Predicting Gestational Diabetes from the First Trimester
– an exploration of alternative screening processes

Dr Siobhan Corcoran Mb Bch BAO MRCPI MRCOG
Department of Obstetrics and Gynaecology
RCSI

A Thesis Submitted to the School of Postgraduate Studies, Faculty of Medical Health Sciences, Royal College of Surgeons in Ireland in fulfilment of the degree of Doctor of Medicine (MD)

Supervisors; Professor Fionnuala Breathnach, Professor Fergal Malone

MD 2017
I declare that this thesis which I submit to RCSI for examination in consideration for award of a higher degree “Doctor of Medicine” is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original and to my knowledge does not breach copyright law and has not been taken from other sources except where such work has been cited and acknowledged within the text.

Signed__________________________________________________________

Student
Number_______________________________________________________

Date___________________________________________________________
# TABLE OF CONTENTS

List of Abbreviations........................................................................................................5
List of Tables.....................................................................................................................7
List of Figures..................................................................................................................8
Summary............................................................................................................................10
Acknowledgements..........................................................................................................11
Dedication..........................................................................................................................12
Introduction.......................................................................................................................13
Materials and Methods....................................................................................................37
Results...............................................................................................................................51
Discussion..........................................................................................................................87
References.........................................................................................................................101
List of Abbreviations

1,5 AG - 1,5 Anhydroglucitol
ACHOIS – Australian Carbohydrate Intolerance Study
ACOG – American College of Obstetricians and Gynaecology
ADA – American Diabetes Association
ADP – Adenosine Diphosphate
ATLANTIC DIP – Atlantic Diabetes in Pregnancy Study
ATP – Adenosine Tri Phosphate
BMI – Body Mass Index
CRP – C-Reactive Protein
CSO – Central Statistics Office
DM – Diabetes Mellitus
ECLIA – Electrochemiluminescence Assay
ELISA - Enzyme-Linked Immunosorbent Assay
EPU – Early Pregnancy Unit
G-6-P – Glucose-6-Phosphate
GDM – Gestational Diabetes
GK - Glucokinase
HAPO - Hyperglycaemia and Adverse Pregnancy Outcome
HbA1c – Glycosylated Haemoglobin type A1c
HIV – Human Immunodeficiency Virus
HRP – Horse Radish Peroxidase
IADPSG – International Association of Diabetes in Pregnancy Study Group
IT – Information Technology
LGI – Low Glycaemic Index
NICE – National Institute for Clinical Excellence
NICU – Neonatal Intensive Care Unit
OGTT – Oral Glucose Tolerance Test
OR – Odds Ratio
PCOS – Polycystic Ovarian Syndrome
PEP – Phosphoenol Pyruvate
PK – Pyruvate Kinase
POD – Peroxidase
PPH – Primary Postpartum Haemorrhage
PROD – Pyranose Oxidase
REC – Research Ethics Committee
ROC – Receiver Operating Characteristics
RR – Relative Risk
SHBG – Sex Hormone Binding Globulin
ST DEV – Standard Deviation
TOP – Termination of Pregnancy
TTN – Transient Tachypnoea of the Newborn
USPSTF – United States Preventative Services Task Force
WHO – World Health Organisation
List of Tables

Table 1 Varying screening protocols and diagnostic criteria for GDM ........ 18
Table 2 Profile of Screen Positive and Screen Negative Patients ........... 57
Table 3 Perinatal Outcome data for entire cohort .......................... 58/59
Table 4 Statistical Parameters of Biomarker results .......................... 60
Table 5 Results of OGTT .............................................................. 65
Table 6 Relationship of each biomarker in the first trimester to the likelihood of a screen positive OGTT as per IADPSG criteria. .......................... 69
Table 7 Mean 1,5 AG results in GDM and Non-GDM patients compared using T test ................................................................. 70
Table 8 Mean CRP results in screen positive and screen negative groups using T Test ................................................................. 70
Table 9 Mean SHBG results in screen positive and screen negative groups using T Test ................................................................. 71
Table 10 Mean Adiponectin results for screen positive and screen negative groups using T Test ................................................................. 71
Table 11 Linear regression analysis to correlate adiponectin values to individual OGTT results ................................................................. 75
Table 12 Low Adiponectin and a screen positive OGTT ..................... 75
Table 13 Positive predictive values of adiponectin >8.9 µg/ml for each serum glucose quartile using the Cochrane Armitage trend test ......................... 76
Table 14 Multiple logistic regression of biomarkers to risk of macrosomia ...... 77
Table 15 Multiple logistic regression the risk of operative vaginal delivery and first trimester biomarkers ................................................................. 78
Table 16 Multiple logistic regression between Biomarker and risk of Emergency intrapartum Caesarean Section ................................................................. 79
Table 17 Correlation between all SHBG values and cord blood c-peptide levels ......................................................................................... 80
Table 18 Logistic regression analysis of SHBG >350nmol/L for cord blood c-peptide <1.7µL ................................................................. 81
Table 19 Logistic regression of each history based risk factor and biomarker results ......................................................................................... 82
List of Figures

Figure 1 Schematic representation of 1,5 AG reaction ........................................44
Figure 2 Number of patients recruited .................................................................52
Figure 3 Distribution of Body Mass Indices of the Cohort ................................53
Figure 4 Risk factor profiling of the cohort ..........................................................54
Figure 5 Risk profile weighting of the cohort .........................................................54
Figure 6 Obstetric Morbidity History of Multiparous Recruits ............................55
Figure 7 Medical Comorbidities in the entire cohort ............................................56
Figure 8 Scatterplot of CRP Results .....................................................................61
Figure 9 Scatterplot of SHBG results .....................................................................62
Figure 10 Scatterplot of 1,5 AG results .................................................................63
Figure 11 Scatterplot of Adiponectin results .........................................................64
Figure 12 Serum glucose following at least 12 hours of fasting at 28 weeks’ gestation. Orange arrow points to diagnostic threshold as per IADPSG criteria ..................................................................................................................66
Figure 13 Serum glucose levels at 1 hour post prandial after ingestion of a 75g oral glucose load at 28 weeks’ gestation following at least 12 hours fasting. Orange arrow points to diagnostic threshold as per IADPSG criteria .................67
Figure 14 Serum glucose results at two hours post ingestion of 75g oral glucose load at 28 weeks following a period of at least 12 hours fasting. Orange arrow points to diagnostic threshold as per IADPSG criteria ..........................................................68
Figure 15 ROC curve of first trimester adiponectin levels as a predictor of a screen positive OGTT at 28 weeks gestation ..............................................................................72
Figure 16 ROC Curve of SHBG measured in the first trimester as a predictor of screen positive OGTT at 28 weeks gestation ........................................................................73
Figure 17 ROC curve of 1st trimester 1,5 AG as a predictor of a screen positive OGTT ..........................................................................................................................74
Figure 18 Linear Regression of SHBG levels and cord blood c-peptide results 80
Figure 19 Convenience of the arrangements for OGTT ........................................83
Figure 20 Overall convenience ..............................................................................84
Figure 21 Physical experiences reported during course of OGTT .......................85
Figure 22 Anxieties reported during OGTT ............................................................86
Summary

Gestational diabetes is recognised to be an increasingly common and treatable cause of maternal and infant morbidity. Screening and formal diagnosis of gestational diabetes have been at the centre of much of the scientific debate. This thesis aims to explore the ability of a panel of biomarkers in the first trimester of pregnancy to predict gestational diabetes and examines their link with the clinical complications of GDM. 248 women with known risk factors for GDM were recruited in the first trimester and had serum samples taken for c-reactive protein, sex hormone binding globulin, adiponectin anhydroglucitol. They underwent oral glucose tolerance testing at 28 weeks gestation and had prospectively collected perinatal outcome data. Cord blood c-peptide levels were drawn as an indicator of fetal hyperglycaemia. Our study found that adiponectin and SHBG demonstrated a correlation to the risk of onset of GDM in the univariate analysis. However, after adjustment for BMI, family history and ethnicity in the multivariate analysis SHBG loses significance. Following adjustment for BMI, family history and ethnicity 1st trimester 1,5 AG becomes a significant predictor of GDM. Mean 1,5 AG levels are significantly lower in the first trimester in women that will go on to develop GDM. However, none of these biomarkers exhibited a specific threshold value at which onset of GDM could be predicted absolutely with acceptable sensitivity and specificity. Our analysis of the relationship between sex hormone binding globulin values in the first trimester and neonatal hyperinsulinaemia (cord blood c-peptide> 90th centile/1.7µL) shows that in women with a high SHBG (>350nmol/L) early in pregnancy have an odds ratio of 2.7 to have a non-hyperinsulinaemic neonate. Our study also explored the patient experience of the OGTT. We found that despite the OGTT having adverse physical effects and being inconvenient, most women find it an acceptable test and would agree to be screened again in the future. BMI >30kg/m² and non-Caucasian ethnicity remain as independent predictors of GDM status in the adjusted analysis of the history-identified risk factors. Adiponectin and 1,5 AG remain independent predictors of GDM status after adjusting for all other variables.
Acknowledgements

Firstly, I wish to acknowledge the unwavering and illuminating support of my supervisors Professors Fionnuala Breathnach and Fergal Malone in guiding me through this process. I also wish to acknowledge the expert and essential help of Dr Elizabeth Tully in bringing the collection and analysis of this data to fruition and Dr Pat Dicker for his invaluable and insightful statistical knowledge and analysis.

I must also acknowledge the dynamic and practical support of Dr John O’ Loughlin at the Rotunda Hospital Laboratory and his staff including Philip Stafford for assistance in analysis of our samples. The excellent and essential help of my research assistants Lucy Shirren, Robin George, Natalie Achamallah and Sami Backley must also be highlighted.

This research would not have been possible but for the support of the Friends of the Rotunda Charity.
Dedication

For Don, for your kindness.
Overview of pathophysiology of hyperglycaemia in pregnancy

Gestational diabetes is hyperglycaemia which first occurs or is first detected in pregnancy (1). Pregnancy itself is shown to be a state of physiological insulin resistance which increases as gestation progresses (2). It is postulated that this is resultant from both hormones secreted by the placenta and increased maternal adiposity (3). In early pregnancy glucose tolerance is normal or even somewhat improved. The peripheral insulin sensitivity (in muscle tissue) is normal and the rate of production of glucose by the liver is steady. This is believed to be caused by the increased production of maternal oestrogen and progesterone in early pregnancy which is known to cause expansion of the beta cell mass in response to pregnancy (4). The hormones produced by the placenta especially human placental lactogen causes a gestation-related rise in insulin resistance beginning to occur notably from 20 to 24 weeks of gestation. As pregnancy advances and the placental mass increases in size, insulin resistance rises and the reserve of pancreatic beta-cells is recruited to help maintain normoglycaemia. In the normal healthy individual, the pancreatic B-cells can increase synthesis of insulin to meet the body’s increasing needs during pregnancy. In gestational diabetes, however, the B-cell function is insufficient to respond to rising insulin resistance and so a hyperglycaemic metabolic state ensues.

It is thought that there are three main causes for the pancreatic beta cell inadequacy that emerges in pregnancy which manifests gestational diabetes. In a small subgroup of women (<10% of those diagnosed with GDM), there are identifiable circulating immune markers or antibodies which point to the underlying development of Type 1 Diabetes Mellitus (T1DM). (5) (6). These patients are seen usually to develop T1DM shortly after pregnancy and the incidence of this subtype usually parallels the incidence of T1DM in the general population. A second, even smaller group (approximately 1-5% of those presenting with gestational diabetes) will have identifiable genetic variants that are responsible for monogenic diabetes such as maturity onset diabetes of the young (MODY) for example (7). The third and largest group by far, those with
beta cell dysfunction that arises in the setting of obesity and insulin resistance. For these women, there is likely a chronic beta cell deficiency which only becomes apparent during pregnancy when rising insulin requirements emerge. This is the group from whom development of Type 2 Diabetes Mellitus (T2DM) is very likely to occur. As a corollary of this, and because beta cell function deteriorates over time, these women are increasingly likely to develop GDM in each subsequent pregnancy and with worsening clinical outcomes successively.

Effects of hyperglycaemia in pregnancy

This transient hyperglycaemia occurring in pregnancy in evolutionary terms was possibly beneficial to the fetus in times of restricted food availability. However, in the modern age with an abundance of food sources surrounding us, this no longer presents an evolutionary advantage. In fact, a diagnosis of gestational diabetes is associated with a plethora of poor obstetric and neonatal outcomes (8).

In the short term, the neonates of mothers with untreated gestational diabetes have been shown to have a significantly increased risk of stillbirth, neonatal macrosomia, neonatal hypoglycemia, erythrocytosis, and hyperbilirubinemia compared to those in the treated group (9). Transient tachypnoea of the newborn (TTN) may occur where delivery has been iatrogenically premature in the setting of profoundly poorly controlled hyperglycaemia. Added to this the hyperinsulinaemic neonate must now physiologically adapt to a new and relatively hypoglycaemic environment. This can lead to hypoglycaemia that is difficult to control, causing jitteriness, poor feeding and sometimes neonatal seizures (10). One large study of 4,190,953 non-anomalous deliveries from gestational ages of 36 to 42 weeks (including 193,028 deliveries to women with GDM) in California showed that the risk of stillbirth from 36-42 weeks was significantly higher in women with GDM when compared with women without diabetes (17.1 vs. 12.7 per 10,000 deliveries, RR 1.34 (95% CI 1.2 – 1.5) (11). The pathophysiology of this occurrence is somewhat unclear and likely multifactorial but there is some evidence that suboptimal placental function coupled with higher incidence of fetal cardiomyopathy and chronic tissue
hypoxia may be at play (12). Macrosomia can lead to birth injuries such as Erb's palsy, clavicular fractures or neurological injury consequent of shoulder dystocia, abrasions, cephalhaematomas and facial nerve palsies from instrumental delivery. In the longer term the children of diabetic mothers have a 21% risk of developing diabetes in later life compared to a 4% risk in the offspring of non-diabetic mothers. A large American study of over 24,000 pregnancies affected by GDM demonstrated increased risk of childhood obesity of 29% in the first 10 years of life even among infants with a normal birthweight (13). This fact alone speaks to the degree of fetal programming that can potentially occur in utero and illustrates how a prenatal diagnosis of GDM can have far reaching and transgenerational consequences.

Fetal macrosomia in turn is responsible for an increase in maternal morbidity also such as increased rates of caesarean and operative vaginal delivery, obstetric anal sphincter injury and prolonged parturition (14). There is also a recognised association with gestational hypertensive disorders however it is unclear if this can be teased out from the fact that many of our patients with GDM are more obese and older than those without and so are more predisposed to hypertensive disorders. It is notable that difficult birth often results in separation of the mother-newborn dyad in the immediate postpartum period which can lead to problems initiating breastfeeding, further compounding the detrimental effects on the infant. Increased rates of intervention such as induction of labour and elective caesarean section are associated with a diagnosis of gestational diabetes. The incidence of type two diabetes in mothers diagnosed with GDM is as high as 50% in the decade after the index pregnancy (15). A history of GDM is associated with increased frequency of cardiovascular events later in life (16) The development of GDM therefore should be an important window into the future health of the patient and offers a real and timely opportunity to initiate education and lifestyle modifications.

Historical Overview of GDM and Controversies Surrounding Diagnosis

Controversy over how to screen for and diagnose gestational diabetes has been a major feature of the scientific debate on this subject for almost as long as
awareness of the condition’s existence (17) This transient glucose intolerance occurring during pregnancy, has been described as far back as 1824 (18) when a Dr Bennewitz from Germany chronicled 3 pregnancies in one woman that were characterised by excessive thirst, recurrent glycosuria and grossly macrosomic infants. In 1882 Duncan presented a case series of 22 pregnancies occurring in 16 women with gestational diabetes (or “puerperal diabetes” as he called it). He accurately concluded that this condition is occurs in pregnancy, resolves with the termination of pregnancy and while the labour and delivery may be unaffected, stillbirth and intrapartum death were more common events in this group (19). Even in the mid 1800’s much debate centred on the thresholds for “physiological glycosuria” in pregnancy at which levels no adverse outcomes would be seen (20). The first reports of employing a glucose tolerance or glucose challenge test in pregnancy were presented by Brocard in 1898 (21) The 1950’s saw the identification for many risk factors for the development of gestational diabetes and the concept of formal screening for GDM began to be explored in the late 1950’s (22,23). O’Sullivan et al described the pregnancy -specific glucose tolerance test in 1964 (24) In their paper they discuss the difficulties associated with stringent diagnostic criteria – over diagnosis leading to an economic healthcare burden and undue psychological morbidity for the patient. As more data became available the debate waged on and in the 1970’s and 1980’s various workshop groups attempted to streamline the thresholds for diagnosis, who should be screened and at what point in pregnancy (25). All failed to reach a worldwide consensus.

The Fourth International Workshop on GDM in 1997 proposed identifying women at low risk and using a “2-step” glucose challenge followed only by an oral glucose tolerance test if challenge-positive thus reserving formal OGTT for all women considered at high risk (26). It is recognised that the process of screening itself not only has resource implications but also may be associated with psychological morbidity and this is explored later in this thesis.

One of the landmark studies in this area came in 2008. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study Group sought to elucidate the risk of adverse outcome which would be associated with various thresholds for glucose intolerance in pregnancy (27). This large multicentre study collated data
from almost 25,000 women including OGTT results, obstetric and neonatal adverse outcomes. The study showed that the risk of adverse outcome was a continuum with maternal glucose tolerance with no clear cut off point above which risk significantly increased. The International Association of Diabetes and Pregnancy Study Groups (IADPSG) came into being in 1998 and in 2008 it developed diagnostic thresholds for GDM based on the results of the HAPO study. It was envisaged that these criteria would provide a worldwide consensus on the diagnosis of GDM however they have been criticised by many diabetes and obstetrical organisations as the incidence of GDM increases dramatically when these thresholds are applied. Echoing O’Sullivan’s concerns back in 1964, they cite an onerous clinical and economic burden of care. Half a century of research has thrown more tinder on a blazing debate.

Current Screening Protocols

A screening tool establishes the risk of disease in the otherwise healthy person. As we have seen above, the timing and mode of administration of a screening test for diagnosis of gestation diabetes has long been a source of controversy. The end of the second trimester/beginning of the third is most frequently chosen (24-28 weeks) as this is felt to give the optimal trade-off between detection of evolving gestational diabetes and time available to manage and treat the condition to prevent adverse outcomes. There is currently insufficient data to support routine OGTT screening prior to 24 weeks’ gestation (28). Different glucose loads and plasma glucose thresholds for diagnosis have been used in various centres over the last number of years. Table 1.1 gives an idea of the varying diagnostic criteria which existed and formed the framework for diabetes research over the last 2 decades. Because of this, reaching a consensus on international research has been marred by inconsistencies in terms of reference.

The IADPSG recommendations for screening use thresholds predictive of adverse pregnancy outcomes and the Endocrine Society advocates using these criteria (28) The Endocrine Society also identifies thresholds for the diagnosis of Overt Diabetes during an OGTT in pregnancy – a fasting plasma glucose of
>7mmol/L or a 2-hour plasma glucose >11.1mmol/L. The most recent WHO guidelines from 2013 endorse the IADPSG criteria also.

**TABLE 1 VARYING SCREENING PROTOCOLS AND DIAGNOSTIC CRITERIA FOR GDM**

<table>
<thead>
<tr>
<th>Organisation</th>
<th>Fasting plasma Glucose nmol/L</th>
<th>Glucose Challenge</th>
<th>1-h Plasma glucose nmol/L</th>
<th>2-h Plasma glucose nmol/L</th>
<th>3-h Plasma glucose nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO 1999</td>
<td>&gt;7</td>
<td>75g OGTT</td>
<td>Not required</td>
<td>≥7.8</td>
<td>Not required</td>
</tr>
<tr>
<td>(one or more values)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>American College of Obstetricians and Gynaecologists</td>
<td>&gt;5.3</td>
<td>100g OGTT</td>
<td>&gt;10</td>
<td>&gt;8.6</td>
<td>&gt;7.8</td>
</tr>
<tr>
<td>(two or more values)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canadian Diabetes Association</td>
<td>&gt;5.3</td>
<td>75g OGTT</td>
<td>≥10.6</td>
<td>&gt;8.9</td>
<td>Not required</td>
</tr>
<tr>
<td>(two or more values)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IADPSG</td>
<td>&gt;5.1</td>
<td>75g OGTT</td>
<td>≥10</td>
<td>&gt;8.5</td>
<td>Not required</td>
</tr>
<tr>
<td>(one or more values)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>American Diabetes Association</td>
<td>&gt;5.3</td>
<td>100g OGTT</td>
<td>≥10</td>
<td>&gt;8.6</td>
<td>&gt;7.8</td>
</tr>
<tr>
<td>(following a 50g non fasting glucose challenge test)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Secondly the question of a risk factor based screening or universal screening of all pregnant patients has been the centre of some debate. Using risk factors identified from the patient’s history to choose who to selectively administer the OGTT gives a 60% detection rate for a 40% false positive rate (29). It has been
shown that the current risk factor based screening misses up to a third of cases of gestational diabetes (30). Griffin et al showed that the incidence of GDM was almost doubled in the universally screened group compared to the “risk-factor” selected group (2.7 vs. 1.45%, p< 0.03). Interestingly the incidence of macrosomia, admission to NICU, hyperbilirubinaemia and preterm delivery were all higher in the “risk-factor” selected group (31). A corollary of this issue is whether to simply universally screen women using a 50g one-hour glucose challenge test (a two-step approach) or to proceed directly to universal diagnosis with a formal OGTT. The glucose challenge test has some benefits. The woman does not need to fast and so opportunistic screening can occur when she presents for antenatal care. However, it does have a significant false negative rate and is described as only “moderately reproducible” as the results are variant upon the time of day and the time elapsed since the last meal (32) (33) (34). Another potential pitfall with a two – step process is that some woman may not engage with both tests. Yapa et al showed that up to 23% of women that screen positive using the glucose challenge test will fail to present for their formal OGTT (35). In the large Toronto Tri-Hospital Gestational Diabetes Project (34) 10% of those screening positive with the GCT did not proceed with the OGTT even though these women gave informed consent and were formally enrolled in a research study.

Most recently universal screening has been increasingly recommended given the estimated prevalence in the population, the incidence of missed diagnosis in the “risk factor” based approach and the problems associated with a two-step procedure as outlined above. It has been shown in a randomised controlled trial that Universal screening using the IADPSG criteria may increase diagnoses of GDM to include up to 15-20% of the obstetric population (36). This raises questions regarding the increased healthcare costs, possible increased intervention and caesarean delivery and psychological burden on the patient with lesser degrees of impaired glucose tolerance.
Prevalence

There is undoubtedly a high and increasing prevalence of gestational diabetes among the obstetric population globally and current estimates put it at between 1 and 14% depending on the population being screened and the screening modality used (37,38,39,40) The ATLANTIC DIP study performed universal GDM screening using a 75g oral glucose tolerance test and the IADPSG criteria at 24-28 weeks’ gestation in five antenatal centres along the Irish Atlantic seaboard. The findings of this study are particularly relevant to ours as it is a similar patient cohort and model of antenatal care. They found the prevalence of impaired glucose tolerance or gestational diabetes to be one in ten pregnant women screened and recommended, based on this high incidence, that universal screening should be adopted (41). Khalifeh et al demonstrated a rising incidence of GDM in a cohort of patients over a 10-year period in Ireland and an increasing need for insulin treatment among those diagnosed. This was not paralleled by a rising incidence of pregnancies complicated by pre-gestational diabetes (42). This rising incidence of gestational diabetes is likely related to the global epidemic of overweight and obesity and in Ireland the changing ethnic backgrounds of antenatal patients have contributed to this. It may also be partially because of more stringent diagnostic criteria and improved multidisciplinary clinical care pathways for those diagnosed, such that problematic refractory hyperglycaemia is recognised and treated aggressively with insulin therapy.

Risk Factors

Risk factors for the development of gestational diabetes are listed in most international guidelines (28,43,44). The most commonly identified risk factors are a body mass index >30 kg/m², family history of type one or type two diabetes, polycystic ovarian syndrome, previous delivery of a macrosomic infant, high risk ethnicity (non-Caucasian eg Asian, Afro-caribbean or Indian), and advanced maternal age (>40 years).

The link between maternal obesity and GDM is long established. Chu et al conducted a meta-analysis of 20 studies on this subject in 2007 to better estimate the magnitude of this association (45). In their study, they found that
the unadjusted odds ratios of developing GDM were 2.14 (95% CI 1.82–2.53), 3.56 (3.05–4.21), and 8.56 (5.07–16.04) among overweight, obese, and severely obese women, respectively, when compared to normal-weight pregnant women. Similarly, the association with advanced maternal age and GDM has been borne out and quantified in other studies. Favilli et al in a study of 630 pregnant women over the age of 40 years described the risk of GDM as an OR of 3.6 (CI 1.232–10.454) (46). Jenum et al showed the GDM prevalence rates to be significantly different among Western European women versus ethnic minority groups at 24 and 36.8% respectively, P<0.001 when using the IADPSG diagnostic criteria (47).

Other non-traditional risk factors for GDM have been reported on also. Wang et al conducted a large study of 13,732 pregnancies arising from artificial reproductive techniques (ART) and 386,660 spontaneous conceptions (non-ART). They found a significantly increased incidence of GDM (7.6% vs. 5% p=0.01 – translating to a 28% increased likelihood) in ART pregnancies compared to non-ART (48). This may be because of the older age of the mothers, ovulation failure secondary to polycystic ovarian syndrome or a combination of both. Currently international guidelines on selection of patients for screening for GDM do not identify a history of ART as a risk factor. The burgeoning increase in ART in the last two decades prompts us to reconsider this. Retnakaran et al performed a systematic review and meta-analysis of 20 studies looking at the risk of GDM dependant on the fetal gender. They conclude that there is a 4% increased risk of GDM in pregnancies where the fetus is male. They propose that there is a hitherto poorly understood interplay between the fetal and maternal metabolism. This association is still seen after adjustment for other risk factors including obesity, age, ethnicity and family history (49). It is unlikely however, even in the era of NiPT (non-invasive prenatal testing), that all parents would elect to know the gender of their baby in the first trimester for the purposes of GDM risk stratification and so it is unlikely to prove a clinically useful pearl to this end.

There is mounting evidence that screening for GDM using maternal characteristics and the personal obstetric history is associated with a higher detection rate for a given false positive rate if the maternal factors are combined
into a multivariate logistic model, rather than if we treat each factor as an independent screening test, as is the current recommendation in the NICE Guideline (50,51,52,53,54) Many authors advocate that using these simple prediction models will decrease unnecessary screening and increase the detection rates currently achieved. Syngelaki et all recently published, in 2015, in a large study of over 71,000 pregnancies on the first trimester screening for gestational diabetes using a maternal characteristics model. They report a detection rate of 83% using a logistic regression incorporating maternal age, weight, height, racial origin, family history of diabetes, use of ovulation drugs, birth weight, and previous history of GDM with a higher area under the receiver operating characteristic curve than all previously reported models (55).

Rationale for treatment of gestational diabetes

It has now definitively been shown that intervention and appropriate treatment of both impaired glucose tolerance and overt gestational diabetes significantly improves perinatal outcomes (56,57). The ACHOIS study group showed infants born to mothers receiving intensive therapy had significantly lower birthweights than those born to women receiving routine care. This study also demonstrated an overall reduction in serious perinatal morbidity and an improved health related quality of life for the mother when diagnosed gestational diabetes is treated and managed appropriately. This study group have also shown that while diagnosis and appropriate management of gestational diabetes increases healthcare costs in the immediate term, the long term cost – benefit analysis also favours treatment (58).

Others have described how the complications of gestational diabetes lead to increased healthcare costs. Kolu at al showed that total mean health care costs adjusted for age, body mass index and education were 25.1% higher among women diagnosed with GDM when compared to those with no GDM (59). The authors conclude that “Effective lifestyle counselling by primary health care providers may offer a means of reducing the high costs of secondary care”. It has also been demonstrated that interventions aimed at preventing the onset of GDM (primary prevention) result in significant healthcare cost savings (60). The National Institute for Health and Clinical Excellence in the UK have undertaken
a large review of screening for gestational diabetes and found it to be cost –
effective (61).

Management of gestational diabetes

The management of gestational diabetes rests initially on dietary (62) and
lifestyle modification to optimize glycaemic control. In those with more overt or
refractory glucose intolerance, the use of insulin becomes necessary. It stands
to reason that earlier diagnosis facilitates earlier treatment and ultimately
improved perinatal outcomes. The benefits of early intervention cannot be
underestimated. 75-80% of women diagnosed with gestational diabetes will be
successfully managed and achieve euglycaemia by adhering to a calorie
controlled, low glycaemic index (LGI) diet (63). Shyam et al described in a
randomized trial of 77 Asian women with a past history of GDM how a LGI diet
resulted in statistically significant improvements in glucose tolerance and body
weight reduction as opposed to a low-fat diet with a similar caloric intake (64).
Mohd Yusof et al found in a review of dietary management of GDM that
adherence to a low glycaemic index diet showed a trend towards lower birth
weight centile and importantly, the effect of the intervention was greater when
instituted earlier in pregnancy (65). Walsh et al conducted a large randomized
controlled trial of 800 women in Ireland with a history of a previous infant
weighing over 4kg being assigned to a low GI diet or no treatment and this
group found significantly less gestational weight gain and improved glucose
tolerance in the intervention arm (66). While a recent Cochrane review of the
benefits of exercise in the management of gestational diabetes found somewhat
limited evidence for the prescription of exercise to manage gestational diabetes
(67), there are several ongoing registered clinical trials regarding this subject
which have yet to conclude and publish results. Physical activity forms a
cornerstone in the management of Type II diabetes and has been shown to
delay the onset and reduce insulin requirements.

Oral hypoglycaemic agents such as metformin have recently been used in
pregnancy for the management of gestational diabetes and also in those with
pregestational type 2 diabetes for some time. Despite the fact that metformin is
known to cross the placenta, there are no serious safety concerns regarding its
use in pregnant patients. The American College of Obstetrics and Gynecology (ACOG) and the UK National Institute of Health and Care Excellence (NICE) have given recommendations that either metformin or glibenclamide can be used as oral hypoglycaemic agents in the management of gestational diabetes. Where dietary and lifestyle modifications have proved insufficient in achieving normo-glycaemia, the use of insulin in gestational diabetes is very common and well described for many years.

INTRODUCTION PART II – Concept of this Thesis

Potential for developing an early screening test

This thesis proposes to investigate if there is a panel of biomarkers which, when analysed early in pregnancy can with good sensitivity and specificity, predict the later onset of gestational diabetes. This information, when gleaned early in pregnancy, facilitates more a targeted screening process, earlier diagnosis and intervention which ultimately translates into better perinatal outcomes and healthcare savings for this group of high risk pregnancies.

Theoretical Framework

The theoretical framework on which this study is founded is the concept of Inversion of the Pyramid of Prenatal Care as proposed by Nicholaides and the Fetal Medicine Foundation (68). Since the early 20th century it had been recommended that women should first be seen for prenatal care by the 16th week of pregnancy and then 24 and 28 weeks, fortnightly until 36 weeks and then weekly until delivery. The rationale for having a high concentration of prenatal care weighted in the third trimester was that most of the serious complications of pregnancy such as gestational hypertension, pre-eclampsia, cholestasis, and fetal growth restriction etc. are seen to emerge by the clinician in the third trimester. There is however a growing body of evidence in the past two decades that suggests the pathogenesis of and predisposition to many of these conditions is detectable early in pregnancy. Thus, by weighting the majority of prenatal investigations early in pregnancy, a select subgroup of the population can be identified which benefits from surveillance and intervention.
whilst allowing the majority of the low risk pregnancies proceed with minimal intervention and cost.

**Gaps in current knowledge**

While numerous studies have recently examined various individual biomarkers and their relationship to onset of gestational diabetes, most have been carried out in a small cohort of patients and not in a group of high risk women. The four biomarkers which we intend to study have not been examined in a single population. The idea of designing an early pregnancy screening test for gestational diabetes, incorporating this panel of biomarkers in conjunction with a risk factor analysis which generates likelihood ratios for onset of disease has not been explored in detail in the literature. Few studies have examined the relationship between the early pregnancy analytes and perinatal outcomes. We will gather all outcome data, both obstetric and neonatal and describe the relationship to the biomarkers as measured in early pregnancy.

Secondly we intend to elucidate the true impact of the current screening OGTT on the pregnant population. As previously mentioned, the psychological burden of screening for GDM has been documented but the physical side effects of the test on pregnant women have not been explored in great detail in the literature. A Cochrane review of alternative screening tests for gestational diabetes identified five small trials of various methods of administering the glucose load but found insufficient evidence in these small trials to recommend the most acceptable method for screening for GDM (69). Taking these concepts on board, we plan to investigate the acceptability of the current IADPSG recommended OGTT screening test to the population concerned.

**Significance of the Study**

This study will not only add to a growing body of evidence for the utility of the individual biomarkers in the prediction of later onset of gestational diabetes but it aims to allow early identification of a sub population that will benefit from timely diet and lifestyle intervention to offset the burden of disease. Clinicians may potentially use a phlebotomy based screening test administered at the
booking visit to identify a group of small cohort very high risk patients who may then go on to receive early intervention (diet and lifestyle modification) and later perform and oral glucose tolerance test. The benefits of this approach would be multiple. Highly selective OGTT screening would result in significant cost savings for the healthcare provider (accepting that administration of universal screening comes at considerable financial cost). It would potentially facilitate earlier administration of the OGTT thus leaving a longer time for close surveillance and intervention in affected pregnancies. Another benefit of this is that a reduced number of women would need to endure the screening test which could reduce anxiety and eliminate the unpleasant physical side effects of the OGTT.

Assumptions, Limitations, and Scope (Delimitations)

We assume that of the patient selected for the study a certain subgroup will screen positive for gestational diabetes.

In terms of the results on the patient experience of current OGTT screening practices it can be assumed that participants will answer truthfully and accurately to the interview questions based on their personal experience, and that participants will respond honestly and to the best of their individual abilities.

The concept of the “fetal glucose steal” phenomenon (70) could potentially affect the results of our study. It is long and well recognised that some patients clinically exhibit the signs of GDM – macrosomic fetus, polyhydramnios and occasional glycosuria but yet they screen negative for GDM when the OGTT is administered. It is postulated that early in gestation when glucose levels have been very high due to maternal insulin deficiency and or resistance, the fetus becomes hyperinsulinaemic. Then at the time of the OGTT, in the late second or early third trimester, since glucose readily and freely crosses the placenta, the fetus metabolises a proportion of it so that the serum glucose levels of the mother are somewhat attenuated and the OGTT is negative. In these cases, therefore, the clinical complications of uncontrolled hyperglycaemia develop in the pregnancy despite a “normal” serum fasting, 1-hour and 2-hour postprandial glucose level. Similarly, the complications of GDM can sometimes occur in patients that seemingly achieve normoglycaemia with the use of oral
hypoglycaemic agents or insulin. Many experienced clinicians will readily identify with this scenario where all of the clinical history and examination findings point to a diagnosis of GDM but yet the patient screens “negative”

It is recognised that glycolysis causes glucose levels in whole blood to decrease over time (71). It is estimated that the levels decrease by 5-7% per hour but that this rate varies in accordance with the temperature (lower temperatures slow the rate of glycolysis), the glucose concentration and the leucocyte count of the sample. In recognition of this it is recommended that plasma be separated from the cells within 60 minutes of phlebotomy or that a tube containing sodium fluoride should be used to reduce the level of glycolysis. Daly et demonstrated in a recent study that when the American Diabetic Association recommendations were strictly adhered to, there was a 2.7-fold increase in the detection of gestational diabetes when compared to standard clinical practice in a cohort of 155 women (72). In our study we used the tubes containing sodium fluoride but undoubtedly some level of glycolysis will occur and this could potentially subtly affect our results. The most recent American Diabetes Association recommendations regarding sample collection prescribe that the sample should be taken in a sodium fluoride tube, placed in an ice-slurry and centrifuged within 30 minutes. The latter recommendations were issued following commencement of this study and therefore did not form part of the protocol.

It is also recognised that glucose intolerance is a spectrum with no particular threshold below which complications of hyperglycaemia do not arise. In our cohort, it is possible that many women may screen negative and have serum glucose levels that are barely just under the threshold for diagnosis. It is possible and indeed likely that these pregnancies may be affected by the complications of hyperglycaemia also. This is a particularly salient point which has informed how we have analysed our results and we discuss this further in the conclusions chapter.

The study may be limited by the number of patients recruited especially if a smaller than expected subgroup screen positive for gestational diabetes. Follow up data in all pregnancies is reliant upon the patient delivering her baby in the
unit to which she first registered her prenatal care and cord blood consistently being taken at the time of delivery.

The scope of results of the study would be applicable to any clinician administering prenatal care to a pregnant woman. In terms of application, the study circumstances are very reproducible in current international obstetric practice.

**What is the ideal biomarker?**

The term “biomarker” refers to a characteristic or substance or gene which may be used objectively to identify a biological process (physiological or pathological). Biomarkers may be used to identify disease or monitor disease process. Ideally the optimally appropriate biomarker would be sensitive, specific, reproducible, easily accessible/acceptable to patients, easy to measure and cost effective. It would also be pertinent that the disease process which one seeks to identify is modifiable. In the setting of capturing women as part of their first visit in routine antenatal care if would be useful if measurement did not require fasting. The focus on identifying and assessing a biomarker capable of predicting GDM has sharpened in recent years.

Since the early 90’s adipose tissue has come to be regarded as an endocrine organ, secreting proteins known as adipokines (73). Examples of these include leptin, adiponectin and resistin. The secretion of these adipokines becomes altered in the obese state such that they cause adipose tissue inflammation and decrease muscle and liver insulin sensitivity creating a vicious circle (74) Compounding this further, adipokines may act on the hypothalmus to drive increased food intake and reduced energy expenditure. Obesity is an inflammatory state where the macrophages of the adipose tissue secrete proinflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and interleukin-12 (IL-12) (75) Pregnancy is a state where the balance between pro-inflammatory and anti-inflammatory mediators is shifted. For example, in pregnancy the T-helper cells produce more T-2 cytokines which are anti-inflammatory in nature and help to mediate the challenge which the fetal allograft presents to the mother (76) It is notable with regard to pregnancy combined with obesity and particularly excessive
gestational weight gain that the balance is flipped in favour of pro-inflammatory cytokines such as TNF-α and IL-6 and this is linked to the development of GDM (77). There are therefore, a large number of potential biomarkers which may be studied with regard to the link with GDM. Below I outline the individual biomarkers selected for interrogation and following on from this we discuss other markers which we did not study.

Biomarkers Chosen

(1) **Sex hormone-binding globulin (SHBG)** or sex steroid-binding globulin (SSBG) is a glycoprotein that binds to the sex hormones, androgen and estrogen. Levels of SHBG have been shown to decrease with high levels of insulin. The normal reference range of adult female premenopausal levels of SHBG are between 40 – 120 nmol/L however data on normal reference values for the pregnant population is scant. SHBG is also produced by the placenta. Smirnakis at al described the use of SHBG measurement in the first trimester as a predictor of later onset of gestational diabetes (78). The study evaluated SHBG taken in the first trimester or early in the second trimester in 35 women with subsequent GDM versus 73 controls and 37 patients with an abnormal glucose challenge test. They note that among three different biomarkers examined in the first trimester, SHBG gave the best performance as a predictor of subsequent diagnosis of gestational diabetes. The authors also highlight how SHBG is a test which most hospital laboratories can do readily and at little cost. This makes it appealing to study its utility in the context of routine antenatal care. Maged at al (79) analysed 269 patients of which 27 developed GDM. They found that SHBG at a cut-off value of 211.5 nmol/l showed a sensitivity and a specificity of 85% and 37%, respectively. They also looked at high sensitivity C-reactive protein (CRP) and note that when the two biomarkers are combined GDM is predicted with a sensitivity and specificity of 74.07% and 75.62%, respectively with an overall accuracy of 75.46%. Both studies were performed using a large cohort of patients and were subject to rigorous statistical analysis. Our study would differ in that ours is a large cohort
already deemed at high risk of developing GDM rather than the unselected population which that group studied.

(2) CRP is a protein found in plasma which rises in response to inflammation and as part of the acute phase response. It is synthesised in the liver and has a half-life of 48 hours which is constant. The levels vary depending on the rate of production and so correlate to the severity of the precipitating cause. High Sensitivity CRP (hs-CRP) measures even low levels of CRP using technique called laser nephelometry. The test gives results in 25 minutes with a sensitivity down to 0.04 mg/L. It is recognised that higher levels of CRP are seen in pregnancy than in the non-pregnant population and that it shows a decreasing trend through pregnancy and post-partum (80). It is also well documented that high levels of CRP correlate with obesity. CRP has also been independently investigated as a biomarker capable of predicting the onset of gestational diabetes. Wolf et al (81) found that in a prospective nested case-control study where 43 women subsequently developed GDM; higher levels of CRP in the first trimester positively correlated with the onset of disease and the difference between the two groups was statistically significant. However once the results were adjusted for obesity the association was attenuated leading the authors to recommend that further larger studies should evaluate this in more detail. Again, CRP is a test which is readily available to the clinician in almost all clinical laboratories and is not expensive to run. Both laboratory and clinical staff would be extremely familiar with CRP and the infrastructure for routine CRP testing would already be largely in place if this marker proved to be of utility. This coupled with the data already available and the biological plausibility made it an attractive biomarker to study in the prediction of GDM. A potential difficulty with using this inflammatory marker as part of a prediction model for GDM however is its non-specific nature. CRP levels may become elevated suddenly and for a whole host of reasons – infections, auto-immune processes and malignancy among others. Elevated CRP results may lead to unnecessary clinical investigations
and undue anxiety to the patient and an increased workload and financial cost to the care-provider if it were routinely employed in otherwise asymptomatic patients.

(3) Adiponectin is a protein which modulates glucose metabolism by affecting insulin sensitivity. It is secreted both from adipose tissue and the placenta in pregnancy (82). It can be found in serum in a variety of different multimers of which the high molecular subtype is the most metabolically active (83). Adiponectin is anti-inflammatory and insulin sensitising. This is achieved by initiating glucose uptake in the skeletal muscle mass and by decreasing glucose production in the liver. Adiponectin levels inversely correlate with body fat percentage and so low levels are seen in obesity which is surprising given that it is produced in adipose tissue. The levels are seen to rise during periods of caloric restriction and females are noted to have higher levels than males. There is an interplay between proinflammatory cytokines such as TNF-α and adiponectin as TNF-α is seen to suppress the transcription of adiponectin by adipocytes and so exacerbate the cycle of inflammation (84). Levels are seen to decline through pregnancy (85) and correlate negatively to BMI and adiposity (86). Low levels of adiponectin have been repeatedly shown to be an independent risk factor for the development of type 2 diabetes (87). There is also good deal of published data surrounding the use of adiponectin as a biomarker linked to the onset of GDM (88,89,90). Low levels of adiponectin are strongly associated with the onset of GDM independently of maternal pre-pregnancy body mass index (BMI) and insulin sensitivity (91,92,93,94). Lain et al demonstrated in a nested case control study of 59 women (30 of whom developed gestational diabetes) that women with a first trimester adiponectin concentration which was below the 25th percentile were ten times more likely to be diagnosed with GDM when compared to women with higher adiponectin levels (95). In 2014 Xu et al published a systematic review and meta analysis of 15 studies showing lowered adiponectin levels in GDM patients compared to controls even after
adjusting for BMI (96). Interestingly there is also evidence that maternal adiponectin may modulate fetal growth patterns by impairing placental insulin signaling and reducing insulin-stimulated amino acid transport (97) and so it is proposed that decreased levels may be implicated in fetal macrosomia seen in both GDM and non GDM women. The known biological actions of adiponectin coupled with emerging evidence regarding its link to GDM identified it as a biomarker we should study in our cohort of high risk patients. Added to this there is a growing body of data suggesting down-regulation of adiponectin occurs well in advance of the emergence of clinically detectable glucose intolerance as seen in GDM (98). This fact lends itself to the concept of a readily detectable pre-clinical phase for a modifiable disease process. Adiponectin is easily accessible in serum and routine phlebotomy occurs as part of standard antenatal care. Adiponectin has the added benefit of being reproducible despite the fasting or non-fasting state of the subject (up to 72hrs) (99). Women presenting for phlebotomy at the first prenatal visit are not given any instruction on fasting. To date the available information on adiponectin as a biomarker for GDM has come from small, retrospective case-control or cross-sectional studies. Different sampling sources (maternal, cord or placental) and different assay methods reported have added further complexity.

(4) 1,5 anhydroglucitol (1,5 AG) is a monosaccharide which is present in almost all foods. 1,5 AG is in a steady state normally and almost completely reabsorbed within the renal tubules. Serum levels decrease during periods of hyperglycaemia due to increased urinary excretion. In the absence of hyperglycaemia the levels return to normal within 14 days. It may be used therefore to detect short term excursions in glycaemia which may not be represented by the traditional methods such as measurement of HbA1c and Fructosamine. It has been shown to more accurately detect hyperglycaemic episodes than other markers when compared to continuous glucose monitoring packs (CGM) (100). Initially this marker was not investigated in pregnancy as it was felt that
physiological changes in kidney function and haemodynamic parameters may invalidate the results but Dworacka et al recently demonstrated it to be a reliable indicator of short term hyperglycaemic episodes in pregnancy in a group of 55 women (both with gestational and pregestational diabetes) and suggests its use as an adjunct to HbA1c (101). Recently a study of 85 patients with a mixture of T1DM, T2DM and gestational diabetes were investigated by Shani et al. They found an inverse relationship between the 1,5 AG levels measured at 28 weeks gestation and the birthweight of the infants. The group conclude that this marker “may be useful in the assessment of glycaemic control in pregnancy in addition to A1c” (102). Nowak et al also published on the inverse relationship between mid-trimester 1,5 AG levels and birthweight in women with T1DM. They found 1,5 AG to be a stronger predictor of macrosomia than HbA1C (103). There is only one study we identified that attempts to determine the normal 1,5 AG values in diabetic, non-diabetic pregnancies and non-pregnant women. Tetsuo et al showed the level of 1,5 AG in the non-pregnant woman was 18.6±5.2 mg/l (mean±SD). While the group noted that levels began to decrease in parallel with gestation from about 9 weeks they do not present reference ranges for pregnancy (104). 1,5 AG was appealing to us as we felt it may help identify a subgroup of patients that already show evidence of glycaemic excursions early in pregnancy and that this group may exhibit different clinical characteristics. Uniquely it had not yet been studied in the first trimester as a predictor of subsequent GDM development.

Other Biomarkers

We now present a series of other biomarkers which we considered but ultimately did not study in our cohort

1. **Triglycerides** and their link to GDM have also been investigated in the literature. Korkmazer et al showed that triglycerides are higher in the serum of women with GDM when measured at 24-28 weeks compared to controls (105). However, Lacroix et al showed in a larger cohort that first and second trimester triglycerides did not differ significantly in those with
GDM compared to controls once confounders such as BMI, HbA1c, age and insulin sensitivity (as measured by the Matsuda Index) were adjusted for (106). This made us reluctant to study this biomarker in early pregnancy.

(2) HbA1c is included in the recent ADA guidelines within the diagnostic criteria for diabetes in non-pregnant individuals. At least one recent robust publication has attempted to outline its utility in the diagnosis of GDM by comparing it to OGTT performed at 28 weeks (107). It is formed by the irreversible binding of glucose to haemoglobin and reflects glycaemic control in the preceding 8 weeks approximately. We declined to study this biomarker in our population as there are concerns frequently cited in the literature regarding its validity in pregnancy – specifically due to the physiological dilutional anaemia and high red cell turnover which occurs in the pregnant state (108). Moreover, the sustained hyperglycaemia which occurs with GDM does not typically develop until the late second and early third trimester and so sampling in the first trimester would likely not have yielded significant results.

(3) Fructosamine is the substance which results from serum protein (mostly albumin) undergoes glycation. It gives a picture of the glycaemic control over the preceding two to three weeks. For this reason and because levels may not be truly reflective in hypoproteinaemic states such as pregnancy we elected not to examine it in the first trimester of pregnancy as a biomarker for subsequent GDM.

(4) Vitamin D has recently been linked to the triad of obesity, insulin resistance and T2DM (109). It is recognised that pregnancy can precipitate a vitamin D deficiency. Those living at latitudes distant from the equator and cultural groups that limit the exposure of female skin are especially prone. The data on early pregnancy Vitamin D levels and
GDM risk is conflicting with at least one major review stating that the observational nature of the studies coupled with the presence of confounders such as ethnicity and adiposity limits the validity of the data (110). Much of the data surrounding Vitamin D and GDM risk was published after our study protocol had been devised however in retrospect it may have been quite an interesting biomarker to interrogate.

(5) **Visfatin** is an adipokine which is produced especially in visceral adipose tissue. It is known to encourage production of pro-inflammatory cytokines (134) and those with T2DM are seen to have elevated levels of visfatin (111). Some studies have reported visfatin levels to be increased in pregnant women with GDM (112,113) However this effect has not been teased out from the confounding factor of obesity in these studies and so further interrogation is necessary

(6) **Resistin** is a hormone whose secretion is related to adiposity that is thought by some to impair glucose tolerance in pregnancy (114). It is produced by the placenta and levels rise in parallel with advancing gestation. The evidence for the link between resistin levels and GDM is controversial and a recent systematic review and meta-analysis of 10 different studies showed no difference overall in the resistin levels of patients with GDM versus healthy controls (115). While it is likely that resistin is involved in insulin resistance in pregnancy, its role in glucose homeostasis is possibly quite tenuous and so we did not pursue this biomarker in this study.

(7) **Leptin** is a hormone secreted by adipose tissue with significant effects on glucose metabolism. Leptin levels correlate directly with the fat mass and the obese state predisposes to leptin resistance (116) Pregnancy is also a state of relative leptin resistance and leptin is known to be secreted by
the placenta (117). It is believed that leptin may be involved in the pathogenesis of GDM by its suppression of action of the pancreatic beta cells in secreting insulin. Several studies have shown elevated leptin levels in women that are known to have GDM compared to BMI matched controls. In a comprehensive review of leptin use as a biomarker for GDM, Abell et al note that to date, the studies have not sufficiently teased out the interplay of BMI and gestational weight gain with leptin levels (118)

(8) Fasting glucose was not chosen as the pathophysiology of true GDM does not manifest until the late second and early third trimester
Materials and Methods Chapter

Study Design

This study was a prospective cohort study.

Setting

This study was conducted at a single centre in the Rotunda Hospital Dublin between January 2014 and October 2015. The Rotunda is a large tertiary and stand-alone maternity unit delivering more than 8,500 births annually. The Rotunda has a joint obstetric – endocrine clinic staffed by a consultant obstetrician and consultant endocrinologist for the management of patients whose pregnancy is complicated by gestational diabetes. Two specialist midwives are involved in delivering care to these women also. Low risk patients have shared care between the general practitioner and the hospital’s perinatal team of midwives, dieticians and doctors. High risk patients are cared for solely by the perinatal team.

Researchers and Roles

Dr Siobhan Corcoran was the lead researcher for this study. She was primarily responsible for candidate recruitment, patient counselling, obtaining informed consent for participation, data collection and organising the analysis by the laboratories and liaising with them through the process. This was a clinical study rather than a basic science or biochemistry study so while Dr Corcoran did not directly perform the assay she was made intimately aware of the laboratory processes through frequent meeting with Mr O’ Loughlin. She was also responsible for the phlebotomy in the majority of cases. Dr Corcoran attended the joint obstetric endocrinology clinic throughout the period of the study, following patients that screened positive, whilst providing clinical care. Dr Corcoran conducted a retrospective chart review at the end of the study on each participant to fill any gaps in data collection.

Prof. Fionnuala Breathnach supervised the project and devised the concept for the study. She also participated in identifying suitable recruits and oversees the Obstetric Endocrine clinic.

Prof. Fergal Malone co-supervised the study.
Mr. John O’Loughlin and Ms. Grainne Kelleher are employed by the laboratory at the Rotunda Hospital and facilitated the analysis of the HS-CRP and SHBG samples. Ms. Kelleher logged, and analysed all samples drawn. Any queries on samples or results were addressed by Ms. Kelleher. Mr. O’Loughlin is the director of the laboratory and facilitated the project, offering guidance on how best to store and report data and also set up the link with Prof Steve Meany and Philip Stafford of Dublin Institute of Technology for analysis of adiponectin and 1,5 anhydroglucitol.

Ms. Lucy Shirrin and Mr. Robin George were employed by the Rotunda as research assistants working on many projects. They were involved in flagging potential patients for recruitment, filing study literature in the patient’s medical record and transporting samples to the lab.

Mr Philip Stafford was a biochemistry Master’s student at Dublin Institute of Technology and an employee of ICON laboratories. He analysed and reported the results of the adiponectin and 1,5 anhydroglucitol assays. He was supervised by Prof. Steve Meany of DIT.

Ms Natalie Achamallah and Mr. Sami Backley are medical students at RCSI who helped with chart review and data collection.

**Ethical Approval**

The project proposal was reviewed by the Research Ethics Committee (REC) of the Rotunda Hospital. The committee meet monthly and require applicants to submit the standardised REC form before meeting the researcher. This committee was comprised of clinicians with a background in Obstetrics, Neonatology and Anaesthesia, a statistician, a solicitor, a representative from the laboratory and the clinical director of the hospital. The lead researcher attended a meeting with the committee where potential problems were identified and addressed. The REC granted ethical approval for the study in November 2013.

**Recruitment of Participants**

All patients presenting for antenatal care at the Rotunda Hospital and indeed across the country, undergo a routine detailed personal, family and medical
history with a midwife at their first booking visit. Patients are not given any instruction on fasting prior to attending this appointment. Midwives performing the booking history were advised of the recruitment criteria for the study during numerous information sessions with the lead researcher. A reminder sheet was placed in the front of all antenatal charts prompting the midwife to call the lead researcher if a patient was deemed suitable for participation. Dr Corcoran met weekly with the midwives of the outpatient’s department to remind them of the recruitment criteria for the study.

Our study focused on patients at risk for GDM. Initially we aimed to recruit patients with a high likelihood of screening positive for gestational diabetes. For this reason, we sought and recruited patients with two or more risk factors for GDM in their history. However, after three months using this approach it was clear that recruitment would be extremely slow and the project would not move along as planned. We then recruited any patient with one or more risk factors for GDM for the remainder of the project. Following a period of slow recruitment due to late booking appointments Dr Corcoran began working at the Early Pregnancy Unit (EPU) to attempt to capture suitable patients in a timely manner.

Patients were considered eligible if they were less than 15 weeks’ gestation and had one or more of the following risk factors identified at the booking visit

**Inclusion Criteria**

- BMI ≥ 30kg/m2
- Maternal age > 40 years
- Ethnicity – Indian, Pakistani, South East Asian, Middle eastern, Afro-Caribbean
- History of Polycystic Ovarian Syndrome (PCOS)
- Family history of first degree relative with type 2 diabetes
- Previous macrosomic baby (> 4 kg birthweight)
• Previous unexplained stillbirth
• Maternal history of prolonged steroid use
• HIV seropositivity (Due to effects of anti-retrovirals on glucose tolerance)

Exclusion Criteria
• Persistent fasting glycosuria (as this would merit first trimester screening for GDM and implies a risk of pre-existing type II diabetes)
• Gestational diabetes in a prior pregnancy (as the recurrence risk for gestational diabetes approximately 65%)

Once a patient was deemed eligible, the midwife contacted the Dr Corcoran who came to interview and consent the patient and arrange phlebotomy. Participants were provided with a patient information leaflet and copies of their signed consent were retained by the researcher. A data collection sheet and information pertaining to the collection of cord blood samples was placed the patient’s medical record.

Each patient was assigned an anonymised study number under which serum samples were processed. The medical record number of the patient was not used by the laboratory or the researchers to protect patient confidentiality. The results of the biomarkers were therefore not reported on the main laboratory reporting IT system in the Hospital to prevent clinicians from acting on results that were of uncertain significance in early pregnancy. A log linking study numbers to actual patient medical record numbers was maintained separately by Dr Corcoran to facilitate collection of perinatal outcome data later on.

The basic demographic data, obstetric and medical history of each participant was recorded by Dr Corcoran at recruitment. Age, parity, gestation, obstetric history, BMI, smoking status, GDM risk factors, medical co-morbidities and antenatal care plan were among the variables recorded at this time.

Power Analysis
We estimated that approximately 20% of our high risk cohort would screen positive for GDM. This study was largely exploratory (most especially with regard to 1,5 AG) which makes a detailed and exact power calculation challenging. We had no pilot study upon which to base our calculations and because the literature is variant and contradictory in some aspects regarding each biomarker, supposing the effect size was challenging. Using a background population incidence of 12% GDM (based on ATLANTIC DIP results) and taking the probability of a type 1 error (α) to be 0.05, while taking the power at 90% we determined we would need a sample size of 206 to show a statistically significant difference between screen positive and screen negative groups. We chose to extend our recruitment significantly beyond this due to the factors discussed above.

**Collection and Analysis of samples**

Cuffed samples were drawn by routine phlebotomy alongside the patient’s standard “booking bloods” for antenatal care (thereby eliminating the need for “extra needles” for the patient). Participants were not required to fast. A 6ml lithium heparin Vacutainer® (Becton Dickinson) biochemistry sample and a 6ml serum sample were drawn and sent the laboratory within 2 hours of collection. CRP and SHBG were analysed contemporaneously on these samples. Samples for adiponectin and 1,5-AG testing were frozen and stored at -80°C following collection for batch testing.

**CRP laboratory analysis**

Quantitative analysis of CRP was performed using a particle enhanced immunotubidometric assay. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate was then determined tubidimetrically. This was done using the Roche/Hitachi Cobas C 501 analyser. Reagents used for this assay were

(R1) TRIS buffer with bovine serum albumin and immunoglobulins (mouse); preservative

(R2) Latex particles coated with anti-CRP (mouse) in glycine buffer; preservative
The expected normal values in a human subject are <5mg/L. The measuring range of the test was 1.00 – 250.00mg/L. Determination of samples with a higher value was performed by the re-run function and a 1:3 dilution. Centrifuged samples were stable for 11 days at room temperature and were all analysed for CRP well within this timeframe. Full calibration of the assay was performed following a reagent lot change and periodically as per the local quality control guideline of the laboratory. In terms of precision the CRP intra assay coefficient of variation (CV) was between 0.9 and 1.5% and the inter assay CV was between 1.3 and 2.5%. The sample stability is given as 11 days at 15 to 25ºC, 2 months at 2 to 8ºC and 3 years at -15 to -25 ºC. Our CRP samples were analysed on the day of phlebotomy and were maintained at room temperature during transport to the laboratory.

**Sex hormone binding globulin laboratory analysis**

Quantitative determination of sex hormone binding globulin was performed using an electrochemiluminescence immunoassay (“ECLIA”) on a cobas immunoassay analyser. The test is performed using the “sandwich principle”. In the 1st incubation 10µL of sample, a biotinylated monoclonal SHBG-specific antibody and a monoclonal SHBG specific antibody labelled with a ruthenium complex form a sandwich complex. In the second incubation, after addition of streptavidin coated micro particles the complex becomes bound to the solid phase through the interaction of the biotin and streptavidin. This reaction mixture is then aspirated into a measuring cell where the micro particles are magnetically captured onto the surface of an electrode. A voltage is applied to the electrode which causes a chemiluminescent emission and this is measured by a photomultiplier. The results of this are then determined on an instrument-specific calibration curve. The measuring range of the assay was 0.350 – 200.00nmol/L. Samples with results above the measurement range were diluted 1:10 and the result was multiplied by the dilution factor. The cobas software automatically takes the dilution into account when calculating the sample concentration. Calibration was performed once per reagent lot and after one month when using the same reagent lot. The intra assay CV was between 1.1 and 1.7 % while the inter assay CV was between 1.8 and 4%. Samples were deemed to be stable for 3 days at 2-8ºC, and one month at -20 ºC. Our samples
for SHBG were not frozen but were rather analysed on the day of venupuncture and transported to the lab at room temperature.

The remaining booking sample was centrifuged, aliquoted, and stored at -80 degrees Celsius at the Rotunda Hospital Laboratory. These samples were then transported by laboratory courier to ICON for analysis of Adiponectin and 1,5 Anhydroglucitol by Philip Stafford.

1,5 Anhydroglucitol (Glycomark®) laboratory analysis

The 1,5 Anhydroglucitol reagents, quality controls and calibrators were obtained from Glycomark Inc. and were tested on the Roche P800 modular® analyser at ICON laboratories. LUCIO®-Medical ELISA Adiponectin (human) kits were purchased from the Nal Von Minden company through Glenbio, their distributor in Ireland. The Glycomark® test for 1,5 AG quantification is an enzymatic method consisting of a two-reagent test kit. The assay is based on a kinetic determination principle. All reagents are used without dilution or other preparation and the reaction is performed at 37°C. This colorimetric assay uses the enzyme pyranose oxidase (PROD) to oxidise the second position hydroxyl group of 1,5-AG and to detect the generated hydrogen peroxide using peroxidase (POD). A pre-treatment step uses the enzyme glucokinase (GK) to convert glucose, a reactant of PROD into non-reactive glucose-6-phosphate (G-6-P). To drive the reaction an adenosine triphosphate (ATP) regenerating system consisting of pyruvate kinase (PK) and phosphoenol pyruvate (PEP) is utilised. As ATP is converted to adenosine diphosphate (ADP), PK, in the presence of PEP, catalyses the phosphorylation of ADP back to ATP. (See schematic below)
The Glycomark kit for measuring 1,5 AG gives equivalent results for wither plasma or serum samples. Fresh serum samples are stable for up to one week at 2-8ºC and beyond this freezing of the sample is recommended by the manufacturer. Our samples were frozen on the day of phlebotomy. The kit insert provides that aliquots may be thawed and re-frozen for up to three cycles whilst maintaining reproducibility. Our samples were only thawed on one occasion. The reagents themselves are stable at refrigerated temperatures (2-8 degrees celcius) for 30 days once opened. Our samples were all analysed together on the same day as the kit was opened. The analytical sensitivity of the Glycomark assay is 0.2µg/mL and the reference range is 6.8 – 29.3µg/mL in healthy non-pregnant females. Linearity is demonstrated to at least 50µg/mL and the high calibrator for the assay was set at 50µg/mL. When considering precision; the within assay/intra assay precision ranged from 1.3 to 3.8% and the between assay/inter assay coefficients of variation ranged from 1 to 4%. This was determined using 2 pools of the Glycomark controls and two serum pools that were assayed twice daily with one reagent lot per the standard procedure for a total of 10 days.

Adiponectin Laboratory Analysis

For the Adiponectin assay the LUCIO®-Medical ELISA Adiponectin (human) kit was used. It is a competitive immunoassay for the quantitative determination of
human adiponectin in serum samples. Standards, quality controls and samples are incubated in microplate wells pre-coated with recombinant human adiponectin together with polyclonal anti-human adiponectin antibody conjugate to horse radish peroxidase (HRP). After a washing step, the HRP conjugate bound to the adiponectin immobilised on the wells is allowed to react with substrate solution (TMB). The reaction is stopped by addition of an acidic solution and the absorption of the colour change is measured. The concentrations of unknown samples are determined using the standard curve.

Philip Stafford conducted the assay at ICON laboratories per the following method. All samples and reagents were brought to room temperature. Samples were diluted 30 fold; 10μl of testing sample was added to 290 μl of dilution buffer. Standards were diluted 3 fold; 50 μl of standard was added to 150 μl of dilution buffer. All dilutions were done in eppendorfs prior to the assay. 50 μl of diluted standards, diluted samples and quality controls were pipetted into appropriate wells. 50 μl of conjugate solution was added to each well. The testing plate was covered with a plate seal and incubated at room temperature for 2 hours on an orbital microshaker at 300 rpm. Following incubation, the wells were washed 3 times (350 μl per well) on the automatic plate washer. After the final wash, the plate was inverted and tapped on absorbent paper to remove residual liquid. 200 μl of substrate solution was added to each well. The plate was sealed and incubated on the bench in the dark using a plate cover for 12.5 minutes. Following incubation, 50 μl of stop solution was added to each well. The absorbance of each well was determined using the SPECTRAmax 384PLUS microplate reader set to 450 nm with a reference range of 650 nm (Subtracting readings at 650 nm from reading at 450 nm). Absorbance readings were done within 5 minutes of adding stop solution. A standard curve was constructed by plotting the known concentrations standards against absorbance readings in logarithmic scale, using a 4-parameter algorithm. Measured concentrations of samples and quality controls calculated from the standard curve were multiplied by dilution factor of 10 (standards diluted 3 fold and samples and quality control diluted 30 fold). The intra-assay CV was 3.3-5% and the inter-assay CV was 6.8-6.9%.
The absorbance is inversely proportional to the adiponectin concentration. Standards and quality control solutions are supplied in the kit.

**Glucose Tolerance Testing**

An oral glucose tolerance test (OGTT) was scheduled for all recruited patients at 28 weeks’ gestation. The OGTT was conducted by an experienced midwife in a dedicated clinical setting in the hospital. All patients presented in the early morning for the test following at least a 12-hour period of fasting (no food or fluids). Each patient was telephoned the afternoon before the test to remind her to fast. A 75g oral glucose load was administered in a carbonated liquid form. The patient was advised to consume the liquid as quickly as was comfortable for her and to remain relaxed and avoid vigorous activity or smoking cigarettes during the test. Repeat phlebotomy for serum glucose levels was performed using sodium fluoride tubes at one and two hours post prandially. Using the IADPSG criteria for screen positive OGTT the patient was considered to have gestational diabetes if the fasting serum glucose was greater than or equal to 5.1mmol/L, the 1-hour serum glucose was greater than or equal to 10.0mmol/L or if the 2-hour post prandial serum glucose was greater than or equal to 8.5mmol/L. One or more of the readings above was sufficient for diagnosis. Samples were collected in sodium oxalate Vacutainer® tubes.

The serum glucose samples were analysed on a Roche cobas c system using a glucose hexokinase assay. The test principal is based on photometrically measuring the rate of NADPH formation during the oxidation of glucose-6-phosphate to gluconate-6-phosphate. The samples are considered stable at 2º-8ºC for a period of 8 weeks however they were all analysed on the same day as they were drawn. Regarding repeatability, the CV in human serum of this assay was 0.7% When considering precision, the CV in human serum was given as 1.2%. The assay was not affected by icterus, haemolysis, lipemia or a panel of common drugs however in rare cases Waldenstroms macroglobulinaemia may cause unreliable results. No patient in our study had this diagnosis.

Results of the OGTT were reported on the hospital laboratory IT system and accessed freely by the clinicians charged with the patient’s antenatal care as per the protocol of the hospital. Patients that screened positive were notified by
telephone on the same day and channelled into the GDM care pathway as per
the protocol of the hospital. Those that met the criteria for diagnosis of overt
diabetes were seen within one week at the joint obstetric endocrinology clinic.
All remaining screen positive patients were reviewed and advised by a
specialist midwife before referral to the clinic. The results were also noted and
recorded by the Dr Corcoran in the database.

Cord Blood Collection

Patients participating in the study were clearly identified by stickers on the front
of the medical record and also by an information leaflet for the healthcare
professional attending the birth contained within. Birth attendants were advised
to double clamp the umbilical cord, obtain samples of cord blood for pH analysis
(which was performed immediately on site on the labour ward) and also for cord
blood c-peptide which was sent to the laboratory, spun and stored for batched
analysis. Pre-labelled vacutainers using the study number, for this purpose
were inserted in the patient’s medical record at the time of recruitment.
Information sessions for birth attendants (mainly midwives) were held on the
labour ward periodically during the course of the study and reminder notices
were placed on the labour ward notice board and staff quarters with the aim of
optimising sample collection

Cord blood C-peptide levels were quantitatively assessed using an
electrochemiluminescence immunoassay (“ECLIA”) on a cobas analyser
similar to SHBG as described above. In the 1st incubation 20µL of sample, a
biotinylated monoclonal C-peptide specific antibody and an antibody labelled
with ruthenium complex interact together to form a sandwich complex. Then in
the second incubation, streptavidin coated antibodies are added and the
complex becomes bound to the solid phase through the interaction of biotin and
streptavidin. The resultant reaction mixture was then aspirated into a measuring
cell where the micro particles are captured magnetically onto the surface of an
electrode. Unbound substances are removed and a voltage is applied which
causes the chemiluminescent emission. The amplitude of this emission is
measured by a photomultiplier. The results are then determined by an
instrument specific calibration curve and reported. The samples were stable for a period of 4 hours at room temperature and so when delivery occurred the on-call lab personnel were contacted and centrifuged, froze and stored the samples for subsequent batched analysis. The measurement range for C-Peptide was 0.003 – 13.3nmol/L. This is a high measuring range and none of the samples from our cohort required further dilution to detect C-peptide levels in excess of this.

Perinatal Outcome Data Collection

A datasheet was filled by the birth attendant detailing the mode of delivery, birthweight, obstetric complications (third degree tear, primary postpartum haemorrhage, instrumental or caesarean delivery) and neonatal data such as Apgar scores and admission to the neonatal unit. The lead researcher then collected and logged the data and later reviewed the medical records of all participants to fill any gaps in data collection 2 months after the final patient had delivered (in order to leave time for any postnatal complications to present). Sources of the data were the written notes, discharge summaries and digital record of laboratory results. We defined macrosomia as a birthweight greater than the 90th centile for gestation.

Statistical analysis

All collected data was compiled to a single excel spreadsheet database which was analysed by in conjunction with a biostatistician, Dr Pat Dicker. We conducted both univariate and multivariate analysis on the correlation between each biomarker and the likelihood of a screen positive OGTT. Univariate analysis is a statistical description of data where only one variable exists (e.g. biomarker versus screen positive or negative OGTT). Multivariate or regression analysis involves observation and analysis of more than one statistical outcome variable at a time considering the effect of all the variables on the outcome (e.g. biomarker and BMI and ethnicity on screen positive OGTT). Receiver operating characteristic (ROC) curves are graphical plot that illustrates the performance of a binary classifier system as its discrimination threshold is varied. ROC curves were generated in an attempt to identify a cut-off point for each biomarker after which the likelihood of screening positive for GDM increased significantly.
Multivariate analysis incorporated age, BMI, ethnicity, and other variables relevant to the individual analysis (e.g. primiparity for instrumental delivery). Due to the continuous and linear relationship between glucose values and adverse pregnancy outcome we also examined the relationship between the individual biomarkers and the uppermost and lowest quartiles of glucose results from the OGTT. We initially analysed covariates such as age (>40 years) and BMI (>30) dichotomously but later expanded our analysis to continuous data as the absolute age and BMI were recorded at booking. This helps to account for the fact that glucose tolerance is likely to be similar in matched controls that are 39 and 40 years old respectively.

Acceptability of glucose tolerance testing methods

Patients with risk factors for gestational diabetes (BMI > 30kg/m2, Maternal age > 40 years, Ethnicity – Indian, Pakistani, South East Asian, Middle Eastern, Afro-Caribbean, History of Polycystic Ovarian Syndrome, Family history of first degree relative with type 2 diabetes, previous macrosomic baby, previous unexplained stillbirth, maternal history of prolonged steroid use, HIV seropositivity) were identified at the first prenatal care visit and scheduled for an OGTT at 28 weeks gestation. All patients attending for OGTT over 7 consecutive working days at the Outpatients Department of Rotunda Hospital were approached and informed of the nature of the study. Inclusion criteria included the ability to speak and read English and age over 18. 80 women were eligible to participate and after reading a patient information leaflet, 57 women agreed to take part. The OGTT was administered using a 75g oral glucose load in the form of 394mls of a carbonated liquid. The midwife oversaw the administration of the test and the collection of samples as per standard hospital protocol.

The study investigator then administered a questionnaire at the end of the OGTT to investigate the physical symptoms (hunger, nausea, vomiting, jitteriness, syncope, reduced fetal movements) and the psychological symptoms (anxiety regarding own health and health of the fetus). A telephone interview was arranged 2 days following the OGTT to determine how the patient felt
about the screening process now in light of the positive or negative results. 50 of the 57 patients were contacted and 7 were lost to follow up at this point. Ethical approval for this part of the study was separately approved by the Rotunda Hospital Research Ethics Committee and the questionnaire was reviewed and edited by a multidisciplinary panel of clinicians, administrators and a legal advisor prior to commencement of the study. Where appropriate, responses to questions for the study were structured in a 5 point “Likert” scale – a widely used approach to scaling responses in survey research.

Numbers recruited, delivering and loss to follow up

248 patients were recruited to the study and serum samples taken before the 15th week of gestation. One was subsequently found to be twin pregnancy and was excluded from the analysis. 23 patients did not attend for OGTT. One of these delivered prematurely before the 28th week. Three patients had a termination of pregnancy (TOP) following recruitment. Four had miscarriages. Four were lost to follow-up – assumed to have delivered in another unit. Ten patients did not show up for oral glucose tolerance testing but did continue their antenatal care at the hospital and so perinatal outcomes are available. One patient’s study number was not linked to the medical record number in the database in error and so the OGTT result could not be retrieved.
Results Chapter

The results of our study are presented as follows

Results 1 – Description of the Cohort

Results 2 – Description of the biomarker and OGTT results

Results 3 – Relationship of each first trimester biomarker to glucose tolerance at 28 weeks

Results 4 – Relationship of each first trimester biomarker to perinatal outcomes

Results 5 – Findings on the Acceptability of the OGTT
Results 1 – Description of the cohort

Figure 2 describes the recruitment to the project. 247 women were approached and gave consent to participate in the first trimester. They all had bloods drawn at their booking visit. 23 of these did not attend the scheduled oral glucose tolerance test at 28 weeks for various reasons outlined above.

Of note while 10 of the women did not attend for OGTT they did deliver at the unit and so perinatal outcome data was available for them. Of those that partook in the OGTT, 46 screened positive by the IADPSG criteria while 178 screened negative.
In Figure 3 we see that 13 women had a BMI less than 20, 78 had a BMI in the normal range, 54 were in the “overweight” category, 84 were in the “class 1 obesity” category and 11 had a BMI in excess of 40 (“class 2 obesity” or above). While it is within the study protocol and is part of the policy of the hospital, 7 women did not have BMI recorded at booking.
**Figure 4 Risk Factor Profiling of the Cohort**

Figure 4 details the history identified risk factors which made the women eligible for participation. The most common risk factor was obesity, followed by a family history, age over 40 years and ethnicity.

**Figure 5 Risk Profile Weighting of the Cohort**

Figure 5 shows that most patients (178) had only a single risk factor identified in the history.
Figure 6 details the obstetric history of the cohort. This naturally only refers to the multiparous patients (n = 131)
Figure 7 outlines the medical co-morbidities of the cohort. The most common co-morbidity was thyroid dysfunction, seen in 15 women. “Other” included diverse diagnoses such as anaemia, scoliosis, irritable bowel syndrome, recurrent urinary tract infections, enrolment in a methadone maintenance programme for opiate addiction and depression.
Table 2 compares the risk factor profile of those that screened positive and negative for GDM. There was a statistically significant higher BMI in the screen positive group.
<table>
<thead>
<tr>
<th>Outcome</th>
<th>All study recruits having delivery outcomes (with one or more risk factors for GDM) n=225</th>
<th>GDM n=40</th>
<th>Non-GDM n=185</th>
<th>All Patients at Rotunda Hospital 2014 n=8787</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Gestation at delivery (weeks)</td>
<td>38.9</td>
<td>38.8</td>
<td>38.9</td>
<td></td>
</tr>
<tr>
<td>Incidence of Macrosomia (birthweight ≥90th Centile)</td>
<td>35 (15.6%)</td>
<td>9 (22.5%)</td>
<td>26 (14%)</td>
<td>14% &gt;4000g p=0.116</td>
</tr>
<tr>
<td>Operative vaginal delivery</td>
<td>42 (18.6%)</td>
<td>8 (20%)</td>
<td>34 (18.3%)</td>
<td>1439 (17%) p=0.189</td>
</tr>
<tr>
<td>Overall Caesarean Section Rate</td>
<td>77 (34.2%)</td>
<td>11 (27.5%)</td>
<td>66 (35.7%)</td>
<td>2724 (31%) p=0.157</td>
</tr>
<tr>
<td>Emergency Intrapartum Caesarean Section</td>
<td>28 (12.4%)</td>
<td>5 (12.5%)</td>
<td>23 (12.4%)</td>
<td></td>
</tr>
<tr>
<td>Elective pre-labour Caesarean section</td>
<td>39 (17.3%)</td>
<td>4 (10%)</td>
<td>35 (18.9%)</td>
<td></td>
</tr>
<tr>
<td>Emergency Pre-labour Caesarean Section</td>
<td>10 (4.4%)</td>
<td>2 (5%)</td>
<td>8 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>Shoulder Dystocia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Primary postpartum haemorrhage &gt;500ml</td>
<td>44 (19.5%)</td>
<td>10 (25%)</td>
<td>34(18.4%)</td>
<td>1545 (17.6%) p=0.23</td>
</tr>
</tbody>
</table>

p<0.05.
Table 3 details the perinatal outcomes for those that underwent OGTT and delivered at the Rotunda Hospital. We present those with a diagnosis of GDM in the second column. This table compares those outcomes to the figures from the whole background population (where available) in the same unit in the same year as the study commenced. These background population figures are gained from the Annual Report of the Rotunda Hospital. The average gestation at delivery in the group was 38.8 weeks. The incidence of macrosomia in the whole cohort was 15% where macrosomia was defined at birthweight over the 90th centile for gestation. The incidence of macrosomia was 22% in those diagnosed with GDM. Since birthweight centile for gestation is not presented in the Annual Report of the Rotunda Hospital, we include instead incidence of birthweight over 4kg and 4.5kg as a reference for the background population. The p-value given refers to the figures highlighted in red text. The operative vaginal delivery, caesarean section, post-partum haemorrhage rates of the entire cohort were similar to that of the background population. There was no incidence of shoulder dystocia in the cohort.
Results 2 – Description of the biomarker and OGTT results

**Table 4 Statistical Parameters of Biomarker Results**

<table>
<thead>
<tr>
<th></th>
<th>SHBG (nmol/L)</th>
<th>CRP (mg/L)</th>
<th>Adiponectin (µg/mL)</th>
<th>1,5 AG (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples analysed</td>
<td>219</td>
<td>222</td>
<td>213</td>
<td>213</td>
</tr>
<tr>
<td>Mean Value (st dev)</td>
<td>281 (88.8)</td>
<td>5.6 (5.8)</td>
<td>8.9 (2.9)</td>
<td>15.9 (6.3)</td>
</tr>
<tr>
<td>Median Value</td>
<td>281</td>
<td>3.9</td>
<td>9</td>
<td>15.9</td>
</tr>
<tr>
<td>Mode Value</td>
<td>282</td>
<td>1</td>
<td>8</td>
<td>10.9</td>
</tr>
<tr>
<td>Range</td>
<td>67-644</td>
<td>1-62</td>
<td>4-31</td>
<td>2-35.3</td>
</tr>
</tbody>
</table>

Table 4 details the mean (and the value of one standard deviation in brackets), median and modal value of each of the 1\textsuperscript{st} trimester biomarkers. The range is also given.
Figure 8 is a scatterplot plot of the values obtained for C-reactive protein in the 1st trimester in our high-risk cohort. A “normal” CRP is taken to be <5mg/L. A raised CRP can be many multiples of this and so, as we see in this graph, there are several outliers. 94 women had a raised CRP (>5mg/L) in the 1st trimester. Our CRP data was not normally distributed with a small number of very high results skewing the data.
Figure 9 is a scatterplot of the results of sex hormone binding globulin results measured in the 1st trimester in our high-risk cohort. The normal reference range of adult female premenopausal levels of SHBG are between 40 – 120 nmol/L. We can see from this plot that our average values for our population are well in excess of this. It is known that SHBG levels are higher in pregnancy however universally agreed pregnancy specific reference values are not available. Our population is also a high-risk population which may go some way to explaining this finding.
Figure 10 is a scatterplot of the 1,5 Anhydroglucitol results of the whole cohort in the 1st trimester. It has been shown that the mean 1,5 AG ± 1SD in the background pregnant population is 18.6±5.2 mg/l by Tetsuo et al (104). Our dataset represented here would echo this.
Figure 11 Scatterplot of Adiponectin Results

Figure 11 is a scatterplot of the adiponectin results of the whole cohort in the 1st trimester. Mean adiponectin levels (+/- 1 SD) in the third trimester in the unselected lean pregnant population was found to be 9.9 +/- 1.4 mg/ml by Catalano et al (85). Our data would not appear to be very different from this. However, our samples were taken in the 1st trimester and there is a recognised gestation-related fall in serum adiponectin levels in pregnancy. The literature does not specifically describe normal 1st trimester levels in a high-risk population.
**Table 5 Results of OGTT**

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Mean nmol/L</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Serum Glucose</td>
<td>212</td>
<td>4.58</td>
<td>0.54</td>
</tr>
<tr>
<td>1hr Serum Glucose</td>
<td>212</td>
<td>8.05</td>
<td>1.96</td>
</tr>
<tr>
<td>2hr Serum Glucose</td>
<td>211</td>
<td>6.04</td>
<td>1.39</td>
</tr>
</tbody>
</table>

Table 5 outlines the mean serum glucose results of the cohort at the 28 week OGTT. The mean fasting serum glucose of the group was 4.58 nmol/L. The mean serum glucose at one hour post-prandial was 8.05 nmol/L and the mean serum glucose at 2 hours post prandial was 6.04nmol/L.
Figure 12 shows the spread of serum glucose results at the fasting time-point of the OGTT in a scatterplot. The orange arrow indicates the diagnostic threshold (5.1 nmol/L) above which GDM is diagnosed. We include this figure to help visually represent and note how many of the values just skim below this diagnostic point, highlighting the spectrum of glucose intolerance.
Figure 13 Serum glucose levels at 1 hour post prandial after ingestion of a 75g oral glucose load at 28 weeks’ gestation following at least 12 hours fasting. Orange arrow points to diagnostic threshold as per IADPSG criteria.

Figure 13 shows the spread of serum glucose results at the one-hour post prandial time-point of the OGTT in a scatterplot. The orange arrow indicates the diagnostic threshold (10 nmol/L) above which GDM is diagnosed. Again, this figure illustrates the numerous cases that come close but do not reach diagnostic thresholds and also the spread of serum glucose values at this timepoint indicating a spectrum of glucose tolerance.
Figure 14 shows the spread of serum glucose results at the two-hour post prandial time-point. This figure shows that the fewest diagnoses were made at this time-point compared to the other two graphs.
**Results 3 – Relationship of each first trimester biomarker to glucose tolerance at 28 weeks gestation**

**Table 6 Relationship of each biomarker in the first trimester to the likelihood of a screen positive OGTT as per IADPSG criteria. Odds ratios correspond to a one standard deviation increase in a biomarker level. “Adjusted analysis” is multiple logistic regression adjusting for BMI, high risk ethnicity and family history of DM.**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Unadjusted Analysis</th>
<th>Adjusted Analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>SHBG</td>
<td>0.64 (0.44 – 0.94)</td>
<td>0.021</td>
</tr>
<tr>
<td>CRP</td>
<td>1.34 (0.98 – 1.82)</td>
<td>0.063</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.47 (0.31 – 0.69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1,5 AG</td>
<td>0.71 (0.50 – 1.02)</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Table 6 describes the relationship of each serum biomarker measured in the first trimester in women considered to be high risk for gestational diabetes to the likelihood of having a screen-positive oral glucose tolerance test at 28 weeks’ gestation. We present this data firstly in an unadjusted analysis and secondly in an adjusted analysis where we account for variables (BMI, high risk ethnicity and a positive family history for diabetes mellitus). Each odds ratio given corresponds to a one standard deviation increase in a biomarker level. With regard to SHBG each one standard deviation increase in serum SHBG in the 1st trimester was associated with a lowered chance of developing GDM (OR 0.64 in the unadjusted analysis) Therefore the lower the SHBG the more likely the patient was to develop GDM. When adjusted for the variables mentioned above this finding loses significance.

With regard to CRP; each one standard deviation increase in CRP in the 1st trimester was associated with an increased chance of developing GDM. This
almost reached significance in the unadjusted analysis however once we adjust for BMI, family history and ethnicity we see the odds ratio is close to 1.

With regard to Adiponectin; each one standard deviation increase in adiponectin levels in the 1st trimester is associated with a lowered risk of developing GDM. This association is independent of BMI, family history and ethnicity as seen in the adjusted analysis.

When looking at 1,5 AG we see that each one standard deviation increase in 1,5 AG measured in the 1st trimester is associated with a lowered risk of developing GDM in later pregnancy. In our unadjusted analysis, this association is not quite statistically significant, however once we adjust for BMI, ethnicity and family history we see the biomarker emerge as a more powerful independent predictor of GDM status (OR of 0.57 for GDM, p=0.016).

**Table 7 Mean 1,5 AG results in GDM and non-GDM patients compared using T-test**

<table>
<thead>
<tr>
<th></th>
<th>Mean µg/mL</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screen Neg (No GDM)</td>
<td>16.39</td>
<td>6.14</td>
<td>p=0.041</td>
</tr>
<tr>
<td>Screen Pos (GDM)</td>
<td>14.23</td>
<td>6.9</td>
<td></td>
</tr>
</tbody>
</table>

Table 7 shows there was a statistically significant lower mean 1,5 AG result in the screen positive (14.23 µg/mL) versus screen negative (16.39 µg/mL) women in the 1st trimester.

**Table 8 Mean CRP results in screen positive and screen negative groups using T Test**

<table>
<thead>
<tr>
<th></th>
<th>Mean mg/L</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screen Neg (No GDM)</td>
<td>5.3</td>
<td>5.85</td>
<td>p=0.2087</td>
</tr>
<tr>
<td>Screen Pos (GDM)</td>
<td>6.3</td>
<td>5.4</td>
<td></td>
</tr>
</tbody>
</table>

Table 8 shows that there is no significant difference between the mean serum CRP levels in the first trimester in the screen negative (5.3nm/L) and the screen positive (6.3mg/L) group.
Table 9 details how there is a statistically significant higher mean sex hormone binding globulin measured in the first trimester in the screen negative (289.5 nmol/L) compared to screen positive (249.9 nmol/L) group.

Table 10 shows that the mean serum adiponectin in the 1st trimester was significantly higher in those that went on to screen negative for GDM (9.38 µg/ml) compared to those that screened positive (7.75 µg/ml)
Figure 15 is a receiver operating characteristic curve for adiponectin levels in the 1st trimester in a high-risk cohort as a predictor of a screen positive OGTT. There is no distinct threshold visualised at which adiponectin functions with acceptable sensitivity or specificity as a predictor of GDM status.
Figure 16 is a receiver operating characteristic curve for sex hormone binding globulin levels in the 1st trimester in a high-risk cohort as a predictor of a screen positive OGTT. Again, there is no distinct threshold visualised here at which SHBG functions with acceptable sensitivity or specificity as a predictor of GDM status.
Figure 17 ROC curve of 1\textsuperscript{st} trimester 1,5 AG as a predictor of a screen positive OGTT.

Figure 17 is a receiver operating characteristic curve for 1,5 AG levels in the 1\textsuperscript{st} trimester in a high-risk cohort as a predictor of a screen positive OGTT. Similarly here we see no threshold at which 1,5 AG functions with acceptable sensitivity or specificity as a predictor of GDM status.
Table 11 is a linear regression analysis correlating adiponectin values to individual OGTT serum glucose results at each time-point. This table illustrates that the association holds strongly across all three measured serum glucose levels.

<table>
<thead>
<tr>
<th>GTT</th>
<th>Pearson Correlation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>-0.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 hour</td>
<td>-0.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2 hour</td>
<td>-0.25</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

In Table 12 we see that having a serum adiponectin less than or equal to 8.9 µg/ml in the first trimester in a high-risk population gives an OR of 3.3 for the subsequent diagnosis of GDM in that pregnancy. Serum adiponectin less than or equal to 12 µg/ml gives an OR of 1.2. These thresholds were selected as they are the mean adiponectin values in our study population and the background unselected population respectively.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Odds-Ratio of Screen positive OGTT</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin ≤8.9 µg/ml</td>
<td>3.3</td>
<td>1.6528 – 6.7</td>
<td>p=0.0008</td>
</tr>
<tr>
<td>Adiponectin ≤12 µg/ml</td>
<td>1.2</td>
<td>0.3297 – 4.4402</td>
<td>p=0.77</td>
</tr>
<tr>
<td>Quartile 1 (Lowest)</td>
<td>Fasting glucose</td>
<td>1hr glucose</td>
<td>2hr glucose</td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td>65%</td>
<td>61%</td>
<td>59%</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>58%</td>
<td>70%</td>
<td>63%</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>53%</td>
<td>47%</td>
<td>47%</td>
</tr>
<tr>
<td>Quartile 4 (Highest)</td>
<td>30%</td>
<td>32%</td>
<td>38%</td>
</tr>
</tbody>
</table>

Table 13 denotes the positive predictive value of having a serum adiponectin higher than the mean (> 8.9 µg/ml) for being in each quartile of glucose tolerance. As expected from previous results those with the higher adiponectin levels had a higher probability of being in the lower quartiles (having better glucose tolerance).
Results 4 – Relationship of each first trimester biomarker to perinatal outcomes

Table 14: Multiple logistic regression of biomarkers to risk of macrosomia* adjusting** for BMI and high risk ethnicity. Odds ratios correspond to a 1 SD increase in a biomarker level.

*Birthweight >90th centile for gestation

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Unadjusted Analysis</th>
<th>Adjusted Analysis**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>SHBG</td>
<td>1.75 (1.17 – 2.61)</td>
<td>0.006</td>
</tr>
<tr>
<td>CRP</td>
<td>1.23 (0.85 – 1.77)</td>
<td>0.265</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.61 (0.39 – 0.95)</td>
<td>0.028</td>
</tr>
<tr>
<td>1,5 AG</td>
<td>1.19 (0.79 – 1.78)</td>
<td>0.416</td>
</tr>
</tbody>
</table>

Table 14 denotes a multiple logistic regression analysis of the 1st trimester biomarker values with the risk of delivering a macrosomic infant. Here we adjusted only for the BMI and ethnicity which we felt to be the most important predictors of fetal size among our known risk factors for GDM.

With regard to SHBG, each one standard deviation increase in SHBG levels was associated with an OR of 1.78 for macrosomia. This surprising result is explored in the discussion section. As for adiponectin, each one standard deviation increase in adiponectin levels in the 1st trimester lowered the risk of delivering a macrosomic infant.
### Table 15

**MULTIPLE LOGISTIC REGRESSION THE RISK OF OPERATIVE VAGINAL DELIVERY AND FIRST TRIMESTER BIOMARKERS ADJUSTING* FOR NULLIPARITY AND BIRTHWEIGHT. ODDS RATIOS CORRESPOND TO A 1 SD INCREASE IN A BIOMARKER LEVEL**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Unadjusted Analysis</th>
<th>Adjusted Analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>SHBG</td>
<td>0.90 (0.59 – 1.36)</td>
<td>0.605</td>
</tr>
<tr>
<td>CRP</td>
<td>1.11 (0.76 – 1.63)</td>
<td>0.584</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>1.00 (0.66 – 1.51)</td>
<td>0.995</td>
</tr>
<tr>
<td>1,5 AG</td>
<td>0.47 (0.30 – 0.74)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 15 shows the multiple logistic regression of the 1st trimester biomarker results for the risk of requiring an operative vaginal delivery. Here in this table we adjust for nulliparity and birthweight as these are felt to have the biggest influence on the likelihood of requiring and instrument such as vacuum or forceps to assist delivery. Here we see that for each one standard increase in 1,5 AG in the 1st trimester there is a significantly lower chance of needing assistance to deliver (OR 0.49).
Table 16 is a logistic regression analysis of the 1st trimester biomarker values and the risk of having an emergency intrapartum caesarean delivery. There is no demonstrable link here and there are far too many variables, many of which we did not collect in our data-set which may influence this outcome.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Odds-Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHBG</td>
<td>0.88</td>
<td>0.57 – 1.38</td>
<td>0.596</td>
</tr>
<tr>
<td>CRP</td>
<td>1.06</td>
<td>0.71 – 1.58</td>
<td>0.783</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.91</td>
<td>0.58 – 1.42</td>
<td>0.686</td>
</tr>
<tr>
<td>1,5 AG</td>
<td>1.06</td>
<td>0.70 – 1.60</td>
<td>0.774</td>
</tr>
</tbody>
</table>

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

<table>
<thead>
<tr>
<th></th>
<th>nshbg</th>
<th>cpep</th>
</tr>
</thead>
<tbody>
<tr>
<td>nshbg</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00000</td>
<td>-0.13939</td>
</tr>
<tr>
<td></td>
<td>218</td>
<td>0.1322</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1322</td>
</tr>
<tr>
<td></td>
<td></td>
<td>118</td>
</tr>
<tr>
<td>cpep</td>
<td>-0.13939</td>
<td>1.00000</td>
</tr>
<tr>
<td></td>
<td>0.1322</td>
<td></td>
</tr>
<tr>
<td></td>
<td>118</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>122</td>
</tr>
</tbody>
</table>
Using Pearson correlation coefficients, Table 17 shows a correlation overall between SHBG and absolute cord blood c-peptide values but it is low.

**Figure 18 Linear Regression of SHBG Levels and Cord Blood C-peptide Results**

Figure 18 shows a linear regression plot of the sex hormone binding globulin levels measured in the first trimester and the cord blood c-peptide levels measured from the umbilical cord at delivery. The wedge shape of the results in Figure 18 prompted us to perform a logistic regression on the link between sex hormone binding globulin levels >350 nmol/L and a cord blood c-peptide level less than the 90th centile (1.7µL) – indicating normal insulin levels in the fetus. The results are shown in Table 18.

**Table 18 Logistic Regression Analysis of SHBG >350nmol/L for Cord Blood C-peptide <1.7µL**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Odds-Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
</table>

---
Table 18 shows that if the SHBG level is >350nmol/L (high) in the 1\textsuperscript{st} trimester, even in a high-risk sub group, the OR of having normal cord blood c-peptide levels (<90\textsuperscript{th} centile/<1.7mL) is 2.7. Therefore, SHBG levels measured in the 1\textsuperscript{st} trimester relate to neonatal hyperinsulinaemia in a population deemed at risk of gestational diabetes.
Table 19 Logistic regression of each history-based risk factor and biomarker results for screen positive OGTT (Adjusted risk is correcting for all patient history risk factors and biomarkers)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Individual Risk1</th>
<th>Adjusted Risk2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>BMI &gt; 30</td>
<td>2.3 (1.2 – 4.6)</td>
<td>0.019</td>
</tr>
<tr>
<td>Age &gt; 40</td>
<td>0.48 (0.14 – 1.7)</td>
<td>0.244</td>
</tr>
<tr>
<td>Family History DM</td>
<td>1.5 (0.7 – 3.0)</td>
<td>0.279</td>
</tr>
<tr>
<td>Ethnicity (non-Caucasian)</td>
<td>3.7 (1.5 – 1.5)</td>
<td>0.006</td>
</tr>
<tr>
<td>Previous macrosomic baby</td>
<td>0.5 (0.1 – 4.5)</td>
<td>0.574</td>
</tr>
<tr>
<td>PCOS</td>
<td>1.0 (0.3 – 3.1)</td>
<td>0.988</td>
</tr>
<tr>
<td>SHBG</td>
<td>0.64 (0.44 – 0.94)</td>
<td>0.020</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.47 (0.3 – 0.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1,5 AG</td>
<td>0.71 (0.50 – 1.02)</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Table 19 looks at each individual history-based risk factor for GDM and the OR of developing GDM. We then adjust for all other history factors and biomarker levels in the adjusted analysis. BMI and ethnicity remain as independent predictors of GDM status in the adjusted analysis of the history-identified risk factors. Adiponectin and 1,5 AG remain independent predictors of GDM status after adjusting for all other variables.
Results 5 – Findings of the Acceptability of the OGTT

Figure 19 explores the convenience of the OGTT to the women sampled. 49% (n=28) took time off work to perform the test and 68% (n=39) the OGTT was not scheduled to coincide with a routine antenatal visit. 26% (n=15) had to organise additional childcare outside of their usual arrangements.
Figure 20 denotes the overall convenience of the test as experienced by the women. 16% (n=9) described the test as “somewhat” or “very inconvenient.”
Figure 21 describes the incidence of the commonly reported side-effects of the OGTT. The most commonly reported physical symptom was hunger. 57% (n=33) reported some degree of hunger however for the majority (27/33) this was experienced “a little” or “moderately”. 28% (n=16) reported feeling lightheaded or dizzy during the screening test while 26% (n=15) reported feeling nauseous. 2 of these reported “severe” nausea. 5 patients (9%) vomited during the glucose tolerance test.
Figure 22 shows 49% (n=28) experienced some level of anxiety regarding the results of the test however most (19/28) described this as “a little”. 25% (n =14) confirmed that the test caused them to worry about their baby’s health and 14% (n=8) worried about their own health as a direct result of performing the test.
Discussion Chapter

Below is a brief description of the results of each section followed by a discussion of the significance of these results and their context within the current literature. We discuss the strengths and inherent flaws in the study design, while outlining a plan for further studies that would build on the data we have collected and examined. We also comment on the clinical utility of these findings as ultimately this is a clinical study.

The first results section of this study examines the relationship between a group of biomarkers measured in the first trimester and the subsequent diagnosis of GDM. Figure 2 describes the number of patients recruited and the reasons for exclusion from analysis. The sample size of the cohort compares favourably to other studies on the individual biomarkers.

Our cohort of patients recruited for this part of the study included any woman identified to have one or more risk factors for the development of GDM at booking.

Figures 3,4,5,6 and 7 describe the profile the cohort in terms of BMI, risk factors for GDM, obstetric history and medical co-morbidities. O'Dwyer et al described the body mass indices of 6000 pregnant women booking for ante-natal care in Ireland in 2010 (119). They found that 54% were normal weight (BMI of ≥ 18.5 and <25), 28% were overweight (BMI of ≥ 25 and <30), 13% were obese (BMI of ≥ 30 and <40), 3% were underweight (BMI of <18.5 kg/m2), and 2% were morbidly obese (BMI of ≥ 40). Our population has a larger proportion of overweight, obese and morbidly obese patients at 21%, 34% and 4% respectively as we sought to study women with risk factors for GDM. Of note, BMI was objectively measured by a midwife in our study and not self-reported. This is a strength of our study compared to some of the other literature where it can be unclear whether BMI is self or objectively reported. O'Dwyer et al showed that self-reporting of height and weight lead to underestimating incidence of overweight and obesity by at least 5%.

In terms of obstetric history, the group of patients studied would not be considered unusual. The average age of the recruited patients (32.8 years) is similar to the average maternal age reported by the Central Statistics Office.
(CSO) for births in Ireland in 2014 which was 32.3 years (120). Table 2 outlines the risk profile of those that screened negative versus those that screened positive. Of the 224 cases formally screened for GDM, 46 (20.5%) screened positive. This is well below the 60% detection rate with a 40% false positive rate quoted by the NICE group for a risk factor based approach to patient selection for screening (121). Unsurprisingly those with GDM were older, more obese and had a higher number of risk factors in their history than those that screened negative.

When examining the medical comorbidities, the incidence of thyroid dysfunction in this group is 6%. This is somewhat higher than the reported incidence in the general pregnant population (overt hypothyroidism 0.3 – 0.5%, sub clinical hypothyroidism 2-3% and hyperthyroidism 0.1–0.4%) (122). There is some evidence to suggest that GDM occurs more frequently in patients with a known diagnosis of hypothyroidism and it is well recognised that thyroid dysfunction is associated with insulin resistance in the non-pregnant state (123). It has been suggested that hypothyroid pregnant women be routinely offered screening for GDM. This may account for the relatively high incidence of thyroid dysfunction in our population as they have many other risk factors for insulin resistance.

The genetic basis for diabetogenesis is complex. It is notable that our population was composed mostly of patients of European descent and that the results may not be wholly applicable to high-risk sub populations based on ethnicity. However, the cohort as a whole is representative of the obstetric population described in the Rotunda Hospital’s Annual Clinical Report of 2014.

When looking at the obstetric and neonatal outcomes of the cohort in Table 3 it is apparent that the rate of macrosomia is higher than would be expected in both the GDM and the non- GDM group (22.5% and 14% respectively). The 14% rate of fetal macrosomia in the screen negative group suggests that some women where the OGTT is normal may have a degree of unrecognized hyperglycaemia leading to this effect.

The rates of operative vaginal delivery in the GDM and non-GDM patients are similar and reflect the total background population of the unit in the year of the
study. There is however an unexplained trend toward a higher caesarean section rate (36%) in the non-GDM group compared to the GDM (28%) or the background population (31%). Dissecting this further, reveals that it is the planned/elective pre-labour caesarean section rate that is so significant in this group but it is unclear why this is so. Table 2 in Results outlines the differences in age, BMI and risk factor profile of the GDM and non GDM group. The GDM group is, on average, older, more obese and has a higher number of GDM risk factors than the non GDM group. However, in spite of the increased fetal macrosomia rate observed in the setting of confirmed GDM, it could be postulated that conferring a diagnosis of GDM instigates a maternal and fetal surveillance regimen, including a formal schedule of departmental sonographic evaluation of fetal weight, that ultimately results in a lower planned Caesarean delivery rate owing to improved prenatal confidence in identification or exclusion of fetal macrosomia. There were 47% primiparous and 53% multiparous patients in the study. In the Rotunda Annual Report there were 43% primips for the year 2014 so this distribution is broadly in keeping with the figures for the background population.

There was an unusually high incidence of primary post-partum haemorrhage in this group as a whole (19.5%) and most particularly in those that screened positive for GDM (25%). For the purposes of our study PPH was defined as an estimated blood loss of 500mls or more at delivery. While blood loss estimation is notoriously inaccurate, the delivery unit and theatre staff try to objectively measure as much as possible by weighing swabs and subtracting amniotic fluid volumes suctioned at delivery. We may postulate that this increased rate of primary post-partum haemorrhage is potentially related to prolonged parturition and macrosomic infants. This association has been borne out in other larger studies also. Matthew et al studied the clinical consequences of macrosomia in over 350,000 pregnancies (both GDM and non GDM) using both crude birthweights and birthweight centiles and found the rate of PPH to be 19% in the macrosomic group. They also found a higher rate of second and third degree perineal trauma overall compared to normal weight infants (an OR of 1.29 CI 1.24- 1.34 and 1.88 CI 1.54-2.31 respectively) (124).
The infants of those mothers that screened positive for GDM had a higher rate of admission to NICU. This is a recognised phenomenon in the literature (125). Interestingly in our cohort there was no infant that required admission due to hypoglycaemia. The protocol of the hospital is that early and frequent feeds be given to infants of mothers with GDM and pre/post feed blood glucose monitoring allows detection and treatment of hypoglycaemia early on the general postnatal wards. Only severe refractory hypoglycaemia would necessitate admission to the NICU. This may explain this finding. It is notable that no case of shoulder dystocia occurred in either group. Shoulder dystocia occurs where additional manoeuvres beyond gentle downward traction are required to facilitate delivery of the infant’s shoulders. A shoulder dystocia may result in serious morbidity such as hypoxia, hypoxic ischaemic encephalopathy and brachial nerve injuries for the infant and perineal trauma for the mother. The occurrence of shoulder dystocia is highly litigious and largely unpredictable (126). The literature quotes a rate of anywhere from 0.1 to 3% of vaginal deliveries complicated by shoulder dystocia in an unselected population. A diagnosis of GDM gives an OR of 1.9 for shoulder dystocia, while a macrosomic birthweight confers an OR of 5.1. (127). The absence of a case of shoulder dystocia in our group may be explained by the high number of caesarean deliveries. The relatively small number of patients recruited and having vaginal deliveries mean that this study was not powered to investigate this outcome.

Table 4 details the results of the measurement of each analyte. The numbers of samples analysed vary since a small number were insufficient samples to permit all four assays to be completed, incorrectly labelled for analysis, incorrectly stored or haemolysed sample unsuitable for analysis. Overall the loss to these effects was small and varied from biomarker to biomarker. The datasets for the results of each biomarker were all normally distributed except for CRP. This data is visually represented in figures 8,9,10 and 11. This relates to the fact that a normal CRP level is considered to be 5 or less, however a raised CRP can be many multiples of this and so one or two raised results can skew the dataset significantly. This characteristic of CRP affected its utility as a biomarker in our study and may undermine its use to this end in other research. We discuss this in greater detail below.
Table 6 examines the correlation between the results of the first trimester biomarkers and a screen positive oral glucose tolerance test. It is statistically described in terms of the odds ratio conferred by a one standard deviation increase in the serum biomarker result. Both sex hormone binding globulin and adiponectin initially show a statistically significant link to the likelihood of a screen positive OGTT. This persists for adiponectin even after adjusting for BMI, ethnicity and family history of GDM. (the three risk factors that were most associated with the likelihood of a screen positive test in our cohort – see table 19 ) The lower the levels of SHBG in the first fifteen weeks of pregnancy the higher the likelihood of screening positive for GDM in the unadjusted analysis. There is a similar recognised strong and consistent link between low levels of SHBG and type II Diabetes in the non – pregnant female population (128). Interestingly when the different genotypes responsible for SHBG production were analysed in this secondary analysis of the Women’s Health Study (129), a very specific subset of patients with low SHBG were identified as being particularly high risk for developing type 2 diabetes. While in in the unadjusted analysis we show a link between lower SHBG levels and high risk of GDM, the ROC curve in figure 16 does not point to a specific cut off value above which a diagnosis of GDM is particularly more likely. In other words, there is no fixed value of SHBG measured in the first trimester below which, with good sensitivity and specificity, we can predict a screen positive OGTT. The graph shows a continuum. These findings echo similar results in smaller studies of use SHBG in this regard (78,79). The fact that SHBG loses significance after adjustment for BMI, ethnicity and family history is interesting. This limits the application of SHBG alone in the clinical setting to absolutely identify a group of “at risk” women early in pregnancy to target for early intervention and screening. Future directions for studies of SHBG and GDM risk could potentially therefore focus on the specific genotypes (e.g. carriers of an rs6257 variant allele) that are strongly linked to the onset of Type II Diabetes. The clinical value of availability and application of this genetic information however is attenuated by the cost of performing this analysis and also by the fact that insurance companies could use this information to affect the price of healthcare insurance for these women in the longer term.
Table 6 shows that adiponectin measured in the first 15 weeks of pregnancy performs well in correlating to the risk of onset of GDM. Furthermore, our results in table 12 show that having a serum adiponectin in the first 15 weeks of pregnancy that measures 8.9µg/mL or below gives an odds ratio for a screen positive OGTT of 3.3 (95% CI 1.6528 – 6.7) and this is highly statistically significant (p=0.0008). This compares very favorably as a significant predictor or risk factor for GDM to having a BMI of >30 at booking (OR of 2.3) as described in Table 19. It is worth noting that our cohort had a lower mean Adiponectin level that that previously shown to be the mean in the healthy pregnant population (130). This is unsurprising given that we deliberately sub selected a cohort that would be at risk of GDM. Similar to the findings with SHBG, reduced levels of circulating adiponectin have been long linked to the onset of Type II Diabetes in the non-pregnant population. LaCroix et al have investigated the link between adiponectin levels and risk of GDM in 445 unselected (i.e. no risk factors) patients in whom 39 developed GDM following a 50g glucose challenge test followed by a formal OGTT(106). Echoing the findings of our study the authors report that while lower adiponectin is associated with a higher likelihood of development of GDM, there was a large overlap in the values for those women that screened negative and those that screened positive. Again, this potentially undermines the clinical utility of adiponectin as a stand-alone biomarker used to accurately and absolutely predict GDM. However, it suggests that adiponectin may have a role in developing an integrated risk assessment model based incorporating patient history risk factors.

Given the highly statistically significant link between adiponectin and GDM we looked at it in relation to each of the individual serum glucose values measured during the OGTT. The biologic rationale for doing this was that those women with an abnormal fasting glucose values are considered more likely to have undiagnosed pregestational type II diabetes. In table 11 we show a linear regression analysis to correlate adiponectin values to individual OGTT results. The association holds strongly across all three measured serum glucose levels. This hints to the value of adiponectin measurement in the 1st trimester for predicting gestational diabetes as distinct from indicating those with pregestational type II diabetes alone. In an attempt to explore the potential of 1st
trimester adiponectin further, we analysed the positive predictive values for each quartile of serum glucose at the 28 week OGTT (table 13). We look in this table at the positive predictive value of adiponectin >8.9µg/L for each quartile of serum glucose values at each timepoint of the test. Division of the glucose values into quartiles was an effort to explore the “spectrum” effect of glucose tolerance.

Another notable point from table 6 is that 1,6 AG becomes a highly statistically significant predictor for GDM once BMI, ethnicity and family history are adjusted for. In the univariate analysis, patient-to-patient variability is unexplained and 1,5 AG is borderline non-significant for first trimester prediction of GDM (p=0.06). However, once this variation is taken in to account, the biomarker signal is stronger. The ROC curve of 1,5 AG as a predictor of GDM (figure 17) fails to point to a distinct threshold which would give acceptable sensitivity and specificity. There is a statistically significant difference in the mean 1,5 AG levels (measured in the 1st fifteen weeks’ gestation) in the pregnancies that went on to develop GDM versus those that did not (table 7). 1,5 AG gives a very accurate picture of the absolute glycaemic control in the preceding 14 days to sampling. Even very short transient spikes in blood glucose levels cause the 1,5 AG levels to drop. It is considered a marker of “short term” glycaemic control (as opposed to HbA1c as a marker of long term and fructosamine as a marker of intermediate term control) It has been validated independently in the pregnant population. We chose to study this particular biomarker for a number of reasons. It had not been investigated in this particular capacity. We felt that a proportion of women that would be diagnosed with GDM would have either very early onset glycaemic instability due to disease severity or even latent type II diabetes and that interrogating 1,5 AG may have highlighted this subgroup. Our results show that indeed, women that go on to develop GDM in later pregnancy have more frequent episodes of hyperglycaemia even in early pregnancy, before the pathophysiological processes of impaired glucose tolerance of pregnancy emerge. These results have not been shown before in the literature to date on 1,5 AG. These data challenge the concept that detectable changes in glucose tolerance only develop late in the second trimester in those with GDM.
Our findings on CRP are at odds with some of the published literature in this field. Ozdu-Erdinc at al looked at CRP concentrations in 450 patients booking for antenatal care in Turkey, with or without risk factors for GDM and found a positive correlation between CRP and development of GDM (131). Wolf et al found that in a prospective nested case-control study where 43 women subsequently developed GDM; higher levels of CRP in the first trimester positively correlated with the onset of disease and the difference between the two groups was statistically significant (81). However, once the results were adjusted for obesity the association was attenuated leading the authors to recommend that further larger studies should evaluate this in more detail. We also must be cognizant of the fact that CRP is a biomarker that can change in great magnitude from day to day and a single measurement at a point in time may not truly reflect the background inflammatory metabolic milieu of the subject. Our CRP data was not normally distributed with a small number of very high results skewing the data. This may have affected our results. Based on our study and the conflicting findings of the current body of available research on CRP use as a biomarker for GDM, we feel it is unlikely to prove a useful tool to this end. A serious draw back to employing CRP as a screening tool is its non-specific nature and the plethora of unnecessary investigations which may ensue following a high result. Routinely measuring CRP in otherwise healthy asymptomatic women would likely lead to unnecessary diagnostics, unfounded patient anxiety and increased healthcare costs.

In summary, of the 2nd and 3rd results section, of the four biomarkers we elected to examine in this study, adiponectin and SHBG demonstrated a correlation to the risk of onset of GDM in the univariate analysis. However, after adjustment for BMI, family history and ethnicity in the multivariate analysis SHBG loses significance. Following adjustment for BMI, family history and ethnicity 1st trimester 1,5 AG becomes a significant predictor of GDM. 1,5 AG is borderline non-significant (p=0.06). However, once other prognostic variables are taken into account, it is highly statistically significant (p=0.01). In the univariate analysis, patient-to-patient variability is unexplained, however once this variation is taken in to account, the biomarker signal is stronger. Mean 1,5 AG levels are significantly lower even in the first trimester in women that will go on
to develop GDM. However, none of these biomarkers exhibited a specific threshold value at which onset of GDM could be predicted absolutely with acceptable sensitivity and specificity. Measurement of these analytes alone in the first trimester is unlikely to accurately pinpoint who will develop GDM or replace the OGTT but the results do allow us to inform the patient of her individual risk of GDM based on her results. It is possible that they may form part of a bio-social risk assessment model which may allow women to be informed of their own personal risk of developing GDM and take steps early in the pregnancy to mitigate against it.

In the 4th results section we examine the relationship between the 1st trimester biomarkers and the perinatal outcomes of the cohort. Table 15 describes a multiple logistic regression of each biomarker for the risk of a macrosomic infant. We defined macrosomia as a birthweight >90th centile for gestation. Contrary to what we would expect, increasing SHBG levels were significantly associated with the risk of a macrosomic infant. This does not fit the hypothesis that lowered SHBG levels are a marker for GDM. It is difficult to explain this finding other than to remind ourselves that in our study after adjustment for BMI, ethnicity and family history, 1st trimester SHBG was not linked to the risk of GDM. Interestingly in table 15 we see that increasing 1st trimester 1,5 AG (indicating better glycaemic control) correlated with a lower chance of operative vaginal delivery. This finding held significance even after adjustment for nulliparity and birthweight- the two most influential factors for risk of operative delivery. Of note fetal position (occipito – anterior/posterior) was not recorded by the birth attendant. This finding is difficult to explain having corrected for birthweight. Could it be that those with improved glycaemic control labour more efficiently? It is, of course, worth noting that this could be a chance finding and may be of no clinical significance. It is also worth noting that the indication for operative vaginal delivery (fetal distress or failure to advance) was not collected by the birth attendant. In retrospect, this would have been a helpful adjunct to dissect this link more carefully. It is difficult to draw any firm conclusions from this finding but this biomarker certainly warrants further interrogation. None of the biomarkers were associated with the risk of emergency intrapartum caesarean section.
Cord blood c-peptide levels were drawn at 128 of the 225 deliveries. 5 of these were insufficient volume so analysis was performed on 123 samples. We firstly performed a linear regression analysis on the 1\textsuperscript{st} trimester serum marker values and absolute cord blood c-peptide values.

Figure 18 is a graph demonstrating the relationship between sex hormone binding globulin and cord blood c-peptide. Using Pearson correlation coefficients; Table 18 shows a low correlation overall between SHBG and absolute cord blood c-peptide values. There is a correlation between sex hormone binding globulin measured in the 1\textsuperscript{st} 15 weeks of pregnancy and absolute cord blood c-peptide levels but it is low.

However, the wedge shape of the results in Figure 18 prompted us to perform a logistic regression on the link between sex hormone binding globulin levels >350 nmol/L and a cord blood c-peptide level less than the 90\textsuperscript{th} centile (1.7µL) – indicating normal insulin levels in the fetus. The results are shown in Table 19. Our analysis of the relationship between sex hormone binding globulin values in the first trimester and neonatal hyperinsulinaemia (cord blood c-peptide> 90\textsuperscript{th} centile/1.7µL) shows that in women with a high SHBG (>350nmol/L) early in pregnancy have an odds ratio of 2.7 to have a non-hyperinsulinaemic neonate. It prompts us to consider and question in future research if a high SHBG (>350nmol/L) measured in the first trimester in women with one or more risk factors for GDM eliminates the need for a formal OGTT? It is regrettable that cord blood was obtained on only 58% of the cohort for whom we have delivery outcomes. This was despite multiple and frequent reminders and information sessions given to birth attendants and written information in all medical records regarding the study. Among the reasons cited for failure to provide the sample were; unintended homebirth/ambulance birth, insufficient blood in the cord following pH analysis and cord blood samples for neonatal rhesus status, attendant omitted to double clamp the cord.

Linear regression analysis of C-reactive protein, adiponectin and 1,5 Anhydroglucitol levels with cord blood c-peptide levels did not demonstrate any relationship and so the graphs are not presented here.
In the 5th results section we examine the acceptability and impact of the current glucose tolerance test on the pregnant population. With regard to convenience; a large proportion of women (49%) had to time off work to perform the test and 26% had to organise additional childcare outside of their usual arrangements. These factors may potentially deter women from attending the OGTT. 16% (n=9) described the test as “somewhat” or “very inconvenient”

Despite reporting the physical and psychological side effects of the test as seen in Figures 21 and 22, when contacted 2 days later by telephone 98% (49/50) were happy to have undergone screening overall. 96% (n=48) would undergo screening in a subsequent pregnancy if recommended to do so by their doctor. Our study shows that despite the OGTT having adverse physical effects on the pregnant population most women find it an acceptable test and would agree to be screened again in the future if it was felt to be necessary or of benefit in their pregnancy. It has previously been shown that pregnant women are motivated to prioritise the health of the fetus even if it is difficult for them (132). Griffiths RD et al conducted a small similar cohort study using a postal questionnaire and found similarly that women were satisfied with the convenience of the test and would be happy to repeat it if necessary (133). Goldstein et al surveyed those with a diagnosis of GDM following OGTT and again found the majority to be satisfied with the screening and diagnostic pathway (134).

The findings regarding anxiety about their own and their baby’s health are supported in the literature by other larger studies on this subject. Kerbel et al describe how women who initially had a positive glucose challenge test but later when on to have a negative OGTT still experienced a significant decline in their perception of their own health (135). Rumbold et al describe an adverse impact on patient’s perception of their own health and quality of life as a direct result of testing for gestational diabetes (136).

A potential criticism is that our study could have been affected by selection bias. Women agreeing to participate in this research may have been more eager to please a healthcare professional and may possibly underreport adverse events encountered during the screening process. It is difficult to quantify the effect of this bias.
In terms of improving the acceptability of the test for patients we have recommend that it be scheduled to coincide with a routine antenatal visit in the future to facilitate convenience. The WHO stipulates that many preparations of 75g of oral glucose are acceptable for the test. Glucose syrup (as found in Lucozade®) has been shown to reduce the incidence of nausea associated with the OGTT. It is far less sweet than other sugars such as sucrose and has been shown in clinical studies to be more acceptable to patients (137) It is also convenient for the care provider. We recommend that this preparation continue to be used.

Previous studies have shown that between approximately anywhere from 10 to 60% of the population selected for OGTT testing by risk factor based selection will test positive. 12% of this particular cohort screened positive for GDM. This would be in keeping with prevalence quoted in the ATLANTIC DIP study where universal testing was employed (41). This underlines the fact that selection for testing based on risk factors alone is crude and rather ineffective, with many patients experiencing nausea, hunger, vomiting, jitteriness and syncopal episodes unnecessarily. If we were to accept the recent USPSTF recommendations for universal screening then (aside from the major clinical impact of treating up to 16% of the pregnant population for GDM) we would also be asking all pregnant women to endure this test. This study finds that women are in fact overwhelmingly satisfied with the experience of being screened. This satisfaction is despite many reporting adverse physical symptoms during the course of the test. Quite apart from the cost/benefit analysis, we also must bear these findings in mind when considering the implications of universal screening for GDM. The prevalence in the population, known benefits of treatment, poor predictive value of risk factor based screening and patient satisfaction with the test all point in favour of universal screening for GDM.

**Directions for further research**

There are a number of important concepts thrown up by the findings of this thesis that warrant further consideration, investigation and development.

(a) Those women considered “at risk” of GDM with a serum adiponectin level <8.9 µg/ml in the first 15 weeks of pregnancy necessitate further scrutiny.
Does randomisation of these women to either early intervention (diet and lifestyle advice/close glycaemic surveillance) or routine care (OGTT at 24-28 weeks and subsequent clinical pathway pending results) impact on (i) the incidence of screen positive OGTT and (ii) their perinatal outcome measures? What is the potential cost/benefit analysis of this early intervention? Obviously, a study of this type would require adherence monitoring (perhaps with diet diaries/periodic interviews with a dietician), patient satisfaction evaluation and postnatal follow-up.

(b) In women “at risk” of GDM with a serum SHBG value of >350nmol/L is screening for GDM even necessary? In this cohort, it would be interesting to see if there is a difference in perinatal outcome in those assigned to “No OGTT” versus those that receive standard care and go for OGTT. There may however be an ethical consideration here to give us pause; is it acceptable to assign a woman and her fetus to the “no screen” group?

(c) Could 1,5 Anhydroglucitol measurement at 24 – 28 weeks of pregnancy substitute for/act as an adjunct to the OGTT for detection of women with impaired glucose tolerance needing intervention in pregnancy? Due to many of the problems with the OGTT outlined in this thesis (the “fetal glucose steal” phenomenon, the unreliability of serum glucose measurements due to sample glycolysis in typical clinical conditions and the somewhat arbitrary nature of the cut-offs taken for diagnosis) it is conceivable that a biomarker such as 1,5 Anhydroglucitol could effectively flag those women with deteriorating glucose tolerance in the late second and early third trimester. It would be a test far simpler to administer and arguably may produce more clinically meaningful results, given its tight correlation with continuous glucose monitoring pack results. It is a particular regret of this study that we did not foresee to repeat the measurement of the 1,5 Anhydroglucitol at the time of the OGTT and compare it to the results. It is felt that this would be an especially clinically applicable study to carry out.
In Summary

Gestational Diabetes is a common condition, amenable to treatment and intervention which undoubtedly improves outcomes. However, screening and diagnosis have long presented a source of controversy. This thesis highlights how measurement of serum adiponectin and 1,5 AG in the first fifteen weeks of pregnancy can identify a particularly “at risk” subgroup which may benefit from early intervention and measurement of SHBG can potentially identify another subgroup of women that are very unlikely to develop the pathophysiology associated with fetal/neonatal hyperinsulinism.

Most certainly, the controversy and many different approaches to screening and diagnosis present a challenge to scientific advancement in this area. However, the vast numbers of women regularly booking for care and undergoing screening presents a fantastic opportunity for further robust research in this area. This is a group that is easy to recruit and follow up for outcomes as each pregnancy has a natural endpoint. While drug trials in pregnancy can prove to have ethical pitfalls, pregnancy lends itself well to prospective observational trials such as ours and the studies we suggest for future work. It is exciting that the information gleaned from this study may potentially help identify women that will benefit from early intervention and experience improved perinatal outcomes as a result.

As we discuss in the opening chapter, a diagnosis of GDM in pregnancy has arms reaching far into the adult life of the fetus by the process of fetal programming. There is a growing body of evidence that the physiological parameters of the fetus can be “reset” by the environment and events in pregnancy. Gene expression is manipulated by these environmental factors and this has consequences into childhood and adult health. Critically important so, is the potential impact of this focus on early intervention on the future generation. In the face of a global obesity and metabolic-syndrome epidemic, it behoves us to focus our scientific energies on breaking the generational cycle of obesity and glucose intolerance. This battle must admittedly be waged on many fronts however the findings of this study may give a starting point and direction to our efforts.
References


(2) Catalano PM, Tyzbir ED, Roman NM et al Longitudinal changes in insulin release and insulin resistance in non-obese pregnant women Am J Obstet Gynaecol 1991 165 1667-1672


(4) Carr DB, Gabbe S Gestational Diabetes detection, management and implications Clinical Diabetes 1998 16 5-19


(8) Negrato CA,Mattar R Gomes MB Adverse pregnancy outcomes in women with diabetes Diabetology and Metabolic Syndrome 2012 DOI: 10.1186/1758-5996-4-41


(11) Melissa G. ROSENSTEIN, MD, Yvonne W. CHENG, MD, MPH, Jonathan M. SNOWDEN, PhD, James A. NICHOLSON, MD, Amy E. DOSS, MD, and Aaron B. CAUGHEY, MD, PhD The Risk of Stillbirth and Infant

(12) N Russell M Higgins M Amaruso M Foley FM McAuliffe Troponin T and pro B typenatiuretic peptide in foetuses of type one diabetic mothers Diabetes Care 2009 Volume 32 Issue 11 2050-2055


(18) Bennewitz HG: De Diabete Mellito, gravidatatis symptomate. 1824, MD Thesis, University of Berlin


(20) Brocard: La glycosuria de al groaesser. 1898, These de Paris


(30) Dahanayaka NJ1, Agampodi SB, Ranasinghe OR, Jayaweera PM, Wickramasinghe WA, Adhikari AN, Chathurani HK, Dissanayaka UT. Inadequacy of the risk factor based approach to detect gestational diabetes mellitus Ceylon Med J. 2012 Mar;57(1):5-9


(33) McElduff A, Hitchman R. Screening for gestational diabetes: the time of day is important. Med J Aust 2002; 176: 136

(34) Sermer M, Naylor D, Gare DJ, Kershole AB, Ritchie JW, Farine D, Cohen HR, McArthur K, Holzapfel S, Biringer A, Chen E, Cadesky KI,


(37) American Diabetes Association Diagnosis and classification of diabetes mellitus Diabetes Care. 2006 Jan;29 Suppl 1:S43-8


(40) Jovanovic L1, Pettitt DJ. Gestational diabetes mellitus. JAMA. 2001 Nov 28;286(20):2516-8


(43) NICE Guideline on Diabetes in pregnancy: management from preconception to the postnatal period February 2015

http://www.nice.org.uk/CG63


Caliskan E, Kayikcioglu F, Ozturk N, Koc S, Haberal A: A population-based risk factor scoring will decrease unnecessary testing for the diagnosis


(60) Gillespie P1, Cullinan J, O'Neill C, Dunne F; ATLANTIC DIP Collaborators. Modelling the independent effects of gestational diabetes mellitus on maternity care and costs. Diabetes Care. 2013 May;36(5):1111-6


(64) Shyam S1, Arshad F, Abdul Ghani R, Wahab NA, Safii NS, Nisak MY, Chinna K, Kamaruddin NA Low glycaemic index diets improve glucose tolerance and body weight in women with previous history of gestational diabetes: a six months randomized trial. Nutr J. 2013 May 24;12:68


(67) Han S1, Middleton P, Crowther CA Exercise for pregnant women for preventing gestational diabetes mellitus Cochrane Database Syst Rev. 2012 Jul 11;7

(68) Nicholaides KH, Turning the Pyramid of Prenatal Care, Fetal Diagnosis and Therapy 2011;29:183-196


(71) N Daly, MJ Turner Laboratory diagnosis of gestational diabetes BJOG 2016 DOI: 10.1111/147-0528.13907


(75) Denison FC, Roberts KA, Barr SM, Norman JE Obesity, pregnancy, inflammation, and vascular function Reproduction. 2010 Sep; 140(3):373-85.


(89) Kinalska M1, Telejko B, Kuźmicki M, Kretowski A, Kinalska I. Tumor necrosis factor alpha system and plasma adiponectin concentration in women with gestational diabetes Horm Metab Res. 2005 Jul;37(7):450-4

(90) Ranheim T, Haugen F, Staff AC, Braekke K, Harsem NK, Drevon CA. Adiponectin is reduced in gestational diabetes mellitus in normal weight women. Acta Obstet Gynecol Scand. 2004 Apr;83(4):341-7


(92) Lain KY1, Daftary AR, Ness RB, Roberts JM. First trimester adipocytokine concentrations and risk of developing gestational diabetes later in pregnancy Clin Endocrinol (Oxf). 2008 Sep;69(3):407-11

(94) Nanda S., Akolekar R., Sarquis R., Mosconi A.P., Nicolaides K.H.
Maternal serum adiponectin at 11 to 13 weeks of gestation in the prediction of macrosomia. Prenat. Diagn. 2011;31:479–483
(95) Lain KY1, Daftary AR, Ness RB, Roberts JM. First trimester adipocytokine concentrations and risk of developing gestational diabetes later in pregnancy Clin Endocrinol (Oxf). 2008 Sep;69(3):407-11
(96) Xu J., Zhao Y.H., Chen Y.P., Yuan X.L., Wang J., Zhu H., Lu C.M.


Elevated serum levels of visfatin in gestational diabetes: A comparative study across various degrees of glucose tolerance. Diabetologia. 2007;50:1033–1037


(118) Sally K. Abell , Barbora De Courten , Jacqueline A. Boyle and Helena J. Teede Inflammatory and Other Biomarkers: Role in Pathophysiology and Prediction of Gestational Diabetes Mellitus Int. J. Mol. Sci. 2015, 16(6), 13442-13473


(120) Central Statistics Office Vital Statistics Yearly Summary 2014

(121) NICE Guideline on Diabetes in pregnancy: management from preconception to the postnatal period February 2015 http://www.nice.org.uk/CG63


(128) Erikson et al Body fat, insulin resistance, energy expenditure and serum concentrations of leptin, adiponectin and resistin before, during and after pregnancy in healthy Swedish women British Journal of Nutrition / Volume 103 / Issue 01 / January 2010, pp 50-57

(133) Griffiths RD1, Rodgers DV, Moses RG Patients’ attitudes toward screening for gestational diabetes mellitus in the Illawarra area, Australia. Diabetes Care. 1993 Feb;16(2):506-8


(136) Rumbold AR, Crowther CA Women's experiences of being screened for gestational diabetes mellitus. Aust N Z J Obstet Gynaecol. 2002 May;42(2):131-

APPENDIX I Delivery Outcome Form

TO BE COMPLETED BY MIDWIFE ATTENDING DELIVERY AND COLLECTED BY RESEARCHER

1. Date of Delivery  
   ___________ / ___________ / ___________  
   dd  
   mm  
   yyyy

2. Time of Delivery  
   _____ : _____

3. Gestational age at delivery  
   ______________ wks ______________ days

4. Stillbirth  
   Y

5. Neonatal Death  
   Y

6. Alive  
   Y

5. Birthweight (grams):  
   ___________________________ g

6. Gender:  
   M  
   F

7. Mode of delivery (circle one):  
   • Normal vaginal delivery / Ventouse/ Forceps / Combined Ventouse/Forceps
     Elective LUSCS / Emergency pre-labour LUSCS / Intrapartum CS/ Classical CS

8. Spontaneous onset of labour  
   Yes  
   No

9. Induction of Labour  
   Yes - Prostaglandin / ARM / Oxytocin  
   No
10. Maternal Complications  3\textsuperscript{rd}/4\textsuperscript{th} degree tear/HDU admission/PPH / Seizure /
Other (specify): ______________________

11. Arterial cord pH  __________, base excess: ______________

12. Venous cord pH  __________, base excess: ______________

13. Cord blood C-peptide taken  YES  NO

14. Colour of Liquor:  Clear
                     Meconium
                     Blood

15. Apgar scores  __________@ 1 min  __________@ 5 min

16. Infant Chart Number ______________________________

17. NICU admission:  Yes  No
  • Indication ________________________________
  • TTN  Yes  No
  • Documented Glycaemic Instability  Yes  No
  • Feeding Difficulties  Yes  No
  • Tremors/Jitteriness  Yes  No
  • Culture proven sepsis  Yes  No
  • Cooling  Yes  No
  • Jaundice requiring treatment  Yes  No
  • Hypocalaemia  Yes  No
  • Hypomagnaesaemia  Yes  No
  • Other ________________________________
  •
Dear Midwife,

This patient is participating in the **PEAR Study** (Predicting geStational diAbetes in the first trimester)

We are examining a panel of biomarkers at booking and their relationship to the subsequent development of gestational diabetes and also the delivery and neonatal outcomes.

Firstly thank you for your participation with the data collection.

For the purposes of this study the umbilical cord will need to be **double clamped**. We will require **cord pH samples** on all infants delivered to mothers enrolled in the study. We will also require a further 10ml sample of that cord blood for analysis of **cord blood c-peptide levels**. This sample is taken with a syringe and sent in a serum (red top) and lithium heparin (green) bottle to the lab. **Please find the bottles and the prefilled forms on the inside of the patients chart**. The Green (biochemistry) lab form should be used and the form should contain one of the study stickers or mark PEAR STUDY clearly on the form. The patients’ MRN is NOT to be used. Please find the anonymised study number on the top right corner of the outcome form in the chart (eg PEAR001) Mark the form and the bottles using the patients’ initials, DOB and study number. Please find a panel of the study stickers in the inner leaf of the front page of this patient’s chart.

Each participating patient will have delivery outcomes datasheet which will take approx. 3-5 minutes of your time to complete what details you can.

Again thank you so much for your help in collecting information for this study.

Please do not hesitate to contact me (at any time) with any queries which arise,

Regards

Siobhan Corcoran
0876560308
siobhancorcoran@rcsi.ie
Lead Researcher
CONSENT FORM

Research title:

Researcher: ________________  Tel: __________  E-mail: ________________

DECLARATION by participant: Please tick (✓) and provide your initials

1. I have read the information leaflet for this research study and I understand the contents.  Yes [ ] No [ ] initials [ ]

2. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction.  Yes [ ] No [ ] initials [ ]

3. I understand that I may be contacted by a member of the research team and requested to participate in an interview(s) on one or more topics covered by this research and I consent to this.  Yes [ ] No [ ] initials [ ]

4. I understand that I will be given an opportunity to review the transcript of such an interview(s) to confirm accuracy.  Yes [ ] No [ ] initials [ ]

5. I understand that the transcript will not identify me by name but will use the study code and that the original digital recording will be erased once the accuracy of the transcript has been confirmed.  Yes [ ] No [ ] initials [ ]
6. I understand that information from this research will be published but that I will not be identified as a participant in this research in any publication. Yes [ ] No [ ] initials [ ]

7. I understand that I will not be identified as a participant in this study (unless a legal requirement) and that the researchers may hold my personal information for 1 year after the study has been completed. Yes [ ] No [ ] initials [ ]

8. I agree that information obtained from me in this research which has been coded so as not to identify me may be stored and used for the purpose of future research which will have obtained Research Ethics Committee approval without the need for further consent from myself. Yes [ ] No [ ] initials [ ]

9. I understand that my personal details (name and address and other identifying information that links my identity to the study data) will be destroyed when this study is complete unless I have agreed to its retention after that date and to being contacted about future research. Yes [ ] No [ ] initials [ ]

10. I consent to my personal details being retained for a further period of 1 year after this study has been completed and used to invite me to participate in future research in accordance with this consent. Yes [ ] No [ ] initials [ ]

11. I understand that the researchers undertaking this research will hold in confidence and securely all collected data and other relevant information. Yes [ ] No [ ] initials [ ]

12. I freely and voluntarily consent to participating in this research study. Yes [ ] No [ ] initials [ ]
PARTICIPANT'S NAME

Contact Address

Phone number: Email:

Participant's signature: Date:

Name of person taking consent: Signature: Date:

Researcher: Signature: Date:
Appendix IV – Questionnaire on Screening

PATIENT’S EXPERIENCE OF GESTATIONAL DIABETES SCREENING

NAME

MRN

TELEPHONE NUMBER

(i) PHYSICAL

Did you experience –

Hunger

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

Lightheadedness /Dizziness

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

Loss of consciousness

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

Nausea

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

Vomiting

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

Pain

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

Anxiety

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

Reduced fetal movements
(2) Jitters/Shakes/Tremors

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

Braxton Hick’s contractions (painless abdominal tightening)

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

(2) ANXIETY

Did you experience;

**Worry/Anxiety about your own general health?**

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

**Worry/anxiety for your baby’s general health?**

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

**Worry/Anxiety about the results?**

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little</th>
<th>A moderate amount</th>
<th>A lot</th>
<th>Very severely</th>
</tr>
</thead>
</table>

Do you think this test will be

- positive (you have gestational diabetes)

- negative (you don't have gestational diabetes)

(3) CONVENIENCE

**Was this test scheduled along with another appointment at the hospital?**

| Yes | No |

| Yes | No |

Did you need to take time off work for this test?

| Yes | No |

| Yes | No |

If so how many hours?

Did you need to organise additional childcare?

**How would you rate the convenience of this test**
<table>
<thead>
<tr>
<th>Very Convenient</th>
<th>Somewhat convenient</th>
<th>Neither convenient nor inconvenient</th>
<th>Somewhat inconvenient</th>
<th>Very inconvenient</th>
</tr>
</thead>
</table>

FOR THE INVESTIGATORS

(4)

<table>
<thead>
<tr>
<th>Screen Positive</th>
<th>Screen Negative</th>
</tr>
</thead>
</table>

(5) Post-natal Perception – TELEPHONE CALL / 6 week postnatal GTT
Knowing what you know now are you happy or unhappy that you had a glucose tolerance test for gestational diabetes?
In a future pregnancy would you undergo screening for gestational diabetes if your doctor advised it?

---

i Rotunda Hospital Annual Clinical Report 2014
ii Rotunda Hospital Annual Clinical Report 2014