037, p=0.169
Model 2	β =0.058, p=0.051
Model 3	β =0.067, p=0.039

Multivariable linear regression analysis using Cortisone : Cortisol ratio as dependent variable and ethnicity as a independent variable and addition of covariates as below.
Model 1: Ethnicity only (0 = WC, 1 = Asian), Model 2: Ethnicity + gestational age + waking time
Model 3: Model 2 + BMI

Further analysis to study the relationship between glycaemia and cortisol metabolites showed that both waking and peak cortisone in early pregnancy were independent predictors of fasting plasma glucose at OGTT even after adjustment for all covariates as shown in table 8. No other cortisol indices were associated with glycaemia.

Table 8: Associations of cortisone with fasting plasma glucose at OGTT

	Cortisone at waking (log cortisone waking)	**Cortisone peak (log cortisone @30min)**
	(β coefficient (95% CI))	
Model 1	0.718 (-0.3, 1.7), p= 0.162	0.389 (-0.119, 0.898), p=0.13
Model 2	1.459 (0.431, 2.487), p=0.006	0.468 (-0.35, 0.970), p=0.067
Model 3	1.469 (0.256, 2.681), p=0.019	0.591 (0.04, 1.13), p=0.035

Multivariable linear regression analysis using waking and peak cortisone as dependent variables and fasting plasma glucose as independent variable and adjustment for covariates as below.
Model 1: Waking time and gestational age only
Model 2: Waking time + gestational age + ethnicity + age
Model 3: Model 2 + BMI

7.7.3 Differences in Urinary cortisol and its metabolites

Table 9 shows differences in urinary cortisol metabolites between SA and WC. Total urinary cortisol, cortisone, total GC metabolites were not different between SA and WC. Multivariable linear regression was performed for using cortisol metabolites as dependent variables and ethnicity as an independent variable and adjusting for covariates such as gestational age at sample collection, maternal age, BMI and smoking (table 10). SA had significantly higher HSD2, lower 5α-reductase activity, and lower total HSD activity compared to WC. The differences in urinary cortisone and 5α-reductase activity were not significant on adjustment for maternal age, and BMI respectively. There were no significant differences in any of the other cortisol metabolites between the ethnic groups.

BMI appeared to be a significant predictor of total GC excretion (table 11) but no significant relation was seen between BMI and other cortisol metabolites like F, E, F:E ratio or 5α-reductase activity either in unadjusted or adjusted models. On repeating the analysis in the two ethnic groups separately, BMI did not predict total GC excretion in SA. (p=0.196) after adjustment for maternal age and gestational age. In WC this relationship remained significant (p=0.02) after adjustment. Other

measures of adiposity, especially abdominal circumferences and skin fold thickness were stronger predictors of urinary GC excretion (table 12) in SA compared to WC.

Table 9: Ethnic differences in urinary cortisol metabolites and enzyme activity

Cortisol metabolites in ug/day	**WC: Mean (SD)**	**SA: Mean (SD)**	**p**
Cortisol (F)	197.35 (114.09)	194.84 (102.89)	0.889
Cortisone (E)	135.70 (83.78)	160.58 (81.05)	0.074
Total GC metabolites	6778.01(4742.71)	6369.62 (4149.74)	0.794
F: E (HSD2)	1.54 (0.59)	1.29 (0.51)	0.016
αTHF: F (5α-reductase)	1.69 (0.93)	1.24 (0.62)	0.019
(THF+ αTHF): THE (whole body HSD1+HSD2)	0.73 (0.31)	0.59 (0.49)	<0.002

Student t test performed for log-transformed variables.
p indicates difference in log transformed variables

Table 10: Relationship between Ethnicity and urinary cortisol metabolites

	24 hour Cortisone	**F: E Ratio**	**5α-reductase activity**	**Total body HSD**
	(β coefficient (95% CI))			
Model 1	0.093 (0.002, 0.184), p=0.045	-0.089 (-0.151, -0.027), p=0.005	-0.157 (-0.295, -0.018), p=0.027	-0.112 (-0.183, -0.041), p=0.002
Model 2	0.081 (-0.009, 0.170), p=0.078	-0.079 (-0.139, -0.019) p=0.010	-0.177 (-0.317, -0.038), p=0.013	-0.112 (-0.185, -0.039), p=0.003
Model 3	0.092 (-0.10, 0.193), p=0.076	-0.069 (-0.137, -0.002), p=0.045	-0.154 (-0.321,0.014), p=0.071	-0.162 (-0.230, -0.094), p<0.0001

Multivariable linear regression using cortisol metabolites or enzyme activity as dependent variable and ethnicity as an independent variable after adjustment for below mentioned variables.
Model 1: Ethnicity + gestational age (WC = reference category), Model 2: Model 1 + age + smoking
Model 3: Model 2 + BMI

Table 11: Relationship between BMI and cortisol metabolites in the whole group:

	Total GC excretion (log) (β coefficient (CI))
Model 1	0.012 (0.003, 0.021), p=0.009
Model 2	0.014 (0.004, 0.024), p=0.005

Multivariable linear regression, using total GC excretion as dependent variable and BMI as independent variable after adjustment for other variables as shown below. Model 1: BMI only
Model 2: BMI + age+ ethnicity + gestational age at urine test (WC reference category)

Table 12: Relationship between urinary GC excretion and measures of adiposity in the two ethnic groups

	SA (β coefficient (95% CI))	**WC (β coefficient (95% CI))**
BMI	0.011(-0.006, 0.027), p=0.196	0.016 (0.003, 0.029), p=0.020
Triceps skin fold thickness	0.012 (0.000, 0.023), p=0.044	0.011 (0.000, 0.022), p=0.047
Subscapular thickness	0.009 (0.001, 0.018), p=0.038	0.004 (-0.005, 0.013), p=0.329
Abdominal circumference	0.010 (0.003, 0.017), p=0.005	0.005 (0.000, 0.010), p=0.063

Multivariable linear regression, using total GC excretion as dependent variable and measures of adiposity as independent variables after adjustment for gestational age and maternal age and smoking. Table shows that measures of adiposity such as skin fold thickness and abdominal circumference were better predictors of urinary GC excretion than BMI in SA.

7.8 Discussion

This study was the first to examine differences in cortisol secretion patterns (with diurnal salivary cortisol) and cortisol clearance (urinary cortisol) between SA and WC women. Our study examined these differences in a high-risk population in early pregnancy.

7.8.1 Diurnal Salivary cortisol patterns

In the initial unadjusted analysis, we found that despite having significantly lower BMI, SA did not differ with respect to cortisol levels either at awakening, peak, 4 pm or bed-time from WC indicating that leaner SA had similar HPA activity to more overweight WC. Studies in WC have indicated that BMI is inversely related with awakening cortisol and AUC of cortisol [227, 243]. Studies in pregnant WC showed that obese women had significantly lower salivary cortisol at all time points compared to lean controls. Despite having significantly lower BMI it was intriguing that SA had similar cortisol secretion and diurnal pattern as WC. However, it was seen in our study that despite BMI differences, SA had similar measures of adiposity to WC as measured by skin fold thickness. It is also well known that SA have greater degrees of adiposity at similar BMI when compared to WC [244]. Therefore is likely that this relationship between cortisol and obesity are mediated by adiposity rather than BMI in SA.

After adjustment for BMI differences SA were shown to have a significantly greater CAR than WC. A higher CAR in SA could be regarded as a clinical predictor of adverse metabolic risk in line with previous observation that has shown an increased CAR in women with metabolic syndrome. However, the significance of CAR in relation to metabolic risk in pre-diabetes states such as metabolic syndrome, insulin resistance and obesity is still unclear. While some studies show a an increased CAR

in women with metabolic syndrome, others have shown a blunting of CAR in insulin resistant adolescents [245], and still others have found no association between CAR and metabolic syndrome or BMI [56] [227]. Bengtsson [228] also showed that the increase in CAR with metabolic syndrome was only seen in women but not in men. This lack of association between CAR and metabolic syndrome in the latter studies could hence be a result of a significant proportion of men in both studies.

Secondly, it was also seen that both waking cortisone and peak cortisone in early pregnancy was closely associated with fasting plasma glucose in later pregnancy and hence could be used as an early first trimester predictor of GDM. Only one other study examined the association between cortisol and hyper glycaemia in pregnancy [229] and showed higher am cortisol in women with GDM. Our results confirm this previous finding using salivary cortisone results and extend this relationship to women without GDM.

It is important to note that all our above results were seen only with respect to salivary cortisone but not with cortisol. As previously explained it has been shown that salivary cortisone but not cortisol was a better marker of free serum cortisol in pregnancy [230] and other states associated with high CBG levels [239]. Therefore, our results with respect to salivary cortisone could be extrapolated to free serum cortisol, and indicate that the HPA activity, i.e hypercortisolemia is closely related to glycaemia in pregnancy.

7.8.2 Urinary clearance of cortisol and its metabolites

We did not observe any significant differences in urinary excretion of cortisol or its metabolites between SA and WC, despite significant BMI differences. It is well known that urinary GC excretion increases with obesity [219, 246, 247] and in those

with metabolic syndrome [248], however again contrary to this observation, leaner SA exhibited similar urinary GC excretion as overweight WC.

However, when this relationship was assessed separately in the two ethnic groups, BMI did not predict urinary GC excretion in SA. Other measures of adiposity, particularly the subscapular skin-fold thickness, which can be regarded, as a marker of central adiposity, was a significant predictor of urinary GC excretion in SA but not WC (table 10). Our results are supported by other observations that showed that central fat distribution was more closely linked to cortisol excretion rates than those with peripheral adiposity [219, 249, 250].

SA appear to have increased urinary clearance of Cortisone and increased HSD2 activity as indicated by reduced urinary F:E ratio compared to WC. The significance of this is unclear. It has been shown that both the expression and activity of renal HSD2 were increased in rodent models of obesity [251]. This was also observed in humans with extreme obesity who had marked elevated renal HSD2 activity compared to lean controls [252]. It appears that SA despite their lower BMIs have similar metabolic clearance of cortisol to more overweight WC women, again reemphasising the possible overriding role of adiposity despite lower BMI in SA.

It must be remembered that the estimated HSD2 activity from the urinary cortisol metabolites represents not only renal HSD2 activity but also placental HSD2 activity. There is evidence to show that placental HSD2 is up regulated and HSD1 is down regulated in the placenta of women with GDM compared to controls [229]. It is possible that a similar picture was seen in SA in early pregnancy given their higher degrees of glucose intolerance.

The significance of reduced 5α-Reductase activity in SA is unclear. 5α-Reductase has an important role in the clearance of cortisol and 5α-Reduced steroids

compromise about a third to a half of all urinary cortisol metabolites [242]. Two isoforms of this enzyme have been detected i.e 5α-Reductase type 1 (5αR1) - mainly present in the liver, adipose tissue and skeletal muscle and type 2(5αR2) - mainly present in the male reproductive tract and to a small extent in the liver [253-256]. Recent evidence in rodents show that reduced 5αR1 activity was associated with hepatic steatosis, weight gain and hyperinsulinemia[257]. This finding was further confirmed in humans where 5αR blockade with duasteride for 3 months was associated with reduced insulin sensitivity and increased body fat by 1.6%[258]. It is possible that reduced 5α-Reductase Type 1 activity could potentially be one of the mechanisms for increased adiposity and higher insulin resistance seen in SA.

In conclusion our study is the first to examine and report novel differences in HPA activity and GC excretion between SA and WC, and the relationship between early pregnancy HPA activity and glycaemia in later pregnancy. SA had a significantly greater awakening response than WC and a more enhanced conversion of cortisol to cortisone because of increased renal HSD2 activity and reduced conversion to 5α-Reduced steroids due to reduced 5α-Reductase activity. Importantly our study also re-emphasises the close relationship between adiposity and the GC excretion in SA, reiterating the poor applicability of BMI in studying metabolic risk in SA. Early pregnancy cortisone independently predicted glycaemia in later pregnancy.

Since these observations were in early pregnancy it can be postulated that similar patterns could be seen in the pre-pregnant state as well. However larger prospective studies are needed in both the pregnant and non-pregnant population to examine the differences in diurnal cortisol pattern in relation to higher metabolic risk in SA.

7.9 Future Directions

- Study the relationship between salivary cortisol metabolites and insulin sensitivity and HOMA-IR at GTT
- Further follow up studies to examine the relationship between cortisol and offspring outcome i.e. adiposity and BW
- Estimation of placental HSD2 activity in SA and WC to measure placental clearance of maternal cortisol and fetal exposure to cortisol
- Larger prospective randomised trials to study the utility of using waking salivary cortisone as a diagnostic tool for GDM

8 Conclusion and summary

In summary, this research addresses important gaps in the diagnosis and follows up of GDM, effect of GDM on fetal growth and birth weight and explores a potential mechanism for the increased risk of GDM in SA and WC. The following are the key findings of our research.

- The new IADPSG criterion for GDM is equally applicable in both SA and WC and is more likely to detect obese women with mild fasting abnormalities. The real benefit of treating mild GDM in these women is not well established. After accounting for BMI differences between SA and WC, it is likely that the IADPSG criteria will identify more SA than the previous mWHO-1999 criteria.
- The uptake of post-natal screening by OGTT is poor overall with no significant ethnic differences. Post-natal screening rates increase significantly with using postnatal HbA1c. SA are more likely to attend postnatal screening by HbA1c than WC. Using FPG during the postnatal period to detect persisting abnormalities fails to detect IGT and diabetes in both SA and WC, however non-WC ethnic minority groups are more likely to be missed out by using FPG.
- Maternal diabetes increases offspring BW in both SA and WC. However it appears that the magnitude of this increase in BW with maternal diabetes is not uniform across ethnic groups with SA having a significantly smaller increase in offspring BW compared to WC. This calls for the consideration of ethnicity specific glycaemic targets in pregnancy, and more studies on adverse fetal outcome in SA.

- SA and WC have different fetal growth patterns in GDM. Despite having higher levels of glycaemia at diagnosis of GDM, SA offspring display patterns of disproportionate fetal growth with patterns of fetal growth restriction, i.e. having smaller abdominal circumference but similar skeletal growth parameters such as Head Circumference and Femur Length. Standard intensive insulin regimens may need to be revisited and ethnic tailored glycaemic targets may need to be considered.
- GDM is associated with disproportionate fetal growth. Fetuses of SA mothers with GDM display increased abdominal adiposity as early as 20 weeks gestation, along with evidence of smaller overall size with reduced AC, HC and FL. Anterior abdominal wall thickness could be used as an early marker of GDM even prior to biochemical diagnosis of GDM. Larger studies are needed in other ethnic groups, to study the effects of treatment, i.e insulin and metformin on fetal growth and the long-term metabolic risk of these offspring.
- There are distinct differences in HPA activity and cortisol clearance between SA and WC in early pregnancy. SA have a significantly greater awakening response than WC, a more enhanced conversion of cortisol to cortisone because of increased renal HSD2 activity and reduced conversion to 5α-Reduced steroids due to reduced 5α-Reductase activity. Early pregnancy waking salivary cortisone was an independent predictor of glycaemia in later pregnancy and could be used as an early predictor of GDM. It is possible that differences in the HPA activity and cortisol clearance could in part explain the higher risk of GDM and overall metabolic risk in SA.

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10 Documents for PRiDE-HPA study

10.1 Consent form

Warwick Medical School

Consent form – PRiDE-HPA Sub-study

PRiDE MAKING YOUR PREGNANCY COUNT

Study title: Role of steroids in gestational diabetes risk

Participant Study ID Number: _____

Name of Midwife/Researcher taking consent: _____

Please initial box

1. I confirm that I have read the Participant Information Sheet (Version 1.0, dated 24th June 2014) for the above study. I have had the opportunity to ask questions and have them answered satisfactorily ☐

2. I understand that my participation is voluntary and that I am free to withdraw from the study at any time without my medical care being affected ☐

3. In addition to PRiDE study samples, urine and saliva samples as outlined in the participant information sheet. ☐

4. I agree to take part in the study ☐

Name of Participant (Print)	Signature of Participant	Date
_____	_____	_____
Name of Midwife/Researcher	Signature of Midwife/Researcher	Date
_____	_____	_____

George Eliot Hospital NHS NHS Trust

Consent form (PRiDE-HPA) ver. 1.1_ 23th July 2014

10.2 Participant information sheet

Warwick Medical School

PRiDE MAKING YOUR PREGNANCY COUNT

Participant Information Sheet

Study Title: PRiDE - HPA study

You are being invited to take part in this PRiDE sub-study that looks at whether steroid hormone imbalance leads to diabetes in pregnancy. Before you decide whether or not you want to take part, you might want to understand what the purpose of the study is and how you will be involved. Please take time to read this information sheet and discuss it with family and friends if you wish.

What is the purpose of the study?

About 5-15% of mothers develop diabetes during pregnancy (known as gestational diabetes, GDM). Currently the commonly used glucose drink test, also called GTT (glucose tolerance test) can only diagnose GDM in late pregnancy (24-28 weeks). Research is ongoing to find a suitable test to detect GDM early in pregnancy.

Cortisol is a natural steroid hormone, also called the "stress hormone" secreted by the adrenal gland. Steroid hormone imbalance is seen in type 2 diabetes. We think that a similar abnormality might also be seen in early pregnancy, in mothers who go on to develop GDM. Therefore, a simple saliva hormone test in early pregnancy could potentially help in easy and early diagnosis of GDM. This could also therefore enable early treatment.

Why have I been chosen for this sub-study?

You have been chosen because you are already part of our PRiDE study.

Do I have to take part in this sub-study?

Participation in this study is entirely voluntary. If you decide not to participate, the standard of your antenatal care and that of your baby will **not be affected** in any way.

What will happen to me if I take part in this sub-study?

We will ask you to sign a consent form to confirm your willingness to participate. Wherever possible the study visits will coincide with your regular antenatal visits to reduce inconvenience. They will be as follows:

At your booking visit - Study visit 1 In addition to the samples collected for the PRiDE study, you will be given some tubes for collection of saliva at home and a urine container to bring back with you on your next routine hospital visit. You will be need to collect saliva at 4 time points during the day i.e. at waking, 30 min after, 16.00 hours and at bed time on any day that is convenient for you in your own home. Urine will need be collected in a container over any 24 hour period at home.

At your GTT visit (24 – 28 weeks) - Study visit 2: This is done as a part of your routine antenatal care. You will receive a separate leaflet from your midwife about this test, where you will have blood tests taken on an empty stomach and again at 2 hours after a glucose drink. For this study, we will in addition take

bloods at 30 min and 1.5 hours. We will place a cannula in your arm to take all the blood samples, in order to avoid repeated needle pricks. You will be given further saliva tubes for saliva collection at home on a day that suits your convenience.

At your delivery: We would take a sample of your baby's saliva using a cotton swab/sponge. Please note that we will **not be taking any blood** or samples directly from your baby at the time of birth.

At 6 weeks post-delivery: We will again need to collect your saliva as detailed above on any one day at home along with **one sample** of baby's saliva. We will send you pre-paid envelopes to post them back.

The samples will be stored in a secure location under the guardianship of University of Warwick indefinitely.

What are the possible advantages and disadvantages of taking part?

Although taking part in the study may not benefit you directly your contribution will provide valuable clues to help detect GDM in early pregnancy and hence help early prevention and treatment of GDM.

What will happen if I change my mind about taking part?

You are free to withdraw from the study at any time, without giving a reason if you do not wish to. All the information and blood samples you have provided us with until that point can be destroyed if you do not want them to be used. The remainder of your and your baby's care will continue as normal

Will my taking part in this study be kept confidential?

Your participation in the study will be kept entirely confidential. Any personally identifiable information will be stored in a password-protected database that only the research staff and study regulatory authorities will have access to. You will be allocated a unique study number and all of your samples will only be identified by this number when processed or stored in the lab.

Who has reviewed this study?

The NHS Research Ethics Committee has approved the study. Your local NHS Trust and antenatal department have also approved it.

What if there is a problem?

Your rights to the regular NHS complaints procedure are not affected in anyway by taking part in this study. If you are concerned about any aspect of the study or unhappy with the research staff at any point, please contact Ms Nicola Owen, University of Warwick Research Support Services (Tel: 024 7652 2785, email: Nicola.Owen@warwick.ac.uk) or your local PALS (Patient Advice and Liaison Service) team.

What will happen to the results of this study?

You will be informed of the summary of the results, after all the data analyses are complete. The scientific results will be published in medical journals and presented in conferences. There will be local and national meetings specifically organised to disseminate the study results.

10.3 Saliva collection instruction leaflet

PRiDE-HPA study – (12/WM/0010)
Instructions for saliva collection

Please collect saliva in the container (salivette) on the day before your study visit. We will provide a telephonic reminder a day or two prior to this.

We will need 4 collections i.e. at waking, 30 min after, 4pm and bedtime in salivettes labelled "waking", "30 min", "4pm" and "bed-time".

Please DO NOT eat or drink anything (including water), take any medications, brush or floss for at least 30 min before saliva collection. Avoid any strenuous activity or exercise on the day of collection.

The following instructions are to help you collect the sample correctly:

Take off the cap from the salivette. Pop the swab from the container into your mouth and chew this for 1 minute. Spit the swab back into the container and put this in your fridge. Refrain from touching the swab as far as possible.

- Repeat this process at

i) Waking ii) 30 min after iii) 4pm iv) Bed time

- **Please record exact date and time of collection on the tube**

Please remember to bring the salivettes with you to your next appointment.

Salivary collection Version 1.0_ 24.09.2014

10.4 Ethics approval documents

NHS
Health Research Authority

National Research Ethics Service

NRES Committee West Midlands - South Birmingham

3rd Floor
Barlow House
4 Minshull Street
Manchester
M1 3DZ

Tel: 0161 625 7815
Fax: 0161 625 7299

22 August 2014

Dr Ponnusamy Saravanan
Associate Clinical Professor & Honorary Consultant Physician, Diabetes, Endocrine & Metabolism
University of Warwick & George Eliot Hospital
Clinical Sciences Research Laboratories
Warwick Medical School
Coventry
CV2 2DX

Dear Dr Saravanan

Study title:	**Micronutrients in Pregnancy as a Risk Factor for Diabetes and Effects on mother and baby: an MRC-funded study (PRiDE)**
REC reference:	**12/WM/0010**
Amendment number:	**Amendment 4**
Amendment date:	**30 July 2014**
IRAS project ID:	**90943**

- The amendment proposes to conduct a sub-study on a selected group of participants from the main study. This sub-study will involve saliva, urine and blood samples.

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

Ethical opinion

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

There were no ethical issues raised.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Notice of Substantial Amendment (non-CTIMP)	Amendment 4	30 July 2014

Participant consent form [PRiDE - HPA Study]	1.1	23 July 2014
Participant information sheet (PIS) [PRiDE - HPA Study]	1.1	23 July 2014
Research protocol or project proposal [PRiDE - HPA Study]	1.1	23 July 2014

Membership of the Committee

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

R&D approval

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

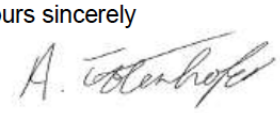
Statement of compliance

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

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

12/WM/0010:	**Please quote this number on all correspondence**

Yours sincerely



Signed on behalf of:
Professor Simon Bowman
Chair

E-mail: nrescommittee.westmidlands-southbirmingham@nhs.net

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

Copy to: Ms Donna McLean - Chelsea and Wesrminster Hospital NHS Foundation Trust

Dr Peter Hedges - University of Warwick

NRES Committee West Midlands - South Birmingham

Attendance at Sub-Committee of the REC meeting on 22 August 2014

Committee Members:

Name	*Profession*	*Present*	*Notes*
Professor Simon Bowman (Chair)	Consultant Rheumatologist	Yes	Chaired the meeting
Reverend Dr Barry Clark (Alternate Vice-Chair)	Retired Hospital Chaplain	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Dr Ashley Totenhofer	REC Manager

Health Research Authority
National Research Ethics Service

NRES Committee West Midlands - South Birmingham
3rd Floor
Barlow House
4 Minshull Street
Manchester
M1 3DZ

Tel: 0161 625 7827
Fax: 0161 625 7299

28 October 2014

Hema Venkataraman
University of Warwick

Dear Hema

Study title:	**Micronutrients in Pregnancy as a Risk Factor for Diabetes and Effects on mother and baby: an MRC-funded study (PRiDE)**
REC reference:	**12/WM/0010**
Protocol number:	**N/A**
Amendment number:	**5**
Amendment date:	**01 October 2014**
IRAS project ID:	**90943**

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

Favourable opinion

Approval was sought for the introduction of a sample collection instruction leaflet for participants in the sub-study.

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

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Notice of Substantial Amendment (non-CTIMP)	5	01 October 2014
Other [Instructions for Saliva Collection]	1.0	24 September 2014

Membership of the Committee

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

R&D approval

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

Statement of compliance

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

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

12/WM/0010:	**Please quote this number on all correspondence**

Yours sincerely

On behalf of
Professor Simon Bowman
Chair

E-mail: nrescommittee.westmidlands-southbirmingham@nhs.net

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

Copy to: Ms Donna McLean, Chelsea and Westminster Hospital NHS Foundation Trust
Dr Ponnusamy Saravanan, University of Warwick & George Eliot Hospital
Dr Peter Hedges, University of Warwick

NRES Committee West Midlands - South Birmingham

Attendance at Sub-Committee of the REC meeting on 27 October 2014

Committee Members:

Name	*Profession*	*Present*	*Notes*
Professor Simon Bowman (Chair)	Consultant Rheumatologist	Yes	
Dr John David Cochrane	Retired GP	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Ms Rachel Katzenellenbogen	REC Manager

11 Published Papers

Postnatal testing following gestational diabetes: time to replace the oral glucose tolerance test?

Lancet Diabetes Endocrinol 2015
Published Online
July 14, 2015
http://dx.doi.org/10.1016/S2213-8587(15)00232-6

Gestational diabetes is associated with up to an eight times increase in risk of future type 2 diabetes: the incidence of type 2 diabetes is 3–24% in the first year postpartum and up to 50% in the first 5 years.[1] Therefore, postnatal testing for these women provides a crucial opportunity for the early detection of diabetes, intervention, and preconceptional care in subsequent pregnancies. Despite this evidence, uptake of postnatal testing for diabetes is poor, with only 23–58% of women with gestational diabetes attending the oral glucose tolerance test (OGTT)[2]—a sharp contrast with the uptake of postnatal cervical screening (94%) and antenatal gestational diabetes screening (98%).[3]

Despite this, most international guidelines,[4] including those of the Fifth International Workshop Conference on Gestational Diabetes, the American Diabetes Association (ADA), the Canadian Diabetes Association, and the Australasian Diabetes in Pregnancy Society continue to recommend postnatal OGTT. However, in 2015, the National Institute of Health and Care Excellence (NICE) recommended either a fasting plasma glucose (FPG) or HbA_{1c} test rather than OGTT for postpartum screening.[5]

We collected contemporary data from 14 477 women who attended antenatal OGTT during 2009–12 across three UK centres. The appendix contains a summary of our methods. Our results further support the need to investigate alternatives to OGTT. Of 1289 (9%) women diagnosed with gestational diabetes, only 630 (49%) attended a postnatal OGTT. Furthermore, non-attenders were more likely to have increased metabolic risk (table), because multiparity (odds ratio 1·80, 95% CI 1·24–2·58), smoking (2·80, 1·58–4·97), and macrosomia (2·52, 1·46–4·34) were independent predictors of non-attendance at postnatal OGTT after adjustment for maternal BMI, age, glucose concentrations at antenatal OGTT, gestational age, and offspring sex. Other studies[6] have reported that women who did not attend postnatal OGTT were more likely to have had worse glycaemic control and needed insulin during pregnancy than were those who attended. Our findings, combined with previous evidence, suggest that women who do not attend a postnatal OGTT are at increased risk for subsequent type 2 diabetes.

We also analysed preliminary data from one of our centres that subsequently adopted HbA_{1c} for postnatal testing in gestational diabetes from December 2013 to assess HbA_{1c} uptake. Of 348 women with gestational diabetes, 217 (62·4%) women attended HbA_{1c} testing, representing an increase of 28% (p<0·0001) compared with OGTT at that centre. Reassuringly, risk characteristics did not differ between those who attended and failed to attend HbA_{1c} tests (unlike OGTT), suggesting increased uptake of HbA_{1c} by women at high risk of type 2 diabetes postpartum.

Barriers to postnatal screening for persisting glucose abnormalities after gestational diabetes mellitus have not been studied extensively. The inconvenience of OGTT and time pressures were the most commonly cited reasons for non-attendance in patient surveys.[7] FPG tests have been recommended as a simpler and cheaper alternative to OGTT. However, FPG requires fasting, is restricted to mornings, and might especially inconvenience mothers with young children. The alternative is a non-fasting HbA_{1c} test.

Both ADA and NICE recommend HbA_{1c} for the diagnosis of diabetes in high-risk non-pregnant adults without symptoms[8] and for preconception risk stratification of women with pre-existing diabetes.[9] Studies[10] to assess the role of HbA_{1c} in the postpartum

	Did attend (n=630)	Did not attend (n=659)	p value
Age (years)	32·3 (5·2)	31·6 (5·7)	0·016
BMI (at booking)	29·6 (6·6)	29·6 (7·2)	0·974
Antenatal FPG (mmol/L)	5·0 (1·0)	5·1 (1·1)	0·106
Antenatal 2 h PG (mmol/L)	9·0 (1·6)	8·8 (1·9)	0·032
Smokers	19/390 (5%)	51/369 (14%)	<0·0001
Ethnic origin	..	..	0·705
South Asian	174/627 (28%)	175/654 (27%)	..
White British	404/627 (64%)	435/654 (66%)	..
Other	49/627 (8%)	44/654 (7%)	..
Multiparity (≥2)	82/391(21%)	118/370 (32%)	0·001
Macrosomia (>4 kg)	23/391 (6%)	47/366 (13%)	0·001

Data are mean (SD) or n/N (%). FPG=fasting plasma glucose. PG=plasma glucose.

Table: **Characteristics of women who attended and did not attend postnatal oral glucose tolerance tests**

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period have reported poor sensitivity of an isolated HbA_{1c} test.[10] However, these studies compared the performance of HbA_{1c} against OGTT with the assumption that the OGTT is the gold standard. This assumption is flawed, as we have previously described for the non-pregnant population.[11] Although HbA_{1c} and OGTT do not necessarily detect the same individuals, the best available data show FPG and HbA_{1c} to be superior to OGTT in signalling the onset of microvascular risk.[12,13] Therefore, it is conceivable that FPG and HbA_{1c} are better choices than OGTT for the screening of women for undiagnosed diabetes in the postpartum period. Although HbA_{1c} and FPG have poor sensitivity to detect impaired glucose tolerance, the existing recommendations state that all women with gestational diabetes, irrespective of postnatal glycaemic status, should be offered the same lifestyle intervention as those with impaired glucose tolerance.[14] Nevertheless, the new NICE guidance[5] also recommends a wide postnatal (measured after 13 weeks) HbA_{1c} window of 5·7% to 6·4% (39 to 47 mmol/mol) to define women at further increased risk of diabetes, at which more intensive interventions should be offered than that offered to other women with gestational diabetes.[5]

Another aim of postnatal testing is to detect undiagnosed diabetes before subsequent pregnancies, thus enabling provision of the best preconception care to reduce risk to offspring. Evidence suggests that a log-linear relationship exists between preconception HbA_{1c} and fetal risk of congenital anomalies, with the highest risk for HbA_{1c} well into the diabetes range.[15] Furthermore, all women with previous gestational diabetes are offered early testing for glucose intolerance in subsequent pregnancies worldwide, irrespective of their postnatal glycaemic status. Therefore, a postnatal HbA_{1c} test also serves as a valuable preconception risk stratification tool for subsequent pregnancies.

HbA_{1c} can be done at any time of the day, at the patient's home, in the non-fasting state, has higher pre-analytical stability and a lower coefficient of variation than does OGTT, and is far easier to repeat. The other advantages of HbA_{1c} compared with OGTT include its established use in diabetes monitoring, and availability of point-of-care testing, especially in resource-limited settings where remote laboratory facilities increase time delays before analysis can be done. Studies[10] to compare the performance of postpartum OGTT with HbA_{1c} have used HbA_{1c} at 3 months postpartum; however, more research is needed to assess the effects of volume shifts in pregnancy and postpartum anaemia on HbA_{1c} and advise the optimum timing of the test. Notably, however, the lowest postnatal HbA_{1c} threshold (5·7% [39 mmol/mol]) recommended by NICE to detect women at high risk of diabetes is lower than that recommended for the general population (6·0% [42 mmol/mol]) to potentially account for any issues linked to red cell turnover at this stage.

In summary, the existing reliance on OGTT to screen for type 2 diabetes after gestational diabetes needs to be reassessed. OGTT has inadequate uptake, especially by women at highest risk. More studies are needed to assess whether changing to HbA_{1c} will indeed improve uptake in such high-risk women and to assess its cost-effectiveness, but preliminary data are encouraging. We believe that changing to a non-fasting HbA_{1c} test will improve screening for long-term outcomes that matter for postpartum women, and that such changes are highly unlikely to adversely affect subsequent pregnancy outcomes. In fact, HbA_{1c} tests might even improve prediction of such outcomes. The new NICE guidelines that recommend either an FPG or HbA_{1c} test in the postpartum period are a step forward in the care of women at high risk of type 2 diabetes and should be welcomed by physicians and patients alike worldwide.

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recommendations to women with previous gestational diabetes and normal glucose tolerance, because they have been shown to benefit from lifestyle intervention or metformin therapy.[3]

Most international guidelines are correct in recommending that women undergo a 75 g OGTT in the early postpartum period, and we believe that this test should not be substituted with other tests that have proven to be less sensitive. Rather, efforts to increase the uptake of postpartum testing with OGTT might be more suitable. Indeed, implementation of such a programme in our group resulted in a postpartum testing rate of 77% among women diagnosed with gestational diabetes.[4]

EN is employed at Steno Diabetes Center, a research hospital integrated into the Danish National Health Service, and owned by Novo Nordisk A/S, and reports previous receipt of an unrestricted educational grant from Novo Nordisk Ireland. FD declares no competing interests.

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Authors' reply

We thank Noctor and Dunne for their interest in our Comment and their correction regarding the timeframe for postnatal HbA_{1c} in their study. Although the local increase in oral glucose tolerance test (OGTT) uptake postpartum reported by the authors' group is commendable, this is the exception to the rule of poor uptake worldwide.

The authors argue that the main reason to do an OGTT is to detect patients with impaired glucose tolerance—a subgroup with a high risk of progression to type 2 diabetes that could merit early intervention. We wish to reiterate that persevering with the more complex and time-consuming postnatal OGTT has no clinical justification for the following reasons.

First, although impaired glucose tolerance (defined on the basis of an OGTT) is thought to be a better predictor of risk of future type 2 diabetes than fasting plasma glucose (FPG) or HbA_{1c} tests, diagnosis of diabetes should be based on glycaemic thresholds commensurate with increased risk of adverse outcomes (such as microvascular complications). Evidence suggests that HbA_{1c} and FPG tests are better than an OGTT at signalling the onset of retinopathy.[1,2] Thus the OGTT definition of diabetes cannot be regarded as the gold standard.[3]

Second, the American Diabetes Association (ADA) guidance cited by Noctor and Dunne recommends lifestyle intervention or metformin not only for individuals with impaired glucose tolerance, but also for those with other glucose abnormalities including impaired fasting glucose or abnormal HbA_{1c} (5·7–6·4%) after gestational diabetes.[4] UK National Institute for Health and Care (NICE) guidance for prevention of type 2 diabetes recommends only FPG or HbA_{1c} tests for risk stratification and intervention in individuals at high risk (eg, previous gestational diabetes).[5] Thus, postnatal risk stratification and intervention to prevent future diabetes can be done using HbA_{1c} or FPG as well.

Third, the Diabetes Prevention Program (DPP)[6] is often cited as interventional evidence to justify the need for detection of impaired glucose tolerance after gestational diabetes; however, this warrants closer inspection. Women with gestational diabetes in the DPP did not have isolated impaired glucose tolerance. Their mean HbA_{1c} was 5·87% (SD 0·5) and 95% had FPG of 4·9–6·9 mmol/L. The mean interval from diagnosis of gestational diabetes to intervention was 12 years. Therefore, in view of the substantial overlap between impaired glucose tolerance, impaired fasting glucose, and prediabetes by HbA_{1c} criteria, evidence from the DPP study cannot be directly extrapolated as evidence for intervention benefit for impaired glucose tolerance alone in the immediate postpartum period.

In conclusion, we believe that the one-step postnatal HbA_{1c} or FPG tests can be used to risk stratify and detect women at increased risk postpartum who would benefit from intensive intervention for type 2 diabetes prevention.

We declare no competing interests.

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CARDIO VASCULAR DIABETOLOGY

ORIGINAL INVESTIGATION Open Access

Vitamin B12 deficiency is associated with adverse lipid profile in Europeans and Indians with type 2 diabetes

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Abstract

Background: Metformin, a standard therapy in type 2 diabetes, reduces vitamin B12 levels. Studies linking low vitamin B12 levels and cardiovascular disease are equivocal and suggest improving B12 levels may help in primary prevention. The role of vitamin B12 deficiency on cardiovascular risk factors, especially in type 2 diabetes has not been explored. The aim of this study is to investigate whether vitamin B12 deficiency in type 2 diabetes patients is associated with cardiovascular risk factors in two different ethnic groups in UK and India.

Methods: Type 2 diabetes patients from two secondary care diabetic centres (Europeans - UK and Indians - India) were studied. Serum vitamin B12, folate and biochemical parameters were measured.

Results: The prevalence rates of vitamin B12 deficiency (<191 ng/L) were 27% and 12% in Europeans and Indians, respectively and higher in metformin treated type 2 diabetes patients. In linear regression analysis, after adjusting for all likely confounding factors, vitamin B12 independently associated with triglycerides in both the populations and cholesterol/HDL ratio in Indians. Logistic regression showed type 2 diabetes patients with vitamin B12 deficiency were at significantly higher odds of having coexisting coronary artery disease (CAD) in Europeans with similar but non-significant trend in Indians, after adjusting for all likely confounding factors.

Conclusions: The prevalence of vitamin B12 deficiency is common in type 2 diabetes patients and is associated with adverse lipid parameters. Type 2 diabetes management guidelines should include the recommendation for regular testing for B12 levels, especially for those on metformin.

Introduction

Vitamin B12 is a key micronutrient responsible for DNA methylation and has various metabolic roles ranging from lipid metabolism to endothelial dysfunction [1]. Studies show association of low vitamin B12 with macro-vascular diseases such as myocardial infarction [2] and cerebral ischemia [3] as well as coronary artery disease (CAD) [4]. However, a systematic review of all published cohort studies was inconclusive [5]. B12 deficiency causes micro-vascular complications such as neuropathy [6] and can worsen the existing neuropathy due to other conditions such as diabetes [7].

Metformin therapy is now considered a standard first line therapy for type 2 diabetes (ADA, NICE, EASD guidelines) [8,9] and is commonly used. Metformin reduces the circulating B12 levels by about 25% [10-12]. One cross-sectional study of 203 type 2 diabetes patients reported the prevalence of B12 deficiency is 22% [13]. However, only 60% of patients with B12 deficiency have anaemia [14] and at milder forms patients with B12 deficiency are asymptomatic. This highlights the importance of regular screening but none of the above mentioned guidelines recommend measuring B12 levels regularly in type 2 diabetes, even when they are on metformin.

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Indians have higher risk of metabolic disorders including type 2 diabetes and cardiovascular diseases (CVD) compared to Europeans [15,16] and these diseases also occur at younger age [17]. They also have higher homocysteine levels, which have been mainly attributed to low B12 levels [18]. Vegetarianism is thought to be cause of such high prevalence of B12 deficiency in this population. Whether high prevalence of B12 deficiency contributes to higher risk of CVD is not known [19].

The purpose of our study is (1) to assess the prevalence of vitamin B12 deficiency in type 2 diabetes patients and (2) its association with cardiovascular risk factors and micro- and macro-vascular diseases in two different ethnic groups in UK and India.

Methods

Study population

Cross-sectional data from two different secondary care diabetic centres were utilized for this study. **(1) UK participants:** 342 consecutive patients of European origin with type 2 diabetes, who had their vitamin B12 and folate levels checked in the George Eliot Hospital (GEH), Nuneaton, UK. **(2) Indian participants:** 321 type 2 diabetes patients of Indian origin had their vitamin B12 checked at the Dr Mohan's Diabetes Specialties Centre were included for the analysis. Patients who were taking vitamin supplements and who were pregnant were excluded from the study. Detailed history, anthropometric and biochemical measures such as age, sex, type of diabetes, duration of diabetes, HbA_1C, smoking status, medications, blood pressure, micro- and macro-vascular complications of diabetes, lipid profile, vitamin B12 and folate levels were collected from both the study population. Information on dietary intake (vegetarian/non-vegetarian) was not collected. These were routine anonymous clinical data extracted from records.

Analytical determinations

Serum glucose, HbA_1C, cholesterol, triglycerides, HDL cholesterol were determined by standard methodologies followed in the respective labs in both the study population. LDL cholesterol was calculated using Friedewald formula. Serum B12 and folate were determined by electrochemiluminescent immunoassay using a Roche Cobas immunoassay analyzer (Roche Diagnostics UK, Burgess Hill, UK). The reference values in both the laboratories were as follows: 191–663 ng/L for vitamin B12 and 2.5-18.7 ug/L for folate. Vitamin B12 and folate deficiencies were defined as levels below 191 ng/L [20] and 2.5 μg/L [21], respectively.

Definition of comorbidity

The following definitions were used to diagnose the comorbidity. Retinopathy: Digital retinal photographs were graded by trained ophthalmologists (India) or retinal graders (UK) by the ETDRS grading system. Neuropathy: Vibratory perception threshold of the great toe > mean + 2SD of healthy non-diabetic study population aged 20–45 years (cut point ≥20 V). Nephropathy: Albumin excretion ≥30 μg/mg of creatinine in urine sample after an overnight fast (microalbuminuria - 30–299 μg/mg of creatinine and macroalbuminuria - ≥300 μg/mg of creatinine). Patients with documented retinopathy, peripheral and autonomic neuropathy, and nephropathy were recorded individually and classified to have microvascular complications. Coronary artery disease (CAD): Past history of documented myocardial infarction, stable and unstable angina, coronary artery bypass graft, stent and/or electrocardiographic changes suggestive of ST segment depression and/or Q-wave changes using appropriate Minnesota codes. Cerebrovascular accidents (CVA): Past history of documented stroke (computed tomography, magnetic resonance imaging, or cerebral angiography). Peripheral vascular disease (PVD): Lack of peripheral pulses or Doppler studies with Ankle Brachial Index <0.9. Those with documented CAD, CVA and PVD were recorded individually and classified to have macrovascular complications.

Statistical analysis

Continuous variables are reported as mean ± standard deviation (SD). Categorical variables are reported in percentages. The distributions of the parameters such as cholesterol, triglycerides, HDL, LDL, vitamin B12 concentrations were skewed; these data were log-transformed. Means of continuous variables were compared using independent t-tests. Bivariate correlations between different variables were done using Pearson correlation test. Risk variables that had significant association were included as independent variables in multiple linear regression analysis. Logistic regression analysis was used to examine the relation between vitamin B12 levels and the risk of micro- and macro-vascular complications. Associations between vitamin B12 and cardiovascular outcomes were adjusted for age, gender, BMI, duration of diabetes, smoking, HbA_1C, cholesterol, HDL, triglycerides, systolic and diastolic pressure, use of metformin, statin and aspirin. p values of <0.05 were considered as statistically significant. All analyses were performed using IBM SPSS Statistics version 19 (IBM Corp, NY, USA).

Results

The clinical characteristics of the study population are shown in Table 1. The use of metformin in Europeans was 65% and in Indians is 75%. The prevalence rates of serum vitamin B12 deficiency (<191 ng/L) in Europeans were 27% and Indians were 12% (Table 1). For those on metformin, these rates were 32.1 and 12.4%, respectively.

Table 1 Basic Characteristics of the study population

Parameters	Europeans total n = 342	Indians total n = 321
Age (years)	63.0 ± 12.3[a]	56.8 ± 10.6
BMI (Kg/m2)	32.8 ± 6.1	28.0 ± 5.7
Duration of diabetes (years)	14.1 ± 9.4	8.4 ± 7.6
HbA1C (%)	7.89 ± 1.62	8.30 ± 2.1
Cholesterol (mmol/L)	4.10 ± 1.10	4.0 ± 1.12
Triglycerides (mmol/L)	2.01 ± 1.48	1.77 ± 0.89
HDL (mmol/L)	1.25 ± 0.35	0.98 ± 0.25
LDL (mmol/L)	1.97 ± 0.81	2.20 ± 0.91
Cholesterol/HDL ratio	3.46 ± 1.19	4.18 ± 1.16
SBP (mmHg)	137 ± 20	132 ± 18
DBP (mmHg)	74 ± 11	81 ± 9.3
Vitamin B12 (ng/L)	290 ± 139	464 ± 228
Vitamin B12 deficiency, n (%)	91 (27)[b]	37 (12)
Folate (ug/L)	7.71 ± 9.77	13.6 ± 5.2
Folate deficiency, n (%)	29 (8.5)	0
Smoking, n (%)	26 (8.5)	75 (24)
Microvascular complications:		
Retinopathy, n (%)	124 (36)	132 (45)
Neuropathy, n (%)	53 (16)	99 (33)
Nephropathy, n (%)	41 (12)	83 (29)
Macrovasuclar complications:		
Coronary artery disease (CAD), n (%)	62 (18)	27 (9)
Cerebro vascular accidents (CVA), n (%)	19 (5.6)	6 (1.9)
Peripheral vascular disease (PVD), n (%)	23 (6.7)	17 (6)
Insulin use, n (%)	215 (63)	149 (46)
Metformin use, n (%)	221 (65)	242 (75)
Statin use, n (%)	286 (84)	154 (48)
Aspirin use, n (%)	246 (72)	44 (14)

[a]Mean ± SD (all such values); [b]Numbers (percentages) (all such values).

There were no gender differences in vitamin B12 and folic acid (Additional file 1: Table S1). The sex specific values of the other variables are also shown in the Additional file 1: Table S1. In both the populations, triglycerides and cholesterol/HDL ratio were significantly and inversely associated with vitamin B12 levels. HDL was positively associated with vitamin B12 levels in Europeans but cholesterol was not significantly associated with vitamin B12 in both the populations (Additional file 1: Table S2). No associations of vitamin B12 with other established cardiovascular risk factors such as BMI, systolic and diastolic pressure and HbA_1C were observed (data not shown).

Linear regression analysis was carried out to assess whether vitamin B12 independently associated with these cardiovascular risk factors in the type 2 diabetes patients by adjusting for all likely confounders. The model included age, gender, BMI, duration of diabetes, smoking, HbA_1C, use of metformin, statin and aspirin as independent variables. After all these adjustments, vitamin B12 independently associated with triglycerides in both the populations (Figure 1a,b) but cholesterol/HDL ratio only in Indians (Figure 1d).

Association of vitamin B12 levels and individual comorbidities were assessed by logistic regression analysis (Table 2). After adjustment for age, gender, BMI, duration of diabetes, smoking, HbA_1C, cholesterol, HDL, LDL, triglycerides, systolic and diastolic pressure, use of metformin, statin and aspirin, type 2 diabetes patients with low vitamin B12 levels were at a significantly higher odds of having coexisting CAD in Europeans (OR = 3.91; 95% CI: 1.09 - 14.05). A similar but non-significant trend of higher risk was observed in Indians (OR = 1.77; 95% CI: 0.376 - 8.33). No associations with other micro- or macro- vascular diseases were observed.

Discussion

Our study involving two different ethnic groups with type 2 diabetes patients had three main findings. Firstly, there was high prevalence of vitamin B12 deficiency in Europeans but interestingly lower than observed prevalence in Indians from South India. Secondly, vitamin B12 deficiency was associated with adverse lipid profiles. Thirdly, low vitamin B12 levels in type 2 diabetes patients were associated with an increased risk of CAD.

Studies in type 2 diabetes patients of European origin on metformin have reported the prevalence of vitamin B12 deficiency to range from 5.8% to 33% [10,11,13,22]. Our study confirms this in the British population, with a prevalence of 27% in all type 2 diabetes and 32.1% in type 2 diabetes with metformin. Our study is the first one to report the prevalence of B12 deficiency in the South Indian population with type 2 diabetes. Previous studies in north Indian population showed much higher rates of 67% in middle-aged men [18] and 54% in diabetes patients [23]. This is likely due to the differences in dietary habits between north and south Indians. South Indians consume higher quantity of fermented foods, which are rich in vitamin B12 [24,25].

In this study, vitamin B12 deficiency independently associated with triglycerides and cholesterol/HDL ratio in type 2 diabetes patients. Our findings were similar to the study in Indians with history of CAD [4]. Similar correlations were also found between B12 levels and total cholesterol and triglycerides in a group of Polish patients with established atherosclerosis, but this relationship was lost in regression analysis, which may be due to the smaller sample size in the study [21]. Vitamin B12 functions as a coenzyme in the conversion of methylmalonyl-

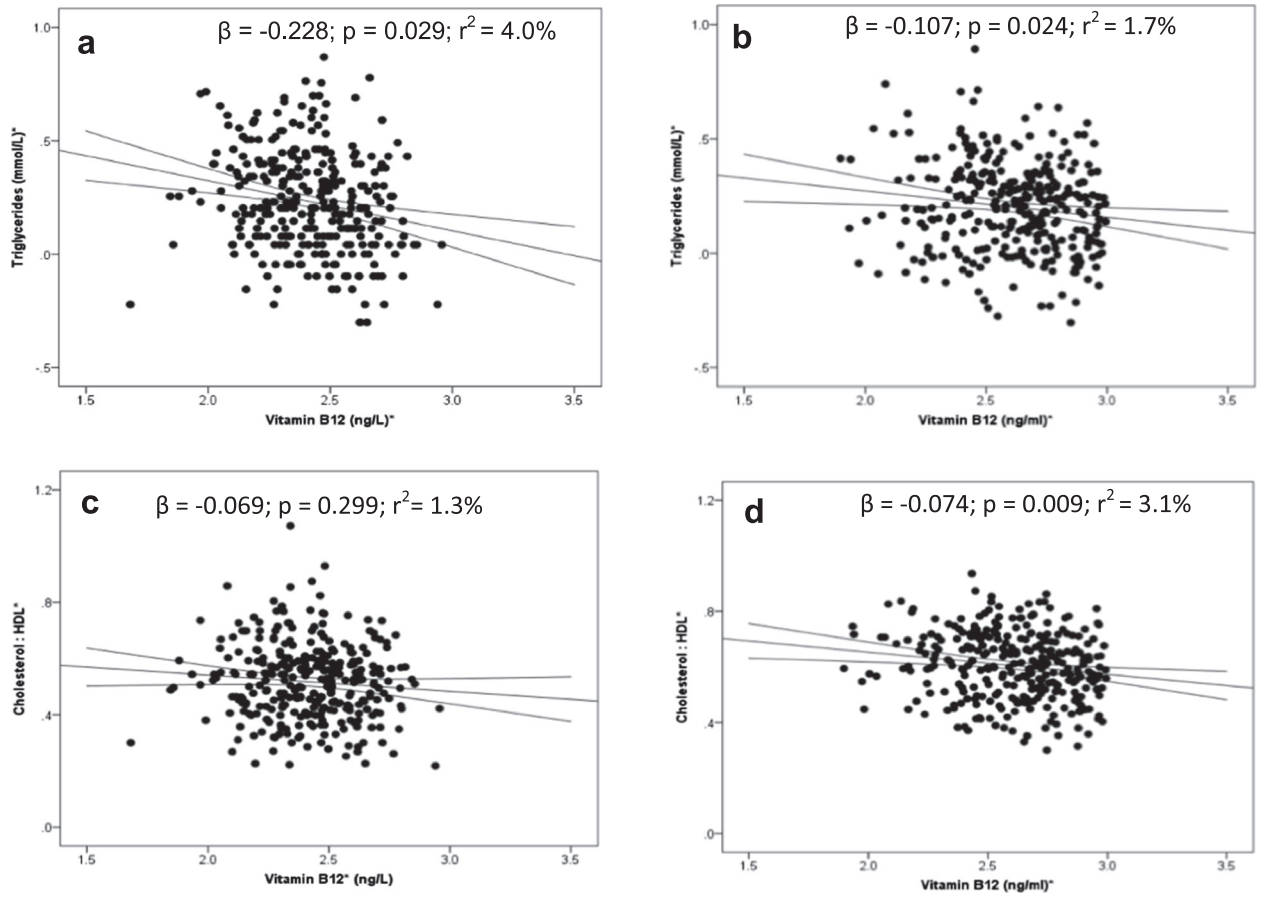

Figure 1 Regression of Vitamin B12 with (a) Triglycerides in Europeans, (b) Triglycerides in Indians, (c) Cholesterol: HDL ratio in Europeans and (d) Cholesterol: HDL ratio in Indians. *Log-transformed for statistical comparisons. Model included vitamin B12, age, BMI, sex, duration of diabetes, HbA_1C, smoking, use of metformin, statin & aspirin.

CoA (MM-CoA) to succinyl-CoA [26,27]. This reaction is blocked if there is vitamin B12 deficiency, resulting in accumulation of MM-CoA which inhibits the rate-limiting enzyme of fatty acid oxidation (CPT1 – carnitine palmitoyl transferase) [28], thus causing lipogenesis. This may be the likely mechanism for the link between B12 deficiency and adverse lipid parameters.

Our observation of an association of increased risk of CAD in type 2 diabetes patients with low B12 levels after controlling for all likely confounding factors is supported

Table 2 Logistic regression analysis of vitamin B12 with co-morbidities

Co-morbidities	Europeans			Indians		
	B (SE)	Odds ratio (95% CI)	p-value	B (SE)	Odds ratio (95% CI)	p-value
Microvascular complications:						
Retinopathy	0.294 (0.604)	1.342 (0.411, 4.386)	0.626	−0.085 (0.440)	0.919 (0.388, 2.176)	0.848
Neuropathy	0.132 (0.663)	1.141 (0.311, 4.186)	0.842	−0.208 (0.558)	0.812 (0.272, 2.424)	0.709
Nephropathy	0.790 (1.132)	2.203 (0.239, 20.267)	0.485	−0.721 (0.519)	0.487 (0.176, 1.346)	0.165
Macrovascular complications:						
Coronary artery disease (CAD)	1.364 (0.653)	3.911 (1.088, 14.054)	0.037	0.571 (0.790)	1.770 (0.376, 8.332)	0.470
Cerebro vascular accidents (CVA)	−1.226 (1.508)	0.294 (0.015, 5.644)	0.416	0.775 (1.584)	2.170 (0.097, 48.369)	0.625
Peripheral vascular disease (PVD)	0.552 (0.608)	1.737 (0.527, 5.722)	0.364	-	-*	-

Model included vitamin B12, age, BMI, sex, duration of diabetes, HbA_1C, Cholesterol, HDL, LDL, triglycerides, SBP, DBP, smoking, use of metformin, statin and aspirin.
*- Odds ratio cannot be computed because in the PVD group, the number of B12 deficient cases were zero.

by other findings in subjects with type 2 diabetes and non-type 2 diabetes. A study by Shargorodsky *et al.* found that vitamin B12 independently correlated with pulse wave velocity in type 2 diabetes patients, an accepted cardiovascular risk factor [29]. Weikert *et al.* [3] in a population-based prospective study showed the association between low vitamin B12 levels and increased risk of cerebral ischemia. Similarly, in south Asian women living in the UK with vitamin B12 deficiency anaemia had a higher prevalence of myocardial infarction and CAD [30]. We did not find any sex specific changes in our study. Thus our findings in support of the previous observations extend the knowledge on the role of vitamin B12 on CAD and its risk factors in type 2 diabetes patients. In spite of the fact that B-vitamins could provide an inexpensive and effective method for the prevention of CVD, their use was rejected, based on the negative results of randomized controlled clinical trials [31,32]. But, when examining the design of these trials, it appeared that concomitant medication such as statin/aspirin therapy applied along with the vitamin substitution could have obscured the separate effects of vitamins in cardiovascular prevention. However, a recent meta-analysis of these vitamin trials suggest that B vitamins are effective in primary prevention of cardiovascular diseases [33]. Similarly, a study in type 2 diabetes patients with another micronutrient supplementation, vitamin D, showed more significant improvements in the cardiometabolic profile [34].

Lipid abnormalities are unique in individuals with T2D and those are at risk of T2D (obesity, metabolic syndrome and pre-diabetes): the total cholesterol and LDL are lower in those with statins but higher in those without. In addition, in both groups the triglyceride levels are higher and the HDL levels are lower as statins have little effect on them [35-37]. In post-menopausal women with T2D and CAD who were not on lipid lowering medications, in addition to higher total and LDL cholesterol and higher triglycerides, homocysteine was also higher, suggesting a potential link between vitamin B12 and folic acid and abnormal lipid profiles [38-41]. It is known that increasing triglycerides and reducing HDL are early features of atherosclerosis, well before increasing LDL [38,42]. Therefore, our findings showing independent association of B12 with triglycerides and HDL in two different ethnic groups provide a possible mechanism how vitamin B12 could offer primary prevention of cardiovascular diseases in type 2 diabetes and may also be an option in the secondary prevention of disease, if statin therapy is accompanied by serious adverse effects.

The strength of this study is the inclusion of two cross-sectional study populations of type 2 diabetes patients from UK and India and comprehensive data from both the groups. However, it also has the following limitations. The study population were based in secondary care and not a true representative sample of all type 2 diabetes. A true primary care representative sampling of type 2 diabetes would have strengthened our findings. However, metformin is routinely prescribed in primary care. This therefore might have under estimated the prevalence of B12 deficiency and may have overestimated its association with CAD as the prevalence of CAD is likely to be higher in secondary care settings. A group of Indian diaspora living in the UK as well as the availability of biomarkers of vitamin B12 deficiency such as MMA and homocysteine would have strengthened the findings. In addition, being a cross-sectional study, it does not prove causality.

In summary, our study demonstrates for the first time that vitamin B12 deficiency in type 2 diabetes patients in two different ethnic groups is associated with adverse lipid parameters and higher risk of CAD. Currently, there are no guidelines advocating for routine screening for vitamin B12 deficiency among patients with type 2 diabetes. While optimal screening frequency remains to be determined, baseline tests at initiation of metformin therapy and subsequent annual testing of B12 levels may be appropriate. Our study also warrants updating of international guidelines for the management of type 2 diabetes.

Statement of human rights

This study was conducted with routine health care data. Therefore, full protocol review was not required. “Ethical approval: For this type of study formal consent is not required”.

Informed consent

Informed consent from all patients in the study was not necessary.

Additional file

Additional file 1: Table S1. Basic Characteristics of the study population. **Table S2.** Correlation between Vitamin B12 and lipid profile.

Competing interests

The authors declare that they have no competing interests.

Authors’ contribution

PS and VM conceived the research question and study design. AA, NS, HV, PGM, GT, VP, SK, PS contributed to data collection, statistical analysis and data interpretation in UK and RJ, RP, KG, RMA contributed in India. AA, PS and VM wrote the manuscript and approved the manuscript for submission. All authors contributed, revised and edited the manuscript. PS and VM is the guarantor of this work and had full access to all the data presented in the study and takes full responsibility for the integrity and the accuracy of the data analysis. All authors read and approved the final manuscript.

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